ENVIRONMENTAL ASSESSMENT

SUPPLEMENT TO NADA 139-472

DENAGARD® (tiamulin) PREMIXES

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ENVIRONMENTAL ASSESSMENT

1. DATE:

2. NAME OF APPLICANT:

Fermenta Animal Health Company

3. ADDRESS:

10150 North Executive Hills Boulevard
Kansas City, Missouri 64153-2314

4. DESCRIPTION OF THE PROPOSED ACTION:

4.1 Requested approval - Need for the action

The proposed action is to add a label claim, for the treatment of swine dysentery associated with *Treponema hyodysenteriae* susceptible to tiamulin, to DENAGARD® (tiamulin)[1] PREMIXES which were earlier approved in NADA 139-472 for (1) control of swine dysentery and (2) for increased rate of gain and feed efficiency from weaning to market weight. The proposed action does not involve a new product form or formulation but only a new use of the previously approved DENAGARD (tiamulin) PREMIXES to provide a therapeutic level of tiamulin hydrogen fumarate in feed for use in treatment of swine dysentery.

In the proposed action tiamulin hydrogen fumarate is administered at 200 g/ton of feed for 14 days for treatment of swine dysentery. This application will be useful where treatments via medicated drinking water or by parenteral injections are not possible or practical. The direct purpose of this use is to treat this debilitating and occasionally fatal disease of swine thus increasing the availability of pork for human consumption. Other benefits include reductions in the cost of swine production and the cost of pork to the consumer.

4.2 Locations where the product is produced

Fermenta Animal Health Company obtains technical tiamulin hydrogen fumarate (THF) from a FDA-approved source outside the USA. The productin of technical THF is described in appropriate DMF's. An updated Environmental Assessment for manufacture of bulk THF (technical) was submitted by the bulk drug supplier to FDA/CVM April 13, 1993.

Tiamulin technical is formulated into a 25% premix at Fermenta Animal Health Company facilities at 15th and Oak, Elwood, Kansas 66024.

The 25% tiamulin premix is made into medicated premixes containing 5 or 10 grams tiamulin hydrogen fumarate per pound at toll blenders. This 10 g/lb

[1] In this Environmental Assessment, tiamulin refers to tiamulin hydrogen fumarate.

premix is incorporated into complete swine feeds by feed manufacturers or swine producers and fed, as needed, to pigs between weaning and market weight.

A Material Safety Data Sheet (MSDS) is found in Appendix I.

4.3 Locations where the product will be used

This product is used throughout the United States on farms involved in swine production. Use in the various states is expected to be largely proportional to the population of swine in those states. States with the largest swine populations are found in the Midwest, however, some other states, e.g., North Carolina, also have significant swine populations.

4.4 Types of environment present at and adjacent to these locations

The Fermenta Animal Health Company pharmaceutical manufacturing facility, used in the manufacture of the 25% active ingredient premix, is located in a semirural area near Elwood, Kansas. Also on this property is a separate facility owned by Fermenta and used for specialty products manufacturing. These Fermenta facilities are surrounded on three sides by largely undeveloped land utilized for crop farming. The fourth side borders some unrelated commercial facilities used largely as warehouse and distribution centers. The terrain at this location is flat and open.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION:

Nomenclature

Tiamulin hydrogen fumarate

[(2-Diethylamino) ethyl) thio] acetic acid 6-ethenyldecahydro-5-hydroxy-4,6,9,10-tetramethyl-1-oxo-3α, 9-propano-3αH-cyclopentacyclopentacycloocten-8-yl-ester

14-desoxy-14-[(2-diethylaminoethyl) mercaptoacetoxy mutilin] hydrogen fumarate

CAS Registry Number

55297-96-6

Molecular Weight

609.8

Structural Formula

Physical Description

Appearance:
Fine crystalline powder

Color:
White to off-white; may be yellow-white

Melting Range:
146±3°C

Additives

None

Impurities

Not more than 3%

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

Information on the environmental assessment of the manufacturing process for DENAGARD Antibiotic PREMIX (25% tiamulin) at the Elwood, Kansas facility follows:

Identification of Pollutants Expected to be Emitted

- Tiamulin hydrogen fumarate: 14-desoxy-14-[(2-diethylaminoethyl) mercaptoacetoxy mutilin] hydrogen fumarate
- Isopropyl alcohol
- Poly (vinyl chloride)

Citation of Applicable Federal, State and Local Emission Requirements

The manufacturing, processing, packaging and holding of the product is performed by Fermenta Animal Health Company, Elwood, Kansas. Responsible personnel from the following regulatory agencies have been contacted. In many instances, operating permits (air, water) have been issued to Fermenta. The Elwood facility is routinely inspected by officials of the State to ensure compliance.

City Administrator of Elwood
6th & Kentucky Street
Elwood, Kansas 66024
(913) 365-6871

Kansas Department of Health and Environment
Bureau of Water Protection
Forbes Field, Building 740
Topeka, KS 66620
(913) 296-1500

Kansas Department of Health and Environment
Bureau of Air and Waste Management
Forbes Field, Building 740
Topeka, KS 66620
(913) 296-1500

Neither the City of Elwood nor the State of Kansas have specific published emission requirements pertaining to components of this formulation.

Fermenta's water discharge is into the City of Elwood's waste system. Requirements for this discharge are determined by the Bureau of Water Protection for the State of Kansas.

The water pollution regulations for the State of Kansas are promulgated under Section 28-16-1 through 28-16-67. These state that no toxic wastes may be discharged in toxic amounts and these amounts will be determined by the State of Kansas.

The state air quality requirements are promulgated under Sections 28-19-6 through 28-19-58. These are concerned with particulate air emissions and those of sulfur dioxide and nitrogen compounds. The primary point of air emissions is from dust collection air stacks which emit essentially particulate free air. The installation and operation of these exhaust systems are covered by permit number 0840016 issued by the Bureau of Air and Waste Management, Kansas Department of Health and Environment, Topeka, Kansas.

Fermenta is classified as an EPA Hazardous Waste Generator under the EPA Resource Conservation and Recovery Act of 1976 (RCRA). Fermenta's EPA identification number is KSD067925347.

Air Emissions

Fermenta utilizes a bag type dust collection system that is equipped with a differential pressure alarm. This is a single pass system that exhausts to the external environment. The differential pressure of the system is routinely monitored and recorded to assure that the system is operating properly. Any observed abnormalities trigger shutdown and inspection of the system with the possible replacement of the bag assemblies. This operation is covered by an internal Standard Operating Procedure.

Waste Water

The average loss of product for this process is ≤ 1%, therefore the manufacturing process does not produce water effluent which is considered potentially dangerous or harmful to the environment.

Fermenta's water discharge is into the City of Elwood's Publicly Owned Treatment Works. Requirements for this discharge are determined by the State of Kansas, Department of Health and Environment, Bureau of Water Protection. Fermenta's Water Pollution Control Permit No. is P-M005-0002, issued July 24, 1991.

From the tiamulin process, some rinse water will enter the process waste system from cleaning operations. Washing will be preceded by thoroughly emptying of vessels and vacuuming where possible.

The process solvents which are evaporated and recovered as a mixture with isopropanol will be treated as a separate waste stream. The mixture will either be reused, recycled off site or incinerated also off site.

The direct discharge to surface water is controlled by engineering design. Indirect discharge is controlled by requirements of accountability and work procedures. There will be no contamination of surface water.

Solid Waste

Material collected from these processes, as well as other chemical wastes, are disposed of in a secured landfill by a permitted facility (currently that facility is USPCI, Inc., a Subsidiary of Union Pacific Corporation and ENSCO Environmental Systems Company) in a manner consistent with applicable federal, state and local regulations.

Solid wastes, such as paper trash, containers, combustible materials, etc. are disposed of by Wheeling Disposal Service, P. O. Box 104, St. Joseph, Missouri 64503, which is an industrial waste disposal company.

Energy

The energy sources which are expended during the mixing, filling and packaging are not retrievable. The sources include natural gas and electricity.

Occupational Standards

Occupational Safety and Health Administration (OSHA) guidelines are maintained as related to the health and safety of all employees. Although no specific limit has been set by OSHA for exposure to tiamulin, personnel who handle the technical product are protected from potential inhalation and dermal hazards by requiring that they wear a dust respirator and protective gloves along with a complete work uniform, safety glasses and safety shoes.

Certification

The methods used in, and the facilities and controls used for the mixing, filling, packaging and holding of the drug product are in conformity with the applicable federal, state and local emission requirements.

Effects of Approval of this Supplemental NADA

Approval of this application is not expected to have any effects on Fermenta's compliance with current emission requirements at their Elwood, Kansas manufacturing location.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT:

Where Drug Will Be Used

DENAGARD (tiamulin) PREMIXES have previously been approved and are currently labeled and marketed in the USA for use in medicating feed at 35 g/ton for the control of swine dysentery and at 10 g/ton for increase in weight gain and feed efficiency from weaning to market weight. The present submission would add the treatment of swine dysentery at 200 g/ton for 14 days to the label uses.

With the addition of the claim for treatment of swine dysentery, it is estimated that an additional 2,464 kg of DENAGARD activity would be used in the USA each year. This is calculated on the number of swine marketed each year as 88 million. It is estimated that 20% of the pigs produced have swine dysentery at some time in their life and that 40% of these might be treated for the disease with medication in the feed. If DENAGARD could penetrate the USA market for feed medication for therapy of swine dysentery to the extent of 5%, it would be used on about 352,000 animals. If the average weight of these pigs was 100 pounds, feed consumption would be approximately five pounds per day for 14 days of therapy and each pig would consume 70 pounds of feed containing a total of seven grams of tiamulin. If 352,000 pigs each

consumed seven grams of tiamulin, a total of 2,464 kg of tiamulin would be utilized for this purpose in one year. This estimated amount would be in addition to the 48,000 kg estimated at product maturity for control of swine dysentery and for increase in rate and efficiency of gain projected in the original NADA 139-472 Environmental Assessment.

Fate of the Drug in Swine

Studies of the metabolism of tiamulin in swine reveal the following:

- Approximately 85-90% of the drug is absorbed.(1,2)[a]
- Peak levels of activity in blood are reached 2-4 hours after an oral dose.(3)
- Daily dosing results in a steady state by 5 days.(1,2)
- The target tissue for residues is liver.(1,2)
- The antibiotic is rapidly absorbed and metabolites are excreted via bile in feces (2/3) and in urine (1/3) in pigs and in rats.(1,2,4,5)
- Only 0.3 to 0.5% of the parent compound is excreted unchanged in the urine.(6,7)
- At least 25 metabolites have been found in urine and bile, 16 of which have been structurally identified.(6,7,8)
- Tiamulin is transformed by N-dealkylation, hydroxylation, oxidation and sulfoxidation in animals.(4,6,8)
- Metabolites of tiamulin have substantially less antibacterial activity than the parent compound and most have little or none.(6,8)
- None of 14 metabolites found in swine urine exceed 6% of the dose and none of 16 metabolites in swine bile exceed 7% of the drug dose administered.(7)
- One metabolite found in swine bile and liver was not present in rats. Neither this metabolite nor any of the many metabolites common to both species exceeded 10% of the total liver residue.(7,9,10)
- The marker metabolite, 8-hydroxymutilin, depletes from swine liver quickly with mean levels of less than one-half the tolerance of 400 ppb reached by 48 hours after the end of feeding tiamulin hydrogen fumarate at 200 g/ton.(11)

[a] Numbers in parentheses refer to abstracts in Appendix II.

In summary, tiamulin is readily absorbed and extensively metabolized in swine. More than half of the 25 metabolites found have been structurally identified, synthesized and tested for antibacterial activity. Most of the metabolites have little or no antibacterial activity. None of the individual metabolites found in swine urine, bile or in swine liver (the target tissue for residues) represents as much as 10% of the dose administered.

Amount of the Drug in Feed Spillage and Swine Excrement

It is estimated that there is a maximum loss of 3% of swine feed due to feed spillage and, therefore, a 3% loss of medication it originally contained. Further, swine appear to excrete 0.3-0.5% of tiamulin hydrogen fumarate unchanged in the urine.[6,7] One could also assume that excretion of tiamulin in swine feces at most may be equivalent to 3% of the amount ingested.

The estimated eventual annual use of tiamulin in medicated feed is 50,464 kg; therefore a 3% loss due to spillage or feed wastage is estimated at 1,514 kg. For use as treatment for swine dysentery at 200 g/ton, 12,500 tons of feed would be medicated. If 24,000 kg of tiamulin were used at 35 g/ton to control swine dysentery, 685,714 tons of feed would be used. For growth promotion and increased efficiency at 10 g/ton, 24,000 kg would be used to medicate 2.4 million tons of feed. Thus, the total amount of feed medicated would be about 3.1 million tons.

If one assumes that swine diets are 80% digestible, swine feces are 76% moisture, and pig excreta is 70% feces and 30% urine, it can be calculated that for every pound of feed consumed 1.19 pounds of excreta (feces plus urine) result. If 3.1 million tons of feed were consumed by pigs fed tiamulin, 3.69 million tons of excreta would be produced.

If 0.5% of the tiamulin ingested by swine is excreted unchanged in the urine and it is assumed that 3% of the tiamulin ingested is excreted in the feces of swine, it can be calculated that about 1,766 kg of tiamulin might be excreted. If this plus the amount of tiamulin lost in feed spillage, 1,514 kg, were uniformly dispersed in the excreta produced, 3.69 million tons, there would be 0.888 grams of tiamulin per ton or 0.976 mg/kg (ppm).

Fate of the Drug in Feed Spillage and Excrement

Most of the tiamulin administered to swine is converted to a large number of metabolites[6,7,8] nearly all of which have little or no antibacterial activity.[6,8] Only the parent compound, tiamulin, from spilled or wasted medicated feed and excreted unchanged in urine and feces might be of environmental concern and this seems unlikely.

a. Air

The vapor pressure of tiamulin is such that it would not be expected to enter the atmosphere. The vapor pressure determined at 100°C, 111°C

and 115°C was 2 x 10^{-5} torr, 4.5 x 10^{-5} torr and 6.0 x 10^{-5} torr, respectively. Under extrapolation of these values, the vapor pressure of tiamulin was calculated to be 0.15 x 10^{-7} torr at 25°C, 0.49 x 10^{-7} torr at 35°C and 1.5 x 10^{-7} torr at 45°C.(12)

Photolysis: According to the first and second laws of photochemistry, light must be absorbed in order to affect photodegradation of a chemical compound. The absorption spectrum of the compound can, therefore, be used to predict the potential of the compound to photodegrade.

The ultraviolet absorption spectrum of tiamulin in aqueous solution was determined. The data show that tiamulin absorbs light in the spectral region between 200 nm and 280 nm and absorbs no light in the wavelength region greater than 280 nm. Natural sunlight has very little energy in the wavelength region less than 280. It is predicted that, under conditions of natural sunlight, tiamulin will not photodegrade.(13)

b. **Freshwater, estuarine and marine ecosystems**

Tiamulin is freely soluble in water and it has been shown that the tiamulin in the DENAGARD premixes is solubilized in water within five minutes; therefore, tiamulin resulting from medicated feed spillage would be expected to be in aqueous solution from exposure to wash water and other sources of moisture in the housing environment.

Adsorption/desorption data show that the binding potential of tiamulin is high and indicates that soil mobility is low. Therefore, based on these properties, tiamulin would not be expected to leach significantly or be subject to run off.(14,17)

In preliminary small-scale environmental impact studies it was shown that at 2.4 ppm in pond water, more than 80% tiamulin was lost when stored for 9 weeks at room temperature; 30% was lost when stored for 2 weeks at 37°C. At a concentration of 600 ppm in pond water, no loss of tiamulin was observed when stored for three months at room temperature; 40 to 50% loss was observed when stored for 10 weeks at 37°C.(15)

Effect on Pond Flora and Fauna

In preliminary small-scale environmental impact studies, it was shown that at concentrations up to 500 ppm tiamulin did not significantly affect the numbers and motility of the flora and fauna in pond water when the water was examined microscopically after one day and one week at room temperature.(15)

Effect on Fish

In preliminary small-scale environmental impact studies, it was shown that no mortality of goldfish was observed at tiamulin concentrations in water less than 160 ppm. At 160 ppm, mortality was observed; surviving fish recovered within 24 hours when exposed to water containing no tiamulin.(15)

No signs of toxicity due to tiamulin were observed when the antibiotic was fed in the diet at 5 and 50 mg/kg for 14 days to rainbow trout infected with *Yersina ruckeri*. In vitro MIC's against 51 strains of seven species of gram-negative bacterial pathogens of fish were all ≥ 6.25 ppm except for *Vibrio anguillarum* which were ≤ 6.25 ppm.(16)

c. Terrestrial Ecosystems

Bioaccumulation factor: It is generally accepted that the octanol/water partition coefficient is an indication of the potential for bioaccumulation. Compounds with partition coefficients greater than 100 have a potential for bioaccumulation while those with partition coefficients, an order of magnitude less, have a low potential for bioaccumulation.

The partition coefficients for tiamulin at two concentrations and at four pH levels have been determined.(13)

Octanol/water partition coefficient for tiamulin:

pH	Concentration (w/v)[1]	Partition Coefficient
4.1	1%	0.90
5.0	1%	3.22
6.0	1%	26.73
7.0	1%	175.14
4.1	0.1%	1.05
5.0	0.1%	1.87
6.0	0.1%	7.98
7.0	0.1%	126.25

[1]Initial concentration in aqueous solution.

At pH levels of 4.1 to 6.0 the partition coefficient is low, thus indicating that tiamulin has a low potential for bioaccumulation. As the pH approaches the pKa2, the base form of tiamulin predominates. Partition coefficients indicate that the base form of tiamulin has a potential for bioaccumulation.

Under field conditions the pH of naturally occurring water will not generally approach the pKa2. It is predicted, therefore, that tiamulin will

have a low potential for bioaccumulation unless the pH does in fact reach the pKa2.

Under the specific conditions used to conduct a half-life study, the calculated half-life of tiamulin was 48, 52, 61 and 97 days for sand, sandy loam, clay and pH 8.0 silt loam soils, respectively.[20] In another test with soils from other sources it was found that in sand, silt loam, clay and pH 8 silt loam the half-life of tiamulin was 43, 100, 150 and 301 days, respectively.[21]

Effect on Plants

In preliminary small-scale environmental impact studies, it was shown that tiamulin did not affect the germination or subsequent development of beans or radishes watered regularly with tiamulin in the water at 5, 50 or 500 mcg/ml.[15]

Germination of lettuce, sugar beets and wheat planted in soil to which the equivalent of 50,000 l of pig slurry (excreta) containing tiamulin at 1.26 ppm was applied per hectare was normal. Plant vigor of sugar beets and wheat may have been reduced. Application of slurry at 100,000 l/ha apparently reduced germination of lettuce and sugar beets and vigor of sugar beets, but these results were confounded by the effects of the excess level of slurry application. No tiamulin activity was detected in any of the crops at either level of slurry application within assay limits of 0.05 ppm.[17]

Effects on Microorganisms

Tiamulin has a high level of activity against many gram-positive bacteria and mycoplasmas and is much less active or inactive against most gram-negative bacteria based on in vitro MIC determinations.[18] Administration of the compound to swine results in the formation of numerous metabolites none of which is as much as 10% of the dose administered.[7] None of the metabolites has as much antibacterial activity as the parent compound and most have little or none.[6,8]

Ultimate Fate of Drug

It would be expected that spilled medicated feed will mix with urine and feces and the final depository of this waste will be the soil.

Decomposition of the waste is accomplished by microorganisms under aerobic and anaerobic conditions. This takes place in the soil or prior to ultimate return to the soil if the excreta is processed

via storage pits, lagoons, oxidation ditches, etc. in which the residence time can be extensive (generally greater than two weeks).

Swine waste is ultimately spread on soil and plowed into the ground. If it is assumed that 10 ton of excreta from swine fed tiamulin is spread per acre and the soil plowed to a depth of 8 inches weighs about 2.7 million pounds, it can be calculated that the resulting tiamulin activity in that soil would be equivalent to 0.0072 mg/kg or 7.2 ppb. This amount is of no environmental concern.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

Pollution

- **Air**

  Use of DENAGARD premixes does not have any effect on air quality, nor will consumption or disposal of the product contribute to air pollution. The vapor pressure of tiamulin is such that even at 45°C the value is 1.5×10^{-7} torr. Tiamulin would not be expected to diffuse into the atmosphere.

- **Soil**

  Although approximately a total of 3,280 kg of tiamulin can be expected to require disposal because of spillage and excreted in urine and feces, a soil concentration of less than eight parts per billion would result. This amount of material will not have any effect on the soil microbial population because of the low concentration[18] and fact that the compound has a strong tendency for adsorption to soil particles.[14]

- **Water**

  Water quality should not be affected by the use of the product. Swine waste is not permitted to be discharged to waterways so there will be no direct addition of the product to the water network. Inadvertent pollution of water streams with swine wastes should not result in any significant contamination with tiamulin because of the very low concentration of tiamulin in swine waste. Tiamulin is unlikely to leach from the soil to any great extent.[14,17]

- **Solid and Liquid Wastes**

  Use of DENAGARD feed additive has a potential beneficial environmental impact by reducing the amount of Treponema-contaminated swine waste for disposal.

- Toxic Substances

Tiamulin is a potent antibiotic used in the treatment and control of swine dysentery and for the improvement of growth and feed efficiency. The toxicology is well defined and it has been shown to be safe for use as directed.

There are no known toxic substances produced by the use, consumption or disposal to tiamulin.

The use of tiamulin does not involve substances such as pesticides or heavy metals.

The occurrence of radiation is not required for any activity associated with tiamulin.

As noted, the potential amount of available tiamulin from medicated feed spillage and in animal excreta is minimal.

Populations

The action will have no direct effect upon any population. The efficacy of the drug may result in a larger swine population. The human population may be positively affected by an increased availability of pork to the consumer.

Humans

Direct contact of tiamulin with skin or mucous membranes may cause irritation. The premix label bears the warning: Avoid contact with the skin. Direct contact with skin or mucous membranes may cause irritation. However, exposure would occur only during formulation and to a much lesser degree when preparing medicated feed. Such exposure only involves an extremely small segment of the human population during formulation and an extremely small exposure during medicated feed mixing.

Subsequent to feeding tiamulin at 200 g/ton, the mean levels of total tiamulin residues in tissues are below the established safe levels in less than three days of withdrawal. Therefore, there is no potential impact from exposure to unsafe residues in swine tissues if the recommended withdrawal (7 days) time is followed.

Plants

In small-scale environmental impact studies, it was shown that tiamulin did not affect the germination of plants.[15,17] Plants do not take up tiamulin from the soil.[17]

- Animals

  - Exposure to Medicated Feed

    Medicated feed containing tiamulin is given only to swine. These animals generally would be confined in areas from which other animals are excluded. Although exposure of other animals to the medicated feed is possible, it would be very limited. The probability of other animals receiving a significant amount of tiamulin is extremely low.

    Tiamulin and most polyether ionophores, e.g., monensin, salinomycin and narasan, are known to be incompatible in animals and poultry when administered above certain dose levels.[19] Gross contamination of feed or errors in medicated feed preparation which result in simultaneous administration of tiamulin at therapeutic levels and the ionophore compounds can result in weakness, ataxia, reduced feed intake, recumbency and death of the animal or bird.

    The medicated feed which is spilled by swine in total confinement housing or which is processed via lagoons, storage pits, oxidation ditches, etc., is usually not physically available to other animals. The only source for potential exposure is that medicated feed deposited in non-confinement housing. The binding potential of tiamulin to soil is high. Therefore, little, if any, of the spilled material would be available to other animals common to the farm environment. Since the availability of tiamulin medicated feed in the non-confinement situation is also limited, the risk to scavenging birds is considered minimal.

    Should any bird or animal consume feed medicated at any approved level for use in swine it is unlikely that any adverse reaction would occur and even less likely that mortality would result. LD_{50} values determined for chickens, turkeys, pigeons and mice administered the compound orally were all over 800 mg/kg body weight.[18]

Exposure to Swine Excreta Containing Tiamulin from Feed Wastage as Well as Nonmetabolized Antibiotic

- Animals

  Animals common to the farm environment do not voluntarily consume swine feces, thus there is no direct exposure to the spilled tiamulin contained therein.

- Birds

  The only sources for potential exposure to swine feces are those deposited in non-confinement housing.

Scavenging fowl and/or wild birds are not recognized to consume feces directly. Birds have an extremely wide range of food preferences, extending from the single-item eaters to the omnivorous species such as crows, gulls and other scavengers which seem to eat almost anything organic. Based on observations and analyses of gastrointestinal contents, great detail has been given of the food eaten by common wild species; feces were not noted as a food item for any of these.

Manure collection and storage areas associated with swine operations are not known to attract birds when other sources of food are available. When food is scarce, however, some species have been observed to pick through freshly deposited feces in search of undigested grain, worms, insects, etc. Under these circumstances and with proper access to feces from treated swine, there does exist the opportunity for scavenging birds to pick up and consume spilled drug adsorbed to fecal materials. However, this amount would be minute and the microbial population in fecal material would quickly degrade the drug.

In view of the LD50 values determined for pigeons, chickens and turkeys[18] it is unlikely that any bird would be seriously effected by consumption of tiamulin found in excreta.

Human Values

Tiamulin is a drug that is used exclusively in animal agriculture. It has no current or planned use in human medicine and the consumer will not be in direct contact with tiamulin.

Food Contamination

The quality of the environment in terms of the human values regarding food contamination will not be affected by the projected uses of DENAGARD. The product is administered only to swine for the treatment and control of swine dysentery or for the improvement of growth and feed efficiency. When used according to the label directions, tiamulin will not become a component of food.

9. USES OF RESOURCES AND ENERGY:

The small amount of isopropanol not recovered and the energy used in the drying process are irreversible and irretrievable losses.

Natural Resources

No change in the use and accessibility of natural resources will result from the proposed action.

Energy

There is no direct impact on the energy supply or utilization that stems from the use of the product.

Other

There are no expected effects upon endangered or threatened species nor upon properties listed in or eligible for listing in the National Register of Historic Places.

10. MITIGATION MEASURES:

Measures to Avoid or Mitigate Potential Adverse Environmental Effects

The only potential adverse environmental consequence would result from inhalation or contact with the skin. In order to avoid or mitigate these adverse effects, the labels bear the warning: Avoid contact with the skin. Direct contact with skin or mucous membranes may cause irritation.

11. ALTERNATIVES TO THE PROPOSED ACTION:

No potential adverse impacts have been identified for the proposed action. Economic benefits to swine producers and to consumers of pork products can result from use of the drug in feed for the treatment of swine dysentery.

12. LIST OF PREPARERS:

Marlin D. Anderson
Director of Feed Additive Development
Qualifications:
Twenty-eight years experience in animal health research and product development.
MS, Ph.D. Animal Nutrition

Robert T. Rossi
Manager, Health/Safety/Equipment
Qualifications:
Twenty-nine years experience in animal health research, product development and quality control.
BS - Chemistry
MS - Analytical Chemistry

13. CERTIFICATION:

The undersigned applicant/petitioner certifies the information furnished in this Environmental Assessment is true, accurate and complete to the best of his knowledge.

1-11-94
Date

John Higgins
Vice President, Operations
Fermenta Animal Health Company
15th & Oak Street
P. O. Box 338
Elwood, KS 66024
(913) 365-9070

APPENDIX I

MATERIAL SAFETY DATA SHEETS FOR

DENAGARD® 10 (TIAMULIN) MEDICATED PREMIX

MATERIAL SAFETY DATA SHEET

DENAGARD® 10 (TIAMULIN) MEDICATED PREMIX

Manufacturer's Name:
FERMENTA ANIMAL HEALTH COMPANY
10150 N. Executive Hills Boulevard
Kansas City, Missouri 64153

Emergency Medical Number:
(800) 530-5432 (24 hours)

Phone Number: (800) 777-7722

Transportation Information: DOT Hazard Classification: Non Hazardous Product

SECTION 1 - IDENTITY

Product Name: DENAGARD 10 (tiamulin) Medicated Premix

NADA 139-472

FDA Regulated Product

Category I Drug Type A Article

Product Use: Antibiotic feed medication for swine.

Chemical Family: Diterpenoid

SECTION 2 - INGREDIENTS

Principal Component(s) (chemical & common names(s))	Mol. Wt.	CAS No.	TLV (units)
Active Ingredient			
TIAMULIN HYDROGEN FUMARATE	609.82	55297-96-6	Not Established
14-desoxy-14-[(2-diethylaminoethyl) mercaptoacetoxy] mutilin hydrogen fumarate			
$C_{28}H_{47}NO_4S,C_4H_4O_4$			
Percentage			
2.25% (10 g/lb) in roughage product carrier			

No hazardous components are contained in this drug product. FDA regulated product. Livestock remedy for use in animal feeds only for indications on label. Observe warnings and note contraindications and cautions on label.

SECTION 3 - PHYSICAL & CHEMICAL CHARACTERISTICS (Fire & Explosion Data)

Boiling Point: N/A Specific Gravity (H_2O=1): N/A Vapor Pressure (mm Hg): N/A

Percent Volatile by Volume(%): N/A Vapor Density: N/A Evaporation Rate: N/A

Solubility in Water: Active ingredient soluble in water. Reactivity in Water: Not Reactive pH: N/A

Appearance and Odor: As finely ground roughage product.

Flash Point: N/A Flammable Limits in Air % by Volume: Lower: N/A Upper: N/A

Extinguisher Media: Any commonly used including water Auto-Ignition Temperature: N/A

Special Fire Fighting Procedures: Use extinguishing agent suitable for surrounding fire. Fire fighters should wear pressure demand self-contained breathing apparatus and impervious clothing in buildings or confined area where chemicals and drugs are stored.

Unusual Fire and Explosion Hazards: Air-borne particles may present dust explosion hazard.

SECTION 4 - PHYSICAL HAZARDS

Stability: Stable Conditions to Avoid: Moisture and high humidity; avoid excessive temperature storage; prevent other than label uses.

Incompatibility (Materials to Avoid): Incompatible with monensin, salenomycin and narasin when administered together to animals and poultry. Avoid cross contamination of feeds with these drugs.

Hazardous Decomposition Products: Not Hazardous Hazardous Polymerization: Will Not Occur

SECTION 5 - HEALTH HAZARDS

Threshold Limit Value: Not Established

Signs and Symptoms of Exposure:

Acute Oral LD_{50} (rat) 2230 mg/kg; (mouse) 710 mg/kg

Product dust may cause sneezing and/or irritation of mucus membranes; skin irritation and/or antibiotic sensitivities in some individuals.

Medical Conditions Generally Aggravated by Exposure: Skin conditions, cuts, wounds. Primary Routes of Entry: Inhalation, ingestion and skin contact.

Chemical Listed as Carcinogen or Potential Carcinogen: Non Carcinogenic
National Toxicology Program: No I.A.R.C. Monographs: No OSHA: No

OSHA Permissible Exposure Limits: None Established ACGIH Threshold Limit Used: None Established
Other Exposure Limit Used: None Established

Emergency and First Aid Procedures:

1. Inhalation: If inhaled, remove victim to fresh air. Apply artificial respiration if needed.
2. Eyes: Flush eyes with plenty of water. Get medical attention if irritation persists.
3. Skin: Wash with soap and water. Get medial attention if irritation persists.
4. Ingestion: Not for human use. If swallowed seek medical assistance.

SECTION 6 - SPECIAL PROTECTION INFORMATION

Respiratory Protection (Specify Type): If dust occurs in use, wear a NIOSH approved dust respirator.

Ventilation: To reduce dust levels. Local Exhaust: N/A

Eye Protection: No Requirement Skin Protection: No Requirement Other Protection: No Requirement

SECTION 7 - SPECIAL PRECAUTIONS AND SPILL/LEAK PROCEDURES

Precautions to be taken in Handling and Storage: Store in a dry, cool place (less than 80° F) in original container. Keep out of reach of children.
Other Precautions: Read complete package label before use. Use only as directed in label. Follow GMP procedures.
Steps to be Taken in Case Material is Released or Spilled: Sweep up. Place in container for disposal.
Waste Disposal Methods: Not a hazardous toxic waste. Dispose of waste and containers in accordance with local, state and federal regulations. Contact manufacturer for disposal of out-dated material.

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APPENDIX II

ABSTRACTS OF STUDY REPORTS

AND PUBLICATIONS REFERENCED

1. Biessels, M. Tiamulin. 10-Day treatment of pigs with ^{3}H and ^{14}C-81.723: Studies of the excretion and tissue residues after oral application. Sandoz Research Institute, G.m.b.H., A-1235 Vienna, Austria. November, 1976.

The objective of this work was to study the excretion and tissue residues of tiamulin with swine after oral dosing with dual labeled compound.

Male and female pigs weighing 11 to 22 kg were given doses of ^{14}C- and ^{3}H-tiamulin hydrogen fumarate at 5 mg/kg twice daily in feed for 10 days. Eighty-four to 104% (average 95%) of the radioactivity administered was recovered in the urine and feces. Twenty-four to 37% of the total ^{3}H-dose was recovered in the urine and 57 to 74% of the ^{3}H-dose was recovered in the feces. Excretion plateaued in both feces and urine by day 5 of treatment. Biexponential decreases in both urine and feces were observed after treatment termination with the major radioactive excretions with half-lives of about 14-16 hours.

The highest concentration of total residues in edible tissue was found in the liver. However, bile contained much higher levels than liver early in the withdrawal period. It was calculated that about 60% of the oral dose was excreted via bile in pigs equal to that found in rats and dogs in other tests. From estimated excretion through the bile and measured excretion in urine, an absorption of about 90% was obtained. It was concluded that the antibiotic was nearly completely absorbed by pigs.

It was shown that the ^{3}H label in the vinyl group was more useful than the ^{14}C label in the side chain in these studies. Tritium exchange with body water was less than 0.3%. ^{14}C activity was found in endogenous substances indicating some degradation of the side chain occurred during metabolism. Calculations indicated that after 4 to 5 days when a plateau was reached, 10% of the daily dose found in the feces and 5% of the daily dose found in the urine was accounted for by metabolites with side chain alterations.

2. Czok, R. Tiamulin (81.723 hfu). Absorption, excretion residues and metabolism in rats, dogs, pigs and chickens. Report of Sandoz Research Institute, G.m.b.H., A-1235 Vienna, Austria. November, 1976.

The objective of these studies with radiolabeled tiamulin was to learn something of absorption and excretion of the antibiotic in several species.

Pigs weighing 10 to 20 kg received 10 mg of ^{3}H-tiamulin hydrogen fumarate per kg daily for 10 days via feed in two equal portions or were administered 2 x 5.5 mg/kg daily for 5 days by gavage with the antibiotic dissolved in water. Urine and feces were collected daily and at the end of the experiment the pigs were bled and various organs and tissues were collected for total residue determinations.

Measurements of ^{3}H showed that excretion in urine and feces, as well as tissue residue levels, plateaued at 5 days. An average of 95% (16 pigs, 86-100%) of the ^{3}H radioactivity administered was found in the excreta. About 29% of the dose was recovered in the urine and about 60% reached the feces via bile. Among edible tissues, liver was shown to have the highest residue level when examined on days 2, 5, 10 and 25 after the end of medication. It was calculated that 90% of the oral dose was absorbed by pigs in these studies.

3. Schutze, E. and G. Laber. 81.723 hfu Blood level findings (pigs). Sandoz Research Institute, Ltd., A-1235 Vienna, Austria. September 1, 1977.

The purpose of this study was to determine the antibacterial activity in the blood of pigs after single oral doses of 10, 25 and 50 mg/kg body weight of tiamulin and two other antibiotics.

Five pigs weighing 12 to 15 kg were used per dose level for each drug tested. The antibiotics were dissolved in water and administered by stomach tube. Blood samples were taken from each animal before and at 0.5, 1, 2, 4, 8, 12 and 24 hours after dosing. Serum was assayed for antibacterial activity by microbiological methods. Tiamulin hydrogen fumarate (81.723 hfu) activity was determined with *Staphylococcus aureus* ATCC 29,067 as the assay organism.

Peak blood activity levels due to tiamulin were reached after 2 to 4 hours. Detected levels were clearly dose dependent.

4. Battig, F. A. Tiamulin. Isolation and structure determination of metabolites from rat bile after daily administration of 50 mg/kg over 6 days. Sandoz Forschungsinstitut, Vienna, Austria. October 13, 1981. (Document number 399-AHD-82-3001-001).

The goal of this study was to elucidate the structure of "major" metabolites eliminated in rat bile after application of ^{3}H-tiamulin to male and female Sprague Dawley rats. This work was part of a project to compare major biotransformation pathways in swine the target species and rats used in toxicity testing.

Male ($n = 7$) and female ($n = 6$) rats were fitted with bile duct cannulas and dosed orally with approximately 50 mg ^{3}H-tiamulin hydrogen fumarate daily for 6 days. Bile was collected continuously until 24 hours after the last dose. An average of 59% of the radioactivity administered was recovered in the bile. No important differences were found between males and females. Less than 0.5% of the parent compound was recovered in the bile.

The metabolites were concentrated and prepurified by filtration and extraction then isolated and purified by preparative reverse phase HPLC.

More than 40 different metabolites occurred in the bile, none of which represented a major portion. The structure of 10 metabolites contained in the "basic" and "neutral" extraction fractions were determined from MS and NMR spectra.

It was concluded that the major pathways of biotransformation appeared to be the same in the rat and swine. These were: N-dealkylation, monohydroxylation, epoxidation and conjugation.

5. Battig, F. A. Tiamulin. Isolation and structure determination of metabolites from rat urine after daily administration of 60 mg/kg over 8 days. Sandoz Foschungsinstitut, Vienna, Austria. October 15, 1981. (Document number 399-AHD-82-3002-001)

The purpose of this study was the elucidation of the structure of "major" metabolites eliminated in rat urine after oral application of ^{3}H-tiamulin to male and female rats. This study was part of a project to compare major biotransformation pathways in swine, the target species, and rats used in toxicity testing.

Twenty male and 20 female Sprague Dawley rats in metabolism cages were dosed orally with 10 mg ^{3}H-tiamulin hydrogen fumarate daily for 8 consecutive days. Doses approximated 63 mg/kg for male and 55 mg/kg for female rats. Urine was collected continuously during dosing and for 24 hours after the end of dosing. The urine was pooled, filtered, extracted and partitioned with several solvents at various pH levels. Metabolite isolation was accomplished with preparative thin layer chromatography and reverse phase HPLC. Metabolite structures were deduced from proton magnetic resonance spectra and from high resolution mass spectra.

About 15% of the radioactivity administered was recovered in the urine. The portion of unmetabolized parent drug in the urine was less than 1% of the total radioactivity.

The "basic" extract urine fraction contained more than 60% of the ^{3}H-activity found in the urine and contained 3 major metabolites. These three metabolites represented 25-50% of the ^{3}H-activity in urine. Two metabolites represented 10-20% of the radioactivity in urine and one other isolate 5-7%. Since about 15% of the oral dose was excreted in the urine, these major metabolites represent 1.5 to 3 and 1 to 1.5% of the oral dose administered. Each of the 3 metabolites had less than 0.3% of the activity of the parent compound, tiamulin hydrogen fumarate.

6. Czok, R. Tiamulin. Isolation and structure determination of metabolites from swine urine after daily administration of 2 x 5 mg/kg over 10 days. Sandoz Forschunginstitut, Gesellschaft m.b.H., A-1235 Vienna, Austria. August 7, 1981.

The purpose of this study was to isolate and determine the structure of tiamulin metabolites found in swine urine resulting from administration of a therapeutic dose daily for 10 days.

Urine was collected from two pigs administered dual labeled (^{3}H and ^{14}C) tiamulin hydrogen fumarate via feed. After adsorption to Amberlite XAD-2 and desorption, the urinary metabolites were partitioned between butyl acetate and alkaline or acid aqueous solutions. In this way groups of acid, alkaline or neutral metabolites were obtained which were then purified by preparative chromatography. The almost pure compounds were analyzed by nuclear magnetic resonance or mass spectroscopy. Data were interpreted with respect to the most likely metabolite structure.

The identification of the pattern peaks was performed by isolation of the corresponding metabolites and by co-chromatography of identified metabolites with urine extracts.

Fourteen of at least 19 metabolites were identified accounting for 83% of the total urinary radioactivity. Only about 0.3% of the total radioactivity was excreted in the urine as parent compound.

In this study the acidic, basic, neutral and nonextractable fractions of radiolabeled urinary metabolites comprised 50, 19.6, 8.4 and 21% of the total radioactivity found in the urine.

It appears that monodethylation of tiamulin results in 30% loss of activity, any hydroxylation results in 95% loss of activity and oxidation to carboxylic acid results in loss of 99% of the activity. There was evidence that sulfoxidation could occur.

7. Tiamulin metabolism in swine: Identification of major metabolites and comparison with the rat. Document number 440-4BE-80-0233-001. Department of Safety Assessment, Diamond Shamrock Corporation., Painesville, Ohio. June 17, 1981.

The purpose of this work was to study the metabolism of tiamulin hydrogen fumarate in swine, to determine metabolic profiles in bile and urine, to make comparisons with profiles in rats and to identify major metabolites.

Two pigs, each with a bile duct cannula, were dosed orally with ^{3}H-tiamulin hydrogen fumarate at approximately 10 mg/kg daily for five days. Urine was collected continuously throughout the dosing period. Bile was collected for a maximum of 15 minutes per hour during the first 8 hours of dosing and for 15 minutes each 16 and 24 hours after dosing. Metabolic profiles were generated from urine after filtration and from bile after fractionation into acidic, basic, neutral and non-extractable portions.

Rats were administered ^{3}H-tiamulin hydrogen fumarate via duodenal cannula as a single dose of approximately 30 mg/kg. Urine and bile were collected continuously for 24 hours after dosing. Metabolic profiles were generated from urine following filtration and from bile after fractionation into acidic, basic, neutral and non-extractable portions using the same methods as for swine.

Metabolic profiles were generated by HPLC. Co-chromatography of samples from these animals with metabolic extracts containing known radiolabeled metabolites from other studies provided identification of major metabolites.

Approximately 14 metabolites were identified in swine urine. None of the metabolites in urine exceeded 6% of the total ^{3}H-tiamulin dose administered. Parent compound, i.e., tiamulin, in swine urine was found to be only 0.32% of the dose administered.

Swine bile contained the same 14 metabolites as swine urine plus two additional metabolites for a total of at least 16. No metabolite in swine bile represented more than 7% of the administered dose.

One metabolite appeared in swine bile but not in the rat represented less than 0.9% of the administered dose in this trial.

It was concluded that swine, the target species, and rat, the species used for toxicity testing, metabolize tiamulin in a qualitatively similar manner.

8. Navarini, I. Determination of the antimicrobial activity of pleuromutilin derivatives formed by the biotransformation of substance 81,723 hfu (tiamulin). Sandoz report number 411. December 13, 1977.

The object of this study was to determine the antibacterial activity of a large number of tiamulin metabolites isolated from the urine and bile of rats and/or pigs.

A plate diffusion test with *Sarcina lutea* as the test organism was utilized to determine the relative bioactivity (antimicrobial activity) of tiamulin metabolites isolated from urine and bile of rats and/or pigs. Twenty-three metabolites, some with unconfirmed structures, were tested. None of the metabolites were as active as the parent compound, tiamulin hydrogen fumarate.

Monodealkylation at the nitrogen in the side chain decreased antimicrobial activity by 30-40% compared to tiamulin. Hydroxylation of otherwise unchanged tiamulin, further degradation of the side chain or additional hydroxylation leads to further decreases or a complete loss of antimicrobial activity of the isolated metabolite. All metabolites tested except for the one previously mentioned had only 3.3% or less of the activity (w/w) of the parent compound and the majority had no measurable activity in this test system.

9. Comparative metabolism of tiamulin between swine liver and rat bile and urine. Document number 371-4TR-82-0043-001. Department of Safety Assessment. Diamond Shamrock Corporation, Painesville, Ohio. August 23, 1982.

The metabolism of tiamulin in swine was investigated by a comparative metabolic study between swine liver and rat urine and bile. Identical methodology and conditions were used to provide a direct comparison of metabolic profiles between the liver of swine, the target species, and bile of rats, the test species for chronic toxicity testing.

Pigs were dosed with ^{3}H-tiamulin hydrogen fumarate at 10 mg/kg by oral gavage daily for 5 consecutive days. Livers from pigs killed 4.5, 24 and 72 hours after the last dose were examined after methanol extraction and fractionation into acidic, basic, neutral and non-extractable portions. The non-extractable fraction was subjected to enzyme digestion before examination.

Bile and urine were collected from rats for 24 hours after dosing with ^{3}H-tiamulin hydrogen fumarate at about 30 mg/kg. Rat bile was fractionated in the same manner as swine liver.

Fractions were examined as HPLC profiles then compared. Known radioactive standards from swine urine extracts, rat bile fractions, swine bile basic fraction and tiamulin base from previous studies were used for co-chromatography purposes of identification of major HPLC generated profile peaks.

In the acidic fraction of swine liver at 4.5 and 24 hours, all major peaks were identified as also being present in rat bile. None of the 7 major peaks (metabolites) represented more than 5.5% of the total residues (^{3}H) present in the liver. At 72 hours some minor unidentified peaks were present but none accounted for as much as 1% of the total residues.

At 4.5 hours there was present in the swine liver basic fraction an unknown peak approximately 5% of the total residue. This metabolite, not seen in rat bile or urine, decreased to 0.61% of the total swine liver residue by 24 hours and to about 0.3% at 72 hours. Some swine liver metabolites at 4.5 and 24 hours may not have been present in rat bile but they represented less than 2% of the total liver residues at these times and less than 1% of the total residues at 72 hours withdrawal.

Most of the metabolites found in the neutral fractions of the three swine liver samples were also found in the rat bile neutral fraction. None of the few unidentified metabolites in swine liver not found in rat bile or urine accounted for as much as 5% of the total liver residue present.

Examination of non-extractable fractions of swine liver after enzymic digestion (β-glucuronidase and arylsulfatase) showed that the main peaks present were also present in the rat bile non-extractable fraction after enzyme treatment. None of the metabolite peaks observed, known or unknown, represented more than about 4% of the total residues in the liver and most were considerably less.

HPLC analyses of the 4 fractions of swine liver represented 93.5, 73.0 and 71.7% of the total liver residue from the 4.5, 24 and 72 hour samples, respectively, in this study. None of the metabolites found in swine liver which were not also present in rat bile accounted for more than 5-6% of the total residues at any time. After 72 hours of withdrawal none of these metabolites comprised 1% of the total residues and it was calculated that none was present in excess of 10 ppb.

It was concluded that the metabolism of tiamulin in pigs and rats is qualitatively similar.

10. Metabolism of tiamulin in swine liver. Document number 371-4TR-81-0234-001. Department of Safety Assessment. Diamond Shamrock Corporation, Painesville, Ohio. January 25, 1982.

This study was conducted to determine if a unique swine biliary metabolite which did not occur in the rat was present in swine liver and, if present, to what extent.

Pigs were administered ^{3}H-tiamulin hydrogen fumarate at 10 mg/kg/day for 5 consecutive days by gavage. Liver samples were collected 4.5, 24, 48, 72, 96 and 120 hours after the last treatment. Liver samples were extracted and metabolic profiles compared with the swine bile basic fraction profile which contained the unique metabolite, designated metabolite #3.

Metabolite #3 was found in liver tissue at 0.5, 2.6, 3.4, 7.8, 9.6 and 6.1% of the total (^{3}H) residues in samples taken 4.5, 24, 48, 72, 96 and 120 hours post treatment, respectively. As this metabolite was never found to be as much as 10% of the total liver residues it was considered a minor component.

11. Rogers, S. and S. Stroh. Residue depletion study in swine fed tiamulin hydrogen fumarate at 200 g/ton. Project number 2223. Fermenta Animal Health Company. November 15, 1991.

Twenty crossbred pigs, 10 gilts and 10 barrows, were fed tiamulin hydrogen fumarate in feed at 200 g/ton for 14 days. Livers were collected from 2 gilts and 2 barrows at 12, 24, 48, 72 and 96 hours after the end of medication and analyzed for the marker substance, 8-hydroxymutilin, by an approved regulatory method.

Group mean marker metabolite concentrations were less than the tolerance of 400 ppb at 48 hours and beyond. At 96 hours the mean concentration was one-half the tolerance.

Statistical analyses demonstrated a calculated withdrawal time of 6.6 days.

12. Analyses of tiamulin hydrogen fumarate. R. S. DePablo. Diamond Shamrock Research Department. January 26, 1981.

The vapor pressure of tiamulin hydrogen fumarate was calculated by extrapolation of vapor pressure values determined at 100, 111 and 115°C by an effusion procedure (J. Chem. Eng. Data 21:141 1976).

Temperature (°C)	Vapor Pressure (Torr)
100.0	2.0×10^{-5}
111.0	4.5×10^{-5}
115.0	6.0×10^{-5}

The above values were used to calculate the vapor pressure at 25°, 35° and 45°C using the following equation:

$$\log_{10} P(\text{Torr}) = 7.6 \pm 0.65 - \frac{4607 \pm 250}{T}$$

Calculated values are listed below.

Temperature (°C)	Vapor Pressure (Torr)
25	0.15×10^{-7}
35	0.49×10^{-7}
45	1.50×10^{-7}

13. Salmon, J. R. Tiamutilin (SQ 22,947) An analytical profile. Squibb IDL Analytical Services report IDL/AP3. December 15, 1975.

The object of this report was primarily to provide quantitative and qualitative information on SQ 22,947 (tiamulin hydrogen fumarate) which would characterize the compound.

Included in the information provided were the following: Appearance, color, odor, IR, UV, NMR and mass spectra, optical rotation, melting point, solubility in various liquids, ionization constants, partition coefficients, crystal properties density, chromatographic characteristics and the like.

The ultraviolet absorption spectrum of tiamulin in aqueous solution was determined. An absorbance peak was found at 202.5 nm. Tiamulin absorbs light in the spectral region of 200 to 280 nm and absorbs little or no light in the wavelength region greater than 280 nm.

Solubility in water has been described as indicated below.

10°C	4.2% W/V
20°C	5.8% W/V
30°C	8.5% W/V
40°C	20.5% W/V

Ionization constants were determined to be:

pK_1	$4.31 \pm .03$
pK_2	$7.64 \pm .03$

Partition into *n*-octanol from aqueous buffers is shown below.

pH	Concentration (w/v)[1]	Partition Coefficient
4.1	0.1%	1.05
5.0	0.1%	1.87
6.0	0.1%	7.98
7.0	0.1%	126.25
4.1	1.0%	.90
5.0	1.0%	3.22
6.0	1.0%	26.73
7.0	1.0%	175.14

[1]Initial concentration in aqueous solution.

14. Adsorption and desorption of tiamulin. Document number 456-3EF-80-0187-002. Department of Safety Assessment. Diamond Shamrock Corporation, Painesville, Ohio. December, 1981.

This report describes work done by a contract laboratory, Raltech Scientific Services, Madison, Wisconsin, on behalf of the drug sponsor, to study the adsorption of tiamulin onto and the desorption of tiamulin from soil.

Clay, loamy sand, silt loam and a high pH (8.0) silt loam soils, characterized by the University of Wisconsin Soil & Plant Analysis Laboratory, Madison, Wisconsin were used in the studies. The soils tested ranged in pH from 5.7 to 8.0; 0.52 to 4.79 in percent organic matter; 5.36 to 32.09 me/100 g exchangable cations (K, Mg, Ca); 10 to 87% sand, 10 to 71% silt and 5 to 41% clay. Tiamulin hydrogen fumarate was quantitated by use of radiolabeled (^3H) material in a 0.01 M calcium nitrate solution at approximately 0.01, 0.1, 1 and 10 ppm.

With three of the soils, approximately 95% of the tiamulin was adsorbed within 1 hour at each of the four concentrations. For the sandy soil with low clay and organic matter content, approximately 60% of the tiamulin in solution was absorbed; 24 hours were required to establish equilibrium. The adsorption constants (K) were 88, 75, 74 and 8 for the pH 8 silt loam, clay, silt loam and loamy sand soils, respectively. The adsorption constant (K) for each soil is related more to clay content than to organic matter content of the soils.

The adsorbed tiamulin was desorbed only to a slight extent. Approximately 1% of the adsorbed material was removed with each solvent dilution for the three strongly adsorbent soils. For the sandy soil, approximately 10% of the adsorbed tiamulin was removed with each solvent dilution.

The data show that the binding potential of tiamulin is high and indicate that soil mobility is low. Based on these adsorption and desorption properties, tiamulin would not be expected to leach significantly.

15. Cosgrove, R. F. Tiamulin: Preliminary, small scale environmental impact studies. Report number IDL/MR/80. Squibb Research and Development. May 16, 1979.

Laboratory studies were conducted to evaluate tiamulin hydrogen fumarate for (1) stability in pond water and pig feces, (2) phytotoxicity and (3) fish toxicity.

(1) Stability studies

Tiamulin hydrogen fumarate was added to pig feces at 30 mcg/g and stored at 4°C, room temperature and 37°C. Loss of activity after 3 weeks storage was not greater than 20% and after 2 months at room temperature and 37°C was no greater than 35%. Loss at 4°C after 2 months was 10-12%.

Pond water containing a variety of organisms such as algae, protozoa, water fleas, desmids, diatoms, etc. and distilled water were both spiked with tiamulin hydrogen fumarate at 600 and at 2.4 mcg/mL and stored at room temperature and at 37°C.

After 90 days of storage there was no significant loss of tiamulin activity at either concentration in distilled water. In pond water the 600 mcg/ml solution lost 10% of its activity after 2 weeks at 37°C and 40-50% of its activity after 10 weeks. No loss was detected at room temperature. The pond water spiked at 2.4 mcg/ml lost 30% of its activity by 2 weeks at 37°C and at room temperature lost 20% at 3 weeks, more than 70% at 7 weeks and more than 80% at 9 weeks.

Doubling dilutions of tiamulin were prepared with pond water and aliquots were examined after 24 hours and one week storage at room temperature. At the highest concentration used, 500 mcg/ml, the numbers and motility of the flora and fauna in pond water were not significantly different from controls as judged by microscopic examination.

(2) Fish toxicity

One group of 5 goldfish were used as controls and one group of 5 were subjected to increasing amounts of tiamulin at 24 hour intervals starting at 2.5 mcg/ml. All fish survived the tiamulin until the 160 mcg/ml level was reached when, after 24 hours, 3 of 5 fish died. The remaining two did not eat or move much, but they appeared to recover completely within 24 hours after removal to nonmedicated water.

(3) Phytotoxicity studies

Radish (French breakfast variety) seeds were sown in plastic containers and watered with 100 ml of water containing tiamulin hydrogen fumarate at 0, 5, 50 and 500 mcg/ml once every 4 days. Seedlings appeared in all groups at 3 days and at test end at 6 weeks, the weight of the radish and of the total

plants were not markedly different. There were no apparent dose related trends.

Dwarf French beans were sown in trays and given 250 ml of water containing tiamulin hydrogen fumarate at 0, 5, 50 or 500 mcg/ml every 3 days. Germination time and plant development were not different to the time of termination at 22 days.

16. Bullock, G. L. and R. L. Herman. 1988. Effects of the antimicrobic tiamulin on seven gram-negative bacterial fish pathogens. Journal of Wildlife Diseases 24:22-24.

In vitro and in vivo tests were carried out on 51 strains of seven species of gram-negative bacterial pathogens of fish. The test organism and number of strains with MIC (ppm) of 6.25 or less over the number tested are shown below.

Aeromonas hydrophila	0/6
Aeromonas salmonicida	1/12
Edwardsiella ictaluri	1/5
Edwardsiella tarda	0/4
Pseudomonas fluorescens	0/6
Vibrio anguillarum	5/5
Yersina ruckeri	0/13

In vivo tests of tiamulin hydrogen fumarate for 14 days at 5 and 50 mg/kg in the diet to rainbow trout infected with *Y. ruckeri* had no apparent effect on mortality due to the disease. No signs of drug toxicity were observed.

It could be concluded that tiamulin has little, if any, effect on the gram-negative fish pathogens tested.

17 Roberts, N. L. and D. M. Cameron. Phytotoxic effects and crop residues of tiamulin derived from slurry. Study 65B/82916. Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. October 22, 1982.

The study was designed as a screening test to determine any phytotoxic effects following application of slurry from pigs receiving tiamulin in the diet and to measure tiamulin residues in soil and crops produced. The crops used in the study were lettuce, sugar beets and wheat. The test was conducted in England.

Three gilts and 3 barrows approximately 12 weeks of age were fed 1.2 to 1.6 kg of feed daily for a total of 28 days. Four of the 6 pigs were fed tiamulin hydrogen fumarate at 30 ppm; two pigs were fed nonmedicated feed (controls). Over the last 7 days of the 28-day period the slurry (excreta) produced by each group was collected and held at ambient temperature in bulk containers until required.

Seventeen days after the end of slurry collection, slurry from medicated and nonmedicated pigs was used to treat soil in seed boxes at a level equivalent to (*n*) 50,000 l/ha incorporated to a depth of 10 cm (considered to be the upper normal maximum limit of application) and also at twice this level (2*n*) equivalent to 100,000 l/ha. The boxes were immediately sown with lettuce, sugar beets and wheat. There were three replications of each crop in control and treated soils at each level of application with a total of 36 boxes. Each box was planted with 100 seeds. Additional boxes were treated with slurry at each application rate but not planted for use in collecting samples of soil, slurry and leached water for residue analysis. Analysis for tiamulin was by microbiological methods.

Slurry from treated pigs was found to contain 1.26 mcg tiamulin-like activity per gram. Control slurry had no detectable activity (i.e., <0.1 mcg/ml).

Low amounts of tiamulin (near the limit of detection 0.05 mcg/g) occurred at all sampling points in soil sampled after the high level (2*n*) of application when assayed at 0, 7, 14, 21 and 28 days after application. Actual values were 0.09 to 0.07 mcg/g. Similar amounts (.08 mcg/g) were found in samples taken at 21 and 28 days after slurry application at the lower level (*n*). No activity was ever found in leached water from the soil samples. When crops (whole plant) were assayed for activity on day 28, none was found within assay limits of <.05 mcg/g.

Germination rate

No adverse effects of treatment or percentage germination were noted in any of the crops tested at the normal application rate (*n*) or in wheat at twice the normal rate (2*n*). Mean germinatin for lettuce at 2*n* was lower in the treated group than the controls and was mainly associated with one replication with the values for the other two replications comparable to control values. There was some indication of a slight reduction in the germination of sugar beets in 2 of 3 treated replications at (2*n*) but this was perhaps confounded by the overall reduction observed at 2*n* in both the control and treated groups compared with application at the normal rate (*n*).

Plant vigor

Plant vigor was subjectively estimated on a scale of 0 to 10 with 10 being the most desirable. Scores for lettuce and sugar beet were generally lower in both treated and control groups at $2n$ vs. the n level of application. Sugar beet plant vigor was apparently reduced at both application rates. At n, there was some indication of a treatment effect in wheat. The $2n$ level control application itself was detrimental indicating over fertilization.

18. Drews, J., A. Georgopoulos, G. Laber, E. Schutze and J. Unger. 1975. Antimicrobial Activities of 81.723 hfu, a new pleuromutilin derivative. Antimicrobial Agents and Chemotherapy 7:507-516.

Work conducted at Sandoz Forschungsinstitut at Vienna, Austria to study the *in vitro* activity of 81.723 hfu (tiamulin hydrogen fumarate) is described.

Tiamulin was found to be extremely active against many gram-positive organisms such as streptococci, staphylococci and against mycoplasmas. A few *Shigella, Klebsiella* and *Escherichia coli* strains were susceptible, but other gram-negative organisms like *Pseudomonas aeruginosa, Proteus* species and *Alcaligenes faecalis* were naturally resistant. Bacteriocidal effects were observed at 50- to 100-fold higher concentrations than bacteriostatic MIC's.

Peroral LD_{50} values for mice (female) was 841 mg/kg, chicken (male) 1,860 mg/kg, pigeon (male) 812 mg/kg and turkey (male and female) was 1,345 mg/kg.

19. Miller, D. J. S., J. J. O'Connor and N. L. Roberts. 1986. Tiamulin/salinomycin interactions in pigs. Veterinary Record 118:73-75.

In a trial conducted in England, pigs 12 weeks of age were administered tiamulin in feed at 30 and 120 ppm, in water at 60 ppm and by intramuscular injection at 15 mg/kg. Some pigs were also given salinomycin concurrently in feed at 80 ppm. Pigs which were given therapeutic levels of tiamulin (120 ppm in feed, 60 ppm in water, 15 mg/kg IM) concurrently with salinomycin at 80 ppm in feed developed signs of toxicity resembling ionophore toxicity. Pigs fed tiamulin at 30 ppm together with salinomycin at 80 ppm and pigs fed salinomycin alone at 80 ppm or treated with tiamulin alone at any level did not show signs of toxicity. Tiamulin-salinomycin incompatibility was considered to be dose related.

20. Half-life of tiamulin in soils. Document number 456-4EF-81-0220-001. Department of Safety Assessment, Diamond Shamrock Corporation, Painesville, Ohio. October, 1982.

This study was conducted to determine the half-life of tiamulin in soils. This report was an interim report.

Sand, sandy loam, silty clay loam and silt loam (pH 7.9) originating at Naples, Florida, Perry, Ohio, Painesville, Ohio and Red River Valley, North Dakota, respectively, were characterized by commercial laboratories. Agvise, Northwood North Dakota determined the properties of the silt loam (pH 7.9); United States Testing Company, Memphis, Tennessee characterized the remaining three soil types.

Radiolabeled tiamulin hydrogen fumarate (^{3}H) was added to each soil sample (about 1 ppm) and the soil adjusted to 75% field moisture capacity. The soil samples were stored under aerobic conditions at 22°C in the dark with distilled water added weekly to maintain moisture content. Samples of each soil type were taken at 0, 14, 30, 60 and 90 days after application of the test material, extracted with methanol and the methanol extract and the post-extraction solids were dried.

Extracts were analyzed by isotopic dilution with unlabeled tiamulin followed by HPLC analysis. All rate calculations were based on the average tiamulin levels in replicate samples for day 0, 14, 30, 60 and 90 samples. Rate constants were calculated using least squares regression analyses and half-life values were then calculated.

Results of this study gave calculated half-lives of 48, 52, 61 and 97 days for tiamulin in sand, sandy loam, silty clay loam and silt loam (pH 7.9) soils, respectively. Tiamulin soil degradation followed first order kinetics. HPLC analyses showed ^{3}H-tiamulin degradation products to be much more polar than ^{3}H-tiamulin.

21. Half-life of tiamulin in soils. Document number 456-3EF-80-0188-002. Department of Safety Assessment. Diamond Shamrock Corporation, Painesville, Ohio. January, 1982.

This study was conducted to determine the half-life of ^{3}H-tiamulin in four soil types. The experimental portion was conducted by Hazelton Raltech, Inc., Madison, Wisconsin.

The four soil types used were Ontonogan clay, Plainfield loamy sand, Elburn silt loam and Wheatville silt loam. The properties of these soils were determined by the University of Wisconsin Soil and Plant Analysis Laboratory. The samples of soil types were obtained in Minnesota, Michigan, Wisconsin and Illinois and ranged in pH from 5.7 to 8.0, in organic matter from .52 to 4.79% and in exchangeable cations (K, Ca, Mg) of 5.36 to 32.09 me/100 g.

^{3}H-tiamulin was dissolved in 95% ethanol and applied to the inside wall of glass jars into which 300 g of soil was added. After 3 hours of tumbling, 90% of the radiolabeled compound was transferred into the soil which was then divided into 40 g portions and placed into foil covered glass jars for storage. The stored samples were adjusted and maintained at a moisture content of 75% of 1/3 bar and stored at room temperature in the dark.

At 0, 7, 14, 21, 30, 60 and 90 days, samples of the soils were dried and a portion oxidized for determination of total ^{3}H. Another portion was extracted eventually into an acetone extract and subjected to thin layer chromatography and bioautography.

The half-life for tiamulin in soils was calculated from the 0, 60 and 90 day data. Under the conditions of this study, the calculated half-life in sand, silt loam, clay and pH 8 soils was determined to be 43, 100, 150 and 301 days, respectively.

Journal of Wildlife Diseases, 24(1), 1988, pp. 22–24

EFFECTS OF THE ANTIMICROBIC TIAMULIN ON SEVEN GRAM-NEGATIVE BACTERIAL FISH PATHOGENS

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ABSTRACT: In vitro and in vivo tests were carried out with tiamulin and gram-negative bacterial pathogens of fish. Determination of minimum inhibitory concentration for 51 strains of seven species of gram-negative bacterial pathogens showed that only strains of *Vibrio anguillarum* were sensitive at 1.6–6.25 ppm, while the rest of test strains required 25–>100 ppm. Control of infection was not achieved when tiamulin was fed for 14 days at 5 or 50 mg/kg to rainbow trout (*Salmo gairdneri*) experimentally infected with *Yersinia ruckeri*.

Key words: Tiamulin, gram-negative pathogens, disease, fish, antibacterial, experimental testing, pharmacology.

INTRODUCTION

The in vitro and in vivo testing by Bosse and Post (1983) of 15 antibacterial drugs for control of enteric redmouth disease indicated that two were the most effective: tiamulin (a semi-synthetic derivative of pleuromulin produced by a basidiomycete) and Tribrissen® (a potentiated sulfonamide). We undertook further testing of tiamulin as part of the research of the U.S. Fish and Wildlife Service in support of registration of chemicals and antibacterials for use in controlling diseases in cultured food fish species. We did not include Tribrissen® because another potentiated sulfonamide, Romet has been registered recently with the Food and Drug Administration for treatment of fish furunculosis, and extension of the registration to include enteric redmouth disease is anticipated.

MATERIALS AND METHODS

The minimum inhibitory concentration (MIC) of tiamulin was determined for several strains of each of seven gram-negative bacterial fish pathogens: *Yersinia ruckeri* (13 strains), *Aeromonas salmonicida* (12 strains), *A. hydrophila* (six strains), *Pseudomonas fluorescens* (six strains), *Edwardsiella tarda* (four strains), *E. ictaluri* (five strains), and *Vibrio anguillarum* (five strains). All test cultures were from the culture collection of this laboratory and were isolated from diseased fish. Test concentrations of tiamulin were prepared by dissolving 0.2 g of 100% active tiamulin (SDS Biotech Corporation, Painesville, Ohio 44077, USA) in 20 ml deionized water. The resulting 10,000 ppm stock solution was filtered through a membrane of 0.45 µm mean porosity (Millipore Corporation, Bedford, Massachusetts 01730, USA) and diluted in Mueller Hinton broth (Difco Laboratories, Detroit, Michigan 48232, USA) to provide final concentrations of 100, 25, 6.25, 1.6, 0.4, 0.1, and 0.025 ppm. The test concentrations were prepared in sterile 100-ml flasks and 5-ml portions were aseptically distributed to sterile test tubes (16 × 125 mm). All cultures were grown in Mueller Hinton broth for 24 hr at 25 C; they were diluted 1:100 in the broth and 0.1 ml of this diluted culture was used to inoculate each test concentration of tiamulin, as well as a tube of the broth (to verify viability of inocula). All tubes were incubated at 25 C and growth was recorded at 24 and 48 hr.

An in vivo trial was conducted to assess the potency of tiamulin in controlling disease in rainbow trout (*Salmo gairdneri*) experimentally infected with *Y. ruckeri*. We transferred about 60 (500 g) rainbow trout fingerlings that were raised at this laboratory (mean weight 2–3 g) to each of 12 tanks (38 liters) supplied with spring-water at 12.5 C, delivered at the rate of 1 liter/min. The trout were challenged for 60 sec in 2 liters of a 24-hr brain heart infusion broth (Difco Laboratories, Detroit, Michigan 48232, USA) culture of *Y. ruckeri*. Water was drained immediately before addition of the culture and the flow was resumed after challenge. Sixty trout in triplicate tanks were fed a diet containing 5 or 50 mg/kg tiamulin or 50 mg/kg Romet (Hoffman LaRoche Company, Inc., Nutley, New Jersey 07110, USA). Trout in triplicate tanks, fed a nonmedicated diet, were the controls. Vegetable oil (Procter and Gamble, Cincinnati, Ohio 45202, USA) was used as a binder (Bullock et al., 1983) to coat tiamulin and Romet onto the pelleted trout feed (Federal formula CR-6). The medicated diets were fed twice daily, beginning

TABLE 1. Minimum inhibitory concentration of tiamulin for seven gram-negative fish pathogens.

Test organism	Number of test strains	ppm Tiamulin				
		>100	100	25	6.25	1.6
Aeromonas hydrophila	6	3*	3	0	0	0
Aeromonas salmonicida	12	0	6	5	1	0
Edwardsiella ictaluri	5	0	2	2	1	0
Edwardsiella tarda	4	0	2	2	0	0
Pseudomonas fluorescens	6	5	1	0	0	0
Vibrio anguillarum	5	0	0	0	4	1
Yersinia ruckeri	13	5	7	1	0	0

*Number of strains showing inhibition.

2 days before the challenge and continuing for a total of 14 days. Mortality was recorded daily for 21 days after the exposure, and we confirmed the presence of *Y. ruckeri* in representative samples of the fish that died by using a direct fluorescent antibody test (Bullock et al., 1980).

RESULTS AND DISCUSSION

Of the seven species of bacteria tested, six showed a rather uniform resistance to tiamulin, but strains of *Vibrio anguillarum* were sensitive at an MIC range of 1.6–6.25 ppm (Table 1). In the 51 strains of the seven species, the MIC was >100 ppm in 13 (25%); 100 ppm in 21 (41%); 25 ppm in 10 (20%); 6.25 ppm in six (12%); and 1.6 ppm in one (2%).

Regardless of the dosage rate and the fact that the feeding of tiamulin was begun 2 days before trout were challenged, mortality in the treated and control groups was essentially the same. Mortality from the replicates 21 days postexposure was 60, 47, and 27 (134 of 200 or 67%) in the 5 mg/kg group; 45, 44, and 20 (109 of 165 or 66%) in the 50 mg/kg group; and 52, 27, and 45 (124 of 198 or 63%) in nonmedicated controls. There was no mortality in trout fed the 50 mg/kg dosage of Romet for 5 days. Although mortality varied among replicates in the tiamulin treatment groups and nonmedicated controls, total mortality in these groups did not differ significantly (chi-square = 1.73, df = 3, $P = 0.6$).

Thus, our results failed to confirm the finding of Bosse and Post (1983) that tiamulin controlled enteric redmouth disease. Only one of the 12 *Y. ruckeri* cultures was slightly sensitive to tiamulin (>6.25 ppm); MIC's in the remaining cultures ranged from 25 to >100 ppm. Similar results were obtained by DeGrandis and Stevenson (1985), who reported a range in MIC of 16–128 ppm for *Y. ruckeri*.

Results from the laboratory in vivo test supported the in vitro sensitivity studies. When tiamulin was fed for 14 days at the recommended daily level of 5 or 50 mg/kg, 10 times the level recommended by Bosse and Post (1983), the mortality was no different from that in nonmedicated controls. There was no mortality in the rainbow trout that received a 5-day treatment of Romet.

The in vitro sensitivities of test strains of *A. salmonicida*, *A. hydrophila*, *E. tarda*, *P. fluorescens*, and *E. ictaluri* indicated that tiamulin would probably not be effective in controlling epizootics caused by these pathogens. In 24 of the 26 strains, the MIC was >6.25 ppm.

The in vitro and in vivo tests strongly indicated that tiamulin has limited (if any) use in treating epizootics in fish caused by gram-negative pathogens. These results are consistent with information listed in the Merck Veterinary Manual (Fraser, 1986), which indicates that tiamulin is active mainly against gram-positive bacteria.

ACKNOWLEDGMENTS

We thank Darlene Bowling for technical assistance during the study, and the SDS Biotech

Corporation, Painesville, Ohio for supplying tiamulin. Use of trade names does not imply U.S. Government endorsement of commercial products.

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Received for publication 15 April 1987.