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Zilpaterol hydrochloride

Residue Monograph

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8. Zilpaterol hydrochloride

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

Background

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

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

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

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

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

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

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

Overview of previous assessment

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

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

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

Radiolabelled residue depletion studies conducted in cattle after treatment at the recommended dose of 0.15 mg/kg bw/day demonstrated that a steady state is achieved by 12 days on treatment. Residues were detected in liver and kidney until 96 h post-dose. No residues were detected in

fat after 12 hours, and no residues were detected in muscle after 48 hours. Extractable residues from liver decreased from 52% to 24 % between 12 h to 96h, and from 89 % to 38 % for kidney over the same time period. Residues in muscle are approximately 100% extractable between 12 and 48 h.

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

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

Residues in food and their evaluation

Pharmacokinetics and metabolism

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

Pharmacokinetics and metabolism in laboratory animals

Rats

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

Note that the same tissues were not always collected between male and female rats. The $R_{t/p}$ results of the study conducted to determine the tissue distribution of zilpaterol as a function of time in the rat after a single oral dose administration showed that the radioactivity concentration of the drug depletes between 0.5 h and 24 h for all tissue matrices and organs of the males or females tested. Kidneys and liver involved in the metabolism and elimination of zilpaterol hydrochloride and its metabolite displayed the highest $R_{t/p}$. At 24 h, the residual radioactivity was low and there was no retention in the organ samples, with no marked difference between male and female rats.

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

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

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

The $R_{t/p}$'s measured for plasma, liver, kidneys, skeletal muscle and lung tissues are given in Table 8.2 for the male rats used in the above study and sacrificed at 0.5 and 24 h after the oral dose. These results show that the concentration of residues likely to be found in muscle are lower than would be found in kidney, liver and lung tissue.

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

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

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

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

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

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

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

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

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

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

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

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

Dogs

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

Humans

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

Pharmacokinetics and metabolism in food producing animals

Cattle

A GLP-compliant study reviewed by the 78th meeting of the Committee was conducted with four Salers steers and four Charolais × Salers heifers averaging 295 kg bw and allotted to four groups of two animals, each group comprising one steer and one heifer (Tulliez, 1992). The first group was kept on feed supplemented with unlabelled zilpaterol and was used as control. Animals in the three other groups were given a single dose of 0.2 mg/kg bw of [^{14}C]zilpaterol hydrochloride by gavage of the pellet and were killed at 12 h, 48 h and 8 days, respectively. Plasma was collected from each animal during the first 10 h and then at the 14th, 21st and 24th hours, and then every day until they were killed. Urine and faeces were collected daily from the individual animals for the 8-day surviving animals. At kill point, liver, kidneys and samples of muscle (*longissimus dorsi*), perirenal and visceral fat and of the four stomachs were excised and frozen until analysis. There was a rapid increase in radioactivity in plasma, which reached a maximal value 12 h and 10 h following drug administration in the male and female, respectively. The corresponding highest concentrations in plasma were 16.8 ng/ml and 22.4 ng/ml zilpaterol equivalents. Depletion of radioactivity in plasma occurred on a biphasic basis. The $T_{1/2}$ for the first phase was observed at 11.9 and 13.2 h for male and female, respectively. The second phase corresponded to a very slow decrease of radioactivity, but could not be described accurately because the signal was not significantly different from the background. Over 90% of the dose (97% in steers and 93% in heifers) was excreted over the 8 days (Table 8.5).

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

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

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

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

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

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

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

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

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

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

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

Tissue residue depletion studies

Radiolabelled residue depletion studies

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

Cattle

A GLP-compliant study reviewed by the 78th meeting of the Committee was conducted in which 17 healthy Hereford cattle (9 steers, 6 heifers) weighing between 200 and 230 kg were allocated into six groups (Tulliez, 1999, V-0238-0158). Group I (1 male and 1 female) was a non-medicated group designed to provide control samples. Each of the remaining Groups (II–VI) comprised 3 animals (2 males and 1 female, or the opposite). During the experimental period, each animal received the radiolabelled [**^{14}**C]-zilpaterol and unlabelled zilpaterol at 0.15 mg/kg bw/day for 12 days. The Group II animals were killed

12 h after the last dose on the 12th day, Group IV 24 h, Group V 48 h and Group VI 96 h after the last dose. Group III animals were fed for 15 days and killed 12 h after the last dose. Liver samples were collected as follows: LL – left lobe; RL – right lobe; CL – caudate lobe; SL – square lobe. Adipose tissue was either PR – perirenal; or SC – subcutaneous. A validated liquid chromatographic/fluorescence method was used for the analysis of zilpaterol and zilpaterol metabolites in edible tissues and fat of cattle. The tissue samples were analysed for total radioactivity (Table 8.8), percentage of extractable radioactivity (Table 8.9), as well as for unchanged zilpaterol and deisopropyl-zilpaterol metabolite by HPLC with radiometric detection (Table 8.10).

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

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

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

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

A mass balance for unchanged zilpaterol and its metabolites in tissues was calculated from the recovery of the radioactivity after different extraction steps. Labelled zilpaterol and labelled metabolites were extracted from liver, kidney and muscle using an ammonia-acetonitrile-methanol mixture and then purified by solid phase extraction. Liver was again the tissue containing the highest total residue concentrations, expressed as zilpaterol HCl-equivalents, with concentrations of 291 ± 56, 205 ± 14, 157 ± 23, and 113 ± 17 µg/kg at 12, 24, 48 and 96 h, respectively, after the last dose for the animals administered 12 daily doses of the drug (Table 8.8). The next highest total residue concentrations were observed in kidney, with concentrations of 184 ± 31, 100 ± 5, 37 ± 25 and 9 ± 4 µg/kg at 12, 24, 48 and 96 h, respectively, after administration of the final dose. The total residue concentration in muscle was already very low 12 h after the last dose, at 22 ± 2 µg/kg, 12 ± 3 µg/kg at 24 h, and depleted to non- detectable concentrations by 96 h after the last dose.

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

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

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

[a] This table contains data previously reported in Table 10.23 published in the 78th JECFA monograph (FAO, 2014).
[1]TRR = Total radioactive residue (as Zilpaterol HCL equivalents). ERR = Extracted radioactive residue (as Zilpaterol HCL equivalents).
[2]MR = Parent zilpaterol (Marker residue) determined by radio-HPLC.
[3]MR = Parent zilpaterol (Marker residue) measured by HPLC/fluorescence. ND = not detected. NQ = Not quantifiable. NA = applicable.

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

zilpaterol were the predominant compounds found in the extractable residues from tissues. Parent zilpaterol was approximately 4–8 times more abundant than the deisopropyl-zilpaterol.

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

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

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

Parent zilpaterol was also measured by a validated HPLC/FL method (Table 8.9). At 12 h, it represented 28 ±7% of the total radioactivity residue (TRR) and 54 ± 8% of extracted radioactive residue (ERR) in liver. The MR:TRR and MR:ERR ratios decreased with time to reach, respectively, 1.2 ± 0.1 and 5.2 ± 0.3% at 96 h. For kidney, a similar trend was observed. Zilpaterol residues in liver show a biphasic curve of depletion for total radioactive residue related to a slow decrease of non-extractable radioactive residue. It should also be noted that there was a difference in the sensitivities of the radiometric versus the fluorescence detection methods used for the quantification of zilpaterol hydrochloride.

Residue depletion studies with non-radiolabelled drug

Cattle

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

the final dose via medicated feed, one group of animals was killed at each of 12, 24, 48 or 96 h post-dose. Two animals were non-medicated control animals. These animals were considered representative of standard feedlot cattle.

Samples of liver, muscle and kidney from the four-day withdrawal study were assayed by the validated HPLC/FL method (Table 8.11). Recoveries of marker residue were 91.8 ±3.72%, 86.1 ±13.9% and 98.4 ±4.57%, respectively, for the liver, muscle and kidney. The LOQs for the method were 3 µg/kg, 1 µg/kg and 1 µg/kg, respectively, for liver, muscle and kidney, while LODs were 1 µg/kg, 0.1 µg/kg and 0.5 µg/kg, respectively, for the liver, muscle and kidney. The mean concentrations of zilpaterol in liver depleted from 28.3 µg/kg 12 h after the last 12th-day dose to 11.4 µg/kg 24 h after the last dose and to 4.5 µg/kg 48 h after the last dose. At 12, 24 and 48 h after the last dose, the concentrations of residues in kidney were 51, 13 and 6 µg/kg, respectively. Notable in this particular study, the residue concentrations in kidney were slightly higher than the residue concentrations in liver. This is contrary to all other zilpaterol residue depletion studies in cattle.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

LOQ = 2.0 µg/kg

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

Another GLP-compliant study was conducted to determine the depletion over time of the marker residue, zilpaterol free base, following administration of Zilmax® to male and female finishing cattle (Crouch et al., 2015). Zilpaterol was administered for 30 days as a medicated complete Type C feed at dietary

concentrations required to provide 60 or 90 mg zilpaterol hydrochloride/head/day. The 84 pool animals from which the study animals were selected were commercial breed steers and heifers (Angus and Angus cross). They received no treatment upon arrival at the testing facility and no treatment was administered at any subsequent time. Animals at arrival to the feedlot ranged from 388-523 kg and were approximately 12 months old.

Thirty eight steers and 38 heifers selected from the pool were randomized by body weight within sex to three Zilmax dose level groups: Group 1 = control (1 steer and 1 heifer plus one spare steer and heifer), Group 2 = 60 mg zilpaterol HCl per head per day (18 steers and 18 heifers), Group 3 = 90 mg zilpaterol HCl per head per day (18 steers and 18 heifers). The medicated groups were further subdivided by post-medicated feed withdrawal time in sub-groups of 3 steers and heifers each. Groups A, B, C, D, E and F corresponded to 12, 24, 48, 72, 120 and 240 hours withdrawal period, respectively (Table 8.15). Treated animals were kept in pens of 18 per sex, and controls in pens of 2 per sex. Animals when assigned to the study groups weighed from 403 - 535 kg. They were fed a complete non-medicated Type C feed *ad libitum* except during the 30 day treatment period for the medicated animals. The complete medicated Type C feed zilpaterol HCL concentration in the daily batches was adjusted as needed to ensure that the average daily zilpaterol consumption per head per pen remained at least at the targeted 60 (Group 2) or 90 mg (Group 3)

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

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

Group ID	Withdrawal Period (h)	Number of animals (Steers / Heifers)	Mean zilpaterol free base ± S.D. (µg/kg)		
			Liver	Muscle	Kidney
Control (0 mg zilpaterol HCl)					
1	NA	2 (1/1)	< LOD	< LOD	< LOD
60 mg zilpaterol HCl/animal/day for 30 days					
2A	12	6 (3/3)	42.0±16.3	5.66±1.97	37.7±4.37
2B	24	6 (3/3)	10.1±5.81	1.23±0.53	10.2±1.46
2C	48	6 (3/3)	1.58±0.97	0.82±0.79	1.93±0.79
2D	72	6 (3/3)	0.48±0.25	0.32±NA	0.46±0.041
2E	120	6 (3/3)	0.38±NA	BLQ±NA	BLQ±NA
2F	240	6 (3/3)	0.29±NA	0.251±NA	BLQ±NA
90 mg zilpaterol HCl/animal/day for 30 days					
3A	12	6 (3/3)	35.9±12.28	4.92±1.42	27.9±0.89
3B	24	6 (3/3)	15.6±5.22	1.84±0.55	9.8±0.21
3C	48	6 (3/3)	4.49±1.54	0.74±0.16	5.47±0.08
3D	72	6 (3/3)	1.22±0.34	0.306±0.04	1.02±0.02
3E	120	6 (3/3)	0.27±0.02	BLQ±NA	0.36±0.03
3F	240	6 (3/3)	0.59±0.14	BLQ±NA	0.47±0.03

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

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

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

Evaluation of zilpaterol residues in lungs and other edible offal

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

Residue data from some cattle tissues other than liver/kidney/muscle/fat are provided in a study evaluated by the Committee (Tulliez J., 1992). In this study, [^{14}C]zilpaterol hydrochloride was adsorbed onto a cellulose plug and administered by oral gavage at a dose of 0.2 mg zilpaterol hydrochloride per kg bw. Animals were sacrificed 12 hours, 48 hours and 8 days after dosing. Total residues were determined in the liver, muscle, visceral fat, perirenal fat; as well as in tripes (rumen, reticulum, omasum and abomasum). Total residues concentrations in the tripes were of the same order of magnitude as in kidneys at 12 and 48 hour withdrawal periods. Residues were not detected in the tripes at 8 days. See Table 8.6 for further details. There are no data for residues in the other tissue matrices listed in the two described definitions (brain, heart, pancreas, etc).

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

Methods of analysis for residues in tissues

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

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

In brief, samples of homogenized bovine tissue (1.00 ± .0500 g) was fortified with a stable label internal standard (d7-zilpaterol free base) and extracted with 2 x 5 mL of methanol. A sub-sample of the extract was purified by cation exchange SPE and then analysed by a validated liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) using electrospray ionization in the positive ion mode. Quantification was performed using a solvent calibration curve with a range of 0.25 to 30 µg/kg tissue

equivalents for all tissues. The limit of quantitation (LOQ) is 0.250 µg/kg for all tissues and the limit of detection (LOD) is 0.0479, 0.0673 and 0.0448 µg/kg for liver, muscle and kidney, respectively. The average recovery of zilpaterol in the methanol extracts was determined to be 76% (liver), 85% (kidney), and 73% (muscle). The analytical parameters of the method in liver, kidney and muscle tissues are summarized in Tables 8.16a-8.16c. The validated method provided by the Sponsor was considered to be adequate for effective residue control of zilpaterol.

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

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

n = 6 per run. QC: quality control.

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

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

n = 6 per run. QC: quality control.

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

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

n = 6 per run. QC: quality control.

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

Comments from the Sponsor:

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

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

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

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

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

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

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

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

JECFA response to request from 22nd CCRVDF

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

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

Before re-evaluation of zilpaterol with the aim of recommending MRLs in lungs and other edible offal of cattle, the Committee would require marker residue depletion data in such tissues over an appropriate

withdrawal period (such as 72 – 96 hours). The Committee noted that the definitions of the tissues comprising offal were not consistent between countries.

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

Appraisal

Evaluation of pharmacological activity of zilpaterol residues

Comment from Sponsor

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

Response from JECFA:

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

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

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

b) Assessment of bioavailability of zilpaterol residues

When assessing the bioavailability of drug residues, the Gallo-Torres model (whereby the bioavailability of non-extractable or bound residues is considered in the human exposure assessment) has been utilized by numerous agencies, including the United States Food and Drug Administration (FDA-CVM, 2006) and the European Medicines Agency (EMA, 2008a; EMA, 2008b). JECFA follows this approach, as described

in Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 8: Maximum Residue Limits for Pesticides and Veterinary Drugs (FAO/WHO, 2009), first used by the 34th meeting of the Committee (FAO/WHO, 1989). The current Committee agreed that such an approach is appropriate for zilpaterol, given the data provided in the study **(**Girkin, 1999) in which rats were fed liver from zilpaterol-treated cattle (see Table 8.4). A bioavailability factor of approximately 5% for bound zilpaterol residues was considered appropriate by the Committee. The bioavailability of non-extractable residues in kidney and muscle was not determined in the study. The Committee however agreed that the same oral bioavailability of 5 % can conservatively be applied for bound residues in kidney and muscle.

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

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

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

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

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

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

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

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

Evaluation of the various zilpaterol residue depletion data sets

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

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

- Only the 90 mg/head/day group was used in the exposure assessment, as this was the highest dose studied (and highest approved label dose). Pooling these data with the other dose group (60 mg/head/d) from the same study was not considered statistically appropriate due to differences in mean concentrations and numbers of concentrations above the LOQ at 120 h. The zilpaterol free base concentrations over time for each tissue are shown in Figures 8.1A-1C.
- Although data were collected at 12, 24, 48, 72, 120, and 240 hour withdrawal times, only data up to and including 120 hours were used to estimate the rate of depletion of free zilpaterol (i.e., used in the regression analysis). As the depletion study with radiolabelled drug (Tulliez, 1999) covers the period up to 96 hours, it was considered acceptable to use the new depletion data using non-pradiolabelled drug (Crouch, 2105) dataset until 120 h to perform the linear regression.
- The data set was sufficient to calculate percentile concentrations and corresponding one-sided 95% confidence interval over the 95th percentile of residue concentrations (95/95 upper tolerance limit, or UTL) associated with the residue depletion profiles, and to assess residue exposure and MRLs consistent with approved uses (Good Veterinary Practices, GVP). The 95/95 UTL were estimated until 96 h, which is consistent with the withdrawal times applied according to current GVP.
- The marker residue data provided (Crouch, 2015) confirm that the depletion curves are parallel for liver, kidney, and muscle, indicating comparable depletion profiles. It was noted that zilpaterol concentrations below 1 µg/kg were observed in liver and kidney at 240 h which suggests a terminal elimination phase with a long half-life.
- The LOQ (0.25 µg/kg) of the analytical method used in this residue depletion study was sufficient to identify/monitor the residue depletion over an adequate time period after the last administration (up to 96 hours). Recoveries of residues from QC samples were typically close to 100 % and, therefore, no recovery correction was deemed necessary. The validated method provided by the Sponsor was considered to be adequate for effective residue control of zilpaterol.

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

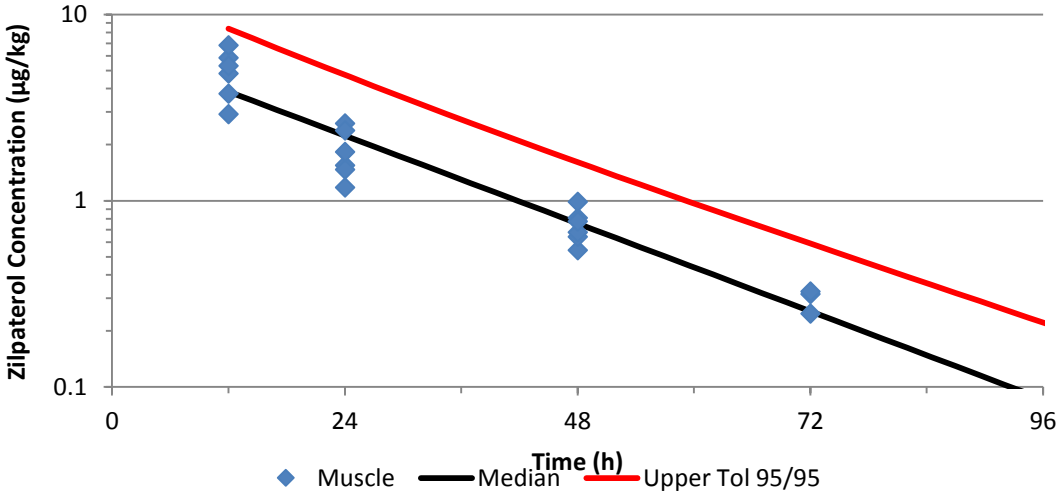


Figure 8.1B: Zilpaterol free base concentrations in kidney from S14078 (90 mg dose).

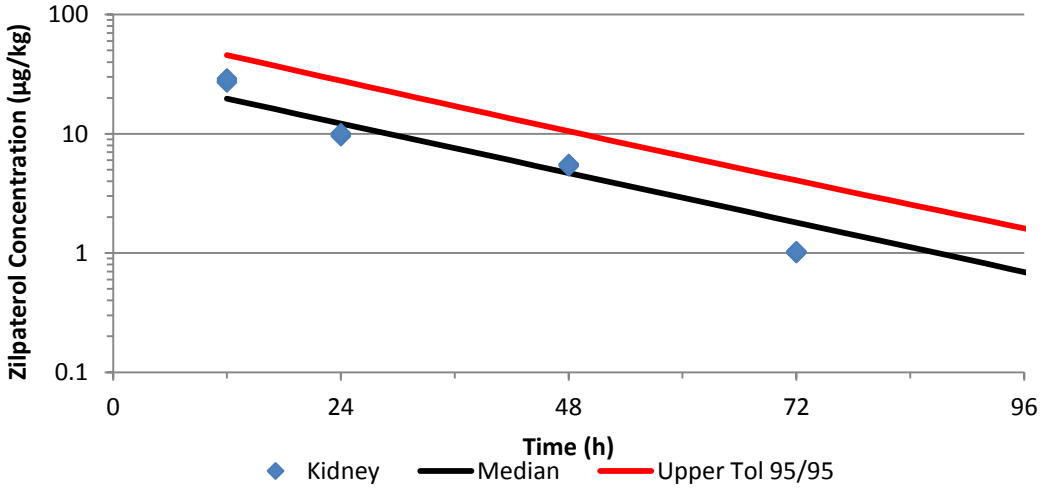
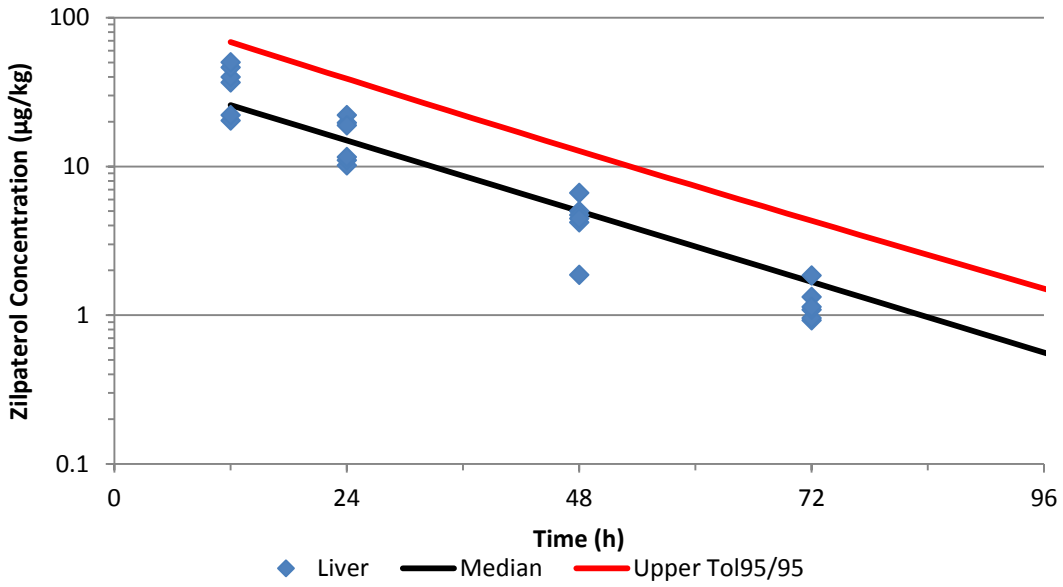


Figure 8.1C: Zilpaterol free base concentrations in liver from S14078 (90 mg dose).



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

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

Evaluation and quantification of the zilpaterol residues of concern

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

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

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

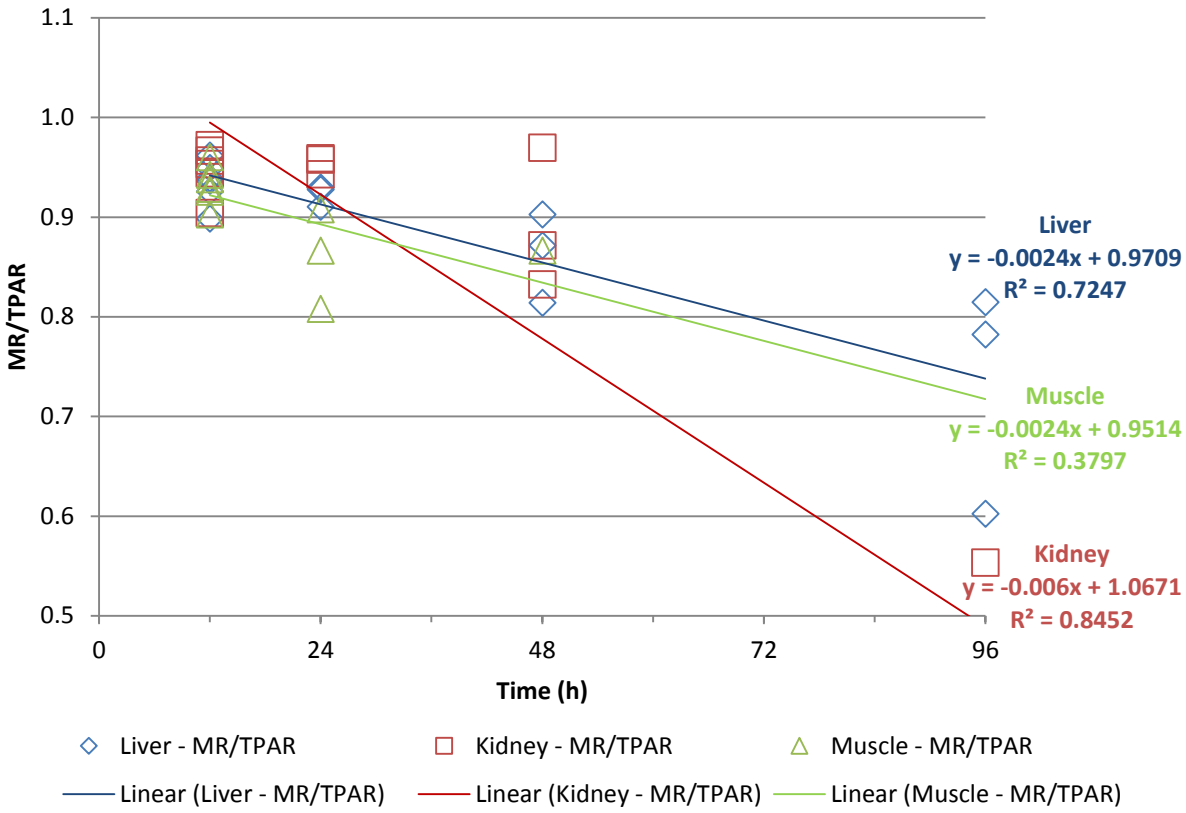
Where:

- Zilpaterol HCl = parent zilpaterol concentration, expressed as zilpaterol hydrochloride;

- 0.1 = relative pharmacological activity correction factor. The activity attributed to zilpaterol HCl was set as 1, whereas activity of all other extractable and bioavailable non-extractable residues was set as 0.1 (i.e., 10% of the parent zilpaterol activity);
- RRExt = sum of other extractable radioactive residue concentrations (including the major metabolite deisopropyl zilpaterol), expressed as zilpaterol HCl-eq;
- RRNonExt= non-extractable (bound) radioactive residue concentration, expressed as zilpaterol HCl-eq
- 0.05 = oral bioavailability of non-extractable residues (as per the Gallo-Torres model used by the 34th meeting of the Committee; FAO/WHO, 1989).

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

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



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

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

Where:

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

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

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

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

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

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

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

Dietary estimates and zilpaterol residue exposure

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

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

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

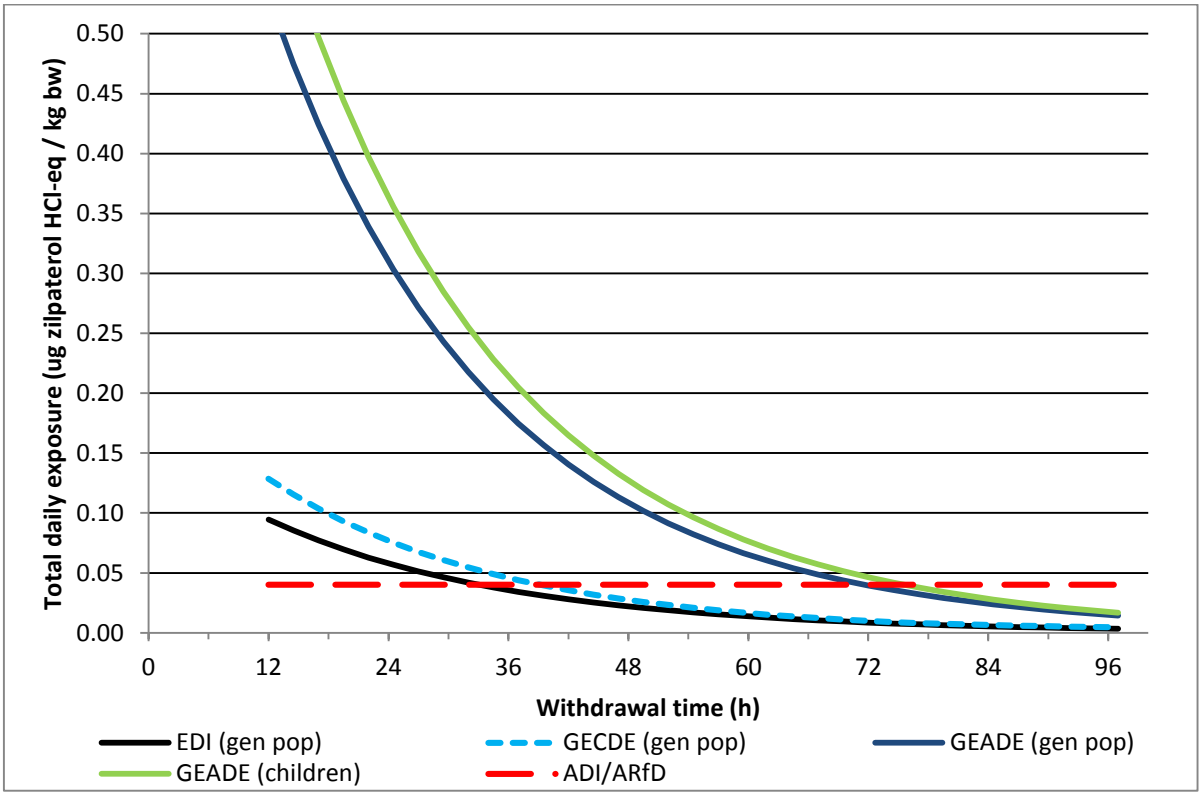
As the ADI for zilpaterol is based on an acute pharmacologic endpoint (immediate β-agonist activity), an acute exposure assessment was deemed most appropriate for the assessment of dietary exposure. The Global Estimated Acute Dietary Exposure (GEADE) approach was therefore used to estimate acute dietary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

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

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

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

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



Estimates of chronic dietary exposure

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

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

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

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

Table 8.18. Estimated Dietary Intake (EDI) of zilpaterol HCl residues at 72 hour withdrawal.

Tissue	Median MR concentration[1] (µg/kg)	MR: TPAR ratio[2]	MW ZHCL/ MW Z[3]	Median total pharmacologically active residue[4] (µg zil HCl-eq/kg)	Standard Food Basket (kg)	Daily intake (µg)
Muscle (Beef&other Bovines)	0.25	0.78	1.1395	0.4	0.3	0.1
Liver (mammalian)	1.67	0.80	1.1395	2.4	0.1	0.2
Kidney (mammalian)	1.81	0.63	1.1395	3.3	0.05	0.2
			TOTAL			0.5

[1]Median zilpaterol free base concentration at 72 hours.
[2]Ratio at 72 hours.
[3] Ratio of molecular weight Zilpaterol HCl to Zilpaterol free base = 1.1395.
[4]Total pharmacologically active residue = 1.1395* [Zilpaterol free base]/$R_{tissue(t)}$

Table 8.19. The global estimated chronic dietary exposure (GECDE) to adjusted zilpaterol HCl median residues (72 hours withdrawal) in the general population and in children.

Category	Type	Mean consumption[1] whole population, g/day	97.5th consumption[2] consumers only, g/day	Exposure (µg/kg bw/day) Mean	Exposure (µg/kg bw/day) 97.5th	GECDE[3] µg/kg bw/day	GECDE[3] %ADI
General population							
Mammalian muscle	Beef/other Bovines	63	291	0.00038	0.00177	0.00038	1.0
Mammalian offal	Mammalian liver	2	111	0.00008	0.00440	0.00008	0.2
Mammalian offal	Mammalian kidney	0.5	166	0.00003	0.00906	0.00906	22.6
TOTAL				0.00046	0.00906	0.00952	24
Children							
Mammalian muscle	Beef/other Bovines	37	159	0.00090	0.00387	0.00090	2.3
Mammalian offal	Mammalian liver	0.5	62	0.00008	0.00983	0.00983	24.6
Mammalian offal	Mammalian kidney	0.05	19	0.00001	0.00415	0.00001	0.0
TOTAL				0.00098	0.00983	0.01074	27

[1] highest mean consumption figures based on whole population considered from the available dataset.

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

Estimates of acute dietary exposure

The definition of high-level consumers is crucial to the outcome of an acute exposure estimate. The reliability of high percentile consumption data is related to the number of subjects used to calculate them; percentiles calculated on a limited number of subjects should be treated with caution as the results may not be statistically robust. When the number of observations is not large enough, the coverage probability may not attain the nominal value, and drops below, for example, 95%. This is more likely to occur at high percentiles such as the 97.5th. Therefore, the coverage probability can be used to set guidelines to determine the minimum number of samples for which 97.5th percentiles can be computed. In the case of significance level (α) being set at 0.05 to determine a 95% confidence interval, the coverage probability should target 95%. This is achieved for observations where n >70 for the 97.5th percentile. Therefore, a cut-off of n =70 has been used for consumption data used as inputs into acute dietary exposure assessment for zilpaterol HCl.

For the purpose of undertaking the acute dietary exposure assessment for residues of zilpaterol HCl, an up-to-date individual food consumption database of animal tissues and food of animal origin expressed on a large portion (LP) sizes values, based on the 97.5th percentile of food consumption, were used by the Committee. The data were derived from records of individual consumer days (i.e. survey days on which the food or foods of interest were consumed) reported in individual-level survey data from 25 countries (Australia, Brazil, China and 22 European countries) and summarized in the EFSA Comprehensive European Food Consumption Database (EFSA, 2015). Those data were previously collected following a request to Member countries as part of the Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs (WHO, 2012). The following rules were followed for selecting consumption amounts as inputs:

- For the complete database, where the highest reported 97.5th percentile tissue consumption reported for a country had consumer numbers larger than 70 this value was selected as an input for acute dietary exposure.
- Where the maximum 97.5th percentile reported had consumer numbers less than 70, the reported observations from the complete database were pooled and treated as independent observations.
- If the total number of consumers of the pooled observations was more than 70, the 97.5th percentile was calculated and used as the input. If the total number of consumers was less than 70, the median was calculated and used as the input.

Table 8.20 shows the consumption data selected for the assessment. The highest 97.5th percentiles reported for individual countries were used as inputs for consumption of muscle for the general population and children. For liver, the highest reported 97.5th percentile for an individual country was used for adults but samples were pooled to derive the 97.5th percentile

for children. For kidney, the observation numbers were low, so pooled observations were used to derive the 97.5th and 50th (median) percentiles for the general population and children respectively.

The acute exposure to total pharmacologically active zilpaterol residues (expressed as zilpaterol HCl equivalents) was estimated from the 95/95 UTLs determined by regression analysis after 72 hours withdrawal (see Tables 8.17 & 8.22, Figure 8.3). The following 95/95 UTLs were derived: 4.1 µg/kg in kidney, 4.3 µg/kg in liver, and 0.6 µg/kg in muscle. Using acute dietary exposure assessments (GEADE), these 95/95 UTLs could lead to an acute dietary exposure of ~ 99% of the Acute Reference Dose (ARfD) in the general population and ~ 117% of the ARfD in children. Note that the Committee established the Acute Reference Dose (ARfD) for zilpaterol at 0.04 µg/kg bw, the same value as the upper bound of the previously-established ADI.

Because the acute exposure in children exceeded the ARfD using the residue depletion data at 72 hrs., the Committee considered a refined assessment with 95/95 UTLs derived at 77 hours post-dose: 3.3 µg/kg in kidney, 3.5 µg/kg in liver, and 0.5 µg/kg in muscle. The GEADE for the general population was approximately 0.032 µg/kg bw/day for the tissue with the highest exposure (beef liver). Exposure from beef kidney was lower (0.019 µg/kg bw/day) and potential exposure from consuming muscle tissue was much lower (0.006 µg/kg bw/day, or 14% of the ARfD) than that from consuming beef liver. For the general population, the GEADE (beef liver) represented 81% of the ARfD of µg/kg bw (Table 8.21).

For children, the GEADE was approximately 0.038 µg/kg bw per day for beef liver. As with the general population, potential exposure from muscle tissue was much lower (0.0001 µg/kg bw/day or 10% of the ARfD). Exposure to beef kidney was also much lower than for the general population because the comparatively lower consumption amount used for the children sub-population (refer to Table 8.20). For children, the acute dietary exposure estimate (beef liver) was 95% of the ARfD (Table 8.21).

Table 8.20. Consumer statistics calculated from 97.5th tissue consumptions (expressed in grams tissue/kg bw/day).

Cattle Tissue	97.5th General population consumption			97.5th Children consumption		
	max	**p97.5**	**Median**	**max**	**p97.5**	**Median**
Muscle	7.7	6.6	3.9	12.7	12.0	7.1
Liver	6.4	5.8	2.0	8.3*	7.5	2.8
Kidney	3.2*	3.0	1.5	12.9*	12.3*	2.1

Bold numbers used as inputs for exposure calculation.

*Number of total consumers <70.

Table 8.21. The global estimated acute dietary exposure (GEADE) to adjusted zilpaterol 95/95 UTL residues (77 hours withdrawal) in the general population and in children.

Category	Type	97.5th Consumption[1] g tissue/kg bw/day	GEADE[2] µg/kg bw/day	%ARfD
General Population				
Mammalian muscle	Beef and other Bovines	7.7	0.0055	14
Mammalian offal	Beef liver	6.4	0.032	81
Mammalian offal	Beef kidney	3.0	0.019	48
Children				
Mammalian muscle	Beef and other Bovines	12.7	0.0091	23
Mammalian offal	Beef liver	7.5	0.038	95
Mammalian offal	Beef kidney	2.1	0.013	33

[1] highest 97.5th food consumption figures considered from the available dataset representing a single eating occasion

[2] GEADE is the product of the 97.5th level of consumption multiplied with the 95/95 UTL pharmacologically active residue (see Table 8.17)

Derivation of 95/95 upper tolerance limits

At the 77 hour withdrawal time point, the 95/95 UTLs for zilpaterol free base as marker residue are 3.5, 3.3, and 0.5 µg/kg in liver, kidney, and muscle, respectively (Table 8.22). These 95/95 UTLs are appropriate for Maximum Residue Limits (MRLs) for zilpaterol.

It is noted that this time point is in the range of approved withdrawal periods for currently approved zilpaterol formulations (2 – 4 days).

Table 8.22. Zilpaterol MRL derivation for acute and chronic dietary exposure estimates.*****

Data from EDI S-14078 (90 mg dose)							**ADI = 0-0.04 µg zilpaterol HCl-eq/day** **ARfD = 0.04 µg zilpaterol HCl-eq/day**			
	Median zilpat free base (µg/kg)			**95/95 UTL (µg/kg)**			***CHRONIC* estimate (µg zilpaterol HCl-eq/kg bw/d)**		***ACUTE* estimate (µg zilpaterol HCl-eq/kg bw/d)**	
Time (h)	**Liver**	**Kidney**	**Muscle**	**Liver**	**Kidney**	**Muscle**	**EDI (median)**	**GECDE (adult)**	**GEADE (adult)**	**GEADE (child)**
12	25.81	19.67	3.85	68.6	45.8	8.4	0.095	0.129	0.531	0.622
14.5	23.03	17.81	3.44	60.9	41.3	7.4	0.085	0.115	0.475	0.556
17	20.55	16.12	3.07	54.1	37.2	6.6	0.077	0.104	0.424	0.497
19.5	18.33	14.59	2.74	48.0	33.6	5.9	0.070	0.093	0.379	0.444
22	16.36	13.21	2.45	42.7	30.3	5.2	0.063	0.084	0.339	0.397
24.5	14.60	11.96	2.19	37.9	27.3	4.6	0.057	0.075	0.303	0.355
27	13.02	10.83	1.95	33.7	24.7	4.1	0.051	0.067	0.271	0.318
29.5	11.62	9.80	1.75	30.0	22.3	3.7	0.046	0.061	0.243	0.285
32	10.37	8.87	1.56	26.7	20.1	3.3	0.042	0.054	0.218	0.255
34.5	9.25	8.03	1.39	23.7	18.1	2.9	0.038*	0.049	0.195	0.228
37	8.26	7.27	1.24	21.1	16.4	2.6	0.034	0.044	0.175	0.205
39.5	7.37	6.58	1.11	18.8	14.8	2.3	0.031	0.040*	0.157	0.184
42	6.57	5.96	0.99	16.7	13.4	2.1	0.028	0.036	0.141	0.165
44.5	5.86	5.39	0.89	14.9	12.1	1.9	0.025	0.032	0.126	0.148
47	5.23	4.88	0.79	13.3	10.9	1.7	0.023	0.029	0.113	0.133
49.5	4.67	4.42	0.71	11.9	9.9	1.5	0.021	0.026	0.102	0.119
52	4.17	4.00	0.63	10.6	9.0	1.4	0.019	0.023	0.091	0.107

54.5	3.72	3.62	0.56	9.5	8.1	1.2	0.017	0.021	0.082	0.096
57	3.32	3.28	0.50	8.4	7.3	1.1	0.015	0.019	0.074	0.087
59.5	2.96	2.97	0.45	7.5	6.6	1.0	0.014	0.017	0.067	0.078
62	2.64	2.69	0.40	6.7	6.0	0.9	0.013	0.015	0.060	0.070
64.5	2.36	2.43	0.36	6.0	5.5	0.8	0.011	0.014	0.054	0.063
67	2.10	2.20	0.32	5.4	4.9	0.7	0.010	0.012	0.049	0.057
69.5	1.88	1.99	0.29	4.8	4.5	0.7	0.009	0.011	0.044	0.051
72	1.67	1.81	0.25	4.3	4.1	0.6	0.009	0.010	0.040*	0.046
74.5	1.49	1.63	0.23	3.9	3.7	0.5	0.008	0.009	0.036	0.042
77	1.33	1.48	0.20	3.5	3.3	0.5	0.007	0.008	0.032	0.038*
79.5	1.19	1.34	0.18	3.1	3.0	0.4	0.006	0.008	0.029	0.034
82	1.06	1.21	0.16	2.8	2.8	0.4	0.006	0.007	0.026	0.031
84.5	0.95	1.10	0.14	2.5	2.5	0.4	0.005	0.006	0.024	0.028
87	0.84	0.99	0.13	2.2	2.3	0.3	0.005	0.006	0.021	0.025
89.5	0.75	0.90	0.12	2.0	2.1	0.3	0.004	0.006	0.019	0.023
92	0.67	0.81	0.10	1.8	1.9	0.3	0.004	0.005	0.018	0.021
94.5	0.60	0.74	0.09	1.6	1.7	0.2	0.004	0.005	0.016	0.019
97	0.54	0.67	0.08	1.4	1.5	0.2	0.003	0.005	0.014	0.017

*Colour denotes first time point at which the exposure estimate falls to the ADI/ARfD for zilpaterol and data used in calculation.

Maximum Residue Limits

In recommending MRLs for zilpaterol, the Committee considered the following factors:

- An ARfD of 0.04 µg/kg bw was established. This is the same value as the upper bound of the ADI previously established by the seventy-eighth Committee and reaffirmed by the present Committee.
- Zilpaterol HCl is registered to be mixed into feed at a level of 7.5 mg/kg on a 90% dry matter basis. This level provides a dose of approximately 0.15 mg/kg bw or 60–90 mg zilpaterol HCl per animal per day.
- Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.
- Zilpaterol HCl is not approved for use in lactating dairy cattle.
- The major metabolite in cattle tissues is deisopropyl zilpaterol.
- Zilpaterol HCl administration in cattle results in non-extractable residues that are poorly bioavailable in laboratory animals. This low oral bioavailability (~5%) demonstrated for liver was assumed to be similar for non-extractable residues in muscle and kidney.
- The most sensitive toxicological end-point is an acute pharmacological effect. It was assumed that zilpaterol HCl has a reference activity of 1. Deisopropyl zilpaterol was shown to have ~10% of the pharmacological activity of parent zilpaterol, with the activity of all other extractable and bioavailable non-extractable residues being equivalent to, or less than, that of deisopropyl zilpaterol.
- Parent zilpaterol (free base) was an appropriate marker residue in muscle, liver and kidney. Fat was not considered relevant for residue monitoring purposes.
- The ratios of zilpaterol (MR) to the total residues of concern (total pharmacologically active residues) for muscle, liver and kidney could be determined with sufficient confidence over a 96-hour period after the last drug administration. The MR:total pharmacologically active residue ratios were between 0.9 and 1.0 for liver, kidney and muscle at 12 hours withdrawal. By 96 hours withdrawal, the MR:total pharmacologically active residue ratios were 0.7 (liver and muscle) and 0.5 (kidney).
- A validated analytical procedure for the determination of zilpaterol in edible bovine tissues (liver, kidney, muscle) is available and may be used for monitoring purposes. The LOQ is 0.25 µg/kg for all tissues.

The MRLs recommended for bovine tissues are based on an acute dietary exposure scenario (GEADE). The Committee initially derived the following one-sided 95% confidence interval over the 95th percentile of residue concentrations (95/95 upper tolerance limit, or UTL) in bovine tissues at the 72-hour time point: 4.1 µg/kg in kidney, 4.3 µg/kg in liver and 0.6 µg/kg

in muscle. Using acute dietary exposure assessments (GEADE), these 95/95 UTLs could lead to an acute dietary exposure of ~ 99% of ARfD in the general population and ~ 117% of the ARfD in children.

Because the exposure in children exceeded the ARfD using the 72-hour data, the Committee considered a refined assessment with 95/95 UTLs derived at 77 hours post-dose: 3.3 µg/kg in kidney, 3.5 µg/kg in liver and 0.5 µg/kg in muscle. These values would result in acute dietary exposure (GEADE of 1.9 µg/day for the general population and 0.57 µg/day for children) of ~94% of the ARfD in children and ~80% of the ARfD in the general population and are recommended as MRLs. It is noted that the time point at which the MRLs are calculated (77 hours) is consistent with currently approved withdrawal times (GVP).

The Committee recognizes that the approach used in this evaluation differs from that of previous evaluations for similar types of veterinary compounds. However, this was appropriate due to the acute nature of the pharmacological end-point and the availability of an appropriate model for acute exposure. Detailed chronic and acute dietary exposure assessments are included in the addendum to the residue monograph to provide additional information to risk managers.

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

Recommendation

The Committee noted that the definitions of the tissues comprising offal were not consistent between countries. Therefore, JECFA requests that CCRVDF provide a definition of edible offal.

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