ENVIRONMENTAL ASSESSMENT

FOR

NARASIN (MONTEBAN®) IN THE FEED OF BROILER CHICKENS

FOR THE PREVENTION OF COCCIDIOSIS

Elanco Products Company
A Division of Eli Lilly and Company
Lilly Corporate Center
Indianapolis, IN 46285

January 1986

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for the Prevention of Coccidiosis

1. DATE May, 1985

2. APPLICANT Elanco Products Company
A Division of Eli Lilly and Company

3. ADDRESS Lilly Corporate Center
Indianapolis, IN 46285

4. DESCRIPTION OF THE PROPOSED ACTION

New Animal Drug approval has been requested for MONTEBAN, a premix incorporated into poultry feeds for the prevention of coccidiosis. Between 60 and 80 ppm (54.5 to 72.6 grams per ton) of narasin, the active ingredient in MONTEBAN, would be used continuously in the feed of broiler chickens. MONTEBAN would be distributed primarily to broiler production companies which prepare the feeds for their chickens.

Approval of the proposed action would authorize the fermentation plants of Eli Lilly and Company at Clinton and Lafayette, Indiana, and at Liverpool, England, to manufacture narasin for sale in the United States as MONTEBAN. Pilot lots and small scale production of narasin may occur at a fermentation facility of Eli Lilly and Company at Kentucky Avenue in Indianapolis, Indiana. Formulation and packaging of MONTEBAN may be performed in Eli Lilly and Company facilities at Omaha, Nebraska.

MONTEBAN® (Narasin, Elanco)

Based on the proposed action narasin could potentially be introduced into the following environments:

a) The environment adjacent to the manufacturing plants.

b) The environment adjacent to facilities which mix MONTEBAN with feed.

c) Chicken farms where residues may be found in animal waste.

d) Agricultural lands where waste products from chickens are used as fertilizer.

e) Aquatic systems where runoff may collect from sites receiving waste products of chickens.

5. INTRODUCTION OF SUBSTANCES IN THE ENVIRONMENT

A. PRODUCT DESCRIPTION

MONTEBAN is a premix to be incorporated into complete feed for broiler chickens. Narasin is the active ingredient in MONTEBAN and is produced in a dried mycelial biomass form by a fermentation process. MONTEBAN contains sufficient quantities of this dried fermentation product to achieve narasin concentrations of 80, 100, 120, 160, or 200 g of activity per kg of premix. MONTEBAN may also contain diluents such as soybean mill run, corn meal, montmorillonite clay, rice hulls, antidusting oil, mineral oil, densifiers, calcium stearate, calcium carbonate, and calcium silicate.

1) Dried Fermentation Product

Narasin is produced by the fermentation of a strain of Streptomyces aureofaciens, an organism isolated from soil (1). The most economical procedure to prepare a usable form of narasin is to harvest the fermentation culture in such a way as to combine narasin with the mycelial cells of the producing organism and the unused components of the feed-stock used in the fermentation to achieve growth of the organism. Thus, the dried mycelial or biomass form of narasin contains nutrients which are commonly found in broiler feedstuffs.

The approximate composition (percent by weight) of a representative lot of dried mycelial narasin is:

Crude Protein	5.84
Crude Fiber	2.70
Ash	52.00
Carbohydrate	1.17
Lipids	21.45
Moisture (Karl Fisher)	4.85
Volatiles (Loss on drying)	4.39
Sodium	0.47
Potassium	0.80
Calcium	2.17
Magnesium	1.27
Phosphorus	0.45
Manganese	0.02
Aluminum	3.21

No aflatoxins were detected in mycelial narasin at an assay sensitivity of 1 ppb.

Fractionation of the "narasin-like" compounds in mycelial narasin showed that narasin accounted for 96.5 percent of the vanillin-positive material and 98 percent of the antimicrobial activity (Appendix A).

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2) Narasin

Narasin is a monocarboxylic polyether compound which complexes with monovalent alkali cations and shows ionophorous activity with a selectivity of $Na^+ > K^+ = Rb^+ > Cs^+ > Li^+$ (2).

Chemical Name: (αβ,2β,3α,5α,6α)-α-ethyl-6-[5-[5-(5α-ethyltetrahydro-5β-hydroxy-6α-methyl-2H-pyran-2β-yl)-3"α,4,4",5,5"α,6"-hexahydro-3'β-hydroxy-3"β,5α,5"β-trimethylspiro]furan-2(3H),2'-[2H]pyran-6'(3'H),2"-[2H]pyran]-6"α-yl]-2α-hydroxy-1α,3β-dimethyl-4-oxoheptyl]-tetrahydro-3,5-di-methyl-2H-pyran-2-acetic acid.

CAS Registry Number	55134-13-9
Molecular formula	$C_{43}H_{72}O_{11}$ (acid form)
Molecular weight	764
Melting point	98-100°C
UV absorption	none above 220 nm
pKa value	7.9 (80% DMF)
Solubility	
water	pH 5 rapid degradation pH 7 102 mg/L pH 8 ca 400 mg/L pH 9 681 mg/L
ethyl acetate	very soluble
chloroform	very soluble
acetone	very soluble
benzene	very soluble
dimethyl sulfoxide	very soluble
hexane	slightly soluble

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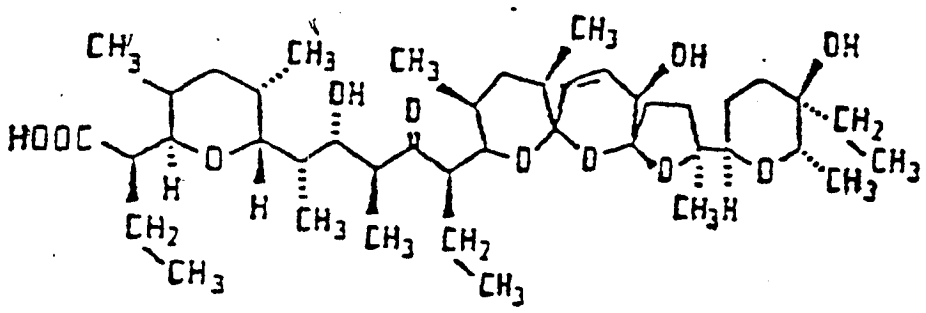
Vapor Pressure	Non-volatile solid based on molecular weight and melting point (thermogravimetric analysis showed no volatilization of narasin up to 220°C, followed by thermal decomposition)
n-Octanol-Water Partition Coefficient	7×10^4 at 236 and 460 mg/L with a pH 8.0 phosphate buffer
Infrared Spectrum in Chloroform	maxima at 2.85, 3.34, 5.83, 6.82, 7.22, 7.53, 7.78, 8.75, 8.95, 9.15, 9.50, 9.55 and 9.60 microns

Structural Formula:



B. INTRODUCTION OF SUBSTANCE FROM THE MANUFACTURING SITE

The manufacturing process for narasin, the active ingredient in MONTEBAN, in conjunction with the corresponding pollution control practices at each of the plant sites, is designed to have minimal environmental impact. Narasin is produced by a fermentation process which also utilizes unit operations such as centrifugation, filtration, concentration, drying, pelletizing, granulation by crushing, screening and blending. MONTEBAN is manufactured using operations such as grinding, mixing and packaging.

The only releases of narasin activity from manufacturing operations will be in the polished centrate or filtrate of fermentation broth and dilute washwaters. At the Indiana plant sites these wastewaters would be treated by wastewater concentration and pyrolysis, by land application or by microbiological degradation, possibly with hydrolysis and photolysis. Manufacture of narasin and discharge of wastewater at the production site in Liverpool, England, are performed in accordance with the pertaining environmental control laws of the United Kingdom, the Control of Pollution Act, 1974, as implemented by national and local authorities.

The production sites at Clinton and Lafayette, Indiana are located along the Wabash River. Either of these facilities could be the major production site for any MONTEBAN manufactured in the United States. Only pilot lots and small scale production of narasin would occur at the Eli Lilly and Company Kentucky Avenue fermentation facility in Indianapolis, Indiana. Formulation and packaging of MONTEBAN may be performed in Eli Lilly and Company facilities at Omaha, Nebraska. The highest discharge rate of narasin from a production facility would be found at the principal manufacturing site along the Wabash River.

The highest expected concentration of narasin in the Wabash River would occur during an acute low-flow period of the river. The three-day-average low flow for the Wabash River that would occur once in ten years is 558 ft^3/sec (U.S. Geological Survey). This is the lower three-day-average flow of the Wabash River at either of the production sites. Process wastewater would carry about 17 kg of narasin per day to treatment facilities. Because the efficiency of the treatment facilities for removal of narasin from the wastewater is not known, it will be assumed that in the worst case, narasin would not be removed by treatment. In this worst case, after the wastewater from the treatment facility mixes with the river at low flow, the highest expected average concentration of narasin in the river would be about 0.012 mg/L.

Calculation:

$$\frac{17 \text{ kg narasin/day} \times 1000 \text{ g/kg}}{558 \text{ ft}^3\text{/sec} \times 28.32 \text{ L/ft}^3 \times 60 \text{ sec/min} \times 1440 \text{ min/day}} = 1.2 \times 10^{-5} \text{ g/L}$$

The highest expected concentration of narasin during an acute low-flow period in the Wabash river is substantially below concentrations which have been found to acutely affect aquatic organisms. The highest expected concentration is about 1/273 of the lowest 96-hr LC_{50} value (3.27 mg/L) for bluegill (Appendices B and Z), at most 1/116 of the lowest 96-hr LC_{50} value (>1.4 <2.0 mg/L) for rainbow trout (Appendices T and AA), and about 1/643 of the lowest 48-hr EC_{50} value (7.72 mg/L) for daphnids (Appendices U and BB). A narasin concentration of 0.19 mg/L did not affect the behavior or survival of any of these species and is 16 times higher than the highest expected narasin concentration in the river water during an acute low-flow period.

The average expected concentration of narasin in the Wabash River from continuous discharge from a production facility can be based on

average river flow. The average river flow over a fifty-six-year period is 6383 ft^3/sec (U.S. Geological Survey). Process wastewater would carry about 17 kg of narasin per day to treatment facilities, and if not removed by treatment, to the river. After the wastewater mixes with the river at average flow, the average expected concentration of narasin in the river would be about 0.0011 mg/L:

Calculation:

$$\frac{17 \text{ kg narasin/day} \times 1000 \text{ g/kg}}{6383 \text{ ft}^3\text{/sec} \times 28.32 \text{ L/ft}^3 \times 60 \text{ sec/min} \times 1440 \text{ min/day}} = 1.1 \times 10^{-6} \text{ g/L}$$

Continuous discharge of narasin could result in aquatic organisms being chronically exposed to an average narasin concentration of 0.0011 mg/L.

The average expected concentration of narasin during average flow of the Wabash River is substantially below concentrations which can be calculated to have no chronic effects on aquatic organisms. An application factor of 100 can be used with the results from acute studies to extrapolate the concentrations which have no observed effects on the test organisms during chronic exposure. The calculated chronic no-observed-effect concentrations for bluegill, rainbow trout, and daphnids are 0.033 mg/L (3.27 mg/L ÷ 100), >0.014 <0.02 mg/L (>1.4<2.0 mg/L ÷ 100), and 0.077 mg/L (7.72 mg/L ÷ 100), respectively. These concentrations are between 12.7 and 70 times higher than the average expected concentration of narasin in the Wabash River.

The highest expected concentration of narasin and the average expected concentration of narasin that might be found in the Wabash River appear to be acutely and chronically safe to aquatic organisms. Even untested aquatic organisms, which could be somewhat more sensitive to narasin than those organisms tested, should be safe.

Residual biodegradable fermentation nutrients from the manufacture of other fermentation products at each of the plant sites are discharged to receiving waters at rates significantly below permitted limitations. Since narasin will not be the only fermentation-based product manufactured at the plant sites, it will account for only a small portion of the permitted discharge of residual nutrients expressed as biological oxygen demand (BOD).

Essentially no other wastewater pollutants or liquid, solid or gaseous pollutants from the manufacture of narasin will be allowed to enter the environment. Therefore, the manufacture of narasin will have a minimal effect on the environment at any of these plant sites.

Limitations for atmospheric pollutant emissions and wastewater pollutant discharges, and disposal practices for other liquid and solid wastes applicable to the Indiana plant sites, are defined by regulations administered by the U.S. Environmental Protection Agency (EPA) and, in certain instances, by Indiana's Air Pollution Control Board (APCB), Stream Pollution Control Board (SPCB), and Environmental Management Board (EMB).

The following list shows the operating permits issued by the APCB for those manufacturing and emission control facilities which would produce narasin and manufacture MONTEBAN at two of the Indiana plants.

Location	Permit Identification No.	Issued	Expiration
Clinton	83-09-87-0067	Dec. 13, 1983	Sept. 1, 1987
Clinton	83-09-87-0068	Dec. 13, 1983	Sept. 1, 1987
Clinton	83-09-87-0073	Dec. 13, 1983	Sept. 1, 1987
Lafayette	79-01-86-0264	Mar. 22, 1982	Jan. 1, 1986*
Lafayette	79-01-86-0277	Mar. 22, 1982	Jan. 1, 1986*

*(These permits are being extended administratively until issuance of the new permits, which is pending.)

The SPCB has issued the following NPDES permits for the discharge of wastewaters from the Indiana plants to the Wabash River.

Location	NPDES Permit No.	Issued	Expiration
Clinton	IN 0002852	September 23, 1985	August 31, 1990
Lafayette	IN 0002861	Issuance Pending*	

*(Previous permit is being extended administratively until the new permit is issued.)

On July 25, 1983, the Indianapolis Department of Public Works issued a permit, No. 283004, for the discharge of wastewaters from Lilly's Kentucky Avenue fermentation facility to the municipal sewer system for treatment. This permit expires on July 31, 1986. Emissions to the atmosphere from this facility would be too low to require a permit.

Limitations for atmospheric pollutant emissions from Nebraska plant sites are defined by regulations promulgated by the U.S. EPA and by Nebraska's Environmental Control Council. Under authority granted by Nebraska to the city of Omaha, the latter's Air Quality Control Division has issued certificates of approval limiting particulate matter emissions to the atmosphere from existing facilities which would be used to formulate and package MONTEBAN at Omaha Laboratories of Eli Lilly and Company. These certificates are as follows:

Certificate No.	Issued	Expiration
2440/CR23545	March 3, 1971	Not Stated
25331/CR95881	January 27, 1982	Not Stated

The Nebraska Department of Environmental Control has provided letter authorization with no stated expiration for Omaha Laboratories of Eli Lilly and Company to dispose of packaging materials and animal health products,

such as antibiotics in feed premixes, by landfilling. No other environmental permits are required for formulating and packaging MONTEBAN at Omaha Laboratories.

No hazardous wastes and essentially no solid wastes will be generated in the manufacture of MONTEBAN. Packaging materials, nonrecyclable tailings and floor sweepings from the Indiana plants would be incinerated at the Clinton plant with industrial and domestic trash from other sources or would be landfilled. Manufacture of narasin at the production facility at Liverpool, England, will comply with all the pertaining environmental control laws of the United Kingdom.

Based on the information above, any atmospheric emissions, wastewater pollutant discharges and disposal practices for other wastes from the manufacturing process for narasin will comply with appropriate statutes, regulations, and permits.

C. INTRODUCTION OF SUBSTANCE FROM THE FEED MIXING LOCATIONS

Virtually all feed mixing would be done by broiler production companies that use MONTEBAN. In order for a feed mixing location to obtain MONTEBAN, it would be necessary to have on file an FDA approved form FDA-1800 and comply with current Good Manufacturing Practices for medicated feeds. With the required manufacturing controls for feed, inventory accountability, and quality assurance procedures, the potential for release of narasin to the environment at these locations would be negligible.

D. INTRODUCTION OF SUBSTANCE FROM THE USE SITE

Virtually all of the broiler chickens produced in the U.S. receive an anticoccidial drug continuously in the feed to prevent or control coccidiosis. Anticoccidial drugs similar in chemical nature to narasin (e.g. monensin, lasalocid and salinomycin) are approved for use. All are produced as mycelial products.

Most broiler chickens are produced in the states of Alabama, Arkansas, California, Delaware, Georgia, Maine, Maryland, Mississippi, North Carolina, Pennsylvania, Texas, and Virginia. Virtually all broiler production is concentrated in approximately 50 companies. MONTEBAN would be marketed directly to these companies. This would minimize potential environmental exposure during the product distribution process.

A broiler chicken could eat up to 3.4 kg of medicated feed with a maximum of 80 mg of narasin activity per kilogram of feed, or a total of 272 mg of narasin activity. The United States Department of Agriculture statistics show that there are approximately 4 billion broilers produced in the U.S. annually. If all of these 4 billion broilers were fed MONTEBAN, as much as 1.09×10^6 kg of narasin activity (272 mg/broiler x 4×10^9 broilers) could be used in a year. This amount of narasin is equivalent to 10.9×10^6 kg of MONTEBAN (with 100 g of narasin activity per kilogram). A more likely market pentetration of 25% would result in the use of 0.272×10^6 kg of narasin activity and about 2.72×10^6 kg of MONTEBAN (with 100 g of narasin activity per kilogram).

Narasin is found in chicken excreta and may be introduced into soil by use of chicken litter as fertilizer. Results from oral dosing of chickens with ^{14}C narasin indicate that narasin is extensively metabolized to 20 or more metabolites (Appendix C) and that only about 5% of the narasin dose is

excreted as parent narasin (Appendix D). Chickens which were fed 80 ppm of ^{14}C narasin had a concentration of 237 ppm total radioactivity (dry matter basis) in the excreta. Only 12 ppm was parent narasin. Most of the narasin metabolites were in concentrations too low to be purified and identified. However, seven metabolites, which comprised about 25% of the radioactivity in excreta from chickens fed ^{14}C-narasin, were isolated and six of these were identified as di- or trihydroxylated narasin congeners (Appendices D and E).

1) Biological Evaluation of Narasin Metabolites in Chicken Excreta

Comparative metabolism of narasin by chickens and rats demonstrated that the narasin metabolite pattern was qualitatively similar for the two species (Appendix C). By inference, the toxicology of narasin metabolites present in chicken excreta has been evaluated in studies in which rats were exposed to narasin. There were more than 20 metabolites in chicken excreta and rat feces. Six hydroxylated narasin metabolites were evaluated with the same thin-layer bioautography technique used to assay narasin activity and they were found to have essentially no antimicrobial activity (Appendix E). The antimicrobial assay for chicken excreta, which contains all identified and unidentified metabolites, showed antimicrobial activity which would occur from the known narasin level alone, indicating that narasin metabolites did not contribute significantly to the biological activity (Appendix D).

Narasin is classified as an ionophore by virtue of its ability to enhance the transport of monovalent metal cations across cellular membranes. Narasin metabolite preparations were evaluated for ionophoric activity as measured by ATPase activity and oxygen uptake in rat liver mitochondria.

The metabolites were essentially inactive (e.g., 200 times less active than narasin) (Appendix F).

Although about 95% of the narasin fed to chickens is excreted as metabolites, these metabolites have a very low level of biological activity. Based on this very low level of biological activity, metabolites of narasin need not be considered in the estimation of environmental concentrations of narasin.

2) Occurrence of Narasin in Litter

In the course of conducting environmental studies, assays for narasin were performed on four separate lots of litter. One lot of litter from chickens fed 80 ppm narasin contained 8.4 ppm (Appendix G). Three lots of litter from chickens fed 100 ppm narasin contained 9.1, 10.2 and 9.8 ppm (Appendix H). These litter samples represent litter from chickens fed for one two-month grow out. In a broiler production facility, several cycles or grow outs of chickens may be produced on the same litter. Therefore, an estimate of the maximum narasin concentration in litter which may be applied to soil is needed. The narasin concentration in air-dry excreta from chickens being fed 80 ppm narasin was 12.1 ppm (Appendix D). Incorporating a factor of ±25% to allow for biological variation gives approximately 15 ppm, which will be used in this report for the maximum narasin concentration in litter.

6. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

The primary manner in which narasin would be introduced into the environment is through chicken litter collected from a broiler production facility and applied to cropland. Because of its large molecular weight and relatively high melting point and because thermogravimetric analysis showed no volatilization of narasin up to 220°C, narasin is a non-volatile solid. Measurable concentrations of free narasin will not, therefore, occur in the atmosphere. It may be possible, however, to find narasin in soil to which it has been applied, and in adjacent aquatic systems.

A. POTENTIAL CONCENTRATION OF NARASIN IN SOIL

The highest expected initial concentration of narasin in soil can be estimated. The highest expected concentration of narasin in chicken litter is 15 mg/kg. A reasonable estimate of the application rate of chicken litter as fertilizer is 10 tons/A (22.4×10^3 kg/ha). It is standard practice to incorporate litter into the top six inches of soil to avoid loss of nutrients in runoff. A six inch deep soil layer in one hectare weighs about 2.25×10^6 kg. The highest initial concentration of narasin in soil is, therefore, about 0.15 ppm ((15 mg/kg x 22.4×10^3 kg/ha) ÷ 2.25×10^6 kg of soil).

This estimate of the concentration of narasin in soil is consistent with the results of a study in which litter from narasin-fed broiler chickens was incorporated into soil (Appendix I). The study was conducted under exaggerated narasin use and litter incorporation conditions compared to those found in practice, but the results of the study are of value for comparative purposes. The chickens during one grow-out were fed diets

containing 100 ppm of narasin. The resulting wet litter, which contained 9.1 ppm of narasin by assay, was incorporated into the top 7 cm (approximately 3 inches) of soil at a rate of 15 tons/A (33.7 metric tons/ha). This is three times the soil incorporation rate described above (15 tons/A in 3 inches vs. 10 tons/A in 6 inches). The initial assayed concentration of narasin in the soil was 0.38 ppm or 2.5 times the value of 0.15 ppm calculated above. Thus, the above value of 0.15 ppm may be considered a realistic estimate of the highest expected concentration of narasin in soil from the use of chicken litter as fertilizer.

The concentration of narasin in soil would rapidly decline from the highest expected value of 0.15 ppm, which might occur directly after application of chicken litter to soil. Studies with crystalline narasin mixed in soil show a rapid decline in narasin activity. The half-life of crystalline narasin in soil, due to degradation under greenhouse conditions, was 8.8 days. Degradation of narasin was considered to have occurred because dissipation by leaching was not possible in this study and narasin activity rapidly declined in the soil, as measured by microbiological assay (Appendix J). When crystalline narasin was mixed in soil and exposed to field conditions, the dissipation half-life was 4.2 days (Appendix K). Dissipation in this study was also probably due to degradation because a substantial amount of narasin activity disappeared from the soil before any rainfall was noted. When litter was incorporated in soil at concentrations that were three and six times higher than would be found under normal agricultural practices, narasin that was in the litter was found to dissipate with half-lives of 5.6 days and 22 days (Appendix I), respectively. The high concentrations of litter were used in order to have narasin concentrations above the detection level long enough to follow the decline in concentrations over time.

Differences were found in half-life values for narasin activity in soil. The faster rate of decline of crystalline narasin in the field soil may have been due to higher microbial activity, since leaching and runoff did not account for the dissipation of crystalline narasin in the field. When litter containing narasin was incorporated into field soil at three times the level used in normal agricultural practice, the dissipation half-life for narasin was nearly the same as that for crystalline narasin in field soil without litter. When the litter concentration was six times the normal level in soil, the dissipation half-life of narasin increased. Narasin dissipation from field soil was also slower than might have been expected in a crop safety study (Appendix Y), where very high levels of litter were spread on the surface of field soil and eventually incorporated to a shallow depth.

Narasin degrades and dissipates rapidly when incorporated in soil that has normal and even somewhat atypically high concentrations of litter. Because of the relatively rapid decline of narasin activity in agricultural soil, nontarget organisms would presumably be exposed to narasin for only a short period of time.

B. POTENTIAL CONCENTRATION OF NARASIN IN AQUATIC SYSTEMS

1) Surface Water

It is possible that runoff water from heavy rainfall could carry some narasin into surface waters which contain aquatic organisms. Because narasin concentrations rapidly decline in soil, a runoff event would have to occur directly after application of chicken litter to soil in order for narasin to reach surface water. An estimate of 0.5% has been made for the loss of soil-incorporated pesticides from application sites during a season into runoff water (3,4). If a large, early runoff event occurs, as much as

three times this loss might be found (3,4). This amount of loss may also serve for narasin incorporated in soil with chicken litter.

A maximum expected concentration of narasin in surface water could be estimated using a 40 acre (16.2 hectares) watershed with a 2.5 acre pond (average depth, 2.5 ft) as the surface water receiving runoff (5). As has been demonstrated, the highest expected concentration of narasin in soil comes from the use of 22.4 metric tons of chicken litter per hectare and, therefore, 336 g of narasin per hectare (22.4×10^3 kg/ha x 15 mg/kg). The 16.2 hectare watershed would contain, at most 5.44 kg of narasin. The pond would contain 7.71×10^6 liter of water (6.25 acre ft x 43,560 ft^3/acre ft x 28.32 liters/ft^3). The losses of narasin from the watershed over a growing season could be as high as 82 g (5.44 kg x 1.5%). If the losses of narasin from an entire season were available in the pond at one time, a maximum expected concentration of narasin in the pond would be 0.0106 ppm (82 g/7.71×10^6 L). Even if it were possible for all of the narasin in this watershed (5.44 kg) to be instantaneously dispersed in the pond, the concentration of narasin would only be 0.706 ppm (5.44 kg/7.71×10^6 L).

Narasin would probably not persist in natural bodies of water for any significant length of time due primarily to its susceptability to photolysis. Narasin has been shown to have a half-life of about 1.5 days when a buffered (pH 7.0) solution of narasin is exposed to ultraviolet light in a laboratory (Appendix L). Narasin may also hydrolyze in more acidic waters. Narasin is stable in aqueous buffered solution at pH 7.0 and 9.0, but it has a half-life of only 3.5 days at pH 5.0 (Appendix L). Concentrations of narasin in test solutions used in aquatic toxicity tests were probably stable because of their slightly basic pH and because the flourescent light in the aquatic laboratory has little ultraviolet light. The intensity of

any ultraviolet light in the flourescent light during the toxicity tests would have been reduced by light diffusers and the glass containers for the test solutions.

Based on the episodic distribution of narasin to surface water from runoff and the short half-life of narasin in water, any exposure of non-target aquatic organisms to narasin can be presumed to be of a short duration.

2) Fate of Narasin in Aquatic Organisms

Aquatic organisms could be exposed to low levels of narasin for short periods of time when runoff occurs from surrounding agricultural fields. Therefore, the potential for organisms such as fish to bioaccumulate narasin from water has been considered.

The octanol-water partition coefficients (K_{ow}) of organic compounds are often predictive of the potential for their bioaccumulation when the compounds are not readily metabolized and excreted. The relatively high K_{ow} of 7×10^4 indicates that, theoretically, narasin has the potential for bioaccumulation.

From a practical standpoint, the metabolism and tissue residue data support the conclusion that narasin would not bioaccumulate significantly. Narasin is biodegradable since it is extensively metabolized by chickens, rats (Appendix C) and cattle (Appendix M) and is rapidly degraded in soil (Appendices J and K). Cattle given oral doses of ^{14}C narasin equivalent to 18 g/ton of feed achieved steady-state concentrations of radioactivity in tissues after about 3 days dosing. Liver contained less than 1 ppm total radioactivity (mean of three animals) and fat contained less than 0.1 ppm (Appendix M). Only about 8% of the liver radioactivity was parent narasin

at zero withdrawal. Similar studies in chickens fed 100 ppm ^{14}C narasin indicate that maximum tissue residues were achieved within four days dosing. Liver contained approximately 0.5 ppm and fat approximately 0.25 ppm total radioactivity at zero withdrawal. Less than 10% of the liver radioactivity and approximately half of the fat radioactivity were parent narasin (Appendix N). After withdrawal of ^{14}C narasin dosing, the parent narasin depleted rapidly (within three days) to nondetectable concentrations (Appendix O). Thus, narasin is not bioaccumulated in the fat of animals and the small concentrations, which do occur while the animal is being dosed, deplete rapidly after withdrawal from treatment.

If aquatic organisms were exposed to the very low levels of narasin in surface water due to runoff from a heavy rainfall event, the exposure would be brief since narasin in solution is susceptible to photolysis and can be susceptable to hydrolysis (Appendix L). Any narasin that might be bioconcentrated during this brief exposure period would probably be rapidly eliminated.

3) Occurrence of Narasin In Groundwater

Mobility of narasin in soil would be low when chicken litter was used as fertilizer under practical conditions. A laboratory study has shown that narasin could be slowly leached with the equivalent of 60 cm of rainfall from certain soil types that would be present in an agricultural area where chicken litter would be incorporated (Appendix P). However, even with moderate mobility in soil, the low initial concentration of narasin in soil and the susceptability to degradation in soil (Appendix J) and acidic water (Appendix L) would render the possibility of narasin contamination of groundwater remote.

7. EFFECTS ON THE ENVIRONMENT OF RELEASED SUBSTANCES

A. MAMMALIAN TOXICITY TESTS

An in-depth testing program has been completed with various laboratory animal species to determine the toxicological properties of narasin and mycelial narasin. Complete reports of all of these studies have been submitted to support the proposed action. Studies which are critical to determine the safety of narasin to the public and the environment are briefly described below. A more complete summary of mammalian toxicology information can be found in a Freedom of Information (FOI) Summary for MONTEBAN (6).

Acute Studies

Oral LD_{50} for ICR Mice: 15.8 mg narasin activity/kg body weight (367 mg dried mycelial narasin/kg body weight) for males and 16.7 mg narasin activity/kg body weight (388 mg dried mycelial narasin/kg body weight) for females

Oral LD_{50} for Wistar Rats: 21.2 mg narasin activity/kg body weight (493 mg dried mycelial narasin/kg body weight) for males and 18.5 mg narasin activity/kg body weight (430 mg dried mycelial narasin/kg body weight) for females

Hazard Evaluation Studies

Guinea Pig Dermal Sensitization: No sensitization with 0.025% crystalline narasin.

Inhalation by Rats: No signs of toxicity found for rats exposed to an aerosol of 9.72 mg narasin activity/M^3 (226 mg dried mycelial narasin/M^3) for 30 minutes.

Ocular Irritation in Rabbits: Severe irritation at 1.72 mg of narasin activity (40 mg dried mycelial narasin). When eyes were rinsed two minutes after exposure, the eye irritation cleared in 48 hours.

Dermal Irritation in Rabbits: No signs of toxicity with 10.75 mg narasin activity/kg (250 mg dried mycelial narasin/kg).

Chronic, Reproduction and Teratology Study

Three-Month Dog Study: No adverse effects at a daily oral dose of 1 mg narasin activity/kg body weight (6.8 mg dried mycelial narasin/kg body weight).

Two-Year Rat Study: No-effect level at a dietary concentration of 15 ppm narasin activity (187.5 ppm dried mycelial narasin).

Rat Multigeneration Reproduction Study: No evidence of reproduction impairment or effect on the offspring at a dietary level of 15 ppm narasin activity (187.5 ppm dried mycelial narasin).

Four Generation Rat Teratology Study: Teratogenic effects were not found at the highest dietary level tested, 30 ppm narasin activity (375 ppm dried mycelial narasin).

Rabbit Teratology Study: No evidence of maternal toxicity with daily oral doses of 0.6 mg narasin activity/kg body weight (7.5 mg dried mycelial narasin/kg body weight) during gestation days 6 through 18 and no evidence of dose-related teratogenic effects up to 1.8 mg narasin activity/kg body weight (22.5 mg dried mycelial narasin/kg body weight).

B. POTENTIAL ADVERSE EFFECTS OF THE PROPOSED ACTION ON HUMAN HEALTH

1) Production of Narasin and Manufacture and Use of MONTEBAN

Production workers would not be exposed to crystalline narasin since narasin is produced as a mycelial product. Mycelial narasin could be produced in only four plants. Engineering controls and personal hygiene precautions are effective in minimizing exposure of workers and users.

Precautionary labeling would advise users to immediately and thoroughly rinse eyes if eye contact is made with MONTEBAN. Considering these measures, the magnitude of the acute LD_{50} values with mycelial narasin, and the fact that in laboratory animals narasin is not a mutagen, teratogen, carcinogen, or a reproductive toxin, it is concluded that workers producing MONTEBAN and users of MONTEBAN would not be adversely affected by the proposed action.

2) Human Exposure to Narasin via the Food Supply

Based on extensive chemistry and toxicology data, the Agency has established a target tissue, marker substance, tolerance, and withdrawal time. Details of these may be found in the Freedom of Information Summary for MONTEBAN (6). Based on this information it may be concluded that the small quantities of residual narasin in food would not cause any adverse effect. It is highly improbable that narasin would occur in groundwater or surface water used for drinking water supplies.

C. EFFECTS OF NARASIN ON NONTARGET ORGANISMS

Studies have been conducted to determine the effects of narasin on nontarget organisms. The results of these studies are summarized below and are listed in detail in the referenced appendices.

Avian Species

Bobwhite quail 14-day acute oral toxicity studies (Appendices Q and DD):

Acute oral studies with mycelial and crystalline narasin and bobwhite quail (Colinus virginianus) have been conducted. In the study with

crystalline narasin, the 14-day LD_{50}, 95% confidence interval, and slope of the dose-response curve for adult male bobwhite were 73.96 mg/kg, 57.41 to 95.33 mg/kg, and 7.44, respectively. The LD_{50} for adult females was > 70 < 100 mg/kg. Male and female mortality levels for mycelial narasin could not be distinguished. For bobwhite dosed with mycelial narasin, the 14-day LD_{50}, 95% confidence interval, and slope of the dose-response curve were 102.9 mg/kg, 46.6 to 227.5 mg/kg, and 1.73, respectively. Dose-related toxic effects included lethargy and ataxia in both studies. Loose feces, ruffled appearance, abnormal posture, and emaciation were also noted for some birds in the study with mycelial narasin. A dose of 6.2 mg/kg was the highest level of narasin activity tested in the study with mycelial narasin which did not result in mortalities, signs of toxicity, or treatment-related reductions in food consumption and body weight.

Bobwhite quail five-day dietary studies (Appendices R and EE): Three five-day dietary studies were conducted with 10 to 15-day old bobwhite quail (Colinus virginianus) and mycelial narasin at nominal dietary concentrations up to 0.500% (w/w). The birds were observed while being fed treated diets for five days, followed by three days of basal diet. Based on nominal dietary concentrations of narasin (assayed levels ranged from 99.2-109% of nominal) in the first study conducted with bobwhite, the five to eight-day LC_{50}, the 95% confidence interval, and the slope of the concentration-response curve were 0.106%, 0.088 to 0.148%, and 3.808, respectively. Based on assayed dietary concentrations of narasin in the second dietary study conducted with bobwhite, the eight-day LC_{50}, the 95% confidence interval, and the slope of the

concentration-response curve were 0.080%, 0.058 to 0.109%, and 3.679, respectively. Based on assayed dietary concentrations of narasin in the third dietary study conducted with bobwhite, the eight-day LC_{50}, 95% confidence limits, and slope of the concentration-response line were 0.063%, 0.046-0.087%, and 3.712, respectively. Based on estimates of total food consumed, the eight-day LD_{50} values (95% confidence limits, slope of dose-response line) for all three studies were 153.7 mg/kg (117.1 to 201.9 mg/kg; 5.127), 575 mg/kg (436 to 758 mg/kg; 4.031), and 270 mg/kg (226 to 322 mg/kg; 6.944). No mortalities or behavioral signs of toxicity were found in any study at a nominal dietary concentration of narasin of 0.005% (assayed as 0.00521%, 0.0048%, and 0.0049% in the three studies). Higher dietary concentrations produced lethargy, loose feces, ataxia, hyperactivity, tremors, wing droop, and mortalities. Concentration-related reductions in food consumption and body weight gain were noted in each study. The nominal dietary level of 0.005% narasin only resulted in a significant decrease in body gain in the third study that was conducted. The body weight gain during the five-day treatment phase of this study was 76% of control when birds were fed a 0.005% (0.0049% assayed) nominal dietary level of narasin. When placed on untreated diet for three days after exposure to a nominal narasin dietary level of 0.005%, body weight gain was 158% of control.

Mallard duck five-day dietary studies (Appendices S and FF): Three five-day dietary studies were conducted with 10-day old mallard ducks (Anas platyrhynchos) and mycelial narasin at nominal dietary concentration up to 0.500% (w/w). The birds were observed while being

fed treated diets for five days, followed by three days of basal diets. In the first study conducted with mallards, nominal narasin dietary concentrations of 0.18% and 0.5% (w/w) resulted in three and four mortalities, respectively, out of 10 birds. No mortalities were found at lower dietary levels of narasin. Based on estimates of food consumption for this study, the highest average total consumption of narasin, 810.8 mg narasin/kg bird, occurred at the highest treatment level. In the second study conducted with narasin, the highest assayed dietary narasin level of 0.4551% resulted in three mortalities out of ten birds. No mortalities were found at lower dietary levels of narasin. Based on estimates of food consumption for the second study, the highest average total consumption of narasin, 2505 mg narasin/kg bird, occurred at the highest treatment level. In the third study conducted with mallards using assayed narasin concentrations, it was possible to calculate the eight-day LC_{50}, 95% confidence limits, and slope of the concentration-response line as 0.38%, 0.259 to 0.557%, and 3.467, respectively. Based on estimates of food consumption, the calculated LD_{50}, 95% confidence limits, and slope of the dose-response line were 2373 mg/kg, 1888 to 2983 mg/kg, and 5.938, respectively. No behavioral abnormalities (lethargy and ataxia) reductions in body weight gain, or mortalities were found in any of the studies for birds that were fed a diet with an assayed narasin level of 0.0205%.

Aquatic Species

96-hour bluegill toxicity studies (Appendices B and Z): Static toxicity tests were conducted to determine the acute effects of crystalline and mycelial narasin on juvenile bluegill. Based on mean measured concentrations of crystalline narasin, the 96 hr LC_{50}, the 95% confidence

limits of the LC_{50}, and the slope of the concentration-response line were 3.27 ppm, 3.04 to 3.55 ppm, and 14, respectively. Based on mean measured concentrations of mycelial narasin in another study, the 96-hr LC_{50}, 95% confidence limit, and slope of the concentration-response line were 5.02 ppm, 4.61 to 5.46 ppm, and about 18.1, respectively. No mortalities or behavioral abnormalities (sluggish movement, hypoactivity, swimming impaired, labored respiration and minimal voluntary movement, and prostration) were found in this study for fish exposed to a measured narasin concentration of 1.66 ppm. No mortalities and no behavioral abnormalities (hypoactivity, minimal swimming behavior, disorientation and/or labored respiration, prostration) were found in another 4 day test with juvenile bluegill at a mean measured concentration of crystalline narasin at 0.58 ppm.

96-hour rainbow trout toxicity studies (Appendices T and AA): Based on nominal concentrations of crystalline narasin, the 96 hr LC_{50} was >1.4<2.0 ppm. No mortalities and no behavioral abnormalities (stressed or prostrate) were found in this study at the nominal narasin concentration of 0.5 ppm. Based on measured concentrations of mycelial narasin in a second study, the 96-hr LC_{50}, 95% confidence limits, and the slope of the concentration-response line were 2.23 ppm, 1.84 to 2.71 ppm, and about 7.0, respectively. No mortalities or behavioral abnormalities (sluggish movement, hypoactivity, swimming impaired, labored respiration and minimal voluntary movement, and prostration) were found in this study at the measured narasin concentration of 0.190 ppm.

48-hour Daphnia toxicity studies (Appendices U and BB): Based on daphnid immobility and nominal concentrations of crystalline narasin, the 48-hr

EC_{50} and the corresponding 95% confidence limits for one acute study with Daphnia magna were 7.72 ppm and 6.84 to 8.72 ppm. The slope of the concentration-response curve was 7.26. No daphnids were found to be immobile nor did any daphnids display abnormal behavior (hypoactivity, prostration) in this study at a nominal narasin concentration of 2.25 ppm (average assayed level of 2.2 ppm). Based on daphnid immobility and measured concentrations of mycelial narasin, the 48-hr EC_{50}, 95% confidence limits, and the slope of the concentration-response line, were 20.56 ppm, 9.19 to 68.1 ppm, and 4.23. Hypoactivity was found in a concentration-related fashion down to the lowest concentration tested in this study, 4.69 ppm, where two daphnids out of 30 were hypoactive and one was immobile.

Terrestrial Species

Earthworm 14-day toxicity studies (Appendices V and CC): Earthworms (Lumbricus terrestris) were exposed for 14 days to nominal soil concentrations of 0.0, 1.0, 5.0, 10.0, 20.0, 40.0, 80.0 and 100.0 ppm of crystalline narasin in study 6018-78, and to concentrations of 0.0, 0.5, 1.0, and 5.0 ppm of crystalline narasin in study 6026-78. Mortalities occurred at exposure levels ≧20.0 ppm and were preceded by weight loss and a decline in physical appearance. Some worms left the test media at these levels. The 3-day, 7-day, and 14-day LC_{50} values for study 6018-78 were >100 ppm, ca 40.0 ppm, and >20.0<40.0 ppm, respectively. In study 6018-78, the following observations were made for worms in soil containing 1.0 ppm of crystalline narasin: day 3, one soft and flaccid worm; day 7, one flaccid worm and another soft and flaccid worm; day 14, one flaccid worm. In this same study, a

reduction in body weight gain was found at 1.0 ppm. In study 6026-78, no mortalities, no reduction in body weight gain, and no changes in physical appearance were found for worms exposed to nominal crystalline narasin concentrations of 0.5 and 1.0 ppm. In study 6026-78, all worms in soil containing 5.0 ppm of narasin were flaccid or soft and flaccid throughout the study and these worms did not gain weight like control worms. The concentration of 0.5 ppm of crystalline narasin in soil did not result in mortalities, reduction in body weight gain, or change in the physical appearance of the worms tested. In another study (W00783) with mycelial narasin, earthworms were exposed to nominal narasin concentrations of 4.5, 10.0, 22.5, 45.0, and .100 ppm. The 14-day LC_{50}, 95% confidence limits, and slope of the concentration-response line for mycelial narasin in this study were 17.9 ppm, 13.2 to 23.6 ppm, and 4.11, respectively. Mycelial narasin affected the physical condition of the worms in a concentration-related manner. Four of the 15 worms exposed to 4.5 ppm of narasin were flaccid. Worms at the 4.5 ppm level did gain about the same amount of weight as control worms and none of the worms at this treatment level died.

Nitrogen-fixing microorganisms (Appendix W): In general, narasin is active in vitro against gram-positive bacteria. In these experiments, the effect of narasin was studied on two gram-negative microorganisms: the bluegreen alga, Anabaena flos-aquae, and a bacterial heterotroph, Azotobacter chroococcum. Growth of the alga was determined by chlorophyll-a content. Optical densities were used to define growth of A. chroococcum. Nitrogenase activity (nitrogen fixation) was measured in both species by acetylene reduction to ethylene.

The highest level of narasin tested against the alga was 1 ppm. There was no effect on growth at this concentration. Nitrogenase activity in the treated systems paralleled nontreated controls but did not reach maximum levels exhibited by nontreated controls. After 48 hours, activities in controls and treated systems were reduced presumably due to nutrient depletion.

The results of the azotobacter studies were more variable. Both growth and acetylene were generally reduced with narasin concentrations of 1 and 10 ppm. In no experiment did narasin exhibit bacteriocidal properties (i.e., complete inhibition). Even at 10 ppm, azotobacter growth remained at 70 percent of controls. No growth reduction was observed at 0.1 ppm.

Phytotoxicity with narasin and chicken litter containing narasin: Narasin was incorporated into soil at levels of 0.15, 1.5, 10, and 40 ppm. Standard greenhouse phytotoxicity tests were run using 14 common species of mono- and dicotyledonous plants (Appendix X). Narasin at 0.15 ppm caused no phytotoxic effects in the test plants. At a level of 1.5 ppm, cucumbers, tomatoes, peppers, barley, soybeans, grain sorghum, and wheat were not affected and alfalfa, fescue, rice, cotton, corn, sugar beets, and oats showed some limited, noncritical stunting. At levels of 10 and 40 ppm, severe phytotoxic effects occurred in all species.

In a second study (Appendix Y), field plots were manured at 67.4 metric tons/ha with litter (containing 9.8 ppm of narasin) from pens of broilers grown on ration containing 100 ppm of narasin, as well as with litter from pens with broilers that received no narasin (control

plots). The litter was applied evenly to the surface of the field plots in December, the plots were tilled to a depth of 10 cm the following May, and in June seeds of alfalfa, corn, cotton, fescue, oats, rice, and sugar beets were planted in the plots. Evaluation of the resulting plants occurred in July and again in August. No phytotoxicity occurred. The stand and quality of growth of all plants and weeds were similar in the narasin and control plots.

D. POTENTIAL ADVERSE EFFECT OF THE PROPOSED ACTION ON AQUATIC AND WILDLIFE ORGANISMS

1) Potential Adverse Effects on Aquatic Organisms

The occurrence of narasin in surface water systems is expected to be acute and episodic, depending on runoff from watersheds fertilized with chicken litter containing narasin. The half-life of narasin in water due to photolysis is 1.5 days and narasin activity disappears in acidic water. A rapid decline of narasin activity in water means that there is little possibility that aquatic organisms would be chronically exposed to narasin. The safety of aquatic organisms can then be assessed by comparing the maximum expected concentration of narasin in an aquatic system to the results of acute studies with aquatic organisms.

In Section 6B(1), a maximum expected narasin concentration of 0.0106 ppm was calculated for a pond. The 96-hr LC_{50} values for rainbow trout and bluegill and the 48-hr EC_{50} value for daphnids range from 1.4 to 7.72 ppm. These acute median lethal and acute median effect concentrations are 132 to 728 times higher than the maximum expected narasin concentration of 0.0106 ppm. In acute laboratory studies, no mortalities or behavioral

abnormalities were found for fish or daphnids at 0.19 ppm, approximately 18 times the maximum expected concentration of narasin in a pond. Even if it were possible for all of the narasin in the fertilizer used in a watershed to be dispersed in a pond (Section 6B(1)), the concentration of narasin would only be 0.706 ppm, resulting only in sluggish or hypoactive behavior of fish.

Based on the low value of the maximum expected narasin concentration in water (0.0106 ppm) and the rapid disappearance of narasin activity from water (half-life about 1.5 days in a laboratory test), the proposed action would not be expected to have an adverse effect on aquatic organisms.

2) Potential Adverse Effects on Earthworms

The 14-day LC_{50} for earthworms exposed to crystalline narasin was between 20.0 and 40.0 ppm. Concentrations of crystalline narasin from 20.0 ppm down to about 1.0 ppm caused reduced body weight gain and change in physical appearance of earthworms in 14 days. In another study, crystalline narasin concentrations at 1.0 ppm and lower did not cause these effects. In a third study with mycelial narasin, the LC_{50} was 17.9 ppm. At the lowest concentration in this test, 4.5 ppm, about 27% of the worms were flaccid and the rest were normal. Narasin at a concentration of 0.5 ppm in soil did not result in mortalities, a reduction in body weight gain, or a change in the physical appearance of the worms tested.

The maximum expected concentrations of narasin in chicken litter and in agricultural soil were estimated to be 15 ppm (Section 5C(2)) and 0.15 ppm, respectively (Section 6A). Narasin concentrations in agricultural soil decline rapidly (Section 6A) in the greenhouse ($t_{\frac{1}{2}}$=8.8 days) and in the field ($t_{\frac{1}{2}}$=4.2 or 5.6 days). Very heavy manuring rates, atypical in normal

agricultural practice, may increase the half-life of narasin in soil ($t_{\frac{1}{2}}$=22 days).

If earthworms do not migrate from piles of chicken litter containing the maximum expected concentration of narasin, their physical appearance and weight gain would probably be affected, and some may die. Based on a low initial concentration of narasin in soil (0.15 ppm is the highest expected initial concentration) and the rapid dissipation rate of narasin in field soil, use of chicken litter on agricultural fields would not be expected to cause earthworm mortality or change the weight gain or physical appearance of earthworms.

3) Potential Adverse Effects on Avian Species

No mortality, significant reduction in body weight gain, change in appearance, or change in behavior occurred for mallard ducks fed diets containing 205 ppm of narasin. No mortality or change in appearance or behavior occurred for bobwhite quail exposed to the lowest dietary level of narasin tested, 49 ppm. Bobwhite quail exposed to 49 ppm narasin did gain significantly less weight and consumed less food than control birds. The birds were observed while being fed treated diets for five days, followed by three days of untreated diet. The dietary LC_{50} values for bobwhite quail and mallard ducks were at least 630 ppm and 3800 ppm, respectively.

The recommended use rates of MONTEBAN in chicken feed would result in a maximum dietary narasin concentration of 80 ppm. Even if wild birds were allowed to only forage chicken feed with the highest expected concentration of narasin for five days, a substantial impact on bird populations would not be expected. The proposed action would not be expected to affect populations of avian species.

4) Potential Adverse Effects on Nitrogen Fixing Organisms

The growth of Anabaena flos-aquae, was not affected by a narasin concentration of 1 ppm, about 94 times the maximum expected concentration in surface water (0.0106 ppm).

A study of the effect of narasin in Azotobacter chroococcum showed some inhibition within the range of 0.1 and 1.0 ppm. Responses within this range were not titrated, but at 0.1 ppm, there was no effect on azotobacter growth. At 1.0 ppm, growth was reduced approximately 15% after 48 hours. At the same time, growth in 10 ppm narasin reached approximately 70% of controls.

A review of microbial susceptibilities (1) indicates a narrow bacterial spectrum. Few aerobic organisms were inhibited above 6.25 ppm. Under anaerobic conditions, some gram-positive organisms were inhibited by narasin below 8 ppm.

Present data would indicate that narasin had bacteriostatic effects on certain nitrogen-fixing organisms. Bactericidal effects were not observed in vitro. The potential is small for significant effects from narasin on nitrogen-fixing organisms in field soil.

5) Potential Adverse Effects on Plants

No phytotoxicity was observed in a greenhouse study in which alfalfa, fescue, cucumber, rice, cotton, tomato, pepper, corn, sugar beets, barley, soybean, wheat, grain sorghum, and oats were planted in soil containing 0.15 ppm of narasin. This concentration is the same as the estimated maximum expected soil concentration of 0.15 ppm. This study was an exaggerated case since 0.15 ppm is the concentration of narasin immediately after the incorporation of narasin-containing litter in soil and studies have shown narasin activity rapidly dissipates from soil.

No phytotoxicity was observed with oats, sugar beets, corn, cotton, rice, fescue, and alfalfa grown in field plots manured at a rate of 67.4 metric tons/ha with litter from pens of chickens fed diets containing 100 ppm of narasin. This crop safety study showed that even when a very high manuring rate was used during the winter with litter that contained a moderate amount of narasin, phytotoxic effects were not found in summer crops. Narasin activity dissipated from soil and only a very low concentration of narasin was in the soil when the crops were grown.

8. UTILIZATION OF NATURAL RESOURCES AND ENERGY

Manufacturing MONTEBAN will require an amount of energy similar to that used to produce and package any conventional fermentation product for animals. Disposal of waste washwater and materials from the manufacturing process will not require use of unusual amounts of energy or natural resources.

9. DISRUPTIONS OF THE PHYSICAL ENVIRONMENT

Manufacture of MONTEBAN will occur at facilities already designed for production of fermentation materials. Unusual levels of noise, odors, construction, or other disruptions should not be required in the manufacture of this product.

10. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. The MONTEBAN label will instruct users that if accidental eye contact occurs with MONTEBAN, the eye should be rinsed immediately and thoroughly with water. The label will also instruct users to wear protective clothing, impervious gloves, and a dust mask when mixing and handling MONTEBAN. The user is instructed to wash thoroughly with soap and water after handling MONTEBAN. The label will also indicate that adult turkeys and horses or other equines must not be allowed access to formulations containing MONTEBAN. Ingestion of MONTEBAN by equines has been fatal. Other than these precautions listed on the label, no mitigation measures are necessary for MONTEBAN.

11. ALTERNATIVES TO THE PROPOSED ACTION

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. Therefore, alternatives to the proposed action do not need to be considered.

12. LIST OF PREPARERS

The following Lilly personnel are responsible for the preparation of this Environmental Assessment:

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13. CERTIFICATION

The undersigned official certifies that the information presented in the Environmental Assessment is true, accurate, and complete to the best of his knowledge.

Merle E. Amundson, Ph.D.
Executive Director
Toxicology Division
Lilly Research Laboratories

January 20, 1987
Date

14. REFERENCES

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6. Freedom of Information Summary for MONTEBAN. 1985. Elanco Products Company. This document is available for review at: Dockets Management Branch (HFA-305), Food and Drug Administration, Room 4-62, 5600 Fishers Lane, Rockville, Maryland 20857.

APPENDIX A: Report Summary

Title: Characterization of Narasin Mycelial Product

Study Dates: February 1976 to October 1976

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Mycelial Narasin

Test System: Chromatographic Separation

Summary:

Three lots of narasin mycelial product, whole dried broth, were fractionated by TLC. Narasin and the non-narasin moieties were separated and recovered. These fractions were examined quantitatively by a vanillin colorimetric procedure and by microbiological turbidimetry.

Results of these studies show that narasin accounted for at least 96.5 percent of the vanillin-positive material and 98 percent of the antimicrobial activity.

Other vanillin-positive compounds were extracted from the mycelial product. The most abundant moiety was factor "I" which is inactive in standard microbiological tests. Factor "I" singly contributed more than 30 percent of the colorimetric vanillin reaction of the non-narasin fraction.

The data demonstrate that narasin-like compounds in the mycelial narasin products are of no practical consequence when measured by chemical or microbiological means.

APPENDIX B: Report Summary

Title: The Acute Toxicity of Crystalline Narasin (Compound 79891) to Bluegill (Lepomis macrochirus) in a Static Test System.

Name and Address of Investigators: D. W. Grothe and P. C. Francis, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140.

Study Dates: September 13 to September 17, 1982 and September 27 to October 1, 1982

Study Numbers: F08182 and F12182

Test Article: Crystalline narasin

Lot Number: X-24458, contains 100% narasin activity

Test Species: Bluegill (Lepomis macrochirus)

Experimental Design:

Study F08182: Groups of ten juvenile bluegill, 0.51 to 1.04 g per fish, were exposed to each of the following average assayed narasin concentrations for 96 hrs: 0.0 (water and acetone controls), 0.82, 1.04, 1.34, 1.38, 2.37, 2.68, 2.87, 3.11, 3.53 and 3.92 mg/L. Jars with 15L of test or control solution were used to contain each group of ten fish. Dissolved oxygen concentration, pH and temperature were recorded daily for each jar. Total hardness, total alkalinity, and conductivity were recorded once for the dilution water. Behavioral signs of toxicity (hypoactivity, minimal swimming behavior,disoriented and/or labored respiration, and prostration) and mortalities were noted for fish in each jar on a daily basis.

Study F12182: This study was conducted using the same experimental design as that used for Study F08182. Average assayed narasin concentrations in this study were lower than those used in Study F08182. The average assayed narasin concentrations used in Study F12182 were 0.0 (water and acetone controls), 0.24, 0.38, 0.58, 0.76, 0.92 and 1.52 mg/L.

Results:

Study F08182: Narasin concentrations were stable throughout this study, presumably because of the slightly basic pH of the water and very low ultraviolet light levels. Narasin dissipates rapidly in water when exposed to ultraviolet light. The flourescent lights in the laboratory produce only low levels of ultraviolet light. The light diffusers and glass containers for the test solutions would further reduce the ultraviolet light levels during this test. The ranges in the water quality characteristics were as follows: pH, 7.7 to 8.6; dissolved oxygen, 8.4 to 9.9 mg/L; temperature, 20 to 21.5°C; total hardness, 137 mg/L (as $CaCO_3$); total alkalinity, 146 mg/L (as $CaCO_3$); and conductivity, 230 µmhos/cm. By the end of this study, at least one fish had died in concentrations as low as 2.68 mg/L. Hypoactivity was noted at the lowest test concentration (0.82 mg/L) within 48 hrs, but by the end of the

APPENDIX B (continued)

study, the behavior of the fish in the two lowest concentrations was normal. Behavior of fish at the end of this study in concentrations ≧1.34 mg/L ranged from hypoactive to prostrate in a concentration related fashion. The 96 hr LC_{50}, 95% confidence limits, and slope of the concentration-response curve for this study were 3.27 mg/L, 3.04 to 3.55 mg/L, and 14, respectively.

Study F12182: Test concentrations were stable throughout the study, presumably due to a low level of ultraviolet light that could reach the test solutions. Narasin dissipates rapidly in water when exposed to ultraviolet light. The ranges in the water quality characteristics were as follows: pH, 8.4 to 8.5; dissolved oxygen, 8.2 to 9.2 mg/L; temperature, 20°C; total hardness 120 mg/L (as $CaCO_3$); total alkalinity 145 mg/L (as $CaCO_3$); and conductivity 225 µmhos/cm. One fish died in this study at the highest concentration tested (1.52 mg/L). Fish in narasin concentrations ≦0.58 mg/L displayed normal behavior throughout the 96-hour study. Fish in narasin concentrations of 0.76 and 0.92 mg/L displayed hypoactive behavior throughout the study. By the end of the study, fish exposed to a narasin concentration of 1.52 mg/L were displaying minimal swimming behavior. No mortalities or behavioral abnormalities were found for bluegill exposed to narasin concentrations ≦0.58 mg/L.

APPENDIX C: Report Summary

Title: Comparative Metabolism of ^{14}C Narasin in the Chicken and the Rat

Study Dates: January 1975 to July 1977

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, A Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Narasin

Test System: Broiler chickens and laboratory rats

Summary of Experimental Design:

Excreta was collected from chickens which were dosed in pilot tissue residue studies and feces were collected from rats dosed for comparative studies. These excreta samples and selected chicken tissues were processed to determine distribution of radioactivity and chromatographic characteristics of metabolites.

Summary of Results:

^{14}C Narasin was extensively metabolized by both the chicken and the rat. More than 20 metabolites were produced by both species. Qualitatively, the metabolite pattern was similar for the two species, but there were some quantitative differences. The most abundant metabolites were a group of three which were designated NM1, NM2, and NM3.

Exhaustive chromatographic clarification of chicken excreta extracts produced a sufficiently concentrated sample of NM3 for mass spectrometric analysis. This compound was tentatively identified as the sodium salt of dihydroxynarasin.

Fractionation of radioactivity in the liver of ^{14}C narasin-fed chickens indicated the presence of numerous radiolabeled metabolites, and no single metabolite accounted for more than 5 percent of the liver radioactivity. The most abundant compound present was unchanged ^{14}C narasin which represented approximately 8.8 percent of the total radioactivity. The metabolite pattern in liver was qualitatively similar to that in chicken and rat excreta. Metabolites NM1, NM2, and NM3 were identified by TLC comparison with excreta metabolites.

APPENDIX D: Report Summary

Title: Chemical and Radiochemical Characterization of ^{14}C Residues in Excreta from Chickens Dosed with Ration Containing 80 ppm ^{14}C Narasin

Study Number: ABC-0260

Study Dates: December 1983 to March, 1984

Name and Address of Investigators: J. A. Manthey, R. J. Herberg, and D. D. Giera, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Narasin

Test System: Broiler Chickens

Summary of Experimental Design:

Male and female broiler chickens were fed a ration containing 80 ppm ^{14}C narasin for seven days. Excreta produced during the fourth through seventh day were collected, air-dried, ground in a laboratory mill to give a homogenous sample, and then frozen. The sample was assayed for total radioactivity by combustion analysis with liquid scintillation counting and for parent narasin by HPLC and microbiological assay. Relative quantities of five metabolites were estimated from column and thin-layer chromatography data.

Summary of Results:

Total radioactivity in the air-dried excreta was equivalent to 237 ppm ^{14}C narasin equivalents. Parent narasin assay values were 12.1 ppm by HPLC and 11.5 by microbiological (turbidimetric) assay. Thus, parent narasin accounted for only about 5% of the total radioactivity. The good agreement between HPLC and microbiological assays indicates that the antimicrobial activity is accounted for by parent narasin and the narasin metabolites have no appreciable antimicrobial activity in this assay system. Column and thin-layer chromatography of an extract of the excreta gave the array of narasin metabolites which has been reported in other studies. The primary metabolites NM-1, NM-2, NM-3, NM-4, NM-5, NM-6 and NM-7 were estimated to constitute approximately 25% of the excreta radioactivity.

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APPENDIX E: Report Summary

Title: Isolation and Characterization of Narasin Metabolites Derived from Excreta of Orally Dosed Chickens

Study Number: Reference No. 636-18C-3565-280

Study Report Dates: November 1976 to November 1982

Name and Address of Investigators: J. A. Manthey, M.S. and G. V. Goebel, B.A., Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Narasin

Test System: Excreta from broiler chickens

Summary of Experimental Design:

Excreta from broiler chickens which had been fed a ration containing 100 ppm ^{14}C narasin was extracted with methanol to recover ^{14}C narasin and metabolites. The extract was subjected to fractionation by liquid-liquid partitioning, silica gel column chromatography, thin-layer chromatography and reversed phase HPLC to characterize the distribution of narasin metabolites. Specific metabolite fractions were purified and subjected to analysis by mass spectrometry.

Summary of Results:

Six labeled narasin metabolites were isolated from chicken excreta as described above. The metabolites were found to be di- or trihydroxylated narasin in which the hydroxy groups were substituted for hydrogen in various positions on the rings of the narasin molecule. Thus, in chickens a primary mode of narasin metabolism is hydroxylation of the narasin molecule.

All six metabolites were evaluated by thin-layer bioautography to determine their antimicrobial activity relative to narasin. The metabolites were inactive, (detection limit for each metabolite was 5% activity when compared to equal amounts of narasin).

APPENDIX F: Report Summary

Title: Effect of Narasin Metabolites on ATPase and Oxygen Uptake in Rat Liver Mitochondria

Report Completion Date: September, 1982

Name and Address of Investigator: D. T. Wong, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Narasin metabolite preparations from chicken and cattle excreta

Test System: Isolated rat liver mitochondria

Summary of Experimental Design:

Four narasin metabolite preparations were evaluated for determination of ionophorous properties by measurement of their effects on ATPase activity and oxygen uptake in rat liver mitochondria.

Summary of Results:

All four of the metabolite preparations were 200 times less active as ionophores than narasin.

APPENDIX G: Report Summary

Title: Greenhouse Test for Phytotoxicity with Litter from Narasin-Fed Chickens

Study Number: B79-3388-218

Study Dates: January 1975 to March 1975

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Floor pen litter from chickens fed 40-80 ppm narasin

Test System: Plants grown from seed in greenhouse flats containing soil-manure mixtures

Summary of Experimental Design:

Floor pen litter containing 5.2 ppm or 8.4 ppm of narasin was collected from chickens which were fed rations containing 40 ppm or 80 ppm of narasin, respectively. This litter was incorporated into soil in amounts corresponding to 1, 2.5, and 5 tons of manure per acre. Fourteen mono- and dicotyledonous crops were planted as seeds in greenhouse flats prepared using this soil. The crops were alfalfa, fescue, cucumber, rice, pepper, cotton, tomato, corn, sugar beet, barley, soybean, wheat, sorghum and oats. The plant growth was evaluated 24 days later. Comparison was made with similar flats prepared from soil which was manured with litter from unmedicated chickens.

Summary of Results:

Growth and development of plants from the treated plots were the same as from the control plots. There was no narasin-related phytotoxicity to the plants.

APPENDIX H: Report Summary

Title: Environmental Studies with Narasin

Report Completion Date: July, 1977

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Summary:

This document is a collection of reports on soil degradation, phytotoxicity, and leaching studies with narasin. Summaries of the pertinent individual studies are reported in other appendices of this Environmental Assessment. Three lots of litter from pens of chickens fed 100 ppm narasin were assayed for narasin concentration by microbiological assay. Narasin concentrations in these three lots were 9.8, 9.1, and 10.2 ppm.

APPENDIX I: Report Summary

Title: Decline of Narasin in Field Soil Manured with Litter from Narasin-Fed Chickens

Study Number: Q61-3452-184

Study Dates: May 1976 to July 1977

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Litter from narasin-fed chickens

Test System: Field soil plot

Summary of Experimental Design:

This study was conducted to quantitate the dissipation of narasin activity from field soil where it can be found when chicken litter is applied as fertilizer. Litter from chickens fed 100 ppm narasin was incorporated into the top 7 cm of soil at application rates of 33.7 and 67.4 metric tons/ha. Because the litter was only incorporated to about half of the normal soil depth, the final concentration of litter in the soil was up to six times higher than levels that would be found in normal agricultural practice. These soil concentrations of litter were used to get soil concentrations of narasin high enough to assay, with dissipation over time. The litter incorporated into the field soil contained 9.1 ppm of narasin, as determined by microbiological assay. Narasin can be extracted from soil with methanol and the extraction efficiency with a spiked standard was used to calculate low concentrations of narasin in soil over time. Residue data from the two field plots were fitted to a line using a non-linear least-squares procedure for regression analysis and the model, $C_t = C_0 e^{-rt}$. The rate constant, r, derived from each regression was used to calculate the dissipation half-life for narasin for each manuring rate.

Summary of Results:

Narasin did dissipate from soil when applied in chicken litter even when soil concentrations were atypically high (Table 1). Narasin dissipated faster from soil when the litter concentration in the soil was only three times the normal level. When the litter concentration in the soil was six times the normal level, the dissipation half-life was longer and the residue data was more varied (R^2=0.65). Since the two manuring rates were used on field plots near each other and since the residue samples were taken at the same time, soil type and weather changes probably do not explain the differences seen in dissipation half-lives in this study. Atypically high levels of litter in soil may have extended the dissipation half-life of narasin in soil.

APPENDIX I (continued)

TABLE 1

Days After Treatment	Narasin Concentration in Soil (ppm)	
	33.7 tons of manure/ha	67.4 tons of manure/ha
0	0.38	0.71
8	0.13	0.59
16	0.05	0.10
23	N.D.	0.53
30	N.D.	0.25
45	N.D.	0.10-0.20
59	N.D.	0.05-0.10
73	Not assayed	0.05-0.10
106	Not assayed	0.10-0.20
134	Not assayed	0.10-0.20
"r", Dissipation Rate constant (day^{-1})	0.123	0.032
Model correlation coefficient (R^2)	0.99	0.65
Dissipation half-life (days)	5.6	22

APPENDIX J: Report Summary

Title: Decline of Narasin in Greenhouse Soil

Study Number: 276A-3480-22

Study Dates: January 1977 to July 1977

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline Narasin

Test System: Soil flats held under greenhouse conditions

Summary of Experimental Design:

Crystalline narasin dissolved in a methanol solution, was blended with air-dried potting soil in order to record the disappearance of narasin activity from soil over time in a relatively controlled situation. The initial nominal concentration of narasin in the soil was 10 ppm. A soil sample was taken on day 0 to confirm this concentration. An initial assayed value of 9.83 ppm indicated that narasin was readily extracted from the soil. The soil consisted of a 1/1 mixture of sand and Brookston loam and it was not sterilized prior to use. The soil was placed in a plastic-lined metal flat to eliminate the possibility of leaching. The moisture level of the soil was brought to field capacity by adding water. The whole metal flat was then placed in a large plastic bag which was subsequently closed to prevent evaporation of the water. The covered soil was then held in a greenhouse for 41 days. Soil samples were taken on days 11, 26 and 41, air dried and assayed for narasin activity. Residue data from soil samples were fitted to a line using a non-linear least-squares procedure for regression analysis and the model $C_t = C_0 e^{-rt}$. The rate constant, r, was used to calculate the degradation half-life for narasin.

Summary of Results:

Narasin activity rapidly dissipated in the soil (Table 1). Less than 7% of the original narasin activity remained after 26 days and less than 3% remained after 41 days. Because of the experimental design, narasin could not leach out of the sampled soil and a good extraction efficiency was demonstrated on day 0. The dissipation of active narasin from the soil was then presumably due to degradation of narasin into inactive substances, based on microbiological assay.

APPENDIX J (continued)

TABLE 1

Sampling Period (Days after initiation)	Narasin Conc. in Soil (ppm)
0	9.83
11	4.70
26	0.62
41	0.26
"r", Degradation rate constant (day $^{-1}$)	0.079
Model correlation coefficient (R^2)	0.99
Degradation half-life (days)	8.8

004

APPENDIX K: Report Summary

Title: Decline of Narasin in Field Soil

Study Number: Q61-3409-24

Study Dates: May to August, 1975

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline Narasin

Test System: Field soil plot

Summary of Experimental Design:

Crystalline narasin was incorporated into the top 7.6 cm layer of a field soil plot in order to assess the dissipation of narasin from soil. Crystalline narasin was incorporated at a concentration of 10 ppm into a Brookston loam soil type (44% sand, 47% silt, 9% clay). The plot was maintained under ambient field conditions but it was surrounded by an earthen dike which reduced runoff and soil erosion. Field soil samples were taken periodically for determination of narasin by microbiological assay. A deep (6.7-15 cm) soil sample was taken at the last sampling time to detect any leaching that may have occurred. Methods of extraction and assay of narasin were the same for all soil samples so the relative rate of dissipation of narasin from soil could be assessed. Residue data from soil samples were fitted to a line using a non-linear least-squares procedure for regression analysis and the model $C_t = C_0 e^{-rt}$. The rate constant, r, was used to calculate the dissipation half-life for narasin.

Summary of Results:

Narasin dissipated quickly from the soil (Table 1). Within seven days, the narasin concentration in the soil declined to about 24% of the original assayed value. Rainfall was noted 13, 17, 20, 21, 30, 33, 34, 36, 44,and 54 days after the study was initiated. Since no rainfall was recorded at the field site before the eight-day sample was taken, it is unlikely that dissipation of narasin up to that sampling time was due to leaching. Dissipation was probably due to degradation of narasin to a form that was not active, based on microbiological assay. Dissipation of narasin in soil 15, 23, 32, 46 and 56 days after initiation of the study due to leaching cannot be completely disproved. Yet no narasin activity was found in the deep core sample taken on day 56. Also, the dissipation rate of narasin is not biphasic, based on the regression correlation coefficient of 0.99. Therefore, degradation was probably the mechanism of dissipation for narasin activity from the soil through the entire study, as it was in the beginning of the study.

005

APPENDIX K (continued)

TABLE 1

Sampling Period (Days after initiation)	Narasin Conc. in Soil (ppm)
0	5.4
8	1.3
15	0.63
23	<0.25
32	N.D.*
46	N.D.
56	N.D.
"r", Dissipation rate constant ($days^{-1}$)	0.165
Model correlation coefficient (R^2)	0.99
Dissipation half-life (days)	4.2

*Detection limit was 0.05 ppm.

006

APPENDIX L: Report Summary

Title: The Solubility, Hydrolysis, and Photolysis of Narasin

Test Article: Crystalline Narasin

Name and Address of Investigators: A. L. Donoho, G. M. Poole, and S. D. West, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Systems: Laboratory Solubility, Hydrolysis, Photolysis

Experimental Design Summary:

Solubility: An excess of narasin was added to sterile 0.01M pH 7.0 and pH 9.0 buffers and stirred at 25°C. At 24-hour intervals samples were removed, filtered, and assayed.

Hydrolysis: Narasin solutions were prepared in sterile 0.01M pH 5.0, 7.0 and 9.0 buffers, sealed in glass ampoules and stored in the dark at 25°C. Samples were removed and assayed at various intervals up to 30 days.

Photolysis: Narasin solution was prepared in sterile 0.01M pH 7.0 buffer and exposed to a combination of sunlamps and black lights which produced an ultraviolet spectral energy distribution similar to natural sunlight. Samples were withdrawn and assayed at various time periods.

Result Summary:

Solubility: Narasin was soluble in water to a maximum concentration of 102 mcg/ml at pH 7.0 and 681 mcg/ml at pH 9.0 in 24 hours or less at 25°C.

Hydrolysis: Narasin was very stable in water at pH 7.0 and 9.0 but hydrolyzed at pH 5.0 with a half-life of approximately 3.5 days.

Photolysis: Narasin photodegraded in water at pH 7.0 with a half-life of approximately 1.5 days.

007

APPENDIX M: Report Summary

Title: A ^{14}C Narasin Tissue Residue and Comparative Metabolism Study in Cattle

Study Number: ABC-0137

Study Dates: July 27, 1981 to August 31, 1982

Name and Address of Investigators: J. A. Manthey, R. J. Herberg and R. L. Van Duyn, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Narasin

Test System: Beef Cattle

Summary of Experimental Design:

Hereford cattle, six steers and three heifers, ranging in weight between 218-319 kg were dosed with ^{14}C narasin equivalent to a feeding level of approximately 18 g/ton of feed. ^{14}C Narasin was administered in gelatin capsules morning and evening for three, five or seven days. Two steers and one heifer from each dosing interval were killed 12 hours after the last dose and edible tissues were assayed for radioactivity. Parent narasin in liver was determined by microbiological assay.

Summary of Results:

Radioactivity in liver was approximately 0.8 ppm calculated as narasin equivalents and there was no significant difference between animals in the three dosing groups. Therefore, steady state concentrations were approximated within three days' dosing. Muscle, kidney and fat residues were all less than 0.025 ppm (mean of all nine animals). Narasin concentrations in livers of the cattle dosed for seven days were approximately 8% of the total radioactivity. Chromatographic profiles of liver radioactivity from the seven-day animals were similar.

008

APPENDIX N: Report Summary

Title: Determination of Residue Levels in Tissues of Chickens Dosed Orally with 100 ppm ^{14}C Narasin Ration for Four or Six Days

Study Numbers: ABC-0059

Study Dates: March 12, 1980 to June 3, 1980

Name and Address of Investigators: J. A. Manthey, P. R. Handy, R. L. Van Duyn and R. J. Herberg, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Narasin

Test System: Broiler Chickens

Summary of Experimental Design:

Seven-week old chickens were fed a ration containing 100 ppm ^{14}C narasin. Three males and three females were killed at a practical zero withdrawal time after receiving the ration for four days. A similar group was killed after dosing for six days. Edible tissues were assayed for total radioactivity. Narasin in fat was determined by microbiological assay.

Summary of Results:

Tissue residue concentrations were the same at the four-day and six-day dosing intervals, indicating that the four-day interval was sufficient to establish steady-state tissue concentrations. Mean net radioactivity concentrations calculated as ppm ^{14}C narasin equivalents were: liver, 0.50; fat, 0.27; skin with subcutaneous fat, 0.16; kidney, 0.13; and muscle, 0.01. There was no apparent sex-related effect on tissue residues. Approximately half of the total residue in fat and less than 10% of the total residue in liver was parent narasin.

009

APPENDIX O: Report Summary

Title: Determination of Levels of Tissue Residues and the Rate of Decline of Residues from Tissues of Chickens Dosed Orally for Five Days with 100 ppm of ^{14}C Narasin Ration

Study Number: ABC-0093

Study Dates: August 1980 to January 1981

Name and Address of Investigators: J. A. Manthey, R. J. Herberg, P. R. Handy and R. L. Van Duyn, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Narasin

Test System: Broiler Chickens

Summary of Experimental Design:

Broiler chickens approximately seven weeks of age were fed a ration containing 100 ppm ^{14}C narasin for a dosing period of five days. Following the dosing period, the chickens were transferred to an unmedicated ration. Three chickens were killed at each of five withdrawal intervals, practical zero-withdrawal (6 hrs), and 1, 2, 3, and 5 days. Edible tissues were assayed for total radioactivity by liquid scintillation counting and for parent narasin by microbiological assay.

Summary of Results:

At practical zero-withdrawal the net mean radioactivity concentrations as ppm narasin were: liver, 0.451; fat, 0.211; kidney, 0.140; skin, 0.136; and muscle, 0.018. At one-day withdrawal, radioactivity in liver was 0.18 ppm and other tissues were below 0.1 ppm. Liver, kidney, and muscle contained no detectable parent narasin at a detection limit of 0.005 ppm. At zero-withdrawal, parent narasin accounted for approximately 40% of the radioactivity in fat and 30% in skin. At three days withdrawal the parent narasin was not detected in any of the five tissues.

010

APPENDIX P: Report Summary

Title: Laboratory Soil Leaching Studies with Narasin

Test Article: Crystalline narasin

Name and Address of Investigators: A. Loh, J. W. Moran, D. G. Saunders, R. E. Stricker, and W. L. Sullivan, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Study Date: October, 1976

Test System: Laboratory Soil Leaching

Summary of Experimental Design:

The design follows protocols as described in Guidelines for Registering Pesticides in the U.S., published in the Federal Register, Vol. 40, No. 123, June 25, 1975, pages 26884-26886. Narasin was applied at a rate equivalent to 25 pounds activity per acre to the surface of 30 cm high by 6.35 cm I.D. columns of four different textures of soil. One control and three treatment columns were prepared from each soil type and leached with the water equivalent of 60 cm of rainfall. The leachates were collected in four increments and analyzed for narasin. At the end of the experiment each soil column was divided into sections for narasin analysis.

Summary of Results:

The results of the laboratory leaching study are summarized in Table 1 and were adjusted for the average standard recovery of narasin from soil and leachate shown in Table 2.

Table 1.
Percent of Narasin Applied to the Column in a Laboratory Soil Leaching Study[1]

Soil Section (cm)	Sand	Sandy Loam	Loam	Clay Loam
0-10	13.5	19.0	26.2	65.1
10-20	14.6	57.5	9.9	33.0
20-30	11.8	21.4	8.7	1.5
Leachate (cm applied)				
0-15	7.2	0	0	0.1
15-30	30.2	0	3.9	0.1
30-45	13.9	0.2	27.9	0.1
45-60	6.6	2.0	23.3	0

[1]Data are averages from three columns.

011

APPENDIX P (continued)

TABLE 2
Narasin Standard Recovery Data

Soil Recoveries

Soil	Amount Added	Amount Found	% of Theory
Sand	500 µg	251 µg	50.2
Sandy Loam	500	422	82.6
Loam	500	269	53.8
Clay Loam	500	301	60.2
		Average Recovery =	61.4%

Soil Leachate Recoveries

Soil Leachate	Amount Added	Amount Found	% of Theory
Sand	100 µg	92 µg	92
Sandy Loam	100	93	93
Loam	100	122	122
Clay Loam	100	90	90
		Average Recovery =	99.3%

Under the conditions of this experiment narasin was found to leach readily from a sandy soil and more slowly from loam, sandy loam, and clay loam soils. Narasin therefore appears to be more mobile in coarse than in fine textured soils, however, other factors such as soil pH also appear to affect mobility. Narasin leached more readily from the two soils exhibiting approximately pH 8 (sand and loam) than from those at pH 5.6 (sandy loam and clay loam). Rate of water percolation through the soils ranged from 1-2 days for sand and sandy loam to 3-5 days for the other soil types. The results of this experiment indicate that narasin is moderately mobile in most soils. Since narasin degrades rapidly in soil (Appendices J and K), the moderate mobility of narasin observed in this severe test with the equivalent of 60 cm of rainfall is not indicative of a potential for narasin to leach into groundwater.

012

APPENDIX Q: Report Summary

Title: The Toxicity of Compound 79891 (Narasin) to Bobwhite Quail in an Acute Oral Study

Name and Address of Investigators: R. E. Karnak, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140.

Study Dates: October 24 to November 7, 1978

Study Number: 7035-78

Test Article: Crystalline narasin

Lot Number: X-24458 (contains 100% narasin activity)

Species/Strain: Bobwhite quail (Colinus virginianus), adult

Number of Animals: 5/sex/group

Levels of Dosing: 0.0, 22.5, 27.5, 36.5, 50, 70, or 100 mg/kg in acacia suspension

Length of Observation: 14 Days

Route: Oral (gavage)

Parameters Studied: Food consumption, body weight, behavioral signs of toxicity (lethargy, ataxia) and mortality.

Results:

The LD_{50}, the 95% confidence interval for the LD_{50}, and the slope of the dose-response curve for adult male bobwhite dosed with crystalline narasin were 73.96 mg/kg, 57.41 to 95.33 mg/kg, and 7.44, respectively. The LD_{50} for adult females was >70<100 mg/kg. Dose-related toxic effects in both male and female birds included lethargy and ataxia. All mortalities occurred within 24 hours following single oral doses. All of the surviving birds were normal within 14 days after treatment. No deaths were found for males or females at a dose of 36.5 mg/kg. Body weights were slightly reduced in groups that received doses ≧50 mg/kg during the first 7 days of observation.

013

APPENDIX R: Report Summary

Title: The Toxicity of Compound 79891 (Narasin) to Bobwhite Quail in a 5-Day Dietary Study.

Name and Address of Investigators: R. E. Karnak, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140

Study Dates: February 14 to February 22, 1979

Study Number: 7002-79

Test Article: Mycelial narasin

Lot Number: X-30694 (contains 15.12% narasin activity)

Species: Bobwhite Quail (*Colinus virginianus*), 10 days old

Number of Animals: 10/treatment

Levels of Exposure: 0.0, 0.005, 0.010, 0.025, 0.050, 0.100, 0.250 or 0.500% w/w (nominal). Assayed values ranged from 99.2 to 109% of nominal values.

Length of Exposure: Treated diets, 5 days; basal diets, 3 days.

Route: Dietary

Parameters Studied:

Food consumption, body weight gain, behavioral signs of toxicity (wing droop, ataxia, lethargy, prostration), and mortality.

Results:

Food consumption decreased as compound concentration in the diet increased during the 5-day treatment period. Birds affected by treatment consumed approximately 50% less food than did control birds. Food consumption during the 3 day basal diet period was only slightly lower among narasin-treated birds than control birds. Statistically significant reductions in body weight gain were observed at dietary concentrations of 0.025, 0.050 and 0.100% ($P \leq 0.05$). Weight gain data for the 0.250 and 0.500% treatment groups were not available because of substantial mortality at these levels. A reduction in body weight gain at the lowest dietary level of narasin was not statistically significant. Behavioral abnormalities (one or more of the following: wing droop, ataxia, lethargy, and prostration) were found in a concentration-related fashion at dietary concentrations of narasin $\geq 0.010\%$ w/w. The 5-8 day LC_{50} and the 95% confidence interval were 0.106% and 0.088% to 0.148%, respectively, expressed as nominal narasin activity in the diet. The slope of the concentration-response curve was 3.808. Based on estimates of food consumption, the nominal 5-day LD_{50}, the 95% confidence limits, and slope of the dose response curve were 153.7 mg/kg, 117.1-201.9 mg/kg, and 5.127, respectively.

014

APPENDIX S: Report Summary

Title: The Toxicity of Compound 79891 (Narasin) to Mallard Ducks in a 5-Day Dietary Study.

Name and Address of Investigators: R. E. Karnak, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140

Study Dates: November 1 to November 9, 1978

Study Number: 7036-78

Test Article: Mycelial narasin

Lot Number: X-30694 (contains 15.12% narasin activity)

Species: Mallard Duck (Anas platyrhynchos), 10 days old

Number of Animals: 10/treatment

Levels of Exposure: 0.0, 0.02, 0.056, 0.18 or 0.5% w/w (nominal)

Length of Exposure: Treated diets, 5 days; basal diets, 3 days.

Route: Dietary

Parameters Studied: Food consumption, body weight gain, behavioral signs of toxicity (ataxia and lethargy), and mortality.

Results:

Diet rejection during the five day treatment period was evident in all treatment groups and was concentration-related. Food consumption during the three-day basal diet period was equal to or greater than food consumed by the control birds. Statistically significant decreases in body weight gain ($P \leq 0.05$) were observed during the five days of treatment in all groups except those fed the 0.02% diet. Weight gains were similar in all groups during the basal diet period.

There were no mortalities or behavioral signs of toxicity among control birds and those fed diets containing nominal concentrations of 0.02 and 0.056% of narasin activity. Birds fed the 0.18 and 0.5% diets appeared normal through day 4. Lethargy and ataxia were observed in both groups on day 5 and mortalities occurred on days 5, 6, and 8. All surviving birds appeared normal at the termination of the study. Three birds died at the 0.18% level and four birds died at the 0.5% level.

The LC_{50} at the end of this 8-day study >0.5% nominal narasin concentration in the diet. At a nominal concentration of 0.02% of narasin (0.0205% based on assayed concentration) in the diet, no mortalities and no reductions in body weight gain or changes in appearance or behavior were found. Based on estimates of food consumption, the nominal LD_{50} value for this study was >810.8 mg/kg.

015

APPENDIX T: Report Summary

Title: The Toxicity of Compound 79891 (Narasin) to Rainbow Trout in a 96-hour Static Study.

Name and Address of Investigators: R. E. Karnak, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140

Study Dates: June 7 to June 11, 1978

Study Number: 2080-78

Test Article: Crystalline narasin

Lot Number: X-24458 (contains 100% narasin activity)

Species: Rainbow trout (Salmo gairdneri)

Experiment Design:

Groups of ten juvenile rainbow trout (mean weight, 0.512 g) were exposed to nominal narasin concentrations of 0.0 (water and solvent controls), 0.5, 0.7, 1.0, 1.4, 2.0, 2.75, 3.65, and 5 mg/L for 96 hours. Jars with 15L of test or control solution were used to contain each group of ten fish. Dissolved oxygen concentrations, pH, and temperature of the solutions were recorded daily. Behavioral signs of toxicity (stressed: hypoactivity, immobilization, loss of equilibrium, and irregular swimming patterns; or prostrate) and mortalities were noted for fish in each jar on a daily basis.

Results:

The temperature of the test solutions was 12.5 ± 0.5°C, pH values ranged from 7.8 to 8.6, and dissolved oxygen concentrations were at least 65% of saturation. Fish exposed to concentrations of narasin ≧0.7 mg/L showed a concentration response pattern of hypoactivity, loss of equilibrium, irregular swimming behavior, prostration and death. The 96 hr LC_{50} >1.4<2.0 mg/L. No mortalities and no behavioral abnormalities were found for fish exposed to a narasin concentration of 0.5 mg/L.

016

APPENDIX U: Report Summary

Title: The Toxicity of Compound 79891 (Narasin) to Daphnia magna in a 48-hour Static Study.

Name and Address of Investigators: R. E. Karnak, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140

Study Dates: November 16 to November 18, 1978

Study Number: 5056-78

Test Article: Crystalline narasin

Lot Number: X-24458 (contains 100% narasin activity)

Species: Daphnia magna

Experiment Design:

Groups of 31 to 36 Daphnia, ≤20 hours old, were exposed to each of the following nominal narasin concentrations: 0.0 (water and solvent controls), 1.1, 2.25, 4.0, 8.0, and 16.0 mg/L for 48 hours. Each of three beakers with 200 ml of solution were used to contain 10 to 13 Daphnia, for each treatment or control solution. Test solutions were maintained at 18°C with pH values between 7.0 and 8.1 and dissolved oxygen concentrations were at least 81% of saturation. Daphnia were assessed for hypoactivity, prostration, and immobility.

Results:

Based on immobility, the 48-hr EC_{50}, the 95% confidence interval, and the slope of the concentration-response curve were 7.72 mg/L, 6.84 to 8.72 mg/L, and 7.26, respectively. By the end of the study, all daphnids exposed to narasin concentrations ≥4.0 ppm were hypoactive, prostrate or immobile. Daphnids exposed to narasin concentrations ≤2.25 ppm were not hypoactive, prostrate, or immobile throughout the study.

017

APPENDIX V: Report Summary

Title: The Toxicity of Compound 79891 (Narasin) to Earthworms in a 14-day Soil Incorporated Study.

Name and Address of Investigators: R. E. Karnak, C. C. Kehr; and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, IN 46140

Study Dates: August 1 to August 15, 1978 and October 10 to October 24, 1978.

Study Number: 6018-78 and 6026-78

Test Articles: Crystalline narasin

Lot Number: X-24458 (contains 100% narasin activity)

Species: Earthworm (Lumbricus terrestris)

Average Initial Weight: Study 6018-78, 3.6 g; Study 6026-78, 4.4 g

Number of Animals: 10-15/group

Levels of Exposure: Study 6018-78 - 0.0 (control and solvent), 1.0, 5.0, 10.0, 20.0, 40.0, 80.0, or 100.0 ppm (nominal). Study 6026-78 - 0.0 (control), 0.5, 1.0, or 5.0 ppm (nominal).

Length of Exposure: 14 days

Route: Incorporated into test media (rabbit feces, water, and non-sterile potting soil).

Parameters Studied: Body weight gain, mortality, physical appearance (flaccid, soft and flaccid, moribund).

Experimental Design: Test media was placed in 2 L cylindrical glass jars. For study 6018-78, three jars were used for control and for the solvent control (0.01% Tween 80 was used as a wetting agent) and two jars were used at each of the treatment levels. For study 6026-78, three jars were used for control (no wetting agent was used for the low concentrations in this study) and two jars were used at the three treatment levels. Five worms were placed into each jar at the beginning of each study. The study was conducted at 10°C.

Results:

Mortalities occurred at exposure levels ≧20.0 ppm and were preceded by weight loss and a decline in physical appearance. Some worms left the test media at these levels. The 3-day, 7-day, and 14-day LC_{50} values for study 6018-78 were >100 ppm, ca 40.0 ppm, and >20.0<40.0 ppm, respectively. Effects observed in this study for worms exposed to concentrations ≧10.0 ppm appeared to be concentration-related in the

018

APPENDIX V (Continued)

following manner: flaccidity, softness and flaccidity, morbidity, and death. In study 6018-78, the following observations were made for worms in soil containing 1.0 ppm of narasin: day 3, one soft and flaccid worm; day 7, one flaccid worm and another soft and flaccid worm; day 14, one flaccid worm. In this same study, a reduction in body weight gain was found at 1.0 ppm. In study 6026-78, no mortality, no reduction in body weight gain, and no changes in physical appearance were found for worms exposed to nominal narasin concentrations of 0.5 and 1.0 ppm. In study 6026-78, all worms in soil containing 5.0 ppm of narasin were flaccid or soft and flaccid throughout the study and these worms did not gain weight like control worms. The concentration of 0.5 ppm of narasin in soil did not result in mortalities, reduction in body weight gain, or a change in the physical appearance of the worms tested.

019

APPENDIX W: Report Summary

Title: The Effect of Narasin on Nitrogen Fixation

Test Article: Crystalline narasin

Name and Address of Investigators: J. S. Peloso and R. M. Kline, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Study Dates: March to April, 1979

Test System: Laboratory cultured nitrogen-fixing microorganisms

Summary of Experimental Design:

Two microorganisms, a blue-green alga (Anabaena flos-aquae) and a bacterial heterotroph (Azotobacter chroococcum), were cultured in the presence of narasin at various test concentrations. Nitrogen-fixing activity and growth were monitored for two to four days.

Summary of Results:

Algal growth was not inhibited by 1.0 ppm narasin, the highest level tested. A slight decrease in nitrogen-fixing activity was detected at the 1.0 ppm level, but it was not sufficient to cause any alterations in the growth pattern.

The azotobacter culture showed slightly depressed levels of growth and nitrogen fixation at 1 and 10 ppm. Although growth of the organisms in highest treatment cultures never reached maximum levels of nontreated controls, there were no indications of bacteriocidal effects. Normal growth occurred at 0.1 ppm.

020

APPENDIX X: Report Summary

Title: Greenhouse Test for Narasin Phytotoxicity

Study Numbers: B79-3328-66, 276A-3482-29, and Q61-3474-287

Study Dates: July-August 1974 for B79-3328-66 and Dec. 1976 to Feb. 1977 for the other two studies.

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline Narasin

Test System: Plants grown from seed in greenhouse soil flats.

Summary of Experimental Design:

Narasin was incorporated into soil at concentrations of 0.15, 1.5, 10 and 40 ppm. The 10 and 40 ppm treatments were in Study B79-3328-66, the 1.5 ppm treatment was in Study 276A-3482-29, and the 0.15 ppm treatment was in Study Q61-3474-287. A standard greenhouse phytotoxicity test was conducted in which fourteen mono- and dicotyledonous plants were grown from seed in the treated and untreated soils. The plant species were alfalfa (Medicago sativa), fescue (Festuca elatior), cucumber (Cucumis sativus), rice (Oryza sativa), cotton (Gossypium hirsutum), tomato (Lycopersicon esculentum), pepper (Capsicum annuum), corn (Zea mays), sugar beet (Beta vulgaris), barley (Hordeum vulgare), soybean (Glycine max), wheat (Triticum aestivum), grain sorghum (Sorghum bicolor), and oats (Avena sativa). Plants were rated for phytotoxic injury (0 = no injury, to 10 = complete kill) and injury, described as chlorosis, burning, stunting, or reduced germination, was noted 18 to 21 days after planting.

Summary of Results:

Narasin at 0.15 ppm caused no phytotoxic effect. At 1.5 ppm there was limited, noncritical stunting of seven of fourteen plant species. These included alfalfa, fescue, rice, cotton, corn, sugar beet, and oats. There were severe phytotoxic effects in all fourteen plant species at 40 ppm and in twelve plant species at 10 ppm. At these two highest concentrations of narasin in soil phytotoxic effects included mild to severe stunting, moderately reduced germination to completely inhibited germination, and mild to severe chlorosis and burning. Only wheat and oats showed no phytotoxic effects at the 10 ppm level. The narasin concentrations which caused phytotoxicity represent artificially high concentrations which would not be encountered in normal agronomic use of chicken litter for manuring agricultural land.

021

APPENDIX Y: Report Summary

Title: Phytotoxicity Test in Field Plots Manured with Floor Pen Litter from Narasin-Treated Broilers

Study Number: 276A-3477-8

Study Dates: December 1976 to August 1977

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Litter from chickens which were fed 100 ppm narasin

Test System: Field soil plots

Summary of Experimental Design:

This crop safety study was conducted to find whether or not a very high manuring rate used during the winter with litter that contained narasin would result in phytotoxic effects to summer crops. Field soil plots, approximately 3.7 x 10 m, were manured with 67.4 metric tons/ha (30 U.S. tons/acre) of either control chicken litter or litter from narasin-treated chickens. The litter from the treated chickens contained 9.8 ppm narasin (on an air-dry basis). The manure applications were made in December, 1976. In May 1977, the plots were tilled to a depth of approximately 10 cm. Oats (Avena sativa), sugar beets (Beta vulgaris), corn (Zea mays), cotton (Gossypium hirsutum), rice (Oryza sativa), fescue (Festuca elatior), and alfalfa (Medicago sativa) were planted as seeds in June after the ground was cultivated to a depth of 2 to 3 cm. In greenhouse conditions, these plant species were the most sensitive to narasin in soil (Appendix X). Evaluation of the resulting plants for phytotoxic injury (0 = no injury, to 10 = complete kill) occurred in July and August.

Summary of Results:

Growth and development of plants in the treated and control plots were equivalent. There was no narasin-related phytotoxicity from manuring with the litter from narasin-treated chickens. Crop injury ratings were all "0" (no injury) for all crops on both observation dates. On site recorded rainfall mesurements were: April, 10.5 cm; May, 6.8 cm; June, 4.3 cm; July, 17.9 cm; and August, 9.3 cm. This rainfall is in the normal range for the geographical area. Three days before the plots were tilled on May 19, narasin activity levels in soil ranged from 0.2-0.4 ppm. Just before the seeds were planted on June 15, detectable levels of narasin (0.05-0.10 ppm) were found in soil. Detectable levels (0.05-0.10 ppm) of narasin were also found on July 15. Narasin concentrations in soil were below detection (0.05 ppm) on August 16.

022

APPENDIX Z: Report Summary

Title: The Acute Toxicity to Bluegill (Lepomis macrochirus) of Narasin (Compound 79891).

Name and Address of Investigator: J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140.

Study Dates: April 11 to April 15, 1983

Study Number: F05183

Test Article: Mycelial narasin

Lot Number: X-40533, contains 10.6% narasin activity

Species: Bluegill (Lepomis macrochirus)

Summary of Experimental Design:

Groups of ten juvenile bluegill, 0.89 to 1.82 g per fish, were exposed to the following average assayed narasin concentrations for 96 hrs: 0.0 (water control), 0.88, 1.66, 2.80, 4.68, 6.00, 6.74, 7.80, 8.70 and 9.55 mg/L. Jars with 15 L of test or control solution were used to contain each group of ten fish. Dissolved oxygen concentration, pH and temperature were recorded daily for each jar. Total hardness, total alkalinity and conductivity were recorded once for the dilution water. Behavioral signs of toxicity (sluggish, hypoactivity, swimming impaired, labored respiration and minimal voluntary movement, and prostration) and mortalities were noted for fish in each jar on a daily basis.

Summary of Results:

Narasin concentrations were stable throughout this study, presumably because of the slightly basic pH of the water and very low ultraviolet light levels. Narasin dissipates rapidly in water when exposed to ultraviolet light. The fluorescent lights in the laboratory produce only low levels of ultraviolet light. The light diffusers and glass containers for the test solutions would further reduce the ultraviolet light levels during this test. The ranges in the water quality characteristics were as follows: pH, 8.05 to 8.6; temperature, 20 to 20.5°C; total hardness, 120 mg/L (as $CaCO_3$); total alkalinity, 152 mg/L (as $CaCO_3$); and conductivity, 250 µmhos/cm. Dissolved oxygen concentrations averaged 8.75 mg/L and were at least 83% of saturation in all test solutions.

No mortalities or behavioral abnormalities were found for fish in control water or for fish in solutions with average narasin concentrations of 0.88 and 1.66 mg/L. Fish exposed to 2.80 mg/L of narasin exhibited sluggish behavior. The behavior of fish exposed to narasin concentrations $\geq$ 4.68 mg/L ranged from hypoactivity to labored respiration with minimal voluntary movement. At least some fish died in narasin concentrations $\geq$ 4.68 mg/L. The 96 hr LC_{50}, 95% confidence limits, and slope of the concentration response curve were 5.02 mg/L, 4.61 to 5.46 mg/L, and about 18.1, respectively.

023

APPENDIX AA: Report Summary

Title: The Acute Toxicity to Rainbow Trout (Salmo gairdneri) of Narasin (Compound 79891).

Name and Address of Investigator: J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Study Dates: April 11 to April 15, 1983

Study Number: F05283

Test Article: Mycelial narasin

Lot Number: X-40533, contains 10.6% narasin activity

Species: Rainbow Trout (Salmo gairdneri)

Summary of Experimental Design:

Groups of ten juvenile bluegill, 0.74 to 2.05 g per fish, were exposed to the following average assayed narasin concentrations for 96 hrs: 0.0 (water control), 0.103, 0.190, 0.316, 0.561, 1.00, 1.82, 3.04, and 5.26 mg/L. Jars with 15 L of test or control solution were used to contain each group of ten fish. Dissolved oxygen concentration, pH and temperature were recorded daily for each jar. Total hardness, total alkalinity and conductivity were recorded once for the dilution water. Behavioral signs of toxicity (sluggish, hypoactivity, swimming impaired, labored respiration and minimal voluntary movement, and prostration) and mortalities were noted for fish in each jar on a daily basis.

Summary of Results:

The temperature, total hardness, total alkalinity, and conductivity of the test solutions were 13°C, 120 mg/L (as $CaCO_3$), 148 mg/L (as $CaCO_3$), and 225 µmhos/cm, respectively. Dissolved oxygen concentrations averaged 10.2 mg/L and were at least 92% of saturation. Measured pH values ranged from 8.2 to 8.6.

No mortalities or behavioral abnormalities were found for fish in control water or for fish in solutions with average narasin concentrations of 0.103 and 0.190 mg/L. Fish exposed to 0.316 mg/L exhibited hypoactive behavior by the end of the study. Fish exposed to narasin concentrations $\geq$ 0.561 mg/L displayed behavior which ranged from sluggish movement to labored respiration and minimal voluntary movement. At least some mortalities were noted for fish exposed for 96 hours to average narasin concentrations $\geq$ 1.82 mg/L. The 96 hr LC_{50}, the 95% confidence limits and the slope of the concentration-response curve were 2.23 mg/L, 1.84 to 2.71 mg/L, and about 7.0, respectively.

024

APPENDIX BB: Report Summary

Title: The Acute Toxicity of Narasin to Daphnia magna in a Static Test System.

Name and Address of Investigators: D. W. Grothe and R. R. Mohr, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Study Dates: April 12 to April 14, 1983

Study Number: C01883

Test Article: Mycelial narasin

Lot Number: X-40533, contains 10.6% narasin activity

Species: Daphnia magna

Summary of Experimental Design:

Groups of 30 Daphnia, ≦ 24 hours old, were exposed to each of the following average assayed narasin concentrations for 48 hrs: 0.0 (water control), 4.69, 7.86, 12.45, 18.96, 35.08, and 42.18 mg/L. The dissolved oxygen concentration, pH, and temperature of each test solution were measured and recorded at test initiation and at each 24-hr interval. Total alkalinity, total hardness, and conductivity of the dilution water were determined once during the test. Daphnia were assessed for hypoactivity, prostration and immobility.

Summary of Results:

Based on immobility, the 48 hr EC_{50}, the 95% confidence limits and the slope of the concentration response curve were 20.56 mg/L, 9.19 to 68.1 mg/L, and 4.23. The following behavior was noted at the end of the study (test concentration, number normal (N), hypoactive (H), prostrate (P) or immobile (I)): 0.0 mg/L, 30 N; 4.69 mg/L, 27 N, 2 H, 1 I; 7.86 mg/L, 22 N, 4H, 4I; 12.45 mg/L, 9 N, 21 H; 18.96 mg/L, 7 N, 14 H, 9 I; 35.08 mg/L, 4 H, 26 I; and 42.18 mg/L, 30 I.

025

APPENDIX CC: Report Summary

Title: The Toxicity of Soil-Incorporated Narasin (Compound 79891) to Earthworms (Lumbricus terrestris) in a 14-Day Test.

Name and Address of Investigators: D. W. Grothe and J. L. Seacat, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Study Dates: April 13 to April 27, 1983

Study Number: W00783

Test Article: Mycelial narasin

Lot Number: X-40533, contains 10.6% narasin activity

Species: Earthworm (Lumbricus terrestris)

Average Initial Weight: 4.46 g

Number of Animals: 15/control or treatment group; 5/replicate

Levels of Exposure: 0.0 (control), 4.5, 10.0, 22.5, 45.0, 100.0 ppm (nominal)

Length of Exposure: 14 days

Route: Incorporated into test media (rabbit feces, water, and non-sterile loamy sand soil).

Parameters Studied: Body weight gain, mortality, physical appearance (flaccid, soft and flaccid, moribund).

Summary of Experimental Design:

Test media was placed in three 2-L cylindrical glass jars for the control group and each treatment group. Five worms were placed into each jar at the beginning of the study. Worms were examined on test days 7 and 14. The study was conducted at 14°C.

Summary of Results:

At the end of the study, mortality frequencies were 7, 0, 20, 53, 100, and 100% at nominal narasin concentrations of 0.0 (control), 4.5, 10.0, 22.5, 45.0, and 100 mg/kg, respectively. The 14-day LC_{50}, 95% confidence limits, and slope of the concentration-response line were 17.9 mg/kg, 13.2 to 23.6 mg/kg, and 4.11.

A statistically significant reduction in weight gain was found for worms exposed to a nominal narasin concentration of 22.5 mg/kg at the end of the study. A reduction in weight gain, although not statistically significant, was also noted for worms exposed to a nominal narasin concentration of

026

APPENDIX CC (Continued)

Summary of Results (Continued):

10.0 mg/kg. Worms exposed to a narasin concentration of 4.5 mg/kg gained about the same amount of weight as did worms in the control media.

At the end of the study, the physical condition of the worms varied in a concentration dependent fashion. Thirteen control worms were normal, one was soft and flaccid, and one was dead. Eleven worms exposed to a narasin concentration of 4.5 mg/kg were normal and four were flaccid. Six worms exposed to a narasin concentration of 10.0 mg/kg were flaccid, five were soft and flaccid, one was moribund, and three were dead. Five worms exposed to a narasin concentration of 22.5 mg/kg were soft and flaccid, two were moribund, and eight were dead. At the end of the study all worms exposed to narasin concentrations of 45.0 and 100.0 mg/kg were dead.

APPENDIX DD: Report Summary

Title: The Toxicity of Narasin (Compound 79891) to Bobwhite in a Fourteen-Day Acute Oral Study

Name and Address of Investigator: R. L. Cochrane, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Study Dates: March 22 - April 5, 1983

Study Number: A00983

Test Article: Mycelial narasin

Lot Number: X-40533, contains 10.6% narasin activity

Species: Bobwhite (Colinus virginianus)

Number of Animals: 6/sex/group

Levels of Dosing: 0.0 (vehicle control, 10% acacia), 6.2, 11.0, 20.0, 33.0, 56.0, and 100 mg/kg

Route: Single oral gavage

Length of Observation: 14 days

Parameters Studied:

Food consumption, body weight, behavioral and physical signs of toxicity (loose feces, lethargy, ataxia, ruffled appearance, emaciation, and abnormal posture) and mortality.

Summary of Results:

The 14-day LD_{50} value, 95% confidence limits, and slope of the dose-response curve for bobwhite were 102.9 mg/kg, 46.6 to 227.5 mg/kg, and 1.73, respectively. There were no sex-related differences in the pattern of mortality. The number of dead birds out of the 12 tested in each group at the end of the study was as follows: 0.0 (control), 0 dead; 6.2 mg/kg, 0 dead; 11.0 mg/kg, 0 dead; 20 mg/kg, 3 dead; 33 mg/kg, 1 dead; 56 mg/kg, 4 dead; and 100 mg/kg, 6 dead.

Mean food consumption values were equivalent for control birds and birds which received a dose of narasin $\leqq$ 20 mg/kg. Significantly reduced food consumption was found on test days three and/or seven for birds which received a dose of narasin $\geqq$ 33.0 mg/kg. Food consumption increased to normal levels between test days seven and fourteen for these birds.

The changes in body weight of birds given a dose of narasin $\geqq$ 33.0 mg/kg appeared to be positively correlated with food consumption. During the study, birds in the 20 mg/kg treatment group had a slight and temporary

APPENDIX DD (Continued)

Summary of Results (Continued):

weight loss. By the end of the study, their final body weight was larger than their initial body weight. No difference in the 14-day trend of mean body weight values was apparent between the vehicle control group of birds and birds that received doses of narasin ≦ 11.0 mg/kg.

No treatment-related signs of toxicity were observed in the vehicle control group or in the 6.2 mg/kg treatment group. Treatment-related loose feces, lethargy, and ataxia occurred in all groups that received a dose of narasin ≧ 11.0 mg/kg. Emaciated birds were noted in groups which received a narasin dose of 20.0, 56.0, and 100.0 mg/kg. Some birds had a ruffled appearance in the 11.0, 33.0, 56.0, and 100.0 mg/kg treatment groups. One bird in the 56.0 mg/kg treatment group temporarily had abnormal posture.

A dose of 6.2 mg/kg was the highest level of narasin activity tested which did not result in mortalities, signs of toxicity, or treatment-related reductions in food consumption and body weight.

029

APPENDIX EE: Report Summary

Title: The Toxicity of Narasin (Compound 79891) to Bobwhite in a Five-Day Dietary Study.

Name and Address of Investigator: R. L. Cochrane, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Study Dates: March 24 to April 1, 1983 and September 22 - 30, 1983

Study Numbers: A01083 and A02183

Test Article: Mycelial narasin

Lot Number: X-40533, contains 10.6% narasin activity

Species: Bobwhite (Colinus virginianus), 11 days old and 15 days old

Number of Animals: 10/group

Levels of Exposure: Study A01083 - 0.0 (control), 0.0048, 0.0098, 0.0194, 0.0393, 0.0809, and 0.1561% (w/w) of diet (assayed);
Study A02183 - 0.0 (control), 0.0049, 0.0100, 0.0197, 0.0347, 0.0800, and 0.159% (w/w) of diet (assayed)

Length of Exposure: Treated diets, five days; basal diet, three days

Route: Dietary

Parameters Studied:

Food consumption, body weight gain, behavioral signs of toxicity (ataxia, lethargy, hyperactivity, tremors, wing droop), and mortality.

Summary of Results:

Study A01083

At the end of this study, the number of dead birds out of a total of 10 in each group was as follows: 0.0% (control), 0 dead; 0.0048%, 0 dead; 0.0098%, 0 dead; 0.0194%, 0 dead; 0.0393%, 2 dead; 0.0809%, 4 dead; 0.1561%, 9 dead. The eight-day LC_{50}, 95% confidence limits, and slope of the concentration-response line were 0.080%, 0.058 to 0.109%, and 3.679. Based on the calculated values of total food consumed in the five-day treatment period per average daily weight of birds in each treatment group, the calculated eight-day LD_{50}, 95% confidence limits, and slope of the dose-response line were 575 mg/kg, 436 to 758 mg/kg and 4.031, respectively.

No significant differences in mean body weight gain occurred between the control group and groups fed diets that contained $\leq$ 0.0098% narasin activity

APPENDIX EE (Continued)

Summary of Results (Continued):

during the treatment phase. A statistically significant reduction in mean body weight gain occurred in treatment groups receiving ≥ 0.0194% narasin in their diet, and the reduction in weight gain or loss of weight was treatment related. During the basal diet phase, the mean body weight gain value for the control group was equivalent to that for each of the treatment groups fed diets with ≤ 0.0194% narasin. Significant reductions in body weight gain were found for birds fed diets with narasin concentrations ≥ 0.0393%. Food consumption by control birds was equivalent to consumption by birds receiving diets with narasin levels ≤ 0.0194% during the treatment and basal diet phase. A treatment-related reduction in food consumption during the treatment and basal diet phase occurred in treatment groups ≥ 0.0393%.

No signs of toxicity were observed in the control group or in groups of birds exposed to dietary concentrations of narasin ≤ 0.0194%. Higher treatment levels produced ataxia and lethargy. The onset and intensity of these clinical signs were related to treatment. Tremors were observed in one bird in the 0.1561% treatment three hours after the start of the study, and wing droop was observed in two additional birds from the 0.1561% treatment group during test-days one through four.

No mortalities, signs of toxicity, or treatment-related changes in food consumption and body weight gain were found for birds exposed to dietary levels of narasin activity ≤ 0.0098%.

Study A02183

At the end of this study, the number of dead birds out of a total of 10 in each group was as follows: 0.0% (control), 0 dead; 0.0049%, 0 dead; 0.0100%, 0 dead; 0.0197%, 0 dead; 0.0347%, 3 dead; 0.0800%, 5 dead; and 0.159%, 10 dead. The eight-day LC_{50}, 95% confidence limits, and slope of the concentration-response line were 0.063%, 0.046-0.087%, and 3.712, respectively. Based on the calculated values of total food consumed in the five-day treatment period per average daily weight of birds in each treatment group, the calculated eight-day LD_{50}, 95% confidence limits, and the slope of the dose-response curve were 270 mg/kg, 226 to 322 mg/kg, and 6.944, respectively.

A treatment-related reduction in food consumption occurred in all treatment groups during the five-day exposure period. The reduction in food consumption was statistically significant in treatment groups ≥ 0.0197%. During the basal diet period food consumption values for birds that survived treatment were equivalent to the control value.

Significant body weight loss or reductions in mean body weight gain values occurred at all treatment levels during the treatment period. Mean body weight gain values for all surviving treated birds were greater than or equal to the control groups value during the basal diet period.

No behavioral signs of toxicity occurred at treatment levels ≤ 0.010%. Higher treatment levels produced ataxia and lethargy. These clinical signs followed a concentration-related pattern.

031

APPENDIX FF: Report Summary

Title: The Toxicity of Narasin (Compound 79891) to Mallards in a Five-Day Dietary Study

Name and Address of Investigator: R. L. Cochrane, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Study Dates: May 26 to June 3, 1983 and August 11 to August 19, 1983

Study Numbers: A01283 and A01983

Test Article: Mycelial narasin

Lot Number: X-40533, contains 10.65 narasin activity

Species: Mallard (Anas platyrhynchos), 10 days old

Number of Animals: 10/group

Levels of Exposure: Study A01283 - 0.0 (control), 0.0154, 0.0302, 0.0619, 0.1231, 0.2282, and 0.4551% of diet (assayed);
Study A01983 - 0.0 (control), 0.0167, 0.0323, 0.0638, 0.1283, 0.2524, 0.5048% of diet (assayed).

Length of Exposure: Treated diets, 5 days; basal diet, 3 days

Route: Dietary

Parameters Studied:

Food consumption, body weight gain, behavioral signs of toxicity (lethargy, ataxia, and labored breathing) and mortality.

Summary of Results:

Study A01283

No mortality occurred in the control group or in treatment groups ≦ 0.2282%. Three of 10 birds died in the 0.4551% treatment group, the highest level tested. The highest average total consumption of narasin, 2505 mg narasin/kg bird, was found for birds in this highest treatment group. No signs of toxicity were observed in birds from the control group or in treatment groups fed diets that contained ≦ 0.1231% narasin activity. Lethargy was observed in birds in the 0.2282 and 0.4551% treatment groups.

A statistically significant reduction in mean body weight gain values occurred for birds exposed to dietary concentrations of narasin ≧ 0.0619% during the five-day treatment phase. No difference in mean body weight gain values were observed during the three-day basal diet phase. There was

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APPENDIX FF (Continued)

Summary of Results (Continued):

no difference in food consumption for birds fed diets that contained 0.0154% and 0.0302% narasin when compared to the control group during the five-day treatment phase. A treatment-related reduction in food consumption occurred in treatment groups ≧ 0.0619% during the treatment phase. Treatment-related reductions in food consumption corresponded to reduced body weight gain or loss of body weight for birds in treatment groups ≧ 0.0619%. No differences in food consumption occurred between the control group and any treatment group during the three-day basal diet phase.

The dietary concentration of 0.0302% was the highest level of narasin activity tested which did not result in mortalities, signs of toxicity or treatment-related changes in food consumption or body weight gain.

Study A01983

At the end of this study, the number of dead birds out of a total of 10 in each group was as follows: 0.0% (control), 0 dead; 0.0167%, 0 dead; 0.0323%, 0 dead; 0.0638%, 0 dead; 0.1283%, 0 dead; 0.2524%, 4 dead; and 0.5048%, 6 dead. The eight-day LC_{50}, 95% confidence limits, and slope of the concentration-response line were 0.38%, 0.259 to 0.557%, and 3.467, respectively. Based on the calculated values of total food consumed in the five-day treatment period per average daily weight of birds in each treatment group, the calculated eight-day LD_{50}, 95% confidence limits, and slope of the dose-response line were 2373 mg/kg, 1888 to 2983 mg/kg, and 5.938, respectively.

No signs of toxicity occurred at treatment levels ≦ 0.0638%. Higher treatment levels produced ataxia and lethargy. These clinical signs occurred during test-days three to eight and followed a concentration-related pattern. Labored breathing was observed in birds in the 0.5048% dietary level during test-days five and six.

Mean body weight gain values for groups fed diets with narasin concentrations ≦ 0.323% were greater than or equal to the control group value for the treatment phase. All other groups either lost weight during the treatment phase or showed significant reductions in mean body weight gain as compared to the control group during the treatment phase. No significant differences in mean body weight gain between the control group and the treatment groups occurred during the basal diet phase.

Food consumption followed a similar pattern. During the treatment phase, food consumption by birds that received diets with narasin concentrations ≦ 0.0323% was greater than or equal to the control group. A treatment-related reduction in food consumption occurred at all other levels, and the reduction was statistically significant in treatment groups ≧ 0.1283%. No statistical differences occurred in food consumption during the basal diet phase; although, food consumption was substantially less than the control group for birds in the 0.2524% treatment group.

APPENDIX FF (Continued)

Summary of Results (Continued):

The dietary concentration of 0.0323% was the highest level of narasin activity tested which did not result in mortalities, signs of toxicity, or treatment-related changes in food consumption and body weight gain.