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Ivermectin

Residue Monograph

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4. Ivermectin

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

Background

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

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

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

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

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

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

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

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

Tissue residue depletion studies

Radiolabelled residue depletion studies

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

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

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

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

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

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

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

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

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

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

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

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

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

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

* Marker residue.

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

Residue depletion studies with non-radiolabelled drug

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

	Concentration of ivermectin B_{1a} residues in tissues									
	Injection Site (µg/kg)		**Regular muscle (µg/kg)**		**Liver (µg/kg)**		**Kidney (µg/kg)**		**Fat (µg/kg)**	
Rec. Factor	**1.198**		**1.198**		**1.392**		**1.385**		**1.215**	
Days Post-Dose	**OC**	**RCC**	**OC**	**RCC**	**OC**	**RCC**	**OC**	**RCC**	**OC**	**RCC**
	25.4	30.4	25.4	30.4	20.2	28.1	15.7	21.8	25.4	30.9
	24.7	29.5	24.7	29.5	42.1	58.6	4.4	6.1	15.1	18.4
	31.3	37.5	31.3	37.5	30.3	42.1	7.2	9.99	10.7	13.0
	40.2	48.2	40.2	48.2	50.5	70.3	8.1	11.2	22.2	26.9
Mean±S.D.	30.4±7.2	36.4±8.6	8.8±3.6	10.5±4.3	35.8±13.3	49.8±18.5	8.9±4.8	12.3±6.6	18.4±6.6	22.3±8.0
	8.3	9.9	8.3	9.9	12.2	16.9	0.5	0.69	14.6	17.8
	24.6	29.5	24.6	29.5	18.4	25.6	3.1	4.3	4.1	5.0
70	15.1	18.0	15.1	18.0	9.6	13.4	6.3	8.7	6.4	7.8
	12.3	14.7	12.3	14.7	4.5	6.3	4.0	5.6	9.3	11.3
Mean±S.D.	15.1±6.9	18.1±8.3	2.1±1.4	2.5±1.7	11.2±5.8	15.6±8.1	3.5±2.4	4.8±3.3	8.6±4.5	10.4±5.5
	3.1	3.7	3.1	3.7	9.9	13.7	0.25	0.35	4.8	5.8

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

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

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

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

ND = Not Detected.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Residues at the injection site

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

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

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

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

Methods of Analysis for Residues in Tissues

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

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

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

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

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

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

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

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

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

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

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

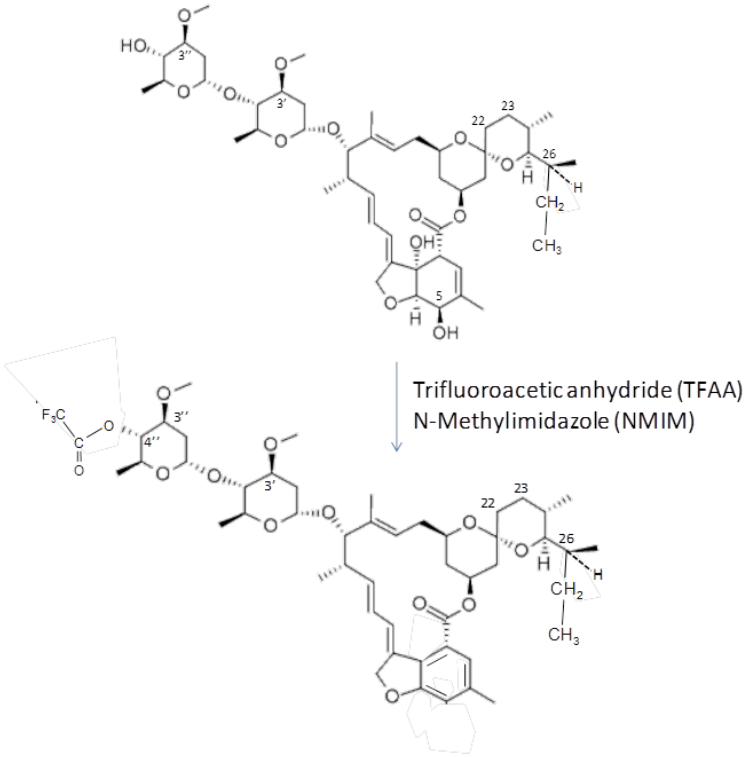

Figure 4.1. Reaction mechanism for the formation of the fluorescent ivermectin derivative.

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

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

Appraisal

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

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

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

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

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

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

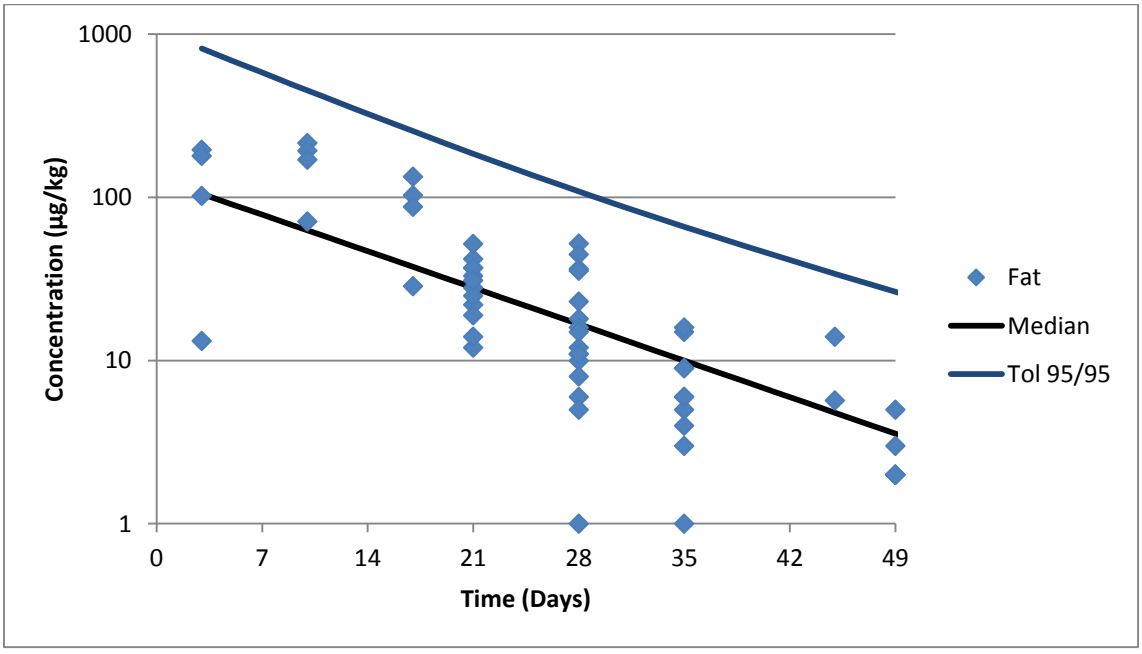

Figure 4.2. Median concentrations and upper tolerance limits of ivermectin B_{1a} in fat.

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

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

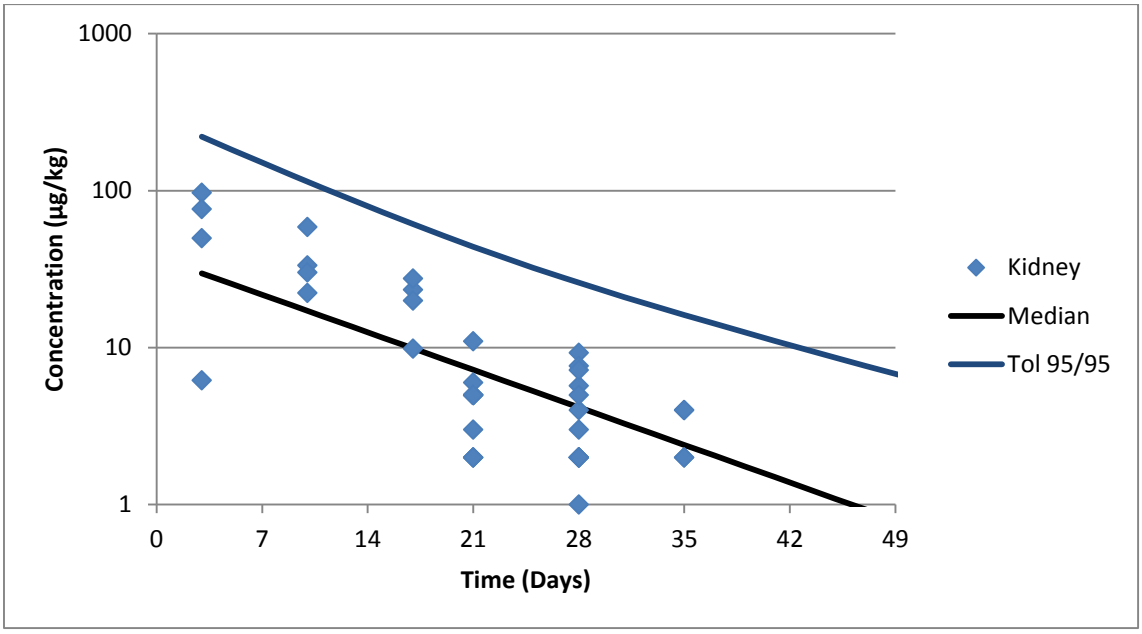

Figure 4.3. Median concentrations and upper tolerance limits of ivermectin B_{1a} in kidney.

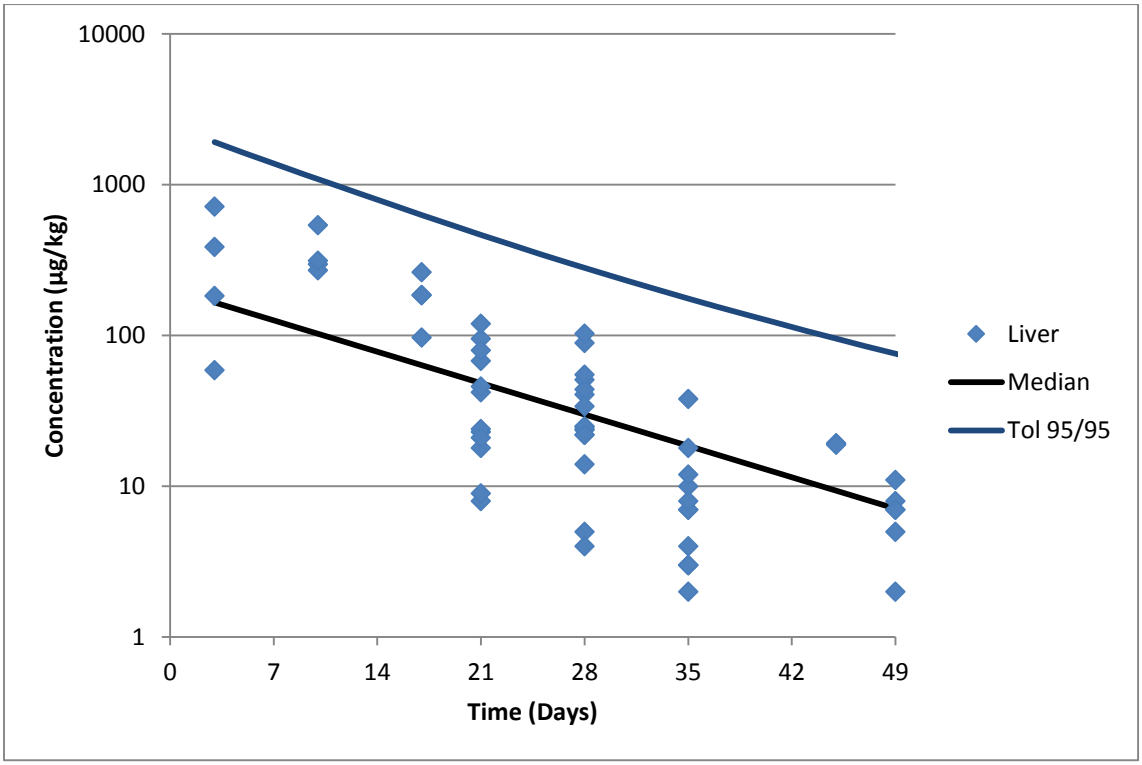

Figure 4.4. Median concentrations and upper tolerance limits of ivermectin B_{1a} in liver.

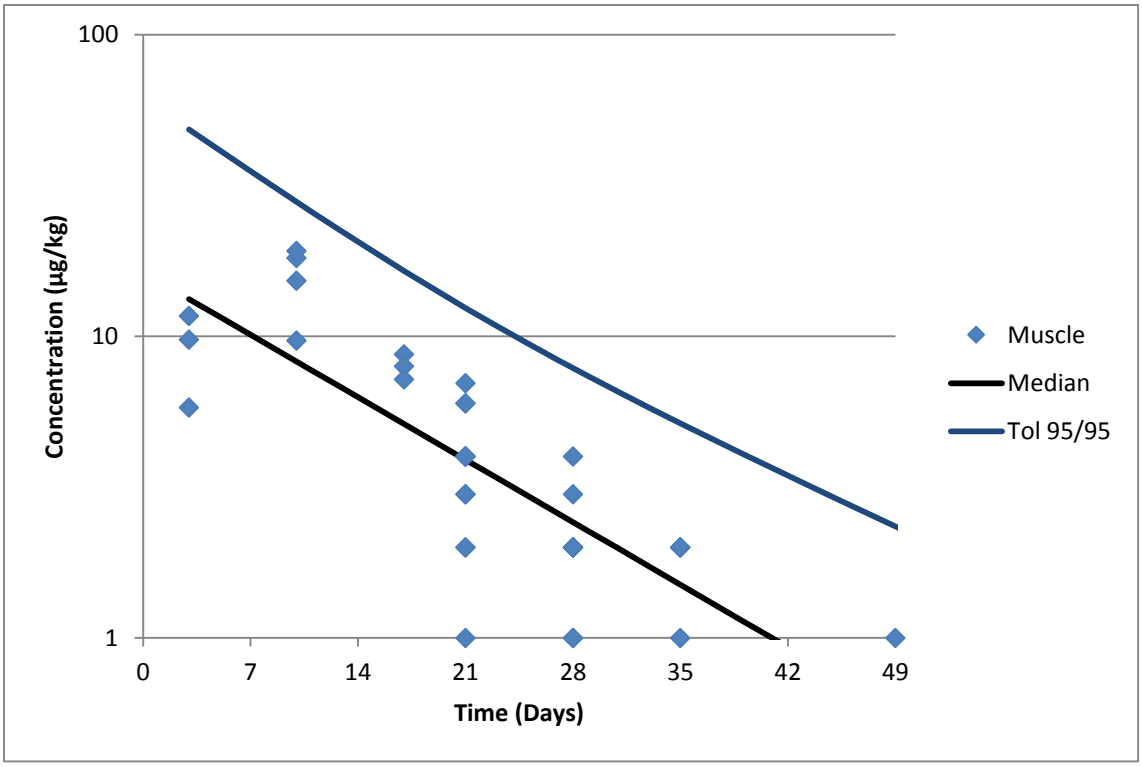

Figure 4.5. Median concentrations and upper tolerance limits of ivermectin B_{1a} in muscle.

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

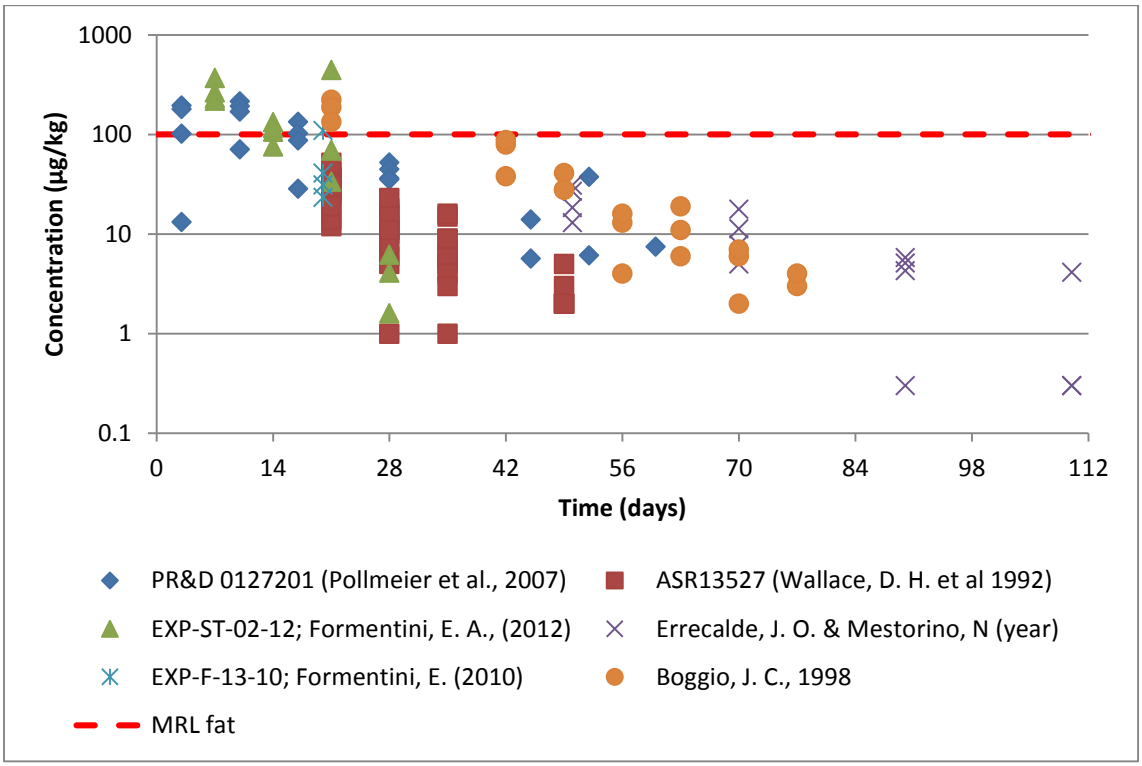

Figure 4.6. Derivation of MRLs from data provided for residues in fat.

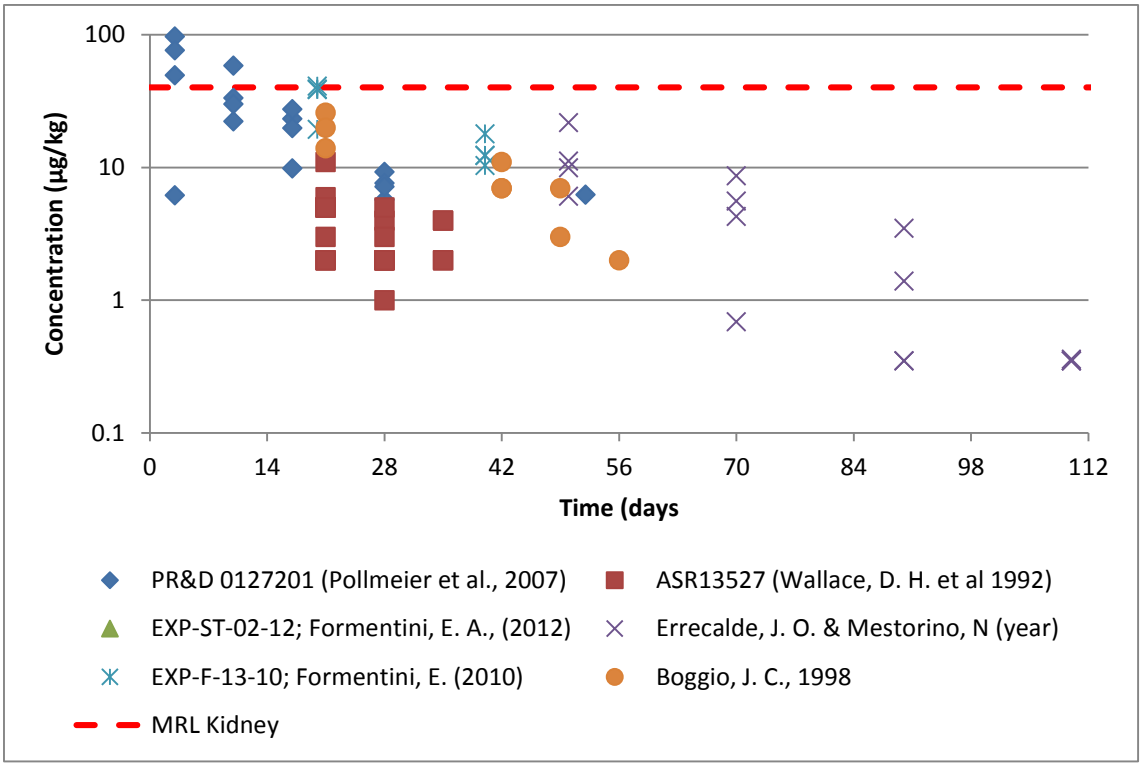

Figure 4.7. Derivation of MRLs from data provided for residues in kidney.

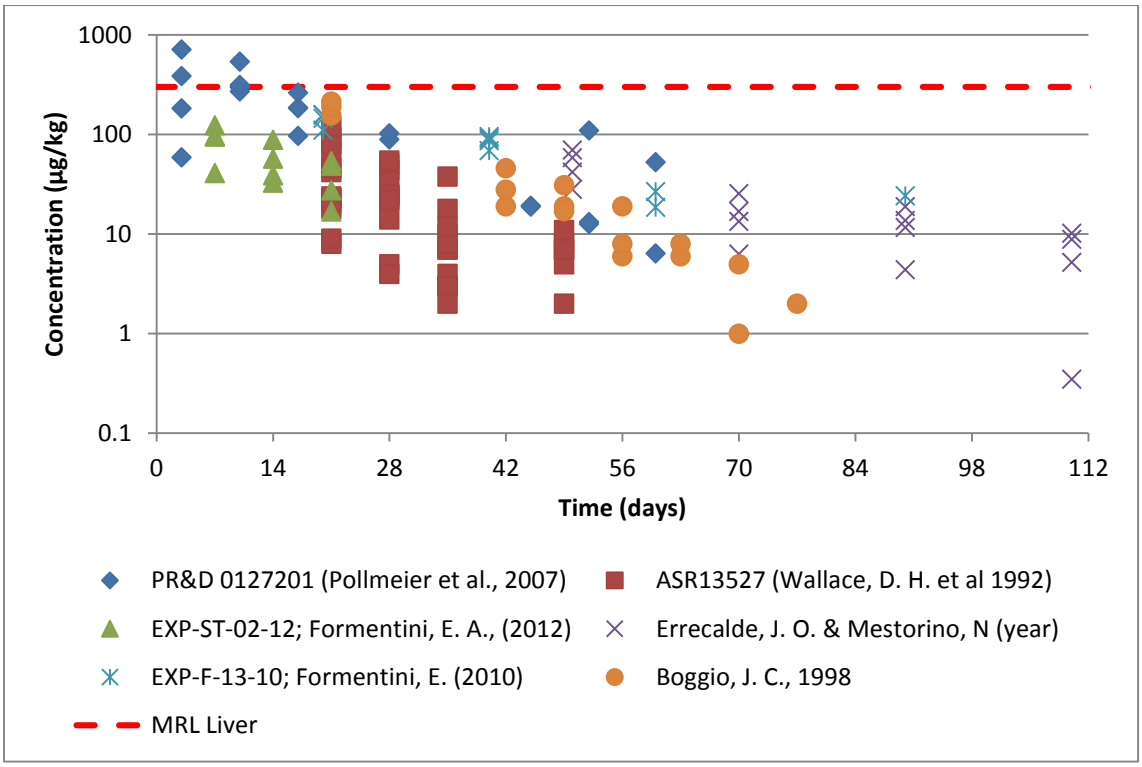

Figure 4.8. Derivation of MRLs from data provided for residues in liver.

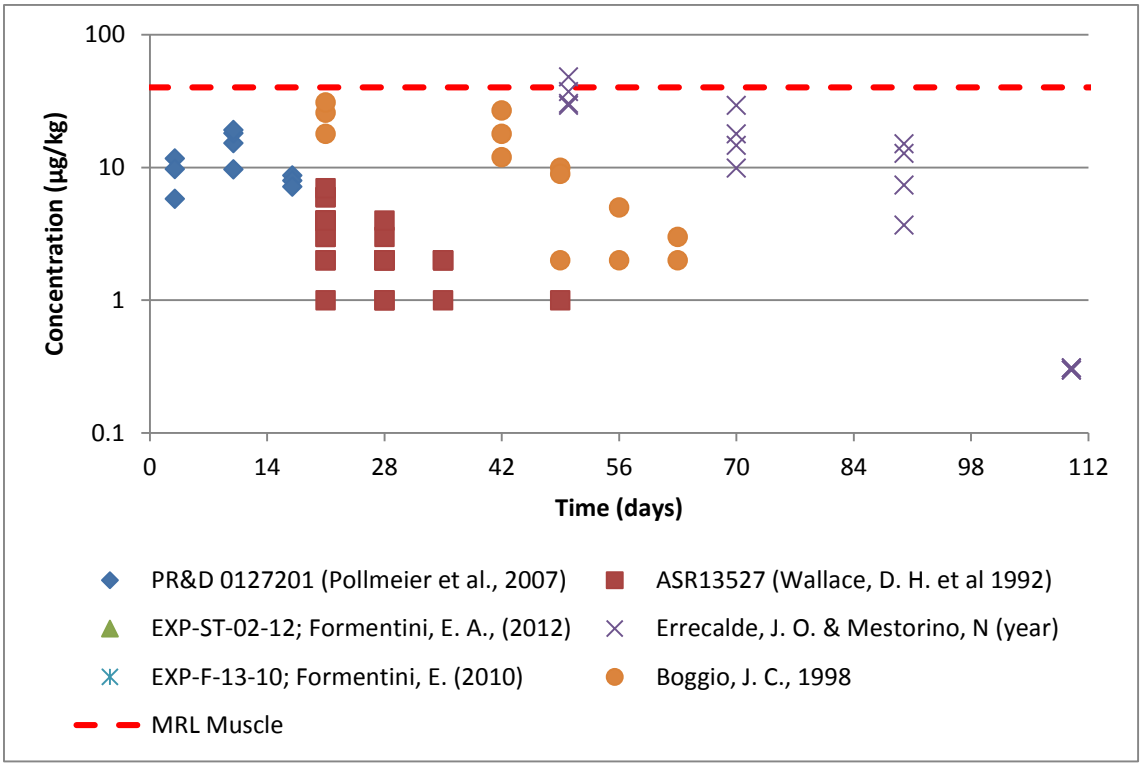

Figure 4.9. Derivation of of MRLs from data provided for residues in muscle.

Residues at the injection site

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

Acute dietary exposure assessment: injection site residues

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

was compared with the acute reference dose of 200 µg/kg bw established by the current meeting of the Committee.

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

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

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

A combined analysis of all studies submitted showed that after 14 days, the maximum concentrations of residues found at injection sites led to a Global Estimate of Acute Dietary Exposure (GEADE) of 52 µg/kg bw for the general population and 87 µg/kg bw for children, corresponding, respectively, to 27% and 43% of the ARfD (Table 4.14) as illustrated in Figure 4.10.

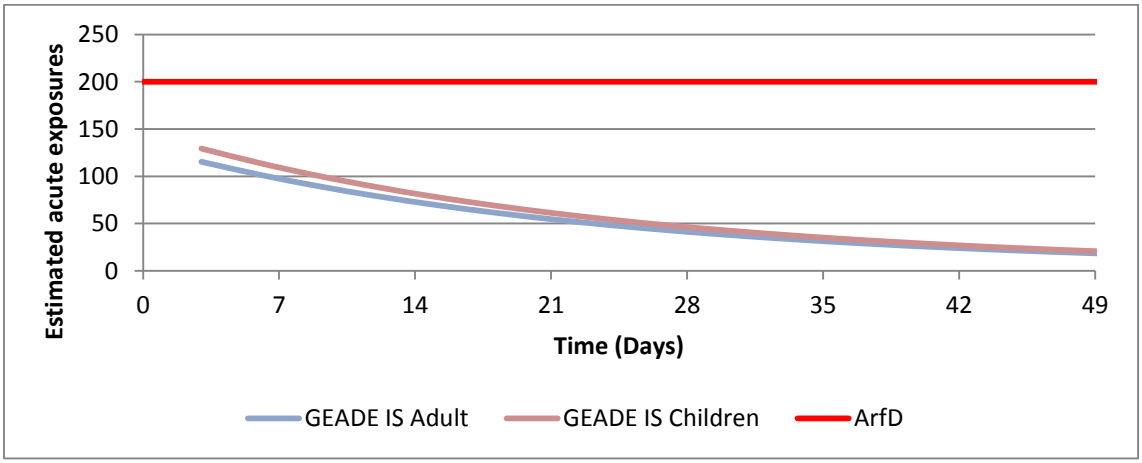

Figure 4.10. Acute reference dose and Global Estimate of Acute Dietary Exposure for total population and children.

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

Chronic dietary exposure assessment

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

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

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

*Median concentration 14 days after treatment.

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

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

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

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

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

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

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

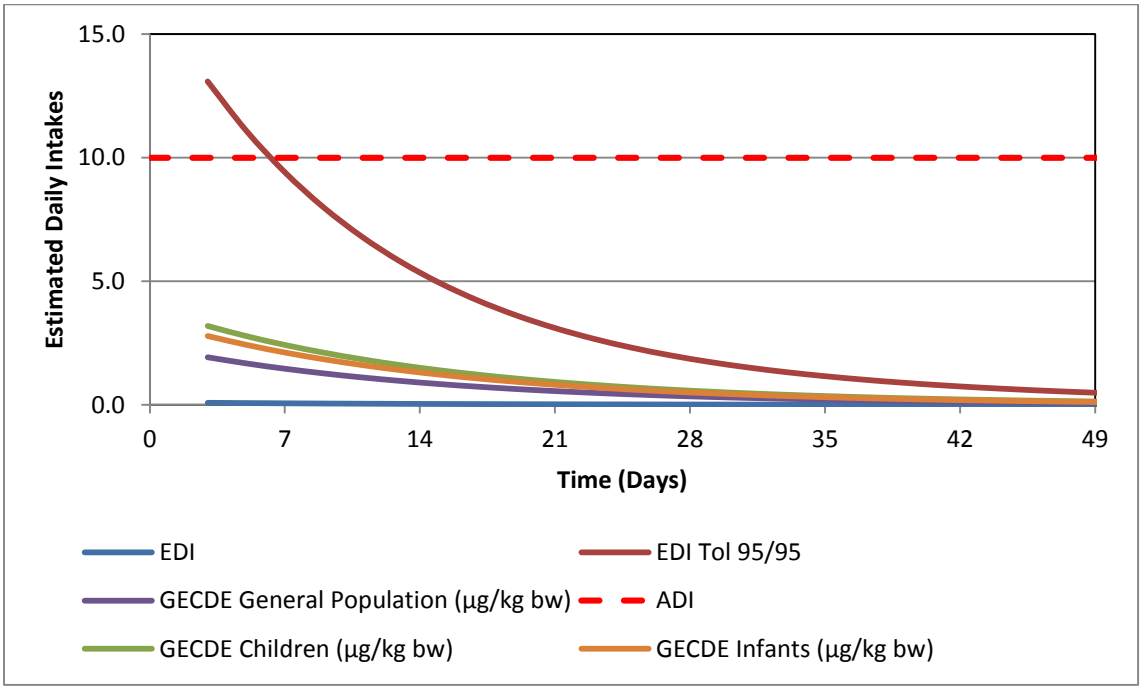

Figure 4.11. Acceptable daily intake, estimated daily intake based on median and upper tolerance limits and global estimated chronic dietary exposure for the general population, children and infants (expressed as µg/kg bw).

Maximum residue limits

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

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

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

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

References

Boggio, J. C. 1998. Determination of residues of ivermectin at 3.15% in excipients of slow release (VERMECTIN L.A.) (LITTORAL, 1998) after subcutaneous administration in neck at a dose of 630 µg/kg body weight.

Chiu S. H. L., Baylis, F. P., Halley, B. A., Eline, D., Rosegay, A., Murphy, T. P., Botto, A., Fink, D., Royce, A., Bloom, A. J., McKissick, G. E., and Sutpin, C. F. 1986. Metabolic Disposition of 22,23-3H-MK0933 (Ivermectin) in Edible Tissue of Steers Dosed Percutaneously at 0.5 mg/kg (EXPT. CA-218), October 1986, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065.

Danaher, M. 2013. Private communication to JECFA reporting the performance characteristics of a validated LC-MS/MS method for ivermectin H_2B_{1a} residues in bovine muscle tissues [TEAGASC, Dublin, Ireland].

Errecalde, J. O., and Mestorino, N. 2007. Brouwer Study "Experiment for the determination of residues of ivermectin after administration to cattle of MR11 3.15% developed by Incam S.A. for Brouwer S.A.

FAO. 1991. "Ivermectin" in *Residues of some veterinary drugs in animals and foods*, FAO Food and Nutrition Paper 41/3, pp. 45-64. Monograph available at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-vetdrugs/en/ Accessed 2016-03-08.

FAO. 1993. "Ivermectin" in *Residues of some veterinary drugs in animals and foods*, FAO Food and Nutrition Paper 41/5, pp. 37-39. Monograph available at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-vetdrugs/en/ Accessed 2016-03-08.

FAO/WHO. 2014. CAC/GL 71-2009, rev. 2012, 2014, Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with

[4] No new data were provided for use of ivermectin in dairy cattle; therefore, the Committee did not recommend any revision to the MRL of 10 µg/kg for ivermectin in milk.

the Use of Veterinary Drugs in Food Producing Animals. Available at http://www.codexalimentarius.org/standards/list-standards Accessed 2016-03-08.

FAO/WHO. 2015. Report of the twenty second session of the Codex Committee on Residues of Veterinary Drugs in Food, San José, Costa Rica, 27 April – 1 May 2015; CAC doc. REP15/RVDF. Available at http://www.fao.org/fao-who-codexalimentarius/meetings-reports/en/. Accessed 2016-03-08.

Formentini, E. A. 2012. EXP-ST-02-12: To determine the withdrawal time for a 1% ivermectin formulation with AD3E vitamins (Bagomectina AD3E Forte/Ivergen Plus AD3E) in cattle using a method with a LOQ of 13.58 and 3.28 µg/kg for liver and fat, respectively.

Formentinin, E. A. 2010. EXP-F-13-10: Determination of residues following subcutaneous administration of a 3.15% ivermectin formulation (Bagomectina LA Star/Ivergen Platimum 3.15) to cattle.

Jacob, T. A., Smith, G. E., Baylis, F. P., Brown, J. E., Green, M. L., Meriwether, H. T., Rosegay, A., and Walsh, M. A. R. 1979. The distribution and depletion of 3H-labeled MK-0933 in cattle dosed subcutaneously at 0.3 mg/kg body weight. Merck Study report RN-190.

Kvaternick, V. J. 1992. Unpublished Report: Method Validation for the HPLC Analysis of Ivermectin Bovine Tissues, ADC Project 1257 A-D, Analytical Development Corporation, Colorado Springs, CO 80907.

Kvaternick, V. J. 1995. Unpublished Report: Validation of the Analytical Method "HPLC-Fluorescence Assay Method for Ivermectin (MK-0933) in Bovine Tissue" for the Determination of Ivermectin Residues in Swine Liver and the Demonstration of Non-interference by Bacitracin, ADC Project 1467S, Analytical Development Corporation, Colorado Springs, CO 80907.

Markus, J. and Sherma, J. 1992. Liquid Chromatography/Fluorescence detection of ivermectin in animal tissue and plasma. *Journal of AOAC International*, 75: 757-767.

Merial Inc. 2015. Re-Evaluation of the JECFA ADI for Ivermectin Residues in the Edible Tissues of Food-Producing Animals. Submitted to JECFA.

Pollmeier, M. 2007 Determination of the depletion of ivermectin and clorsulon in bovine tissues following a single administration of Ivemec-F (PR&D 0127201).

VICH. 2015. Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Marker residue depletion studies to establish product withdrawal periods; VICH GL48(R) (MRK) - February 2015 - For implementation at Step 7 by January 2016. Available at http://www.vichsec.org/guidelines/biologicals/bio-quality/impurities.html Accessed 2016-03-08.

Wallace, DH., Kunkle, B. N., Maddox, R., Wooden, J. W., Malinski, T. J., Green, S. A., Fox, A., Wehner, T. A., and Krupa, D. 1992 Final report Animal Science Research, Merck Sharp and Dohme Research Laboratories. ASR13527: HPLC Fluorescence determination of ivermectin in bovine tissues (ADC Project #1257 Completed 1992).

Wehner, T. A. 1990. Unpublished Report: Ivermectin (MK-0933). Report for Study CA-270 - A Study to Deterrnine Ivennectin Residues in Liver, Kidney, Fat and Muscle Tissue From Cattle Dosed Orally with a Sustained-release Runtinal *Bolus.* Merck Sharp and Dohme Research Laboratories, Rahway, *NJ* 07065.

Wehner, T.A. 2004. HPLC-Fluorescence Assay Method for Ivermectin in Edible Tissue. Bioanalytical Method Merial Limited, 17 August 2004.

WHO. 1990. "Ivermectin" in Evaluation of certain veterinary drug residues in food, (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives), *WHO Technical Report Series* No. 799, pp 23-31.

WHO. 1993. "Ivermectin" in Evaluation of certain veterinary drug residues in food. Ivermectin (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives), *WHO Technical Report Series* No. 832, pp 17-20.

WHO. 2002. "Ivermectin" in Evaluation of certain veterinary drug residues in food. (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives), *WHO Technical Report Series* No. 911, pp 10-12.

WHO. 2012a. "Ivermectin" in Evaluation of certain veterinary drug residues in food. (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives), *WHO Technical Report Series* No. 969, pp 52-54.

WHO. 2012b. Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs, Final Report including Report of Stakeholder Meeting, 7–11 November 2011, Rome. Available at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/ Accessed 2016-03-08.

WHO. 2014. "Ivermectin"in Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives), *WHO Technical Report Series* No. 988, pp 54-56.

Wood, J. S. 1980. Unpublished report: "Ivermectin (MK-0933): Tissue Residue in cattle Subcutaneous Injection. Study CA-129 [0.3 mg/kg Formulation B]"J. Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065.

Wood, J. S. 1981. Unpublished Report: Ivermectin (MK-0933): Tissue Residue in Swine Dosed Subcutaneously, Study SW 304 (0.4 mg/kg Formulation B). Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065. 574.