ENVIRONMENTAL ASSESSMENT

1. April, 1992

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4. DESCRIPTION OF PROPOSED ACTION

The proposed action is the approval of a Supplemental New Animal Drug Application. NADA 128-686 is presently approved for use of salinomycin continuously at 40-60 g/ton in poultry feeds for the prevention of coccidiosis in broiler chickens caused by Eimeria tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti, and E. mivati. Salinomycin is fed to broiler chickens from approximately day-old to final slaughter weight. This supplemental application is for the continuous use of salinomycin at 40-60 g/ton for the prevention of coccidiosis caused by E. tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti, and E. mivati in roaster, breeder replacement and layer replacement chickens. Labeling is for birds up to 16 weeks of age. The drug will be used in intensive poultry operations where broiler, roaster, breeder replacement and layer replacement chickens will likely be exposed to a coccidial challenge. Preparation of salinomycin-medicated feed will require the use of a premix. The premix will contain 30 g of active drug ingredients per pounds of premix. No preslaughter withdrawal period is required.

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

Generic Name: Salinomycin

Trade Name: BIO-COX (poultry)

a. Chemical and Physical Properties of Salinomycin

Salinomycin is a naturally occurring monocarboxylic polyether antibiotic elaborated by a strain of Streptomyces Albus (ATCC-21838). The producing organisms was initially isolated from soil (Addendum I).

(αR,2R,5S,6R)-α-ethyl-6-[(1S,2S,3S,5R)-5-(2S,5S,7R,9S,10S,12R,15R)-2-((2R,5R,6S)-5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5.3]pentadec-13-en-9-yl}-2-hydroxy-1,3-dimethyl-4-oxoheptyl]tetrahydro-5-methyl-2H-pyran-2-acetic acid

Pure salinomycin (free-acid or Na salt) is a fine white powder. Less pure salinomycin has a tan color. Salinomycin has a faint odor of streptomyces (fresh soil). Salinomycin and its sodium salt are high molecular-weight nonvolatile solids. Attempts to perform vaporphase chromatography have been unsuccessful as we have unable to accomplish elution, either before or after treatment with various silylating reagents.

	Salinomycin	
	Free Acid	Na Salt
CAS Register Number	53003-10-4	55721-31-8
Molecular Formula	$C_{42}H_{70}11$	$C_{42}H_{69}O_{11}Na$
Molecular Weight	750	772
Melting Point (°C)	112.5-113.5	140-142
pka'	6.4 (DMF)	-
UV absorption	Figure 1	Figure 1

Attached is the UV spectrum of salinomycin-free acid and salinomcyin sodium salt. A 2.47 nM solution of the sodium salt was prepared in acetonitrile, and the UV spectrum was determined directly. To prepare the free acid form of salinomycin, the sodium salt was dissolved in methylene chloride, washed with dilute glacial acetic acid, evaporated to dryness, and reconstituted in acetonitrile to give a final concentration of the free acid form of salinomycin of 2.47 nM. The UV spectrum was then determined for the free acid. The spectrophotometer was a SLM AMINCO Model DW2C with a 1-cm path length. The calculated molar extinction coefficient (ϵ) for the sodium salt and free acid were 73 and 526, respectively. The λmax for the sodium salt was approximately 288 nm and the λmax for the free acid was approximately 284 nm (Notebook Reference: 81421-163).

Stability (Na Salt)	Very stable from natural to alkaline, rather unstable in acid. Stable at temperature of 50°C for extended period (Unpublished, Hoechst AG, Germany).
Solubility (Na Salt):	Readily soluble in methanol, acetone, ethyl acetate, chloroform, benzene, and ethylether; water solubility approximately 3.4 mg/mL. Studies of water solubility of salinomycin indicate that the value of 3.4 mg/mL is a maximum value attainable only under conditions of excess solute. In these studies varying amounts of salinomycin were added to a constant volume of distilled water and equilibrated for 18 to 180 hours at room temperature. Water solubility of salinomycin is greatly decreased by acidification of an aqueous salinomycin solution or by addition of excess salinomycin solution.
Ion affinity and transport:	The relative affinity of salinomycin for complex formation with various cations is $K+>Na>Cs+>Sr^{2+}>Ca^{2+},Mg^{2+}$. Salinomycin has been shown to transport monovalent cations more effectively than divalent cations from aqueous buffer into organic solvent (Addendum II).

Octanol/Water Partition Coefficients (P) (Addendum III) determined by using ^{14}C salinomycin Na are as follows:

TABLE 1

Effect of Salinomycin Concentration, pH, and Mixing Time on the n-Octanol/Water Partition Coefficient (P)[a]

Salinomycin Added (M)	Buffer	pH	Log P
64.2	Water	-	3.457
64.2	Borate	9.0	2.578
67.0	Phosphate	7.0	2.901
67.0	Citrate	5.0	3.456
6.7	Water	-	3.444
6.7	Borate	9.0	2.552
6.7	Phosphate	7.0	2.886
6.7	Citrate	5.0	3.331

[a]The values presented for P are a mean of 3 determinations and expressed as the log.
[b]The concentration of salinomycin in 10 mL of n-octanol before being mixed with the aqueous phase.

The NADA is for use of salinomycin in biomass form (i.e., total mass from fermenter is dried with no subsequent purification). The proximate composition of a typical batch of salinomycin biomass after drying is as follows:

Crude protein	7.8
Crude ash	26.1
Crude fat	26.8
Crude fiber	2.8
Nitrogen-free extract	31.8
Water	4.7

The dried biomass contains approximately 20% salinomycin by weight.

b. Pharmacological/Toxicological Properties of Salinomycin

Subject addressed in sections 1 b.(1) and 1 b.(2), pages 4-12 of the September 1981 EA of salinomycin.

FIGURE 1

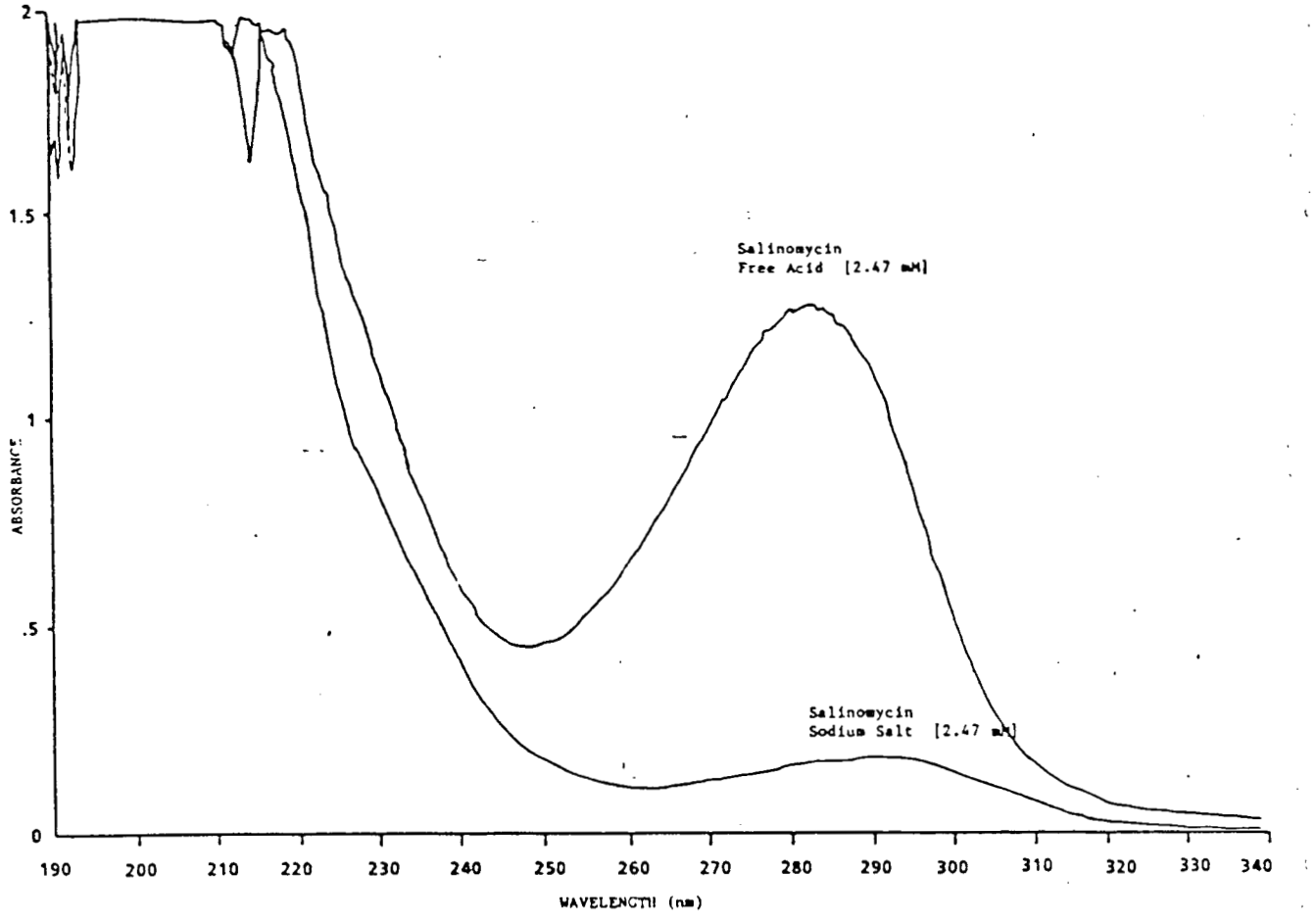
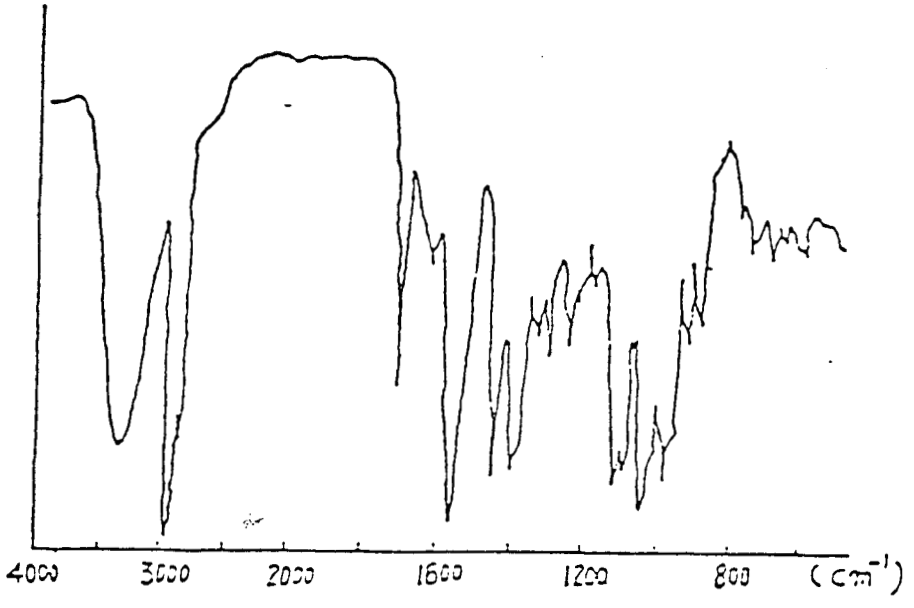


FIG. 2 I.R. SPECTRUM



6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

Introduction of salinomycin into the environment can occur from 3 sources: 1) the primary manufacturer of the bulk drug, Hoechst AG Frankfurt, West Germany; 2) Premix manufacturer, Agri-Bio Corporation, Van Buren, AR; and 3) site of intended use in poultry. The environmental impact of the manufacturing process (i.e., production of salinomycin and production of the medicated premix) is addressed in 1 e., pages 13-14 of the September 1981 EA of salinomycin.

a. Bulk Drug

Hoechst AG confirms that the manufacturer of salinomycin sodium is in compliance with the following Environmental Control laws and regulations in the Federal Republic of Germany:

1) Air Emissions

2) Water Conservation

3) Solid Waste

4) Clean Air

5) Protection Against Dangerous Chemicals

6) Regulations for Working Places

7) Federal Law for Handling Drugs

Official regulatory citations are found in Addendum IV.

b. Description of Salinomycin Sodium Premix Manufacture

Agri-Bio Corporation, Van Buren, AR

1) Liquid Waste Stream

There are no liquid wastes generated in this process.

2) Air Emissions

Air emissions from the production of salinomycin, Type A, Medicated Article that escape the production system consist of dust that contains salinomycin and the inert carrier. Only negligible amounts of this dust escape outside the plant. The dust is contained inside the plant by keeping the manufacturing system closed as much as possible and using a central Cyclone System dust collector to extract dust that escapes the system. The dust collected by the system is deposited in a central container and is disposed of at the Crawford County landfill by Altus Refuge Service. This system meets the requirements of the Arkansas Department of Pollution Control and Ecology and has been issued permit No. 92A, Modified, subject ot the provisions of the Arkansas Water and Air Pollution Control Act. Air emissions associated with the production of salinomycin, Type A, Medicated Articles, contain a hazardous materials regulated by the State of Arkansas; therefore, the state does not require a permit. Nor are there any hazardous materials listed under the Superfund Amendment Reauthorization Act of 1986 (SARA Title III).

3) Dry Solid Waste

Dry solid waste is disposed of at the Crawford County Landfill. The municipal landfill is regulated by the Arkansas Department of Pollution Control and Ecology, Chapter 6 - Disposal of Solid Wastes and Other Refuse. The dry solid waste consist of flush material used to clean equipment, floor sweepings, and dust the dust collector (approximately 200 pounds per year), which contains approximately 50 pounds of salinomycin biomass. This waste is disposed of in accordance with the above referenced laws and regulations.

4) Employee Protection

Material Safety Sheets (Addendum XIII) are available for employees who work in the production area. In addition, employees in the production and packaging areas wear protective clothing and dust respirators as needed, to ensure compliance with OSHA standard, CFR 29, Part 1900 to 1910 and OSHA's Hazard Communication, CFR 29, Part 1910. Employee training and industrial hygiene programs are routine plant operations.

5) Calculation of Environmental Exposure

The amount of salinomycin contained in liquid waste is nil. Approximately 200 pounds per year of dust containing approximately 50 pounds of salinomycin biomass will be disposed of at the landfill on a yearly basis in accordance with the referenced laws and regulations.

By signing this environmental assessment, the Agri-Bio Corporation, subsidiary of the A. H. Robins Company, representative certifies that Agri-Bio Corporation complies with the cited emission requirements.

c. Theoretical Amounts of Salinomycin Eliminated by Poultry

According to recent figures, there were approximately 5.6 billion broilers, 57 million broiler breeders, and 226 million replacement pullets either marketed for slaughter or raised for breeding or replacement purposes per year in the United States. Ninety-nine percent of the broiler chickens produced in the United States receive an anticoccidial drug continuously in the feed to prevent or control coccidiosis. It is estimated that one-half of the replacement pullets are fed anticoccidials. The total dollar volume is estimated to be 89 million for broilers and 4.1 million for replacement pullets and broiler breeders. Salinomycin has been marketed in the United States in broilers since July 5, 1983. Currently, salinomycin (brand name BIO-COX) is being used for prevention of coccidiosis in broiler chickens at 40-60 g/ton. In 1991, there was 14,660,700 pounds of premix (6541 metric tons of premix containing 30 grams of salinomycin per pound of premix, brand name Bio-Cox) containing 969,623 pounds of salinomycin activity (432 metric tons) marketed in the United States. It is estimated that the proposed use of salinomycin at 40-60 g/ton for use in the prevention of coccidiosis caused by E. tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti, and E. mivati in roaster, breeder replacement, and layer replacement chickens will expand the present usage by no more than 5 percent. The highest usage is estimated to be 454 metric tons of salinomycin activity.

Most broiler type chickens currently receive an anticoccidial during part or all of their life. Most breeder replacement and approximately one-half of the layer replacement birds receive an anticoccidial from up to 16 weeks of age. The use of salinomycin as an anticoccidial to the extent that it is adopted by the poultry industry, will substitute for a currently approved anticoccidial. Approval of this supplemental NADA is not expected to substantially increase total feed additive usage by the poultry industry. The estimated 5% increase in the present market is reasonable in the highly competitive poultry feed additive market for anticoccidials.

Data in the supplemental application prove that the level of 40-60 g/ton is effective against E. tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti, and E. mivati for the class of birds discussed above. An informal survey supports the statement that the present use of salinomycin will be expanded by no more than 5 percent with the following facts: 1) there will be increased competition in the coccidiostat market in future years that will ultimately split the market share among more drugs; 2) the market share of the leading drugs of the last few years will diminish for the presently approved claims and the new claims obtained will not significantly increase the total marketed; 3) producers will be weighing better control of coccidiosis vs. the cost of medication; 4) approximately one-half of the layer replacement birds are raised on wire and do not receive an anticoccidial; and 5) some medication programs will be a shuttle between presently approved levels of other drugs and thus there will not be a continuous usage of salinomycin.

As discussed in an environmental assessment dated September, 1981, the major source of salinomycin entry into the environment would be from excreta of chickens fed diets containing salinomycin. As previously mentioned, the amount of salinomycin entering the environment by this route would be extremely small as salinomycin is extensively metabolized by chickens. Studies that utilize ^{14}C-salinomycin (Addendum V) indicate that mature chickens excreted no intact salinomycin following medication with either 80 or 100 g/ton salinomycin. Results of an additional study (Addendum V) to investigate the biotransformation of salinomycin by young chicks agree with previous results with mature chicks as only about 2% of the salinomycin administered to young chicks appeared to be excreted as unchanged salinomycin. Chromatographic separation of radioactivity in excreta from

chicks fed ^{14}C-labeled salinomycin (Addendum VI) has shown that salinomycin metabolism in the chicken yields a large number of metabolites with no single metabolite accounting for more than 16% of the initial radioactive dose. The 3 major metabolites--X, Y, and Z-accounted for 12%, 11%, and 16%, respectively, of the administered radioactivity. Therefore, no single metabolite would be expected to be present in the excreta in very large amounts. (The structures of metabolites X, Y, and Z are presented in Addendum VI.)

When broiler chickens were fed salinomycin 60 g/ton and hte litter accumulated for a period of approximately 9 months (4 grow-outs), there was 9 ppm salinomycin in the litter (Addendum VIII).

Salinomycin is also approved for use as a cattle feed additive (Bio-Gro, NADA D-137-654) in the range of 5-10 g/ton of complete feed. However, because of possible patent infringement, the NADA has been withdrawn. Efficacy of salinomycin as a feed additive to increase rate of weight gain and improve feed efficiency in swine is being investigated. There are no plans to use salinomycin in human medicine.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

Salinomycin and its sodium salt are high molecular-weight, nonvolatile solids. Attempts to perform vapor-phase chromatography have been unsuccessful as we have been unable to accomplish elution, either before or after treatment with various silylating reagents.

Salinomycin is not likely to be dispersed to any significant extent in air. Dispersion in air is a potential hazard to humans during production or biomass and subsequent premix manufacture, and appropriate safety precautions are instituted during these phases. The marketed premix, which is 6.6% (w/w), bears a caution statement concerning use of protective clothing, respirator, etc., to avoid possible effects from airborne particles that may or may not contain salinomycin activity on feed-mixing personnel.

Environmental fate of salinomycin was investigated in a study conducted at the University of Illinois that used a laboratory "feed-lot" model ecosystem (Addendum X). Their initial investigation of salinomycin degradation products with the use of one-day-old chicks indicated the presence of 10 different ^{14}C-labeled degradation products in excreta of chicks following a single, oral dose of ^{14}C-salinomycin. Results from the ecosystem from spilled feed and excreta from chicks fed a diet with ^{14}C-salinomycin added at a level of 100 ppm are shown on the next page.

Environmental Fate of ^{14}C-Salinomycin in the Laboratory Model "Feed-Lot" Ecosystem[a]

	H_20	Oedogonium (alga)	Physa (snail)	Culex (mosquito)	Gambusia (fish)
Total extractable ^{14}C	0.1636	0.0370	0.6986	0.1748	0.0329
Salinomycin (R_f 0.93)[b]	0.0073	0.0349	0.6329	0.1435	0.0291
Polar (R_f 0.0)	0.1089	0.0198	0.0657	0.0313	0.0038
Unextractable	0.0474	0.0479	0.0519	0.0353	0.018

[a]Values in the table are expressed as ppm of ^{14}C-salinomycin equivalents

[b]TLC with ammonium hydroxide:
methanol:chloroform = 1:10:90 by volume

Intact salinomycin was the major fraction (approximately 80-90%) of the total radioactivity accumulated in key organisms of the ecosystem food chain. Salinomycin persisted throughout the key organisms of the food chain: however, salinomycin was not biomagnified to high levels of ecological magnification. Intact salinomycin showed moderately low biodegradability in the ecosystem whereas degradation products (metabolites) were short lived. Sorghum plants grown in the terrestrial area of the ecosystem showed only trace uptake of radioactivity throughout the study. Roots of the sorghum plants were judged to be more brittle than normal, possibly a drug effect.

> "In summary salinomycin showed substantial persistence in the food chain organisms and moderately low biodegradability. It is moderately degradable, however, in the target organisms, the chicken. The degradation products appear to be short lived or subject to photodegradation. Salinomycin is not readily translocated in sorghum in the "feed-lot" area. Therefore, the environmental impact of application of salinomycin as coccidiostat to poultry can be minimized if precautions are taken."

Data from biotransformation studies conducted in our laboratory (Addenum V) indicate that essentially no intact salinomycin is excreted by chickens following oral administration of 14C-salinomycin. Therefore, it appears that the major portion of intact salinomycin detected and shown to be persistent in the ecosystem was probably introduced via spilled feed, which contained intact ^{14}C-salinomycin. Therefore, the ecosystem evaluation appears to be an extreme "worse case" situation and would suggest that the drug can be used with minimal effect on the environment.

The major source of salinomycin entry into the environment would be from excreta of chickens fed diets containing salinomycin. As previously mentioned, the amount of salinomycin entering the environment by this route would be extremely small as salinomycin is extensively metabolized by chickens. Studies that utilize ^{14}C-salinomycin (Addendum V) indicate that mature chickens excreted no intact salinomycin following medication with either 80 or 100 g/ton salinomycin. Results of an additional study (Addendum V) to investigate the biotransformation of salinomycin by young chicks agree with previous results with mature chicks as only about 2% of the salinomycin administered to young chicks appeared to be excreted as unchanged salinomycin. Chromatographic separation of radioactivity in excreta from chicks fed ^{14}C-labeled salinomycin (Addendum VI) has shown that salinomycin metabolism in the chicken yields a large number of metabolites with no single metabolite accounting for more than 16% of the

initial radioactive dose. The 3 major metabolites--X, Y, and Z-accounted for 12%, 11%, and 16%, respectively, of the administered radioactivity. Therefore, no single metabolite would be expected to be present in the excreta in very large amounts. (The structures of metabolites X, Y, and Z are presented in Addendum VI).

Water Supply - Poultry are always fed in covered confined buildings, so the potential for environmental contamination via runoff of rainwater is nil. The extremely small quantity of salinomycin expected to gain entry into the environment via spreading of excreta on soil would not be expected to adversely affect the water supply. The very rapid degradation of salinomycin in soil (half-life of 40-50 hours, Addendum VII and its water solubility of 3.4 mg/mL (Addendum IX) suggest that the possibility of ground water contamination by salinomycin would be very unlikely. Likewise, the possibility of contamination of streams, lakes, etc., as a result of surface runoff from fields where the excreta from broilers fed salinomycin have been spread does not appear to be a serious threat to the environment.

Food Supply - Metabolism and tissue residue evaluation indicate that the amount of salinomycin entering the feed supply as a result of its use as an anticoccidial agent for broiler chickens would be extremely minute as salinomycin is almost completely metabolized by the chicken. The amount of residual salinomycin in tissues is extremely small with the proposed use. In addition bioaccumulation studies indicate that salinomycin does not accumulate in plants grown in soil fortified with salinomycin.

Agricultural Land - The major route of entry of salinomycin into the environment would be via the spreading of excreta on soil. The amount of salinomycin entering the environment by this route should be extremely small as metabolism studies have demonstrated that little intact salinomycin is excreted by chickens fed diets containing salinomycin. Soil application of excreta from chickens fed salinomycin resulted in no phytotoxicity.

Experiments conducted by Hoechst AG, Germany, (Addendum VII) indicate that salinomycin added to soil is rapidly degraded in soil with a half-life of 40-50 hours based on the disappearance of salinomycin as determined by microbiological assay. In the same experiment droppings from broilers fed salinomycin were added to soil, and the disappearance of salinomycin activity was monitored by a microbiological assay with a limit of determination of 0.01 ppm in soil. Fresh droppings (assay of dried droppings indicated 3 ppm salinomycin in the droppings) were mixed with soil (fresh droppings/soil - 1:10), and samples of the mixture were assayed periodically through Day 49. The level of

salinomycin in the soil decreased to an undetectable level in less than 7 days. Investigations conducted by A. H. Robins that used ^{14}C-labeled salinomycin sodium (Addendum XI) also indicate that salinomycin is rapidly degraded in soil. These data suggest that the small amount of salinomycin entering the environmental through spreading of poultry litter on cropland would be rapidly degraded.

Bioaccumulation of salinomycin by bush bean and corn plants has been investigated (Addendum X). Salinomycin was mixed in soil at levels of 0.1 and 14 ppm, and bush bean and corn seeds were planted in the soil (in pots). Temperature was maintained at 22°C, and soil was moistened daily. Ten days after planting all seeds had sprouted, and the sprouts (10-15 cm height) were harvested. The root and aerial portion of the sprouts were separated, and each portion was assayed for salinomycin content (limit of detection 0.02 ppm in the plant material). No salinomycin was detected in the root or aerial portions of bean or corn sprouts grown in control soil (no salinomycin added) or soil with salinomycin incorporated at 0.1 ppm. No salinomycin was detected in the aerial portion of beans or corn grown in soil with salinomycin incorporated at 14 ppm. Assay of the root portion of these plants indicated levels of 0.14 ppm in corn roots and 0.3 ppm in bean roots; however, these levels appear to be the result of soil contamination, as no salinomycin was detected in aerial portions of either corn or bush beans.

The absorption-desorption characteristics of salinomycin in 4 different soil types have been investigated by using salinomycin-^{14}C sodium (Addendum VI). Salinomycin ^{14}C sodium was adsorbed in the range 0.01 to 0.2 μ mole/g of soil. Adsorption of salinomycin-^{14}C sodium on each of the soil types examined was considerably greater than that of benzoic acid ^{14}C, which was used as a model compound. Salinomycin-^{14}C sodium was desorbed into deionized water from the 4 soil-types to an extent of 35-97% at equilibrium. Adsorption isotherms for salinomycin-^{14}C sodium are included in the study report (Addendum XI).

When chickens were fed salinomycin 60 g/ton and the litter accumulated for a period of approximately (four grow-outs), there was 9 ppm in the litter. This demonstrates the maximum expected microbiologically active residue in poultry feces. The maximum concentration of bioactive salinomycin related residue expected to be attained in agricultural soil immediately following incorporation of poultry waste at a rate of 4.5 metric tons of dry matter/acre into the upper 6 inches of soil as fertilizer is approximately 0.045 mg/kg of soil or 0.045 ppm or 45 ppb (assuming the top six inches of soil weighs 909,000 kg/acre).

Conc.* of bioactive residue in X excreta (mg/kg)	Application rate** pf excreta into - soil kg/acre	Weight of soil*** per unit area at 6" depth of incorporation (kg soil/acre)	=	mg of bioactive residue per kg of soil

*Dry weight basis = 9 mg/kg of DM (as per microbiological assay salinomycin activity in feces from poultry fed 60 g/ton salinomycin and the litter accumulated for a period of approximately 9 months, 4 grow-outs)

**Application rate = 4500 kg dry matter/acre

***909,000 kg soil per acre

9 mg/kg DM x 4500 kg DM/acre - 909,000 kg/acre = 0.045 mg/kg soil

Additionally, a worst case estimate of the salinomycin concentration in runoff from soil with salinomycin from poultry waste incorporated at approximately 40.5 g/acre (same rate of application as the above example) is approximately 0.2 ppm on the assumption that l inches of rainfall leaches the entire amount of salinomycin incorporated into the soil; none is degraded; and the 2 inches of rain falling on an acre weighs about 205,000 kg.

Conc.* of bioactive residue in x excreta (mg/kg)	Application rate** of excreta into - soil (kg/acre)	Weight of 2 inches of rainfall*** = leaching salinomycin (kg/acre)	Worst case run-off from soil with salinomycin incorporated in poultry litter (ppm, mg/kg)

*Dry weight basis = 9 mg/kg DM (as per microbiological assay of salinomycin activity in feces from poultry were fed 60 g/ton and the litter accumulated for a period of approximately 9 months (4 grow-outs)

**Application rate = 4500 kg dry matter/acre

***205,000 kg/acre = weight of 2 inches of rain/acre

9 mg/kg DM x 4500 kg DM/acre - 205,000 kg/acre = 0.20 ppm

The aerobic biodegradation of sodium salinomycin was determined in 3 different soil types by Westinghouse Environmental and Geottechnical Services, Inc. (Addendum VII). The specifications of the 3 soil types are listed in Table 2.

TABLE 2

Soil Parameters

Sample No.	% Organic Matter	CEC* mEq/ 100 g	pH	% Sand	% Silt	% Clay	Textural Classification	Moisture gH_2O/ 100 g/ Soil	Field Capacity gH_2O/ 100 g/ Soil
2	3.4	8.6	5.2	59	33	8	Sandy Loam	14.2	26.8
3	5.0	8.9	5.4	59	25	16	Sandy Loam	16.1	38.0
W2	5.8	7.1	7.1	63	24	13	Sandy Loam	22.8	53.1

*CEC = Cation Exchange Capacity

Carbon dioxide production from salinomycin and dextrose (reference) was measured over a period of 64 days. The study was conducted as described in aerobic biodegradation in soils, Environmental Assessment Technical Assistance Handbook, FDA, March 1987, PB-87-175345. Net carbon dioxide production from salinomycin in soil nos. 3 and W2 reached 50% well within 64 days of exposure of drug to soil. However, carbon dioxide production from salinomycin relative to controls for soil no. 2 was zero throughout the duration of the study.

The results obtained from this study suggest that sodium salinomycin is generally, biodegradable in soils, although the pattern and kinetics of biodegradation are likely to vary from 1 soil type to another. Indeed, there is little reason to suspect salinomycin would be resistent to aerobic biodegradation. Salinomycin is a biogenic compound and, in general, biologically synthesized organic materials tend to be inherently biodegradable in aerobic environments (Alexander, M., 1977. Soil Microbiology. New York: John Wiley and Sons). Moreover, salinomycin is a polyether ionophore, and ether linkages typically are subject to cleavage by microbial enzymes (Prof. Frederic K. Pfaender, Univ. of North Carolina, personal communication). Although the relatively low aqueous solubility of salinomycin may limit its biological availability, there appear to be no factors related to the molecular structure or origin of the compound that would cause it to persist in the environment.

This study has demonstrated a positive result for salinomycin biodegradation in 2 of the 3 soils tested, i.e., net carbon dioxide production exceeded 50% of the carbon supplied by the test compound. The reason for the negative response in the one soil is unclear. Soil No. 2 exhibited the lowest pH (5.2) of the 3 samples, and it is known that the biodegradation of hydrocarbons in soil can be severely inhibited at acidic pH (Atlas, R.M. 1984. Petroleum Microbiology. Macmillian Publishing Co., New York). However, the pH of Soil No. 3 was only slightly higher (5.4) than Soil No. 2 but showed a much greater net carbon dioxide production. There are no other obvious parameters related to the composition of Soil No. 2 that suggest an explanation for this result.

Carbon dioxide production in the control flasks of Soil No. 2 and those exposed to dextrose was quite similar to the responses noted for the other 2 soils. Furthermore, the close agreement between the carbon dioxide evolution measured in the control flasks and those receiving salinomycin indicates that the addition of salinomycin did not inhibit or intoxicate the microorganisms or otherwise alter the rate or amount of carbon dioxide produced in Soil no. 2.

It must be recognized that this test procedure provides an indirect measurement of biodegradation; at no time is the concentration of the test compound actually determined. It must be inferred, therefore, that the production of carbon dioxide is the result of metabolism of the added substrates. Unfortunately, the control flasks demonstrate that substantial carbon dioxide evolution occurs even in the absence of supplemental organic carbon. As a result, net carbon dioxide production must be calculated from a relatively small difference between 2 much larger numbers. This difference could conceivably be magnified by increasing the amount of substrate (and carbon) added to the flasks. However, it is generally understood by biochemists and microbial ecologists that the rate and extent of substrate metabolism by microbial communities is directly influenced by the concentration of the substrate. It is essential, therefore, that the concentrations of test chemicals used in biodegradation studies of this type be kept to environmentally realistic levels. Doubling the addition of salinomycin would raise its initial soil concentration to more than 600 ppm (mg/kg), a concentration well in excess of what could be expected from reasonable environmental deposition scenarios.

Recently published studies from Hoechst AG, Germany (Addendum VII) report isolation of 2 strains of bacteria from soil, which are capable of deactivating salinomycin. The strains identified as Pseudomonas stutzeri and Enterobacter agglomerans were shown to cleave the salinomycin molecule to nonionophoric products without detectable antibiotic activity. The identified decomposition product was not toxic at an oral dose of 4 g/kg BW in mice as compared to LD_{50} values in the ranges of 60-100 mg/kg for orally administered salinomycin in mice.

Additional bioconcentration factors (BCF_f-flowing water system; and BCF_t-terrestrial-aquatic system) can be calculated from water solubility (WS) and the Soil Organic Carbon Sorption Coefficient (Koc) for salinomycin using regression equations presented by Kenaga and Goring in Aquatic Toxicology, Eaton, Parrish and Hendricks, Eds., American Society for Testing and Materials, Philadelphia, 1980, p. 103. The bioconcentration factors (BCF) can also be calculated from the octanol-water partition coefficient (Kow) for salinomycin using the regression equation presented by Veith et al. (Drug Metabolism Reviews. 1984-85 15:1295-303).

Parameter	Calculated From	Predicted Value(s)
$BCF_{(f)}$	WS = 1-3.4 mg/mL	12-6
$BCF_{(f)}$	Koc (Rains silt loam) = 78.8	4
$BCF_{(t)}$	WS = 1-3.4 mg/mL	2-1
$BCF_{(t)}$	Koc (Rains silt loam) = 78.8	2
BCF	Kow@ pH 5.0 = log 3.456	234
BCF	Kow@ pH 7.0 = log 2.901	78
BCF	Kow@ pH 9.0 = log 2.578	45

Studies conducted by Kaken Chemical Company (Addendum VI) indicate that salinomycin metabolites extracted from chicken excreta show much less antimicrobial activity than intact salinomycin against Bacillus subtilis, Staphylococcus aureus, and micrococcus flavus. The studies further demonstrated in an acute toxicity evaluation that a mixture of salinomycin metabolites extracted from chicken excreta was tolerated at much higher doses by mice than intact salinomycin.

Examination of workers in Japan involved in salinomycin manufacturing and research for a period of 1 year revealed no abnormal changes in general medical condition or clinical findings (clinical chemistry, urinalysis, blood pressure, and electrocardiogram). Further, eye and chest x-ray examinations of workers handling salinomycin revealed no changes that could be attributed to salinomycin exposure (Addendum XII).

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

The potential antibacterial effect of salinomycin in the environment would be against Gram-positive bacteria (Addendum I). Staphylococci Bacilli, and similar microorganisms are inhibited by salinomycin in vitro at levels of 0.39 mcg/mL and above; and this action may have an effect on the population levels of these bacteria. However, in in vivo studies the intestinal flora of chickens fed a diet with salinomycin added at a level of 80 ppm for 6 weeks was not different from that of controls receiving no salinomycin. Staphylococcus and other Gram-positive bacteria passed a number of times in the presence of sublethal concentrations develop little or no resistance to salinomycin, and cross-resistance with other antibiotics is not routinely seen.

Gram-negative bacteria and fungi are insensitive to salinomycin at levels below 25-100 mcg/mL (Addendum I) and should not be affected in the environment. In a study of chickens artificially infected with Salmonella typhimurium and fed a control diet (no salinomycin) or the same diet with salinomycin added at a level of 80 g/ton, no significant difference between groups was seen in the incidence and shedding of Salmonella and the development of resistance to this or other antibiotics. Coliform bacteria from salinomycin-treated birds developed slight, but significant, resistance to streptomycin and tetracycline (but not to 10 other antibiotics); the reverse pattern was seen in recovered streptococcidi, in that birds receiving the control diet yielded bacteria with greater resistance to assorted antibiotics than did birds receiving the salinomycin (80 g/ton) diet. Results of a similar study in calves artificially infected with Salmonella typhimurium and fed an unmedicated basal diet or the basal plus salinomycin at 22 ppm for 8 weeks, similarly showed no effect of salinomycin on salmonella shedding (i.e., duration, prevalence, or quantity). In addition, dietary salinomycin at 22 ppm had no effect on antimicrobial resistance of fecal coliforms or test salmonella isolated from feces of test calves. Pigs experimentally colonized with Salmonella typhimurium and dosed continuously at a dietary salinomycin level of 75 g/ton did not differ from controls with respect to quantity or prevalence of fecal salmonella shedding. Antimicrobial susceptibility of fecal isolates of the test salmonella were not affected by drug treatment. Salinomycin dosing did result in a statistically significant increase in the estimated mean duration of salmonella shedding compared to control pigs. Dietary salinomycin at 75 g/ton had no effect on antimicrobial resistance of fecal coliforms isolated from test pigs.

Salinomycin has been shown to be active against a number of species of coccidia, but showed very slight to no effect against several other pathogenic protozoans. Similarly, free-living protoza are fairly resistant to the drug, the minimum inhibitory concentrations being 144 mcg/mL for Naegleria grumberi and Paramecium caudatum and more than 481 mcg/mL for Amoeba proteus and Tetrahymena piriformis. By using the worst case estimate of salinomycin concentration (0.2 ppm) in runoff from agricultural soils following normal application of poultry litter (Section 7c), an acute toxicity safety factor of approximately 700-2400X can be estimated for these free-living protozoa. In summary, after salinomycin is fed to chickens at a level of 66 ppm, any level that reaches the environment may have some effect on Gram-positive bacterial populations on the basis of the evidence at hand; but this effect should be temporary because of the drug's instability in this circumstance (Addendum III) and would not affect other kinds of microorganisms.

Data collected by Kaken Chemical Company demonstrated no effect on germination of seed or growth of kidney bean, cucumber, sweet pepper, tomato, or cabbage plants when excreta from chickens fed salinomycin at 75 ppm in the diet were incorporated into the upper 5 cm of soil at a rate of approximately 4 tons/acre. Surface application of salinomycin at rates as high as approximately 800 g/acre 1 day after planting tomato, eggplant, sweet pepper, cucumber, chinese leek, great burdock, carrot, kidney bean, or soybean seeds resulted in no phytotoxicity to any of these plants during the 18-day observation period. The only untoward effects noted were slight growth retardation in turnips with salinomycin application at approximately 400 or 800 g/acre and a similar mild growth retardation in cabbage and chinese mustard with salinomycin application at approximately 800 g/acre. The investigators noted that the mild growth retardation appeared similar to fertilizer injury. The slight growth retardation observed at the extremely high salinomycin rates employed appears to be of no practical significance as the "worse case" litter previously described (Addendum VIII) containing less than 11.25 ppm of salinomycin would have to be added at rates of greater than 40 and 80 tons/acre to result in salinomycin applications of 400 and 800 g/acre, respectively. Poultry manure is normally applied to soil at a rate of approximately 5 tons/acre.

Toxicity of salinomycin to fish (Red Killifish, Oryzias latipes) was investigated by Kaken Chemical Company, Tokyo, Japan. Data for calculating the TLm (median tolerance limit) by the Probit method was obtained by observing fish in water containing salinomycin at levels ranging from 0 to 237 ppm. The TLm (medium tolerance limit) for salinomycin was calculated to be 63.5 ppm.

For comparison, median tolerance limits for sodium penta-chlorphenol and simetryne (herbicide) in the same test system were 0.58 ppm and 9.3 ppm, respectively. All fish survived a salinomycin concentration of approximately 32 ppm during the 72-hour observation period. By using the worst case estimate of the concentration of salinomycin that may be present in runoff from agricultural soils of 0.2 ppm (Section 7c), an acute toxicity safety factor of approximately 160X can be calculated for this species of fish (assuming no dilution of the runoff and no salinomycin degradation).

Safety of salinomycin to the target species and various laboratory animals has been evaluated in both acute and chronic studies (pages 4-12 of the September 1981 EIAR on salinomycin). Data relevant to evaluation of occupational safety of persons involved in the manufacture and handling of salinomycin biomass and premix are summarized below.

Acute toxicity of metabolites of salinomycin and degradation products of salinomycin following acid hydrolysis was investigated by Kaken Chemical Company, Tokyo, Japan. In associated studies Kaken investigated the metabolism and excretion of ^{14}C salinomcyin in chickens. Numerous metabolites were separated via two-dimensional thin-layer chromatography (TLC). Utilizing TLC, the end products of ^{14}C salinomycin hydrolysis in a 0.1 N HCl 20% methanol aqueous solution at 37°C for 30 minutes were demonstrated to be simnlar to those present in gastric contents of chickens dosed via capsule with ^{14}C salinomycin. As an adequate quantity of the metabolite mixture present in gastric contents could not be prepared for acute toxicity testing, nonlabeled salinomycin was hydrolyzed as described above and acute toxicity of the resulting mixture of degradation products, containing no intact salinomycin was investigated. In addition, 50 chickens were dosed once by capsule with 2 mg of salinomycin and their excreta (feces-urine mixture) collected for 72 hours post-dosing. A salinomycin metabolite mixture was extracted and partially purified by TLC. Acute toxicity of the salinomycin acid-hydrolysis product mixture and the metabolite mixture extracted from chicken excreta was investigated. The antimicrobial activity of either mixture against Bacillus subtilis, Staphylococcus aureus, and Micrococcus flavus was decreased relative to that of intact salinomycin. Likewise, acute oral toxicity of either metabolite mixture in mice was much lower than that of intact salinomycin. LD_{50} values for mice were >700 mg/kg BW for the salinomycin acid-hydrolysis product mixture and >1000 mg/kg BW for the metabolite mixture extracted from chicken excreta, as compared to an oral LD_{50} of approximately 70 mg/kg BW for intact salinomycin in mice.

Salinomycin tolerance by swine and chickens is decreased by concurrent administration of tiamulin. Administration of therapeutic levels of tiamulin to swine consuming a diet containing salinomycin at 80 ppm resulted in signs of ionophore toxicity (muscle tremors, inappetence, hindlimb ataxia, or incoordination and labored breathing).

Salinomycin toxicity to horses has been documented. A single horse (gelding) tolerated a single oral dose of 0.15 and 0.20 mg/kg of body weight without signs of toxicity. A single oral dose of 0.6 mg/kg of body weight to the same horse resulted in overt toxicity. At 54 hours after dosing, the horse was completely immobilized. The animal was sacrificed and necropsy indicated cardiac insufficiency with degeneration of myocardial and skeletal musculature.

The mutagenicity of salinomycin was studied in the Ames Salmonella/Microsome Plate Test, the Morse Lymphoma Forward Mutation Assay, Sex-Linked Recessive Lethal Test in Drosphelia Melanogaster, and Primary Rats Hepatocyte Unscheduled DNA Synthesis Assay. Results indicate salinomycin is not mutagenic.

Special Toxicity

Data relevant to evaluation of occupational safety of persons involved in the manufacture and handling of salinomycin biomass and premix are summarized below.

Acute dermal toxicity of salinomycin biomass was evaluated in female SPF-Wistar rats. Groups of 6 rats each were exposed (i.e., by using depilated intact skin) to salinomycin at dosages of 500, 1000, and 2000 mg/kg, respectively, for a period of 24 hours. After 24 hours the test area was washed with water. Lethally intoxicated rats were necropsied, and surviving rats were observed for 21 days. Signs of intoxication included

narrowed eyelid opening, disturbance of equilibrium, accelerated respiration, and weight loss. Necropsies of lethally intoxicated rats showed a yellow discoloration of the internal organs located directly under the application site, severe erythema of the small bowel, and pulmonary hyperemia. A dermal LD_{50} of 1030 mg/kg (95% confidence limits 611-1760 mg/kg) was calculated by using data from the exposed rats.

Acute skin irritation by salinomycin biomass was studied in Albino-Himalayan rabbits. Two rabbits per time period were exposed (skin patch test with depilated intact skin) for periods of 0.5, 1, 2, or 4 hours to 500 mg salinomycin biomass paste (paste prepared by moistening salinomycin biomass, 10.2% salinomycin by weight, with physiological saline). Mild erythema and edema were observed with 0.5- to 2-hour exposure, and moderate to severe erythema and moderate edema were observed with 4-hour exposure. The salinomycin biomass was evaluated as a mild skin irritant for the 0.5- to 2-hour exposure and as a moderate to severe skin irritant for the 4-hour exposure.

Inhalation of salinomycin biomass dust (particle size range 0.3-6 mm) was evaluated in 6 adult male and female SPF-Wistar rats per treatment group. Rats were exposed individually for 4 hours in a cyclindrical, plastic, inhalation chamber to 1 of 3 salinomycin biomass dust concentrations ranging from approximately 33-3500 mg/M^3 of air and observed for 14 to 21 days postexposure. All except 1 male rat exposed to the highest salinomycin dust concentration (2020-3530 mg/M^3 of air) survived the 4-hour exposure period. Concentration-related symptoms observed in the rats during and after exposure included irregular respiration, narrowed opening of the eyelids, tremor, and crouching position. Necropsy of the rat that died showed focal dark red spots in the lungs. All rats in groups exposed to salinomycin biomass dust concentrations ranging from 33-1140 mg/M^3 of air gained weight during a 14-day, postexposure, observation period; surviving rats in the highest treatment group (dust concentration ranging from 2020-3530 mg/M^3) had recovered to their initial weight or greater by Day 21 postexposure. Necropsy of all surviving rats (approximately 21 days postexposure) revealed no abnormal findings.

The health status of 22 workers of Kaken Chemical Co., LTD., in Japan was evaluated prior to their involvement in salinomycin manufacturing and research and again after approximately 1 year. Examination of the workers who usually handled salinomycin for the purpose of manufacturing and research for a period of 1 year revealed no abnormal changes in general medical condition or clinical findings (clinical chemistry, urinalysis, blood pressure, and electrocardiogram). Further, eye and chest x-ray examinations of workers handling salinomycin revealed no changes that could be attributed to salinomycin exposure (Addendum XII).

To mitigate potential skin irritant and salinomycin dust inhalation injury, workers involved in manufacture of Bio-Cox premix are required to wear respirators, eye protection, and protective clothing. As a further safeguard the following caution statement has been included on labeling of Bio-Cox premix to mitigate potential health hazards to persons using the marketed Bio-Cox premix.

> WHEN MIXING AND HANDLING BIO-COX® PREMIX, USE PROTECTIVE CLOTHING, IMPERVIOUS GLOVES, EYE PROTECTION, AND AN APPROVED DUST RESPIRATOR. OPERATORS SHOULD WASH THOROUGHLY WITH SOAP AND WATER AFTER HANDLING. IF ACCIDENTAL EYE CONTACT OCCURS, IMMEDIATELY RINSE THOROUGHLY WITH WATER.

An appropriate Materials Safety Data Sheet (Addendum XIII) will be provided to manufacturers, processors, and formulators whose personnel may be exposed to Bio-Cox premix.

Overall Conclusions

Although there is the potential for biomagnification in the aquatic ecosphere, there is little if any real significance to the effects upon the environment from the proposed use of salinomycin in poultry. Degradation in the soil is rapid and poses no threat to the microbial populations of the soil and hence will not interfere with microbial-medicated soil processes. There should be no effect on terrestrial wild life or avain species. Overall, there should be no adverse effects on the envirorunent.

9. USES OF RESOURCES AND ENERGY

The only irretrievable commitment of resources (gas, oil, and elecricity) would be those involved in the production of salinomycin and during the premix manufacturing process (page 20 of the September 1981 EIRA of salinomycin).

There are no anticipated effects upon endangered or threatened species and upon property listed in or eligible for listing in the National Register of Historic Places.

10. MITIGATION MEASURES

Adverse environmental effects expected to result from use of salinomycin in poultry diets as an anticoccidial agent are minimal.

The short-term improvement in poultry health and productivity will obviously benefit poultry producers and consumers. The minimal adverse environmental effects of salinomycin use as an anticoccidial agent for poultry and the lack of adverse toxicological findings suggest no long-term adverse effects on the environment or human health.

The use of salinomycin as an anticoccidial agent will contribute to more efficient production of food for humans through control of coccidiosis. The amount of feed grains, protein supplements, etc., required to produce a quantity of poultry products is less in the absence of clinical or subclinical coccidiosis. In addition the more efficient utilization of feed in the absence of the disease will result in some reduction in the amount of animal wastes to be disposed of in the environment.

The major portion of the use of salinomycin would be as a replacement for currently marketed anticoccidial agents since most commercially produced poultry receive an anticoccidial agent in their diet. The most widely used anticoccidial agents are of the same general class of compounds (polyether ionophores) so one would expect the use of salinomycin to add very little to the total environmental impact resulting from commercial use of anticoccidial agents in poultry production.

Labeling of salinomycin (Trademark Bio-Cox for poultry) bears caution statements concerning toxicity to equines and precautions to be taken by persons mixing and handling the premix. A Materials Safety Data Sheet is provided to manufacturers, processors, and formulators whose personnel may be exposed to Bio-Cox.

11. ALTERNATIVES TO THE PROPOSED ACTION

On the basis of the information included in this report, it appears that approval of the proposed action would not result in any substantial adverse impact on the environment. The proposed action will expand the present use of salinomycin in poultry by no more than five percent.

The considerable benefit that can result from use of salinomycin as an anticoccidial agent for poultry appears to overshadow any minimal adverse effect on the environment, which may result from approval of the proposed action. Improved health and efficiency of poultry production through the control of clinical or subclinical coccidiosis would directly benefit poultry producers and indirectly benefit consumers.

Widespread use of salinomycin (Bio-Cox) by the poultry industry for the past 8 1/2 years without obvious adverse environmental impact supports the contention that the product can be used safely and without adverse environmental impact.

12. LIST OF PREPARERS

This environmental assessment was prepared by Donald L. Gilbert, B.S., M.B.A., Director, Regulatory Affairs and Product Development. The preparer has 28 years of work experience with pharmaceutical companies with expertise in toxicology, biology, and the development of animal health products. The preparer has studied salinomycin for 15 years.

Data included in this EA was collected and interpreted by toxicologists, pharmacologists, chemists, biochemists, microbiologists, environmental scientists, veterinarians, and pathologists at the A. H. Robins Company, Hoechst AG (Germany), Kaken Chemical Company (Japan), and cooperating university and contract laboratories.

13. CERTIFICATION

The undersigned official certifies that the information is true, accurate, and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

April 1, 1992
Date

Donald L. Gilbert
Director, Regulatory Affairs and
Product Development
Special Products Division
A. H. Robins Company

LIST OF ADDENDA

Hoechst

Hoechst AG · Postfach 800320 · D-6230 Frankfurt am Main 80

Hoechst Aktiengesellschaft
Pharma-Produktion Hoechst
Referat Inspektionen und GMP D610

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(BLZ 50070010) Kto. Nr. 926006
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Dr. Robert L. Miller
A.H. ROBINS CO.
1407 Cummings Drive
P.O. Box 26609
Richmond, Virginia 23261-6609
U. S. A.

Ihre Zeichen	Ihre Nachricht vom	Unsere Zeichen	Telefon Durchwahl	Frankfurt am Main
		Dr. Ho/CT	(069) 305- 6831	June 16, 1989

Re: Salinomycin-Sodium
Environmental Protection Statement

Dear Dr. Miller,

please find attached as requested the statement of HOECHST AG that they are in compliance with all applicable laws and guidelines for protection of the environment including a list of these laws and guidelines.

With best regards
HOECHST AKTIENGESELLSCHAFT

(Dr. Schikorr) (ppa. Dr. H. Hoffmann)

Hoechst

Hoechst AG · Postfach 800320 · D-6230 Frankfurt am Main 80

Hoechst Aktiengesellschaft
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TO WHOM IT MAY CONCERN

Ihre Zeichen	Ihre Nachricht vom	Unsere Zeichen	Telefon Durchwahl	Frankfurt am Main
		Dr. Ho/CT	(069) 305- 6831	June 9, 1989

Re: Salinomycin-Sodium

Environmental Assessment

HOECHST AKTIENGESELLSCHAFT, as the producer of Salinomycin-Sodium at its factory:

HOECHST AKTIENGESELLSCHAFT
Höchst Works
Brüningstrasse 50
Postfach 80 03 20
D-6230 Frankfurt am Main-Höchst 80
Federal Republic of Germany (West)

confirms herewith that Salinomycin-Sodium is manufactured in the above mentioned plant in compliance with the existing environmental control laws and regulations of the Federal Republic of Germany.
Environmental protection in the Federal Republic of Germany is subject to a number of laws and regulations which are strictly enforced.
The most important ones are listed below:

- Immissions (Air etc.):
  "Gesetz zum Schutz vor schädlichen Umwelteinwirkungen durch Luftverunreinigungen, Geräusche, Erschütterungen und ähnliche Vorgänge" ("Bundesimmissionsschutzgesetz"),
  (Federal Law for Protection of the Environment against the Adverse Influences Caused by Contamination of the Air, by Noise, Vibration, and Similar Events).
  March 15, 1974,
  published in Federal Law Gazette I, 721, corrected 1193,
  amended August 12, 1980/Federal Law Gazette I, 1310.

- Water Conservation:
  "Gesetz zum Schutze des Wasserhaushaltes" ("Wasserhaushaltsgesetz"),
  (Federal Law for Protection of the Water Houshold).
  October 16, 1976,
  published in Federal Law Gazette I, 3017,
  amended March 28, 1980/Federal Law Gazette I, 373.

- Solid Wastes:
  "Gesetz zur Vermeidung und Entsorgung von Abfällen" ("Abfallgesetz"),
  (Federal Law for Avoidance and Disposal of Wastes).
  August 27, 1986,
  published in Federal Law Gazette I, 1718.

- Technical Instructions for Maintaining Clean Air:
  "Technische Anleitung zur Reinhaltung der Luft" ("TA Luft"),
  (Technical Instructions for Maintaining Clean Air).
  February 27, 1986,
  published in Joint Ministerial Gazette 95, 202.

- Technical Instructions for Noise Protection:
  "Technische Anleitung zum Schutz gegen Lärm" ("TA Lärm"),
  (Technical Instructions for Protection Against Noise).
  July 16, 1986,
  published in "Beilage zum Bundesanzeiger No. 137",
  July 26, 1968.

- Chemicals:
  "Gesetz zum Schutz vor gefährlichen Stoffen" ("Chemikaliengesetz"),
  (Federal Law for Protection Against Dangerous Chemicals).
  September 16, 1980,
  published in Federal Law Gazette I, 1718.

- Regulations for Dangerous Products:
  "Gefahrstoffverordnung",
  (Regulations for Dangerous Products).
  August 28, 1986,
  published in Federal Law Gazette I, 1470.

- Containers for Compressed Gases:
  "Druckbehälterverordnung",
  (Regulations for Containers for Compressed Gases).
  February 27, 1980,
  published in Federal Law Gazette I, 184.

- Regulations for Notifications of Immissions:
  "Zwölfte Verordnung zur Durchführung des Bundesimmissionsschutzgesetzes" ("Störfallverordnung"),
  (12th Regulation for the Inplementation of the Federal Law for Protection of the Environment Against the Adverse Influences Caused by Contamination of the Air, by Noise, Vibration, and Similar Events).
  June 27, 1980,
  published in Federal Law Gazette I, 772.

- Storing of Inflammable Liquids:
  "Verordnung über Anlagen zur Lagerung, Abfüllung und Beförderung brennbarer Flüssigkeiten zu Lande",
  (Regulations for Facilities for Storage, Filling, and Transport of Inflammable Liquids on Land).
  February 27, 1980,
  published in Federal Law Gazette I, 229,
  amended May 3, 1982/Federal Law Gazette I, 569.

- Regulations for Transportation of Dangerous Products (road, railway, sea, river, air):

-- For Transport by road:
"Gefahrgutverordnung Straße",
(Regulations for Transport of Dangerous Products by Road).
July 22, 1985,
published in Federal Law Gazette I, 1550.

-- For Transport by Railway:
"Gefahrgutverordnung Eisenbahn",
(Regulations for Transport of Dangerous Products by Railway).
July 22, 1985,
published in Federal Law Gazette I, 1560.

-- For Transport by Sea:
"Gefahrgutverordnung See",
(Regulations for Transport of Dangerous Products by Sea).
July 5, 1978,
published in Federal Law Gazette I, 1917,
amended July 27, 1986.

-- For Transport on Waterways within Germany:
"Gefahrgutverordnung Binnenschiffahrt",
(Regulations for Transport of Dangerous Products on the Waterways within the Federal Republic of Germany).
March 24, 1983,
published in Federal Law Gazette I, 1977.

-- Dangerous Products Regulations of the IATA:
"IATA - DGR"
(Dangerous Goods Regulations),
28th edition.

- Drinking Water:
  "Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe",
  (Regulations for Drinking Water and for Water to be Used in Food Handling Factories).
  May 22, 1986,
  published in Federal Law Gazette I, 760.

- Feedstuffs (Animal Nutrition):
  "Futtermittelgesetz",
  (Federal Law on Feedstuffs).
  July 2, 1975,
  published in Federal Law Gazette I, 1745,

  and:

  "Futtermittelverordnung",
  (Regulations on Feedstuffs).
  April 8, 1981,
  published in Federal Law Gazette I, 352.

- Working Places:
  "Verordnung über Arbeitsstätten" ("Arbeitstättenverordnung"),
  (Regulations for Working Places).
  May 20, 1975,
  published in Federal Law Gazette I, 729.

Hoechst

Empfänger	Unsere Zeichen	Datum	Blatt
TO WHOM IT MAY CONCERN	Dr. Ho/CT	June 9, 1989	6

- Drug Law:
  "Gesetz über den Verkehr mit Arzneimitteln" ("Arzneimittelgesetz"),
  (Federal Law for Handling of Drugs).
  August 24, 1976,
  published in Federal Law Gazette I, 2445, 2448, (and amendments);

  and:

  "Betriebsverordnung für pharmazeutische Unternehmer",
  (Operations Ordinance for Pharmaceutical Entrepreneurs).
  March 8, 1985,
  published in Federal Law Gazette I, 546, (and amendments).

Yours faithfully
HOECHST AKTIENGESELLSCHAFT

(Dr. Schikorr) (ppa. Dr. H. Hoffmann)

PRODUCT SAFETY DATA SHEET

Agri-Bio Corporation, A Subsidiary of A. H. Robins Company, P. O. Box 897, Gainesville, Georgia 30503 (800) 247-4246

BIO-COX®

Bio-Cox is the trade name for salinomycin sodium. Bio-Cox is fed to broiler chickens as an aid in the prevention of coccidiosis caused by *Eimeria necatrix, E. tenella, E. acervulina, E. brunetti, E. mivati,* and *E. maxima.*

I. PHYSICAL AND CHEMICAL PROPERTIES

a. **Active Ingredient**
salinomycin sodium (an ionophore)

b. **Premix Formula**
Thirty grams of salinomycin activity (as mycelial salinomycin sodium) per pound mixed with diluents such as calcium carbonate, mineral oil and up to 1% silicon dioxide. May also contain calcium silicate. Packaged in 50 lb. bags.

c. **Auto Ignition Temperature**
410° F (210° C)

d. **Solubility**
Insoluble in water

e. **Appearance and Odor**
Free flowing light brown, granular material with slight odor of amyl alcohol.

f. **DOT Classification**
Nonregulated

g. **Threshold Limit Value**
Exposure to all dusts should not exceed 10 mg/m^3

h. **Flash Point**
No ignition up to 180° F (82.2° C)

II. STABILITY AND STORAGE

Store in properly closed bags under normal storage conditions. Bags should be stored in a cool, dry place. Product has an 18-month dating from date printed on top/tape part of the bag.

III. UNUSUAL FIRE AND EXPLOSION HAZARDS

Relatively non-combustible, requires continuous ignition source to support flames. Dust explosion potential is less than that of grain dusts.

IV. FIRE FIGHTING INFORMATION

Extinguish with water. Wear protective clothing.

V. HEALTH HAZARD DATA

Threshold Limit Value — Exposure to all dusts should not exceed 10 mg/m^3.

EMERGENCY INFORMATION

In the event that an individual accidentally consumes or has been overly exposed to Bio-Cox, follow the first aid procedures set forth in this document. If further information is required, have a physician contact the Medical Department, A. H. Robins Company, Richmond, VA (804) 257-2000. In case of a large chemical spill or contamination, call (800) 247-4246. In Georgia, call (404) 536-0111.

This Product Safety Data Sheet is directed to manufacturers, processors and formulators whose personnel may be exposed to this product. It is intended for use by manufacturers, safety hygiene and medical personnel.

All information contained herein is offered in good faith and with the belief that it is accurate. As of this date of issuance, we are providing all the information that we have or are aware of that is relevant to the foreseeable use or handling of the product. However, in the event of an adverse incident associated with this product, this Safety Data Sheet is not, and is not intended to be, a substitute for consultation with appropriately trained personnel

Effects of Overexposure

BIO-COX Premix is considered a skin irritant and a severe eye irritant. Other symptoms, as described below, have been observed.

Acute toxicology signs (see page 3 for LD_{50} values) in various animal species by various routes of administration (i.e., oral, dermal, subcutaneous, intraperitoneal, and inhalation) of salinomycin or salinomycin biomass include: depression of spontaneous movement, decreased rate of respiration, salivation, ataxia (total or partial inability to coordinate voluntary bodily movement, especially muscular movement). Transient tingling sensations in worker's fingers and toes have been reported after heavy exposure to Bio-Cox dust. Salinomycin premix (40 gm/lb premix) at a level of 100 mg/kg or greater was lethal to rats and produced depression, decreased pain, and slowed reflexes.

Male workers exposed to salinomycin sodium during manufacturing and research for a period of one year, with the exposure varying from small amounts of drug to kilogram quantities for periods of time ranging from a few hours daily to the entire workday, were monitored for effects on clinical chemistry values, urine analysis values, blood pressure, electrocardiograms, and general health. Workers wore protective clothing, glasses and respirators. No abnormal changes were observed.

Chronic exposure of mice to a diet containing 100 ppm (13 mg/kg/day) of salinomycin sodium, the active ingredient of Bio-Cox, for two years produced no adverse effects.

Chronic exposure of rats to a diet containing 100 ppm (5.5 mg/kg/day) of salinomycin biomass for 30 months produced no adverse effects.

Dogs exposed to salinomycin at a dose of 3 mg/kg/day for 13 weeks developed neurological abnormalities characterized by transient and reversible hind limb weakness/paralysis and muscular incoordination. Histopathological examination showed sciatic nerve injury.

Chronic oral exposure of dogs to salinomycin biomass for one year produced no adverse effects at a dosage of 2.5 mg/kg/day. A higher dosage (12.5 mg/kg/day) produced peripheral sciatic nerve injury resulting in transient inability to coordinate voluntary muscular movements. Dosage-related depressed or nonexistent responses were noted during neurological examination for: blink reflex; the flexor, extensor, and patellar reflexes; the tonic neck reflex; the exterior postural thrust; righting reaction; visual and tactile placing reactions; and hopping reflex.

An inhalation study in rats exposed for 4 hours to salinomycin biomass at levels to 1137 mg of salinomycin per m^3 of air produced no deaths, but rats showed narrow opening of the eyelids, ruffled fur, and irregular respiration. A concentration of 2020 mg/m^3 and greater was lethal and produced irregular respiration, tremor, narrow opening of eyelids, ruffled fur, and a crouching position.

Dermal application of salinomycin (as either premix, biomass, or sodium salt) to rabbits, rats, guinea pigs, and mice produced dose-related edema, erythema, and eschar formation in rats and mice. Death occurred in rats and mice at high exposure levels.

Salinomycin sodium, applied as a solution to the eyes of rabbits, produced conjunctival injection, edema, and hypersecretion. In powder form, salinomycin caused severe injury to the conjunctiva and damage to the cornea and iris.

Salinomycin sodium did not produce either immediate or delayed type hypersensitivity when applied to the skin of rabbits, rats, or guinea pigs.

Emergency First Aid

EYES — If accidental eye contact occurs, flush eyes immediately with water and seek medical attention if irritation persists.

INGESTION — In case of ingestion, induce vomiting and call a physician.

SKIN — For severe skin exposure, remove clothing and shower thoroughly; if irritation persists, seek medical attention.

INHALATION — In the event a person becomes weak, dizzy, acquires a headache or hyperventilates due to increased breathing from Bio-Cox Premix, the person should leave the work area and call a physician if symptoms persist.

Acute Toxicity Studies

Species	Sex	Age	Route	LD_{50}(mg/kg)
		SALINOMYCIN BIOMASS[a]		
Mice	M	Adult	Oral	612(480-780)[b]
Mice	M	Adult	Oral	>350 >630
Mice	F	Adult	Oral	293 (225-383)
Rat	M	Young	Oral	503(474-533)
Rat	M & F	Adult	Inhalation	>2020 mg/m^3
Rat	F	Adult	Dermal	1030(611-1760)
Rat	M & F	Adult	Oral	434(342-551)
Rabbit	M & F	Adult	Oral	21(11-28)[c]

[a] Doses expressed as Biomass except as noted
[b] 95% Confidence Limits
[c] Doses expressed as Salinomycin

VI. SPILL OR LEAK PROCEDURES

Sweep or clean with vacuum. Control the dust by use of oils or water during clean up. Follow self-protective measures as indicated under Special Protection Information.

Dispose of spills in a chemical waste system, in accordance with federal, state and local regulations.

VII. SPECIAL PROTECTION INFORMATION

Respiratory Protection
Use NIOSH approved respiratory protective devices such as 3M brand disposal respirator, part #8710 for toxic dusts and mist having a time weighted average not less than 0.05 mg/m^3. In areas of heavy dust exposure, respiratory protection such as Racal Breathe Easy #5 (NIOSH approval TC-21C277) equipped with high efficiency filters is recommended.

Protective Clothing
Use protective clothing to prevent contact of Bio-Cox Premix with skin. In heavy dust areas, disposable coveralls are recommended. Impervious gloves should be used to protect hands.

Eye Protection
Use appropriate eye protection such as goggles or a face shield to prevent accidental eye contact.

Ventilation
Local exhaust is the preferred method for removal of Bio-Cox Premix dust.

Other Protective Measures
Workers exposed to heavy concentrations of Bio-Cox dust should shower and change clothes prior to leaving work.

VIII. SPECIAL PRECAUTIONS

Precautions To Be Taken In Handling — Storing
Store in properly closed bags or containers.

Other Precautions
Do not feed to laying chickens or chickens over 16 weeks of age.

Do not allow turkeys, horses or other equines access to formulations containing Bio-Cox Premix. Ingestion may be fatal.

Do not feed Bio-Cox Premix undiluted. Always mix in feeds before use.

SECTION J. ENVIRONMENTAL IMPACT ANALYSIS

RE: 21 CFR PART 25

1. An identification of pollutants expected to be emitted.

   The only pollutant expected to be emitted would be dust. Each plant and production area has adequate dust control equipment to prevent emission of dust.

2. A citation of applicable Federal, State and Local Emission requirements.

   NutriBasics certifies that the facility complies with all local, state and federal requirements since no pollutants are released.

3. Certification

   To the best of my knowledge and belief the above statements are true

Signed Janet R Churchill 8/6/86
Quality Control Manager