MONENSIN

First draft prepared by
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IDENTITY

International Non-proprietary names (INN): Monensin sodium

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

International Union of Pure and Applied Chemistry (IUPAC) Names: Stereoisomer of 2-[2-ethyloctahydro-3′methyl-5′[tetrahydro-6-hydroxy-6-(hydroxymethyl)]-3,5-dimethyl-2H-pyran-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 Abstract Service (CAS) Number: Monensin 17090-78-8
Monensin Sodium 22373-78-0

Structural formula of main components:

Figure 1:

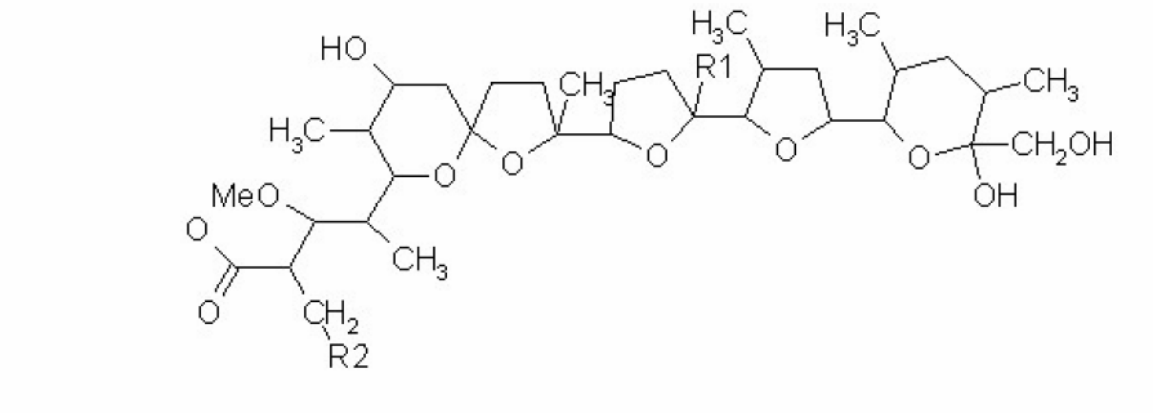

Table 1: Summary of Monensin Factors.

Monensin Factor	R_1	R_2	R_3
A	C_2H_5	H	H
B†	CH_3	H	H
C*	C_2H_5	H	CH_3
D*	Not supplied		

†Factor B accounts for less than 4% of the total composition.
*The relative biological activity of trace factors C and D assayed against *Streptococcus faecium* are negligible (Haney and Hoehn., 1967; Agtarap and Chamberlin, 1967; Chamberlin and Agtarap, 1970).

Molecular formula: Monensin A (sodium salt): $C_{36}H_{61}O_{11}Na$
Monensin B (sodium salt): $C_{35}H_{59}O_{11}Na$

Molecular weight: Monensin A (sodium salt): 692
Monensin B (sodium salt): 678

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: Off-white to tan crystalline powder

Melting point: 267-269˚ C (sodium salt); 103-106° C (acid)

Solubility: Soluble in ethyl acetate, acetone, chloroform and dimethyl sulphoxide. Essentially insoluble in water and petroleum ether.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Monensin sodium is used for the control of coccidiosis in chickens, turkeys, and quail. In feedlot and pasture cattle, it is used to improve the efficiency of rumen fermentation, increase rate of weight gain and for the prevention and control of coccidiosis. In feedlot and lactating and non-lactating dairy cattle, it is used to control ketosis. In dry and lactating dairy cows, it is used to increase milk production efficiency (production of marketable solids-corrected milk per unit of feed intake). In calves, non-lactating goats, and sheep it is used for prevention and control of coccidiosis.

Dosage

Monensin sodium is provided in a complete feed at maximum use concentrations of 125 mg/kg feed for broiler chickens and 120 mg/kg feed for replacement layers. The maximum dose for turkeys is 100 mg/kg feed; for quail, the maximum dose is 73mg/kg feed.

To improve the efficiency of rumen fermentation, monensin sodium is provided at a maximum dose of 360 mg/animal/day or 40 mg/kg in complete feed for feedlot cattle and 200 mg/animal/day in a 0.45 kg feed supplement for pasture cattle. For control of ketosis in feedlot cattle, the maximum dose is 480 mg/animal/day. To control ketosis in lactating dairy

cattle, monensin is administered as a controlled-release capsule providing a maximum daily dose of 400 mg/animal, released into the rumen. It may be given to dry and lactating dairy cows continuously as a total mixed ration containing 11 to 22 g monensin/ton (12 to 24 mg/kg). In calves, it is provided at a maximum dose of 200 mg/animal/day. In the USA, it is explicitly labelled not to be used in calves raised for veal. In non-lactating goats, it is provided as the sole ration containing 20 g monensin/ton (22 mg/kg). In sheep, it is provided in the total ration at a rate of not less than 11 and not more than 22 g monensin/metric ton.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics in Laboratory Animals

Rats

The disposition of orally administered [^{14}C] monensin was determined in rats treated for 13 days with feed containing 100 mg unlabelled monensin/kg feed. On day 14, rats received a single oral dose of radiolabelled monensin, 2.15 mg (specific activity 0.0266 μCi/mg), by gavage. Thereafter, for the remainder of the study (12 days), rats again received unlabelled monensin in the feed. Unlabelled feed was provided *ad libitum*. Urine and faeces were assayed daily for radioactivity. Within three days after dosing with the labelled drug, 92.0% of the total radioactivity was recovered. The majority of the radioactivity was recovered in the faeces (91.5%) but a small amount was recovered in urine (0.5%). Selected tissues and organs were assayed for radioactivity but the detected radioactivity was not different from that measured in pooled control tissues (Herberg, 1973a). Monensin and monensin metabolites were isolated from the liver and faeces of male and female rats treated orally with 5 mg [^{14}C] monensin/kg body weight (Donoho, 1985).

In another study (Howard and Lobb, 1981), tissue distribution and biliary elimination of radioactivity was evaluated in male and female rats following oral administration of [^{14}C] monensin. Radiolabelled monensin (0.614 μCi/mg) was administered orally at doses ranging from 5 to 40 mg/kg body weight in male rats and 2 to 16 mg/kg body weight in female rats. The doses were based on previously determined oral toxicity data (Broddle and Worth, 1976) for male (40.1±3.0 mg/kg) and female (24.3±2.7 mg/kg) rats. After administration, radioactivity was eliminated rapidly and extensively in faeces during a 72-hour collection period. Faecal elimination accounted for 83.6-87.4% and 70.8-87.2% of the dose in male and female rats, respectively. Urinary excretion represented only a minor route of elimination representing 1.0-1.6% and 1.0-1.3% of the dose in male and female rats, respectively. Biliary secretion was the primary excretory pathway following oral administration in rats. There were no differences in the urinary, faecal, or biliary excretions of radioactivity between male and female rats. At toxic doses, there was an initial delay in the excretion of radioactivity in faeces and bile considered secondary to the toxicity. Further support for partial gastrointestinal absorption of monensin in male and female rats was found in studies that showed that 31 to 53% of a radioactive dose (32.8-46.6% in males and 30.7-53.2% in females) of 2 to 40 mg/kg body weight was collected in bile within 72 hours of administration (Howard and Lobb, 1981).

Pharmacokinetics in Food Animals

Cattle

Gastrointestinal absorption of monensin in cattle has been evaluated. In one study, absorption of [^{14}C] monensin in calves was evaluated by measuring radiolabelled residues in bile (Davison, 1984). The amount of radiolabelled material recovered in bile can serve as an estimate of the amount of material absorbed because little monensin is excreted in cattle urine. Two calves, one male and one female, were fitted with bile duct cannulae. Each calf

received a single oral dose of 10 mg [^{14}C] monensin/kg body weight in a gelatine capsule. Bile was collected continuously for 72h. Approximately 35 and 37% of the administered radioactivity was recovered in the bile from the male and female calf, respectively. The presence of monensin or monensin metabolites in plasma (Donoho, 1984), liver and milk (Herberg, et al., 1978; Kline and Wicker, 1975; Kennington, et al., 1995) from orally treated animals provides supporting evidence for absorption.

Chickens and Turkeys

The pharmacokinetic profile of monensin was evaluated in broiler chickens (Atef, et al., 1993) following administration by gavage and intravenously as a single dose of 40 mg/kg body weight. Following intravenous administration, disposition of monensin followed a two-compartment open model. The absorption half-life was 0.6 hours, the volume of distribution was 4.1 L/kg, and the total body clearance was 28.4±0.2 ml/kg/min. The highest serum concentration (4.1±0.05 µg/ml) was reached after 0.4 hours following administration by gavage. The absorption half-life was 0.3 hours and the elimination half-life was 2.1 hours. A somewhat longer terminal elimination half-life (3.1 to 5.6 hours) has been determined recently (Henri, et al*,* 2008a). *In vitro* serum protein binding was calculated to be 22.8%. Bioavailability following administration by gavage was 65.1% (Atef, et al., 1993). In chickens, monensin concentrations in serum and tissues were higher after administration by gavage (40 mg/kg body weight) than after feeding a diet containing 120 mg monensin/kg for 2 weeks (average daily consumption was 24 mg monensin based on a daily feed consumption of 200 g of medicated feed).

The rate of faecal excretion and quantitation of orally administered monensin in chickens were determined. Chickens received an oral dose of [^{14}C] monensin (7.36 mg, specific activity 0.018 µCi/mg). Seventy-five percent of the administered dose was eliminated in excreta within 3 days and was eliminated completely within 12 days (Herberg, 1973b). In another study, three chickens were treated *ad libitum* with feed containing 120 mg unlabelled monensin/kg feed. Chickens were then dosed by oral capsule with a single dose of [^{14}C] monensin. More than 75% of the radioactivity was recovered within 3 days following the dose. Radioactivity in excreta returned to background levels in 4, 5, and 12 days (Herberg, 1975a). In another study (Grundy, *et al.*, 1998), chickens were fed a ration containing 125 mg [^{14}C] monensin/kg feed for 6 days then slaughtered 6h, 1, 3 or 5 days after the treated feed was withdrawn (3 male and 3 female chickens per withdrawal group). Bile contained approximately 87mg monensin/kg after 6h withdrawal. By 5 days withdrawal, bile contained approximately 0.4mg monensin/kg and approximately 76% of the dose had been recovered in excreta. These data indicate that radiolabelled monensin is eliminated rapidly and quantitatively by chickens.

In turkeys, the evidence of intestinal absorption is analogous to that for chickens. Monensin and metabolites were found in turkey liver at zero withdrawal following *ad libitum* access feeding with 110 mg [^{14}C] monensin/kg feed for five days (Donoho, et al., 1982a). The reported terminal elimination half-life in turkeys is 1.4 to 1.6 hours (Henri, et al*,* unpublished 2008a).

Metabolism in Laboratory Animals and Humans

Rats

Data from studies in laboratory animals indicate that monensin is extensively metabolized prior to excretion. The *in vitro* metabolism of monensin has been evaluated in several studies (Ershov, et al., 2001; Nebbia, et al., 1999; Nebbia, et al., 2001). In liver microsomes induced by dexamethasone, monensin is metabolized by the 3A family of cytochrome P-450. Inducers of O-demethylation enhance and inhibitors reduced monensin metabolism. Although

monensin is a substrate for P-450, it does not appear to be a direct *in vitro* inhibitor of rat liver microsomes (Ceppa, et al., 1997). Studies concluded that for drugs commonly administered to humans, monensin is unlikely to inhibit human P-450 directly and would not affect drug metabolism attributable to this family of enzymes (Ueng, et al., 1997). However, it is hypothesized that drugs that inhibit P-450 enzymes could result in toxic interactions with monensin, including drugs potentially administered concurrently with the ionophore (tiamulin or several macrolides) (Nebbia, et al., 1999). Metabolites M-1, M-2, M-3, M-6, and M-7 have been identified in liver and/or excreta from monensin-exposed rats (Donoho, 1985).

Mice

No data were provided on metabolism in mice.

Dogs

The *in vitro* metabolism of monensin also has been evaluated in microsomal incubates from dogs using monensin concentrations of 0.5, 1.0, and 10 µg/mL. The microsomes were sourced from pooled samples comprising more than one donor. An HPLC/MS with electrospray ionisation was used to measure the disappearance of parent drug (monensin A) at multiple time points following incubation. Data indicate that monensin is metabolized by first order kinetics in all cases, consistent with a metabolic pathway involving phase I metabolism due to cytochrome P450 (Herrera, et al., 2005). Because this comparative study also included an assessment of human microsomal activity (pooled from Caucasian, Hispanic and African American donors from 15 to 66 years of age), it was possible to conclude that metabolism in dogs and humans is similar.

Horses

In vitro metabolism of monensin also has been evaluated in microsomal incubates from a horse. Compared to the values obtained for humans and dogs, metabolic stability was highest in the horse and intrinsic clearance was lowest (Herrera, et al., 2005). This effect was exacerbated at high concentrations and reflects the toxicity seen in horses. The catalytic efficiency (chickens >> cattle >> rat/pig > horse) was found to correlate inversely with the interspecies differences in the susceptibility to toxic effects (Nebbia, et al., 2001).

Metabolism in Food Producing Animals

Cattle

Monensin is converted to a large number of metabolites in steers, with the most abundant (M-6) representing approximately 6% of the liver [^{14}C] residue (Donoho, et al,, 1978). A subsequent study in dairy cows reported a similar pattern of metabolites with the most abundant (again M-6) representing 24% of the liver total radioactivity (Kennington, et al., 1995). Six faecal metabolites (M-1 to M-6) were isolated and tentatively identified based on their mass spectral comparison to monensin (Donoho, et al., 1978). In faeces, the predominant residue is monensin (50%), followed by M-6 (4%) and M-2 (2%), respectively. O-demethylation is a major metabolic pathway (Donoho, et al., 1978; Kennington, et al., 1995).

Three steers were fed 300 mg unlabelled monensin per day for at least 15 days. The steers were then given single doses of approximately 300 mg [^{14}C] monensin (specific activities 0.027 - 0.030µCi/mg). Animals received unlabelled monensin for the final 14 days. Radioactivity in faeces remained above background for seven to 11 days. The proportion of the dose recovered was 88.6% - 102.3%. Urine contained no radioactivity above the pre-dose level (Herberg, 1973c; Herberg, 1974a).

Pigs

Monensin and metabolites M1, M2, and M8 were identified in liver of pigs (Giera, et al., 1984a).

Two balance-excretion experiments were conducted in pigs using barrows that had been conditioned to diets containing 50 mg monensin/kg feed then given single doses of [^{14}C] monensin. Doses used were 10.4 mg (specific activity 0.576 µCi/mg) and 5.23 mg (specific activity 0.608 µCi/mg). Recoveries were 78.1% and 54.9% of the doses over ten and 13 days, respectively (Donoho and Herberg, 1977; Herberg and Donoho, 1977a). In the high dose study, 75.0% of the dose was recovered in faeces and 3.1% in urine. In the low dose study, recoveries were 53.9% in faeces and 1.0% in urine. In both studies, excretion was rapid, with approximately 92% of the total in faeces recovered within the first 3 to 3½ days.

Sheep/Goats

In lambs dosed orally with [^{14}C] monensin, liver residues of monensin (6-9% of total radioactivity), M-6 (4-6% of the total), M-1 (5-10% of total) and M-2 (5-9% of total) were identified by TLC radioautography and comparison with standards (Giera, et al., 1984b). In faeces, the major residue was determined to be parent monensin (approximately 75% of the total radioactivity). Small amounts of M-1 (4% of the total) and M-2 (5% of the total) also were identified in sheep faeces (Giera, et al., 1984b).

A wether lamb was given a single dose of 50 mg unlabelled monensin for two weeks. The lamb then received a single 50 mg dose of radiolabelled monensin (0.027µCi/mg). For the final two weeks, the lamb again received unlabelled monensin. Radioactivity in faeces remained above background for nine days. The total amount of radioactivity recovered was 102.0% of the dose. Urine contained no radioactivity above the pre-dose level (Elanco, 1998).

Monensin also was detected in the liver of goats (Handy and Rea, 1984).

Chickens/Turkeys

In chickens, radiochromatograms show extensive metabolism of monensin. The identification and quantification of all metabolites was not possible. Liver (at practical zero withdrawal), bile (at zero and one day withdrawal) and excreta (on the sixth day of dosing) were collected for analysis. Monensin metabolites in liver, bile and excreta included O-demethylation and oxidation (hydroxylation) products. Parent monensin was present in liver and excreta but not bile. Monensin metabolites M-1, M-2, M-6, M-7, and M-9 were identified in liver, bile, and excreta (Grundy, et al., 1998). Metabolites M-7 and M-9 had not been identified in earlier TLC/autoradiography determinations (Donoho, et al., 1980a; Donoho, et al., 1982b).

Figure 2:

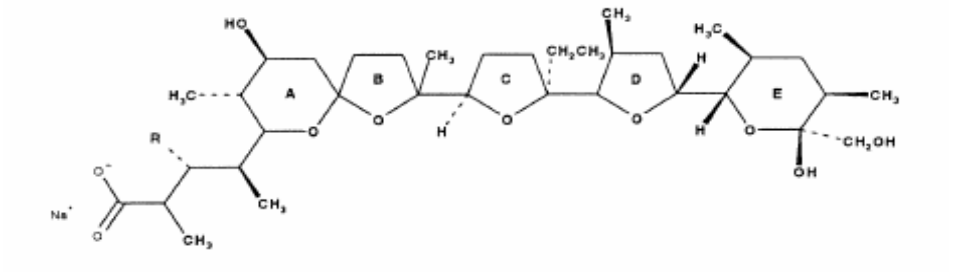

Table 2: Summary of monensin metabolites isolated from animal tissues and excreta. (Structural positions refer to Figure 2)

Metabolite	Molecular weight	Properties	R	Other	Source
M-1	678	O-demethylated monensin (hydroxylated)	OH	-	C, R, T, Ch, Sh, S
M-2	694	O-demethylated monensin (hydroxylated in two positions)	OH	Additional OH on ring E	C,R, T, Ch, Sh, S
M-3	694	M-2 epimer (hydroxylated in two positions)	OH	Additional OH on ring E	R,T, Ch
M-4	694	O-demethylated monensin (hydroxylated in two positions)	OH	Additional OH on ring D	C, R, T
M-5	708	Monensin sodium + oxygen (hydroxylated)	OCH_3	Additional OH on ring D	C
M-6	610	O-demethylated monensin with oxidation of the OCH_3 group to a ketone	keto	Carboxyl group absent	C, R, T, Ch, Sh
M-7	694	Isomeric with M-2, M-3, M-4 but with the oxygen at a different location (hydroxylated in two positions)	OH	Additional OH on ring B, C, or D	R, Ch
M-8	-	O-demethylated monensin (hydroxylated in two positions)	OH	Additional OH on ring B, C, or D	S
M-9	-	O-demethylated monensin with oxidation of the OCH_3 group to a ketone + additional OH	keto	No carboxyl group; additional OH on ring E	Ch

C = Metabolite identified in cattle liver, bile and/or excreta
R = Metabolite identified in rat tissues and/or excreta
T = Metabolite identified in turkey tissues and/or excreta
Ch = Metabolite identified in chicken tissues and/or excreta
S = Metabolite identified in pig tissues and/or excreta
Sh = Metabolite identified in sheep tissue and/or excreta

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

Cattle

A number of early studies in steers orally dosed with 300 mg [^{14}C] monensin/kg body weight indicated that essentially all of the radioactivity could be recovered in the faeces within 11 days after dosing, but no radioactivity above the predose period could be recovered in the urine (Herberg, 1973c; Herberg,1973d; Herberg, 1974a; Herberg, 1974b; Herberg, 1974c). A subsequent study with similar doses of [^{14}C] monensin for a five-day period indicated that the liver contained the greatest concentration of radioactivity 12 hours after the last dose (Herberg, et al., 1978).

The distribution of radiolabelled monensin (Day, et al., 1973) residues in cattle tissue obtained at zero withdrawal is summarized in Table 3 (Herberg, 1975b; Herberg, et al., 1978,

Donoho, et al., 1978; Donoho, 1979). Animals were slaughtered after a practical zero withdrawal, six to twelve hours after the last dose. Cattle were dosed orally by gavage with a gelatine capsule, twice daily for two to five days. The daily dose was 0.71 to 0.83 mg [^{14}C] monensin/kg body weight, corresponding to 300 to 330 mg [^{14}C] monensin per day. An equivalent dose of monensin in feed would be 33 mg monensin/kg feed to 44 mg monensin/kg feed. One steer was preconditioned for twelve days with an equal dose of unlabelled monensin provided in feed. The remaining cattle were withdrawn from monensin-treated feed three days prior to receiving [^{14}C] monensin.

Table 3: Summary of total radioactive residues (mg equivalents/kg) at zero withdrawal in tissues of cattle dosed orally with [^{14}C] monensin.

Dose Equivalent	Dosing Interval	Animal	Total Radioactive Residue (mg monensin equivalents/kg)				
			Liver	Kidney	Heart	Muscle	Fat
44 mg monensin/kg feed	2 days	Steer	0.59	0.03	0.01	0.01	0.05 (back)
							0.04 (kidney)
		Steer 518	0.43	0.01	0.01	0.01	NDR[1]
33 mg monensin/kg feed	5 days	Steer 558	0.36	0.01	NDR	0.01	0.02
		Heifer 074	0.21	0.01	0.01	0.02	NDR

[1] No detectable residue (not statistically different from control tissues).

A radiolabelled residue study was conducted in five lactating dairy cows. Animals received 0.9 mg [^{14}C] monensin/kg body weight in a gelatine capsule twice daily *via* rumen cannulae for 9½ days (Kennington, et al., 1995). The total daily dose ranged from 918 to 1125 mg [^{14}C] monensin/day corresponding to approximately 36 mg monensin/kg feed (1.5 times the labelled dose). As in other studies, liver was the edible tissue with the highest mean residue at practical zero withdrawal times (Table 4). Mean muscle residues were below the assay limit of detection (28.4 cpm in muscle). Total residues in milk are reported below.

Table 4: Mean radioactivity (mg monensin equivalents/kg) at zero withdrawal in tissues of dairy cows administered 918 to 1125 mg [^{14}C] monensin/day.

Tissue	Total Radioactive Residue (mg monensin equivalents/kg)
Liver	1.28
Kidney	0.07
Muscle	NDR
Fat	0.02

Pigs

In an early study, [^{14}C] monensin, at a nominal concentration of 55 mg monensin/kg feed, was fed to one barrow and three gilts for five days (Herberg and Donoho, 1977b). One barrow and one gilt were sacrificed after five days of feeding as a zero-time withdrawal pair. The remaining two gilts were sacrificed after 24 and 48 hours withdrawal. At all withdrawal times, liver had the greatest radioactivity concentration. Zero-withdrawal time liver concentrations were 1.67 and 1.20 mg monensin equivalents/kg for barrow and gilt, respectively. The net radioactivity concentrations in muscle tissue were < 0.05 mg monensin/kg at all times. All tissues other than intestine and pancreas at all withdrawal times contained some residue of radioactivity (<0.09 mg monensin equivalents/kg).

In a preliminary study, one male and one female pig were dosed orally for two and one-half days with [^{14}C] monensin at an equivalent to 50 mg monensin/kg in feed (Herberg and Donoho, 1978). Four hours after the final dose, the pigs were sacrificed and edible tissues were assayed for residual activity. Liver contained the highest net residue, 1.02 mg monensin equivalents/kg in the male and 1.44 mg monensin equivalents/kg in the female. Residues in the other tissues were less than 0.09 mg monensin equivalents/kg.

Three grower pigs of each sex were fed [^{14}C] monensin-fortified ration (110 mg monensin/kg feed) for five consecutive days (Giera, et al., 1984a). One male and one female were slaughtered at six hours, three days and five days withdrawal. Liver, kidney, fat, and muscle were assayed for total radioactivity (limit 0.05 mg/kg). Bioautography was used for detection of monensin in those tissues. The bio-autographic method (Rea, 1976) has a limit of detection of 0.025 mg/kg for liver and muscle and 0.05 mg/kg for fat and kidney. Selected samples of liver and faeces were characterized chromatographically (LOD = 0.005 mg/kg).

The radioactive residues found in pig tissues are summarized in Table 5. Liver from the male and female zero-time withdrawal animals contained approximately 2.26 mg monensin equivalents/kg total residue. Residues decreased to approximately 0.44 mg monensin equivalents/kg by 5 days withdrawal. Kidney contained approximately 0.17 mg monensin equivalents/kg total residue at zero withdrawal. Residues decreased to approximately 0.05 mg monensin equivalents/kg after 5 days. The radioactive residues in fat and muscle were approximately 0.044 and 0.037 mg monensin equivalents/kg, respectively, at zero withdrawal. After five days of withdrawal, fat and muscle ^{14}C residues were approximately 0.05 and 0.02 mg monensin equivalents/kg, respectively. Monensin was not detected in the tissues of any of the treated animals by a microbiological assay (sensitivity 0.025-0.050 mg/kg) or HPLC assay (sensitivity 0.005 mg/kg).

Table 5: Net mg ^{14}C-monensin/kg equivalents in pig tissues following oral administration of radiolabelled monensin in feed (110 mg/kg).

	0-Day (6hr) Withdrawal		3-Day Withdrawal		5-Day Withdrawal	
	F 126	M 127	F 122	M 125	F 121	M 120
Liver	2.08	2.45	0.88	1.03	0.35	0.53
Kidney	0.16	0.18	0.08	0.09	0.04	0.06
Fat	0.04	0.05	0.05	0.05	0.03	0.06
Muscle	0.04	0.04	0.02	0.02	0.02	0.02

Sheep

Lambs were fed [^{14}C] monensin equivalent to a feeding level of 15g per ton (16.5 mg/kg) of complete ration (Giera, et al., 1984). Groups of lambs (two wethers and one ewe) were dosed for 3, 5, or 7 days and killed at zero withdrawal (12 hours) after the last dose. Edible tissues were assayed for total radioactivity (assay reliability = 0.1 mg/kg or lower). Liver samples were assayed for parent monensin, and selected samples of liver and faeces were characterized chromatographically. After dosing for 3, 5, or 7 days, liver contained mean residues of 0.36, 0.32, and 0.20 mg/kg radioactivity equivalents, respectively (Table 6). Parent monensin concentrations in liver were determined using bioautography (Kline, et al., 1975) and were less than 0.05 mg/kg. There was no accumulation of residues for either total radioactivity or parent monensin with longer dosing intervals. Residues in kidney and fat were all less than 0.03 mg monensin equivalents/kg and muscle all less than 0.01 mg monensin equivalents/kg.

Table 6: Summary of ^{14}C (mg monensin equivalents/kg) in sheep tissues following oral administration of radiolabelled monensin at 15 g/ton in feed.

	3 Day Dosing Period			5 Day Dosing Period			7 Day Dosing Period		
	M 578	F 579	M 583	M 580	M 581	F 582	M 577	M 584	F 585
Liver[1]	0.50	0.18	0.39	0.40	0.29	0.285	0.32	0.19	0.11
Kidney	0.01	0.004	0.01	0.02	0.01	-0.01	0.01	-0.01	0.01
Fat	0.01	0.01	0.01	0.01	0.02	0.01	0.03	0.01	0.01
Muscle	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Chickens/Turkeys

The distribution of radiolabelled residues at zero withdrawal has been extensively evaluated in chickens and turkeys.

In several studies, chickens were treated with [^{14}C] monensin in feed at 110-125 mg/kg, *ad libitum*, for four to six days. The first study (Table 7) established that steady-state equilibrium occurred within four days in chickens receiving 120 mg [^{14}C] monensin/kg in feed (Donoho, et al., 1980a). In all [^{14}C] monensin residue studies, liver tissue had the highest amount of total residues at a practical or true zero withdrawal. Muscle had the least residues at zero withdrawal. This was consistent with the distribution pattern seen in rats (Howard and Lobb, 1981).

Table 7: Mean radioactivity (mg monensin equivalents/kg) at zero withdrawal in tissues of chickens fed 110 to 125 mg [^{14}C] monensin/kg feed.

Study No.	Dose (in feed)	Dosing Interval	Total Radioactive Residue (mg monensin equivalents/kg)				
			Liver	Kidney	Muscle	Skin/Fat	Fat
ABC-0043[1]	120 mg/kg	4 days	0.56	0.12	0.01	0.09	0.14
		6 days	0.43	0.12	0.01	0.07	0.07
ABC-0080[2]	120 mg/kg	5 days	0.83	0.22	0.05	0.29	0.23
ABC-0092[3]	110 mg/kg	5 days	0.53	0.18	0.02	0.12	0.07
T1F759701[4]	125 mg/kg	6 days	0.94	0.20	0.06	0.29	0.48

[1]Donoho, et al., 1980a; [2]Donoho, et al., 1980b; [3]Donoho, et al., 1980c; [4]Grundy, et al., 1998

The depletion of [^{14}C] monensin is summarized in two studies (Donoho, et al., 1980c; Grundy, et al., 1998). In the first study (Table 8), fat and skin/fat had slowly depleting residues. The relatively high and persistent radioactivity in abdominal fat was due to incorporation of radioactivity into endogenous fatty acids (Grundy, et al., 1998). Total residue in liver was approximately 2-15 times greater than residues in kidney, muscle, abdominal fat, and skin/fat at zero withdrawal. After three days, liver residues depleted to less than the fat and skin residues.

Fat was also a slowly depleting tissue in the second study (Table 9, Donoho, et al., 1980c). In the liver, the percentage of bound (unextracted) radioactivity increased from 38 to 69% during the 5-day withdrawal even as the total liver radioactivity decreased. Over the 5-day study, the absolute amount of bound residue in liver decreased from approximately 0.20 mg/kg to 0.07 mg/kg.

Table 8: Summary of total radioactive residues (mg monensin equivalents/kg) in edible tissues, fat, and bile of chickens after *ad libitum* access to 125 mg [^{14}C] monensin/kg feed.

Treatment Group	Withdrawal Time (days)	Total Radioactive Residue (mg monensin equivalents/kg)					
		Liver	Kidney	Muscle	Skin/Fat	Abdominal Fat	Bile
01	0	0.94	0.20	0.06	0.29	0.48	87
02	1	0.49	0.14	0.05	0.17	0.31	26
03	3	0.27	0.09	0.05	0.23	0.47	1.3
04	5	0.14	0.05	0.04	0.17	0.26	0.4

Table 9: Summary of total radioactive residues (mg monensin equivalents/kg) in tissues of chickens fed 100 mg [^{14}C] monensin/kg in feed.

Withdrawal Time (days)	Total Radioactive Residue (mg monensin equivalents/kg)				
	Liver	Kidney	Muscle	Skin	Fat
0	0.53	0.18	0.02	0.12	0.07
1	0.27	0.08	0.01	0.05	0.07
2	0.22	0.06	0.01	0.06	0.07
3	0.15	0.05	0.01	0.04	0.05
5	0.11	0.04	0.01	0.03	0.05

In turkeys fed 110 mg [^{14}C] monensin/kg in feed for five days, radiolabelled residues were evaluated in the edible tissues of birds killed 6 hours (practical zero withdrawal) after the removal of medicated feed (Donoho, et al., 1982a). As in the chicken studies, turkey liver and muscle had the most and least amount of radioactive residue, respectively (Table 10).

Table 10: Total radioactive residue in turkey tissues at zero withdrawal following treatment with 110 mg [^{14}C] monensin/kg feed for five days.

Tissue	Total Radioactive Residue (mg monensin equivalents/kg)
Liver	0.91
Kidney	0.16
Muscle	<0.03
Skin/Fat	0.10
Fat	0.14

Cows' Milk

Radiolabelled residues in milk were determined in a study conducted in lactating dairy cows (Kennington, et al., 1995). Cows were treated intra-ruminally *via* gelatine capsule for 9 days. Within approximately five days, radiolabelled milk residues had reached a steady state and averaged 0.045 mg/kg during the final three days of dosing. No residues of monensin were detected in the milk of any treated animals (LOQ = 0.005 mg/kg in milk). The radioactivity was distributed approximately 30% in the cream, 50% in the whey, and 20% in the casein. Fractionation of the milk and the use of a sensitive LC/ESP-MS/LSC analytical technique allowed for the detection of parent monensin in milk at low concentrations (<0.001 mg/kg). As in chickens (Grundy, et al., 1998), it was determined that a significant proportion (26.5%) of the radioactivity in milk from an animal treated with [^{14}C] monensin was due to the incorporation of the radioactivity into endogenous fatty acids (myristic, oleic, palmitic and stearic acid), rather than resulting from monensin-related residues (Grundy and Bewley, 2003).

Residue Depletion Studies with Unlabelled Drug

Residues in Tissues

Cattle

Monensin was fed to cattle at levels of 100 and 500 mg/animal/day for 148 days and at 750 mg/animal/day for 106 days (Kline, 1973). Animals were slaughtered at 0, 48, 120 and 240 hours withdrawal for the 100 and 500 mg/animal/day treatments and at 0 and 48 hours withdrawal for the 750 mg/animal/day treatment. No monensin residues (assay sensitivity 0.05 mg/kg) were detected in animals receiving 100 or 500 mg monensin/day at any withdrawal period. One kidney sample from a zero withdrawal animal in the 750 mg/animal/day treatment group contained a detectable residue. At 48 hours withdrawal, tissues in the 750 mg/animal/day treatment group were free of residual monensin.

Lactating dairy cows were treated intra-ruminally with two controlled release capsules (32 g monensin in a hexaglycerol distearate matrix into a plastic tube) and fed a medicated ration containing 24 mg monensin/kg feed for 10 days and then fed 36 mg monensin/kg for 21 days (Bagg and Dick, 1999). 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. At zero withdrawal, animals were slaughtered and liver and kidney samples were analyzed using a validated HPLC method with post-column derivatization. There were no detectable monensin residues in kidney tissue (<0.025 mg/kg). Monensin residues were detected in 4 of 6 liver samples (detected residues ranged from 0.05 mg/kg to 0.09 mg/kg).

To determine monensin residues in the tissues of lactating dairy animals, Holstein cows were fed medicated rations containing 0, 24, or 36 mg monensin/kg feed or with 1.8 mg monensin/kg body weight *via* gelatine capsule through a rumen fistula (Dick, et al., 1994). At the completion of feeding periods, liver was analyzed using a validated HPLC method with post-column derivatization. No residues were found above the method LOQ of 0.025 mg/kg.

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 body weight for seven consecutive days (Bassissi and Larvor, 2007). Gelatine capsules containing equal doses were administered at approximately 12-hour intervals. Tissues were collected at 6, 18 and 30 hours after the final dosing. Monensin residues (Table 11) were determined using a validated HPLC-MS/MS method with a LOQ of 1 µg/kg.

Table 11: Residues of monensin in the tissues of dairy cows treated *via* gelatine capsule at 0.9 mg/kg body weight in two equal doses for 7 days.

		Concentration (µg/kg)			
Animal No.	Time after last dosing (hours)	Muscle	Fat	Liver	Kidney
25	6	BLQ	5.2	9.6	1.03
32		BLQ	3.2	9.4	BLQ
39		ND	BLQ	6.4	BLQ
43		BLQ	1.1	10.8	BLQ
16	18	ND	BLQ	4.8	BLQ
71		ND	1.4	5.2	BLQ
15		BLQ	BLQ	5.4	BLQ
72		ND	BLQ	6.7	BLQ
3	30	ND	BLQ	2.2	ND
11		ND	BLQ	2.3	ND
75		ND	BLQ	5.4	ND
77		ND	BLQ	2.4	ND

ND = below the limit of detection. BLQ = below the lower limit of quantification (LOQ = 1 µg/kg). Bassissi and Larvor, 2007.

Pigs

Growing-finishing pigs received a medicated feed containing 100 mg monensin/kg feed for 98 days (Handy and Rea, 1976). Randomly selected animals were continued on medicated feed for two days or transferred to a non-medicated diet for two days. Animals were then slaughtered (effectively zero and 48 hours withdrawal). Edible tissues were analyzed for monensin by bio-autography (Rea, 1976). No monensin residues were detected in any of the tissues assayed. The assay has a sensitivity of <0.05 mg/kg for muscle and <0.025 mg/kg for liver, kidney, and fat.

Sheep/Goats

Wether lambs were treated *ad libitum* for 118 days with a medicated ration containing 0, 10, 20, or 30 g monensin/ton (11, 22 or 33 mg/kg) (Kline et al., 1975). Medicated feed was replaced with non-medicated feed and animals were withdrawn for 0, 24, or 48 hours. Samples of muscle, fat, liver, and kidney were collected and analyzed using a bioautography procedure (Kline and Wicker, 1975). No detectable monensin residues were found in samples of muscle, fat, and kidney from animals at any of the withdrawal times for any of the doses. Monensin was detected in liver samples collected at zero withdrawal. Activity below the test sensitivity of 0.05 mg/kg also was found in several liver samples at 24 h withdrawal. No residues were detected at 48 hours withdrawal.

Male Angora goats were fed rations containing 0, 20, or 30 g monensin/ton (11, 22 or 33 mg/kg) of feed for 56 days (Handy and Rea, 1984). Animals were withdrawn from medicated feed for zero or five days. At slaughter, liver samples were collected and analyzed by a bioautography procedure (Kline and Wicker, 1975). Monensin was detected in half of the 33 mg monensin/kg treatment liver samples and about 20% of the 22 mg monensin/kg treatment samples collected at zero withdrawal. One sample contained 0.04 mg/kg monensin (LOQ = 0.04 mg/kg). No monensin was detected in any of the 5-day samples.

Chickens

In one study (Callender, et al., 1980), male Hubbard chickens were reared for 45 days on feed containing 110 g monensin/ton (120 mg/kg). The birds were then placed on feed containing 15, 45, or 110 g monensin/ton (16.5, 50, or 120 mg/kg) for five days. At slaughter, abdominal fat, liver, and breast muscle tissues were assayed for monensin residues. No residues of monensin were found in liver and muscle tissue from any of the birds (LOQ = 0.04 mg/kg). Concentrations of monensin <0.04 mg monensin/kg were detected in fat tissue from birds in the 120 mg monensin/kg treatment group. No residues were found in the fat tissues from birds treated with feed containing 16.5 or 50 mg monensin/kg.

In an earlier study, chickens were fed monensin (120 mg monensin/kg) alone or in combination with other feed additives (Callender, 1978). Tissues were analyzed with a bioautography method with a sensitivity of approximately 0.05 mg/kg (Donoho and Kline, 1967). Residues are reported as samples positive/total samples. More than 2000 samples were analyzed. Although a few samples were positive at 0 and 24 hours withdrawal, samples from chickens withdrawn for 48, 72, and 96 hours were all negative. The study concludes that there was little potential for residues to exceed the 0.05 mg/kg concentration when chickens were fed monensin and withheld for 24 hours or more.

In studies (Pankhurst, 1981) where monensin was fed to boiler chickens at the highest recommended level (120 mg monensin/kg feed), the concentrations of monensin were determined with a bioautography method (Donoho and Kline, 1967). Samples were reported as positive/negative for monensin. While a limited number of fat (18/22), muscle and liver (2/12), and kidney (1/16) contained detectable concentrations of monensin at zero withdrawal, all samples at 24 or 48 hours were negative.

In another study (Okada, et al., 1980), chickens received medicated feed containing 80, 100, or 120 mg monensin/kg feed for 9 weeks. Thin-layer bioautography was used to determine the concentrations of monensin in edible tissues (Donoho and Kline, 1967). The minimum detection limits for the method used were 0.01 mg/kg in fat and 0.0125 mg/kg in liver, kidney, and muscle. Concentrations of monensin residues at zero withdrawal were 0.06 to 0.11 mg/kg in fat, undetectable to 0.04 mg/kg in muscle, undetectable to 0.04 mg/kg in liver and undetectable to 0.01 mg/kg in kidney. No detectable residues of monensin were found in fat at 48 hours or longer withdrawal times, or in liver, muscle, and kidney at 24 hours or more after withdrawal. Tissue residue concentrations did not increase proportionally when feed concentrations were increased to 300 and 600 mg monensin/kg feed.

The depletion of monensin from edible tissues was evaluated in broiler chickens (Atef, et al., 1993) following administration by gavage as a single dose of 40 mg monensin/kg body weight. Monensin residues were detected in all tested tissues (liver, kidney, fat, skin, thigh and breast muscle, plus heart) collected 2, 4, 6, and 8 hours after administration. The highest concentrations of monensin residues were found in liver. Twenty-four hours after administration, monensin residues were detected in liver, kidney and fat. Monensin residues were detected only in liver 48 hours after administration.

In a recent study, 30 chickens were treated with monensin sodium in the diet (nominal level of 125 mg monensin/kg in feed) for 42 consecutive days (Walker and McLean, 2007). The birds were sacrificed at specified time points after removal of the medicated diet. Samples of liver, kidney, muscle, and skin with fat were collected for analysis using a validated HPLC method with post-column derivatization and UV detection at 520 nm. The assay LOQ was 0.025 mg/kg. At zero withdrawal, the highest concentration of monensin (factor A) was found in skin with fat (0.02 mg/kg) followed by liver (0.02 mg/kg) and kidney (0.01 mg/kg). No monensin residues were detected in muscle samples. There were no detectable residues in any

tissues collected at 12 or 48 hours withdrawal. Samples collected at 48 and 72 hours withdrawal were not analyzed.

In another recent study, a sensitive LC-MS/MS method was developed and validated for the quantitation of monensin in the edible tissues of chicken (Chéneau, et al., 2007). In a subsequent depletion study, 68 chickens were treated orally with monensin (121 mg monensin/kg feed) for 33 days. The residues declined rapidly and were only observed in fat at the 18 hour sampling.

Unpublished data also are available (Sanders, 2008a; Henri, et al., 2008b). In this study, samples were collected at close intervals. The data are presented in Table 12.

Table 12: Tissue residues (µg/kg) in chickens after feeding monensin in the diet at the rate of 125 mg/kg (mean ± SD).

Tissue	0 h	2 h	4 h	6 h	8 h
Liver	17.0 ± 6.4	4.9 ± 3.9	3.8 ± 1.8	1.5 ± 0.3	<LOQ
Fat	49.1 ± 20.5	29.2 ± 7.0	28.8 ± 10.2	10.5 ± 7.8	5.0 ± 2.1
Muscle	5.8 ± 2.0	6.35	<LOQ	3.4 ± 0.3	<LOQ
	10 h	12h	23 h	35 h	71 h
Liver	<LOQ	<LOQ	NA	NA	NA
Fat	9.3 ± 2.5	7.1 ± 5.4	<LOQ	<LOQ	<LOQ
Muscle	<LOQ	<LOQ	<LOQ	ND	<LOQ

LOQ: 1 µg/kg for liver, 2.5 µg/kg for fat and muscle. NA=not analysed. ND = not detected.

Turkeys

In a residue depletion study, turkeys received medicated feed containing monensin at 120 mg monensin/kg for approximately 17 weeks. Birds were withdrawn from medicated feed for 0, 24, 48, 72 and 96 hours. Edible tissues were collected at slaughter and analyzed for monensin using a bioautography method (Donoho, 1972). Detectable residues were found in all tissues at zero withdrawal. No residues of monensin were detected in fat and kidney samples beyond 24 hours withdrawal. Muscle and skin were free of residual monensin 48 hours post-treatment. Liver samples were negative at 72 hours (Donoho, 1972). Additional residue data for turkeys are provided in an unpublished study (Sanders, 2008b), Table 13.

Table 13: Tissue concentrations of monensin (µg/kg) in turkeys after feeding at the rate of 100 mg/kg monensin (mean ± SD).

Tissue	0 h	2 h	4 h	6 h
Liver	3.5 ± 1.2	1.7 ± 0.1	1.8 ± 1.0	1.8 ± 1.0
Fat	40.5 ± 7.4	33.8 ± 19.2	35.6 ± 14.1	42.4 ± 37.1
Muscle	4.5 ± 1.4	4.1 ± 0.0	2.5 ± 0.0	4.3 ± 1.6
	8 h	10 h	12 h	24 h
Liver	ND	ND	ND	ND
Fat	9.8 ± 4.3	6.1 ±3.3	4.2 ± 0.2	3.4 ± 0.6
Muscle	ND	ND	ND	ND

NA=not analyzed. ND=not detected.

Quail

Two groups of quail were reared for eight weeks. One group received non-medicated feed while the treatment group received feed containing 80 mg monensin/kg feed continuously throughout the growth period (Handy and Rea, 1985). At the end of the feeding period, the

birds were sacrificed with no withdrawal period. Liver tissues were pooled to provide 15 g samples from each group. Monensin residues were determined using a thin-layer bio-autography method with a LOQ of 0.04 mg monensin activity/kg in chicken and bovine tissues (Kline and Wicker, 1975). No monensin was detected in any of the liver samples from monensin-treated birds.

Residues in Milk and Eggs

Cows' Milk

In a milk residue depletion study, lactating dairy cows were treated with two monensin controlled release capsules and fed a medicated feed containing 36 mg monensin (Bagg and Dick, 1999), resulting in an average daily dose of 1804 mg monensin per cow. There were no detectable residues of monensin in milk (<0.005 mg/kg) while on treatment.

In another study, Holstein cows, approximately 80 to 120 days in milk with four functional quarters, were treated with a total mixed ration containing 0, 24, or 36 mg monensin/kg feed or administered a gelatine capsule (through a rumen fistula) containing 1.8 mg monensin/kg body weight. At the completion of feeding periods, the milk was assayed for monensin using a validated HPLC method with post-column derivatization (Dick, et al., 1994). No milk samples contained residues of monensin at or above the LOQ of the method (0.005 mg/kg).

Residue depletion in the milk of 12 lactating dairy cows was determined after dosing with monensin at 0.9 mg/kg body weight for seven consecutive days (Bassissi and Larvor, 2007). Gelatine capsules containing equal doses were administered at approximately 12-hour intervals. Milk samples were collected prior to the first treatment, on second milkings and on the third milkings after the last treatment. Monensin residues were determined using a validated HPLC-MS/MS method with a LOQ of 0.25 µg/kg. See Table 14.

Table 14: Concentrations of monensin in the milk of individual dairy cows treated *via* gelatine capsule at 0.9 mg/kg body weight in two equal doses for 7 days.

	Monensin residues (µg/kg)			
		Post-final-treatment		
Animal number	Pre-treatment	Milking 1	Milking 2	Milking 3
3	ND	0.54	BLQ	NSC
75	ND	0.38	BLQ	NSC
77	ND	0.32	BLQ	NSC
11	ND	0.39	BLQ	NSC
12	ND	0.41	BLQ	BLQ
19	ND	0.41	ND	ND
52	ND	0.48	0.32	BLQ
76	ND	BLQ	ND	ND

ND = below the limit of detection. BLQ = below the lower limit of quantification (LOQ = 0. 25 µg/kg). NSC = no specimen collected. (Bassissi and Larvor, 2007).

Effect on dairy starter cultures

Although no data on the effect of monensin on dairy starter cultures were included in the dossier, information is available in the EMEA summary report (EMEA, 2007). According to the summary report, monensin was tested against a panel of dairy starter cultures. *Lactobacillus acidophilus* La-5 (MIC equal to 1 µg/ml) and *Streptococcus thermophilus* TH-4 (MIC equal to 2 µg/ml) cultures were found to be the most sensitive to monensin. For all

other cultures, monensin MICs were 4 μg/ml or above. The summary notes that *Lactobacillus lactis* was not included in the test panel. The EMEA established the NOEL of monensin for commercial dairy starter cultures at 0.1 μg/ml (EMEA, 2007).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The earliest semi-quantitative method for the analysis of monensin in animal tissues and fluids was based on thin layer chromatography/bio-autography (Donoho and Kline, 1967; Donoho, 1984). It has a limit of detection of 0.025 mg/kg and a routine performance limit of 0.05 mg/kg. It has been refined to have a detection limit of 0.01 mg/kg (Okada, et al., 1980).

Residues of monensin in milk and tissues also are analyzed using an HPLC method with post-column derivatization with vanillin and detection at 520 nm (Elanco AM-AA-CR-R174-AA791 for bovine tissues and milk; Elanco AM-AA-CR-R152-AA-791 for poultry tissues). The limit of quantification is 0.025 mg/kg for tissues and 0.005 mg/kg for milk.

More recently, a method utilizing extraction with an organic solvent and clean-up on solid-phase extraction columns followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using C_{18} columns and electrospray detection methods (Dubois, *et al.*, 2004) has been developed. This multi-residue LC-MS/MS method, which uses dinitrocarbanilide-d8, diclazuril-bis and nigericin as internal standards, is suitable for the determination of monensin residues in whole eggs and residues in bovine, porcine, and avian tissues including muscle, liver and fat with a sensitivity of ≤0.001 mg/kg. Chéneau, et al. also have validated an HPLC/MS/MS method for analysis of chicken tissues and plasma using a narasin internal standard (Chéneau, et al., 2007), Tables 15 and 16.

Table 15: Results of the regression analysis of the data of the standard calibration graphs.

Matrix	Curve	Intercept	Slope	Quadratic term	Weight	R^2
Plasma	1	0	9.160E−05	–	1	–
	2	0	9.475E−05	–	1	–
	3	0	9.125E−05	–	1	–
			RSD: 2.1%			
Muscle	1	0	8.785E−05	-	1	-
	2	0	8.205E−05	-	1	-
	3	0	7.975E−05	-	1	-
			RSD: 5.0%			
Fat	1	0	3.058E−05	-	1	-
	2	0	2.955E−05	-	1	-
	3	0	2.893E−05	-	1	-
			RSD: 2.8%			
Liver	1	4.813E−04	1.168E−04	2.457E−09	$1/x^2$	0.9987
	2	6.231E−04	1.338E−04	2.073E−09	$1/x^2$	0.9997
	3	5.383E−04	1.246E−04	1.784E−09	$1/x^2$	0.9978
			RSD: 6.8%			

Most recently, a validated HPLC-MS/MS method with ESI tandem mass spectrometry was developed in accordance with Good Laboratory Practice and European Guidelines for the establishment of MRLs for residues of veterinary medicinal products in foodstuffs of animal origin (Cordroc'h, 2007). Using liquid phase extraction and a narasin internal standard, separation is achieved with a reversed phase column and gradient elution. The method is summarized in Table 17.

Table 16: Detailed results of validation for all matrices (plasma, muscle, fat, and liver).

Mean introduced concentration (g/L (plasma) or (g/kg)	Trueness		Precision		Accuracy	
	Absolute bias (g/L or g/kg)	Recovery (%)	Repeatability (RSD%)	Intermediate precision (RSD%)	β-Expectation tolerance limit (g/L or g/kg)	Risk (%)
Plasma						
2.5	-0.002	99.2	8.5	13.3	[1.85, 3.11]	18.3
5	-0.07	98.5	8.8	8.8	[4.24, 5.62]	2.1
10	-0.30	97.0	3.7	3.7	[9.12, 10.29]	<0.1
25	-0.40	98.4	3.4	3.4	[23.27, 25.93]	<0.1
100	1.91	101.9	4.5	4.5	[94.87, 109.00]	<0.1
Muscle						
0.5	-0.21	58.7	10.9	10.9	[0.16, 0.43]	94.1
2.5	0.05	102.2	5.2	5.7	[2.34, 2.77]	0.1
5	-0.59	88.2	4.6	4.6	[4.07, 4.75]	0.6
10	-0.99	90.1	3.2	6.5	[7.78, 10.24]	12.1
100	-1.72	98.3	2.8	7.7	[82.87, 113.70]	10.1
Fat						
2.5	0.11	104.3	3.3	4.0	[2.34, 2.88]	<0.1
5	0.35	107.0	6.5	8.1	[4.23, 6.47]	6.4
10	0.05	105.4	3.0	4.3	[9.24, 11.85]	0.2
100	0.07	100.7	4.8	4.8	[88.73, 112.60]	<0.1
200	-14.00	93.0	1.9	2.8	[168.90, 203.10]	<0.1
Liver						
1	-0.02	97.9	6.4	6.4	[0.82, 1.14]	<0.1
2.5	0.12	105.0	8.3	8.3	[2.12, 3.13]	<0.1
5	0.23	104.5	8.9	9.4	[4.05, 6.41]	0.1
10	-0.80	92.0	11.8	11.8	[6.32, 12.09]	1.2
100	0.69	100.7	3.5	3.5	[92.18, 109.20]	<0.1

Table 17: Summary of performance characteristics for the validated HPLC-MS/MS method (Cordroc'h, 2007).

	Kidney	Liver	Fat	Muscle	Milk
Interference and carry over	No interference				
Selectivity	Selective against tylosin, tilmicosin, tulathromycin, salinomycin, amoxicillin, ampicillin, cloxacillin, benzylpenicillin, cefoperazone and thiopental				
Linearity (µg/kg; ng/ml for milk)	1.0 to 250.0	1.0 to 250.0	1.0 to 250.0	1.0 to 50.0	0.25 to 50.0
Regression (weighting factor)	$1/x^2$	$1/x^2$	$1/x^2$	$1/x^2$	$1/x^2$
LOQ (µg/kg; ng/ml for milk)	1.00	1.00	1.00	1.00	0.25
LOD (µg/kg; ng/ml for milk)	0.22	0.24	0.15	0.12	0.06
Within-run precision (%)	3.0 to 6.5	2.5 to 4.2	2.9 to 4.8	0.7 to 5.0	5.8 to 8.2
Between-run precision (%)	4.2 to 9.3	2.5 to 5.2	3.3 to 6.3	3.0 to 5.5	8.7 to 19.3
Accuracy (%)	-3.0 to +6.3	-2.0 to +0.2	+1.6 to +8.0	+1.0 to +2.0	-0.7 to +5.1
Stability in extract during analysis (*ca.*5°C)	24 hours	48 hours	48 hours	48 hours	48 hours
Stability after freeze-thaw cycles	3 cycles				
Stability in extract during analysis (*ca.*20°C)	72 days	71 days	64 days	93 days	89 days
50-fold dilution test Precision (%) Accuracy (%)	 15.6/19.2* -17.9/-18.7*	 4.5 -11.1	 7.9 -11.5	 2.9 -12.3	 2.6 +1.2

*Not validated

APPRAISAL

Monensin has not been reviewed previously by the Committee. Monensin is a polyether ionophore produced by *Streptomyces cinnamonensis*. It exhibits both antibacterial and anticoccidial activities. Monensin is used for the control of coccidiosis in poultry, cattle, sheep, and goats. It is used to improve the efficiency of rumen fermentation, increase weight gain and to control ketosis. In dry and lactating dairy cows, it is used to increase milk production.

Monensin is metabolized extensively; the metabolic profiles are qualitatively similar across many tested species. The rat appears to be a suitable species for toxicity testing of monensin and its metabolites. Metabolism rates vary by species, with horses showing slow metabolism and high sensitivity to monensin. Animal species have been classified as relatively insensitive (mice and poultry), moderately sensitive (rats, rabbits, pigs, and ruminants) and extremely sensitive (horses). Monensin is eliminated rapidly, primarily in the faeces.

Radiolabelled studies were conducted in cattle (including lactating dairy cows), pigs, sheep, chickens, and turkeys. Radiolabelled total residues in muscle were uniformly low, in some studies less than the method LOQ. At zero withdrawal, residues were highest in liver in all species. In most species, at most doses, residues at early withdrawal times were highest in liver, followed by kidney and fat. In

chickens, at a dose of 125 mg monensin/kg feed, residues in fat exceeded those in kidney at all withdrawal times and, at withdrawal periods greater than one day, residues in fat also exceeded those in liver. In lactating dairy cows, radiolabelled residues in milk reached steady state (0.045 mg/kg) after five days dosing. There were no detectable residues of monensin (LOQ = 0.005 mg/kg). Using a sensitive LC/ECP-MS/LSC method, parent monensin was detected at low concentrations (<0.001 mg/kg). Much of the detected radioactivity in milk is attributed to incorporation of the radiolabel into endogenous fatty acids.

Monensin is an appropriate marker residue for monensin residues in tissues and milk. It represents approximately 5% of the total residues in tissues and 2.7% in milk.

Residue depletion studies using unlabelled monensin have been conducted in cattle, pigs, sheep, goats, chickens, turkeys, and quail.

In early studies in cattle, few if any detectable monensin residues were found. An early study in Holstein cows treated with monensin in feed or capsules, no detectable residues were found in liver tissues (LOQ = 0.025 mg/kg). In a more recent study, lactating cattle were treated with controlled release capsules and fed a medicated ration. Low but quantifiable residues were found in liver tissues. Kidney residues were less than the LOQ of <0.025 mg/kg. In the most recent study in lactating dairy cows, monensin was administered in gelatine capsules administered twice daily. Tissues were analyzed with an HPLC MS/MS method (LOQ = 0.001 mg/kg). Muscle residues were below the LOQ at all sampling times as were most of the kidney residues. Residues in fat were detectable at 6 hours withdrawal (3 of 4 samples) and 18 hours withdrawal (1 of 4 samples) but not at 30 hours withdrawal. Detectable residues were found in all liver samples at all withdrawal times.

No detectable residues were found in pigs treated with monensin. The studies are more than 30 years old and the method had limited sensitivity (LOQ range 0.025-0.050 mg/kg).

In sheep and goats, detectable residues of monensin were found in liver at zero withdrawal. Residues also were detected in sheep liver samples collected at 24 hours withdrawal. In goats, only one liver sample at zero withdrawal had quantifiable residues (LOQ = 0.040 mg/kg). There were no detectable residues at withdrawal times greater than 24 hours.

Several residue depletion studies were conducted in chickens. In the earliest studies, more than 2000 samples were analyzed. Only a few samples were positive at 0 and 24 hours withdrawal (sensitivity = 0.05 mg/kg). In a subsequent study, no residues were detected in muscle and liver samples. At the highest dose, 110 g monensin/ton, detectable residues were found in fat samples. When chickens were treated with medicated feed containing 120 mg monensin/kg feed, limited numbers of zero withdrawal samples contained detectable residues. All of the samples collected at 24 and 48 hours withdrawal were negative for monensin. In still another study, TLC bio-autography was used to assess monensin residues. While detectable residues were found in fat at zero withdrawal, residues were significantly lower in muscle, liver, and kidney. No detectable residues were found after 24 hours (liver, muscle and kidney) or 48 hours (fat) withdrawal. Following administration by gavage, monensin residues were detected in all tested samples collected at 2, 4, 6, and 8 hours withdrawal. Highest concentrations were found in liver. At 24 hours, monensin residues were detected in liver, kidney and fat. Only the liver contained detectable residues at the 48-hour withdrawal time. In a recent study, monensin residues were determined using a validated HPLC with post-column derivatization and UV detection. At zero withdrawal, highest residues were found in skin with fat followed by liver and kidney. There were no detectable residues in muscle. There were no detectable residues in any tissues collected at 12 or 48 hours withdrawal. In another recent study, an HPLC-MS/MS method was used to measure monensin residues in the edible tissues of chickens. Residues declined rapidly and were detectable only in fat at the 18-hour sampling time.

Depletion studies also were conducted in turkeys and quail. In turkeys, no residues of monensin were detected in fat and kidney samples beyond 24 hours withdrawal; muscle and skin were free of residual

monensin 48 hours post-treatment; liver samples were negative at 72 hours withdrawal. In quail, no monensin was detected in any of the liver samples from monensin-treated birds using a thin-layer bioautography method.

Three milk residue studies were conducted. In the oldest study, none of the milk samples contained residues of monensin at or above the LOQ of the method (0.005 mg/kg). In a more recent study, cows were treated via controlled release capsules and medicated feed. There were no detectable (LOQ = 0.005 mg/kg) residues in milk, even for milk samples collected while on treatment. In the most recent study, cows were treated by gelatine capsule and monensin residues were determined using an HPLC-MS/MS method (LOQ = 0.25 µg/kg). Only in the first milking were residues consistently above the LOQ.

MAXIMUM RESIDUE LIMITS

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

- An ADI of 0–10 µg/kg bw was established by the Committee based on a chronic toxicological end-point. This ADI is equivalent to up to 600µg monensin for a 60-kg person.
- Monensin is the marker residue in both tissues and milk.
- Monensin is extensively metabolized; monensin represents, conservatively, 5% of total residues in tissues and 2.7% in milk.
- Liver contains the highest concentration of total residues at zero withdrawal in all species tested. In chickens treated at the maximum dose of 125 mg/kg in feed, total residues in abdominal fat exceed those in liver at 3 and 5 days of withdrawal. Liver can serve as the target tissue.
- While residue data in the studies submitted were determined using several methods, newer methods include a validated HPLC method with post-column derivatization and a validated HPLC-MS/MS method. Both of these newer methods are suitable for routine monitoring.
- The MRLs recommended for poultry tissues were based on residue data from the unlabelled residue depletion studies. For cattle, the residue concentrations were determined using the validated HPLC with post-column derivatization method. For chickens and turkeys, the residue concentrations were determined using the validated HPLC-MS/MS method.
- The MRL recommended for cows' milk was based on unlabelled residue depletion data determined using the validated HPLC-MS/MS method. The recommended milk MRL is 8 times the LOQ (0.25 µg/kg) for that method.
- Because monensin is not currently approved for use in pigs, no MRLs were recommended for monensin residues in pig tissues.

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, and 100 µg/kg in fat, and 2 µg/kg in milk. Residues in all species are determined as monensin.

It was not possible to do an intake estimate for monensin because of the small number of residue data points. Using the model diet and marker to total residue ratios of 5% for tissues and 2.7% for milk, the MRLs recommended above would result in an intake of 301 µg/person per day (poultry tissues plus milk) or 321 µg/person per day (ruminant tissues and milk), which represent 50% and 54% of the upper bound of the ADI, respectively.

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