Monensin

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Addendum to the monograph prepared by the 70th meeting of the Committee
and published in FAO JECFA Monograph 6

Identity

International Non-proprietary Name(s) (INN): Monensin sodium

Synonyms: Monensin A sodium salt; Monensin sodium; Monensin sodium salt; NSC 343257; Sodium monensin; Elancoban®; Elancogran®, Coban®, Rumensin®, Coxidin®

IUPAC Names: Stereoisomer of 2-[2-ethyloctahydro-3′methyl-5′[tetrahydro-6-hydroxy-6-(hydroxymethyl)]-3,5-dimethyl-2Hpyran-2-yl] [2,2′-bifuran′5′yl]]-9-hydroxy-β-methoxy-a,γ,2,8,-tetramethyl-1,6-dioxaspiro[4.5]decan-7-butanoic acid.

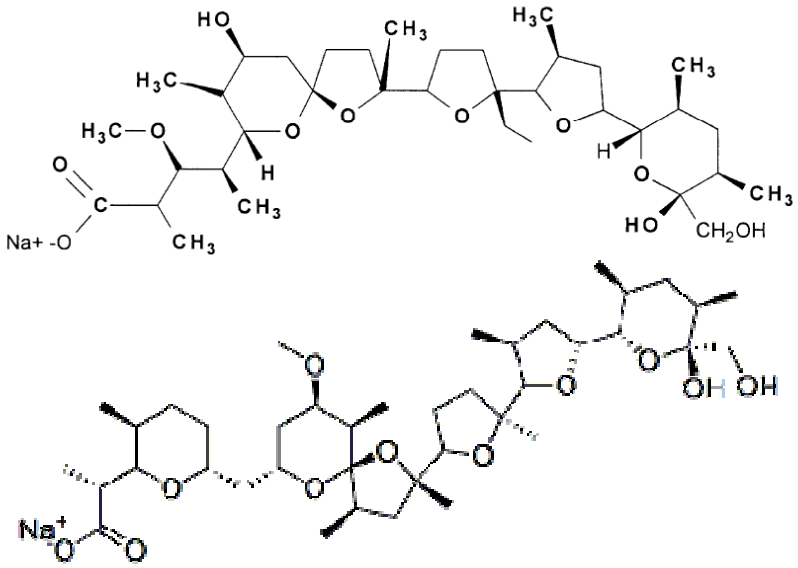
and: 4-[2-[5-ethyl-5-[5-[6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-oxan-2-yl]-3-methyl-oxolan-2-yl]oxolan-2-yl]-9-hydroxy-2,8-dimethyl-1,6-dioxaspiro[4.5]dec-7-yl]-3-methoxy-2-methyl-pentanoic acid

Chemical Abstracts Service Number: Monensin 17090-78-8; Monensin Sodium 22373-78-0

Molecular Formula: Monensin A $C_{36}H_{61}O_{11}Na$

Molecular Mass: 693 g/mol

Chemical structures: Monensin A as sodium salt (upper); nigericin as sodium salt used as internal standard (lower).



Background

The Committee evaluated the residue safety of monensin in different species of food animals at its 70th meeting (FAO/WHO, 2009). In the evaluation, the Committee considered Monensin A (shown above) to be a suitable marker residue for monensin in milk and tissues in all species. At the 18th meeting of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), draft maximum residue limits (MRLs) for monensin in various species as recommended by the 70th meeting of JECFA were proposed for Step 5/8 of the evaluation procedure. The Committee recommended permanent MRLs for monensin in poultry (chicken, turkey and quail) tissues of 10 µg/kg in liver, kidney and muscle, and 100 µg/kg in fat. The Committee recommended permanent MRLs for monensin in ruminant (cattle, sheep and goat) tissues of 10 µg/kg in kidney and muscle, 20 µg/kg in liver, 100 µg/kg in fat and 2 µg/kg in milk. The original assessment of the MRL was based on a limited dataset (Bassissi and Larvor, 2007; Bagg and Dick, 1999, 2000); much of the residue data were below the limit of quantitation (50 µg/kg or 25 µg/kg, depending on the method used). In consequence, a re-evaluation of the monensin MRL in cattle liver was requested and monensin was added to the priority list of veterinary drugs for evaluation or re-evaluation of the cattle liver MRL by JECFA.

The sponsor submitted additional data and requested that the new data and one previously submitted study be considered in conjunction with that of Bassissi and Larvor (2007). MacDougall and Roberts published in 2011 a paper indicating that the Codex MRL (20 µg/kg) would not be compatible with a zero time withdrawal period. Elanco has submitted a variation of the EU MRLs requesting an increase in the MRL for bovine liver from 30 µg/kg to 50 µg/kg and for kidney from 2 µg/kg to 10 µg/kg.

The sponsor submitted four main documents in support of the expected additional data for monensin in cattle tissues and milk. The first was a copy of the method formatted according to the ISO 78/2 format (Analytical Method 1775 ISO 78/2). This is an improved method for the determination and confirmation of marker residue (monensin A) in cattle tissues. The second document is a report for a GLP-compliant validation study conducted for monensin A in cattle tissues and milk (Bassissi and Larvor, 2007). The third document is a validation data of an analytical method for the determination of Monensin A in bovine liver, kidney, muscle, fat and milk by LC-MS/MS (MacDougall, 2011). The fourth document (presented in three volumes) corresponds to a non-clinical laboratory study (GLP) on tissue and milk residue in dairy cows following a single oral dose administration of monensin controlled release capsule (MacDougall and Roberts, 2011). Monensin is approved for administration to cattle in a variety of formulations, and including in particular an intra-ruminal controlled release capsule (CRC).

The sponsor requests that any proposed changes to the cattle liver MRL be extended to other ruminants (i.e. goats and sheep) as well.

Approved uses for monensin in cattle

The sponsor clarified the currently labelled uses for monensin. Canada, USA, Australia and New Zealand have label uses for monensin that could result in a monensin dose that exceeds 1 mg/kg bw, and under some circumstances be as high as 2 mg/kg bw.

Monensin Capsule

The monensin CRC is a winged plastic delivery device for cattle which is introduced into the rumen via the mouth. It contains a stack of monensin tablets (total of 32 g of monensin) which slowly release monensin into the rumen via an orifice in the device. In most countries

where the CRC is registered, the label stipulates a 200 kg minimum body weight for cattle that can be dosed with the capsule. This means that at the start of the treatment period, these 200-kg cattle would be receiving 320 mg monensin/head/day or 1.6 mg/kg bw/day (based on an average payout period of 100 days). In Canada, differences in beef cattle breeds and diet has resulted in a labelled average capsule payout period of 80 days. Thus the dose for 200-kg beef cattle in Canada is almost 2 mg monensin/kg bw/day at the start of the treatment period.

In New Zealand, the CRC label has a minimum weight of 300 kg (to account for the smaller frames of Jersey cattle, a common dairying breed in New Zealand); this still delivers a monensin dose of 1.07 mg monensin/kg bw/day.

On the Australian CRC label, the restriction has been removed, allowing the capsule to be re-dosed at less than 100 days. The purpose of the removal of this restriction and the removal of other restrictions on some labels for concurrent use (see below) was to ensure continuous protection of cattle from bloat and ketosis during periods of high challenge. This means that cattle dosed with a monensin CRC, could be provided with a second CRC, at the same time as the first dose is completing its payout period, resulting in a short time where cattle could be getting twice the usual dose, or 640 mg monensin/head/day, or up to 2 mg monensin/kg bw/day.

Concurrent use of monensin capsule and monensin premix

In New Zealand, the monensin CRC label permits cattle in excess of 600 kg to receive additional monensin product (according to the New Zealand label for other monensin products, the additional dose would be 300 mg monensin/head/day). Thus a 600 kg animal could receive 620 mg monensin/head/day or 1.03 mg monensin/kg bw/day.

In Canada, concurrent dosing of the monensin CRC with monensin premix is also permitted for dairy cattle. Thus, assuming a minimum weight of 550 kg for a Canadian dairy cow and an upper feed intake of 4.5% bw per day, concurrent use of monensin (95-day payout period for Canadian dairy cattle) and 396 mg monensin/head/day from the feed (16 ppm in the feed – upper limit). The total dose of monensin would be 1.3 mg monensin/kg bw/day.

The Australian CRC label also has no restrictions preventing concurrent use with in-feed monensin premix.

Monensin premix

In Canada, mature Holstein dairy cattle weigh approximately 800 kg and can have a daily feed intake greater than 4.0% body weight in dry matter. At the upper inclusion rate (24 ppm in the feed) on the Canadian premix label (monensin sodium) for dairy cattle, the intake of monensin in some cows could be about 800 mg/head/day, or approximately 1 mg monensin/kg bw/day. In the United States, dairy cattle may be dosed up to 660 mg monensin/head/day, which could result in a dose of approximately 1 mg/kg bw/day in smaller (600 kg) cattle. The upper dose limit for monensin in beef cattle in the United States is 480 mg/head/day, which also approximates to 1 mg/kg bw/day in many cattle at finishing.

Summary of approved uses

The permitted label uses for the monensin CRC and in-feed monensin premix could result in a significant number of cattle (beef and dairy) receiving doses of monensin from 1.0–2.0 mg/kg bw/day. Based on data submitted for evaluation to the 70th meeting of the Committee and submitted for evaluation by the 75th meeting of the Committee, there is a

high probability that some cattle treated with monensin in accordance with approved uses will exceed the liver MRL currently adopted by Codex, although the ADI would not be exceeded. Given the safety of monensin in edible tissues, the current adopted Codex MRLs have a potential negative impact on beef trade for major cattle exporting countries. The maximum dose rate used in the world for monensin in cattle is estimated to be 2 mg/kg bw/day.

Residue studies

Two studies were conducted to determine the monensin milk and tissue residues in lactating dairy cattle at zero time withdrawal following the administration of two CRCs and the feeding of premix. In both studies, lactating dairy cows were treated intra-ruminally with two controlled release capsules (32 g monensin in a hexaglycerol distearate matrix in a plastic tube) at day 0. In the first study, previously submitted, cows were fed a medicated ration containing 24 mg monensin/kg feed from day 11 to day 20, and then fed a 36 mg monensin/kg feed for 21 days (Bagg and Dick, 1999). MISSING In the second study (Bagg, 2000) MISSING, submitted for the current evaluation, cows were fed a medicated ration containing 24 mg monensin/kg from day 14 to day 35. After measurement of the monensin release rate from the controlled release capsules, the resulting daily dose ranged from 1537 to 1804 mg monensin per cow (equivalent to 2.4 to 3 mg/kg bw) in the first study and from 778.2 to 1384 mg per cow (equivalent to 1 to 2.4 mg/kg bw) in the second study. At zero time withdrawal, animals were slaughtered and liver and kidney samples were analysed using a validated HPLC method with post-column derivatization (Method AM-AA-CR-R174-AA-791). In the two studies, there were no detectable monensin residues in kidney tissue (<0.025 mg/kg). Monensin residues were detected in 6 of 6 liver samples with two values below the LOQ (detected residues ranged from 0.02 mg/kg to 0.09 mg/kg) collected in the first study, and in one of six livers (25.8 µg/kg) was just above the LOQ (25 µg/kg) in the second study (Table 4.1).

Table 4.1. Relationship between monensin A dosage regimens (2 CRC + premix) and liver residue levels in two studies made available by the sponsor (Bagg, 1999, 2000)

	From Bagg, 1999		From Bagg, 2000	
	Dose (mg/kg bw)	Liver concentration (µg/kg)	Dose (mg/kg bw)	Liver concentration (µg/kg)
	2.7	55.1	2.0	<25
	2.5	20.3*	1.9	25.8
	2.9	69.6	2.0	<25
	2.9	45.8	2.4	<25
	3.0	23.8*	1.0	<25
	2.4	84.5	1.9	<25
Mean	2.7	49.8	1.9	
Min.	2.4	20.3	1.0	
Max.	3.0	84.5	2.4	

NOTES: * = below LOQ (25 µg/kg).

Another study (Terhune, 2007) was completed in 2007. In this study, 9 cattle approximately 18 months of age and weighing from 406 to 537 kg were fed a medicated ration containing 40 mg monensin/kg feed for 24 days with a daily feed consumption around 5 kg per day (estimated to be around 0.4 mg/kg bw). At zero withdrawal time, animals were slaughtered and liver was analysed for monensin using the method: 'Determination of Monensin in Tissues and Eggs (Modified Method 5801654)', a semi-quantitative thin-layer chromatography (TLC) autobiographic method with a LOQ of 50 µg/kg. One of the cattle had a monensin liver residue with an estimated concentration of 0.0533 mg/kg (between 0.05 and 0.07 µg/kg). The limit of quantitation for this study was 0.05 mg/kg using a TLC autobiographic method.

Table 4.2. Residues of monensin in dairy cows tissues treated via gelatin capsule at 0.9 mg/kg bw/day as 2 equal doses daily for 7 days

Animal	Time after last dosing	Muscle (µg/kg)	Fat (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)
25	6 h	BLQ	5.24	9.63	1.03
32		BLQ	3.23	9.39	BLQ
39		ND	BLQ	6.42	BLQ
43		BLQ	1.08	10.76	BLQ
16	18 h	ND	BLQ	4.84	BLQ
71		ND	1.41	5.24	BLQ
15		BLQ	BLQ	5.39	BLQ
72		ND	BLQ	6.70	BLQ
3	30	ND	BLQ	2.23	ND
11		ND	BLQ	2.27	ND
75		ND	BLQ	5.43	ND
77		ND	BLQ	2.36	ND

NOTES: BLQ = below limit of quantitation; ND = not detected.

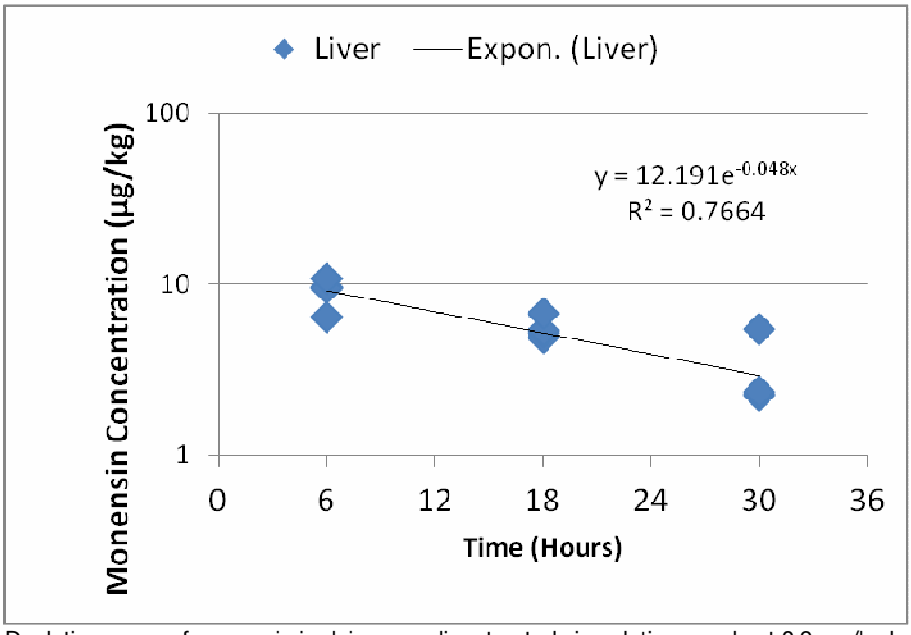


Figure 4.1. Depletion curve of monensin in dairy cows liver treated via gelatin capsule at 0.9 mg/kg bw in 2 equal doses for 7 days

The depletion of monensin was determined in the edible tissues (liver, muscle, kidney and fat) of 12 lactating dairy cows after dosing with monensin at 0.9 mg/kg bw/day for seven consecutive days (Bassissi and Larvor, 2007). Gelatin capsules containing equal doses were administered at approximately 12-hour intervals. Tissues were collected at 6, 18 and 30 h after the final dosing. Monensin residues were determined using a validated HPLC-MS/MS method with a LOQ of 1 µg/kg (Table 4.2).

The depletion curve obtained in liver is reported in Figure 4.1. The half-life of monensin in liver is about 14 h.

Finally, a study was recently completed to support the registration of a newly designed CRC in Europe (MacDougall and Roberts, 2011). The object of this study was to evaluate the residues of monensin in milk (24 dairy cows) and tissue (10 dairy cows) after 14 days following oral administration of a single monensin CRC. The target dose release for each capsule was 335 mg/day over a 95-day period. Using the daily target dose release and the overall average body weight for cattle in the study, the dose rate was calculated to be 0.53 mg/kg bw/day. Milk samples from each animal were collected twice daily, at 12-hour intervals, immediately prior to treatment (pre-trial) and for 28 consecutive milkings up to 336 h after treatment. Following the morning milking on study day 15, ten animals were slaughtered and tissue samples were collected: liver, kidneys, muscle and fat. All milk samples were initially stored refrigerated until transfer to the analytical laboratory. All tissue samples were stored frozen until analysis. Milk and tissue residue concentrations were evaluated for monensin A using a validated LC-MS/MS method (Charles River Analytical Method No. 1775 Version 1). Due to the nature of the intraruminal CRC, a residue decline phase was not included in this study because the device cannot be removed once administered. Thus, this study provides only one data point, at zero withdrawal. Only tissue sample results are reported.

Table 4.3. Residues of monensin (µg/kg) in the tissues of dairy cows treated via a single CRC device at approximately 0.5 mg/kg bw

Animal	Liver	Kidney	Muscle	Fat
2	2.54	<LOQ	ND	<LOQ
8	23.1	1.29	0.752	5.32
10	18.8	<LOQ	ND	1.07
11	11.2	<LOQ	<LOQ	2.57
13	13.6	<LOQ	ND	<LOQ
17	9.64	<LOQ	ND	1.28
18	26.3	<LOQ	ND	<LOQ
21	13.6	1.45	0.836	2.90
22	22.4	1.37	<LOQ	4.04
24	7.99	<LOQ	ND	<LOQ
Mean	14.9	0.936	<LOQ	2.12
SD	7.53	0.302	0.261	1.55
CV%	50.4	32.2	55.5	73.1

NOTES: ND = not detected.

Samples with no monensin A detected or with a found concentration less than the validated LOD are reported as ND. When a data point is assigned as <LOQ, the LOQ value is assigned for derivation of means and standard deviations. When a data point is assigned as ND the LOD value is assigned for derivation of means and standard deviations. In this study, while the average concentration in liver was below the Codex MRL, several liver samples were determined to be greater than the Codex MRL, and the Upper Tolerance Limit

calculated using the arithmetic mean for the 95% confidence limit of the 95th percentile was 36 µg/kg. All other tissue types, including milk, were well below the JECFA MRLs. Results are summarized in Table 4.3.

No new residue data were provided for goat and sheep.

Analytical methods

A method for the determination and confirmation of monensin A and narasin A in cattle liver was validated (MacDougall, 2011). Only the monensin data are relevant to this submission. The laboratory method (CRM 1775) has been formatted according to the ISO 78/2 format. Liver samples are extracted twice with iso-octane/ethyl acetate (90/10). A portion of the combined supernatant is purified by silica solid phase extraction (SPE) before detection and quantification by HPLC with tandem mass spectrometry detection (LC-MS/MS) in the Selected Reaction Monitoring (SRM) mode. Data presented has been quantified for a single transition (693.4>675.6 *m/z*). Data was also collected for confirmatory transitions (693.4>501.6 and 693.4>479.4 *m/z*) but no data was processed or reported for these transitions. Quantification was from a matrix matched calibration line, with 1/x weighting. During the validation work, the European Union MRLs were used as the target concentrations (tissue residue depletion study for submission to the European Medicines Agency, EMA). The Codex MRL (20 µg/kg) is within the validated range for the method (EMA MRL for liver is 30 µg/kg).

System linearity was demonstrated over the range 0.5–100 ng/ml in liver for matrix match calibration standards prepared in extracted control samples for each matrix.

The inter-day assay accuracy and precision was determined for each matrix at their respective ½MRL, MRL and 2MRL (European Union) levels on 3 occasions. The mean intra-day assay accuracy and precision for monensin fortified liver ranged from 89.3–103%, with a precision ranging from 2.13 to 9.36%. The inter-day accuracy and precision for liver fortified with 15, 30 and 60 µg/kg monensin was 95.2–101% with a precision no greater than 6.47%.

The specificity of the LC-MS/MS was investigated in liver; the assay was shown to be sufficiently specific. No interference was noted in control samples with a peak area greater than 10% for either test item at the respective LOQ for the matrix. The assay was also shown to be specific against solution standards of penicillin, tylosin, tilmicosin, tetracycline, lasalocid, ceftiofur, ractopamine and ketoprofen.

Figure 4.3. Representative ion chromatogram of a control liver extract for monensin (MacDougall, 2011 [217751 Tissue Method Validation, page 147])

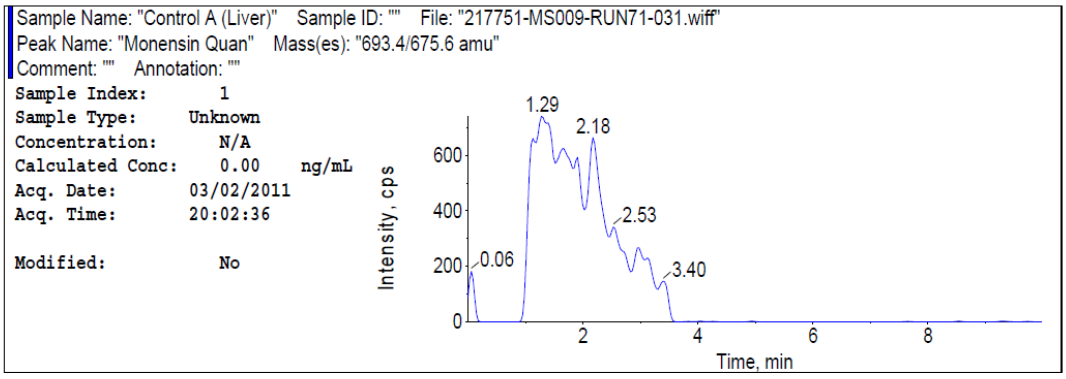


Table 4.4. Limits of quantitation and limits of detection are given for Monensin A in liver, kidney, muscle and fat

Matrix	MRL (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)
Liver	30	0.750	0.0823
Kidney	2	0.750	0.0379
Muscle	2	0.750	0.269
Fat	10	1.00	0.0852

The assay LOD was determined by extraction and analysis of 20 aliquots (4 extractions from each of 5 different animals) of the matrix to determine the mean background noise. The LOD was defined as the concentration of each test item equivalent to the mean background noise plus 3 times the standard deviation. The LOQ for detection of each test item was determined by the extraction and analysis of replicate (n=6) aliquots of control matrix fortified with decreasing concentrations of each test item, and assaying these samples with the standard method. The target intra-day assay accuracy at the LOQ (defined as the mean percentage determined concentration/actual concentration) was 70–110%. The precision at each concentration (defined as the coefficient of variation of the mean determined concentration) was ≤20%. Results are shown in Table 4.4 and Figures 4.4 and 4.5. MRL values referred to are those of the European Union.

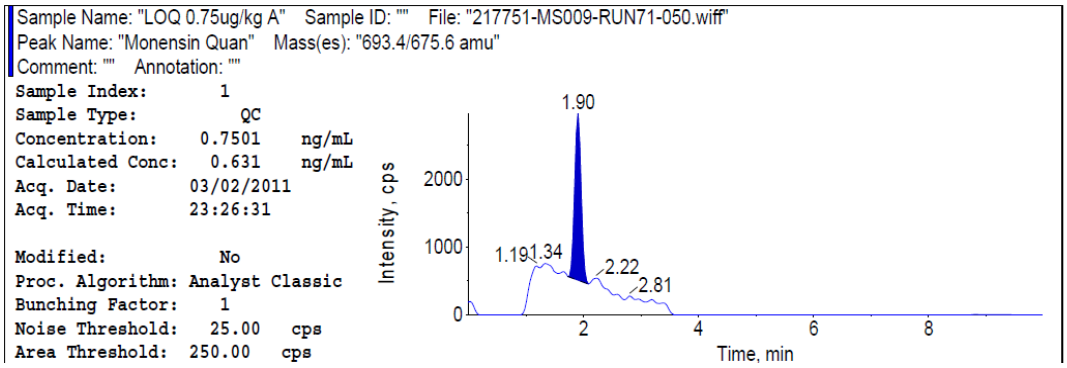


Figure 4.4. Representative ion chromatogram of a liver assay sample fortified with monensin at 0.75 µg/kg (MRL) (MacDougall, 2011 [217751 Tissue Method Validation, page 129])

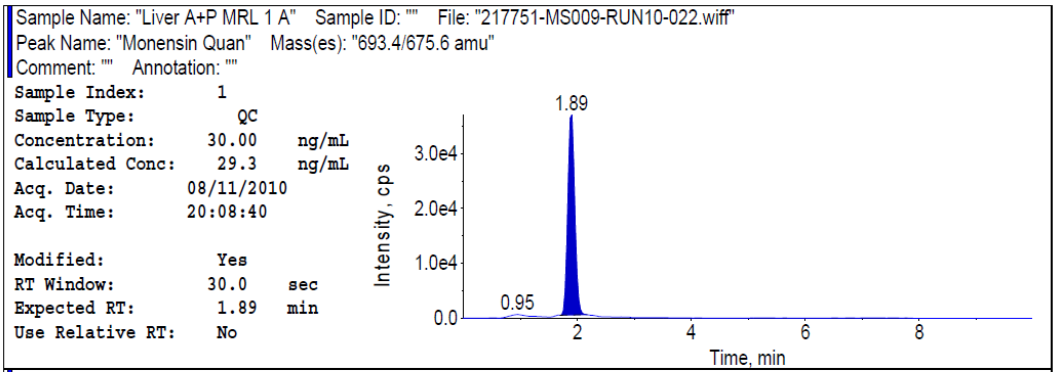


Figure 4.5. Representative ion chromatogram of a liver assay sample fortified with monensin at 30µg/kg (MRL) (MacDougall, 2011 [217751 Tissue Method Validation, page 138])

Stability for monensin A was found acceptable during storage for each tissue at room temperature (i.e. 4 h) and for long (i.e. up to 2 months) frozen storage at -20°C. Its stability after three freeze-thaw cycles was observed for liver. After 1, 2 and 3 freeze-thaw cycles in liver the found concentration difference for monensin at the tested MRL was -14.2, -14.5 and -15.7% respectively compared with freshly extracted samples.

Appraisal

The sponsor provided updated information on the different market authorization and practices in cattle using a Controlled Release Capsule (CRC) or medicated feed, or both. The maximum dose rate used in the world for monensin in cattle is estimated by the sponsor to be 2 mg/kg bw/day. The sponsor provided two new study reports and informed the Committee that an additional study is on-going with animals treated with medicated feed, but this study was not available for the present evaluation. The most recent study was based on a new CRC with residue determined by LC/MS-MS.

The sponsor provided residue data based on different modes of treatment and analytical methods. Two studies (Bagg and Dick, 1999, Bagg 2000) combined 2 CRCs with medicated feeding to lactating cows, resulting in 2 dosage regimens (mean exposure: 2.4 mg/kg bw/day and 1.9 mg/kg bw/day). The analytical method was characterized by a LOQ of 25 µg/kg for monensin in liver. In one other study (Bassissi and Larvor, 2007), monensin was administered in gelatin capsule (0.45 mg/kg bw every 12 h for 7 days) to lactating cows. Monensin residues were determined using a validated HPLC-MS/MS method with an LOQ of 1 µg/kg in liver, and a depletion curve obtained.

In a new study (MacDougall and Roberts, 2011), monensin was administered as one CRC to lactating cows, resulting in a mean dosage regimen of 0.53 mg/kg bw/day. The analytical method had a LOQ of 1 µg/kg for monensin in liver. The sponsor hypothesized a linear relationship between dose and monensin concentration in liver. Assuming the same dosage regimen of 1 mg/kg bw/day, Figure 4.6 shows that this assumption is not fully valid. Monensin bio-availability varies according to the administration protocol (CRC alone; 2×CRC+premix; gelatine capsule). Therefore, the concentration of monensin in liver of lactating cows differs between the studies.

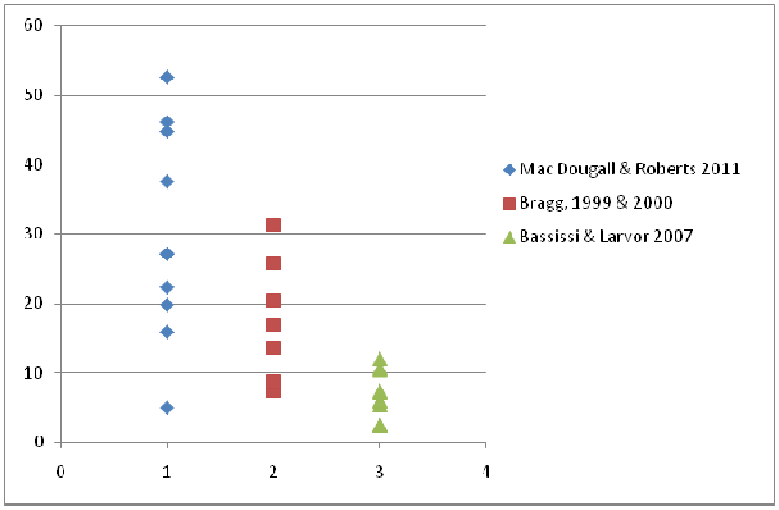


Figure 4.6. Extrapolated monensin concentration in liver (y-axis; µg/kg) for a theoretical dosage regimen of 1 mg/kg bw/day (x-axis) based on studies by MacDougall and Roberts (2011), Bagg (1999, 2000) and Bassissi and Larvor (2007)

The difference in distribution should be related to differences in residue kinetics. The study performed by Bassissi and Larvor (2007) follows an experimental design with a gelatin capsule. The data obtained in the study by MacDougall and Roberts (2011) corresponds to use of a CRC alone in lactating cows. The liver tissue of two animals had monensin concentrations higher than 20 µg/kg. The tolerance limit (the upper limit of the one-sided 95% confidence interval over the 95th percentile of the linear regression line, the "95/95 tolerance limit") was calculated using the logarithmic transformed monensin concentrations and reached a value of 129 µg/kg at zero withdrawal time. There is uncertainty in the calculation due to the limited number of animals (n=10).

The data obtained in the study (Bagg *et al*, 1999, 2000) are used to describe the concomitant use of monensin CRC and premix. The monensin concentrations in liver were estimated for a maximal dose of 2 mg/kg. The 95/95 tolerance limit was calculated using the logarithmic transformed monensin concentrations, and reached a value of 222 µg/kg at zero withdrawal time. Again, there is uncertainty in the calculation due to the limited number of animals with quantifiable liver concentrations (n=5).

The study of MacDougall and Roberts (2011) employed an approved use of CRC. The monensin concentrations in liver were higher than the MRL of 20 µg/kg in three lactating cows. The 95/95 tolerance limit calculated with logarithmic concentration was 129.6 µg/kg. The uncertainty in the calculation is explained by the limited number of animals (n=10).

The effect of combination of CRC and medicated feed at a maximal dose of monensin 2 mg/kg bw/day on monensin residue in liver is estimated using experimental data (Bagg and Dick, 1999; Bagg, 2000). This scenario is realistic because it is very close to the actual dose administered. However, the dataset is limited (n=5 quantifiable values; 7 values below the LOQ of 25 µg/kg). Consequently, the 95/95 tolerance limit (222 µg/kg) calculated using logarithmic concentrations is far from 100 µg/kg. Using arithmetic concentrations, the 95/95 tolerance limit is calculated to be 115 µg/kg.

Maximum residue limits

In recommending a revised MRL for monensin for cattle liver, the Committee considered the following factors:

- An ADI of 0–10 µg/kg bw was established by the 70th meeting of the Committee based on a chronic toxicological end-point. This ADI is equivalent to up to 600 µg monensin for a 60 kg person.
- Monensin A is a suitable marker residue in liver.
- Monensin A is extensively metabolized and represents conservatively 5% of total residues in tissues.
- Different oral formulations of monensin and intra-ruminal CRCs are approved for use in cattle or lactating cows. Concomitant administration of these formulations and intraruminal controlled release capsules would lead to a maximum daily dose regimen of up to 2 mg/kg bw, according to the sponsor.
- At zero withdrawal time, one GLP study based on the administration of one CRC to lactating cows showed that the existing MRL for liver, originally set at 20 µg/kg, was exceeded. The 95/95 tolerance limit of monensin A in cattle liver was calculated as slightly above 100 µg/kg. This value is explained partly by the uncertainty associated with the low number of animals (10) slaughtered at zero withdrawal time.
- Using the data issued from the over-dosage studies conducted with a combination of two CRCs and medicated feed, liver concentrations higher than 25 µg/kg were reported for dose rates higher than 2 mg/kg bw/day. Under the assumption of dose linearity, for a maximum daily dose of 2 mg/kg bw the 95/95 tolerance limit leads to a value significantly higher than 100 µg/kg. This value is explained by the uncertainty associated with the low number of reported values and lack of information on the residue concentration below the method LOQ (25 µg/kg).
- For goat and sheep, no additional information was provided by the sponsor. Without any additional data, the Committee was unable to revise its recommendation on liver MRLs for goat and sheep.
- A validated HPLC-MS/MS complete method with adequate performance parameters and method validation was provided and was considered suitable for routine monitoring of monensin A as marker residue. On the basis of the residue study performed with the CRC administered alone to lactating cows, the Committee recommended a revision for MRL for cattle liver to 100 µg/kg, determined as monensin A.
- Using the model diet, these MRLs would result in an intake of 481 µg/day per person, which represents 80% of the upper bound of the ADI.
- The combined use of the controlled release capsule and premix in cattle at the highest dosage reported by the sponsor will be likely to result in a residue in excess in liver, over the MRL of 100 µg/kg.

Table 4.5. Maximum daily intake calculated for the standard food basket for the recommended MRLs in bovine tissues

Tissue	Consumption factor (kg)	Codex MRL (µg/kg)	Ratio (MR:TRR)	Quantity ingested (µg total residue)
Muscle	0.300	10	0.05	60
Fat	0.050	100	0.05	100
Liver	0.100	100	0.05	200
Kidney	0.050	10	0.05	10
Milk	1.500	2	0.027	111
			TDMI (µg/person)	481
			ADI (µg/person)	600
			% of ADI	80

References

Bagg, R. 1999 Determination of monensin residues in lactating dairy cattle at zero time withdrawal following administration of two Rumensin Control Release Capsules and feeding of Rumensin Premix at 36 ppm total matter intake. Unpublished Study No. T1FCA9804. Elanco Animal Health.

Bagg, R. 2000. Determination of monensin residues in lactating dairy cattle at zero time withdrawal following the administration of two rumensin controlled release capsules (CRC) and the feeding of rumensin premix at 24 ppm of the total dry matter intake. Unpublished Study no. T1FCA0001. Elanco Animal Health.

Bagg, R. & Dick, P. 1999. Determination of monensin residues in lactating dairy cattle at zero time withdrawal following administration of two Rumensin controlled release capsules (CRC) and the feeding of Rumensin premix at 36 ppm of the total dry matter intake. Unpublished study No. T1FCA9804 from Elanco, Guelph, Ontario, Canada. Submitted to FAO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Bassissi, F. & Larvor, A. 2007. Non-clinical laboratory study: Residue depletion study of monensin in milk and edible tissue from dairy cattle following oral administration of monensin at 0.9 mg per kg bodyweight for seven consecutive days. Unpublished GLP study No. A061486 from Avogadro, Parc de Genibrat, Fontenilles, France. Submitted to FAO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

FAO/WHO. 2009. Evaluation of certain veterinary drug residues in food. Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series*, 954.

MacDougall, J. 2011. Validation of an analytical method for the determination of Monensin A and Narasin A in bovine liver, kidney, muscle, fat and milk by LC-MS/MS. Analytical method number 1775. Version 1A. (2011) Unpublished Study Number 217751. Conducted for Elanco Animal Health by Charles River Laboratories, Tranent, Edinburgh, UK.

MacDougall, J. & Roberts, S. 2011. Non-clinical laboratory study (GLP): Tissue and milk residues in dairy cows following a single oral dose administration of Monensin controlled release capsule. Unpublished Study Number 286139 (Elanco Number T1FGB100014). Conducted for Elanco Animal Health by Charles River Laboratories, Tranent, Edinburgh, UK.

Terhune, T. 2007. Non-clinical laboratory study: Residue depletion in beef cattle following oral administration of monensin, ractopamine, tylosin and melengestrol acetate (heifers only) for 24 days. Unpublished Study Number HMS 041004. Conducted for Elanco Animal Health by HMS Veterinary Development, Tulare, CA, USA. [Includes Final Study Report Amendment #1.]