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Toxicological evaluation of certain veterinary drug residues in food

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MONENSIN

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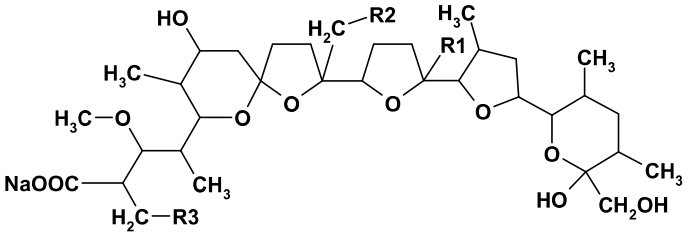
1. EXPLANATION

Monensin is a polyether carboxylic ionophore antibiotic produced by *Streptomyces cinnamonensis* ATCC15413. It is used for the treatment of coccidiosis in poultry (chickens, turkeys and quail) and ruminants (cattle, sheep and goats) (Shumard & Callender, 1967; Anderson et al., 1976; Calhoun, 1986; USFDA, 1986). Monensin is also used to control ketosis and bloat in cattle (Shumard & Callender, 1967; Anderson et al., 1976; Calhoun, 1986; USFDA, 1986) and as a growth promoter feed additive in cattle and sheep (Goodrich et al., 1984). Monensin

is mainly effective against Gram-positive bacteria (Haney & Hoehn, 1967). Monensin is not used in human medicine and was therefore not classified as a critically important antibiotic for humans by the 2007 World Health Organization (WHO) expert meeting on categorization of critically important antimicrobials for human medicine for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use (WHO, 2007). Monensin acts by interfering with ion flux across bacterial membranes, causing reallocation of bacterial energy resources to maintaining cellular pH and ion balance rather than growth and reproduction (Haney & Hoehn, 1967; Pressman & Fahim, 1982; Russell, 1987). Effects on feed conversion efficiency may arise from the ability of monensin to shift rumen fermentation towards the more energetically efficient propionate pathway, reduce methane production and increase nitrogen retention by reducing dietary protein deamination and urinary ammonia excretion (Van Nevel & Demeyer, 1977; Russell, 1987; Russell & Strobel, 1989).

Monensin (2-(5-ethyltetrahydro-5-(tetrahydro-3-methyl-5-(tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl)-2-furyl)-2-furyl)9-hydroxy-β-methoxy-a,g,2,8-tetramethyl-1,6-diaoxaspiro(4,5)decane-7-butyric acid) (Chemical Abstracts Service No. 22373-78-0) is generally used as the sodium salt. Monensin is a mixture of four analogues, A, B, C and D, which are produced during fermentation, with monensin A being the major component (98%) (Haney & Hoehn, 1967). The chemical structures of monensin A, B and C are shown in Figure 1. Depending on the method of purification, monensin can exist in mycelial, crystalline and recrystallized forms.

Figure 1. Chemical structures of monensin A, B and C



Factor	R1	R2	R3
A	$-CH_2CH_3$	-H	-H
B	$-CH_3$	-H	-H
C	$-CH_2CH_3$	-H	$-CH_3$

Monensin has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Codex Committee on Residues of

Veterinary Drugs in Foods decided at its 17th Session (Codex Alimentarius Commission, 2007) to put monensin on the priority list for evaluation by JECFA.

The present Committee considered data on the pharmacokinetics (including metabolism), acute toxicity, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity, immunotoxicity, cardiovascular and respiratory toxicity, epidemiological findings and microbiological effects of monensin. Additionally, residue depletion studies and analytical methods were reviewed. Many of the studies were conducted prior to the introduction of Good Laboratory Practice (GLP).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Studies in laboratory and domestic animal species have demonstrated that orally administered monensin sodium is readily absorbed and extensively metabolized, mainly in liver, followed by biliary excretion and elimination in the faeces. Absorption is significantly greater in monogastric species than in poligastric species (cattle or sheep), which absorb only about 50% of the dose (Donoho, 1984).

(a) Rats

In a non-GLP-compliant study examining the absorption of monensin in Wistar rats, three animals per sex per dose with exteriorized bile duct cannulae were orally administered ^{14}C-labelled monensin at doses of 5 or 40 mg/kg body weight (bw) in males and 2 or 16 mg/kg bw in females. Recovery of the radioactivity in the bile within 72 h was independent of dose and ranged from 32.8% to 48.6% in males and from 30.7% to 53.2% in females (Howard & Lobb, 1981a).

In another study that was performed prior to the development of GLP regulations, Wistar rats were exposed to [^{14}C]monensin at 4 and 16 mg/kg bw (females) or 5 and 20 mg/kg bw (males) by oral gavage for 4 or 24 h, with five animals per sex per dose per exposure time. Following 4 h of exposure, radioactivity could be detected in all tissues examined from both sexes, with concentrations in the liver, duodenum, jejunum, ileum and colon more than 10-fold higher than serum concentrations. Females receiving 4 mg/kg bw also had elevated levels of radioactivity in the adrenal glands. The concentration of radioactivity in the serum and tissues significantly declined by 24 h, but a greater than 10-fold higher concentration was observed in the liver, ileum and colon of all animals relative to the serum. Greater than 10-fold higher [^{14}C]monensin concentrations were also observed at 24 h in the duodenum and jejunum of males given 20 mg/kg bw, the adrenal glands, pituitary gland, thyroid gland and jejunum of females given 4 mg/kg bw and the adrenal glands, duodenum and jejunum of females given 16 mg/kg bw (Howard & Lobb, 1981b). There was no indication that any tissue accumulated a large percentage of the dose.

Two studies provided evidence that monensin is primarily excreted in the faeces of rats. In the first non-GLP-compliant study, a male Harlan rat was administered 2.15 mg of [^{14}C]monensin by gavage. The single dose was preceded by 13 days of exposure to unlabelled monensin at 100 mg/kg in the diet (equivalent to 10 mg/kg bw per day), followed by 12 days of exposure to the dosed feed. Radioactivity was detected in the faeces for 3 days following exposure, with a recovery of 91.47% of the dose. Only 0.48% of the dose was recovered in the urine, and radioactivity in the urine was detectable only for 1 day following exposure (Herberg, 1973c).

In a second study that was conducted prior to GLP regulations, Wistar rats were administered a single oral dose of [^{14}C]monensin by gavage. Male rats received doses of 5, 10, 20 or 40 mg/kg bw, and females received 2, 4, 8 or 16 mg/kg bw, with five rats per treatment group. Excretion of radioactive monensin within 72 h was independent of dose, with 84.7–88.9% and 71.8–88.2% excreted in males and females, respectively. In males, 83.6–87.4% of the dose was excreted in the faeces and 1.0–1.6% in the urine, whereas females excreted 70.8–87.2% in the faeces and 1.0–1.3% in the urine. At 24- and 48-h time points, there was a significantly lower percentage of monensin excreted in males and females that received the two highest doses, which may have been due to toxicity observed at higher doses (Howard & Lobb, 1979).

(b) Dogs

Blood samples from a dog dosed orally with [^{14}C]monensin at 1 mg/kg bw were assayed by extraction with carbon tetrachloride and scintillation counting. [^{14}C]Monensin was rapidly absorbed, with the highest concentration of 0.056 mg [^{14}C]monensin/l detected in the blood 15 min after dosing. The radioactivity declined rapidly to less than 0.01 mg/l by 3 h. In a separate experiment, an unspecified dose of intravenously administered [^{14}C]monensin was recovered primarily in the faeces. Fractionation of faecal radioactivity indicated that approximately 6% was the parent compound monensin and the remainder had been metabolized, providing indirect evidence for rapid biliary excretion as a major route of elimination (Donoho, 1984).

(c) Chickens

Ten White Leghorn roosters and two White Leghorn hens were exposed orally to a single dose of [^{14}C]monensin in a gelatine capsule (dose range: 2.6–100 mg). Some birds were colostomized, whereas others had bile cannulae inserted. Absorption in the chickens ranged from 11% to 31% of the ingested [^{14}C]monensin. The primary route of excretion was in the faeces, with a small proportion excreted in the urine and by respiration (Davison, 1984).

In a non-GLP-compliant study, broiler chickens were administered [^{14}C]monensin sodium at a concentration of 120 mg/kg in the diet for 4 days (two males, three females) or 6 days (three males, three females). Six hours after withdrawal from the treated feed, radioactivity was detected in the liver, kidney, fat and skin, with the highest level detected in the liver (0.5 mg/kg liver). No radioactivity was detected in the muscle tissue (Donoho et al., 1980).

In a non-GLP-compliant study, six chickens were exposed to [^{3}H]monensin sodium at 121 mg/kg in the diet for 2 days. Only 52–73% of the radioactivity was recovered; of this, 97% was found in the faeces. The reason for poor radioactivity balance was unknown (Herberg, 1967). In a second non-GLP-compliant study, a broiler-type cockerel was exposed to unlabelled monensin sodium at 110 mg/kg in the diet for 15 days, followed by a single exposure to a capsule containing 7.4 mg of [^{14}C]monensin. Seventy-five per cent of the radioactivity was recovered in the excreta within 3 days, 90% within 6 days and 100% within 12 days of exposure (Herberg, 1973a). In an additional study, three Leghorn chickens were exposed to monensin sodium at 120 mg/kg in their feed for 35 days, receiving a single dose of [^{14}C]monensin on day 15 by capsules. The monensin capsule doses were equivalent to an average monensin feed concentration of 120 mg/kg. The radioactive dose was recovered in the faeces, with more than 75% recovered within 3 days and a total of 85–101% recovered by the end of the study (Herberg, 1975).

(d) Cattle

In a non-GLP-compliant study, two 3-month-old bile-cannulated calves (one male Shorthorn, one female Angus) were administered a single dose of [^{14}C]monensin sodium at 10 mg/kg bw orally in a gel capsule. Absorption in the calves was calculated to be 36–40% of the dose, and most of the absorbed ^{14}C was recovered in the bile. The primary route of ^{14}C excretion was in the faeces, with a small proportion excreted in the urine (Davison, 1984).

Serum levels of monensin remained low following oral administration in cattle. Administration of an intraruminal dose of 60 mg/kg bw in three fistulated animals resulted in plasma concentrations below 0.02 mg/l, when determined by semiquantitative autoradiography, even though all three animals eventually died from the dosing. Correspondingly, steers given an oral dose of 30 mg/kg bw had essentially no detectable monensin in the plasma at a limit of detection of approximately 0.05 mg/l. In addition, plasma concentrations of monensin rapidly declined when six steers received 0.15 mg monensin/kg bw intravenously. Monensin could not be detected in the serum 1 h after treatment (Donoho, 1984).

Low concentrations of monensin could be detected in the tissues of cattle shortly after monensin administration. In a non-GLP-compliant study, three animals were treated daily for 5 days with an oral capsule containing 330 mg of [^{14}C]monensin, and tissues were collected 12 h after the last capsule. The highest concentration of net radioactive residues was detected in the liver (0.2–0.4 mg/kg liver), whereas the muscle, fat, kidney and heart tissue had less than 0.021 mg/kg tissue (Herberg et al., 1978).

Additional experiments have demonstrated that the faeces are the primary route of excretion of monensin sodium in cattle. In a non-GLP-compliant study, a single 260-kg steer was fed 300 mg of unlabelled monensin for 15 days prior to administration of a capsule containing 299.8 mg [^{14}C]monensin by gavage. After administration of the capsule, the steer returned to the diet containing unlabelled monensin for 14 days while the excretion of radioactivity in the urine and faeces was monitored. Within 7 days, 93.7% of the radioactive dose was recovered in the

faeces, with no radioactivity detected in the urine (Herberg, 1973b). When this study was repeated using two additional Black Angus steers, 88–102% of the dose was recovered in the faeces within 8–11 days, with no radioactivity found in the urine (Herberg, 1974b). In a third non-GLP study using three Black Angus steers, 300 mg of unlabelled monensin was provided in the feed for 4 weeks, with a single 300 mg dose of [^{14}C]monensin in a capsule on day 14 of the study (equivalent to 1 mg/kg bw). Between 88% and 102% of the radioactivity was recovered in the faeces within 7–11 days after administration. No radioactivity was detectable in the urine (Herberg, 1974a).

(e) Sheep

In a non-GLP-compliant study to determine the rate and route of excretion of monensin, a wether lamb was exposed to unlabelled monensin at 50 mg/kg bw per day in the diet for 4 weeks, receiving 50 mg of [^{14}C]monensin in two oral capsules on day 14 of the study. Within 9 days, 101.96% of the radioactive dose was recovered in the faeces, whereas no radioactivity was detectable in the urine (Elanco Animal Health, 1998). In a separate study, groups of three finishing lambs were dosed daily for 3, 5 or 7 days with a gel capsule containing the equivalent of 16.5 mg [^{14}C]monensin/kg feed. Twelve hours after the last dose, radioactivity concentrations of 0.20–0.35 mg/kg tissue were detected in the liver; however, concentrations in the kidney, fat and muscle were less than 0.027 mg/kg tissue. The faeces were the primary route of excretion (Giera et al., 1984a).

(f) Pigs

In a non-GLP-compliant study, six pigs were exposed to [^{14}C]monensin at 110 mg/kg in the diet for 5 consecutive days. Six hours after the final feeding, the highest concentration of radioactivity was in the liver (2.5 mg/kg tissue), followed by the kidney (0.17 mg/kg tissue). Concentrations in the fat and muscle were less than 0.045 mg/kg tissue (Giera et al., 1984b).

Two non-GLP-compliant studies have been performed in pigs to examine the route and rate of excretion of monensin sodium (Donoho & Herberg, 1977; Herberg & Donoho, 1977). In the first, a 54.5-kg barrow was conditioned to a diet containing unlabelled monensin at 50 mg/kg for 2 weeks and was then administered a capsule containing 5.23 mg [^{14}C]monensin (equivalent to 0.1 mg/kg bw). Urine and faeces were collected for 13 days following the radioactive dose. Only 54.87% of the dose was recovered, with 53.89% in the faeces and 0.98% in the urine. Excretion occurred rapidly, with 92% of the ^{14}C in the faeces recovered in 3 days. The reason for the non-quantitative recovery of radioactivity in this experiment was unknown (Herberg & Donoho, 1977).

In the second study, a 50.5-kg barrow was conditioned to a diet containing monensin sodium at 50 mg/kg for an unspecified amount of time and then administered a capsule containing 10.4 mg [^{14}C]monensin (equivalent to 0.2 mg/kg bw). Urine and faeces were collected daily for 10 days following exposure to radioactive monensin. In 10 days, 78.14% of the dose was recovered, with 75.04% in the faeces and 3.10% in the urine. A majority of the ^{14}C in the urine was recovered

within the first 2.5 days after exposure, whereas most of the ^{14}C in the faeces was detected during the first 3.5 days (Donoho & Herberg, 1977).

2.1.2 Biotransformation

Monensin is extensively metabolized in the liver, producing more than 50 different metabolites that have been detected in the liver, bile and faeces of chickens, cattle, rats, pigs, dogs, turkeys, sheep and horses (Donoho et al., 1978, 1982a,b; Donoho, 1984, 1985; Grundy et al., 1998). In most species (chickens, rats, dogs, turkeys and pigs), less than 10% of monensin is excreted as the parent compound (Donoho, 1984), whereas a study in calves indicated that 50–68% of the ^{14}C identified in the faeces was unmetabolized monensin (Davison, 1984). This difference in amount of metabolized monensin may have been a result of differences in absorption of the molecule in different species (Donoho et al., 1978). Total microsomal monensin metabolism, estimated by measuring the rate of substrate disappearance by a high-performance liquid chromatographic (HPLC) analytical method, is highest in cattle, intermediate in rats, chickens and pigs, and lowest in horses (Nebbia et al., 2001). The pattern of metabolites is qualitatively similar between laboratory and non-laboratory animal species, although quantitative differences exist. No single metabolite dominates the metabolic profile.

Monensin metabolites result mainly from *O*-demethylation at the methoxylic group and/or hydroxylation at several places on the ionophore backbone (Donoho, 1984). To date, no metabolites representing fragmentation or conjugation of monensin have been identified. Although it is difficult to obtain sufficient monensin metabolites to test activity, four metabolites generated by rat liver microsomes, including a by-product of monensin production (*O*-desmethylmonensin), have been tested and have at least 10- to 20-fold less antibacterial, anticoccidial, cytotoxic, cardiotonic and ionophoric activity than the parent compound, indicating that metabolism eliminates most of the biological activity of monensin (Donoho et al., 1979; Donoho, 1984; Halstead et al., 2007).

The *O*-demethylation of monensin is greater in microsomes from phenobarbital-treated rats than in untreated rats and is dependent on reduced nicotinamide adenine dinucleotide phosphate (NADPH), suggesting that monensin is a cytochrome P450 (CYP) enzyme substrate (Ceppa et al., 1997). The oxidative metabolism of monensin appears to occur at least in part by CYP3A, since treatment of rat hepatic microsomes with chemical inducers of CYP3A significantly increased monensin *O*-demethylation (Nebbia et al., 1999). It has been speculated that competition between monensin and other CYP3A substrates may explain accidental poisonings that have occurred in several domestic species following co-administration of monensin and other chemotherapeutic agents, since monensin metabolism is significantly decreased in the presence of other CYP3A substrates in rats (Anadón & Reeve-Johnson, 1999; Nebbia et al., 1999).

The metabolism of monensin sodium in human liver microsomes has been compared with metabolism in the microsomes of horses and dogs (Herrera et al., 2005; Holmstrom, 2007). A pooled human microsomal sample from multiple donors (male and female, Caucasian, Hispanic and African American, 15–66 years old),

pooled dog microsome sample and equine microsomes from a single donor were incubated with 0.5, 1 and 10 µg monensin/ml in the presence or absence of NADPH. The metabolite profiles were examined at 0, 5, 10, 20, 40 and 60 min by liquid chromatography/mass spectrometry (LC-MS) analysis. Monensin was metabolized by first-order kinetics in all species, and metabolism was extensive (93–99% by 60 min). The turnover of monensin in humans was similar to that in dogs, whereas the turnover in horses was only 10% of that in dogs and humans (Herrera et al., 2005; Holmstrom, 2007).

2.2 Toxicological studies

2.2.1 Acute toxicity

The studies of acute toxicity were all conducted prior to the adoption of GLP. In all studies, monensin was administered as a single oral dose, the most relevant route of exposure for humans. The median lethal doses (LD_{50} values) for oral administration of monensin ranged from 22 to 96 mg/kg bw, with rats displaying more sensitivity than mice or rabbits and female rats being more sensitive than males (Table 1). Death was preceded by hypoactivity, ataxia, dyspnoea, ptosis, loss of righting reflex and muscle weakness in all species tested.

Table 1. Acute toxicity of a single dose of monensin sodium in laboratory species

Species	Strain	Sex	Route/form	LD_{50} (mg/kg bw)	Reference
Mouse	Cox ICR	M	Oral/mycelial	70	Broddle & Worth (1976)
Mouse	Cox ICR	F	Oral/mycelial	96	Broddle & Worth (1976)
Mouse	Not specified	Not specified	Oral/mycelial	44	Haney & Hoehn (1967)
Rat	Harlan Wistar	M	Oral/mycelial	40	Broddle & Worth (1976)
Rat	Harlan Wistar	F	Oral/mycelial	24	Broddle & Worth (1976)
Rat	Harlan Wistar	M	Oral/mycelial	50 (estimate)	Pierson (1981)
Rat	Harlan Wistar	F	Oral/mycelial	22	Pierson (1981)
Rat	Harlan Wistar	F	Oral/Coban premix	22	Arthur & Downs (1979)
Rabbit	New Zealand albino	M & F	Oral/crystalline	42	Arthur et al. (1976)

F, female; M, male.

Acute toxicity was also examined in a non-GLP-compliant study in mature rhesus monkeys. Pairs of monkeys were exposed to a single dose of 20, 40 or 60 mg monensin activity/kg bw by gavage and were monitored for 7 days. Thirty-four days later, five of the monkeys were dosed again by gavage with 80, 110 or 160 mg monensin activity/kg bw and were monitored for 7 days. No mortality was observed during the study. All animals developed diarrhoea within 24 h after dosing. Vomiting was observed in one animal receiving 110 mg monensin activity/kg bw and both animals receiving 160 mg/kg bw, and suppressed appetites were seen for one female monkey receiving 160 mg/kg bw and one receiving 110 mg/kg bw (Gossett et al., 1977a). The LD_{50} value for mycelial monensin in rhesus monkeys is greater than 160 mg/kg bw.

2.2.2 Short-term studies of toxicity

(a) Mice

In a GLP-compliant study, 5- to 6-week-old male and female B6C3F1 mice (15 mice per sex per dose) were fed diets containing 0, 37.5, 75, 150 or 300 mg mycelial monensin sodium/kg for 3 months (equivalent to 0, 5.6, 11.2, 22.5 and 45 mg/kg bw per day). Body and organ weight, haematology, clinical chemistry and histopathology were examined. A dose-dependent decrease in body weight gain occurred in all dose groups. At the end of the study, the decrease ranged from 27% and 21% in the lowest dose group in females and males, respectively, to 99% in the highest dose group in both sexes. Mean body weights also declined by 5% and 8% in the lowest dose group up to 29% and 35% in the highest dose group in females and males, respectively. Except for the declines in body weights and body weight gains of males in the lowest dose group, all changes were statistically significant. Doses of 75, 150 or 300 mg/kg in the diet also led to significant reductions in liver, kidney plus adrenal, and heart weight of both sexes, spleen and ovary weight of female mice, and testes weight of male mice, but this reduction in organ weight was considered to be due to the dose-dependent decrease in body weight. Decreased leukocyte counts were observed for female mice in all four treatment groups and in males receiving 75 mg/kg in the diet. Decreased erythrocytes, haemoglobin and haematocrit values were seen for all animals in the highest dose group, as well as many animals in the second highest dose group. Males in the 75 mg/kg dose group also had reduced leukocytes and per cent lymphocytes and increased per cent neutrophils. Differences in haematology were considered secondary to profound effects on growth, as were most observed changes in clinical chemistry. An increased incidence of elevated serum creatine kinase values for the males in the two highest dose groups and for the females receiving the highest dose was evident. Mild diffuse vacuolation of cardiac myofibres occurred in eight males and two females receiving 300 mg/kg in the diet and one male receiving 37.5 mg/kg in the diet. Since weight gain was affected in all dose groups, a no-observed-adverse-effect level (NOAEL) could not be identified (Howard, 1981a).

(b) Rats

In four parallel subchronic toxicity studies that were conducted according to GLP regulations, 15 Wistar rats per sex were exposed in their diet to crystalline, double-drum-dried, azeotrope or flash-dried mycelial monensin sodium at concentrations of 0, 50, 200 or 400 mg/kg (equivalent to 0, 2.5, 10 and 20 mg/kg bw per day) for 3 months. Mortality occurred in four females exposed in their diet to either double-drum-dried mycelial monensin at 400 mg/kg or azeotrope mycelial monensin at 400 mg/kg, one male exposed to flash-dried mycelial monensin at 400 mg/kg and one female exposed to azeotrope mycelial monensin at 200 mg/kg. The cause of death could not be determined; however, a relationship with the treatment could not be ruled out. Decreased weight gain was observed in all rats exposed to at least 200 mg monensin/kg in the diet and in female rats exposed to the mycelial forms of monensin at 50 mg/kg in the diet. Females consuming 200 or 400 mg mycelial monensin/kg in the diet consumed less food than females consuming the crystalline form, but weight gain was similar for both forms of monensin. Males receiving 200 or 400 mg mycelial monensin/kg in the diet consumed less food and gained less weight than males receiving crystalline monensin. Observed changes in haematology, clinical chemistry and organ weights were considered secondary to effects on growth. Histopathological examination identified focal interstitial myocarditis and myocardial degeneration; however, there was no difference in incidence between control animals and animals exposed to the various forms of monensin. Focal degeneration and interstitial myositis of the diaphragm and skeletal muscle occurred in higher incidence in female rats of the highest dose group than in controls; however, overall incidence and severity were low. Owing to a decreased body weight gain at the lowest dose, a NOAEL could not be identified (Howard & Young, 1981).

In a study conducted prior to the effective date of GLP regulations, the subchronic oral toxicity of mycelial monensin sodium was examined in Harlan Wistar rats. Fifteen 4- to 6-week-old animals per sex were exposed to mycelial monensin sodium at nominal concentrations of 0, 25, 50, 80 or 125 mg/kg in the diet for 3 months. Based on measured concentrations in the feed and feed consumption, this was equal to 0, 0.89–2.45, 1.83–4.63, 3.02–7.71 and 4.54–12.05 mg/kg bw per day in males and 0, 1.30–2.55, 2.75–5.83, 4.04–12.83 and 10.17–20.21 mg/kg bw per day in females. Physical and behavioural changes, growth, food consumption, terminal haematology, clinical chemistry, organ weights and histopathology were examined. All animals survived the study and were normal in appearance and behaviour. A transient dose-dependent decrease in mean body weight was observed in females receiving 50, 80 or 125 mg monensin/kg in their diet, and weight gain was reduced in these animals during the first 2 weeks of the study. Males in the highest dose group also had reduced weight gain during the first 2 weeks. During the 1st week of the study, decreased food consumption was seen in all animals in the highest dose group and in females exposed to 50 and 80 mg/kg in the diet, with the high-dose females also having decreased food consumption in the 2nd and 3rd weeks. No changes in haematology or clinical chemistry were attributed to monensin consumption. Minute lesions were observed in the heart and skeletal muscle of both control and treated animals, particularly in males, but the incidence

and severity of these lesions were not dose dependent. Based on the effects of mycelial monensin on body weight and food consumption, the NOAEL for this study was the nominal concentration of 25 mg/kg in the diet. An exact dose could not be determined owing to the wide range of measured concentrations of monensin in the feed (Howard, 1980a).

In order to better compare the toxic effects of crystalline and mycelial monensin, a non-GLP-compliant study comparing both forms of monensin was performed in Wistar barrier-reared 4- to 6-week-old rats. Twenty-five animals per sex were fed a control diet, and 15 animals per sex were exposed to crystalline or mycelial monensin sodium in their diet at concentrations of 50, 200 or 400 mg/kg (equivalent to 2.5, 10 and 20 mg/kg bw per day) for 3 months. Animals were examined for changes in physical condition, behaviour, body weight, haematology, clinical chemistry, organ weights, and gross and microscopic pathology. One control male and three high-dose females (one in the crystalline group, two in the mycelial group) died during the study. A severe reduction in body weight gain was observed for both formulations starting at 200 mg/kg in the diet. A slight, transient decrease in body weight gain was observed for females in the lowest dose groups for the first 2 weeks of the study. Decreases in organ weight were also observed in the highest and middle dose groups, but these appeared to be related to the decreased body weight gain. Haematology was normal in all animals except for white blood counts, which were decreased in males receiving either type of monensin at the highest dose. Analysis of clinical chemistry indicated an increase in total bilirubin and alkaline phosphatase levels and a decrease in mean serum glucose and creatinine levels in males and females receiving either preparation of monensin at 400 mg/kg in the diet. Similar changes were also observed in female rats receiving 200 mg monensin/kg in their diet. Female rats in all treatment groups also had decreased serum alanine aminotransferase. Initial histopathological examination revealed an incidence of scattered foci of a few myocardial fibres with degeneration, necrosis and infiltration of mononuclear cells, particularly in males, in all three dose groups for both forms of monensin. A second independent pathology evaluation concluded that the scattered myocardial lesions were not adverse and that the incidence was similar to the control incidence. A NOAEL could not be identified because of a slight, transient reduction in body weight gain in females in the lowest dose group, which became severe and non-transient in the next higher dose group (Gossett et al., 1977b).

(c) Dogs

In a study that was not compliant with GLP, mongrel dogs (two per sex per dose, age unknown) were administered monensin sodium orally in capsules at daily doses of 0, 2.5, 5, 11 or 25 mg/kg bw for 90 days. Deaths attributed to treatment with monensin occurred in one female of the second highest dose group and two males of the highest dose group. Females of the highest dose group developed ataxia, tremors, loss of muscular control and slight relaxation of the nictitating membrane; therefore, treatment was stopped after 5 days. There were no signs of toxicity in surviving male and female dogs receiving 11 and 5 mg/kg bw per day or less, respectively. Haematology, clinical chemistry, urinalysis, organ weights and

gross pathology of all animals were normal, with the exception of transiently elevated serum alanine aminotransferase levels in the second highest dose group. The NOAEL was 5 mg/kg bw per day (Worth et al., 1967).

In a second non-GLP-compliant study, mycelial monensin sodium manufactured using a new method was administered to Beagle dogs in gel capsules. Two 12- to 18-month-old dogs of each sex were administered 0, 5, 15 or 50 mg monensin activity/kg bw per day for 91 days. Two males in the highest dose group died, and one male in the middle dose group was sacrificed within the first 2 weeks of the study. Necropsy on these animals revealed myopathy of the heart with degeneration of the muscle fibre, macrophage infiltration and visceral congestion. Animals dosed with 15 and 50 mg/kg bw per day vomited more frequently, lost body weight and developed muscular weakness, ataxia, arrhythmias, convulsions and mydriasis. Haematology, urinalysis and clinical chemistry were normal for all animals, with the exception of transiently increased serum lactate dehydrogenase and alanine aminotransferase in animals in the two highest dose groups. Pathology results indicated that middle- and high-dose males and high-dose females had degenerative changes in striated muscle, including diffuse degeneration of muscle fibres and infiltration of histiocytes, at the end of the study. A slight loss of body weight was observed in dogs treated in all dose groups, but no other effects were seen. Since toxic effects were seen at the lowest dose, a NOAEL could not be identified (Gibson et al., 1974).

In a GLP-compliant study, mycelial monensin sodium was orally administered as an equally divided dose in gel capsules twice daily to Beagle dogs (5–6 months old, four per sex per dose) for 1 year at doses of 0, 1.25, 2.5, 5 or 7.5 mg/kg bw per day. The animals were observed for changes in physical appearance, behaviour, body and organ weights, ophthalmology, haematology, clinical chemistry, urinalysis, and gross and microscopic pathology. No data on feed intake were reported. Two dogs receiving 5 mg/kg bw per day and four dogs receiving 7.5 mg/kg bw per day showed signs of toxicity, which included hypoactivity, muscle weakness (particularly legs and neck), stilted gait, difficulty standing and anorexia, but they recovered within a few days. Increased alanine aminotransferase and creatine kinase levels were observed during the first 2 weeks of monensin administration in dogs receiving 5 and 7.5 mg/kg bw per day, and several dogs in these groups also had periodic increases in alanine aminotransferase and creatine phosphokinase throughout the entire study period. Decreased mean total protein in 5 mg/kg bw per day females and 7.5 mg/kg bw per day males during week 45 and elevated mean serum calcium in females receiving 5 or 7.5 mg/kg bw per day during weeks 45 and 52 may have been treatment related. No statistically significant decrease in weekly mean body weight was observed, although a decrease in body weight gain was seen in male dogs receiving 2.5, 5 or 7.5 mg/kg bw per day, which exceeded 10% for the highest dose. No changes were observed in ophthalmology, haematology, urinalysis or electrocardiography (ECG) results that could be directly attributed to monensin administration. Organ weights were unaltered by treatment, and no pathological changes were seen that were related to monensin exposure. Since body weight gain was decreased at the next higher dose, a NOAEL of 1.25 mg/kg per day was identified (Howard, 1980b).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

In an experiment that complied with GLP, groups of 60 male and 60 female 5- to 6-week-old B6C3F1 mice received diets containing mycelial monensin at concentrations of 0, 10, 25, 75 or 150 mg/kg for 2 years, equal to 0, 1.2, 3.1, 10.2 and 22.6 mg/kg bw per day for males and 0, 1.4, 3.5, 11.7 and 25.6 mg/kg bw per day for females. Clinical signs and histopathological changes were examined. No substance-related deaths, physical signs or behavioural changes were observed. A statistically significant decrease in body weight and body weight gain occurred in mice receiving the three highest doses. Decreased body weight gain interfered with the development of meaningful conclusions regarding the significant effect of monensin on the weight of multiple organs. A statistically significant, dose-dependent decrease in leukocyte counts was observed in males receiving monensin at concentrations of 25, 75 or 150 mg/kg in their diet. Minimal increases in urea nitrogen, creatinine, bilirubin, alanine aminotransferase and creatine phosphokinase occurred with the highest dose. No evidence of carcinogenicity was observed at the gross or microscopic level that could be attributed to monensin. A NOAEL of 10 mg/kg in the diet (equal to 1.2 mg/kg bw per day) was identified, based on the effects of mycelial monensin on body weight gain and leukocyte counts (Howard & Usher, 1984).

(b) Rats

In a study conducted according to GLP guidelines, 5- to 6-week-old male and female Wistar rats (80 animals per sex per dose group) were maintained on a diet containing 25, 56 or 125 mg crystalline monensin sodium/kg, whereas control rats (120 per sex) received a normal diet for 2 years (Howard et al., 1981). The monensin concentrations in the diet were equal to a time-weighted average daily dose of 1.14, 2.57 and 5.91 mg/kg bw per day in males and 1.46, 3.43 and 8.68 mg/kg bw per day in females. All of the rats were monitored for changes in physical appearance, behaviour, body and organ weight, food and water consumption, efficiency of feed conversion, haematology, clinical chemistry, urinalysis and pathology. Survival was not adversely affected by crystalline monensin sodium administration. Body weight and weight gain were significantly decreased in animals receiving 125 mg monensin/kg in their diet and were transiently decreased during the first 4 months in rats in the middle dose group. Feed conversion efficiency was decreased in the animals receiving 56 or 125 mg monensin/kg in the diet, and mean feed consumption was decreased in animals in the highest dose group during the first 5 weeks of the trial. No physical signs of toxicity were observed that were attributable to monensin administration, and no differences in haematology or clinical chemistry values were observed at 6, 12, 18 or 24 months that were specific to monensin exposure. Urinalysis at 12 months was also normal, and absolute and relative organ weights were unaffected by treatment. Pathology revealed skeletal muscle degeneration and cardiomyopathy in control and treated animals, with no bias towards monensin-treated animals. Similarly, benign and malignant neoplasms were observed in treated and untreated animals, with no association between monensin

administration and neoplasm type or severity. It was concluded that lifetime exposure of rats to diets containing up to 125 mg crystalline monensin sodium/kg did not produce carcinogenicity. A NOAEL of 25 mg/kg (equal to 1.14 mg/kg bw per day) was identified based on the effects on body weight (Howard et al., 1981).

In a second GLP-compliant study conducted in Wistar rats, groups of 100 male and 100 female rats that were exposed to monensin in utero were further exposed to 0, 33, 50 or 80 mg mycelial monensin sodium/kg in their diet for 2 years. These monensin concentrations were equal to a time-weighted average daily dose of 0, 1.40, 2.18 and 3.60 mg/kg bw per day in males and 0, 1.72, 2.86 and 5.02 mg/kg bw per day in females. Rats were examined for changes in physical condition, body and organ weights, food consumption and efficiency of feed conversion, haematology, clinical chemistry, urinalysis results and pathology. Survival in both sexes increased following monensin exposure in a dose-dependent manner. A transient decrease in body weight was observed at the beginning of the study in all animals consuming 80 mg monensin/kg in the diet and in females receiving 50 mg/kg in the diet. Body weight gain was also significantly decreased during the 1st week in males receiving monensin in the diet at concentrations of 33 and 80 mg/kg and during the first 2 weeks in females receiving the highest dose. There was a statistically significant increase in feed intake in females in the highest dose group. As was observed for crystalline monensin (Howard et al., 1981), no differences in haematology, clinical chemistry, urinalysis or organ weights were observed that could be linked to monensin exposure, and no signs of physical toxicity occurred. Non-neoplastic lesions were observed in the muscle and cardiac tissues; however, the incidence and severity were not influenced by monensin exposure. As well, the latency and prevalence of benign and malignant neoplasms did not differ in treated and untreated rats. It was concluded that in utero exposure followed by 2 years of exposure to mycelial monensin sodium at doses up to 80 mg/kg in the diet did not lead to carcinogenicity. As the observed decrease in body weight gain was transient and restricted to the first few weeks of the 2-year study, the effect was not considered to be adverse. The NOAEL was identified to be the highest dose, 80 mg/kg in the diet, equal to 3.60 mg/kg bw per day (Howard, 1981b).

2.2.4 Genotoxicity

The genotoxic effects of monensin sodium were investigated in vitro and in vivo in several experiments that were conducted according to GLP. The results of these studies are summarized in Table 2. No evidence of genotoxicity was observed.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

(i) Rats

In a GLP-compliant multigeneration study, mycelial monensin was administered to three generations of Wistar-derived rats (25 of each sex) and their offspring at doses of 0, 33, 50 or 80 mg/kg in the diet (equivalent to 0, 1.6, 2.5 and

Table 2. Results of genotoxicity tests for crystalline monensin sodium

End-point	Test object	Concentration/ dose	Result	Reference
Reverse mutation[a,b]	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; *Escherichia coli* WP2uvrA	312.5–5000 µg/ plate	Negative	Garriott & Schwier (2001)
Chromosomal aberrations[a,c]	Chinese hamster ovary cells	25–100 µg/ml (4 h) 5–25 µg/ml (19 h)	Negative for chromosomal aberrations; increased diplo-chromosomes observed in 4-h studies	Garriott & Gilbert (2002)
In vivo micronucleus test[d]	Male and female ICR mice (5 per sex per group)	181.3, 362.5 and 725.0 mg/kg for 2 days by oral gavage	Negative	Phelps & Murphy (2002)

[a] Conducted with and without activation with S9 (9000 × *g* rat liver supernatant) fraction from Aroclor 1254–induced rat livers.
[b] Positive controls were *N*-methyl-*N*′-nitro-nitrosoguanidine for TA1535, TA100 and WP2uvrA, 2-nitrofluorene for TA98 and 9-aminoacridine for TA1537 in the absence of S9; 2-amino-anthracene for all strains in the presence of S9.
[c] Positive controls were mitomycin C for non-activated assays and cyclophosphamide for activated assays.
[d] Positive control was 50 mg cyclophosphamide/kg.

4 mg/kg bw per day). In male rats, a reduction in body weight gain during the growth phase was observed at all doses in the F_0 generation and at the middle and high doses in F_2 animals. In females, 80 mg monensin/kg in the diet reduced weight gain during the growth phase in F_0, F_1 and F_2 animals, whereas there was also reduced weight gain in F_2 females in the middle and highest dose groups. Mean body weight was reduced in pregnant and lactating females in the middle and highest dose groups, in F_1, F_2 and F_3 progeny in the highest dose groups and in F_2 progeny in the middle dose group. No statistically significant differences between the control and treatment groups could be found for the parameters of reproductive performance, including fertility, litter size, gestation length, parent and progeny survival, and sex distribution. No evidence of embryotoxicity or teratogenicity was observed. A NOAEL for parental and offspring toxicity could not be identified, owing to the reduction in body weight gain in both sexes in every generation and at all doses. The NOAEL for reproductive toxicity was 80 mg/kg feed, equivalent to 4 mg/kg bw per day, the highest dose tested (Adams, 1981).

(b) Developmental studies

(i) Rats

In a non-GLP-compliant study, the effects of exposure to monensin during development were studied using 28-day-old Wistar rats. Groups of 15, 14 and 12 female rats received powdered diets containing a non-specified preparation of monensin at concentrations of 0, 100 or 300 mg/kg (equivalent to 0, 5 or 15 mg/kg bw per day) until premating weights achieved 185 g and during pregnancy and lactation. Gestation length, maternal weight gain (difference in body weight from gestation days 3 to 10 and from gestation days 0 to 18), litter size, presence of external malformations, sex ratio and pup weight were examined. All pups were monitored for altered development, including time to development of primary coat of downy hair, ear unfolding, fur development, incisor eruption, ear opening and eye opening. The surface righting and negative geotaxis reflexes of all pups were also examined. Female body weight was significantly decreased in the highest dose group after 8 days of treatment and remained lower throughout the study. No significant differences in female fertility were observed. All females became pregnant, with the exception of two in the high dose group, which did not show vaginal opening and could not be mated. Weight gain by monensin-treated dams during pregnancy did not differ significantly from that of control dams. Gestation length, litter size and number of stillborn pups were also unaltered by monensin treatment. The body weights of male and female pups in the highest dose group were reduced from postnatal day 10 until postnatal day 21. Male offspring in the low dose group showed body weight reduction only on postnatal day 21. No external signs of malformation were detected in the pups. Females perinatally exposed to 100 mg monensin/kg in the diet showed a delay of incisor eruption; this effect was not seen in the high dose group. No other treatment-related effects were observed. Owing to effects on body weight in males in the low dose group on postnatal day 21, a NOAEL for developmental toxicity could not be identified (de Souza Spinosa et al., 1999).

(ii) Rabbits

In a study that was not conducted according to GLP, monensin sodium was administered by gavage to groups of 15 pregnant Dutch Belted rabbits at doses of 0.076, 0.38 or 0.76 mg/kg bw per day from gestation days 6 to 18, whereas 25 control rabbits received 5% vehicle. At gestation day 28, all animals were killed and examined for clinical condition and reproductive performance, and all progeny were weighed, sexed and examined for developmental defects. Maternal mean daily food consumption decreased in animals receiving 0.76 mg/kg bw per day during treatment only, but there was no effect on mean body weight. No differences were observed in litter size, corpora lutea number, implantation occurrence, fetal viability or resorption occurrence. In addition, sex distribution, progeny survival and mean fetal weights did not differ between groups. Fetal abnormalities occurred in low incidence and were unrelated to monensin treatment. The NOAEL for maternal toxicity was 0.76 mg/kg bw per day, the highest dose tested. The NOAEL for teratogenicity was 0.76 mg/kg bw per day, the highest dose tested (Gossett et al., 1974).

2.2.6 Special studies

(a) Dermal irritation

(i) Rabbits

In an experiment that was not GLP compliant, the exposed skin of three male and three female New Zealand albino rabbits was exposed to monensin in the feed premix Coban at a dose of 0.2 mg/kg bw and occluded for 24 h. Three rabbits had their skin abraded prior to application of the Coban. Toxicity was monitored for 2 weeks. Erythema occurred in only one animal. All animals lost between 50 and 1340 g bw during the study (Arthur & Downs, 1979). To confirm that the observed weight loss was due to dermal exposure and not due to oral ingestion, the study was repeated following the same protocol in six additional animals, which were collared to prevent licking of the exposure site. No dermal toxicity was observed, but weight losses still occurred and ranged from 20 to 370 g (Arthur & Downs, 1979). An additional study to ensure that weight loss was not due to procedural trauma confirmed that a very high dose of Coban placed on abraded skin of rabbits for 24 h leads to a transient loss in body weight (Arthur, 1980).

In another non-GLP-compliant dermal toxicity study, the fur of three male and three female New Zealand albino rabbits was clipped, and the skin was abraded in three rabbits. Mycelial monensin at 500 mg/kg (equivalent to 42 mg/kg bw) was applied to the abraded area, which was occluded for 24 h. Toxicity was evaluated for 2 weeks. Slight erythema was observed in a single rabbit 4 days after treatment. No other signs of toxicity were observed (Pierson, 1981).

(ii) Guinea-pigs

In a non-GLP-compliant study, groups of 12 albino guinea-pigs (males and females) were exposed on the anterior portion of their backs to mycelial monensin at 0 or 2 g/kg (equivalent to 0 and 220 mg/kg bw) for 4 h/day, 5 days/week, for a total of 15 treatments. The animals were examined throughout the study for signs of toxicity, including primary irritation, and six animals in each group underwent histopathological examination at the conclusion of the study. The remaining six animals in each group were held without treatment for 17 days and then were challenged with 2 g monensin/kg. No skin irritation was observed following initial treatments, and there was no evidence of contact sensitization following the challenge treatment. Four control and eight treated animals developed transient signs of lacrimation and eye irritation after 12 treatments. Body and organ weights and pathology were normal for all animals tested (Arthur, 1968).

(b) Ocular irritation

(i) Rabbits

In a non-GLP-compliant experiment, six New Zealand albino rabbits were treated in one eye with 53 mg of feed premix Coban (premix containing 9.9% monensin sodium). Corneal dullness, mild corneal opacity, marked iritis and

moderate conjunctivitis were observed within 1 h of treatment. Within 24 h, well defined to severe corneal opacity and severe conjunctivitis developed. Corneal changes appeared irreversible. Three additional rabbits were treated and then had their eyes rinsed after 2 min. Slight conjunctivitis developed in all animals, with corneal dullness and slight iritis observed in one animal. Ocular irritation was reversed within 48–72 h (Arthur & Downs, 1979).

In a non-GLP-compliant study, nine New Zealand albino rabbits were treated in one eye with 59 mg of monensin. Three rabbits had their eyes rinsed 2 min after treatment with 300 ml saline. One hour after treatment, slight corneal opacity, marked iritis and moderate conjunctivitis were observed in unrinsed eyes. In five rabbits, symptoms subsided by 7 days. Staphyloma with corneal perforation was observed in a single rabbit within 7 days. During healing, vascularization developed and involved 50% of the cornea by 21 days. Animals with rinsed eyes exhibited corneal dullness, moderate iritis and mild conjunctivitis. Evidence of irritation disappeared by 7 days (Pierson, 1981).

(c) Inhalational toxicity

(i) Rats

In a non-GLP-compliant inhalational exposure study, two groups of 10 male and 10 female 6- to 8-week-old Harlan Wistar SPF rats were exposed to either normal air or air containing particulate mycelial monensin sodium at a mean concentration of 79 mg/m^3 for 2 weeks (1 h/day, 5 days/week). All animals were observed for changes in physical appearance, behaviour, body weight, haematology, clinical chemistry and histopathology. All animals survived the study. Nine of 10 treated females became anorexic and lost weight during the 2nd week of the study. Several clinical chemistry values were altered by monensin treatment, including elevated creatinine levels in males, decreased serum alkaline phosphatase in females and increased serum alanine aminotransferase in females, but these changes were not considered biologically significant. Haematology was normal for all animals. Slight focal myositis of the skeletal muscle was seen in two males and two females but none of the controls. Multifocal myocardial changes, which were more pronounced than in the control group, were observed in four male rats treated with monensin; these changes included eosinophilic myocardial fibres, a few pyknotic nuclei and increased mononuclear cells (Arthur et al., 1976).

In two additional non-GLP-compliant inhalational exposure studies, the effects of mycelial and crystalline monensin dust were examined in groups of 10 male and 10 female Harlan Wistar SPF or barrier-derived rats. In one study, the animals were exposed (head only) to mycelial monensin sodium dust at concentrations of 0, 9.83, 18.14 or 33.33 mg/m^3 for 1 h/day, 5 days/week, for 2 weeks. In a second study, animals received mycelial monensin at 28.86 mg/m^3 or crystalline monensin dust at 0, 8.19, 12.83 or 23.93 mg/m^3 using the same dosing regimen. One male and two females receiving 33.33 mg mycelial monensin/m^3 and 30% of the females receiving 23.93 mg crystalline monensin/m^3 did not survive the study. Dose-dependent chromorhinorrhoea was observed, with a significantly greater incidence in males receiving 18.14 or 33.33 mg mycelial monensin/m^3, in

all females receiving mycelial monensin and in females receiving the high dose of crystalline monensin. A dose-dependent decrease in body weight was observed for females exposed to mycelial and crystalline monensin. Histological changes seen with both mycelial and crystalline monensin included scattered degeneration and regeneration of skeletal muscle fibres (particularly in females) and/or necrosis of myocardial fibres, although myocardial effects were infrequent. A no-observed-adverse-effect concentration (NOAEC) of 8.19 mg/m^3 was identified for inhalational exposure to crystalline monensin dust (Gossett et al., 1976).

In another inhalational exposure experiment that was not conducted using GLP, five female and five male Fischer 344 rats were exposed (head only) to a gravimetric concentration of 0.37 mg mycelial monensin sodium/l (8.88 mg monensin/l nominal) for 1 h/day for 14 days. Three animals showed signs of chromodacryorrhoea during the exposure period. Following exposure, all animals appeared normal. At necropsy, 9 of 10 animals had an enlarged caecum (Pierson, 1981).

(ii) Dogs

In a GLP-compliant subchronic inhalation study in Beagle dogs, males and females were exposed to a sub-80 sieve fraction of mycelial monensin sodium for 6 h/day, 5 days/week, for 90 days. Two animals per sex were exposed to gravimetric concentrations of 0, 0.23, 0.61 and 2.3 µg mycelial monensin sodium/l air (equivalent to 0, 0.08, 0.15 and 0.84 µg monensin activity/l). Animals in the highest exposure group were observed to have ocular irritation, bloody diarrhoea, excessive salivation and hypoactivity. These dogs also had elevated serum alanine aminotransferase, aspartate aminotransferase and creatine kinase (owing to skeletal muscle isoenzyme) and lactate dehydrogenase from study days 8 to 22 in males and from study days 8 to 29 in females. ECG effects, including tachycardia, R-wave suppression, altered T-waves and premature ventricular repolarization, were also observed in the high exposure group only. Elevated mean platelet counts in high-dose females were also observed on two sampling dates, but haematological parameters were otherwise normal for all animals tested. Myeloid:erythroid ratios calculated from examining terminal bone marrow samples were normal for all animals. No treatment-related effects were seen in organ weights, and there were no treatment-related pathological lesions. Since toxicity was observed only at 0.84 µg monensin activity/l, a NOAEL of 0.15 µg monensin activity/l (0.61 µg mycelial monensin sodium/l) was identified for both sexes (Dorato & Howard, 1983).

(d) Immunotoxicity

(i) Mice

In two separate GLP-compliant studies, the potential for delayed contact hypersensitivity was investigated in female CBA/J mice using the local lymph node assay (Griffon, 2002a,b). A 10% weight by volume (w/v) extract of monensin-containing feed premix (ELANCOBAN®200) in ethanol:water (50:50 by volume) was prepared, and groups of four mice were treated over the ears with 0.5, 1.0,

2.5, 5, 10, 25, 50 or 100% solutions of this extract, the positive control (25% α-hexylcinnamaldehyde) or vehicle alone for 3 consecutive days. After 2 days of resting, proliferation of cells in the lymph nodes draining the ears was determined using tritiated methyl thymidine and used to calculate stimulation indices. No cutaneous reactions were observed, and there was no significant change in ear thickness during either study. A dose-related increase in stimulation index was observed in the study using concentrations ranging from 5% to 100%, but not in the study with doses ranging from 0.5% to 10%. A significant increase in stimulation index occurred at doses greater than or equal to 5%. This increase was attributed to delayed contact hypersensitivity, and it was determined that feed premix ELANCOBAN®200 is a weak sensitizer (Griffon, 2002a,b).

(e) Cardiovascular and respiratory effects

(i) Dogs and pigs

The cardiovascular and respiratory effects of intravenous administration of monensin sodium in male mongrel dogs (conscious and anaesthetized, 11–23 kg) and pigs (anaesthetized, 19–27 kg) were investigated in a non-GLP-compliant study. Dogs were exposed to doses ranging from 0.69 µg/kg bw to 1.4 mg/kg bw, and pigs received between 0.0069 and 0.69 mg/kg bw. The exact dosing regime was not reported. In anaesthetized dogs, monensin significantly and dose-dependently increased left ventricular contractility (0.035 mg/kg bw), blood pressure (0.014 mg/kg bw), heart rate (0.035 mg/kg bw) and left anterior coronary artery blood flow (0.0069 mg/kg bw). Exposure to 0.035 mg/kg bw resulted in premature ventricular contractions and ventricular tachycardia. Respiration rate was also significantly increased in animals receiving at least 0.14 mg monensin/kg bw, and 50% of the animals receiving 1.4 mg monensin/kg bw died of respiratory arrest. The NOAEL for anaesthetized dogs was 0.0035 mg/kg bw (Holland, 1978).

To confirm that cardiovascular effects could be produced in conscious dogs, two mongrels were exposed intravenously to increasing doses of monensin. The exact dosing regime was not reported. A dose of 0.21 mg/kg bw or greater was required to cause premature ventricular contractions and ventricular tachycardia in these animals, with occasional premature contractions occurring up to 7 days following exposure. The dogs also became hyperactive, vomited, defecated and hyperventilated following administration of the highest doses of monensin. The NOAEL for conscious dogs was 0.0345 mg/kg bw (Holland, 1978), suggesting that concurrent administration of anaesthetics may potentiate the effects of monensin in dogs by a factor of 10.

Similar cardiovascular effects were observed in five anaesthetized pigs (7/8 Yorkshire, 1/8 Hapshire, 19–27 kg). The exact dosing regime was not reported. A monensin dose of 0.035 mg/kg bw administered intravenously caused increased left ventricular contractility, heart rate, coronary blood flow and premature ventricular contractions. Effects on left ventricular contractility were less pronounced than in dogs, whereas the effects on heart rate were greater in the pigs. The lowest effective dose in pigs was 0.0069 mg/kg bw, which significantly increased mean

blood pressure. Since this was the lowest dose used for the study, no intravenous NOAEL could be identified in pigs (Holland, 1978).

As acute overdose is more likely to occur through oral exposure than through intravenous exposure, a second non-GLP-compliant study was performed in conscious Beagle dogs to determine whether oral administration will have effects on cardiovascular and respiratory function similar to those observed following intravenous administration (Holland et al., 1981). The effects of oral exposure by gavage to 0, 0.138, 0.345, 0.690 or 1.38 mg monensin sodium/kg bw in 15 ml of 10% acacia were examined in four, four, four, six and four dogs, respectively, and compared with the effects in three male and three female dogs, weighing 8.5–15.2 kg, which were intravenously administered bolus doses of monensin sodium every 10 min to give cumulative doses of 0.0069, 0.0138, 0.0345, 0.069 and 0.138 mg/kg bw. In dogs that received monensin orally, coronary artery flow was significantly increased with 0.69 and 1.38 mg/kg bw, whereas heart rate and blood pressure remained unchanged. The elevation in coronary blood flow was maximal 13–17 min after dosing and returned to normal by 30 min. Intravenous doses of 0.069 and 0.138 mg/kg bw significantly increased coronary blood flow, and mean blood pressure increased with a dose of 0.138 mg/kg bw. No changes in heart rate were observed. When the dose required to cause 100% increase in coronary flow was estimated using log-linear interpolation, the intravenous route was approximately 11 times more active than the oral route in increasing coronary blood flow (Holland et al., 1981). The threshold for pharmacological effects on the heart following oral administration was 0.345 mg/kg bw, based on increased coronary blood flow at 0.690 and 1.38 mg/kg bw. The observed transient increase in coronary blood flow in dogs given a single oral dose of monensin was considered treatment related but not adverse, owing to the absence of effects on blood pressure or heart rate.

(f) Microbiological effects

A JECFA decision tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with Guideline 36 of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL36) (VICH, 2004) was used by the Committee to determine the need to establish a microbiological acceptable daily intake (ADI) for impact of monensin residues on the intestinal microbiota. The decision tree approach initially seeks to determine if there may be microbiologically active monensin residues entering the human colon. If the answer is “no” to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: 1) disruption of the colonization barrier and 2) increase of the population(s) of resistant bacteria. At Step 4 of the decision tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both end-points. Step 5 is where a microbiological ADI would be determined. Should a microbiological ADI not be necessary, then the toxicological or pharmacological ADI would be used. The Committee evaluated minimum inhibitory concentration (MIC) susceptibility, faecal

binding interaction and the biological activity of monensin metabolites and used the decision tree to answer the following questions in the assessment of monensin.

Step 1: Are residues of the drug, and (or) its metabolites, microbiologically active against representatives of the human intestinal flora?

Yes. Monensin is microbiologically active against some bacterial genera and species representative of the human intestinal flora.

The antimicrobial spectrum of activity for monensin was first reported by Haney & Hoehn (1967). Monensin is active against some Gram-positive bacteria and some Gram-negative anaerobes and has some limited activity against mycoplasma, fungi and viruses. Monensin is inactive against Gram-negative, aerobic enteric bacteria, including *Pseudomonas* spp., and Gram-negative, facultative anaerobic enteric bacteria, including Enterobacteriaceae (*Escherichia coli*, *Salmonella* spp.) and *Vibrio* spp. MICs of monensin against 68 strains of 18 species of *Clostridia* isolated from the digestive tract of cattle, poultry and pigs, as well as human strains of *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Bacteroides*, *Peptostreptococcus* and *Eubacterium*, have been determined (Dutta et al., 1983; Scott et al., 1999).

In a more recent GLP-compliant study, the MIC of monensin was determined against 100 bacterial strains, comprising 10 isolates from each of 10 groups of genera representing the normal human intestinal microbiota (Pridmore, 2004a). All strains were sourced from the faecal microbiota of healthy unmedicated humans. The test system was standardized agar dilution MIC methodology using quality control strains as described in the Clinical and Laboratory Standards Institute guidelines (CLSI, 2004). To assess the effect of bacterial density on monensin activity, each MIC was determined using two inoculum levels of 10^9 and 10^5 colony-forming units (cfu)/ml for each strain. Monensin activity against each bacterial group is summarized in Table 3. MIC_{50}, MIC_{90} and geometric mean MIC were calculated for each bacterial group. In tests using the higher bacterial inoculum density, monensin exerted little or no antibacterial activity against *Bacteroides fragilis*, other *Bacteroides* species or *E. coli*. This is consistent with the known spectrum of activity for this compound, which has low activity against Gram-negative bacteria. Monensin also exerted very weak activity against *Bifidobacterium* species. Monensin activity was clearly demonstrable against the other six bacterial groups tested at the higher inoculum density. *Peptostreptococcus* was the most susceptible group (MIC_{50} of 0.5 µg/ml), whereas *Fusobacterium* was the least susceptible. At the lower inoculum density, monensin MICs against *E. coli*, *Enterococcus* and *Peptostreptococcus* were similar to those obtained using the higher inoculum density. Thus, monensin activity against these organisms was not affected by bacterial density. Conversely, *B. fragilis*, other *Bacteroides* species and *Bifidobacterium* demonstrated a large "inoculum effect"; that is, susceptibility of these organisms to monensin was greatly enhanced when tested at a lower inoculum density. A moderate inoculum effect—i.e. monensin MICs were reduced by 1–3 doubling dilutions at the low inoculum level compared with those obtained using the high inoculum level—was seen in *Clostridium*, *Eubacterium*, *Fusobacterium* and *Lactobacillus*.

Table 3. Summary of monensin activity against bacterial groups representing the normal human intestinal microbiota[a]

Bacterial group	Summary of MIC parameters (µg/ml)							
	High inoculum density				Low inoculum density			
	MIC_{50}	MIC_{90}	Geometric mean MIC[b]	MIC range	MIC_{50}	MIC_{90}	Geometric mean MIC[b]	MIC range
Bacteroides fragilis	>128	>128	128	All >128	8	16	10.6	4–16
Other *Bacteroides* spp.	>128	>128	128	All >128	8	16	7.5	2–16
Bifidobacterium	128	>128	52	2–>128	2	4	1.9	0.5–4
Clostridium	1	4	1.6	0.5–>128	0.5	0.5	0.5	0.125–4
Enterococcus	8	8	7.5	4–8	8	8	6.5	4–8
Escherichia coli	>128	>128	128	128–>128	>128	>128	128	All >128
Eubacterium	2	4	2.3	1–4	0.5	1	0.7	0.5–1
Fusobacterium	16	128	19.7	0.5–>128	2	16	2	ND
Lactobacillus	8	>128	12.1	2–>128	2	>128	4	0.5–>128
Peptostrepto-coccus	0.5	2	0.6	0.25–4	0.25	4	0.5	0.125–4

ND, not determined (number of results <10).

[a] From Pridmore (2004b).

[b] To calculate the geometric mean, MIC results of >128 µg/ml were treated as being 128 µg/ml.

Step 2: Do residues enter the human colon?

Yes. A number of residue studies using ^{14}C radiolabelling to detect total residues or analytical methods to detect parent monensin have been conducted in chickens, turkeys, quail, pigs, ruminants, sheep, goats and milk, as described in section 2.1.1. Muscle contains little or no monensin-derived residue, regardless of the period between withdrawal of medication and slaughter. However, residues may be present at low levels in offal, fat and skin. Therefore, monensin-related residues could enter the colon of a person ingesting tissues or milk from treated animals.

Step 3: Do the residues entering the human colon remain microbiologically active?

No. Monensin residue will be extensively transformed to metabolites with very reduced activity prior to entering the colon of the consumer; within the colon, it will become substantially bound to faecal material.

To determine the effect of faecal binding on the antibacterial activity of monensin, selected monensin concentrations of 0, 1, 2, 5, 10, 20, 50 and 100 mg/ml were incubated with increasing concentrations of sterilized human faeces (0, 10, 20 and 50% w/v in Mueller Hinton Broth), collected from three individual donors (Pridmore, 2004b, 2007a). Monensin activity was determined using *Bacillus subtilis* ATCC 6633 as an indicator organism, as it is susceptible to monensin. All three faecal samples had maximal binding of monensin (>90% binding) at 50% concentration. The 50% faecal concentration provided the closest representation of the in vivo situation (Table 4). The results demonstrated the rapid and extensive binding of monensin to human faeces. Based on this in vitro study, it can be estimated that the binding of monensin residues to undiluted faecal material would be highly likely to exceed 90%. An additional faecal interaction study was conducted, incorporating the microbiological assay methodology and HPLC/MS chemical assays (Pridmore, 2007b). The mean proportion of monensin that became unavailable after 12 h interaction with faeces, as determined by the growth inhibition assay (n = 3) and chemical assay (n = 5), was 96.8% and between 94.3% and 98.6%, respectively. This confirmed the conclusions from the earlier study that the antibiotic activity of monensin in the colon would be reduced by >90% by contact with faecal material.

Table 4. Determination of monensin availability after interaction with faeces: definitive experiment with faecal sample 012/06/008[a]

Interaction time (h)	Broth only (no faeces)		50% faeces by weight	
	Initial monensin concentration (µg/ml) required to inhibit growth ("a")	Percentage of monensin "unavailable" after interaction with faeces	Initial monensin concentration (µg/ml) required to inhibit growth ("b")	Percentage of monensin "unavailable" after interaction with faeces: $[(b - a)/b] \times 100$
Incubation for 24 h				
0	10	0	100	90.0
1	10	0	100	90.0
2	10	0	100	90.0
4	10	0	100	90.0
6	10	0	120	91.7
8	10	0	120	91.7
12	10	0	120	91.7
Incubation for 48 h				
0	10	0	100	90.0
1	10	0	100	90.0
2	10	0	100	90.0

Table 4 (contd)

Interaction time (h)	Broth only (no faeces)		50% faeces by weight	
	Initial monensin concentration (µg/ml) required to inhibit growth ("a")	Percentage of monensin "unavailable" after interaction with faeces	Initial monensin concentration (µg/ml) required to inhibit growth ("b")	Percentage of monensin "unavailable" after interaction with faeces: $[(b - a)/b] \times 100$
4	10	0	100	90.0
6	10	0	120	91.7
8	10	0	120	91.7
12	10	0	120	91.7

[a] From Pridmore (2007b).

Monensin is extensively metabolized and converted to numerous metabolites by cattle, pigs and rats. *O*-Demethylation and hydroxylation appear to be the major metabolic pathways. Antimicrobial activity of *O*-desmethylmonensin was determined by bioautography against *Bacillus subtilis* and by turbidimetric assay against *Streptococcus faecalis*. In these systems, *O*-desmethylmonensin was only 5% as active as monensin (Boder et al., 1979). Most of the monensin is metabolized to products without antimicrobial activity.

The antibiotic potency of monensin metabolites was further investigated by Pridmore (2007c). Based on a zone inhibition assay, the antibacterial activity of metabolite M1 (*O*-desmethylmonensin) was 19–26.6% of the activity of monensin. MIC values for metabolites M2 and M6 were 2–3 twofold dilutions higher than those for monensin, suggesting that their activity was 12.5–25% of the parent compound activity.

Step 4: Is there any scientific justification to eliminate testing for either one or both end-points of concern, i.e. disruption of the colonization barrier or resistance development?

Monensin does not appear to select for true acquired resistance in bacteria and is not a drug used in human medicine. Results from the microbiological studies suggest that the development of resistance to monensin and cross-resistance to a number of commonly used antimicrobials in veterinary and human medicine is unlikely (Callaway et al., 2003). Thus, the only potential adverse effect on human intestinal microbiota would be disruption of the colonization barrier as the end-point of concern for determining the microbiological ADI. However, since the majority of monensin residues in the colon are bound to faeces and are biologically inactive, the bioavailable concentration is below the lowest MIC_{50} of any of the representative human intestinal bacteria listed in Table 3. Therefore, monensin residues are unlikely to disrupt the colonization barrier of the human intestine. Consequently, there is no need to determine a microbiological ADI for monensin residues.

2.3 Observations in humans

No controlled studies have been performed in which humans have been intentionally exposed to monensin sodium. Two case-reports were found in the literature that described the effects of human monensin intoxication. In the first case, a 17-year-old boy ingested an unknown amount of monensin sodium (Kouyoumdjian et al., 2001); in the second, a 16-year-old boy consumed approximately 500 mg of monensin (Caldeira et al., 2001). In both cases, a similar pattern of toxicity was observed as has been seen previously during overdosing in domestic animal species. Early symptoms included nausea, loss of appetite and abdominal pain, followed by muscle weakness, severe pain, primarily in the lower limbs, and dark brown urine. Clinical chemistry results revealed highly elevated serum creatine kinase, lactate dehydrogenase and aspartate aminotransferase levels, and creatinine and potassium levels were also elevated. The haemogram revealed leukocytosis and a very high erythrocyte sedimentation rate. In both cases, monensin caused rhabdomyolysis, which led to acute kidney failure and in one case led to heart failure. Death occurred in both patients within 11 days of consumption. The primary targets of monensin overdose in humans appear to be skeletal and heart muscles.

The health effects of occupational exposure to monensin during production have also been documented (Twenty, 2001). During the 30-year period that was reviewed, irritant conjunctivitis was observed in several individuals who received a direct splash of monensin in the eye, and irritant contact dermatitis was also observed in one individual. Six employees developed an immunoglobulin E (IgE)-mediated allergic response to monensin, with symptoms including transient urticaria, swelling of the face or tongue, pruritus, chest congestion and chest tightness. These symptoms resolved upon the removal of the employees from the monensin manufacturing area (Twenty, 2001).

3. COMMENTS

3.1 Biochemical data

The absorption and excretion of monensin have been studied in a variety of species, including rats and dogs. The recovery of monensin in the bile was independent of dose (33–49% in male rats receiving 5 or 40 mg monensin/kg bw and 31–53% in female rats receiving receiving 2 or 16 mg monensin/kg bw).

Monensin rapidly disappears from the serum in rats and dogs, and the highest concentration of monensin is attained in the liver.

In rats and dogs, less than 10% of excreted monensin is the parent compound. Monensin is extensively metabolized in the liver in the majority of animals. In human microsomes, monensin sodium is extensively metabolized (93–99% by 60 min), and its turnover is similar to that in dogs.

Monensin metabolism occurs primarily through *O*-demethylation or hydroxylation of the ionophore backbone and is believed to occur at least in part by

CYP3A. Tested monensin metabolites have decreased antibacterial, anticoccidial, cytotoxic, cardiac and ionophoric activity.

The primary excretion route for monensin is the faeces. In rats, a majority of the administered dose (up to 10 mg/kg bw in males, up to 4 mg/kg bw in females) was excreted within 72 h (70–91%), and excretion was independent of dose. Higher doses in rats of both sexes led to decreased excretion of monensin.

3.2 Toxicological data

Crystalline monensin and several preparations of mycelial monensin were tested for toxicity. The Committee evaluated the toxicity of monensin on the basis of the specific form utilized in the studies.

The acute oral toxicity of monensin was moderate to high. The LD_{50} values in mice, rats and rabbits ranged from 22 to 96 mg/kg bw. Signs of toxicity included hypoactivity, ataxia, dyspnoea, ptosis, loss of righting reflex and muscle weakness. Pairs of monkeys given a single dose of monensin in a range from 20 to 160 mg/kg bw by gavage developed diarrhoea within 24 h at all doses and vomiting and appetite suppression starting at 110 mg/kg bw, but no mortality occurred. The LD_{50} value for mycelial monensin in monkeys is greater than 160 mg/kg bw.

In a GLP-compliant study, male and female mice fed diets containing 0, 37.5, 75, 150 or 300 mg mycelial monensin sodium/kg for 3 months (equivalent to 0, 5.6, 11.2, 22.5 or 45 mg/kg bw per day) showed a dose-dependent decrease in body weight gain at the end of the study, ranging from 27% and 21% in the lowest dose group in females and males, respectively, to 99% in the highest dose group in both sexes. Mean body weights also declined, with decreases ranging from 5% and 8% in the lowest dose group to 29% and 35% in the highest dose group in females and males, respectively. Except for the declines in body weights and body weight gains in the lowest-dose males, all changes were statistically significant. Elevated creatine phosphokinase values were observed for the males in the two highest dose groups and for the females receiving the highest dose. Mild diffuse vacuolation of cardiac myofibres occurred in the highest dose group. Since weight gain was affected in all dose groups, a NOAEL could not be identified from this study.

Several GLP-compliant and non-GLP-compliant oral subchronic toxicity studies were conducted in rats using mycelial or crystalline monensin. In a non-GLP-compliant study, rats were fed diets containing mycelial monensin sodium for 3 months at nominal concentrations of 0, 25, 50, 80 or 125 mg/kg (based on feed analysis, equal to 0, 0.89–2.45, 1.83–4.63, 3.02–7.71 and 4.54–12.05 mg/kg bw per day in males and 0, 1.30–2.55, 2.75–5.83, 4.04–12.83 and 10.17–20.21 mg/kg bw per day in females). Transient dose-dependent decreases in body weight gain, mean body weight and feed consumption were observed in females receiving 50, 80 or 125 mg/kg in the diet. Males receiving 125 mg/kg in the diet also had a transient decrease in body weight gain. The NOAEL for this study was the nominal concentration of 25 mg/kg in the diet. An exact dose could not be determined owing to the wide range of measured concentrations of monensin in the feed.

In four parallel subchronic toxicity studies conducted according to GLP, rats were fed diets containing crystalline monensin sodium or three different preparations of mycelial monensin sodium at 0, 50, 200 or 400 mg/kg (equivalent to 0, 2.5, 10 and 20 mg/kg bw per day) for 3 months. Mortality occurred in four females and one male exposed to the highest dose of mycelial monensin and in one female in the middle dose group. The cause of death could not be determined; however, a relationship with the treatment could not be ruled out. Decreased body weight gain was observed for all forms of monensin starting at 200 mg/kg in the diet and in female rats exposed to the mycelial forms of monensin at 50 mg/kg in the diet. Doses of 200 and 400 mg mycelial monensin/kg in the diet reduced feed consumption compared with crystalline monensin and led to decreased body weight gain in male rats. Slight quantitative differences in body weight gain and feed consumption between the crystalline and mycelial forms were not considered biologically significant. Focal degeneration and interstitial myositis of the diaphragm and skeletal muscle occurred in higher incidence in treated female rats than in controls; however, overall incidence and severity were low. Owing to the decreased body weight gain at the lowest dose, a NOAEL could not be determined.

In a non-GLP-compliant study, rats of both sexes were exposed in feed to crystalline or mycelial monensin sodium at 0, 50, 200 or 400 mg/kg (equivalent to 0, 2.5, 10 and 20 mg/kg bw per day) for 3 months. The toxicological responses to both forms of monensin did not differ. Three high-dose females (one in the crystalline group, two in the mycelial group) died during the study. A severe reduction in body weight gain was observed for both formulations starting at 200 mg/kg in the diet. A slight, transient decrease in body weight gain was observed for females in the 50 mg/kg diet group for the first 2 weeks of the study. Increased total bilirubin and alkaline phosphatase levels and decreased mean serum glucose and creatinine levels were seen in all animals receiving either form at 400 mg/kg in the diet and in females receiving 200 mg/kg in the diet. Female rats in all treatment groups also had decreased alanine aminotransferase levels. Initial histopathological examination revealed a non-dose-dependent incidence of scattered foci of a few myocardial fibres, with degeneration, necrosis and infiltration of mononuclear cells, particularly in males, in all three dose groups for both forms of monensin. A second independent pathology evaluation concluded that the scattered myocardial lesions were not adverse and that the incidence was similar to the control incidence. The Committee concluded that a NOAEL could not be identified because of a slight, transient reduction in body weight gain in females in the lowest dose group, which became severe and non-transient in the next dose group.

In a non-GLP-compliant study, two dogs per sex per group were administered monensin sodium orally in capsules at daily doses of 0, 2.5, 5, 11 or 25 mg/kg bw for 90 days. Deaths attributed to treatment with monensin occurred in two males of the highest dose group and one female of the second highest dose group. Females of the highest dose group developed ataxia, tremors, loss of muscular control and slight relaxation of the nictitating membrane; therefore, treatment was stopped after 5 days. There were no signs of toxicity in surviving male and female dogs receiving 11 and 5 mg/kg bw per day or less, respectively. Haematology, clinical chemistry, urinalysis, organ weights and gross pathology of

all animals were normal, with the exception of transiently elevated serum alanine aminotransferase levels in the second highest dose group. The NOAEL was 5 mg/kg bw per day.

In a second non-GLP-compliant study, dogs were orally administered monensin sodium at doses of 0, 5, 15 or 50 mg/kg bw per day for 91 days in gel capsules. Two males in the highest dose group and one male in the middle dose group did not survive the study and exhibited myopathy of the heart with degeneration of the muscle fibre, macrophage infiltration and visceral congestion. Dogs dosed with 15 and 50 mg monensin sodium/kg bw per day vomited more frequently, lost body weight, had transient increases in lactate dehydrogenase and aspartate aminotransferase and developed muscular weakness, ataxia, arrhythmias, convulsions and mydriasis. Degenerative changes in striated muscle were observed starting at 15 mg/kg bw per day. A slight loss of body weight was observed in dogs in all dose groups. Since toxic effects were seen at the lowest dose, a NOAEL could not be identified.

In a 1-year study, mycelial monensin sodium was orally administered as an equally divided dose in gel capsules twice daily to dogs at doses of 0, 1.25, 2.5, 5 or 7.5 mg/kg bw. No data on feed intake were reported. Dogs receiving 5 and 7.5 mg/kg bw per day showed transient signs of toxicity, which included hypoactivity, muscle weakness (particularly legs and neck), stilted gait, difficulty standing and anorexia. Increased alanine aminotransferase and creatine phosphokinase levels were observed during the first 2 weeks of monensin administration in dogs receiving 5 and 7.5 mg/kg bw per day, and several dogs in these groups also had periodic increases in alanine aminotransferase and creatine phosphokinase levels throughout the entire study period. A decrease in body weight gain was seen in male dogs receiving 2.5, 5 or 7.5 mg/kg bw per day, which exceeded 10% for the highest dose. No histopathological changes were seen that were related to monensin exposure. Since body weight gain was decreased at the next higher dose, the NOAEL was 1.25 mg/kg bw per day.

Long-term (1- and 2-year) studies were carried out in mice and rats orally administered mycelial or crystalline monensin sodium. All long-term toxicity studies were conducted according to GLP guidelines.

Mice were given mycelial monensin sodium in the diet at concentrations of 0, 10, 25, 75 or 150 mg/kg (equal to 0, 1.2, 3.1, 10.2 and 22.6 mg/kg bw per day for males and 0, 1.4, 3.5, 11.7 and 25.6 mg/kg bw per day for females) for 2 years. Significant decreases in body weight and body weight gain occurred in mice receiving 25 mg/kg in the diet or greater, and a statistically significant, dose-dependent decrease in leukocyte counts was observed in males receiving monensin at concentrations of 25, 75 or 150 mg/kg in the diet. Minimal increases in urea nitrogen, creatinine, bilirubin, aspartate aminotransferase and creatine phosphokinase occurred with the highest dose. No substance-related deaths, physical signs or behavioural changes were reported. There was no evidence of carcinogenicity that could be attributed to monensin. A NOAEL of 10 mg/kg in the diet (equal to 1.2 mg/kg bw per day) was identified.

Rats were given crystalline monensin sodium in the diet at 0, 25, 56 or 125 mg/kg (equal to 0, 1.14, 2.57 and 5.91 mg/kg bw per day in males and 0, 1.46, 3.43 and 8.68 mg/kg bw per day in females) for 2 years. Body weight and body weight gain were significantly decreased in animals receiving 125 mg/kg in the diet, and a decrease in these parameters was observed during the first 4 months in rats receiving 56 mg/kg in the diet. Feed conversion efficiency was decreased in the animals receiving 56 or 125 mg/kg in the diet, and mean feed consumption was decreased in animals in the highest dose group during the first 5 weeks of the trial. Crystalline monensin sodium did not produce carcinogenicity. The NOAEL was 25 mg/kg in the diet (equal to 1.14 mg/kg bw per day).

In a second study, rats that were exposed to monensin in utero were further exposed to 0, 33, 50 or 80 mg mycelial monensin sodium/kg in the diet for 2 years (equal to 0, 1.40, 2.18 and 3.60 mg/kg bw per day in males and 0, 1.72, 2.86 and 5.02 mg/kg bw per day in females). Survival in both sexes increased in a dose-dependent manner. A transient decrease in body weight was observed at the beginning of the study in all animals receiving 80 mg/kg in the diet and in females receiving 50 mg/kg in the diet. Body weight gain was also significantly decreased during the 1st week in males receiving monensin at 33 and 80 mg/kg in the diet and during the first 2 weeks in females receiving the highest dose. There was a statistically significant increase in feed intake in females in the highest dose group. In utero exposure followed by 2 years of exposure to mycelial monensin sodium at doses up to 80 mg/kg in the diet did not lead to carcinogenicity. As the observed decrease in body weight gain was transient and restricted to the first few weeks of the 2-year study, the effect was not considered to be adverse. The NOAEL was 80 mg/kg in the diet (equal to 3.60 mg/kg bw per day).

Monensin produced negative results in an adequate range of in vitro and in vivo genotoxicity studies. The Committee concluded that monensin had no genotoxic potential.

In a GLP-compliant multigeneration study, mycelial monensin was administered to three generations of rats and their offspring at doses of 0, 33, 50 or 80 mg/kg in the diet (equivalent to 0, 1.6, 2.5 and 4 mg/kg bw per day). Reduced body weight gain was seen in animals of both sexes in every generation and at all doses. There were no significant differences in fertility, litter size, gestation length, parent and progeny survival or sex distribution, and no evidence of teratogenicity was observed. A NOAEL for parental and offspring toxicity could not be determined owing to the reduction in body weight gain in both sexes in every generation and at all doses. The NOAEL for reproductive toxicity was 80 mg/kg in the diet (equivalent to 4 mg/kg bw per day), the highest dose tested.

In a one-generation, non-GLP-compliant reproduction study, female rats received diets containing a non-specified preparation of monensin at concentrations of 0, 100 or 300 mg/kg (equivalent to 0, 5 or 15 mg/kg bw per day) until premating weights achieved 185 g and during pregnancy and lactation. Female body weight was significantly decreased in the highest dose group after 8 days of treatment and remained lower throughout the study. No significant differences in female fertility were observed. All females became pregnant, with the exception of two in the high

dose group, which did not show vaginal opening and could not be mated. Weight gain during pregnancy was not affected by treatment. Gestation length, litter size and number of stillborn pups were also unaltered by monensin treatment. The body weights of male and female pups in the highest dose group were reduced from postnatal day 10 until postnatal day 21. Male offspring in the low dose group showed body weight reduction only on postnatal day 21. No external signs of malformation were detected in the pups. Females perinatally exposed to 100 mg monensin/kg in the diet showed a delay of incisor eruption; this effect was not seen in the high dose group. No other treatment-related effects were observed. Owing to effects on body weight in males in the low dose group on postnatal day 21, a NOAEL for developmental toxicity could not be identified.

In a non-GLP-compliant teratogenicity study, pregnant rabbits received monensin sodium at doses of 0, 0.076, 0.38 or 0.76 mg/kg bw per day from gestation days 6 to 18. Decreased maternal feed consumption was observed in the high dose group during treatment only. Doses up to 0.76 mg/kg bw per day did not affect litter size, corpora lutea number, implantation occurrence, fetal viability or resorption occurrence. In addition, sex distribution, progeny survival and mean fetal weights did not differ between groups. Fetal abnormalities occurred in low incidence and were unrelated to monensin treatment. The NOAEL for maternal toxicity was 0.76 mg/kg bw per day, the highest dose tested. The NOAEL for teratogenicity was 0.76 mg/kg bw per day, the highest dose tested.

In dogs that received monensin sodium by gavage at a single dose of 0, 0.138, 0.345, 0.690 or 1.38 mg/kg, coronary artery flow was significantly increased at 0.690 and 1.38 mg/kg bw, whereas heart rate and blood pressure remained unchanged. The elevation in coronary blood flow was maximal 13–17 min after dosing and returned to normal by 30 min. The threshold for pharmacological effects on the heart was 0.345 mg/kg bw. The observed transient increase in coronary blood flow in dogs given a single oral dose of monensin was considered treatment related but not adverse, owing to the absence of effects on blood pressure or heart rate.

Monensin is not used in human medicine. An evaluation of the medical records of employees involved in the manufacture of monensin from 1968 to 2001 provided no evidence of chronic diseases that could be related to monensin exposure. Several employees developed IgE-mediated allergic responses, including transient urticaria, swelling of the face or tongue, pruritis, chest congestion and chest tightness, which resolved upon their removal from the monensin manufacturing area.

Two case-reports are available in the literature regarding accidental exposure of humans to monensin. In the first case, a 17-year-old boy ingested an unknown amount of monensin sodium; in the second, a 16-year-old boy consumed approximately 500 mg of monensin. In both cases, a similar pattern of toxicity was observed as has been seen previously during overdosing in domestic animal species. Monensin caused rhabdomyolysis, which led to acute kidney failure in both patients and heart failure in the 16-year-old boy. Death occurred in both patients

within 11 days of consumption. The primary targets of monensin overdose in humans appear to be skeletal and heart muscles.

3.3 Microbiological data

A JECFA decision tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with VICH GL36 (VICH, 2004) was used by the Committee to determine the impact of monensin residues on the intestinal microbiota.

The Committee evaluated MIC susceptibility, faecal binding interaction and biological activity of monensin metabolites in the assessment of monensin.

Monensin is microbiologically active against some bacterial genera and species representative of the human intestinal flora.

In tests using the higher bacterial inoculum density of 10^9 cfu/ml, monensin exerted little or no antibacterial activity (MIC_{50} values >128 µg/ml) against *Bacteroides fragilis*, other *Bacteroides* species and *Escherichia coli*. This is consistent with the known spectrum of activity for this compound, which has low activity against Gram-negative bacteria. Monensin also exerted very weak activity against *Bifidobacterium* species. Monensin activity was clearly demonstrable against the other six bacterial groups tested at the higher inoculum density. *Peptostreptococcus* was the most susceptible group (MIC_{50} of 0.5 µg/ml), whereas *Fusobacterium* was the least susceptible.

To determine the effect of faecal binding on the antibacterial activity of monensin, selected monensin concentrations of 0, 1, 2, 5, 10, 20, 50 and 100 µg/ml were incubated with increasing concentrations of sterilized human faeces (0, 10, 20 and 50% w/v in Mueller Hinton Broth), collected from three individual donors for time periods between 0 and 12 h. All three faecal samples had maximal binding of monensin (>90% binding) at 50% concentration. The results demonstrated the rapid and extensive binding of monensin to human faeces. An additional faecal interaction study was conducted, incorporating the microbiological and chemical assays. The mean proportions of monensin that became unavailable after 12 h interaction with faeces, as determined by the growth inhibition assay and chemical assay, were 96.8% and 94.3–98.6%, respectively. This confirmed the conclusions from the earlier study that the antibiotic activity of monensin in the colon would be reduced by >90% by contact with faecal material.

In terms of resistance development, monensin does not appear to select for true acquired resistance in bacteria and is not a drug used in human medicine. Results from the microbiological studies suggest that the development of resistance to monensin and cross-resistance to a number of commonly used antimicrobials in veterinary and human medicine is unlikely. Thus, the only potential adverse effect on human intestinal microbiota would be disruption of the colonization barrier as the end-point of concern for determining the microbiological ADI. However, since the majority of monensin residues in the colon are bound to faeces and are biologically inactive, the bioavailable concentration is below the lowest MIC_{50} of any of the representative human intestinal bacteria. Therefore, monensin residues are

unlikely to disrupt the colonization barrier of the human intestine. Consequently, there is no need to determine a microbiological ADI for monensin residues.

4. EVALUATION

Oral exposure to monensin results in skeletal and cardiac muscle damage and a decrease in leukocyte count and body weight gain. The effects on leukocytes and body weight gain occur at similar doses, which are lower than those that induce the effects in muscle. The effect on body weight gain was consistent, at similar doses, across studies in mice, rats and dogs; based on feed intake data in a rat study, it was considered to be a true treatment-related effect. The observed transient increase in coronary blood flow in dogs given a single oral dose of monensin was considered treatment related but not adverse, owing to the absence of effects on blood pressure or heart rate.

The Committee considered the effects of high doses of monensin on muscle tissue to be important adverse effects. The Committee also considered the consistent decrease in body weight gain at lower doses as a conservative indicator of monensin toxicity, even though the exact mechanism of this effect is not known. On the basis of the toxicological findings, the Committee selected the lowest relevant NOAEL of 1.14 mg/kg bw per day in the 2-year oral rat study, based on a decrease in body weight gain at the next higher dose, as the basis for derivation of the ADI. The Committee noted that this NOAEL was supported by similar NOAELs for this effect in other species. An ADI of 0–10 µg monensin/kg bw was established by applying a safety factor of 100 to this NOAEL and rounding to one significant figure.

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