



COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

MONENSIN **(Cattle, including dairy cows)**

SUMMARY REPORT

1. Monensin (CAS 17090-79-8, CAS 22373-78-0 for sodium salt) is a polyether antibiotic from the group of carboxylic ionophores produced by *Streptomyces cinnamonensis*. Monensin is used as the sodium salt. It is composed of the analogues A, B, C and D with monensin A being the major component (equivalent to 98%). The intended use as a veterinary medicinal product in lactating dairy cattle is as an oral device (controlled release capsule) that will release monensin in the rumen at a maximum rate of 400 mg/day over about 100 days to control ketosis.

Monensin was previously authorised in the European Union as a feed additive to improve growth and feed efficiency in cattle, except lactating cows (Council Regulation (EC) No 1831/2003). This use in cattle was phased out as of January 2006. Monensin has been re-evaluated and authorised as a feed additive for the control of coccidiosis in poultry (Council Regulation (EC) No. 1356/2004; amended by Council Regulation EC No 108/2007). The European Food Safety Authority (EFSA) Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has produced an Opinion on the safety and efficacy of monensin sodium as a feed additive for calves for rearing and cattle for fattening.

Monensin is not used in human medicine.

2. Monensin exhibits both antimicrobial and anticoccidial activity. It acts via complexation of monovalent cations. Its lipophilic structure facilitates transport of cations through biological membranes altering normal concentration gradients. Monensin is mainly active against Gram-positive bacteria. Therapeutic effects of monensin in cattle are explained as a result of a shift in ruminal fermentation characteristics leading to higher propionate concentrations at the expense of other volatile fatty acids (e.g. acetate, butyrate) and effects on energy metabolism including reduced blood ketones and increased serum glucose. These changes may also result in growth efficiency enhancement through improvements in energy capture from feed and protein utilisation.
3. The ionophoretic activity of monensin is also responsible for secondary pharmacological effects in the target species. The main target of secondary pharmacological activity in mammalian species is the cardiovascular system (inotropic activity via membrane current mechanisms) and heart/skeletal muscle (changes in subcellular organelles/cell damage). Susceptibility for effects was found to vary remarkably between species and the therapeutic margin is narrow. Cardiovascular effects were investigated following oral as well as intravenous administration in dogs (conscious and anaesthetised) and pigs (anaesthetised). In the oral study in dogs (0.138, 0.345, 0.69, 1.38 mg/kg bw) transient increases in coronary blood flow were observed while heart rate and mean blood pressure did not change significantly at the dose levels tested. The NOEL was established at 0.345 mg/kg bw. In the intravenous studies in dogs and pigs (doses ranging from 0.00069 to 1.4 mg/kg bw), monensin resulted in increased coronary flow, blood pressure and heart rate. The NOEL for intravenous treatment was identified as 0.0035 mg monensin/kg in anaesthetised dogs and 0.0345 mg/kg in conscious dogs. No intravenous NOEL could be identified in pigs. Concurrent administration of anaesthetics appeared to potentiate cardiovascular

effects of monensin in dogs by a factor of 10. It is also known that simultaneous use of monensin with other medicinal products (e.g. macrolides) can inhibit its metabolism resulting in increased toxicity from accumulation of the ionophore.

No cardiovascular effects of monensin were noticed in cats at oral doses of 30 mg/kg bw, which is in contrast to observations in other animal species. Studies on other secondary pharmacological effects in laboratory species such as mice and cats indicated no relevant effects on the central, peripheral, and autonomic nervous systems or respiratory and digestive systems at oral doses of 10 mg/kg or higher.

The oral NOEL for cardiovascular effects observed in dogs is quite close to oral doses that have potentially lethal effects in horses (about 4-fold margin), from the species studied, the most sensitive for monensin. Publicly available scientific literature suggests that differences between species in the potential of monensin to induce acute effects are possibly correlated with the rate and capacity of monensin turnover by microsomal liver enzymes (cytochrome P450). New *in-vitro* studies concerning comparative interspecies metabolic clearance (using horse, pooled dog and pooled human microsomal preparations) indicate that in humans and dogs monensin is metabolized more rapidly than in the horse. Due to experimental shortcomings no conclusions can be drawn on interindividual variability in humans to metabolize monensin. It was concluded that the average intrinsic clearance of monensin in humans and dogs may be comparable thereby providing support for the conclusion that the dog is an appropriate species in which to assess the pharmacological NOEL.

4. Single dose toxicity studies for a wide range of species indicated a high potential of monensin for producing acute toxic effects. Though susceptibility for effects between species was remarkably different, signs of toxicity were shown to be similar in all animals tested and comprised death, anorexia, hypoactivity, skeletal muscle weakness, ataxia, diarrhoea and decreased weight gain. Reported oral LD₅₀ values (mg/kg bw) in laboratory species were as follows: rats 21.7 to 50, dogs more than 10 (female) to more than 20 (male), rabbits 41.7, mice 70 to 96, monkeys more than 60 to more than 110. In non-laboratory species LD₅₀ values (mg/kg bw) were: horses 1.3 to 3, pigs 17 to 50, cattle 22 to 80, sheep about 12, goats 26.4, guinea fowl 95, chickens 130 to 250, and turkeys 346 to 416. Generally, female animals were more sensitive than males. There was no significant difference in the acute toxic response to either crystalline or mycelial forms of monensin.
5. The pharmacokinetics of monensin following oral administration have been evaluated in cattle and rats using ¹⁴C-monensin. Monensin was shown to be rapidly absorbed and extensively metabolised, mainly in liver in both species. Monensin and its metabolites were shown to be predominantly excreted in the bile. Biliary excretion in cattle and rats was reported to correspond to about 35% and 40% of the oral dose, respectively. Orally dosed radioactivity was nearly completely recovered in faeces, whereas urinary excretion was negligible in both cattle and rats. Metabolites were found to be produced by either single or combined demethylation, decarboxylation and hydroxylation at various positions along the ionophore backbone, resulting in a number of mainly unknown polar metabolites in low concentrations. Parent monensin and five metabolites resulting from O-demethylation and oxidation reactions were identified in liver, bile and/or faeces from monensin treated cattle. The compounds represented about 30% of total radioactivity in liver and 67% in faeces. Unchanged monensin represented only a limited fraction of the monensin-related compounds in liver and faeces (less than 10 %) and 2 % in milk. The pattern of metabolites in cattle and rats was found to be qualitatively comparable. Similar routes of metabolism were reported for other species including poultry. *In vitro* studies in rats, cattle, pigs, chickens, and horses provided some evidence for the involvement of cytochrome P450, and probably the cytochrome P4503A subfamily, in the oxidative metabolism of monensin. While the rate of monensin O-demethylation was nearly of the same order of magnitude in all species tested, total monensin metabolism was highest in cattle, intermediate in rats, chickens and pigs and lowest in horses. The catalytic efficiency (expressed as nmol of metabolized monensin/min nmol/CYP450) was in the following order: chicken > cattle > pig ≈ rat > horse, which correlated inversely with known interspecies differences in the susceptibility to the toxic effects of the ionophore moiety. Information on metabolism of monensin in dogs was not available. The

metabolite O-demethyl monensin was reported to be about 20-times less active than parent monensin in respect to ionophoretic and antibiotic activity in *in vitro* test systems. In a new study, rat microsomal incubates were used to generate the major monensin metabolites M1 (demethylated and hydroxylated derivative), M2 (demethylated and hydroxylated derivative with additional hydroxylation ring E), M5 (monohydroxylated ring D) and M6 (demethylated keto derivative, decarboxylated). Chromatographic techniques and LC/MS were used to separate and identify the metabolites generated. Monensin and metabolites M1, M2 and M6 were evaluated for antimicrobial and ionophoretic activity; metabolite M5 was evaluated for ionophoretic activity. The data provided indicate a loss of activity for tested metabolites of 75 % or more and seem to confirm previous results that major metabolic pathways (oxidative modification) of monensin significantly modify the polarity or complexing ability of the molecule and thus impairs the biological properties. Due to several experimental limitations (number of samples/statistical analysis) a precise figure for activity of each individual metabolite could not be obtained. However, a conservative estimate based on all available information is that major monensin metabolites retain no more than 50% of the pharmacological and microbiological activity of the parent compound.

6. Several subchronic repeated dose oral toxicity studies were conducted in rodents and dogs. In 5 GLP-compliant and 3 older non-GLP subchronic studies (90 days duration), rats were fed daily doses of monensin beginning with 25 to 50 mg/kg feed to up to 500 mg/kg feed, either as crystalline or mycelial form (equivalent to approximately 2 mg/kg bw or 3.5 mg/kg bw to up to approximately 35 mg/kg bw). Major signs of toxicity included death, decrease in body weight gain and organ weights and skeletal and cardiac muscle degeneration. Reported NOELs ranged from 2 to approximately 3.5 mg/kg bw based on growth retardation and changes in absolute or relative organ weights. In some studies slight effects were still observable in the lowest dose groups at about 3.5 mg/kg bw and NOELs could not be identified. In mice (37.5, 75, 150, or 300 mg/kg monensin equivalent to approximately 5 to 42 mg/kg bw), determination of a NOEL for subchronic toxicity (90 days duration) was not possible because growth was affected in all dose groups and minimal cardiac muscle degeneration was found on histopathological examination in one male of the lowest dose group.

Two non-GLP compliant 3-month subchronic toxicity studies were available in dogs. In the first study, five groups of dogs (2 per sex per group) were administered daily doses of 2.5, 5, 11, or 25 mg crystalline monensin/kg by capsule while doses of 5, 15, or 50 mg monensin/kg as mycelial monensin (2 per sex per group) were used in the second study. Doses of 11 mg monensin/kg or higher were not tolerated by dogs and caused death, ataxia and loss of muscular control, relaxation of the third eyelid and liver toxicity. Dogs given doses of 15 and 50 mg/kg bw in the second study showed anorexia, weakness, ataxia, laboured respiration, body weight loss and dose related degeneration of striated muscle. Aspartate transaminase values were significantly elevated during weeks 1 to 4 in dogs from the two highest dose groups and returned to normal ranges for the remainder of the study. In the study with crystalline monensin no treatment related effects were observed at a dose of 5 mg/kg bw or lower. In the study using mycelial monensin, significant effects were still observed at the lowest dose of 5 mg/kg and an overall NOEL for subchronic toxicity in dogs could therefore not be derived. Interpretation of results for the second study was further hampered by the fact that some animals received less than half of the nominal daily doses only, due to food refusal.

In a one-year GLP compliant chronic oral dog study (4 per sex per dose), beagle dogs were given nominal daily doses of 1.25, 2.5, 5, or 7.5 mg monensin/kg bw/day (half of the dose in the morning and half in the afternoon). No animal died during the study and there were no statistically significant differences in mean body weights of treated dogs. There were no observable physical signs in the two lowest dose groups (1.25 and 2.5 mg/kg). Dogs in the 5 and 7.5 mg/kg bw groups had transient signs of anorexia, hypoactivity and weakness, leg weakness, and weakness in neck musculature. Both groups of dogs showed monensin-related alterations in clinical chemistry parameters, including elevations in serum creatine phosphokinase and serum alanine transaminase. These elevations were transient and returned to normal values with continued administration of monensin. The clinical signs and clinico-pathologic findings were indicative of muscle related toxicity, consistent with previous findings in the subchronic toxicity

evaluation. Monensin-related effects on haematology, urinalysis, organ weights, electrocardiograms and pathologic evaluation were not observed. The NOEL was considered to be 1.25 mg/kg bw based on minimal, non-significant body weight gain decrease in male dogs from the 2.5 mg/kg group.

7. In two multigeneration reproduction studies, crystalline (non-GLP compliant study) or mycelial (GLP-study) monensin was administered via diet to three generations of rats. Crystalline monensin levels of 2.5 mg/kg (equivalent to 0.14 to 0.2 mg/kg bw/day), 12.5 mg/kg (equivalent to 0.74 to 0.97 mg/kg bw/day), and 25 mg/kg (equivalent to 1.43 to 2.3 mg/kg bw/day) for 30 parent and 20F₁/40F₂ animals per sex per dose were used. No treatment related changes were reported for the groups receiving crystalline monensin up to the highest dose tested. Thus the NOEL was 25 mg/kg corresponding to 1.43 to 2.3 mg/kg bw/day. In the multigeneration study with mycelial monensin 30 parent animals and 25 F₁/25F₂ animals/sex/dose group received dose levels of 33 mg/kg (equivalent to 1.6 to 2.2 mg/kg bw/day), 50 mg/kg and 80 mg/kg (equivalent to 4 to 8 mg/kg bw/day). In this study weight gain was depressed during the growth phase in F₀ males of all monensin-treated groups and F₂ males of middle and high dose groups, and in F₂ females of the low and middle doses and F₀, F₁, and F₂ females of the high dose groups. Reductions in weight of pregnant and lactating females of the middle and high dose groups were observed. A NOEL in relation to maternal toxicity could not be identified since slight body weight effects were still observable at a dose of 33 mg/kg (equivalent to 1.6 to 2.2 mg/kg bw/day). Reproductive performance including fertility, litter size, gestation length, parent and progeny survival and sex distribution was unaffected by any form of monensin treatment. There was no evidence of teratogenicity in the progeny from parents treated with either crystalline or mycelial monensin. It was concluded that monensin up to a dose of 80 mg/kg (equivalent to 4 to 8 mg/kg bw/day) did not adversely affect reproductive processes.
8. In a non-GLP compliant teratology study in rabbits, 15 pregnant rabbits were given daily oral gavage doses of 0.076, 0.38, or 0.76 mg monensin/kg on gestation days 6 through 18. On gestation day 28, the females were evaluated for reproductive performance and the foetuses were examined for abnormalities. Monensin treatment had no effect on the reproductive parameters and indices. Foetal viability, sex distribution, and weight were normal. Developmental deviations were observed in a relatively small number of progeny with no evidence of a treatment effect. Based on these results it was concluded that monensin administered orally to pregnant rabbits at doses up to 0.76 mg/kg affected neither the reproductive performance of the doe nor the development of the foetus. Treatment-related maternal effects included a reduction in mean daily food consumption in the highest dose group which was however not accompanied by body weight changes. There was no indication of embryo/foetotoxicity up to the highest dose tested (0.76 mg/kg bw). The apparent NOEL for maternal and embryo/foetotoxicity in this study was 0.76 mg/kg bw (higher doses were not tested).
9. A series of several recently conducted GLP compliant mutagenicity studies was provided (Ames test, chromosome aberration test *in vitro* and a micronucleus test *in vivo*). Monensin was negative in the Ames test, was not clastogenic *in vitro* and did not increase the incidence of micronucleated cells *in vivo*. In the *in vitro* cytogenetic assay the relevance of the occurrence of diplochromosomes remained unclear. However endoreduplication is normally not associated with the production of cancer, unlike imbalanced numbers of chromosomes (aneuploidy). Therefore it is considered that the increased number of diplochromosomes had no relevance to processes involved in cancer development. Overall monensin was not considered mutagenic.
10. The potential for monensin to induce carcinogenicity was assessed in 2-year carcinogenicity studies in rats and mice. In rats, one study used mycelial monensin and the other crystalline monensin, while a single study using mycelial monensin was conducted in mice. In the first study, groups of 100 male and 100 female rats, 6 to 8 weeks of age and derived from parents given diets containing mycelial monensin, were maintained for 2 years on diets containing mycelial monensin at 0, 33, 50, and 80 mg monensin/kg feed. The average daily doses were 1.40, 2.18, and 3.60 mg monensin/kg in males and 1.72, 2.86, and 5.02 mg monensin/kg in females. In the second study, groups of 80 male and 80 female rats were maintained on diets containing, 0, 25, 56, and 125 mg/kg feed crystalline monensin for 2 years. The average daily

doses were 1.14, 2.57, and 5.91 mg monensin/kg for males and 0, 1.46, 3.43, and 8.63 mg monensin/kg per day for females, respectively. Mice were maintained for 2 years on diets containing mycelial monensin at 0, 10, 25, 75, and 150 mg monensin/kg feed (1.2, 3.1, 10.2, and 22.6 mg monensin/kg bw for males and 0, 1.4, 3.5, 11.7, and 25.6 mg monensin/kg bw for females). Monensin did not influence the occurrence of non-neoplastic alterations, including cardiac and skeletal muscle lesions and other inflammatory and degenerative conditions in rats and mice.

The latency and prevalence of benign and malignant neoplasms were similar in control and monensin-treated animals. Survival was not adversely affected by treatment. It was concluded that monensin was not carcinogenic to rats and mice.

11. Minimum Inhibitory Concentrations (MIC₅₀) of sodium monensin against 100 bacterial strains representative of human gut microorganisms were determined (10 isolates from each of 10 genera). The results (medians for each genus) were as follows: *Bacteroides fragilis* 12 µg/ml, *Bacteroides* 8 µg/ml, *Bifidobacterium* 2 µg/ml, *Clostridium* 0.5 µg/ml, *Enterococcus* 8 µg/ml, *Escherichia coli* more than 128 µg/ml, *Eubacterium* 0.5 µg/ml, *Fusobacterium* 2 µg/ml, *Lactobacillus* 1.5 µg/ml, *Peptostreptococcus* 0.25 µg/ml. The lower 10% confidence limit of the geometric mean MIC₅₀ over all genera was calculated as 0.9860 µg/ml. MIC values above 128 µg/ml were defined as non-susceptible and were not considered in this calculation.

Two new studies investigated the fate of monensin in the presence of human faeces using either microbiological assay techniques and or (complementary) chemical assays based on direct monensin determination by HPLC/MS. The studies used slurry samples of sterilized human faeces incubated with monensin at concentrations ranging from 0.5 to 520 mg/ml. The results indicated substantial interaction between monensin and faecal matter at the concentrations tested. More than 90% of the monensin added to faecal suspensions disappeared from the soluble fraction (supernatants) and was found to be associated with solid faecal material. Due to model inherent limitations, the nature of this interaction remains unknown (degree of reversibility) and use of sterilized faecal material (in which enzyme activity is destroyed) makes interaction via covalent enzyme-catalyzed binding highly unlikely. In the absence of other explanations, strong non-specific adsorption of low (hydrophobic) monensin concentrations in the presence of excess faecal surface material may be seen as the most plausible mechanism. The results suggested an adsorption-desorption equilibrium with only a small fraction of freely available ionophore.

In vitro studies, in which microbiological activities of metabolites (M1, M2 and M6) were tested against human gut bacteria and other bacteria, suggested that residues of the substance would have a significantly reduced bioactivity compared to parent monensin.

For the establishment of the microbiological ADI the following standard formula was used:

$$\text{ADI} = \frac{\frac{\text{10\% lower conf. limit of MIC}_{50}\text{'s} \times \text{CF2}}{\text{CF1}} (\mu\text{g/ml}) \times \text{daily faecal bolus (220 g)}}{\frac{\text{fraction of an oral dose available for microorganisms}}{\text{x weight of human (60 kg)}}} (\mu\text{g/kg bw})$$

and the microbiological ADI was calculated as follows:

$$\text{ADI} = \frac{\frac{0.9860 \times 2}{1} \times 220}{0.5 \times 60} = 14.46 \mu\text{g/kg bw i.e.} = 867.7 \mu\text{g/person}$$

The following assumptions were made:

- CF1 = 1, unique mode of action, no use in human medicine, no evidence for transferable resistance, no cross-resistance with other antibiotics, no resistant strains isolated in host animals, no resistance genes identified,
 - CF2 = 2 to correct for the lower density in strains used in the test system compared to the higher density in the gut, MIC data showed a significant decrease in activity with increasing bacterial densities
 - 220 g was the weight of the colonic content,
 - 0.5 conservatively estimated factor reflecting the results of the microbiological and chemical faecal bioavailability studies which, despite some model inherent limitations, indicated a significant reduction of the free bioavailable fraction of monensin through strong association with solid faecal matter
12. Monensin was tested against a panel of dairy starter cultures. Cultures found to be most susceptible to monensin were *Lactobacillus acidophilus* La-5 (MIC equal to 1 µg/ml) and *Streptococcus thermophilus* TH-4 (MIC equal to 2 µg/ml). Monensin MICs against all other cultures were 4 µg/ml or above. *Lactobacillus lactis* was not included in the testing programme. The NOEL of monensin with regard to milk acidification rate of commercial dairy starter cultures was established as 0.1 µg/ml.
13. The potential for crystalline monensin and the feed premix formulation to induce skin sensitisation was evaluated in CBA/J mice using the local lymph node assay. No clinical signs of toxicity, mortality, cutaneous reactions, and clinically significant increase in ear thickness were observed. However, there were dose-related increases in indices for lymphoproliferative response. It was concluded, that monensin induced delayed contact hypersensitivity in the local lymph node assay and would be classified as a weak sensitizer by skin exposure.
14. Monensin is not used in humans and there is very limited data on effects following direct exposure of humans to monensin. A report of intentional exposure to monensin in a 17-year old male who died from acute rhabdomyolysis with renal failure after ingesting monensin premix were available. Although the amount of monensin ingested in this case was not estimated, in another case cited, two deaths among 6 people who consumed baked goods made with monensin premix was attributed to monensin exposure of at least 10 times daily dose fed to cattle. Allergic symptoms including urticaria, swelling of the face or tongue, pruritus, nasal congestion, contact dermatitis and local respiratory irritation have been reported in workers handling monensin.
15. Based on the results described above the following ADIs were calculated:
- a pharmacological ADI of 3.45 µg/kg bw i.e. 207 µg/person was derived, using an uncertainty factor of 100 from a NOEL of 0.345 mg/kg in an acute oral study of cardiovascular system end-points in the dog.
 - a toxicological ADI based on the NOEL of 0.76 mg/kg bw, corresponding to toxicity in the teratogenicity studies in rabbits, was retained. This value represents the lowest NOEL observed in toxicology studies and, given the sensitivity of the species, provides a conservative basis for the toxicological ADI. Use of an uncertainty factor of 100 resulted in a toxicological ADI of 7.6 µg/kg bw or 456 µg/60 kg person, respectively.
 - a microbiological ADI was calculated as 14.46 µg/kg bw or 867.7 µg/60 kg person.
- The CVMP concluded that the relevant ADI for the assessment of consumer safety is the pharmacological ADI.
16. In the pivotal radiometric residue study in cattle (5 animals), tissues and milk were investigated following twice daily oral doses of 0.9 mg ¹⁴C-monensin/kg bw in gelatine capsules via a rumen fistula over a period of 9.5 days (918 to 1125 mg ¹⁴C-monensin/day), which is 3 times the daily dose from the control release device. Cows were milked twice daily at approximately 12 hour intervals. Animals were slaughtered 6 hours after the last dose. Milk and tissues were assayed for total radioactivity (LSC). In addition, milk, liver, and kidney were assayed for parent monensin A by HPLC, and milk and liver were analysed for parent monensin A and major metabolites by LC/MS. Radioactivity in kidney, muscle and fat was considered too low to allow identification of

metabolites. Total residues in milk reached steady-state after 5 days of administration (average concentration range 43 to 48 µg/kg). Parent monensin concentrations in milk collected at steady-state were below the limit of quantification of 5 µg/kg of the HPLC method. Mass spectrometry analysis of milk extracts identified monensin and the metabolite M-6 (demethylated keto derivative, decarboxylated). Monensin represented approximately 2% of the total radioactivity in milk. Approximately 26.5% of the total radioactivity in milk was determined to be incorporated into endogenous fatty acids. Of the tissues collected, liver had the highest concentration of mean total residues (1280 µg/kg), followed by kidney (70 µg/kg), fat (20 µg/kg) and muscle (lower than the limit of detection of 20 µg/kg). Parent monensin concentrations in liver and kidney were below the 25 µg/kg limit of quantification of the HPLC method. Mass spectrometry analysis of LC fractions of liver extract identified monensin, and the metabolites M-1 (demethylated and hydroxylated derivative), M-2 (demethylated and hydroxylated derivative with additional hydroxylation ring E) and M-6 (demethylated keto derivative, decarboxylated) which were estimated to account for approximately 6.8%, 4.5%, 4.5%, and 18% of the total radioactivity (about 75% of extractable radioactivity). Most of the metabolites in tissues and milk were reported to be polar derivatives of monensin and remained unidentified. In addition, two pre-GLP radiometric studies in steers and heifers were presented in which oral daily doses of 0.76 to 1.4 mg/kg bw and 0.83 mg/kg bw in gelatine capsules (300 to 330 mg ¹⁴C-monensin/animal) were given for 5 days or 2 days. Animals were slaughtered 12 hours after administration. Results for total residues were qualitatively similar to those obtained in the pivotal study with residues being highest in liver and lowest in fat. Parent monensin as determined by TLC/microbiological methods accounted for 3 to 6 % of total residues in liver.

17. All residue studies in which total tissue residues and/or monensin and metabolites were investigated used single point slaughter times immediately after administration. Neither studies on total residues nor non radiolabelled residue depletion studies using the intended preparation over the full 100-day period of drug release have been performed. However, in one residue study 6 dairy cows received two controlled release capsules for 22 days. The release rates ranged from 408.5 to 469.9 mg/day. Simultaneously, the animals were fed a total mixed ration containing 36 mg monensin/kg in the feed (equivalent to a dose of 1536.8 to 1803.7 mg monensin/cow per day or 4 to 5 times the recommended daily dose as feed additive). Cows were milked at day 0, 15 and 22 during treatment. Liver and kidney tissue were collected at day 22 (zero withdrawal). Milk and tissues were assayed for monensin A by HPLC. Monensin A concentrations in milk were all below the limit of quantification of 5 µg/kg of the HPLC-method. Monensin A was also detected in the livers of 4 cows at concentrations of 55.1 µg/kg, 69.6 µg/kg, 45.8 µg/kg, and 84.5 µg/kg. The monensin A concentrations in kidney were below the limit of quantification of 25 µg/kg of the HPLC-method. In an old non-GLP study, cattle were treated with monensin via feed at doses of 750 mg/day for 148 days and at 500 mg/day for 106 days. Tissue samples were analysed at 48 to 240 hours after cessation of treatment. Monensin concentrations were reported to be below the test sensitivity of the TLC/microbiological method used (50 µg/kg). The validation of this method was, however, not complete.
18. In a new residue study a total of 16 lactating cows were orally treated with monensin at the maximum therapeutic dose rate of 0.9 mg/kg bw/day for 7 consecutive days. Monensin was administered via gelatine capsules at twice daily doses of 0.45 mg/kg bw at approximately 12 hour intervals. Tissue samples (liver, kidney, muscle, fat) were collected from 4 animals at 6, 12 and 24 hours after the last treatment, respectively. Milk samples were collected from 8 cows at the milkings before the first treatment and at 3 milkings (6, 12, 24 hours) following the last treatment. Monensin A was assayed in bovine tissues and milk using a validated HPLC/MS/MS method with a limit of quantification (LOQ) of 1 µg/kg for tissues and 0.25 µg/kg for milk. Monensin A was detected in tissues and milk at very low concentrations only. Highest concentrations were observed in liver with maximum values of 10.46 µg/kg (6 hours), 6.70 µg/kg (12 hours) and 5.43 µg/kg (24 hours) followed by fat with 5.24 µg/kg and 1.41 µg/kg at 6 and 12 hours, respectively and kidney with 1.03 µg/kg at 6 hours (one sample). In kidney and fat monensin A concentrations were below the limit of quantification in all samples at 12 hours and 24 hours, respectively, while in muscle no monensin could be detected at any time point. In milk parent monensin could be detected up to the 2nd milking at very low concentrations (0.54 µg/kg

to below the limit of quantification in the 1st milkings and 0.32 µ/kg to below the limit of quantification in the 2nd milkings).

19. Residue studies suggested that monensin A is the most reasonable choice for the marker residue. The ratio of marker to total residues as determined in the pivotal radiolabelled study was approximately 6.8% in liver. For the purpose of calculations a uniform lower ratio of 5 % was assumed for all tissues (marker residue proportions/concentrations were generally too low to determine a more precise ratio). The ratio in milk was 2.7%. This factor already included correction for the portion of radiolabelled milk residues incorporated into endogenous fatty acids.
20. A routine analytical method for the determination of monensin A in bovine tissues and milk based on HPLC coupled with ESI tandem mass spectrometry (HPLC/MS/MS) using narasin A as internal standard was available. The limit of quantification was 1 µg/kg in all tissues and 0.25 µg/kg in milk. Recovery was in the range of 97 to 108 % in tissues and milk. The specificity of the method against other ionophores as salinomycin and narasin as well as the antibiotics tylosin, tilmicosin, tulathromycin, penicillines, cefoperazone, and thiopental was demonstrated. The method was validated according to Volume 8 of the Rules Governing Medicinal Products in the European Union and presented in the ISO 78/2 format.
21. In 2004, monensin was evaluated by the FEEDAP Panel of the EFSA for use as feed additive for the control of coccidiosis in poultry. The EFSA proposed an ADI of 3 µg/kg bw i.e. 180 µg/person based on a NOEL for pharmacological effects in dogs rounded to 0.3 µg/kg bw/day and applying an uncertainty factor of 100. In 2006 MRLs of 0.008 mg/kg have been established for poultry liver, kidney, muscle and 0.025 mg/kg for skin plus fat of chicken and turkey.
22. CVMP and the FEEDAP Panel of the EFSA concluded that the relevant NOEL for monensin is the one derived from pharmacological end-points in the dog based on the same pivotal study. Based on this NOEL an ADI of 3.45 µg/kg bw using an uncertainty factor of 100 was calculated by the CVMP. When calculating the ADI, the FEEDAP Panel of the EFSA rounded the NOEL to 0.3 mg/kg bw/day and obtained an ADI of 3 µg/kg bw i.e. 180 µg/person.

For the benefit of harmonisation, the CVMP agreed to retain the same ADI for monensin as calculated by the FEEDAP Panel of the EFSA i.e. 3 µg/kg bw (180 µg/person).

Conclusions and recommendation

Having considered that:

- a pharmacological ADI of 180 µg/person was established as the overall ADI for monensin,
- monensin is extensively metabolised,
- monensin A was identified as the marker residue and the ratio of marker to total residues is approximately 5 % in tissues and 2.7 % in milk,
- ionophoretic and microbiological activity of monensin derived metabolites was largely reduced, a conservative estimate of 50% of activity was considered appropriate for intake calculations,
- a validated routine analytical method for monitoring residues in bovine species is available;

The Committee for Medicinal Products for Veterinary Use recommends the inclusion of monensin in Annex I of Council Regulation (EEC) n° 2377/90 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal Species	MRL	Target tissue(s)	Other provisions
Monensin	Monensin A	Bovine	2 µg/kg 10 µg/kg 30 µg/kg 2 µg/kg 2 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRLs, the theoretical maximum daily intake from tissue and milk of cattle represents 57% of the ADI.

Taking into account the MRLs established by the FEEDAP Panel of the EFSA for chicken tissues and the CVMP MRLs for milk and the consideration of 50% activity of monensin metabolites, the theoretical maximum daily intake from chicken tissue and milk represents 82% of the ADI.