Environmental Assessment

in Support of

an Import Tolerance

for

Monensin in Food Derived from Sheep

March 14, 2016

Elanco Animal Health
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Table of Contents

1. General Information

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Drug Established Name: Monensin

2. Purpose and Need for the Proposed Action

Monensin is the active pharmaceutical ingredient (API) in several drug products used in ruminants and poultry animal feeds for the prevention and control of coccidiosis and for increased rate of weight gain. It is also used to increase milk production efficiency in dairy cows. Drug products containing monensin are currently approved for marketing and use in the United States (U.S.), and globally, for use in cattle, goats, chickens, turkeys, and quail.[1] Monensin is also approved for use in sheep in Australia (e.g., Rumensin® 100[2]). However, it is currently not approved, nor is it conditionally approved, in the U.S. as a drug substance for use in sheep. Therefore, Elanco Animal Health is requesting establishment of an import tolerance for residues of monensin in edible tissue from sheep so that imported food derived from sheep treated with, and containing residues of, monensin may be legally marketed in the U.S. for human consumption.

The act of establishing an import tolerance is an agency action requiring preparation of an environmental assessment (EA) unless the action is one that meets criteria for categorical exclusion under FDA regulations in 21 CFR Part 25, Subpart C, which is currently not the case. Therefore, this EA has been prepared to address and evaluate the potential direct and indirect environmental impacts in the U.S. due the action of establishing an import tolerance for residues of monensin in edible tissue from sheep.

The impact on the environment of the U.S. arising from the occurrence of monensin residues in edible tissue from sheep will be evaluated herein based on the primary pathways for environmental exposure and the available physical-chemical properties (Section 3) and fate data for the drug (Section 5).

3. Identification of the Substance

Monensin is a polyether ionophore antibiotic produced by the fermentation of the bacterium species *Streptomyces cinnamonensis*. It is used in food-producing animals to prevent coccidiosis and for improved weight gain and feed efficiency[2]. Table 1 summarizes the most relevant physical-chemical properties of monensin.

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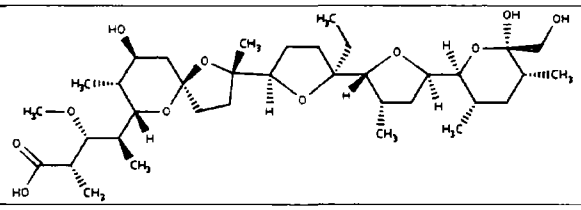
[1] Examples of monensin products approved in the U.S. for ruminants and poultry are Rumensin® 90 and Coban® 90 Type A Medicated Articles, respectively.

[2] See Appendix 1 for additional information on the use of monensin in sheep.

Table 1: Physical-chemical properties of monensin

Drug established (nonproprietary) name	Monensin	
CAS Registry Number	17090-79-8	
Chemical structure[3]		
Molecular formula[3]	$C_{36}H_{62}O_{11}$	
Molecular weight[3]	670.87 g/mole	
Log P (octanol-water partition coefficient) at 25°C (Donoho et al., 1989)	4.24 at pH 5 2.75 at pH 7 3.79 at pH 9	
Solubility (mg/L)	@30°C; Measured by high performance liquid chromatography (HPLC) method (Hogg, 2002)	@25°C; Measured by turbidimeter (Poole et al., 1982)
	109 mg/L (Milli-RO water) Degraded (pH 4) 4.80 mg/L (pH 7) 8.91 mg/L (pH 9)	 63.0 mg/L (pH 7) 0.85 mg/L (pH 9)
Hydrolysis (Poole et al., 1982)	Little or no degradation was noted within 30 days at pH 5, 7, and 9.	
Photolysis (Poole et al., 1982)	Some photolysis was observed at pH 7 with an estimated half-life of 43.9 days	
Vapor pressure[3]	3.20×10^{-23} mm Hg	

4. Sites of Introduction and Exposure Pathways

There are two general types of exposure pathways for monensin to the U.S. environment that could potentially exist due to the establishment of an import tolerance for this drug in edible tissue from sheep: 1) pathways arising from the release of drug residues, if present, from imported food derived from treated sheep, or 2) pathways arising from use of the drug on sheep in countries where it is legally authorized. With respect to the first of the two general types of exposure pathways, i.e., following the importation of food derived from monensin-treated sheep, release of monensin into the U.S. environment (e.g., soil, surface water, air) may potentially occur through two points of introduction:

- through release from landfills that may hold seized materials (edible tissue/meat from treated sheep) containing residues of the drug;

[3] Data from ChemIDplus, a TOXNET database (http://chem.sis.nlm.nih.gov/chemidplus/name/monensin), accessed March 3, 2016. Data provided to ChemIDplus by Syracuse Research Corporation.

- through wastewater treatment plants and their effluents that may contain residues of the drug in human excreta as a result of consumption of imported food from treated sheep.

The potential introduction of drug residues into soils and surface waters from landfills and wastewater treatment facilities strongly depends on the inherent physical-chemical and fate properties of the respective drug (e.g., water solubility, adsorption coefficients, biodegradation rates, etc.), as well as on numerous factors specific to the landfills (e.g., liner thickness, soil type, proximity to surface water) and wastewater treatment facilities themselves (e.g., removal efficiencies, flow rates, dilution factors). In general, only in cases where the substance is volatile or highly mobile (i.e., will migrate out of the compartments at the site of introduction), and present at high enough concentrations to cause effects, is it possible that environmental impacts on the circumjacent ecosystems could become evident.

The environmental exposure and likelihood of monensin to cause impacts on the ecosystems at the sites of introduction is evaluated in Section 5.

5. Analysis of Exposure and Risk

The potential exposures due to the pathways listed in Section 4 will be evaluated based on available residue and environmental fate data for monensin, which is described below. Information on the residues of monensin in the edible tissues of sheep will help to determine the types and magnitude of residues, if any, that could potentially be present in imported meats (which could possibly be disposed of in landfills in the U.S.), as well as the amount of the drug (and/or its potential metabolites) consumed by humans in the U.S., which could then be present in sewage, be processed by wastewater treatment facilities, and subsequently be discharged into surface waters. The environmental fate information will help to determine if monensin is likely to migrate out of landfills, and whether it will likely be persistent in terrestrial and aquatic environments.

A. Residues and Fate of Monensin

Residues in sheep

Giera et al. (1984) have conducted a study to determine the concentration of radioactive residue in edible tissues of sheep following oral administration of radiolabeled monensin at 15 g/ton in feed. In this study, groups of lambs were dosed for 3, 5, or 7 days and sacrificed at 12 hours post final dose. Liver contained the highest mean total residue concentrations (0.36 ± 0.16, 0.33 ± 0.06 and 0.21 ± 0.11 mg monensin equivalents/kg after 3, 5, or 7 day dosing, respectively). Muscle, kidney, and fat contribute minor amounts to total residues. Residues in kidney and fat were all less than 0.03 mg monensin equivalents/kg, and those in muscle were all less than 0.01 mg monensin equivalents/kg. Data indicated that the largest extractable portion of radioactivity is present as the parent compound. These are the types of edible tissues from sheep that could be imported into the U.S. and could end up in landfills or wastewater treatment facilities.

Adsorption, Desorption, and Mobility in Soil

Several studies implementing different experimental and analytical methods are available for estimating the soil organic carbon adsorption coefficient (K_{oc}) of monensin. In a laboratory leaching study, leaching was found to occur in sand and sandy loam soil and little leaching was found to occur in loam and clay loam soils (Decker and Day, 1976), but soil K_{oc} values were not determined. In another study, the adsorption and desorption coefficients and constants were determined according to the Organization of Economic Cooperation and Development (OECD) Guideline 106 (Ponizovsky and Schaefer, 2007). Soil suspensions of five soil types were dosed with monensin and equilibrated for up to 48 hours. The concentrations and stability of monensin in the test system (i.e., aqueous and solid phases) were analyzed by liquid scintillation counting (LSC) and HPLC with radioactivity detector (HPLC/β-RAM), respectively. The adsorption K_{oc} for monensin in the five soils ranged from 369 to 555 mL/g (log K_{oc} 2.57 to 2.74). The desorption K_{oc} for monensin in the five soils ranged from 502 to 807 mL/g (log K_{oc} 2.70 to 2.91). Freundlich adsorption and desorption isotherm coefficients (log K_F) ranged from 0.590 to 1.282 and -0.768 to -1.497, respectively. Monensin is, therefore, considered to have a moderate mobility in soils and adsorption can be reversible.

Degradation in Soil and Water

Soil biodegradation of ^{14}C-monensin was determined in sandy loam, silt loam, and clay loam soils at application rates of 1.50 to 1.53 µg/kg wet weight (Lowrie and Clayton, 2002). The degradation half-life (DT_{50}) values were 18, 13, and 15 days, respectively. $^{14}CO_2$ evolution was observed accounting for 81, 43, and 63% of the applied radioactivity at the end of the 12-week study in sandy loam, silt loam, and clay loam, respectively. Based on these data, monensin is not considered to be persistent in soil and will rapidly degrade with a large fraction of residues mineralized to carbon dioxide.

In water, little or no hydrolytic degradation of monensin was noted within 30 days and the photolytic degradation half-life was reported to be approximately 43.9 days (Poole et al., 1981).

B. Exposure and Risk for Pathways Arising from the Release of Monensin Residues from Imported Food Derived from Treated Sheep

As discussed previously, there are two possible pathways to the environment at large for monensin residues originating in imported food (e.g., edible sheep tissue):

- through release from landfills that may hold seized materials (edible tissue/meat) containing residues of the drug;
- through discharges from wastewater treatment plants via effluents that may contain residues of the drug in human excreta as a result of consumption of imported food from treated sheep.

The amounts of monensin introduced into the U.S. environment from food derived from sheep from both of these pathways are expected to be extremely low. As observed by Giera et al (1984), residues of monensin in edible sheep tissues are low shortly after treatment (e.g., <0.01 mg/kg in muscle within 12 hours post final dose). These reside levels will

continue to decline thereafter as a result of metabolism and excretion. Additionally, several countries including Australia have established an export slaughter interval[4] to mitigate trade risk in other countries that may have different residue standards. For sheep that have been administered monensin, Australia has established a withholding period[5] of no less than 24 hours before slaughter and an export slaughter interval of no less than seven days. Therefore, any residues in the edible sheep tissues imported into the U.S. from Australia are expected to be *de minimus* and likely below detection limits.

Further, although monensin is not approved for use in sheep in the U.S., it is approved for use in the U.S. in cattle, goats, chickens, turkeys, and quail. Under these approvals, neither a preslaughter withdrawal period nor a milk discard period is required for the use of monensin in dairy cows (21 CFR 558.355) because total resides in the edible tissues at practical zero withdrawal (6 hours) were considered to be far below the safe concentrations[6] of 1.5, 3.0, 4.5 and 6.0 ppm established for muscle, liver, kidney and fat of cattle, respectively. The codified tolerances[7] for monensin in cattle are 0.10 ppm in liver and 0.05 ppm in muscle, kidney, and fat; but it has been determined by FDA that a tolerance for residues of monensin in chickens, turkeys and quail is not needed (21 CFR 556.420). Because the residues levels of monensin in most sheep tissues measured by Giera et al (1984) at 12 hours post final dose were already below the tolerances set for monensin in the U.S. for other food producing animals, it is expected that residue levels in imported sheep tissue following an export slaughter interval of no less than seven days, would be insignificant and even further below tolerance limits and safe concentrations.

The potential for impacts of monensin in imported edible sheep tissues to the U.S. environment through these two exposure pathways is evaluated further below.

Landfill Pathway

Landfills in the U.S. are highly regulated by local, state, and federal authorities to prevent environmental contamination. For example, most landfills are required to have caps and liners of clay or an impermeable membrane to prevent leaching of water or fluids therein (and any contaminants they may contain) to groundwater and/or local surface waters (e.g., rivers and lakes). As a result of these controls and the fact that the amounts of residues in sheep tissue will be very low to begin with, there is expected to be minimal or no movement of monensin out of U.S. landfills and into the adjacent U.S. environment (groundwater or surface water). In addition, because monensin has a low vapor pressure, it is not expected to volatilize from landfills and enter air to any significant extent. Due to the low

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[4] The export slaughter interval is the minimum time which should elapse between administration of a veterinary chemical to animals and their slaughter for export. This interval manages differences between maximum residue limits allowed for chemicals in Australia and its trading partners.

[5] The withholding period is the minimum period which must elapse between last administration or application of a veterinary chemical product, including treated feed, and the slaughter, collection, or harvesting or use of the animal commodity for human consumption. Withholding periods are mandatory for domestic slaughter in Australia.

[6] A *safe concentration* is the amount of residue that can be eaten in any edible tissue each day for an entire lifetime without exposing the consumer to residues in excess of the allowable daily intake (ADI). For monensin, the ADI is 12.5 µg per kg body weight per day (21 CFR 556.420).

[7] A *tolerance* is the concentration of the marker residue in the target tissue the concentration of the marker residue in the target tissue at the time the total radiolabelled residues in the target tissue has depleted to less than the target tissue safe concentration.

concentration of resides in the edible tissues and rapid degradation in soils, there is expected to be minimal movement of monensin from the edible tissue of monensin-treated sheep out of a U.S. landfill and into the adjacent U.S. environment. Therefore, based on a lack of exposure, significant environmental impacts on the terrestrial and aquatic environments are not expected from residues of monensin in imported food derived from treated sheep that are disposed of in U.S. landfills.

Wastewater Discharge Pathway

The concentrations of drug residues introduced into the U.S. environment from wastewater treatment facilities as a result of human consumption of imported food containing residues of monensin is expected to be extremely low for several reasons. First, the export slaughter interval is no less than seven days. As previously described, the residues of monensin in sheep tissues are low shortly after administration (within 12 hours of treatment), and will continue to decline thereafter due to metabolism and excretion; therefore, the amounts of residues in sheep tissues at the time of export to the U.S. are expected to be *de minimus*. Additional reasons why this exposure pathway is not expected to be significant include: 1) consumption rates of sheep in the U.S. are low compared to those for most other types of meats; 2) further metabolism of monensin residues, if present, is likely to occur in humans after consumption; 3) the distribution of the excreted residues, if any, in the U.S. environment will likely be spatially and temporally variable (i.e., it is very unlikely that enough humans will consume sheep in the same region on the same day and have their excreta enter the same wastewater treatment facility); and 4) additional degradation/transformation and removal of monensin in wastewater treatment facilities. As a result, the expected concentrations of monensin residues originating from the consumption of edible sheep tissue in effluents entering aquatic systems as a result of discharge from wastewater treatment facilities are expected to be extremely low, approaching zero, with further dilution and degradation of monensin expected to occur in effluent receiving waters. Therefore, no significant environmental impacts on the aquatic environment are expected from this exposure pathway.

C. Exposure and Risk to the U.S. Environment from Use of Monensin on Sheep in Countries Where it is Legally Authorized or Use is Reasonably Foreseeable

Typical Use Conditions

Monensin is approved for use in sheep in Australia for the prevention of ovine coccidiosis and for improved weight gain and feed efficiency and should be administered orally at a rate of 5 to 20 mg/kg in complete feed, or at a dose of 5 to 40 mg monensin/head/day as a supplement. Although monensin is currently approved for use in sheep only in Australia, future drug use in sheep could potentially occur in other countries, including those adjacent to the U.S., Canada in particular. Thus, consideration is given to the potential for effects on the U.S. environment as a result of monensin use in these other countries.

Monensin has been approved in Canada (e.g., Rumensin Premix) and other countries for use in food producing animals other than sheep, such as cattle, chickens, and turkeys. Therefore, it has presumably undergone an evaluation as part of the approval processes in those countries to determine its potential impact on the terrestrial and aquatic environments. In order for monensin to be approved without restrictions by regulators in

these countries, the environmental evaluations would need to demonstrate that significant effects of monensin on terrestrial or aquatic organisms in the vicinity of treated animals would be highly unlikely. If there are no significant effects on sensitive terrestrial or aquatic life in Canada (or other countries) from the use of monensin in these countries, there should be no resulting effects from this use on U.S. environments. In addition, even if effects were to occur in Canada or other countries, effects in the U.S. are still highly unlikely because there will be additional degradation, dispersion, and dilution of any drug residues before they would reach the U.S. border and enter the U.S. environment.

D. Potential Cumulative Exposure and Risk of Monensin on the U.S. Environment from the Establishment of an Import Tolerance for Monensin in Sheep

Although monensin is not approved for use in sheep in the U.S., it is approved for use in cattle, goats, chickens, turkeys, and quail. As part of the approval process in the U.S., monensin has undergone several evaluations to determine the potential impacts on terrestrial and aquatic environments. The Center for Veterinary Medicine (CVM) considered the potential environmental impacts of these actions and concluded that the use of monensin in the U.S. would have no significant effect on the quality of the human environment. The additional residues that may be present in the edible tissue of imported sheep will have a negligible impact on the overall environmental concentrations of monensin in the U.S. Therefore, there would be no significant cumulative environmental impact if an import tolerance were established for monensin in food derived from sheep.

6. Description of Any Alternatives to the Proposed Use

Elanco Animal Health is proposing to establish a tolerance for monensin in edible tissue from sheep that is imported into the U.S. for human consumption. The only alternative to the proposed action is the 'no action' alternative, which would be the failure to establish a tolerance for monensin in sheep. Based on our analysis in this EA, we do not believe that significant environmental impacts will occur from this action; therefore, the preferred alternative is the establishment of a tolerance for monensin in sheep imported into the U.S. and the no action alternative was eliminated from consideration.

7. Conclusions

Based on the available information on the residues in edible tissue, environmental fate, and exposure of monensin originating from the edible tissue from treated sheep, there is expected to be little or no exposure to monensin residues in the U.S. environment for the two exposure pathways evaluated that originate from the importation of meat from monensin-treated sheep. Although monensin is approved for use in other food producing animals in the U.S., the establishment of an import tolerance for monensin in sheep is not expected to have a cumulative impact on the U.S. environment. In addition, there is expected to be no significant impact on the U.S. environment from the use of monensin in sheep (or other food producing animals) in Australia or in other countries where use is reasonably foreseeable, such as Canada. Therefore, it is concluded that the proposed action

of establishing an import tolerance for monensin residues in sheep will not result in significant environmental impacts in the U.S.

8. Agencies and Persons Consulted

This EA was prepared with input and assistance from members of the Environmental Safety Team in the Office of New Animal Drug Evaluation in FDA's CVM.

9. Preparers

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10. Signatures of Responsible Officials

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11. References

Decker OD and Day EW (1976). Laboratory soil leaching study with monensin. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA.

Donoho AL and Ruggles DE (1989). Octanol-water partition coefficient for monensin. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA, Study No. ABC-0438.

Giera DD, Herberg MS, Thomson TD, and Handy PR (1984). ^{14}C-Monensin Tissue Residue Study in Sheep. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA, Experiment No. ABC-0270.

Hogg A (2002). Physico- chemical testing with monensin: validation of analytical method and determination of water solubility. Unpublished report from Inveresk Research, Tranent EH33 2NE, Scotland, Study Project No. 341613, Study Report No. 21247.

Lowrie C and Clayton MA (2002). The degradation of [^{14}C]-monensin in soil under aerobic conditions. Unpublished report from Inveresk Research, Tranent EH33 2NE, Scotland, Study Project No. 802395, Study Report No. 21631.

Ponizovsky AA and Schaefer EC (2007). Adsorption/desorption characteristics in five representative soils following OECD guideline 106. Unpublished report from Wildlife International, Ltd., Easton, Maryland, USA, Study No. 151E-106.

Poole GM, West SD, and Donoho AL (1982). The solubility, hydrolysis, and photolysis of monensin in aqueous solution. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA, Study No. S-AAC-81-13.

Appendix 1

Drug Information for Rumensin® 100, Rumensin® 200, Rumensin® Technical, and Rumensin® Granular for use in Sheep

Monensin is a polyether ionophore antibiotic. Monensin sodium is the active ingredient in Rumensin® 100, Rumensin® 200, Rumensin® Technical, and Rumensin® Granular. These products are produced in crystalline or granules forms at concentrations ranging from 100 g/kg in Rumensin® 100 to 800 g/kg in Rumensin® Technical. Rumensin® 100 and Rumensin® 200 are mixed into an intermediate premix before mixing into the complete feed while Rumensin® Technical and Rumensin® Granular are not mixed into an intermediate premix prior to use in complete feed.

Rumensin® 100 and Rumensin® 200, Rumensin® Technical, and Rumensin® Granular are currently registered in Australia are approved for use in feedlot cattle, pasture cattle, dairy cows, cattle, sheep, goats, and chickens. For use in sheep, monensin is administered orally at a rate of 5 to 20 mg/kg in complete feed or at a dose of 5 to 40 mg monensin/head/day as a supplement for the prevention of ovine coccidiosis and for improved weight gain and feed efficiency.

Monensin should not be administered to sheep which are producing, or may in the future produce, milk where the milk or milk products may be used for human consumption. The meat withholding period for sheep is no less than 24 hours before slaughter for human consumption. The export slaughter interval is no less than 7 days.

Appendix 2

Executive Study Summaries

1. **Decker OD and Day EW (1976).** Laboratory soil leaching study with monensin. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA.

This study determined the leaching characteristics of monensin in four types of soil (sand, sandy loam, loam, and silty clay loam). Leaching columns (approx. 30 cm high x 6.35 cm i.d.) for each soil type contained 100 g of soil. An additional 100 g of soil containing 10 ppm monensin was then added to each column for a total of 200 g of soil. One control and three treatment columns were prepared for each soil type and leached with the water equivalent of 25 inches of rainfall (2,000 mL deionized water). Leachate and soils (analyzed in 5 cm segments) were collected, extracted, and analyzed for monensin by microbiological assay. The mean recovery of the monensin standard used to fortify the samples was of 81 ± 6.8% of theoretical amount. Recoveries from sand, sandy loam, loam, and silty clay loam test soils fortified at 10 ppm were 62.6, 78.0, 63.5, and 84.9% of the theoretical amount added, respectively.

Under the conditions of this experiment, the application of the equivalent of 25 inches of rain caused leaching of monensin from the sand and sandy loam soil while there was very little leaching from the loam and silty clay loam. The total amount of monensin observed in the soils and leachate for each column was substantially less than the amount added to each column. It was presumed that degradation accounts for the loss of monensin in this leaching study, with greater losses occurring in soils which required longer time periods for leaching. The results of this experiment indicate that monensin is moderately mobile in coarse textured soils.

Mean Percent of Theoretical Amount of Monensin Added to Triplicate Leaching Columns for Each Soil[†]

	Sand	Sandy Loam	Loam	Silty Clay Loam
Leachate	31.6	29.1	1.1	1.4
Soil	10.3	6.9	7.5	4.7
Degraded[*]	58.1	64.0	91.4	93.9
Time required for leaching (days)	0.25-1	3-5	4-10	12-17

† No correction was made for recovery efficiency

* The amount presumed to be degraded is the difference between the amount applied and the total observed in the soil and the leachate.

2. **Donoho AL and Ruggles DE (1989)**. Octanol-water partition coefficient for monensin. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA, Study No. ABC-0438.

The purpose of the study was to determine the partition coefficient of 0.0002M ^{14}C-monensin between n-octanol and water (K_{ow}) at pH 5, 7, and 9 at 25°C using the shake flask method in the FDA Environmental Technical Assistance document 3.02. It was also determined at pH 7 at a concentration of 0.00002 M. The concentrations of monensin in both the n-octanol and water phases were determined using LSC. The log K_{ow} of the test item (0.0002 M monensin) was estimated to be 4.24, 2.75, and 3.79 at pH 5, 7, and 9, respectively. The log K_{ow} of 0.00002 M monensin was estimated to be 2.87 indicating that the test concentrations were sufficient for accurate K_{ow} determination.

3. **Giera DD, Herberg MS, Thomson TD, and Handy PR (1984).** ^{14}C-Monensin Tissue Residue Study in Sheep. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA, Experiment No. ABC-0270.

Lambs were fed ^{14}C-monensin equivalent to a feeding level of 15 g/ton (16.5 mg/kg) of complete ration. Groups of lambs (two wethers and one ewe) were dosed for 3, 5, or 7 days and sacrificed at 12 h post dose. Edible tissues were assayed for total radioactivity (assay reliability = 0.1 mg/kg or lower). Liver samples were assayed for parent monensin, and selected samples of liver and feces were characterized chromatographically. Liver contained the highest mean total residue concentrations with mean total residue concentrations ranging from 0.21 to 0.36 mg monensin equivalents/kg. Residues in kidney and fat were all less than 0.03 mg monensin equivalents/kg and muscle all less than 0.01 mg monensin equivalents/kg. The largest extractable portion of radioactivity is parent. Mean Resides are shown in the table below.

Mean concentrations of ^{14}C-(mg monensin equivalents/kg) in sheep tissues following oral administration of radiolabelled monensin at 15 g/ton in feed.

	3 day dosing period	5 day dosing period	7 day dosing period
Liver	0.36 ± 0.16	0.33 ± 0.06	0.21 ± 0.11
Kidney	0.01 ± 0.005	0.01 ± 0.02	0.003 ± 0.01
Fat	0.01 ± 0.0	0.01 ± 0.01	0.02 ± 0.01
Muscle	<0.01	<0.01	<0.01

4. **Hogg A (2002).** Physico-chemical testing with monensin: validation of analytical method and determination of water solubility. Unpublished report from Inveresk Research, Tranent EH33 2NE, Scotland, Study Project No. 341613, Study Report No. 21247.

The purpose of the OECD Guideline 105 GLP-compliant study was to develop an analytical method for monensin sodium based on HPLC and to determine the aqueous solubility of monensin in Milli-RO water and pH 4, 7, and 9 aqueous buffers. The method is based on the flask method. Samples were removed from an orbital shaker after incubations of 24, 48 and 72 h in a water bath at 29.4°C and then placed in a water bath at 20°C to equilibrate for 24 h. Samples were analyzed by HPLC. The solubility of monensin sodium in Milli-RO water and aqueous media buffered to pH 7 and pH 9 was determined to be 109 mg/L, 4.81 mg/L, and 8.91 mg/L, respectively. Monensin sodium degraded in aqueous medium buffered to pH 4 after 24 hour incubation at 29.4°C.

5. **Lowrie C and Clayton MA (2002).** The degradation of [^{14}C]-monensin in soil under aerobic conditions. Unpublished report from Inveresk Research, Tranent EH33 2NE, Scotland, Study Project No. 802395, Study Report No. 21631.

The purpose of this study was to evaluate the degradation of ^{14}C-monensin in three fresh field soils (sandy loam, silt loam, and clay loam) at 20°C. Treatment solutions were prepared corresponding to treatment application rates of 1.50 to 1.53 mg/kg wet weight for each soil. Single incubates from each soil were sampled over the course of the 12-week study. The rate of degradation was assessed in each soil type. The route of degradation was investigated only in the sandy loam. The radioactivity associated with the soil extracts was quantified by LSC. Characterization of radioactivity in soil extracts was carried out using reverse-phase HPLC and normal-phase thin layer chromatography (TLC). Volatile transformation products were also collected and analyzed. Monensin degraded rapidly in all three soils. The principle degradation

product was $^{14}CO_2$, accounting for 81%, 43%, and 63% of applied radioactivity at study termination in the sandy, silt, and clay loam, respectively. The degradation pathways to CO_2 included up to 27 unknown components, with up to 16 individual unknown components in samples at any one time point. One unknown degradation product greater than 20% was noted at time point in one soil (i.e., clay loam on day 14 accounted for 36% of applied radioactivity). The DT_{50} values were estimated as 18, 13, and 15 days for sandy loam, silt loam, and clay loam soils, respectively. Based on the results of this study, it is unlikely that monensin will persist in soil for a prolonged period of time

6. **Ponizovsky AA and Schaefer EC (2007).** Adsorption/desorption characteristics in five representative soils following OECD guideline 106. Unpublished report from Wildlife International, Ltd., Easton, Maryland, USA, Study No. 151E-106.

An OECD 106 guideline compliant GLP-study investigated the adsorption and desorption kinetics and isotherms of monensin in five soil types (clay loam, sandy clay loam, clay loam, loamy sand, and clay) at ambient temperature (20.8 to 21.5°C). The soil types varied in organic carbon content, pH, and texture.

In the preliminary test, sorption of monensin to the test vessel, stability of monensin in the solution and test vessel, and optimal soil:solution ratio for subsequent tests was determined. In the adsorption/desorption kinetics test, the equilibration time for sorption and desorption in the test systems and blank were assessed. Soil suspensions were dosed with monensin and incubated until adsorption and desorption equilibrium was achieved. Concentrations of the test substance in the aqueous phase were determined using LSC. Equilibrium was achieved for adsorption and desorption after ~24 hours when mixing by orbital shaker and ~0.5 hours when mixing by vortexer.

In the isotherm tests, adsorption and desorption isotherms of monensin (five concentration levels) in each soil was assessed. After equilibrium, monensin concentrations and stability in the aqueous phase were analyzed by LSC and HPLC/β-RAM, respectively. The solids of the highest test concentration (two replicates for each soil and the blank) were extracted and the supernatant was analyzed by LSC and HPLC/β-RAM. Samples of the remaining extracted solids were then combusted with subsequent analysis by LSC to determine the concentration of sorbed material. The mass balance in each test system at each concentration was verified. The following sorption coefficients and isotherms were found for the five soils tested.

Distribution Coefficients (K_d)* and organic carbon normalized distribution coefficients (K_{oc}) of Monensin Estimated from Adsorption and Desorption Isotherm Tests

Sample	K_d^{ads} mL/g	K_{OC}^{ads} mL/g		K_d^{des} mL/g	K_{OC}^{des} mL/g
Clay Loam (TB-PF)	20.4	408		26.8	537
Sandy Clay Loam (MSL-PF)	10.5	555		14.2	745
Clay Loam (DU-Loam)	15.1	369		20.6	502
Loamy Sand (Roger Myron)	5.7	439		8.9	683
Clay (Montana Clay)	3.7	524		5.6	807

* K_d values reported in this table were calculated by fitting the isotherms with linear regressions

Parameters of the Freundlich Isotherm Equation for Adsorption and Desorption of Monensin*

Sample	$\text{Log}\,K_F^{ads}$	n	R		$\text{Log}\,K_F^{des}$	n	R
Clay Loam (TB-PF)	1.2816	1.06	0.998		-1.4974	0.917	0.994
Sandy Clay Loam (MSL-PF)	1.0708	0.97	0.998		-1.1886	0.896	0.999
Clay Loam (DU-Loam)	1.1516	1.07	0.996		-1.3928	0.861	0.997
Loamy Sand (Roger Myron)	0.7521	1.04	0.996		-0.9467	0.919	0.995
Clay (Montana Clay)	0.5898	0.95	0.972		-0.7682	0.989	0.993

* Soil:solution = 1:5; temperature 20.8 to 21.5 °C; concentration of the sorbed test substance approximately 0.022 to 2.61 mg kg^{-1}

7. **Poole GM, West SD, and Donoho AL (1982).** The solubility, hydrolysis, and photolysis of monensin in aqueous solution. Unpublished report from Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN, USA, Study No. S-AAC-81-13.

The purpose of this study was to determine the 1) aqueous solubility of monensin following sterile filtration of buffer solutions of pH 7 and 9 at 25°C, 2) hydrolytic stability of monensin in aqueous solutions in pH 5, 7, and 9 in sterile buffer solutions stored in the dark at 25°C over 30 days, and 3) photolytic stability of monensin in pH 7 aqueous solution over 30 days. Endpoints were determined turbidimetrically. The average solubility of monensin was 63 and 0.85 mg/L at pH 7 and 9, respectively. The hydrolysis of monensin was slow at pH 5, 7, and 9 with little or no degradation noted within 30 days. The photolytic degradation of monensin at pH 7 was moderate with an estimated half-life of 43.9 days. The rate constant was estimated to be 0.0158 day^{-1}.