ENVIRONMENTAL ASSESSMENT
FOR THE USE OF
PAYLEAN® TYPE A MEDICATED ARTICLE
(RACTOPAMINE HYDROCHLORIDE)
IN THE FEED OF SWINE

NADA 140-863

ELANCO ANIMAL HEALTH
A DIVISION OF ELI LILLY AND COMPANY
LILLY CORPORATE CENTER
INDIANAPOLIS, IN 46285

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Environmental Assessment
For the Use of
Paylean® In the Feed of Swine

1. DATE November 1995

2. APPLICANT Elanco Animal Health
A Division of Eli Lilly and Company

3. ADDRESS Lilly Corporate Center
Indianapolis, Indiana 46285

4. DESCRIPTION OF THE PROPOSED ACTION

A new animal drug approval has been requested for the use of the premix Paylean in the feed of swine in finishing operations. Ractopamine hydrochloride is the active ingredient in Paylean. Between 5 and 20 ppm (4.5 to 18.2 g/ton) of ractopamine hydrochloride will be used continuously in the feed of finishing swine to improve feed efficiency and increase carcass leanness. Approval of this new animal drug would authorize Dista Products Limited (Fleming Road, Speke, Liverpool L24 9LN), a production facility of Eli Lilly and Company in England, to formulate and package Paylean for sale within and outside the United States. Production of the bulk active ingredient will be done at the facilities of Eli Lilly S. A. (Dunderrow, Kinsale) in Ireland for Eli Lilly and Company.

Based on the proposed action, ractopamine hydrochloride could potentially be introduced into the following environments:

a. The environment adjacent to the manufacturing plant and the formulating and packaging plant.
b. The environment adjacent to facilities which mix Paylean with feed.
c. Finishing operations for swine where residue may be found in animal waste.
d. Agricultural lands where waste products from swine are used as fertilizer.
e. Aquatic systems where runoff may collect from sites receiving waste products from swine.

5. IDENTIFICATION OF THE CHEMICAL SUBSTANCE

A. PAYLEAN

Paylean will be incorporated into the complete feed used for swine in finishing operations. Two percent of Paylean will be the active ingredient, ractopamine hydrochloride. Paylean also contains ground corn cobs and soybean oil. Soybean oil is used to produce a low-dust product.

B. RACTOPAMINE HYDROCHLORIDE

Ractopamine hydrochloride is manufactured in a one-step process. The bulk drug is produced as an aqueous solution containing 3 to 20 percent ractopamine hydrochloride. Without the water, not less than 96.0 percent will be ractopamine hydrochloride when determined by HPLC. Not more than 1.5 percent of the dry material will be an individual related substance, with no more than 4.0 percent total related substances.

Chemical Name (ractopamine hydrochloride): d,1 4-Hydroxy-α-[[[3-(4-hydroxyphenyl)-1-methyl-propyl]amino]methyl] benzenemethanol, hydrochloride.

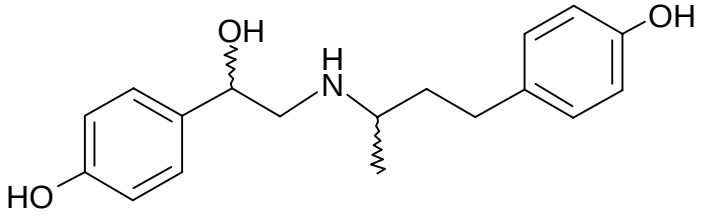
CAS Registry Number: 99095-19-9

Molecular Formula: $C_{18}H_{23}O_3N \cdot HCl$

Molecular Weight: 337.8

Structural Formula:

HCl



MIXTURE OF ALL STEREOISOMERS RR, SS, RS, SR

Solubility (Appendix A):	Water	pH 5	51.9 g/L
		pH 7	31.0 g/L
		pH 9	41.2 g/L

Melting Point: 163.9 to 164.6°C

UV Absorption: Peak absorptions occur at 225.8 and 277.6 nm with molar extinction coefficients of 463 and 93 L/cm/mole, respectively, in methanol at room temperature.

Vapor Pressure: Ractopamine hydrochloride is a nonvolatile solid. Differential thermal analysis indicated a strong endotherm at 180°C and an exotherm at 188°C coinciding with decomposition. Thermogravimetric analysis of ractopamine hydrochloride shows no weight loss until 176°C, where loss begins and continues through decomposition.

n-Octanol/Water Partition Coefficient (Appendix B): The n-octanol/water partition coefficients for ractopamine hydrochloride were 1.75, 1.02, and 17.4 in pH 5.0, 7.0, and 9.0 buffer solutions, respectively.

Related Substances in Production Lot (AJK63348):

4-(p-hydroxyphenyl)butan-2-ol	0.09%
4-(p-hydroxyphenyl)butan-2-one	0.56%
1,6-bis(p-hydroxyphenyl)-4-methyl-3-azahexane	0.02%
1,6-bis(p-hydroxyphenyl)-4-methyl-3-aza-1-methoxyhexane*	0.20%
Ractopamine dimer*	0.58%

* Exist as diastereoisomers and their relative abundances are totaled (i.e. 0.20% and 0.58%)

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

A. INTRODUCTION OF SUBSTANCES FROM THE MANUFACTURING SITES

1. LOCATION OF FACILITIES USED FOR MANUFACTURING, FORMULATING, AND PACKAGING

The processes for manufacturing ractopamine hydrochloride, the operations for formulating and packaging Paylean, and pollution control practices at the corresponding facilities are designed, constructed, or are being constructed to result in minimal environmental impact. Production of ractopamine hydrochloride will occur at the production facilities of Eli Lilly S.A. (Dunderrow, Kinsale) in Ireland for Eli Lilly and Company. The Paylean premix will be manufactured at the facilities of Dista Products Limited (Fleming Road, Speke, Liverpool L24 9LN) in England for Eli Lilly and Company. These facilities will effectively contain and control the liquid, solid, or gaseous pollutants from the production of ractopamine hydrochloride or manufacture of Paylean premix.

2. ENVIRONMENTAL REGULATORY REQUIREMENTS

Treatment, storage and disposal practices for the Kinsale facility are defined by an Integrated Pollution Control license administered by the Irish E.P.A. by the authority of the Environmental Protection Agency Act of 1992 (Appendix C). Licenses and permits granted under the authorities of the Water Pollution Act of 1977, the Air Pollution Act of 1987, the European Communities (Waste) Regulations of 1979, and the European Communities (Toxic and Dangerous Waste) Regulations of 1982 were incorporated into one integrated pollution control license granted by the Irish E.P.A.

Treatment, storage, and disposal practices for the Dista facility are defined by the regulations administered by the North West Water PLC, the Merseyside Waste Disposal Authority, and the Metropolitan Borough of Knowsley. The Metropolitan Borough of Knowsley grants a license allowing construction of facilities under the authority of the Town

and Country Planning Act of 1990 and issues a license stipulating the allowable air emissions under the Environmental Protection Act of 1990 Part 1. The North West Water PLC administers a Consent to Discharge license under the Public Health Acts of 1937 and 1961, and the Water Act of 1973. The Merseyside Waste Disposal Authority administers a Waste Disposal Facility license under the Control of Pollution Act of 1974. Permits related to the formulation and packaging of ractopamine hydrochloride issued by these regulatory agencies for the discharge of wastewater, the treatment, storage, and disposal of materials, and air emissions are listed in Appendix C.

3. PROCESS CHEMICALS AND MATERIALS USED IN MANUFACTURING

Ractopamine hydrochloride is produced in a one-step chemical process. Paylean premix is manufactured by using a controlled spraying process that attaches the active ingredient via a water carrier to minigranules (corn cob grits). A list of materials used, consumed, and discharged in the process to manufacture ractopamine hydrochloride is provided in a confidential appendix.

4. WASTE STREAM TREATMENT, CONTROL, AND HANDLING

a. Wastes from Manufacturing at Kinsale Facility

Aqueous waste streams from processes, tank and equipment washings, and floor washings will be generated from the manufacture of ractopamine hydrochloride. These aqueous wastes will be pumped to the on-site biotreatment balance tank prior to treatment through a decant vessel, which is designed to remove traces of nonmiscible solvents that may get washed into the aqueous waste streams.

All aqueous waste streams received by the treatment plant will be treated in an activated sludge biotreatment system where organic carbonaceous material can be broken down by microorganisms to yield end products of carbon dioxide and water, plus new

cellular material (sludge). All off-gases from the treatment plant will be collected from the roofed tanks and will be routed to a fume incinerator.

The activated sludge system consists of a 1,000 cubic meter holding tank where all incoming wastes will be jet mixed and pH adjustment will be carried out. From this tank, the wastewater will be forwarded to the biotreatment tanks at a rate based on the characteristics of the waste (average throughput is estimated to be 300 to 500 cubic meters per day). The wastewater will be forwarded through a tank with pH adjustment to a range of 6.5 to 8.0. If the pH is outside control limits, the forward feeding of wastes is controlled and the system recirculates waste streams until pH is within the control limits.

The activated sludge tanks consist of two tanks with a total usable capacity of 1,000 cubic meters. In these tanks, the mass of the microorganisms will be kept in suspension and will be supplied with oxygen by means of a jet aeration system. The air will be supplied by three 100 horsepower blowers (one on standby). In-line dissolved oxygen monitoring will be conducted to facilitate air control to the system to ensure that adequate residual dissolved oxygen is maintained.

Effluent will be routed to clarifiers where biomass in suspension will be allowed to settle. The supernatant from the clarifiers will overflow to final holding tanks before discharge. The settled biomass (sludge) will be returned to the activated sludge system for further treatment. If excess sludge is present in the system, sludge holding tanks will be used to stabilize the material prior to dewatering with a separator. Solids produced from the treatment plant will be taken off site to an approved facility. Supernatant from the dewatering process will go to polishing clarifiers.

The facility Wet Well will collect the effluents from the clarifier, sludge dewatering, and the thermal oxidizer scrubber. The thermal oxidizer wastewater will contain inorganic salts and fine particulate materials. This wastewater will be passed through clarifiers and a sump before discharge to cooling ponds, which will feed the facility Wet Well. Before

discharge to the cooling ponds, approximately 70% of the effluent will be recycled back to the thermal oxidizer units for reuse.

The final effluent will be pumped through a 5 mile pipeline which will transport it to the outer Kinsale harbor. The pipeline extends approximately 1000 meters out to sea. The effluent will be discharged through a diffuser. Based on a survey of tidal movement and mixing rates in the general harbor area, the effluent will be diluted and have no measurable impact on the receiving waters.

Numerous samples will be taken within the treatment plant and from the final effluent, and assayed on-site. The final effluent will be assayed for the emission limit values as stipulated in the discharge license. The final effluent will be assayed for biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, total suspended solids (TSS), ammonia as nitrogen, phenol and cyanide. Parameters that will be measured across the treatment system include the Settled Sludge Volume Index (SSVI), the Food to Microorganism (F/M) ratio, and the percentage of COD reduction.

The manufacturing of ractopamine hydrochloride requires use of 4 chemicals on the OSHA Air Contaminants List (ethyl acetate, hydrochloric acid, methanol, and potassium hydroxide). Only small quantities (50 kg or less) of methanol , hydrogen, and nitrogen are emitted to the atmosphere from the process steps.

The facility will utilize a fume oxidizer, which is a regenerative thermal oxidizer unit. It will be a tertiary treatment device operated at about 850 °C and used to incinerate fumes and to control odor. Process and scrubber vents will be ducted from the production buildings and will be routed to the fume oxidizer before discharge to the atmosphere. Within the unit, volatile organic compounds, other hydrocarbons and odor-causing constituents will be converted to carbon dioxide and water vapor.

The facility will employ the use of one liquid thermal oxidizer and one solids incinerator onsite to treat solvent and solid waste generated by the production processes. Both units will be down-fired incinerators with vertical combustion chambers operated at

1,000 °C. The units will have a minimum residence time of 1.5 seconds. The combustion gases leaving the chambers will be quench cooled before being directed to the gas cleaning plant. The liquid thermal oxidizer will also have two stages comprised of a condenser/adsorber for acid gas removal and a hydrosonic scrubber for particulate and droplet removal. The cooled and cleaned gases form the liquid thermal oxidizer will be combined with the fume oxidizer discharge.

Both units will be computer controlled. Any deviations outside the acceptable limits will be alarmed. If an alarm on a unit is unresolved, an automatic shutdown of the unit will occur. The computer ensures that waste will only be burned when the units are running under optimum conditions.

Two types of liquid waste will be fed into the units. Primary waste will be comprised of flammable solvent and waste material. Secondary waste will be comprised mainly of water with small amounts of organics. The wastes will be stored in tanks for characterization before being fed into the units. Diesel fuel will be used to heat the units and to maintain combustion temperature if there is insufficient energy available from the wastes.

Continuous monitoring of oxygen, water vapor, carbon monoxide, total organic carbon and hydrogen chloride levels will be carried out on the stack gas. The units will also be monitored for hydrogen fluoride, particulates, sulfur dioxide, and nitrogen dioxide on a quarterly basis. Performance tests indicate a destruction and removal efficiency of greater than 99.99%.

In addition, the solids unit will be provided with an inclined rotary kiln which will be used to burn solid waste (such as contaminated packaging, fiber drums, etc.) from the site. Solid waste will automatically be loaded using a ram feeder. The combustion gas will be routed through the afterburner of the solids unit.

The final product from this bulk manufacturing facility will be a 3 to 20 percent slurry of ractopamine hydrochloride in water. This product form eliminates the need for concern

about particulate air emissions of ractopamine hydrochloride that might have been present if the final product were a dry bulk material.

b. Wastes From Formulating and Packaging at the Dista Facility

At Dista, commissioning of a new state-of-the-art facility is being completed. The facility will have isolation rooms, localized venting, and devices to further reduce the emissions or discharge of wastes during formulation and packaging of Paylean. All process air will pass through high performance particle (HEPA-like) filters and dust collectors which will be packaged with solids, particulates, and dust for approved disposal. Where appropriate, air filtration systems are designed such that they are equipped with multiple filter systems. The HEPA-like filter systems that will be used throughout this facility will have removal efficiencies of 95 percent or more for particulates of 0.7 micron in size or larger.

These facilities include corn grit silos equipped with filters to minimize particulate emissions. Corn grit from these silos will transfer to hopper systems that will feed wet premixers. At this point, a solution of ractopamine hydrochloride will be added to the corn grit. This mixture will then be transferred to a fluid bed dryer that will feed nitrogen blanketed blenders. An auger weigher will receive the product from the blenders and will feed a bagging machine. The bagging machine will be composed of a bag feed system that will form bags from laminate reels, and will employ local exhaust ventilation systems to fill the bags with little or no displacement of the final product. The filled bags will pass through a conveyer system that will leak test the bag, check the bag weight, and transfer the bag to a palletizing robot which will load the bags into boxes. The palleted boxes of Paylean will be stretch wrapped on a rotating table and then stored in the warehouse section of the packaging building. Facility engineering controls, use of wet bulk material and corn grits in the mixing process, and bags with little or no volumetric displacement will minimize the potential for emission of aerosolized particles from the formulation and packaging of Paylean.

Authorization for processes at this contained fill/finish facility has been issued by the Metropolitan Borough Council of Knowsley (Appendix C).

Packaging materials and nonrecyclable wastes from the formulation and packaging areas will be disposed of at an approved off-site facility.

Nitrogen will be emitted to the atmosphere from the packaging and formulating process steps. The average and peak rate of nitrogen emission will be about 4000 lb/hr and 4400 lb/hr, respectively. This amount of nitrogen is required due to the type of containment operations necessary to control the atmospheric conditions inside the formulating and packaging equipment.

Liquid wastes (e.g., floor washings) from the area will be collected into effluent storage tanks where the material will be checked before being either pumped to a tanker for disposal at an approved facility or discharged to the sewer. If the liquid waste is discharged to the sewer, trace amounts of ractopamine hydrochloride may appear in the water contained in these storage tanks (i.e., <10 ppm). Up to 125 gallons of this water could be discharged in a 15-minute period with the rest of the wastewater from the entire site, which is discharged at a rate of approximately 1.2 million gallons/day. If this discharge occurred, a maximum concentration of 0.1 ppm ((125 gal/12500 gal/15 min) x 10 ppm) could be found in wastewater before dilutions in receiving waters.

Four consents to discharge trade effluent from the waste treatment tanks have been provided by the North West Water PLC. These consents contain provisions which limit the total COD discharged in any 24-hour period, the total flow in any 24-hour period, the pH range of the effluent, and the concentrations of various metals. Some classes of substances are restricted or prohibited in the discharge. North West Water PLC monitors compliance with the consent conditions normally on a weekly basis and retains the right to prosecute under the Control of Pollution Act of 1974 for any breach of compliance. Only negligible amounts of COD and suspended solids will be discharged from the floor washing operation at the formulating and packaging facility.

5. COMPLIANCE WITH ENVIRONMENTAL REGULATORY REQUIREMENTS

All production, formulation, and packaging facilities will comply with applicable regulations concerning emission control and waste treatment. Since it is not the practice of the regulatory authorities to issue letters of compliance, letters from Kinsale and Dista confirming the intent to comply with relevant environmental regulations and the intent to comply with Good Manufacturing Practices are provided in Appendix D. Letters indicating intent to comply with British and Irish occupational safety laws are also provided in Appendix D.

B. INTRODUCTION OF SUBSTANCE FROM FEED MIXING LOCATIONS

Feed mixing will be done by commercial feed vendors and by swine finishing operations. Commercial feed vendors are required to meet current USDA and FDA approved Good Manufacturing Practices for feed additives. Based on the required manufacturing controls for feed inventory accountability and on the low-dust formulation, the potential for release of ractopamine hydrochloride into the environment from feed mixing locations would be low.

Exposure monitoring studies have been conducted in various feed mills with two premix formulations, marumerized pellets and corn cob grits. The change from a marumerized product to the current corn cob grit formulation was made to ensure better homogeneity in the final feed. Another advantage of the corn cob formulation is that the premix will be manufactured directly from the aqueous ractopamine bulk slurry, thus eliminating exposure to airborne dust that could occur when handling dry technical material. Exposure monitoring studies in feed mills with the old marumerized product form are summarized in Appendices E through G.

A study was conducted at a moderately sized feed mill to assess the concentration of ractopamine hydrochloride in the air during repeated weighing of the marumerized premix and during mixing, bagging, and stacking operations (Appendix E). This feed mill has been

used for several years to periodically mix experimental feeds containing ractopamine hydrochloride. Dust respirators were worn by personnel during this monitoring study. Concentrations of ractopamine hydrochloride ranging from 0.003 to 0.008 mg/m^3 were found during the weighing of the marumerized premix for 4 minutes or 14 minutes. In 29 samples collected from personal and area samplers associated with all other operations, which lasted 30 minutes or less, concentrations of ractopamine hydrochloride were almost always at or below the detection limit (0.0002 to 0.0004 mg/m^3). Exceptions were noted in one of 14 samples in the bagging area (0.0056 mg/m^3) and in one of five personal samples in the bag stacking area (0.0046 mg/m^3).

Another study was conducted with the marumerized product at a small commercial feed mill to measure the concentration of ractopamine hydrochloride in the air during weighing, mixing, and bagging operations (Appendix F). This feed mill was not technically advanced nor automated. Samples were collected from the weighing, mixing, and bagging areas. All the marumerized premix needed to mix three separate batches was weighed into three small bags at one time in a small room with very little ventilation. Dust respirators were worn during the weighing operation which was completed within 6 minutes. Personal and area samplers indicated ractopamine hydrochloride concentrations of 0.042 and 0.0119 mg/m^3, respectively, were present during the weighing operation. Ractopamine hydrochloride could not be detected in 19 of the 20 samples collected during and after the mixing and bagging operations, which took 30 minutes or less to complete.

A third study was conducted with the marumerized premix at an automated and computerized feed mill to measure the concentrations of ractopamine hydrochloride in the air during weighing and mixing operations (Appendix G). Feed containing the marumerized premix was not bagged, but was delivered directly to a feed storage tank. Two one-ton batches of feed were mixed on three different days. Weighing operations were completed in 5 or 6 minutes and all weighing, mixing, and storage operations for both batches of feed were finished in about 50 minutes. Dust respirators were worn by personnel during this

monitoring study. Personal samplers indicated ractopamine hydrochloride concentrations ranged from <0.0017 to 0.0043 mg/m^3 during the weighing operations. The concentration of ractopamine hydrochloride in the air near the mixing area was 0.008 mg/m^3 on one occasion, but was not detected (<0.0002 or <0.0003 mg/m^3) in most air samples from this area.

With the change to the corn cob grit formulation, several preliminary studies were conducted to compare the general airborne concentrations of ractopamine hydrochloride from the weighing operations in a feed mill. The weighing operation was chosen since it routinely yielded the higher detectable levels of ractopamine in personal samplers from the marumerized premix formulation. Exposure monitoring for the formulations with and without soybean oil provided information for a decision to add soybean oil to reduce dust levels.

An initial study with four formulations (Appendix EE) demonstrated that premix with soybean oil resulted in lower mean levels of particulate air suspensions containing ractopamine hydrochloride. Weighing of the 2 percent premix without oil for one minute yielded a mean atmospheric exposure of ractopamine hydrochloride to operators of 0.0689 mg/m^3, while weighing of the 2, 5, and 10 percent premixes with 1 percent oil yielded concentrations of 0.0092, 0.0116, and 0.0234 mg/m^3, respectively. Weighing the 2 percent formulation without oil for 15 minutes resulted in a mean ambient air concentration of 0.0912 mg/m^3, but with oil the concentration was only 0.0069 mg/m^3.

In another study (Appendix FF), ambient levels of ractopamine hydrochloride from weighing of the 2 percent premix without oil were high, indicating substantial variation from study to study. Mean exposure from one-minute weighing operations was 1.575 mg/m^3. Weighing 2 percent premix without oil for 15 minutes resulted in an ambient level of 5.123 mg/m^3. The 2 percent premix with 1 percent oil yielded exposure levels quite close to those found in the previous study. Weighing the oiled formulation for 1 minute produced a mean air concentration of 0.015 mg/m^3, with the highest single measurement of 0.032 mg/m^3. A

fifteen-minute weighing operation led to an ambient air level of 0.014 mg/m^3 for the oiled formulation.

The 2 percent premix with 1 percent soybean oil was chosen as the final formulation for Paylean since initial studies just described indicated that exposure levels from handling ractopamine hydrochloride would be significantly lowered with addition of oil.

Once the final formulation was identified, a definitive exposure monitoring study (Appendix GG) was designed by an industrial hygienist to determine ractopamine hydrochloride concentrations that could exist in personal and area samples around the weighing, mixing, and bagging operations. Short-term (15 min) and long-term (8 hr) samples were collected in a feed mill that prepared 15 batches of medicated feed. Premix containing 2 percent ractopamine hydrochloride and 1 percent soybean oil was added to each 750-pound batch to achieve a concentration of 20 ppm in the feed. Each day for 3 days, 5 lots of premix were weighed out at one time and then subsequently added to feed before mixing and bagging. Full-shift personal monitoring encompassed all tasks, while short-term personal monitoring was conducted on the tasks of premix weighing and bagging. Mixing was not assessed with short-term personal monitoring due to the short duration of the task. Ractopamine hydrochloride could be detected (detection range 0.000105 to 0.000155 mg/m^3) in only one sample (0.000182 mg/m^3) from full-shift personal monitoring. Measurements from personal samplers active during short term weighing and bagging operations yielded mean exposure values of 0.00092 and 0.00049 mg/m^3, respectively. Ractopamine hydrochloride could not be detected in most personal samplers or any area samplers from the bagging operation. Area samplers next to the mixing operation collected from <0.00041 to 0.00116 mg/m^3, while ractopamine hydrochloride could not be found in area samplers about 15 feet away from the mixing operation.

The corn cob grit formulation of Paylean containing 1 percent soybean oil appears to have a consistently low potential for producing aerosolized particles in the workplace.

C. INTRODUCTION OF SUBSTANCE FROM THE USE SITE

Information from the United States Department of Agriculture (USDA Economic Reporting Service, 1988) indicates that there were about 77 million pigs raised as barrows and gilts for slaughter in the United States in 1987. About one-half of this production was centered in the states of Iowa, Illinois, Indiana, Ohio, Minnesota, Missouri, Kansas, Nebraska, North Carolina, and Georgia (USDA Agricultural Statistics Board, 1988).

There is substantial variation in the numbers of swine finished on farms and production facilities in the United States. Hundreds of facilities produce over 5000 head each year (Rhodes and Grimes, 1983). A pig in the finishing stage of growth fed ractopamine hydrochloride for 50 days has a weight increase of about 100 pounds. Feed conversion at this stage is about 3.3 pounds of feed/pound of growth (Watkins et al., 1987). A pig in the finishing stage of growth could then eat 330 pounds of feed with, at most, 18.2 g of ractopamine hydrochloride/ton of feed. A pig could then consume a total of about 3.0 g of ractopamine hydrochloride. A facility producing 5000 head could use as much as 15 kg of ractopamine hydrochloride or 750 kg of Paylean in a year.

If ractopamine hydrochloride were used for all of the swine produced in the United States, at most, 231,000 kg of ractopamine hydrochloride would be used each year (3.0 g ractopamine hydrochloride/pig x 77 x 10^6 pigs). This is equivalent to the use of 11.6 x 10^6 kg of Paylean each year. An optimistic market penetration of 30% could result at most, in an annual use of about 70,000 kg of ractopamine hydrochloride or 3.5 x 10^6 kg of Paylean each year.

Little, if any, ractopamine hydrochloride would be introduced from treated feed into the air of a swine feedlot facility. An air monitoring study was conducted in an enclosed swine facility to measure concentrations of ractopamine hydrochloride in the air before, during, and after feed treated with the marumerized premix was dispensed to the pigs (Appendix H). Over 5600 pounds of feed were dispensed during this study. Samples were collected from a remote area and from an enclosed wing of a building where pigs were held

and given feed treated with the marumerized premix. Dust respirators were worn by personnel during this study. Ractopamine hydrochloride could not be detected in 33 of the 45 samples collected. Measurable levels of ractopamine hydrochloride were found in both the remote area and the swine enclosure. These levels were at or just above the levels of detection. The highest concentration of ractopamine hydrochloride measured in this study, 0.0014 mg/m^3, was found in an area sample during the dispensing of the treated feed, which took about 20 minutes to complete. Personal samples collected during the study with marumerized product in the swine barn routinely contained lower ractopamine hydrochloride levels than those collected from personal samplers at the feed mill.

Levels of ractopamine hydrochloride found in personal samplers during a definitive study (Appendix GG) at a feed mill with the new premix (corn cob grits, 2 percent ractopamine, and 1 percent oil) were lower than levels found in personal samplers from most of the feed mills studied earlier for exposure from the marumerized product. Since levels found at the swine barn for the marumerized product were normally below detection limits, a study for exposure in the swine barn with the new premix was not conducted. Exposure levels from use of the new premix in the swine barn should be at least as low, and probably lower than, those found for the old marumerized product.

Ractopamine hydrochloride may be introduced into the environment via waste products from swine. The major metabolites excreted along with ractopamine hydrochloride by pigs are glucuronide conjugates of ractopamine hydrochloride (Appendix I). Because the biological activity of these conjugates is unknown, it will be assumed that the metabolites have the same biological activity as ractopamine hydrochloride. Essentially, all of the ^{14}C-ractopamine hydrochloride fed to a pig is excreted in the urine and feces, with an average of 91.3% in urine (Appendix J). For pigs fed a diet with 30 ppm (1.5 x the highest recommended rate) of ^{14}C-ractopamine hydrochloride, the average concentration of ractopamine hydrochloride residue (parent ractopamine hydrochloride plus metabolites) in undried excreta was found to be 17.9 ppm (Appendix K). Based on this study, pigs fed a diet

with the maximum recommended concentration of ractopamine hydrochloride (20 ppm) should produce undried excreta with 11.9 ppm (20 ppm/30 ppm x 17.9 ppm) of ractopamine hydrochloride residue.

This concentration (11.9 ppm) in undried excreta is about the same as the highest theoretical concentration calculated for ractopamine hydrochloride in undried excreta from the pigs in a finishing operation. A pig could consume and excrete up to about 3.0 g of ractopamine hydrochloride during the finishing stage of growth, or about 60 mg/day. A pig in the finishing stage of growth excretes an average of 5.2 kg of urine and feces each day (Midwest Plan Service, 1983), resulting in a theoretical concentration of ractopamine hydrochloride in undried excreta of 11.5 ppm (60 mg ractopamine hydrochloride/5.2 kg of excreta). The highest expected ractopamine hydrochloride concentration in undried excreta was assumed to be 11.9 ppm.

7. FATE OF EMITTED SUBSTANCE IN THE ENVIRONMENT

Several studies have been conducted to evaluate the characteristics of ractopamine hydrochloride which may influence its fate in the environment. Ractopamine hydrochloride is soluble in water (Appendix A) and hydrolytically stable in the normal environmental range of water temperatures (Appendices L and M). Photodegradation of ractopamine hydrochloride in water does occur so it will not accumulate in the aquatic environment. Photodegradation occurred with half-lives of 16.3, 10.5, and 0.64 days at pH values of 5.0, 7.0, and 9.0, respectively (Appendix N). Quantum yield and photolysis products were not determined in this study. Accurate estimates of half-lives at other latitudes are not available. Ractopamine hydrochloride also degrades (66%) over 28 days in an aqueous media with activated sewage sludge (Appendix JJ). Ractopamine hydrochloride strongly adsorbs to soil with adsorption coefficients (Kd) of 36.0, 29.6, and 14.5 for clay loam, loam, and sandy loam, respectively. Ractopamine hydrochloride does not readily desorb from soil. The adsorption coefficients (Koc) expressed on the basis of organic content of the soils are 2007, 2698, and 2090 for clay loam, loam, and sandy loam, respectively (Appendix O). Ractopamine hydrochloride is substantially biodegraded in soil resulting in evolution of CO_2 and volatile organic residues from the molecule (Appendix P). Degradation products which do not volatilize are strongly adsorbed to soil and cannot be extracted with acetone or methanol. The decline in the amount of ractopamine hydrochloride which can be chemically extracted from soil occurs in two phases (Appendix Q). The half-life for the first phase is about 1.1 days. The half-life for the second phase is about 51 days.

The primary manner in which ractopamine hydrochloride would be introduced into the environment is through use of swine excreta as fertilizer on cropland. Based on its relatively high melting point and on thermogravimetric analysis, measurable concentrations of ractopamine hydrochloride will not occur in the atmosphere. Based on the strong adsorption of ractopamine hydrochloride and its degradation products to soil, it is very unlikely that residues of ractopamine hydrochloride would leach through soil into groundwater.

Ractopamine hydrochloride could be found in cropland soil to which it is applied with swine excreta and in adjacent aquatic systems. It could also be possible to find measurable concentrations in the runoff from open-front confinement facilities for swine.

A. POTENTIAL CONCENTRATIONS OF RACTOPAMINE HYDROCHLORIDE IN SOIL

1. POTENTIAL CONCENTRATION OF RACTOPAMINE HYDROCHLORIDE IN THE FEEDLOT

Typical grower/finisher swine operations have enclosed confinement facilities or open-front confinement facilities on concrete. Swine excreta is collected from these facilities for later use as fertilizer on cropland. Few production facilities confine pigs in the open on soil. Open-front facilities would probably only occur in small operations. Production at these smaller operations could be limited by winter weather. The finishing stage of growth in swine takes about 60 days. Pigs in the finishing stage of growth in an open-front facility would have an average area of about 15 ft^2 each (Midwest Plan Service, 1983a). Each pig would be fed a total of 3 g of ractopamine hydrochloride (about 0.06 g/day for 50 days) and would excrete about the same amount of residue. The highest expected concentration of ractopamine hydrocholoride at a feedlot would be found in swine excreta. Any excreta mixed into the soil of a small feedlot would have a lower concentration of ractopamine hydrochloride.

If dissipation of ractopamine hydrochloride from excreta occurred at about the same rate as it does from soil, only 29% would be left in the excreta after two days (Appendix Q). Further dissipation would take several weeks. After initial dissipation, the concentration of ractopamine hydrochloride in excreta would be 3.45 ppm (11.9 ppm x 0.29). The total amount of ractopamine hydrochloride remaining from each pig in a feedlot at the end of a finishing period would be 0.87 g (3 g x 0.29).

2. POTENTIAL CONCENTRATION OF RACTOPAMINE HYDROCHLORIDE IN CROPLAND SOIL

The highest initial concentration of ractopamine hydrochloride in cropland soil can be estimated from the highest expected concentration in swine excreta (11.9 ppm, Section 6C), and from the use rate of swine excreta on cropland. A reasonable estimate of the application rate of swine excreta as fertilizer is 10 tons/acre (22.4×10^3 kg/ha). It is standard practice to incorporate manure 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. If ractopamine hydrochloride did not dissipate from excreta before it was added to soil, the initial concentration of ractopamine hydrochloride in cropland soil can be calculated to be as high as 0.12 ppm ((11.9 mg/kg x 22.4×10^3 kg/ha) ÷ 2.25×10^6 kg of soil/ha).

Ractopamine hydrochloride would dissipate from soil at a rapid rate (half-life, 1.1 days). Residues of ractopamine hydrochloride would dissipate from field soil by degradation and volatilization of degradation products. Ractopamine hydrochloride would not be expected to accumulate from year to year in cropland soil.

B. POTENTIAL CONCENTRATIONS OF RACTOPAMINE HYDROCHLORIDE IN AQUATIC SYSTEMS

Movement of ractopamine hydrochloride through runoff into aquatic systems could occur from cropland soils or, although less likely, from open-front feedlots with no containment systems. The highest possible aquatic concentrations of ractopamine hydrochloride would be found in runoff water before it is diluted by streams or ponds where aquatic organisms dwell. The half-lives for aqueous photolysis of ractopamine hydrochloride are 0.64 days at pH 9.0 and 10.5 days at pH 7.0. Ractopamine hydrochloride does degrade in soil and in an aqueous media with sewage sludge. Although the solubility of ractopamine hydrochloride varies with the pH of water, the solubility levels at different pH values are high enough to have no impact on the concentration of the compound in runoff water. Based on episodic introduction of ractopamine hydrochloride to surface water from runoff and the

moderately short half-life of the compound due to biodegradation in soil and photolysis, any exposure of nontarget aquatic organisms to ractopamine hydrochloride would be of short duration.

1. POTENTIAL CONCENTRATION OF RACTOPAMINE HYDROCHLORIDE IN RUNOFF FROM FEEDLOTS

The highest theoretical concentration of ractopamine hydrochloride in two inches of runoff from a feedlot can be calculated. Assuming all the ractopamine hydrochloride fed to the swine was excreted as parent material, assuming this material did not dissipate from excreta throughout the feeding period, and assuming all the ractopamine hydrochloride could be extracted from the excreta from an entire finishing period into the water of one two-inch runoff event, the highest theoretical concentration would be the total amount of ractopamine hydrochloride contributed from each pig (3 g) in a feedlot area (15 ft^2) divided by the amount of water in runoff from that area ((28.32 L/ft^3)(15 ft^2 x 2 in x 1 ft/12 in)). The highest theoretical concentration of ractopamine hydrochloride in runoff from a feedlot is 42.4 ppm. Assuming ractopamine hydrochloride does dissipate from excreta, the highest expected concentration in runoff from a feedlot is 12.3 ppm (0.87 g ractopamine hydrochloride in a 15 ft^2 area). Well designed feedlots have catchment systems for this runoff. In some cases, however, this runoff may reach surface waters.

2. POTENTIAL CONCENTRATION OF RACTOPAMINE HYDROCHLORIDE IN RUNOFF FROM CROPLAND

The highest expected concentration of ractopamine hydrochloride in cropland soil is 0.12 ppm. Runoff water from rainfall could carry some ractopamine hydrochloride from cropland into surface waters containing aquatic organisms. Because ractopamine hydrochloride dissipates from soil with an initial half-life of 1.1 days, significant amounts of this compound would not be extracted by runoff events which occur more than a week after manure is applied to the soil. If it were possible for all of the ractopamine hydrochloride incorporated into cropland with swine excreta to be extracted into runoff from one rainfall

event, a two-inch runoff event would carry 107.9 g of ractopamine hydrochloride or 0.52 mg/L ((10 tons of manure/acre x 907 kg/ton x 11.9 mg ractopamine hydrochloride/kg manure) ÷ (2 in x 102,794 L/acre-inch)). All ractopamine hydrochloride in the soil would not, however, be available for extraction into runoff.

The soil-adsorption coefficient (Kd) would limit the amount of residue extracted from the cropland soil. The lowest Kd found for ractopamine hydrochloride in soil was 14.5, which is the equilibrium ratio of the concentration of ractopamine on soil compared to the concentration in aqueous solution. Assuming ractopamine is at equilibrium in a slurry with equal amounts of water and soil (g/g), the amount of ractopamine in the water would be about 7% of the total (1/14.5 +1). In a slurry with less water than soil, a lower amount of ractopamine would be found in the water. If the level of ractopamine hydrochloride that could be extracted from soil is, at most, 7%, then the highest concentration of ractopamine hydrochloride in two inches of runoff would be about 0.037 ppm (0.07 x 107.9 g/205,588 L). This estimated maximum concentration of ractopamine hydrochloride in runoff water is based on the unlikely assumption that the runoff water would be in contact with the upper six inches of cropland soil long enough to allow concentrations of ractopamine hydrochloride in the soil and water to come to equilibrium.

A better estimate of the maximum expected concentration of ractopamine hydrochloride in runoff from cropland was calculated using the CREAMS (Chemicals, Runoff, and Erosion from Agricultural Management Systems) model developed by the USDA Agricultural Research Service (Appendix R). The model estimates chemical yield in runoff from field-sized areas using daily rainfall records. Input parameters and the geographical locations used for the model were selected to maximize the potential for runoff from cropland (Appendix R). The model simulation was conducted for a 20-year period, with 10 tons of swine manure containing 11.9 ppm of ractopamine hydrochloride incorporated into the soil each year. Conventional tillage, a high average field slope, and row-cropped corn were used in the simulation to maximize runoff. Manure application was

selected for the 20-year simulation to coincide with the date (April 30, Julian Day 120) of the second largest spring rainfall (3.58 in) and runoff (2.0 in) in 20 years for the selected site. The maximum concentration of ractopamine hydrochloride in the aqueous phase of runoff for the 20-year simulation was 0.0027. This maximum concentration was projected to be in runoff water from rainfall that occurred the same day (Julian Day 120) that ractopamine hydrochloride was incorporated into the field. The maximum annual yield of ractopamine hydrochloride in runoff of sediment and water occurred in the year with the second largest spring runoff event in 20 years and was 0.7% of the total applied on Julian Day 120 (April 30). This calculated maximum annual yield is within the annual yield values (<1.5%) suggested by Wauchope (1978) and Willis and McDowell (1982) for agricultural chemicals.

It is unlikely that ractopamine hydrochloride would persist in natural bodies of water for any significant length of time due to its susceptibility to photolysis and biodegradation. The photolysis half-life of ractopamine hydrochloride in sunlight ranges from 0.64 days to 10.5 days in water with pH values from 9.0 to 7.0, respectively (Appendix N). Ractopamine hydrochloride does biodegrade (Appendix P) and has an initial dissipation half-life of 1.1 days in soil (Appendix Q).

Based on the episodic introduction of ractopamine hydrochloride into surface water from runoff and the moderately short half-life of the compound, the duration of any exposure of nontarget aquatic organisms to ractopamine hydrochloride is probably short.

3. FATE OF RACTOPAMINE HYDROCHLORIDE IN AQUATIC ORGANISMS

Aquatic organisms could be exposed to very low levels of ractopamine hydrochloride when runoff occurs from surrounding agricultural fields. Substantial bioconcentration of ractopamine hydrochloride would not be expected based on the relatively low n-octanol/water partition coefficient. Neely, Branson, and Blau (1974) developed a regression equation for projected steady-state residue concentrations in trout muscle versus measured n-octanol/water partition coefficients for a variety of synthetic compounds.

$$\text{Log BCF (bioconcentration factor)} = 0.542 (\log K_{ow}) + 0.124$$

Using this equation, and the highest experimentally derived log K_{ow} value of 1.24 (Appendix B), the predicted BCF for ractopamine hydrochloride is 6.3. This calculated BCF indicates that 6.3 times more ractopamine hydrochloride might be found in fish muscle than in the surrounding water. If fish only lived in cropland runoff water containing the highest expected concentration of ractopamine hydrochloride (0.0027 ppm), the highest concentration of ractopamine hydrochloride in fish tissue would be 0.017 ppm (6.3 x 0.0027 ppm). Dilution of runoff from cropland soil and the short half-life of ractopamine hydrochloride in water, would combine to rapidly reduce the actual concentration of ractopamine hydrochloride to which fish could be exposed. Given the episodic nature of exposure, it is unlikely that substantial amounts of ractopamine hydrochloride would be bioconcentrated by aquatic organisms.

8. EFFECTS ON THE ENVIRONMENT OF RELEASED SUBSTANCES

A. MAMMALIAN TOXICITY STUDIES

An in-depth testing program has been completed with various laboratory animal species and ractopamine hydrochloride. Complete reports of all of these studies have been submitted to support the proposed action. Studies which help determine the safety of ractopamine hydrochloride to the public and the environment are briefly described below.

Acute and Subchronic Studies With Ractopamine Hydrochloride

Oral Median Lethal Dose for ICR Mice: 3547 mg/kg for males, 2545 mg/kg for females.

Oral Median Lethal Dose for Fischer 344 Rats: 474 mg/kg for males, 367 mg/kg for females.

Intraperitoneal Median Lethal Dose for Fischer 344 Rats: 132 mg/kg for males, 122 mg/kg for females.

Intravenous Injection in Beagle Dogs: Intravenous infusion of 0.035 mg/kg over 10 minutes produced increased tachycardia and decreased blood pressure.

Intravenous Infusion in Rhesus Monkeys: Intravenous infusion of 0.035 mg/kg over 10 minutes produced modest tachycardia and blood pressure was maintained.

Dermal Toxicity and Irritation in Rabbits: No dermal irritation, no mortality, and no overt signs of systemic toxicity were observed for 14 days after a dose of 2000 mg/kg body weight was applied topically to the skin. No compound-related lesions were found in a gross pathological examination of the test animals.

Ocular Irritation in Rabbits: Mild corneal opacity, slight to marked iritis, and slight to moderate conjunctivitis occurred within 1 hour after doses of 23 mg of ractopamine hydrochloride were placed in the eyes of rabbits. Corneal and iridal irritation cleared in five of six treated eyes within 7 days. All irritation cleared within 14 days.

Inhalation by Fischer 344 Rats: Twenty rats were exposed for 4 hours to each ractopamine hydrochloride concentration used in this study. Two, eight, and seven rats died from

exposure to ractopamine hydrochloride levels of 1.13, 1.96, and 2.50 g/m^3, respectively. No rats died from exposure to a ractopamine hydrochloride concentration of 0.583 g/m^3. The 4-hour median lethal concentration of ractopamine hydrochloride was 2.8 g/m^3. Signs of toxicity included hypoactivity, dyspnea, weight loss, poor grooming, and dry nasal exudate. Hepatic congestion and focal thymic hemorrhages were present only in animals which died following exposure to 2.50 g/m^3. Five female rats which died during exposure to 1.96 g/m^3 displayed dilatation of the vaginal orifice and vagina. No gross lesions were observed in rats which survived the full two weeks of the study.

15-Minute Inhalation by Rhesus Monkeys: Six conscious male rhesus monkeys were exposed to ractopamine at average activity concentrations of 0, 2.4, 13.9, and 27.4 mg/m^3 for 15 minutes. The mass median equivalent aerodynamic diameters of the aerosols were 1.4, 1.64, and 1.48 µm for exposure concentrations of 2.4, 13.9, and 27.4 mg/m^3, respectively. The activity median equivalent aerodynamic diameters were 1.40, 1.63, and 1.56 µm. Heart rates were monitored before, throughout, and after the exposure period. Significant increases in heart rates occurred at 13.9 and 27.4 mg/m^3. The no-observed- effect concentration for a 15-minute exposure to ractopamine aerosol was 2.4 mg/m^3.

Comparative Bioavailability of ^{14}C-Ractopamine Hydrochloride: Young female beagle dogs and rhesus monkeys were administered a single oral dose of 0.125 mg/kg ^{14}C-ractopamine hydrochloride. During the first 72 hours after administration, 79.4% and 69.8% of the radioactive dose was recovered from the dog and monkey, respectively. More than 90% of the recovered radioactive dose was cleared during the first 24 hours. Urine was the major route of clearance in both species.

Plasma and whole blood levels after administration of ^{14}C-ractopamine hydrochloride to dogs and rats indicate that absorption and elimination of the radioactive doses were fairly rapid. Peak blood levels occurred 1 to 2 hours after orally dosing dogs with 0.05, 0.5, or 5 mg/kg (except females at 5 mg/kg peaked 4 to 8 hours after dosing).

The elimination half-life for radiolabelled material from the plasma and whole blood of dogs was approximately 6 hours. Peak blood levels occurred within 2 hours after orally dosing rats with 0.5, 2.0, or 20 mg/kg. The elimination half-life from plasma and whole blood of rats was about 7 hours, except for males dosed at 20 mg/kg (about 15 hours).

Acute Oral Study for Cardiovascular Effects in the Beagle: Left ventricular inotropic state, systemic arterial pressure, heart rate, and electrocardiograms were recorded for conscious, instrumented beagles exposed to doses of ractopamine hydrochloride. Animals were tested using a double latin square design that allowed for assessing residual effects. The doses of ractopamine were 2, 50, and 125 µg/kg. Dose-dependent increases in heart rate and left ventricular inotropic state were found at the 50- and 125-µg/kg doses. There was no residual carry-over effect from one treatment to the next in the latin square design. Aortic pressure, both systolic and diastolic, decreased at the two highest doses. The highest dose resulted in a decrease in aortic pulse pressure. Treatment-related effects on electrocardiograms were not found at any dose. No cardiovascular effects were found for dogs treated with ractopamine hydrochloride at a dose of 2 µg/kg.

Acute Studies With a Marumerized Formulation Containing 10% Ractopamine Hydrochloride

The formulation used in these acute studies contained five times the concentration of ractopamine hydrochloride present in Paylean.

Oral Median Lethal Dose for Fischer Rats: A single dose of 2000 mg of formulation/kg body weight resulted in no mortality. Some leg weakness and hypoactivity were observed on the day of dosing.

Dermal Toxicity and Irritation in Rabbits: No mortality and no overt signs of toxicity were observed when a dose of 5000 mg/kg was applied topically to the skin. Very slight

erythema did occur in 4 of 10 animals, but this sign of irritation cleared within 7 days. A gross pathological examination showed no evidence of compound-related lesions.

Ocular Irritation in Rabbits: Corneal dullness, slight iritis, and slight to moderate conjunctivitis occurred within 1 hour after a dose of 61 mg of formulation was placed in the eyes of rabbits. All irritation cleared within 7 days.

Inhalation by Fischer 344 Rats: Because of low aerosolization of material from a granular formulation, a powdered blend of the formulation, before marumerization, was tested. Using appropriate dust-generating procedures, the highest concentration that could be tested was 3.34 gm formulation/m^3 (0.294 gm of ractopamine hydrochloride/cubic meter). Exposure to this concentration for 4 hours resulted in the death of 3 of 10 male rats and 2 of 10 female rats. Signs of toxicity included dyspnea, hypoactivity, dry nasal exudate, and poor grooming. All surviving rats appeared normal 2 days after exposure. A gross pathological examination revealed red and wet lungs in animals which died during exposure and dark livers in 4 of the 5 animals which died. No gross lesions were present in surviving animals.

Chronic, Reproduction, and Teratology Studies

Three-Month Rat Study: Rats were fed diets containing 0, 0.002, 0.02, and 0.2% ractopamine hydrochloride. The time-weighted average daily dose of ractopamine hydrochloride was 0, 1.3, 13.4, or 152.9 mg/kg for males and 0, 1.4, 15.3, or 156.8 mg/kg for females. No treatment-related effects were observed in rats fed a diet containing 0.002% of ractopamine hydrochloride. No mortalities were observed up to the highest dietary level tested, 0.2%.

Three-Month Mouse Study: In this supplemental study, mice were fed diets containing 0.0, 0.02, 0.14, and 1.0% ractopamine hydrochloride. This resulted in estimated time-weighted average daily doses of 0.0, 25, 175, and 1250 mg/kg/day. All the mice survived without clinical signs of toxicity. Only subtle effects on the weight of testes were found

for mice maintained on the diet containing the lowest level of ractopamine hydrochloride tested.

Three-Month Oral Monkey Study: Ractopamine hydrochloride was administered by nasogastric gavage to monkeys in doses of 0.125 mg/kg one time/day for 3 months. No effects were found on body weight, food consumption, heart rate, or electrocardiogram wave forms.

One-Year Dog Study: Oral doses of 0.112, 0.224, and 5.68 mg ractopamine hydrochloride/kg were administered daily as the 2% marumerized premix. Treatment-related effects on clinical observations, hematology and clinical chemistry parameters, organ weights, and pathology were limited to the high dose group. Except for resting bradycardia which returned to near normal in all dose groups during the last 6 months of the study, the no-effect dose was 0.224 mg/kg/day. Compared to primates, the dog was more sensitive to the cardiovascular effects of ractopamine hydrochloride.

One-Year Monkey Study: Rhesus monkeys (4/sex/dose) were dosed daily by nasogastric gavage with ractopamine hydrochloride (0.125, 0.5, or 4.0 mg/kg) for one year. Controls received purified water. All monkeys survived the treatment period and no clinical signs attributable to treatment were observed. Food consumption, physical and ophthalmic examinations, hematology, clinical chemistry, urinalysis, and gross and microscopic pathology were not affected by treatment. A significant increase in body weight occurred in the monkeys at the high dose group. Treatment-related increases in heart rate were found for the two high dose groups. Resting or nighttime heart rates were also significantly increased at these doses compared to controls. Heart weight relative to body weight was lower in females in the top two dose groups and a similar trend for heart weight was observed in males. The number and affinity of heart beta adrenergic receptors for [^{3}H]dihydroalprenolol was not affected. There was a decrease in the number of lung beta adrenergic receptors in monkeys of both sexes in the high dose group. The no-effect dose for this study was 0.125 mg/kg/day.

Two-Generation Reproduction and Teratology Study with Rats: Two generations of male and female rats were maintained on diets containing 0, 0.0002, 0.002, 0.02, or 0.2% ractopamine hydrochloride. Time-weighted estimates for consumption of ractopamine hydrochloride by both generations of males were 0, 0.15, 1.4, 15, and 160 mg/kg/day. Ranges of time-weighted estimates for consumption of ractopamine hydrochloride by both generations of females during different life-stages were 0, 0.12 to 0.17, 1.3 to 1.6, 13 to 17, and 140 to 190 mg/kg/day. Treatment-related toxicity was found only for rats at the highest treatment level, 0.2%. Two adult males and one adult female died at the highest treatment level. Treatment-related depressions in body weight, body weight gain, and efficiency of food utilization at the highest treatment level were expected because ractopamine hydrochloride is a thermogenic sympathomimetic amine. Mating performance and fertility were not adversely affected. The proportion of live fetuses was significantly depressed in the 0.2% group due to increases in both early and late resorptions. Developmental toxicity seen at the 0.2% level was attributed to physiological changes that may be associated with diminished uterine blood flow and/or maternal and fetal brown adipose thermogenesis. In F_{1a} and F_{2b} litters, litter size, gestation survival, progeny survival, and body weights were significantly depressed in the 0.2% group.

Pallor, apparent hypothermia, thinness, dehydration, and rough haircoat occurred with the highest frequency in neonatal and postnatal progeny of the 0.2% group of the F_{1a} and F_{2a} litters. In addition, the incidences of abnormalities, which included edema, cleft palate, limb and shoulder anomalies, brachygnathia, protruding tongue, and open eyelids, were increased.

Frequently observed abnormalities in the 0.2% group of the F_{2b} litters included edema, hydramnios, misshapen scapula, and limb anomalies. Other frequently observed developmental variations in the 0.2% group of the F_{2b} litters included incomplete

ossification of the calvaria, ribs, vertebral arches, ischium, and pubis; adrenal hemorrhaging; wavy ribs; and misalignment and incomplete fusion of sternal bars.

Parental and developmental toxicity were not found at dietary concentrations of ractopamine hydrochloride $\leq$0.02%. Time-weighted average consumption of ractopamine hydrochloride for males exposed to the 0.02% diet was 15 mg/kg/day. For females exposed to the 0.02% diet, the time-weighted consumption of ractopamine hydrochloride ranged from 13 to 17 mg/kg/day.

Subchronic Inhalation Toxicity Study in Rhesus Monkeys: In a pilot study, rhesus monkeys (2/sex/treatment) were exposed to aerosols of ractopamine hydrochloride for 4 hours/day for 2 to 8 days. Exposure to 23.8 mg/m^3, the highest concentration tested, was stopped after 2 days because of a near maximal (250 bpm) increase in heart rate that persisted until the second day. Exposure to treatment levels of 6.42 and 1.69 mg/m^3 were stopped after seven exposures. The lowest exposure level, 0.38 mg/m^3, also resulted in significant increases in heart rate both during exposure (daytime) and after exposure (nighttime). The increased heart rates at the three highest levels persisted after treatment was stopped and required approximately 2 weeks to return to normal values. No treatment-related changes occurred in body weights, organ weights, food consumption, hematology, or clinical chemistry parameters. No treatment-related gross or microscopic lesions were observed.

In the definitive study, rhesus monkeys (2/sex/treatment) were exposed to aerosols of ractopamine hydrochloride for 4 hours/day for 8 days. In order to detect any increased sensitivity following an exposure free period and to simulate potential exposure to humans, the monkeys were exposed daily for 5 days, not exposed for 2 days, then exposed again for 3 days. Aerosol exposure concentrations of ractopamine hydrochloride in the aerosols were 0 (air control), 0.05, 0.17, or 0.44 mg/m^3.

All animals survived to the end of the study. No clinical signs of toxicity were observed. No toxicologically important changes occurred in body weights, food

consumption, hematology or clinical chemistry parameters, or in organ weights. There were no gross or microscopic tissue changes related to exposure to ractopamine hydrochloride.

Heart rates were monitored during the 4-hour exposure period (daytime values) and during 6-hour postexposure intervals (nighttime values). A slight, but significant increase in heart rate was found for nighttime, but not daytime values, from monkeys exposed to a ractopamine hydrochloride concentration of 0.44 mg/m^3. There was no evidence of a cumulative effect on heart rate following repeated exposures to ractopamine hydrochloride. No significant changes in heart rate were found for daytime or nighttime measurements at ractopamine hydrochloride treatment levels of 0.17 or 0.05 mg/m^3.

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

1. EXPOSURE DURING PRODUCTION AND USE OF PAYLEAN

Ractopamine hydrochloride will be produced as a 3 to 20 percent aqueous slurry. This eliminates the need for special containment of dust that could come from the dry bulk drug. New state-of-the-art facilities and equipment will be designed and built to formulate and package Paylean. The solution side of the process is a closed system. The premixers maintain the integrity of the contained process during all phases of operation (loading, mixing, and unloading). Advances in the fluid bed dryer selected for this process, such as the metal cartridge filter, HEPA filter, and clean-in-place system, allows for containment of materials. The blender inlet and outlet conveyors will be suitably sealed to prevent release of materials. Blenders are totally contained. Paylean will be loaded into bags with little or no air displacement. Given that an aqueous slurry of ractopamine hydrochloride will be used to formulate Paylean, that the formulation and bagging processes will be contained, and that soybean oil will be added to reduce potential for dust formation, the potential for exposure to ractopamine hydrochloride during the production and formulation processes is quite low.

Engineering controls, personal protective equipment, and personal hygiene precautions will be used to minimize exposure. During the production processes, when appropriate, workers may wear personal protective gear such as foot covers, gloves, eye protection, protective clothing, or respirators. Short-term and long-term exposure guidelines of 0.24 mg/m^3 and 0.017 mg/m^3, respectively, for ractopamine hydrochloride have been recommended to allow safe exposure for 15 minutes and up to 12 hours. The background for selecting these guidelines has been covered in an overview of occupational exposure (Appendix HH).

The Paylean label will instruct people to routinely wear protective clothing, impervious gloves, and a NIOSH-approved dust mask when mixing and handling Paylean and to wash thoroughly after handling the product (Appendix II). The time for greatest potential exposure to ractopamine hydrochloride would be during the weighing of Paylean at feed mills, but this only requires a few minutes. Concentrations from 0.000432 to about 0.032 mg/m^3 have been measured in personal samplers during this weighing operation. A dust mask should reduce concentrations during this short exposure by a factor of at least 5, to levels below 0.010 mg/m^3. Detectable concentrations were not normally found around operations such as feed mixing and bagging. Concentrations associated with using treated feed in a swine barn were also normally below detection limits (<0.0004 mg/m^3) with a marumerized formulation, and are expected to be just as low for the new low dust formulation with corn cob grits. No special precautions will be recommended to handle treated feed in the swine barn. Exposure levels will be well below the short-term (0.24 mg/m^3) and long-term guidelines (0.017 mg/m^3) recommended for occupational safety.

Based on proposed safety measures and expected exposure concentrations, the production, formulation, and use of ractopamine hydrochloride is not expected to result in adverse affects on human health.

2. EXPOSURE VIA THE FOOD SUPPLY

Exposure of humans to biologically active amounts of ractopamine hydrochloride via the food supply is not expected. As has already been indicated (Section 7), it is highly improbable that measurable amounts of ractopamine hydrochloride would occur in drinking water from groundwater or surface water sources. Details of any exposure of humans to ractopamine hydrochloride in meat are listed in a Freedom of Information (FOI) Summary. The proposed action is not expected to adversely affect human health through the food supply.

C. EFFECTS OF RACTOPAMINE HYDROCHLORIDE ON NONTARGET ORGANISMS

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

Avian Species

Bobwhite Quail 14-Day Acute Oral Toxicity Study (Appendix S): Adult bobwhite quail (*Colinus virginianus*) were given a single oral dose of 0.0, 20, 40, 90, 200, 400, 900, and 2000 mg ractopamine hydrochloride/kg body weight at the beginning of the study. Three of 10 birds which received a dose of 2000 mg/kg died during the study. Another bird was judged to be moribund when the study was terminated. No mortality occurred in any other treatment group or in the control group. Loose feces occurred in a treatment-related fashion in all dose groups tested. Lethargy was noted at doses of 20, 40, 900, and 2000 mg/kg. Tremors were observed during the study in the bird judged to be moribund at the end of the study. Food consumption and body weight were reduced in birds given doses $\geq$200 mg/kg. Reductions in body weight gain, reductions in food consumption, and mortality were not found in the 20-mg/kg dose group. Loose feces and one lethargic bird were found in the 20-mg/kg dose group.

Bobwhite Quail 5-Day Dietary Study (Appendix T): Bobwhite quail, 10 days old, were fed diets containing ractopamine hydrochloride at average measured concentrations of 0.0, 0.0017, 0.0044, 0.01, 0.027, 0.067, 0.19, and 0.47% (w/w), resulting in average total consumption levels of 0.0, 0.018, 0.048, 0.106, 0.289, 0.909, 2.31, and 4.99 g ractopamine hydrochloride/kg body weight, respectively. Three of 10 birds died in the 0.47% treatment group and one of 10 birds died in the 0.01% treatment group. The highest dietary concentration of ractopamine hydrochloride tested which did not result in mortality, signs of toxicity (ataxia), reduced mean body weight gain, or reduced mean food consumption was 0.0044% (44 ppm).

Mallard Duck 5-Day Dietary Study (Appendix U): Mallard ducks (*Anas platyrhynchos*), 10 days old, were fed diets containing ractopamine hydrochloride at average measured concentrations of 0.0, 0.0091, 0.0165, 0.0356, 0.0672, 0.145, 0.291, and 0.596% (w/w), resulting in average total consumption levels of 0.0, 0.151, 0.268, 0.615, 1.12, 2.15, 4.07, and 10.0 g of ractopamine hydrochloride/kg body weight, respectively. No mortality or signs of toxicity were observed for birds from the control group or from any treatment group. The 0.0672% (672 ppm) dietary concentration was the highest treatment level tested which did not result in treatment-related reductions in mean body weight gain or food consumption.

Aquatic Species

Rainbow Trout 96-Hour Toxicity Study (Appendix V): Rainbow trout (*Salmo gairdneri*) were exposed to average measured ractopamine hydrochloride concentrations of 0.0, 23.2, 48.2, 94.7, 598, 672, 772, 870, and 971 ppm. The 96-hour median lethal concentration and its 95% confidence limits were 693 ppm and 523 to 918 ppm, respectively. No mortalities or behavioral signs of toxicity were found for fish exposed to ractopamine hydrochloride concentrations $\leq$48.2 ppm.

Bluegill 96-Hour Toxicity Study (Appendix W): Bluegill (*Lepomis macrochirus*) were exposed to average measured ractopamine hydrochloride concentrations of 0.0, 90.0, 191, 381, 482, 539, 591, 668, and 761 ppm. The 96-hour median lethal concentration, the 95% confidence limits for the median lethal concentration, and the slope of concentration-response curve were 544 ppm, 473 to 610 ppm, and 7.48, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to ractopamine hydrochloride concentrations ≤191 ppm.

Daphnia 48-Hour Toxicity Study (Appendix X): First-instar *Daphnia magna*, <24 hours old, were exposed to average measured ractopamine hydrochloride concentrations of 0.0, 4.47, 9.34, 23.1, 46.9, 71.3, and 93.3 ppm. The 48-hour median effective concentration, the 95% confidence limits of the 48-hour median effective concentration, and the slope of the concentration-response curve were 34.5 ppm, 27.9 to 41.0 ppm, and 4.81, respectively. No immobilization or other physical signs of toxicity were observed in animals exposed to ractopamine hydrochloride concentrations ≤9.34 ppm.

Selenastrum capricornutum 72-Hour Toxicity Study (Appendix KK): The green alga (*Selenastrum capricornutum*) was exposed to mean assayed ractopamine hydrochloride concentrations of 0.0, 25.4, 51.0, and 101.2 ppm for 3 days. Terminal cell count, maximum cell count, maximum specific growth rate (µ-max), and area under the curve (AUC) were significantly reduced relative to water control cultures at the highest treatment level, 101.2 ppm. The average specific growth rate (µ-reg) and the terminal biomass were not significantly affected at any concentration tested. Based upon these results, the NOEC for reduced population growth of this green alga was 51.0 ppm. The EC50 values based on µ-reg and growth of cell populations (AUC) were higher than the highest concentration tested, 101.2 ppm.

Activated Sludge Respiration Inhibition Study (Appendix LL): The respiration rate of activated sludge, obtained from a municipal sewage treatment facility, was measured in the presence of ractopamine at concentrations of 0.0, 10, 30, 100, 300, and 1000 ppm.

After 3 hours, the respiration rate was inhibited by about 39% at the highest concentration tested. No inhibitory effects on respiration rate were observed at the remaining treatment levels. An EC50 value of 1413 ppm was estimated based on linear regression analysis.

Terrestrial Species

Earthworm 28-Day Toxicity Studies (Appendix Y): Earthworms (*Lumbricus terrestris*) were exposed to ractopamine hydrochloride for 28 days in two separate studies. In the first study, earthworms were exposed to ractopamine hydrochloride concentrations of 0.0, 30.9, 63.1, 341, and 747 ppm in soil. In the second study, earthworms were exposed to ractopamine hydrochloride concentrations of 0.0, 1.35, and 8.11 ppm in soil. In the first study, treatment-related mortality was only observed at the 341 and 747 ppm levels, although reductions in body weight gain were noted down to the lowest level tested, 30.9 ppm. All earthworms exposed to mean ractopamine hydrochloride concentrations of 8.11 and 1.35 ppm in the second study appeared normal and in good physical condition throughout the study. No mortality, physical signs of toxicity, or statistically significant reductions in body weight gain were observed at the 8.11 or 1.35 ppm treatment levels.

Seed Germination and Radicle Development in Plants (Appendices Z and AA): Seeds of corn (*Zea mays*), cucumber (*Cucumis sativus*), turnip (*Brassica rapa*), and wheat (*Triticum aestivum*) were pretreated for 24 hours in aqueous solutions which contained 0, 1, 10, and 100 ppm ractopamine hydrochloride. The seeds were then washed and allowed to germinate for 3 to 5 days between moist layers of filter paper in Petri dishes. The results show that the seeds of wheat and corn at all treatment levels of ractopamine hydrochloride had the same extent of germination as controls. Reduced germination occurred in turnips and cucumbers at 100 ppm. Reduced radicle length occurred in turnips exposed to 10 and 100 ppm and in cucumbers exposed to 100 ppm. None of the four test species were affected at the ractopamine treatment level of 1 ppm.

In a second study, seeds of cucumber (*Cucumis sativus*), turnip (*Brassica rapa*), barley (*Hordeum valgare*), and soybean (*Glycine max*) were soaked in distilled water, then placed in test solutions containing ractopamine hydrochloride. The concentrations of the ractopamine hydrochloride were 1, 10, and 100 ppm for barley and soybean; 50, 75, and 100 ppm for cucumber; and 5, 7.5, and 10 ppm for turnips. The seeds were allowed to germinate for 4 to 5 days. The results of these studies show that ractopamine hydrochloride at all treatment levels had no effect on seed germination of the four cultivars and no effect on the development of the radicle of cucumber, barley, and soybeans. Reduced radicle length was found for turnip exposed to 10 ppm, but the radicle length was unaffected at concentrations $\leq$7.5 ppm.

Seedling Growth (Appendix BB): Seeds of corn, barley, cucumber, soybean, turnip, and wheat were germinated in sand and irrigated for 21 days with nutrient solutions containing 0, 1, 10, 50, and 100 ppm ractopamine hydrochloride. No significant effects were noted for the shoot length or the shoot and root weight of any species exposed to ractopamine hydrochloride concentrations $\leq$100 ppm.

Antimicrobial Activity (Appendix CC): A group of 35 gram-positive and gram-negative animal pathogens and 19 gram-positive and gram-negative anaerobes were inoculated onto the surface of agar plates containing ractopamine concentrations ranging from 0.5 to 256 ppm. Antimicrobial activity was not found against any of the animal pathogens or anaerobes at a ractopamine hydrochloride concentration $\leq$64 ppm.

D. POTENTIAL ADVERSE EFFECTS OF RACTOPAMINE HYDROCHLORIDE ON NONTARGET ORGANISMS

1. POTENTIAL ADVERSE EFFECTS ON AQUATIC ORGANISMS

The occurrence of ractopamine hydrochloride in surface water systems is expected to be acute and episodic, depending on runoff from cropland soils or feedlots containing ractopamine hydrochloride in swine manure. Any release into surface waters from the formulation facility would also be episodic. Biodegradation and photolysis of ractopamine

hydrochloride are relatively rapid so there is little possibility that aquatic organisms would be chronically exposed to ractopamine hydrochloride. The safety of aquatic organisms can then be assessed by comparing the concentration of ractopamine hydrochloride in wastewater effluent or runoff water from a large rainfall event to the results of acute studies with aquatic organisms. Although the concentration of ractopamine hydrochloride in runoff from a feedlot could theoretically be as high as 42.4 ppm, the highest expected concentration of ractopamine hydrochloride extracted into runoff water from a feedlot is 12.3 ppm. The maximum expected concentration of ractopamine hydrochloride in runoff from cropland soil is 0.0027 ppm. No mortalities or behavioral abnormalities were found for rainbow trout, bluegill, or *Daphnia* at measured ractopamine hydrochloride concentrations of 48.2, 191, or 9.34 ppm, respectively. *Selenastrum* was not affected by concentrations up to 51.0 ppm. These values are at least 3460 times higher than the maximum expected concentration of ractopamine hydrochloride in runoff water from cropland soil. These values are also substantially higher than the highest concentration expected in wastewater (0.1 ppm). The maximum expected concentration (12.3 ppm) of ractopamine hydrochloride in runoff from a feedlot is lower than the acute concentration test effect on fish and algae, but slightly higher than 9.34 ppm. *Daphnia* exposed only to the maximum expected concentration of ractopamine hydrochloride in runoff from a feedlot for 24 hours could experience some hypoactivity. Once the feedlot runoff water is diluted only slightly by surface waters where invertebrate species normally live, ractopamine hydrochloride concentrations would probably be too low to cause acute effects in *Daphnia*. Exposure to ractopamine hydrochloride delivered by runoff water into surface waters is not expected to have adverse effects on populations of aquatic organisms.

2. POTENTIAL ADVERSE EFFECTS ON EARTHWORMS

The highest expected concentrations of ractopamine hydrochloride in swine manure and cropland soil are 11.9 ppm and 0.12 ppm, respectively. Earthworm survival, appearance, and

growth are not significantly affected when worms are exposed to a ractopamine hydrochloride concentration of 8.11 ppm in soil. Earthworm growth rate is affected when worms are exposed to 30.9 ppm in soil. Earthworm survival, appearance, and growth would not be affected if worms were exposed to cropland soil containing the highest expected concentration of ractopamine hydrochloride. Earthworms in piles of swine manure may not grow as fast as other worms, but their survival and appearance would be unaffected by ractopamine hydrochloride. The use of ractopamine hydrochloride in swine feed is not expected to substantially affect populations of earthworms.

3. POTENTIAL ADVERSE EFFECTS ON AVIAN SPECIES

No mortality, reduction in body weight gain, change in food consumption, change in appearance, or change in behavior occurred for mallard ducks or bobwhite quail fed diets containing ractopamine hydrochloride concentrations $\leq$44 ppm. The recommended use rates of ractopamine hydrochloride in swine feed would result in a maximum dietary level of 20 ppm. Even if wild birds were allowed to forage in swine feed for several days, an 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 TERRESTRIAL PLANTS

Phytotoxicity from exposure to ractopamine hydrochloride is unlikely. Seed germination and the radicle length of corn, turnips, cucumbers, barley, soybeans, and wheat were assessed after exposure to solutions containing ractopamine hydrochloride. No effects were found on any cultivar at an exposure concentration of 7.5 ppm of ractopamine hydrochloride. This concentration is about 62 times higher than the highest expected concentration of ractopamine hydrochloride in cropland soil, 0.12 ppm. Seedlings of all species germinated and grew normally when irrigated with nutrient media with $\leq$100 ppm ractopamine hydrochloride. This concentration is 833 times higher than the highest expected

concentration in cropland soil. Since the initial half-life of ractopamine hydrochloride in soil is 1.1 days, this highest expected exposure concentration would quickly decline. The proposed action would not be expected to result in phytotoxicity to seeds grown in soil containing ractopamine hydrochloride.

5. POTENTIAL ADVERSE EFFECTS ON MICROBIAL ACTIVITY

None of the gram-negative or gram-positive microbes tested were inhibited by concentrations of ractopamine hydrochloride ≤64 ppm. This concentration is about 5 times higher than the highest expected concentration of ractopamine hydrochloride in runoff (12.3 ppm) from a feedlot and about 23,700 times higher than the maximum expected concentration of ractopamine hydrochloride in runoff (0.0027 ppm) from cropland soil. These aqueous concentrations are at least 81 times lower that the concentration (1000 ppm) shown to inhibit microbial respiration in an aqueous suspension of sewage sludge. The highest expected concentrations of ractopamine hydrochloride in excreta and cropland soil are 11.9 and 0.12 ppm. These concentrations are at least five times lower than the highest ractopamine hydrochloride concentration which resulted in no inhibition of the growth of gram-negative or gram-positive microbes. The proposed action would not be expected to affect the activity of the tested aerobic or anaerobic microbes in soil or water.

9. UTILIZATION OF NATURAL RESOURCES AND ENERGY

Production of ractopamine hydrochloride will occur at facilities already designed for safe and efficient production processes. Formulation and packaging of Paylean will occur in new facilities built specifically to safely contain dust from this process. The new building will be constructed at a large manufacturing plant that already produces pharmaceutical products. All of these facilities are operated according to Good Manufacturing Practices.

In general, process streams from the production of ractopamine hydrochloride only utilize a portion of the waste treatment or recovery facilities already installed for these and other process wastes. Disposal of waste from the manufacturing processes and operations will not require use of unusual amounts of energy or natural resources.

Estimation of natural resources and energy used in the production of Paylean included fixed costs and other miscellaneous energy usage not directly related to production, such as administrative office use. Production for the final liquid bulk drug substance at the facility in Kinsale will use about 10 percent of the natural resources (i.e., electricity, fuel oil, and propane gas) used by the entire production facility. At the Dista facility, formulation and packaging Paylean will use about 3.8 percent of the natural resources (i.e., electricity, oil, and coal) used by the entire facility.

10. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. Extensive state-of-the-art engineering controls in the formulation facility will reduce exposure to ractopamine hydrochloride. Personal protective gear will be worn, when appropriate, in production facilities. Protective measures recommended for feedmill operators are described in Section 8B1. The Paylean label (Appendix II) will instruct people to routinely wear protective clothing, impervious gloves, protective eyewear, and a NIOSH-approved dust mask when mixing and handling Paylean, and to wash thoroughly after handling the product. The label will also warn people that ractopamine hydrochloride is a beta-adrenergic agonist and that individuals with cardiovascular disease should exercise special caution to avoid exposure. A material safety data sheet describing Paylean has been included in Appendix DD.

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 personnel of Eli Lilly and Company are responsible for the preparation of the Environmental Assessment:

Roger D. Meyerhoff* — November 14, 1995*

Roger D. Meyerhoff, Ph. D. — Date
Head, Environmental Science and Hazard Communications

Gail D. Williams* — November 21, 1995*

Gail D. Williams, D.V.M., Ph. D. — Date
Senior Pathologist
Pathology

Neil J. Parke* — November 15, 1995*

Neil J. Parke, M.A. — Date
Senior Environmental Affairs Representative
Environmental Affairs

*** Documents on file with Elanco contain the original signatures.**

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:

Douglas M. Morton*

Douglas M. Morton, Ph. D.
Vice President
Lilly Research Laboratories

November 19, 1995*

Date

*** Documents on file with Elanco contain the original signatures.**

14. REFERENCES

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Appendix A: Report Summary

Title: Solubility of Ractopamine Hydrochloride in Aqueous Buffers

Study Number: JJL8603

Study Dates: September 5 to September 10, 1986

Name and Address of Investigators: J. J. Lewis and T. D. Macy, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Crystalline Ractopamine Hydrochloride

Test System: Laboratory apparatus, including high-performance liquid chromatographic (HPLC) assay of samples.

Summary of Experimental Design: Excess ractopamine hydrochloride was added to sterile, 0.1 M pH 5.0, 7.0, and 9.0 aqueous buffer solutions. One set of samples at each pH were heated overnight at 37°C and the remaining set of samples was left at room temperature (23°C). Both sets of samples were then equilibrated with continuous shaking at 23°C for 2 days. Samples were removed, filtered, and assayed by HPLC.

Summary of Results: In 48 hours at 23°C, ractopamine hydrochloride was highly soluble in water to a maximum concentration of 51.9, 31.0, and 41.2 g/L at pH 5.0, 7.0, and 9.0, respectively.

Appendix B: Report Summary

Title: N-Octanol-to-Water Partition Coefficient of Ractopamine Hydrochloride

Study Number: EWD8513

Study Dates: July 8 to July 31, 1985

Name and Address of Investigator: K. S. Cocke, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Crystalline ^{14}C-Ractopamine Hydrochloride

Test System: Laboratory apparatus for mixing and partitioning phases in centrifuge tubes, including liquid scintillation analysis of each phase.

Summary of Experimental Design: Solutions of ^{14}C-radiolabeled ractopamine hydrochloride in n-octanol were equilibrated with pH 5.0, 7.0, and 9.0 aqueous buffer. The ractopamine hydrochloride concentration in each phase was determined by radiochemical analysis.

Summary of Results: At 25°C, the n-octanol/water partition coefficients (K_{ow}) were determined to be 1.75, 1.02, and 17.4 for the pH 5.0, 7.0, and 9.0 aqueous buffer solutions, respectively. These low values indicate that ractopamine hydrochloride would not bioaccumulate in lipoid tissue.

Appendix C: Environmental Regulations Affecting the Dista Formulation Facility and the Kinsale Production Facility

Environmental Regulations Affecting the Dista Products, Ltd. Formulation Facility in England and the Eli Lilly S.A. Manufacturing Facility in Ireland.

Legislation	License	License Ref. No.	Date of Issue	Admin. Agency
Dista Products Ltd.				
Town and Country Planning Act 1990	Planning permission	K/APP/14656	21 Jan 1993	Metropolitan Borough of Knowsley
Environmental Protection Act 1990 Part 1	Environmental Protection (Applications, Apeals & Registers) Regs 1991 SI No. 507	B3/1194/6.9/24	13 Jan 1995	Metropolitan Borough of Knowsley
Public Health (Drainage of Trade Premises) Act 1937 Public Health Act 1961 Water Act 1973	Consent to Discharge	-	1 June 1981	North West Water PLC (formerly Authority)
Control of Pollution Act 1974[1]	Waste Disposal Facility Licence[2]	WDL 292/02	13 Mar 1989	Merseyside Waste Disposal Authority
Eli Lilly S.A. – Kinsale				
Environmental Protection Agency (EPA) Act of 1992	Integrated Pollution Control License	9	7 April 1995	Environmental Protection Agency

[1] Solids will be disposed of in accordance with the requirements of this act.
[2] Not specifically required for the formulating and packaging operations for Paylean, but applicable to other operations at the facility.

Appendix D: Letters Assuring Compliance with Regulations and Permits.

Dista Products Limited

Fleming Road, Speke
Liverpool L24 9LN
Telephone (051) 486 3939
Telex 627 178
Fax (051) 486 8740

Neil J. Parke,
Environmental Affairs
Eli Lilly.

10th Oct 1994

Dear Neil,

This letter of confirmation is provided in answer to your request in connection with the Paylean/Pulmotil submission to the US Food and Drug Administration. The request was for official confirmation that our planned facility at Dista Products Ltd., Speke, Liverpool for the manufacture of Paylean, will comply with the relevant health and safety laws and regulations, as applied to the united kingdom.

While it is not possible to obtain official confirmation that we are complying with these regulations, we can however confirm that we have received no official notification that our proposed operations in the manufacture of Paylean or Pulmotil will not comply with the relevant occupational health and safety regulations in the UK, and that we intend to manufacture the products mentioned in compliance with the laws and regulations, as detailed below.

The main pieces of legislation which apply are as follows:

Health and Safety at Work Act 1974
Control of Substances Hazardous to Health Regs 1988
Notification of New Substances (93) Regs.
Chemicals (Hazard Information and Packaging) Regs 1993
Noise Regulations 1989
Management of Health and Safety at Work Regulations 1992
Workplace (Health, Safety and Welfare) Regs 1992
Factories Act 1961
Fire Precautions Act 1971
First Aid Regs 1981

Yours sincerely,

D. Johnson*

Dr. D. Johnson
Operations Coordinator, Paylean/Pulmotil Production
Dista Products Ltd.,
Speke Operations.

*** Original signed document is on file with Elanco.**

Dista Products Limited

Fleming Road, Speke
Liverpool L24 9LN
Telephone (051) 486 3939
Telex 627 178
Fax (051) 486 8740

12th March 1993

TO: Dr. Derek Johnson,
Paylean/Pulmotil Project Manager,
Dista Products Limited.
Speke Operations.

Dear Dr. Johnson,

This letter of confirmation is provided in answer to the request in connection with the Paylean/Pulmotil submission to the U.S. Food & Drug Administration. The request was for official confirmation that our planned facility at Dista Products, Speke for the manufacture of Paylean/Pulmotil, will comply with the relevant environmental regulations of the U.K., especially as detailed in Appendix A of this letter.

From our enquiries, it appears that it is not possible to obtain such official confirmation. We can however, confirm that we have received no official notification that our operations in the manufacture of Paylean/Pulmotil will not comply with the relevant environmental laws of the U.K. as detailed, and that we intend in the manufacture of Paylean/Pulmotil, to comply with the relevant environmental laws of the U.K especially those mentioned in Appendix A of this letter. It is also our intention that the production of Paylean/Pulmotil will comply with Good Manufacturing Practices.

Yours sincerely,

Alan Tinsley*

Mr. Alan Tinsley
Director H.R. & Environmental Services
Dista Products Limited,
Speke Operations.

*** Original signed document is on file with Elanco.**

Eli Lilly S.A. Irish Branch, Dunderrow, Kinsale, Co. Cork.

Lilly

ELI LILLY S.A. – IRISH BRANCH

Mr. Mark Owens,
Director Corporate Environmental Affairs,
Eli-Lilly and Co.,
Indianapolis,
Indiana, 96285.

November 3, 1995

Dear Mr. Owens,

This letter of confirmation is provided in answer to your request in connection with the Paylean/Pulmotil submission to the U.S. F.D.A. The request was for official confirmation that our facility at Eli-Lilly, Kinsale for the manufacture of Paylean/Pulmotil will comply with the relevant health and safety laws and regulations of Ireland.

We confirm that our manufacture of Paylean/Pulmotil will be carried out in compliance with all applicable Health and Safety legislation and regulations in Ireland.

The main pieces of legislation which apply are as follows:

Safety, Health and Welfare at Work Act 1989
Safety, Health and Welfare (Chemical agents) Regulations, 1994
European Communities (Classification, Packaging, Labelling and Notification of Dangerous substances) Regulations, 1994
EC (Protection of workers exposure to noise) Regulations, 1993
Factories Act, 1955 & 1980
Safety in Industry Act, 1980
Fire Services Act, 1981
Dangerous Substances Act, 1972
Safety, Health & Welfare at work (General Application) Regulations, 1993.

Yours sincerely,

William Barrett*

William Barrett,
Director of Human Resources, Safety & Environmental Control

Tel: (021) 772699
Telex: 75900 LILY EI
Fax: (021) 755152

* Original signed document is on file with Elanco.

Eli Lilly S.A. Irish Branch, Dunderrow, Kinsale, Co. Cork.

Lilly

ELI LILLY S.A. – IRISH BRANCH

Mr. Mark Owens,
Director Corporate Environmental Affairs,
Eli-Lilly and Co.,
Indianapolis,
Indiana, 96285.

November 3, 1995

Dear Mr. Owens,

This letter of confirmation is provided in answer to your request in connection with the Paylean/Pulmotil submission to the U.S. F.D.A. The request was for official confirmation that our facility at Eli-Lilly, Kinsale for the manufacture of Paylean/Pulmotil will comply with the relevant environmental regulations of Ireland.

We confirm that our operations in the manufacture of Paylean/Pulmotil will comply with the requirements set out in our current Environmental licence, Integrated Pollution control licence (ref. 9) as issued by the Environmental Protection Agency on 7th April, 1995. This superceded the previous licences, namely, Air Pollution Licence A.P. 3/93 (R), Water Pollution Licence W.P. (W) 6/91, Toxic & Dangerous waste permit 2 TDW/1993. It is our intention that the production of Paylean/Pulmotil will comply with Good Manufacturing Practices.

Yours Sincerely,

William Barrett*

William Barrett,
Director of Human Resources, Safety & Environmental Control

Tel: (021) 772699
Telex: 75900 LILY EI
Fax: (021) 755152

* Original signed document is on file with Elanco.

Appendix E: Report Summary

Title: Air Monitoring Survey for Ractopamine Hydrochloride in a Feed Mill at Greenfield, Indiana

Study Number: T4V908806

Study Date: June 23, 1988

Name and Address of Investigators: J. S. Rybka and L. B. Hunt, Lilly Research Laboratories, Lafayette, Indiana 47902

Test Article: Paylean (2% ractopamine hydrochloride)

Test System: Moderately sized feed mill used to mix experimental feeds. This includes a weighing room and a room containing a 3000-lb capacity, tilt-tub mixer and a bagger.

Study Design: Paylean was mixed with five separate 3000-lb batches of swine feed to achieve a final ractopamine hydrochloride concentration of 20 ppm in the diets. Paylean was weighed (1361 g) for each individual batch of feed. Each batch was mixed, bagged, and stacked before the next batch was started to provide five replicates for each operation. Air samples near the weighing, mixing, bagging, and stacking operations were pumped through filters and these filters were analyzed for their content of ractopamine hydrochloride. Samples were also collected through filters attached to personnel in some of these areas. Since the weighing operation for any one batch of feed took less than 4 minutes, a special weighing study was monitored in which an individual weighed Paylean for 14 minutes. The analytical detection limit was 0.1 μg/filter pad. The actual ambient levels of ractopamine hydrochloride in the air were calculated based on the volume of air pumped through the filters. Dust respirators were worn by personnel during this monitoring survey.

Appendix E: Continued

Summary of Results:

Results of the survey are presented in Table 1. The weighing operation was consistently at exposure concentrations between 0.003 and 0.006 mg/m^3. Since some feed mills would weigh the Paylean into several containers at one time for all the feed batches produced during a day, Paylean weighing operations were repeated 14 times in a row. This special repeated weighing process resulted in an ambient ractopamine hydrochloride concentration of 0.008 mg/m^3 in the air.

In 29 samples collected from personal and area samplers associated with all other operations within the feed mill, concentrations of ractopamine hydrochloride were almost always below the detection limits (0.0002 to 0.0004 mg/m^3). One of five personal samples in the bag stacking area collected 0.0046 mg/m^3. The rest of the samples in this area were below detection. In the bagging area, one sample contained 0.0056 mg/m^3, another sample contained 0.0004 mg/m^3, and the other 12 samples had levels that were below detection. In the feed mixing area, one sample contained a ractopamine concentration of 0.0003 mg/m^3, while the remaining nine samples from this area contained no ractopamine hydrochloride.

One of nine samples not associated with the operations of feed production contained a detectable level of ractopamine hydrochloride. This sample was collected near the mixer before operations started. The sample collected near the bagger before operations began had no ractopamine hydrochloride. This active ingredient was also below detectable concentrations in samples collected outside the feed mill and in samples collected near the mixing and bagging equipment after all the batches of feed had been mixed and bagged.

Appendix E: Continued

Table 1. Assay Results of Samples from the Air Monitoring Survey for Ractopamine Hydrochloride in a Feed Mill at Greenfield, Indiana. Dust Respirators Were Worn by Personnel During this Survey.

		Results (mg/m^3)							
	Sample		Replicate No.						
Operation	Type	Before	1	2	3	4	5	After	Special
Weighing (4 min; Special – 14 min.)	Pers	--	0.0056	0.0030	0.0057	0.0060	0.0056	--	0.0080
Mixing – Near (30 min.)	Area	0.0003	<0.0002	<0.0003	0.0003	<0.0002	<0.0002	<0.0002	--
Mixing – Remote (30 min.)	Area	--	<0.0002	<0.0003	<0.0002	<0.0002	<0.0002	--	--
Bagging – Near (15 – 30 min.)	Area	<0.0002	*	0.0056	0.0004	<0.0003	<0.0003	<0.0002	--
Stacking (15 – 20 min.)	Pers.	--	0.0046	<0.0004	<0.0005	<0.0004	<0.0004	--	--
Bagging (15 – 20 min.)	Pers.	--	<0.0004	<0.0004	<0.0004	<0.0003	<0.0004	--	--
Bagging – Rem (15 – 20 min.)	Area	--	<0.0004	<0.0004	<0.0005	<0.0004	<0.0004	--	--
Outside (30 min.)	Area	--	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	--	--

Note: *No sample was collected because the pump had not been turned on due to a confusion in the sampling procedure.

Appendix F: Report Summary

Title: Air Monitoring Survey for Ractopamine Hydrochloride in a Feed Mill at Terre Haute, Indiana

Study Date: December 15, 1988

Name and Address of Investigators: J. S. Rybka, Lilly Research Laboratories, Lafayette, Indiana 47902

Test Article: Paylean (2% ractopamine hydrochloride)

Test System: Small commercial feed mill located in a pole barn

Study Design: Paylean was mixed with three separate 2000-lb batches of swine feed to achieve a final ractopamine hydrochloride concentration of 20 ppm. All the Paylean needed for each batch of swine feed was weighed at the beginning of the day in a small room with very little ventilation. Each separate batch was prepared by transferring the Paylean to the mixing equipment, mixing the diet in the mixer, and bagging the resulting diet. Batches of feed were prepared sequentially, so that three replicates could be monitored. Air samples near the weighing, mixing, and bagging operations were pumped through filters and these filters were analyzed for their content of ractopamine hydrochloride. Samples were also collected through filters attached to personnel in the operational areas. Weighing Paylean for all three batches took 6 minutes. Mixing and bagging operations took about 8 minutes and 20 minutes, respectively. The analytical detection limit was 0.01 μg/filter pad. The actual ambient levels of ractopamine hydrochloride in air were calculated based on the volume of air pumped through the filters. Dust respirators were worn by personnel during this monitoring survey.

Summary of Results: Results of the survey are presented in Table 1. Except for one sample collected from a remote location outside the building, ractopamine hydrochloride was only detected during the weighing operation. The personal sampler collected 0.042 mg/m^3, whereas the area sampler near the weighing operation collected 0.0119 mg/m^3.

Appendix F: Continued

Table 1. Results of Samples from the Air Monitoring Survey for Ractopamine Hydrochloride in a Feed Mill at Terre Haute, Indiana. Dust Respirators were Worn by Personnel During this Survey.

Sample Description (Duration)		Sample Type	Results (mg/m^3)
Weighing (6 min)		Area	0.0119
		Personal	0.0420
Desk Near Mixing Station (30 min)	Before	Area	<0.0003
	Rep 1	Area	<0.0003
	Rep 2	Area	<0.0003
	Rep 3	Area	<0.0003
	After	Area	<0.0003
Mixing (8 min)	Rep 1	Area	<0.001
	Rep 2	Area	<0.001
	Rep 3	Area	<0.001
	Rep 1	Personal	<0.001
	Rep 2	Personal	<0.001
	Rep 3	Personal	<0.001
Bagging (20 min)	Rep 1	Area	<0.0004
	Rep 2	Area	<0.0004
	Rep 3	Area	<0.0004
	Rep 1	Personal	<0.0004
	Rep 2	Personal	<0.0004
	Rep 3	Personal	<0.0004
Outside Remote (30 min)	Before	Area	<0.0003
	Rep 1	Area	0.001
	Rep 2	Area	<0.0003
	Rep 3	Area	<0.0003
	After	Area	<0.0003

Appendix G: Report Summary

Title: Air Monitoring Survey for Ractopamine Hydrochloride in a Feed Mill at Sheridan, Indiana

Study Date: January, 1989

Name and Address of Investigators: J. S. Rybka, Lilly Research Laboratories, Lafayette, Indiana 47902

Test Article: Paylean (2% ractopamine hydrochloride)

Test System: Automated and computerized feed mill

Study Design: Paylean was mixed with two 1-ton batches of swine feed. About 4 lbs of Paylean was weighed in 5 or 6 minutes for each batch. Each batch of feed was mixed so as to contain about 20 ppm of ractopamine hydrochloride. Each batch of feed was mixed and then augured directly to a feed storage tank. The feed was not bagged. All operations to prepare both batches lasted about 50 minutes. These operations were monitored three times over a 2-week period for replication. Air samples were collected from the weighing and mixing operations by pumping ambient air through glass fiber filters and these filters were analyzed for their content of ractopamine hydrochloride. The actual ambient levels of ractopamine hydrochloride in air were calculated based on the volume of air pumped through the filters. Dust respirators were worn by personnel during this air monitoring survey.

Summary of Results: Results of the survey are presented in Table 1. Ractopamine hydrochloride concentrations of <0.0017, 0.0017, and 0.0043 mg/m^3 occurred when Paylean was weighed out before being mixed into the feed of swine. The concentration of ractopamine hydrochloride in air during the mixing operation was 0.008 mg/m^3 on one occasion, but concentrations were generally not detectable.

Appendix G: Continued

Table 1. Assay Results of Samples From the Air Monitoring Survey for Ractopamine Hydrochloride in a Feed Mill at Sheridan, Indiana. Dust Respirators Were Worn by Personnel During This Survey.

Sample Operation (Duration)	Replicate Type	Day #	Results (mg/m^3) Before	During	After
Weighing (5-6 min)	Pers	1	--	0.0043	--
		2	--	<0.0017	--
		3	--	0.0017	--
Mixing (Approx. 26 minutes)	Area	1	<0.0003	<0.0002	<0.0003
		2	<0.0003	0.008	<0.0003
		3	0.0003	<0.0002	<0.0003
Feed Pump Area	Area	1	--	<0.0002	<0.0003
		2	<0.0003	<0.0002	0.0004
		3	<0.0003	<0.0002	0.0003
Outside	Area	1	<0.0003	<0.0002	<0.0003
		2	<0.0003	0.0013	0.0003
		3	<0.0003	<0.0002	<0.0003

Appendix H: Report Summary

Title: Air Monitoring Survey for Ractopamine Hydrochloride in a Swine Barn at Greenfield, Indiana

Study Number: T4V908807

Study Dates: September 12, 1988; September 18, 1988; and September 21, 1988

Name and Address of Investigators: J. S. Rybka, Lilly Research Laboratories, Lafayette, Indiana 47902 and W. P. Waitt, Lilly Research Laboratories, Greenfield, Indiana 46140

Test Article: Swine feed containing Paylean (2% ractopamine hydrochloride)

Test System:

This study was conducted in one of six enclosed wings attached to a moderately sized swine facility. This wing was 120 ft long, 26 ft wide, and 8 ft high with a concrete path down the center of the entire length of the building. Pig pens located on both sides of the path were separated by metal bar partitions and a solid sheet of metal from the floor up to 1 ft above the floor. The floor of each pen was slotted concrete which allowed disposal of manure into a pit below the floor. Fans were located in the walls and the pit to draw air from the edges of the ceiling through the facility to help control the air temperature and odor within the facility. Feeders that held about 150 lbs of feed were located in a corner of each pen next to the central aisle.

A total of 18 pens were used to contain 72 pigs in this study. Four pigs were placed in each of nine pens on each side of the central aisle. All pigs occupied pens at one end of the facility. The initial average weight of crossbred barrows and gilts used in this study was 180 lbs. All animals were allowed to feed *ad libitum* and fresh water was available at all times. The general health of all pigs was monitored by the station veterinarian.

Study Design: Pigs in an enclosed facility were provided final feed containing Paylean. The active ingredient in Paylean, ractopamine hydrochloride, was at a concentration of 20 ppm (18.14 g/ton) in the feed. A total of 5650 lbs of feed was dispensed by hand from bags into the pig feeders on three separate dates. Air samples were pumped through filters fitted to the neck and shoulder region of the person dispensing the feed and through filters placed between and only slightly above feeders in three sets of adjacent pens (areas 1, 2, and 3). The analytical detection limit was 0.1 μg/filter pad.

The actual ambient levels of ractopamine hydrochloride in the air were calculated based on the volume of air pumped through the filters. Samples were collected before, during, and after the feed was dispensed. Dust respirators were worn by personnel during this monitoring study.

Appendix H: Continued

Summary of Results: Results of the survey are presented in Table 1. Five of the 12 samples collected in the remote office area had ractopamine concentrations that ranged from 0.0003 to 0.0011 mg/m^3. Ractopamine hydrochloride was not detected in the remaining samples from the remote area. In the swine holding area, ractopamine hydrochloride concentrations were generally near or below detection limits.

Appendix H: Continued

Table 1. Assay Results of Samples from the Air Monitoring Survey for Ractopamine Hydrochloride in a Swine Barn at Greenfield, Indiana. Dust Respirators Were Worn by Personnel During the Survey.

Operation	Sample Type	Results (mg/m^3) Sample Date 9/12/88	9/18/88	9/21/88	Detection Limit	Average Sampling Duration
Before	Remote	ND	ND	ND	0.0003	30 min
	Area 2	ND	ND	0.0004		
Dispensing	Remote	ND	0.0004	0.0011	0.0004	20 min
	Area 1	0.0007	ND	ND		
	Area 2	0.0007	0.0005	0.0014		
	Area 3	ND	ND	ND		
	Personal	ND	ND	ND		
Dust Settling	Remote	ND	ND	ND	0.00003	240 min
	Area 1	ND	ND	ND		
	Area 2	ND	ND	0.00003		
	Area 3	ND	ND	ND		
Dust Settled	Remote	0.0003	0.0003	0.0004	0.0003	30 min
	Area 1	ND	ND	ND		
	Area 2	0.0004	ND	ND		
	Area 3	ND	ND	ND		

Appendix I: Report Summary

Title: Characterization of ^{14}C-Residues in Tissues and Excreta from Swine Fed ^{14}C-Ractopamine Hydrochloride

Study Number: ABC-0355

Study Dates: June 27, 1984 to August 21, 1986

Name and Address of Investigator: J. E. Dalidowicz, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: ^{14}C-Ractopamine Hydrochloride

Test System: Swine

Summary of Experimental Design: Liver, kidneys, and urine from swine receiving 30 ppm of ^{14}C ractopamine HCl in the feed were used to identify and estimate the ^{14}C-ractopamine residues. Ractopamine used in this study had a uniform ^{14}C-label on both rings. Parent material and metabolic products were identified by high-performance liquid chromatography, thin-layer chromatography, nuclear magnetic detection, and mass spectroscopy.

Summary of Results:

At steady-state tissue residue concentrations (4 days of dosing), the mean amount of ractopamine as a percent of total residues present in urine from six pigs was 58.8%. The rest of the urinary residues were essentially all glucuronic acid conjugates of ractopamine. Since the residues in feces were small (~9% of the total excreta residues), they were not characterized.

Close to 75% of the liver and 85% of the kidney residues were extractable. Of the extractable liver residues, 30% to 50% were ractopamine and the rest glucuronic acid conjugates of ractopamine. The kidneys contained higher amounts of glucuronic acid conjugates (64%) with a concomitant decrease in the amount of ractopamine (20% to 30%).

Appendix J: Report Summary

Title: ^{14}C-Ractopamine Hydrochloride Balance-Excretion Study in Swine

Study Number: ABC-0330

Study Dates: November 5 to November 21, 1985

Name and Address of Investigators: J. E. Dalidowicz, T. D. Thomson, and R. J. Herberg, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: ^{14}C-Ractopamine hydrochloride

Test System: Swine

Summary of Experimental Design: Three crossbred swine (two barrows and one gilt) were each fed 1.0 kg of feed containing 20 ppm of unlabeled ractopamine hydrochloride twice daily for 5 days. At the end of the predosing period, each swine received a one-time dose of 40 mg of ^{14}C-ractopamine hydrochloride (0.5 μCi/mg) incorporated into control feed. The swine then continued to receive 1.0 kg of ration containing 20 ppm of unlabeled ractopamine hydrochloride twice daily until termination of the experiment. The entire urinary and fecal output of each animal was collected at 24-hour intervals.

Summary of Results: During the 7-day collection period, the three animals excreted an average of 96.5% of the theoretical dose of ^{14}C-ractopamine hydrochloride administered. This is considered to be essentially complete recovery for an experiment of this kind. A total of 91.3% of the recovered ^{14}C-ractopamine hydrochloride was found in urine and 8.7% was in feces. The bulk of the ^{14}C-ractopamine dose (95.4%) was excreted in the first 3 days with 84.7% eliminated during the first day.

Appendix K: Report Summary

Title: ^{14}C-Ractopamine Residue Concentrations in Swine Excreta

Study Number: ABC-0377

Study Dates: January 17, 1985 to February 1, 1985

Name and Address of Investigator: J. E. Dalidowicz, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: ^{14}C-Ractopamine hydrochloride

Test System: Crossbred barrow and gilt

Summary of Experimental Design: A barrow and gilt were fed ^{14}C-ractopamine hydrochloride at 30 ppm for 4 days. On Days 3 and 4, the entire 24-hour urinary and fecal output was collected and analyzed for radioactivity.

Summary of Results: The amount of urine and undiluted feces and the concentration of ^{14}C-ractopamine hydrochloride in urine and feces are listed in Table 1 for this study. The average concentration of ^{14}C-ractopamine hydrochloride in the undried excreta can be calculated as 17.9 ppm, based on average amount of ^{14}C-ractopamine hydrochloride excreted in the urine and feces (59.4 mg) divided by the average weight of the urine and feces (3.32 kg). If these pigs were fed the maximum recommended concentration (20 ppm) of ^{14}C-ractopamine hydrochloride in their diet, the calculated concentration of this material in undried excreta is 11.9 ppm (20 ppm/30 ppm dietary concentrations x 17.9 ppm concentration in undried excreta in this study).

Appendix K: Continued

Table 1. Excretion of ^{14}C-Ractopamine Hydrochloride in the Feces and Urine of Swine.

	Pig 181		Pig 187		
	Day 3	Day 4	Day 3	Day 4	Mean
Feces Excreted (g)	683	438	656	914	673
Concentration in Feces (ppm)	7.7	9.8	6.3	6.4	7.6
Total Compound Excreted in Feces (mg)	5.3	4.3	4.4	5.8	4.9
Urine Excreted (ml)	2010	2760	3025	2800	2649
Concentration in Urine (ppm)	25.8	18.2	20.4	19.4	21.0
Total Compound Excreted in Urine (mg)	51.9	50.2	61.7	54.3	54.5

Appendix L: Report Summary

Title: Hydrolysis of Ractopamine Hydrochloride in Aqueous Buffer Solutions

Study Number: EWD8509

Study Dates: June 1985 to July 1985

Name and Address of Investigators: K. S. Cocke, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Crystalline Ractopamine Hydrochloride

Test System: Laboratory hydrolysis rate test with sterile buffer solutions and high-performance liquid chromatographic (HPLC) assay of samples.

Summary of Experimental Design: Sterile, aqueous buffer solutions of pH 4.0, 7.0, and 9.0 were fortified with 100 μg/mL ractopamine hydrochloride and maintained in the dark at 52°C. Samples at each pH were periodically removed for up to 7 days after initiation of the study and analyzed by HPLC.

Summary of Results: At pH 4.0 and 7.0, ractopamine hydrochloride was hydrolytically stable in water after 7 days at 52°C. At pH 9.0, the results at 52°C indicated hydrolysis of ractopamine hydrochloride with a first-order rate constant of 0.0364 day^{-1}. This corresponds to a half-life of 19 days. Hydrolysis at environmentally significant temperatures should be much slower.

Appendix M: Report Summary

Title: Definitive Hydrolysis Study of Ractopamine Hydrochloride in pH 9.0 Aqueous Buffer Solution

Study Number: JJL8601

Study Dates: July 1986 to August 1986

Name and Address of Investigators: J. J. Lewis and T. D. Macy, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Crystalline Ractopamine Hydrochloride

Test System: Laboratory hydrolysis rate test with sterile, pH 9.0 buffer solutions at room temperature and high-performance liquid chromatographic (HPLC) assay of samples.

Summary of Experimental Design: To further define the extent of base promoted hydrolysis, sterile aqueous buffer solutions of pH 9.0 were fortified with 100 μg/mL ractopamine hydrochloride and maintained in the dark at 25°C. Samples at pH 9.0 were periodically removed for up to 28 days after initiation of the study and analyzed in triplicate.

Summary of Results: The results of this definitive hydrolysis study demonstrated that ractopamine hydrochloride was hydrolytically stable at pH 9.0 and 25°C with a half-life of 653 days.

Appendix N: Report Summary

Title: Sunlight Photodegradation Study of Ractopamine Hydrochloride

Study Number: EWD8625

Study Dates: July 17, 1986 to August 21, 1986

Name and Address of Investigators: J. R. Koons and T. D. Macy, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Crystalline Ractopamine Hydrochloride

Test System: Summer sunlight on aqueous solutions in quartz tubes.

Summary of Experimental Design:

Solutions containing 10 μg/mL of ractopamine hydrochloride were prepared with sterile aqueous buffer solutions of pH 5.0, 7.0, and 9.0. Aliquots of these sterile test solutions were poured into sterile quartz tubes, sealed, and exposed to summer sunlight at approximately 30° from the vertical. Test samples at pH 5.0 and 7.0 were exposed to sunlight for 21 consecutive days. Test samples at pH 9.0 were exposed to sunlight for one day. At each pH, identical positive control solutions contained in quartz tubes were wrapped with aluminum foil to exclude sunlight and were exposed for slightly longer times than the test samples. Buffer solutions (blanks) were also exposed to sunlight to check for any interferences. At the initiation of the sunlight photolysis study, zero-time samples for each pH were placed in the dark for subsequent assay at the end of each test period. At certain intervals and at the end of each test period, the ractopamine hydrochloride concentrations were measured by high-performance liquid chromatography.

The relative sunlight intensity during the study was monitored using a chemical actinometer, p-nitroacetophenone (PNAP). PNAP solutions at 5 μg/mL in 0.063 M aqueous pyridine were exposed to sunlight as previously described for the ractopamine hydrochloride samples.

Summary of Results: No degradation of ractopamine hydrochloride or PNAP was observed in positive control solutions. Both ractopamine hydrochloride and PNAP photodegraded in sunlight at all pH levels. The average aquatic photodegradation rate constants (k) for ractopamine hydrochloride at pH 5.0, 7.0, and 9.0 were 0.0425, 0.0657, and 1.086 $days^{-1}$, respectively. The corresponding half-lives for ractopamine hydrochloride in aqueous solutions exposed to sunlight were 16.3, 10.5, and 0.64 days at pH 5.0, 7.0, and 9.0, respectively. Quantum yield was not determined in this study, so accurate estimates of photolysis half-lives at other latitudes are not available. Photolysis products were not identified in this study. Based on these data, however, ractopamine hydrochloride should not accumulate in the aquatic environment.

Appendix O: Report Summary

Title: Soil Sorption/Desorption Study with Ractopamine Hydrochloride

Study Number: JJL8602

Study Dates: July 9, 1986 to August 5, 1986

Name and Address of Investigators: J. J. Lewis and T. D. Macy, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Crystalline ^{14}C-Ractopamine Hydrochloride

Test System: Laboratory kinetics, sorption/desorption, and isotherm determination tests with sandy loam, loam, and clay loam soils.

Summary of Experimental Design:

In the soil kinetics test, 8 g of each soil type were equilibrated in glass centrifuge tubes with 40-mL solutions fortified with 1.0 µg/mL ^{14}C-ractopamine hydrochloride in 0.01 M $CaCl_2$. Blanks containing soil and 0.01 M $CaCl_2$, yet no ractopamine hydrochloride, were also prepared. All samples were stoppered and secured on a mixing wheel to keep the soil in suspension. At zero-time and at the end of 24-, 48-, and 72-hour mixing periods at room temperature, fortified samples and blanks from each soil type were removed and centrifuged. Aliquots were assayed by radiochemical analysis and high-performance liquid chromatography.

In the sorption/desorption test, 8 g of each soil type were equilibrated with 40-mL solutions of 1.0 µg/mL ^{14}C-ractopamine hydrochloride in 0.01 M $CaCl_2$ for 24 hours on a mixing wheel at room temperature. Blanks containing no ractopamine hydrochloride were also prepared. After the equilibration period, samples were centrifuged and an aliquot from the aqueous layer from each tube was analyzed radiochemically to measure the extent of soil sorption. The remaining aqueous layer was decanted and another 40 mL of 0.01 M $CaCl_2$ solution was added to each of these tubes. These samples were agitated an additional 16 hours and centrifuged. The aqueous layer was then analyzed radiochemically to measure the extent of soil desorption. The remaining aqueous layer was decanted as before and the desorption process was repeated one additional time. For the determination of mass balance, the aqueous layer was decanted and the soil samples remaining in each of these tubes were analyzed by ^{14}C-combustion.

In the isotherm determination, 40-mL solutions of 0.2, 1.0, 5.0, and 25.0 µg/mL ^{14}C-ractopamine hydrochloride in 0.01 M $CaCl_2$ were equilibrated with 8 g of soil in centrifuge tubes on a mixing wheel. Blanks were also prepared. After a 24-hour equilibration period, the samples were centrifuged and an aliquot of the aqueous layer from each tube was analyzed radiochemically.

Appendix O: Continued

Summary of Results:

The data obtained in the soil kinetics test indicated that the time required for ractopamine hydrochloride to achieve an equilibrium concentration was 24 hours for all three soil types. The results of the sorption/desorption test are presented in Table 1. The mass balance data in Table 2 were determined to be within experimental error of theoretical values.

Table 1

Soil Sorption and Desorption
of Ractopamine Hydrochloride

		% Desorbed	
Soil Type	% Sorbed	1st Desorption	2nd Desorption
Clay Loam	83	9.4	7.0
Loam	78	8.6	4.8
Sandy Loam	59	16	9.9

Table 2

Mass Balance Data Summary

Soil Type	% (Observed/Expected)	% Relative Standard Deviation
Clay Loam	89.4	2.7
Loam	85.6	9.4
Sandy Loam	93.5	9.4

A summary of the isotherm determination is presented in Table 3. The high K_d and K_{OC} values indicate that ractopamine is quite tightly bound to organic matter in soil and is considered immobile[1].

Appendix O: Continued

Table 3

Sorption Coefficients from Isotherm Determination

Soil Type	K_d[a]	K_{oc}[b]
Clay Loam	36.0	2007
Loam	29.6	2698
Sandy Loam	14.5	2090

[a] K_d is the soil/water distribution (sorption) coefficient

[b] K_{oc} is the sorption coefficient expressed on an organic carbon basis

Reference:

1 KENAGA, E. E. (1980). Predicted bioconcentration factors and soil coefficients of pesticides and other chemicals. Ectox. Environ. Saf. 4:26-38.

Appendix P: Report Summary

Title: Biodegradation in Soil of ^{14}C-Ractopamine Hydrochloride by the Soil Incubation Flask System

Study Number: ABC-0332

Study Dates: November 4, 1985 to January 21, 1986

Name and Address of Investigator: L. L. Zornes, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: ^{14}C-Ractopamine Hydrochloride

Test System: Soils contained in closed incubation flasks.

Summary of Experimental Design: The biodegradation of ^{14}C-ractopamine hydrochloride in soil was determined by procedures described in the Environmental Assessment Technical Handbook, Food and Drug Administration, Center for Veterinary Medicine. Three replicates in each of three soil types were fortified with ^{14}C-ractopamine hydrochloride at a concentration of 0.53 mg per 50 g of soil (approximately 10.6 ppm). The fortified soils were incubated in triplicate in flasks in the dark at 22°C and 60% of field moisture capacity for a period of 64 days. Volatiles and $^{14}CO_2$ were collected periodically by means of a specially designed incubation and trapping apparatus. Radioactivity, evolved as $^{14}CO_2$ and ^{14}C volatile products, was assayed by liquid scintillation counting.

Summary of Results:

In the positive control samples there was greater than 50% CO_2 production for glucose, the reference compound, in the three soil types within the 64 days of incubation. In the ^{14}C-ractopamine hydrochloride-treated soils, the mean total radioactivity evolved as $^{14}CO_2$ was 8.5, 8.9, and 7.0% of the initial radioactivity for sandy loam, loam, and clay loam soils, respectively. The total radioactivity in the volatile products was less than 1% in all soil types. The acetone and methanol extractable radioactivity for each soil type was less than 2% of the initial radioactivity. The nonextractable radioactivity was more than 70% of the initial radioactivity and was not primarily associated with parent ractopamine HCl.

Results of this experiment indicate that ^{14}C-ractopamine hydrochloride did undergo biodegradation to $^{14}CO_2$ slowly and without a measurable lag time. It also suggests that the parent compound was substantially degraded and its products were bound (more than 70%) to the soil. Less than 0.5% of the total radioactivity was confirmed as ractopamine hydrochloride when the spent soil after methanol and acetone extraction was assayed for parent ractopamine hydrochloride.

Appendix Q: Report Summary

Title: A Greenhouse Study to Determine the Decline of Soil-Incorporated Ractopamine Hydrochloride

Study Number: ABC-0335

Study Dates: December 19, 1985 to May 1, 1986

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

Test Article: Crystalline Ractopamine Hydrochloride

Test System: Soil flats maintained in the greenhouse.

Summary of Experimental Design:

This study was conducted in a greenhouse under ambient temperatures and photoperiods. The test article was blended into weighed portions of air-dried, nonsterile coarse-textured, sandy-loam soil at a concentration of 5 ppm. The dry, blended soil replicates were assayed for ractopamine hydrochloride at the start of the experiment.

Two equal portions of treated soil and one of control soil were placed in foil-lined metal flats and brought to a moisture content of approximately 70% of field capacity. Each flat was covered with aluminum foil which prevented adventitious growth of weed seedlings. The flats were then each enclosed in a plastic bag to reduce the evaporation of water from the soil and prevent rapid fluctuations of soil moisture levels. Initial and periodic samples of the control and test soils were taken, air-dried, finely ground with a glass mortar and pestle, and assayed for ractopamine hydrochloride.

Summary of Results:

Results are summarized in Table 1. The decline of soil-incorporated ractopamine hydrochloride occurred in two phases. There was a rapid initial decline (half-life, 1.1 days; rate constant, 0.622 day^{-1}) of 71% from the initial level of 5.62 ppm to 1.62 ppm during the first 2 days. In the second phase, from Days 2 through 15 weeks, the decline occurred more slowly with a half-life of approximately 51 days. Eight weeks were required for the test compound to degrade by 90% to a soil concentration of 0.55 ppm. At the final 15-week soil sampling only 0.36 ppm of ractopamine hydrochloride remained. Thus, 94% of the test compound had degraded by the end of 15 weeks.

Appendix Q: Continued

Table 1

Decline of Ractopamine Hydrochloride in Soil

Sampling Interval	ppm	% of Initial
Initial (after mixing)	5.62	100
Initial (after hydration)	2.53	45.0
2 days	1.62	28.8
5 days	1.60	28.5
1 week	1.44	25.6
2 weeks	1.18	21.0
3 weeks	1.31	23.3
4 weeks	0.92	16.4
6 weeks	0.84	14.9
8 weeks	0.55	9.8
10 weeks	0.45	8.0
15 weeks	0.36	6.4

Appendix R

Title: Use of the CREAMS (Chemicals, Runoff, and Erosion from Agricultural Management Systems) Model to Estimate the Maximum Concentration of Ractopamine Hydrochloride in Runoff Water from Cropland

Author: P. J. Cocke

Introduction:

The use of ractopamine hydrochloride in the feed of swine could result in concentrations of ractopamine hydrochloride of up to 11.9 ppm in the wet feces from swine. If this wet manure were applied to cropland as fertilizer, runoff occurring soon after application could transport small amounts of ractopamine hydrochloride into surface waters. The variable dilution available in receiving waters makes it difficult to estimate the highest expected ractopamine hydrochloride concentration to which aquatic organisms might be exposed. Aquatic organisms would certainly never be exposed to ractopamine hydrochloride levels greater than the maximum expected concentration in runoff water.

The highest expected concentration and total annual yield of ractopamine hydrochloride in runoff from cropland was estimated using the CREAMS (Chemicals, Runoff, and Erosion from Agricultural Management Systems) model developed by the USDA Agricultural Research Service. CREAMS is a daily simulation model that estimates runoff, erosion/sediment transport, and chemical yield from field-sized areas (1,2). The model uses the SCS curve number method to estimate surface runoff from daily rainfall data (3). Evapotranspiration and percolation algorithms are included to maintain a daily water balance of the agricultural system. The Universal Soil Loss Equation is used in the model to incorporate the effects of topology, soil characteristics, crop cover, and meteorological conditions on soil erosion and sediment transport (4). In estimating the loss of a chemical from cropland, CREAMS accounts for the processes of sorption and degradation in the soil. A thorough discussion of the conceptual basis for the CREAMS model is provided in the user's manual (1).

Parameter selection, site selection, and model assumptions were generally made to maximize model estimates of the loss of ractopamine hydrochloride in runoff. A 20-year simulation was run for the annual application of 10 tons/acre (2.24 x 104 kg/ha) of wet swine manure containing 11.9 ppm ractopamine hydrochloride. The manure was incorporated into the soil each spring prior to planting straight-row corn. It was assumed that the corn was maintained on a field having a 5% slope using conventional tillage practices without contouring.

The 20-year simulation was run for an annual manure application on April 30 (Julian day 120). This application date coincided with the second largest spring rainfall (9.1 cm) and runoff (5.2 cm) events that occurred in the 20-year precipitation record selected for the site.

Appendix R: Continued

Methods:

The selection of the geographical location of the simulation was based on three criteria: a) it must be representative of a major swine production area; b) it must be representative of major cropland to which swine manure can be applied; and c) it must have meteorological and hydrologic conditions that are conducive to runoff. The first two criteria were met by visually comparing U.S. maps showing the geographical distribution of major crops and swine production (5,6). This comparison identified corn as the representative crop. Corn grown in straight rows using conventional tillage without contouring was selected to maximize the potential loss of ractopamine hydrochloride in runoff.

Swine and corn production overlap in several states. In Table 1, information on rainfall, hydrologic conditions, and soil type are compared for representative sites in the states where the largest production of corn and swine occur (6). The Illinois site was selected for the runoff simulations because of relatively high annual precipitation and slow infiltration rates which result in a moderate to high potential for runoff.

Operation of the CREAMS model required the estimation of parameters for three model components: hydrology, erosion/sediment transport, and chemistry. Once a particular geographical setting, soil type, and cropping pattern were selected, the CREAMS User's Guide provided most of the parameters needed for the hydrology and erosion/sediment transport components (1,2). Parameters for the chemistry component of the model were obtained from environmental fate studies summarized for ractopamine hydrochloride in the adjoining appendices. Below is a description of the selection of model parameters.

Hydrology: Table 1 lists the general characteristics of the representative site in Illinois. A 20-year record of daily rainfall was obtained for meteorological station #8179 (Springfield, IL) from the National Center for Atmospheric Research database developed by EPA-Athens, GA. This database was also used to obtain monthly mean temperature values. Monthly mean solar radiation values for Illinois were obtained from the CREAMS User's Guide (2).

The physical setting for the simulation was a square 16.2 ha (40 acre) field planted in continuous corn with no winter cover crop. The field was assumed to have a high uniform 5% slope with a slope length of 402 m. The soil was a silt loam with 2.5% organic matter in the 1-cm surface zone and 1.25% organic matter through the 90-cm root zone. The hydrologic soil group was C which represents a slow infiltration rate (3). The remaining hydrology parameters (leaf area index, soil drainage parameters) were taken from the CREAMS User's Guide. The hydrology parameter data set used in the simulation is listed in Table 2. This data set corresponds to the format specified in the CREAMS User's Guide (2).

Appendix R: Continued

Erosion: Parameters for the erosion/sediment yield component were obtained from the CREAMS User's Guides (1,2). Soil loss ratios, contouring factors, and soil roughness factors were chosen to represent conventional tillage, moderate yields of corn, and partially shredded stalks. The particle size distribution for the silt loam soil was 20% sand, 60% silt, and 20% clay. The annual corn crop was planted on June 1 and harvested on October 30. The erosion parameter data set used in the simulation is listed in Table 3 in the format specified in the program User's Guide (2).

Chemistry: The chemistry component of the CREAMS model required parameters describing the use pattern and environmental behavior of ractopamine hydrochloride. Application of swine manure with a ractopamine hydrochloride concentration of 11.9 ppm was assumed to occur at a rate of 10 tons/acre (2.24 x 104 kg/ha) on April 30 of each year of the 20-year simulation. This is equivalent to a ractopamine hydrochloride application rate of 0.27 kg/ha. The material was uniformly incorporated into the soil to a depth of 15 cm (6 inches). The degradation rate constant of ractopamine hydrochloride in soil was obtained from the results of a greenhouse study (Appendix Q). The decay rate constant was 0.622/day, which corresponds to a half-life of about 1.1 days. The aqueous solubility of ractopamine hydrochloride was set at 52 ppm. Model results are only affected by solubilities <1 ppm. The soil/water distribution coefficient (K_d) for ractopamine hydrochloride was estimated from an average organic carbon partition (K_{OC}) of 2265 determined in a laboratory sorption study (Appendix O). The value of K_d was computed as:

$$(K_{OC}) \times (\% \text{ organic matter} / 100) / 1.73 =$$
$$2265 \times 0.025 / 1.73 = 33.$$

The factor of 1.73 was used to convert the organic matter content in the upper 1 cm of soil to an equivalent organic carbon content (1). The chemical parameter data set used in the simulation is listed in Table 4 in the format specified in the program User's Guide (2).

Results and Discussion:

The results of the 20-year runoff simulation are summarized in Table 5. Total annual precipitation ranged from 63.2 to 109.2 cm and averaged 86.4 cm. Total annual runoff ranged from 3.5 to 22.1 cm and averaged 12.7 cm. An average of 27 runoff events occurred each year. However, over the 20-year simulation period, only eight runoff events contained ractopamine hydrochloride levels greater than or equal to 0.0001 ppm.

Total annual loss of ractopamine hydrochloride in runoff ranged from 0.0% to 0.7% of the amount applied (Table 5). The maximum annual loss of 0.7 percent corresponded to a year in which the application date coincided with the second largest spring rainfall (9.1 cm) and runoff (5.2 cm) events in the 20-year simulation period. This maximum annual loss was within the annual yield values (<1.5%) suggested by Wauchope (7) and Willis and McDowell (8) for soil-incorporated compounds with characteristics similar to those of ractopamine hydrochloride.

Appendix R: Continued

Table 6 summarizes the six runoff events containing aqueous ractopamine hydrochloride concentrations of at least 0.0001 ppm. The maximum concentration of 0.0027 ppm was associated with runoff events that occurred on the day of a manure application (Julian day 120). The aqueous concentration of ractopamine hydrochloride in runoff was highly dependent on the amount of time between a manure application and the occurrence of runoff. Runoff events that occurred more than 5 days after an application did not contain aqueous ractopamine hydrochloride concentrations greater than 0.0001 ppm.

These simulation results indicate that the application to cropland of swine manure containing up to 11.9 ppm ractopamine hydrochloride should not result in the contamination of nearby surface waters. Due to the rapid degradation of ractopamine hydrochloride in soil, the compound is only transported in runoff events occurring within a few days of a manure application.

References:

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Appendix R: Continued

Table 1. Meteorological and Soil Characteristics of Representative Sites Associated With the Production of Swine and Corn[a].

State	Annual Precip (in)	Mean Temp. (°C)	Soil Group[b]	Percent Organic Carbon	Predominant Soil Type
CO	16.3	10.6	C	1-2	Loamy sand
NE	23.2	10.6	D	1-2+	Silty clay loam
IL	34.0	11.9	C	1-2+	Silt loam
MI	34.6	9.4	B	1-2+	Loam
OH	36.1	9.2	B	1-2	Silt loam

[a] From Dean *et al.*, 1984 (6).

[b] Hydrologic soil group as defined in SCS, USDA, 1972 (3).
A = high infiltration rate - low runoff potential
B = moderate infiltration rate
C = slow infiltration rate
D = very slow infiltration rate - high runoff potential

Appendix R: Continued

Table 2. Parameter Set for the Hydrology Component of the CREAMS Model Used to Simulate Ractopamine Hydrochloride Loss in Runoff Following an Application of Swine Manure on April 30. This Data Set Corresponds to the Format Specified in the CREAMS User's Guide (2).

HYDROLOGY PARAMETERS - RACTOPAMINE HYDROCHLORIDE / SWINE
ILLINOIS / SILT LOAM
CLIMATE STATION #8179, 1/54 - 12/73

54001	0	1	1	0					
40.0	0.10	0.64	0.50	4.50	0.43	0.12			
0.2	86.0	0.05	1.0	36.0					
0.31	1.55	1.86	1.86	1.86	1.86	1.86			
25.1	30.0	39.8	54.0	63.5	72.8	76.3	74.0	67.6	56.1
42.2	30.5								
170.0	242.0	340.0	402.0	506.0	553.0	540.0	498.0	398.0	275.0
165.0	138.0								
1.0									
001	0.00								
150	0.00								
165	0.09								
180	0.19								
195	0.23								
210	0.49								
225	1.16								
240	2.97								
255	3.00								
270	2.72								
285	1.83								
303	0.00								
366	0.00								
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
-1	0	0							

Appendix R: Continued

Table 3. Parameter Set for the Erosion Component of the CREAMS Model Used to Simulate Ractopamine Hydrochloride Loss in Runoff Following an Application of Swine Manure on April 30. This Data Set Corresponds to the Format Specified in the CREAMS User's Guide (2).

EROSION PARAMETERS - RACTOPAMINE HYDROCHLORIDE / SWINE
ILLINOIS / SILT LOAM
CLIMATE STATION #8179, 1/54 - 12/73

54	73	0	1	0	1	0			
0.20	0.60	0.20	0.025	20.0	4.0	0.05	1000.0		
40.0	320.0	0.05	0.05	0.05	0.05	1320.0	0.0	1320.0	0.0
1	1.0	0.40							
1	MANAGEMENT PARAMETERS								
001	120	150	199	228	234	240	255	303	
1	1.0								
0.27	0.43	0.64	0.56	0.43	0.32	0.25	0.21	0.27	
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.03	

Appendix R: Continued

Table 4. Parameter Set for the Hydrology Component of the CREAMS Model Used to Simulate Ractopamine Hydrochloride Loss in Runoff Following an Application of Swine Manure on April 30. This Data Set Corresponds to the Format Specified in the CREAMS User's Guide (2).

CHEMICAL PARAMETERS - RACTOPAMINE HYDROCHLORIDE / SWINE
ILLINOIS / SILT LOAM
CLIMATE STATION #8179, 1/54 - 12/73

54001	0	0	1	0	3	1			
0.430	0.320	1.250							
1	54119	73365							
1	EL-737		52.	0.0	0.622	33.	0.000	0.000	0.000
1120	2119	1							
1	0.267	15.24	1.000	0.000	1.000	0.000			
	0								

Appendix R: Continued

Table 5. Annual Summary of Rainfall, Estimated Runoff, and Estimated Loss of Ractopamine Hydrochloride.

Year	Rainfall (cm)/ # of Storms	Runoff (cm)/ # of Events	Total Annual[a] Loss (%)
1	63.2 / 101	3.5 / 18	0.0
2	91.2 / 101	16.0 / 11	0.0
3	76.9 / 99	9.7 / 25	0.01
4	104.1 / 117	13.3 / 34	0.0
5	81.5 / 104	13.6 / 24	0.0
6	89.7 / 98	20.5 / 22	0.0
7	99.9 / 99	16.7 / 35	0.01
8	95.5 / 102	13.2 / 27	0.0
9	78.6 / 101	11.9 / 26	0.01
10	74.1 / 80	11.4 / 23	0.0
11	78.3 / 90	12.6 / 25	0.0
12	95.3 / 98	17.4 / 30	0.0
13	82.1 / 97	8.5 / 24	0.0
14	84.8 / 117	9.0 / 26	0.0
15	82.4 / 105	12.3 / 30	0.0
16	92.2 / 107	13.5 / 30	0.0
17	96.6 / 115	15.3 / 28	0.7[b]
18	70.6 / 94	7.4 / 23	0.0
19	81.6 / 116	7.3 / 24	0.0
20	109.2 / 119	22.1 / 28	0.0
Means:	86.4 / 103	12.7 / 27	0.04

[a] Total annual loss of ractopamine hydrochloride in runoff as a percent of the amount applied.

[b] The manure application date coincided with the second largest spring rainfall (9.1 cm) and runoff (5.2 cm) events that occurred in the 20-year precipitation record selected for the site.

Appendix R: Continued

Table 6. Summary of Aqueous Ractopamine Hydrochloride Concentrations in Runoff for an April 30 (Julian Day 120) Application of Swine Manure Containing Ractopamine Hydrochloride.

DATE	JULIAN DATE[a]	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS CONC.[b] (ppm)
29APR56	56120	1.37	0.05	0.0027[c]
03MAY58	58123	1.90	0.05	0.0004
29APR60	60120	1.63	0.05	0.0027[c]
05MAY61	61125	2.62	0.23	0.0001
01MAY62	62121	1.96	0.10	0.0014
06MAY67	67126	3.89	1.07	0.0001
30APR70	70120	9.09	5.16	0.0026[c]
06MAY71	71126	2.08	0.03	0.0001

[a] First two digits represent the year and the last three digits represent the day of the year.

[b] Runoff events containing aqueous ractopamine hydrochloride concentrations $\geq$0.0001 ppm.

[c] Runoff event that occurred on the day of a manure application (Julian Day 120).

Appendix S: Report Summary

Title: The Toxicity of Ractopamine Hydrochloride to Bobwhite in a 14-Day Acute Oral Study

Name and Address of Investigators: R. L. Cochrane and R. D. Meyerhoff, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Study Dates: February 18, 1986 to March 4, 1986

Study Number: A00586

Test Article: Ractopamine Hydrochloride

Lot Number: X-44156 (purity, 92.8%)

Species: Bobwhite quail (*Colinus virginianus*)

Age: 21 Weeks

Number of Animals: 5/Sex/Dose

Dose Groups: 0.0 (vehicle control, 10% acacia), 20, 40, 90, 200, 400, 900, and 2000 mg ractopamine hydrochloride/kg body weight. A single dose was administered at the beginning of the study.

Route: Oral (gavage)

Length of Study: 14 days

Parameters Studied: Food consumption, body weight, behavioral signs of toxicity (i.e., lethargy, hyperactivity, ataxia, tremors, etc.) and mortality.

Results: Three of 10 birds which received a dose of 2000 mg/kg died during the study. Another bird was judged to be moribund at this dose when the study was terminated. No mortality occurred in any other treatment group or in the control group. Loose feces occurred in a treatment-related fashion in all dose groups tested. Lethargy was noted at doses of 20, 40, 900, and 2000 mg/kg. Tremors were observed during the study in the bird judged to be moribund at the end of the study. Food consumption and body weight were reduced in birds given doses $\geq$200 mg/kg. Reductions in body weight gain, reductions in food consumption and mortality were not found in the 20 mg/kg dose group. Loose feces and one lethargic bird were found in the 20 mg/kg dose group.

Appendix T: Report Summary

Title: The Toxicity of Ractopamine Hydrochloride to Juvenile Bobwhite in a 5-Day Dietary Study

Name and Address of Investigators: R. L. Cochrane and R. D. Meyerhoff, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Study Number: A01186

Study Dates: June 25, 1986 to July 3, 1986

Test Article: Ractopamine Hydrochloride

Lot Number: X-44156 (purity, 92.8%)

Species: Bobwhite quail (*Colinus virginianus*)

Age: 10 days

Number of Animals: 10/group

Levels of Exposure: Average measured concentration of ractopamine hydrochloride in diets: 0.0, 0.0017, 0.0044, 0.01, 0.027, 0.067, 0.19, and 0.47% w/w.

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

Route: Dietary

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

Results: The average total consumption of ractopamine hydrochloride was 0.018, 0.048, 0.106, 0.289, 0.909, 2.31, and 4.99 g/kg of body weight for the 0.0017, 0.0044, 0.01, 0.027, 0.067, 0.19, and 0.47% treatment groups, respectively. Three of ten birds died in the 0.47% treatment group and one of ten birds died in the 0.01% treatment group. No mortalities were found in the 0.0017, 0.0044, 0.027, 0.067, and 0.19% treatment groups. Ataxia was observed in four birds in the 0.47% treatment group. Food consumption by birds in the 0.47% treatment group was considerably lower than food consumption by control birds. Treatment-related reductions in body weight gain were noted during the 5-day treatment phase of the study for birds exposed to diets with >0.01% ractopamine hydrochloride. Detailed observations of body weight and food consumption are shown in Tables 1 and 2. The highest dietary concentration of ractopamine hydrochloride tested which did not result in mortality, signs of toxicity, reduced mean body weight gain, or reduced mean food consumption was 0.0044% (44 ppm).

Appendix T: Continued

Table 1. Body Weight Data for Juvenile Bobwhite (*Colinus virginianus*) Fed Diets Containing Ractopamine Hydrochloride. Study A01186.

Measured Dietary Concentration (%)	Number Of Birds	Mean Body Weight +/- SD (g)			Mean body Weight Gain +/- SD (g)	
		Test Day			**Treatment Phase**	**Basal Diet Phase**
		Initial	5	8	(5 Days)	(3 Days)
0.0 (Control)	10	22.4 +/- 0.8	36.1 +/- 2.0	44.8 +/- 3.1	13.7 +/- 1.9	8.7 +/- 1.5
0.0017	10	22.7 +/- 1.1	37.1 +/- 2.7	45.2 +/- 3.6	14.3 +/- 2.1	8.2 +/- 1.2
0.0044	10	21.9 +/- 0.6	35.1 +/- 2.6	44.0 +/- 3.5	13.2 +/- 2.7	8.9 +/- 2.9
0.01	10	22.1 +/- 0.7	33.7[a] +/- 3.2	41.5 +/- 3.9	11.6[a] +/- 3.0	7.8 +/- 1.4
0.027	10	22.8 +/- .06	33.2 +/- 3.4	44.1 +/- 3.2	10.4* +/- 3.1	10.9 +/- 1.5
0.067	10	22.5 +/- 1.1	30.5* +/- 3.4	40.4 +/- 6.1	8.0* +/- 2.9	9.9 +/- 3.0
0.19	10	22.4 +/- 1.1	25.2* +/- 3.2	36.1* +/- 5.6	2.8* +/- 3.2	10.9 +/- 3.0
0.47	10	22.4 +/- 0.9	22.8[b]* +/- 2.2	33.2* +/- 3.4	0.5[b]* +/- 1.7	10.4 +/- 3.3

* Statistically significant difference between this value and the corresponding control ($P < 0.05$).

[a] N = 9 birds

[b] N = 7 birds

Appendix T: Continued

Table 2. Food Consumption for Bobwhite (*Colinus virginianus*) Fed Diets Containing Ractopamine Hydrochloride. Study A01186.

Measured Dietary Concentration (%)	Number of Pens	Mean Food Consumption (g/bird/day) ± SD			
		Treatment Phase (5 Days)	% of Control	Basal Diet Phase (3 Days)	% of Control
0.0 (Control)	2	6.7 ±1.0	--	9.8 ±1.7	--
0.0017	2	6.3 ±0.1	86	8.5 ±0.5	90
0.0044	2	6.2 ±0.2	86	9.5 ±0.9	100
0.01	2	5.9 ±0.4	86	9.7 ±1.4	100
0.027	2	6.0 ±0.2	86	9.2 ±0.2	90
0.067	2	7.2 ±0.6	114	10.0 ±1.0	100
0.19	2	5.8 ±0.4	86	8.1 ±1.4	80
0.47	2	4.8 ±1.6	71	11.0 ±0.1	110

Appendix U: Report Summary

Title: The Toxicity of Ractopamine Hydrochloride to Juvenile Mallards in a 5-Day Dietary Study

Name and Address of Investigators: R. L. Cochrane and R. D. Meyerhoff, Toxicology Division, Lilly Research Laboratories,
Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Study Number: A00986

Study Dates: April 10 to April 18, 1986

Test Article: Ractopamine hydrochloride

Lot Number: X-44156 (purity, 92.8%)

Species: Mallard duck (*Anas platyrhynchos*)

Age: 10 days

Number of Animals: 10/group

Levels of Exposure: Average measured concentration of ractopamine hydrochloride in diets: 0.0, 0.0091, 0.0165, 0.0356, 0.0672, 0.145, 0.291, and 0.596% w/w.

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

Route: Dietary

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

Results: The average total consumption of ractopamine hydrochloride for birds fed 0.0091, 0.0165, 0.0356, 0.0672, 0.145, 0.291, and 0.596% treated diets were 0.151, 0.268, 0.615, 1.12, 2.15, 4.07, and 10.0 g of ractopamine hydrochloride/kg body weight, respectively. No mortality or signs of toxicity were observed for birds from the control group or from any treatment group. Reduced body weight gains during the 5-day treatment phase were found in treatment groups fed diets containing $\geq$0.145% ractopamine hydrochloride. Reduced body weight gain was associated with reduced food consumption at the same dietary concentrations of ractopamine. Detailed observations of body weight and food consumption are shown in Tables 1 and 2. The 0.0672% dietary concentration was the highest level of ractopamine hydrochloride tested which did not result in treatment-related reductions in mean body weight gain or food consumption.

Appendix U: Continued

Table 1. Body Weight Data for Juvenile Mallards (*Anas platyrhynchos*) Fed Diets Containing Ractopamine Hydrochloride. Study A01186.

Measured Dietary Concentration (%)	Number of Birds	Mean Body Weight +/- SD (g)			Mean body Weight Gain +/- SD (g)	
		Test Day			**Treatment Phase**	**Basal Diet Phase**
		Initial	5	8	(5 Days)	(3 Days)
0.0 (Control)	10	121 +/- 19	239 +/- 36	321 +/- 50	117 +/- 25	82 +/- 16
0.0091	10	125 +/- 13	261 +/- 43	346 +/- 35	136 +/- 35	85 +/- 13
0.0165	10	130 +/- 14	267 +/- 28	346 +/- 35	137 +/- 16	80 +/- 16
0.01	10	125 +/- 12	247 +/- 16	329 +/- 25	123 +/- 14	81 +/- 12
0.027	10	128 +/- 18	248 +/- 42	334 +/- 54	120 +/- 27	86 +/- 16
0.067	10	131 +/- 19	240 +/- 21	343 +/- 26	109 +/- 14	103 +/- 13
0.19	10	128 +/- 23	225 +/- 37	312 +/- 53	98 +/- 21	87 +/- 18
0.47	10	125 +/- 19	208 +/- 28	296 +/- 44	83* +/- 21	89 +/- 21

*Statistically significant difference between this value and the corresponding control ($P \leq 0.05$).

Appendix U: Continued

Table 2. Food Consumption for Juvenile Mallards (*Anas platyrhynchos*) Fed Diets Containing Ractopamine Hydrochloride. Study A00986.

Measured Dietary Concentration (%)	Number of Pens	Mean Food Consumption (g/bird/day) ± SD			
		Treatment Phase (5 Days)	% of Control	Basal Diet Phase (3 Days)	% of Control
0.0 (Control)	2	64 ±11	--	100 ±18	--
0.0091	2	64 ±6	100	88 ±4	88
0.0165	2	65 ±1	102	96 ±22	96
0.0356	2	64 ±10	100	99 ±4	99
0.0672	2	63 ±13	98	93 ±5	93
0.145	2	55 ±6	86	94 ±16	94
0.291	2	50 ±6	78	80* ±7	80
0.596	2	56 ±8	88	89 ±13	89

*Statistically significant reduction in mean food consumption ($p \leq 0.05$).

Appendix V: Report Summary

Title: The Toxicity of Ractopamine Hydrochloride to Rainbow Trout 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, Box 708, Greenfield, Indiana 46140

Study Number: F03286

Study Dates: April 28, 1986 to May 2, 1986

Test Article: Ractopamine hydrochloride

Lot Number: X-44156 (purity, 92.8%)

Species: Rainbow trout (*Salmo gairdneri*)

Experimental Design: Groups of 10 juvenile rainbow trout (mean individual weight, 1.03 g) were exposed to average measured ractopamine hydrochloride concentrations of 0.0 (water control), 23.2, 48.2, 94.7, 598, 672, 772, 870, and 971 ppm. Jars with 15 L of test or control solution were used to contain each group of 10 fish. Dissolved oxygen concentrations, pH, and temperature of the solutions were recorded daily. Total alkalinity, total hardness, and conductivity of the dilution water were determined. Behavioral signs of toxicity (hypoactivity, minimal swimming behavior, labored respiration, and prostration) and mortality were monitored for fish in each jar on a daily basis.

Results: Water quality characteristics were as follows: pH, 7.9 to 8.6; dissolved oxygen, at least 96% of saturation in all test solutions; temperature, 11.6 to 11.9°C; total hardness, 154 mg/L (as $CaCO_3$); total alkalinity, 168 mg/L (as $CaCO_3$); conductivity, 300 µS/cm. Detailed records of behavioral observations and mortalities are shown in Tables 1 to 5. Fish exposed to ractopamine hydrochloride concentrations $\geq$94.7 ppm showed behavioral signs of toxicity in a concentration related fashion, from hypoactivity to prostration. The 96-hour median lethal concentration and its 95% confidence limits were 693 ppm and 523 to 918 ppm, respectively. No mortalities or behavioral signs of toxicity were found for fish exposed to ractopamine hydrochloride concentrations $\leq$48.2 ppm.

Appendix V: Continued

Table 1. Physical Condition/Behavior of Rainbow Trout (*Salmo gairdneri*) Exposed for 24 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 24 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
25	23.2	10	-	-	-	-	-	-
50	48.2	10	-	-	-	-	-	-
100	94.7	10	-	-	-	-	-	-
620	598	10	-	-	-	-	-	-
700	672	-	10	-	-	-	-	-
800	772	-	10	-	-	-	-	-
900	870	-	-	10	-	-	-	-
1000	971	-	-	8	-	1	1	-

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix V: Continued

Table 2. Physical Condition/Behavior of Rainbow Trout (*Salmo gairdneri*) Exposed for 48 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 48 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
25	23.2	10	-	-	-	-	-	-
50	48.2	10	-	-	-	-	-	-
100	94.7	-	10		-	-	-	-
620	598	-	-	9	-	-	-	1
700	672	-	-	8	-	-	-	2
800	772	-	-	10	-	-	-	-
900	870	-	-	8	-	-	-	2
1000	971	-	-	7	1	-	-	2

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix V: Continued

Table 3. Physical Condition/Behavior of Rainbow Trout (*Salmo gairdneri*) Exposed for 72 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 72 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
25	23.2	10	-	-	-	-	-	-
50	48.2	10	-	-	-	-	-	-
100	94.7	-	-	10	-	-	-	-
620	598	-	-	8	-	-	-	2
700	672	-	-	4	2	-	-	4
800	772	-	-	4	2	-	-	4
900	870	-	-	5	2	-	-	3
1000	971	-	-	1	4	-	-	5

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix V: Continued

Table 4. Physical Condition/Behavior of Rainbow Trout (*Salmo gairdneri*) Exposed for 96 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 96 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
25	23.2	10	-	-	-	-	-	-
50	48.2	10	-	-	-	-	-	-
100	94.7	-	-	10	-	-	-	-
620	598	-	-	8	-	-	-	2
700	672	-	-	4	-	-	-	6
800	772	-	-	5	-	-	-	5
900	870	-	-	4	-	-	-	6
1000	971	-	-	2	2	-	-	6

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L

Appendix V: Continued

Table 5. Cumulative Mortality Frequencies for Rainbow Trout (*Salmo gairdneri*) Exposed for 96 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Averaged Analyzed Ractopamine Hydrochloride Concentration (mg/L)	Cumulative Mortality (%)			
		24 Hr	48 Hr	72 Hr	96 Hr
0.0	ND[a]	0	0	0	0
25	23.2	0	0	0	0
50	48.2	0	0	0	0
100	94.7	0	0	0	0
620	598	0	10	20	20
700	672	0	20	40	60
800	772	0	0	40	50
900	870	0	20	30	60
1000	971	0	20	50	60
	96-Hr Median Lethal Concentration (mg/L)			693	
	95% Confidence Limits			523, 918	

[a] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix W: Report Summary

Title: The Toxicity of Ractopamine Hydrochloride to Bluegill 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, Box 708, Greenfield, Indiana 46140

Study Number: F03186

Study Dates: April 21 to April 25, 1986

Test Article: Ractopamine Hydrochloride

Lot Number: X-44156 (purity, 92.8%)

Species: Bluegill (*Lepomis macrochirus*)

Experimental Design: Groups of 10 juvenile bluegill (mean individual weight, 0.74 g) were exposed to average measured ractopamine hydrochloride concentrations of 0.0 (water control), 90.9, 191, 381, 482, 539, 591, 668, and 761 ppm. Jars with 15 L of test or control solution were used to contain each group of 10 fish. Dissolved oxygen concentrations, pH, and temperature of the solutions were recorded daily. Total alkalinity, total hardness, and conductivity of the dilution water were determined. Behavioral signs of toxicity (hypoactivity, minimal swimming behavior, labored respiration, and prostration) and mortality were monitored for fish in each jar on a daily basis.

Results: Water quality characteristics were as follows: pH, 7.6 to 8.6; dissolved oxygen, at least 95% of saturation; temperature, 21.4 to 21.8°C; total hardness, 137 mg/L (as $CaCO_3$); total alkalinity, 155 mg/L (as $CaCO_3$); conductivity, 325 μS/cm. Detailed records of behavioral observations and mortalities are shown in Tables 1 to 5. Fish exposed to ractopamine hydrochloride concentrations $\geq$381 ppm exhibited sluggish behavior, hypoactivity, or impaired swimming. The 96-hour median lethal concentration, its 95% confidence limits, and the slope of the concentration-response curve were 544 ppm, 473 to 610 ppm, and 7.48, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to ractopamine hydrochloride concentrations $\leq$191 ppm.

Appendix W: Continued

Table 1. Physical Condition/Behavior of Bluegill (*Lepomis macrochirus*) Exposed for 24 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 24 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
100	90.9	10	-	-	-	-	-	-
200	191	10	-	-	-	-	-	-
400	381	10	-	-	-	-	-	-
500	482	-	-	9	1	-	-	-
560	539	-	-	-	7	-	-	3
620	591	-	-	5	-	-	-	5
700	668	-	-	6	-	-	-	4
800	761	-	-	3	-	-	-	7

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix W: Continued

Table 2. Physical Condition/Behavior of Bluegill (*Lepomis macrochirus*) Exposed for 48 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 48 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
100	90.9	10	-	-	-	-	-	-
200	191	10	-	-	-	-	-	-
400	381	-	9	-	-	-	-	1
500	482	-	8	-	-	-	-	2
560	539	-	-	6	-	-	-	4
620	591	-	-	3	1	-	-	6
700	668	-	-	4	-	-	-	6
800	761	-	-	3	-	-	-	7

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix W: Continued

Table 3. Physical Condition/Behavior of Bluegill (*Lepomis macrochirus*) Exposed for 72 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 72 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
100	90.9	10	-	-	-	-	-	-
200	191	10	-	-	-	-	-	-
400	381	-	9	-	-	-	-	1
500	482	-	8	-	-	-	-	2
560	539	-	-	4	-	-	-	6
620	591	-	-	3	-	-	-	7
700	668	-	-	3	-	-	-	7
800	761	-	-	2	-	-	-	8

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix W: Continued

Table 4. Physical Condition/Behavior of Bluegill (*Lepomis macrochirus*) Exposed for 96 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03286.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/mL)	Individual Fish Condition/Behavior Value at 96 Hours [a]						
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	ND[b]	10	-	-	-	-	-	-
100	90.9	10	-	-	-	-	-	-
200	191	10	-	-	-	-	-	-
400	381	-	9	-	-	-	-	1
500	482	-	8	-	-	-	-	2
560	539	-	-	3	-	-	-	7
620	591	-	-	3	-	-	-	7
700	668	-	-	3	-	-	-	7
800	761	-	-	2	-	-	-	8

[a] Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from the probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix W: Continued

Table 5. Cumulative Mortality Frequencies for Bluegill (*Lepomis macrochirus*) Exposed for 96 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study F03186.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Averaged Analyzed Ractopamine Hydrochloride Concentration (mg/L)	Cumulative Mortality (%)			
		24 Hr	48 Hr	72 Hr	96 Hr
0.0	ND[a]	0	0	0	0
100	90.9	0	0	0	0
200	191	0	0	0	0
400	381	0	10	10	10
500	482	0	20	20	20
560	539	30	40	60	70
620	591	50	60	70	70
700	668	40	60	70	70
800	761	70	70	80	80

96-Hr Median Lethal Concentration (mg/L)	544
95% Confidence Limits	473, 610
Slope	7.48

[a] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix X: Report Summary

Title: The Acute Toxicity of Ractopamine Hydrochloride to *Daphnia magna* 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, Box 708, Greenfield, Indiana 46140

Study Number: C00786

Study Dates: March 11 to March 13, 1986

Test Article: Ractopamine Hydrochloride

Lot Number: X-44156 (purity, 92.8%)

Species: *Daphnia magna*

Number of Animals: 5/replicate; 4 replicates/treatment.

Experimental Design: A group of 20 *Daphnia*, <24 hours old, were exposed for 48 hours to control water and to solutions of ractopamine hydrochloride with measured concentrations of 4.47, 9.34, 23.1, 46.9, 71.3, and 93.3 ppm. Each replicate beaker contained 200 ml of test solution. Temperature, dissolved oxygen, and pH of the test solutions were measured daily. Total alkalinity, total hardness, and conductivity were measured in the diluent water and the test solutions. *Daphnia* were assessed for hypoactivity, prostration, and immobility.

Results: The water quality characteristics were as follows: pH, 8.1 to 8.3; dissolved oxygen concentration, at least 92% of saturation; temperature, 20.3 to 21.0°C; total alkalinity, 117 mg/L (as $CaCO_3$); total hardness 106 mg/L (as $CaCO_3$); and conductivity, 237 μS/cm. At ractopamine hydrochloride concentrations ≥23.1 ppm, exposure related signs of toxicity ranged from hypoactivity to immobility. Detailed records of the physical conditions noted in this study are shown in Tables 1 and 2. The 48-hour median lethal concentration, the 95% confidence limits, and the slope of the concentration-response curve were 34.5 ppm, 27.9 to 41.0 ppm, and 4.81, respectively. No immobilization or other physical signs of toxicity were observed in animals exposed to ractopamine hydrochloride concentrations ≤9.34 ppm.

Appendix X: Continued

Table 1. Physical Condition of *Daphnia magna* Populations Exposed for 48 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study C00786.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/L)	Physical Condition[a] 24 Hr N	24 Hr H	24 Hr P	24 Hr I	48 Hr N	48 Hr H	48 Hr P	48 Hr I
0.0 (Control)	ND[b]	20	-	-	-	20	-	-	-
5.0	4.47	20	-	-	-	20	-	-	-
10	9.34	20	-	-	-	20	-	-	-
25	23.1	14	6	-	-	4	11	-	5
50	46.9	13	7	-	-	-	7	-	13
75	71.3	6	12	-	2	-	1	-	19
100	93.3	-	18	-	2	-	-	-	20

[a] Expressed as the number of test organisms that exhibited one of the following general physical conditions: N - normal, H - hypoactive, P - prostrate, I - immobilized.

[b] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix X: Continued

Table 2. Cumulative Immobilization Frequencies for *Daphnia magna* Exposed for 48 Hours to Ractopamine Hydrochloride (EL-737, Compound 031537). Study C00786.

Nominal Ractopamine Hydrochloride Concentration (mg/L)	Average Analyzed Ractopamine Hydrochloride Concentration (mg/L)	Cumulative Immobilization (%)	
		24 Hr	48 Hr
0.0 (Control)	ND[a]	0	0
5.0	4.47	0	0
10	9.34	0	0
25	23.1	0	25
50	46.9	0	65
75	71.3	10	95
100	93.3	10	100
48-Hr Median Effective Concentration (mg/L)		34.5	
95% Confidence Limits		27.9, 41.0	
Slope		4.81	

[a] ND = None detected. The analytical detection limit was 1.0 mg/L.

Appendix Y: Report Summary

Title: The Toxicity of Soil-Incorporated Ractopamine Hydrochloride to the Earthworm in a 28-Day Test

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

Study Numbers: W00986 and W01186

Study Dates: June 26, 1986 to July 24, 1986 and August 7, 1986 to September 4, 1986

Test Article: Ractopamine Hydrochloride

Lot Number: X-44156 (purity, 92.8%)

Species: Earthworm (*Lumbricus terrestris*)

Experimental Design:

Study W00986 - Ractopamine hydrochloride was blended weekly with pulverized rabbit feces, sandy loam soil, and water to achieve average measured ractopamine hydrochloride concentrations of 0.0, 30.9, 63.1, 341, and 747 ppm. Four replicates, each containing 2.0 kg of test media and 10 earthworms, were used for a control and at each treatment level. Every 7 days the earthworms were observed (normal, flaccid, prostrate, or dead), individually weighed, and transferred to freshly prepared medium. Earthworms were exposed to the test media for 28 days.

Study W01186 - Methods used in this study were the same as those used in Study W00986. The average measured concentrations of ractopamine hydrochloride in the soils tested were 0.0, 1.35, and 8.11 ppm.

Appendix Y: Continued

Results:

Study W00986 - Detailed results from this study are shown in Tables 1 to 5. One earthworm out of a total of 40 worms died in the control and at the 30.9 and 63.1 ppm treatment levels. At the end of the study, 87.2% and 77.5% of the worms were alive at the 341 and 747 ppm treatment levels, respectively. The physical condition of worms throughout the study at the 341 and 747 ppm treatment levels ranged from normal to prostrate. The body weight of control earthworms increased by 36.8% by the end of the study. Body weights of earthworms exposed to 30.9 and 63.1 ppm treatment levels increased 28.4% and 17.8%, respectively, by the end of the study. Earthworms exposed to ractopamine hydrochloride at the 341 ppm treatment level essentially gained no weight. The body weight of worms at the 747 ppm treatment level decreased 19.7% by the end of the study. Although the 30.9 ppm treatment level did not result in significant mortality, earthworms exposed to the lowest treatment level tested did not gain as much weight as control worms by the end of the study.

Study W01186 - Detailed results from this study are shown in Tables 6 to 10. All earthworms exposed to mean ractopamine hydrochloride concentrations of 8.11 and 1.35 ppm appeared normal and in good physical condition throughout the study. No mortality, physical signs of toxicity, or statistically significant reductions in body weight gain were observed at either of these two treatment levels.

Appendix Y: Continued

Table 1. Analyzed Concentrations of Ractopamine Hydrochloride in the Test Soil. Study W00986.

Nominal Ractopamine Hydrochloride Concentration	Analyzed Ractopamine Hydrochloride Concentration (mg/kg)										
	Day 0 [a]	Day 7		Day 14		Day 21		Day 28 [a]	Mean +/- SD		
(mg/kg)	New	Old	New	Old	New	Old	New	Old	New	Old	Overall
0.0 (control)	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
50	43.1 +/- 1.2 (41.7–44.3)	15.1	40.7	15.9	42.2	17.6	45.7	26.8 +/- 4.0 (21.1–30.4)	42.9 +/- 2.1	18.8 +/- 5.4	30.9 +/- 13.4
100	90.0 +/- 10.0 (81.1–98.9)	36.2	84.3	38.6	85.5	42.4	84.2	43.9 +/- 7.5 (38.2–54.8)	86.0 +/- 2.7	40.3 +/- 3.5	63.1 +/- 24.6
500	445 +/- 28 (406-468)	227	441	247	442	220	436	269 +/- 27 (248-309)	441 +/- 4	241 +/- 22	341 +/- 108
1000	895 +/- 69 (803-961)	568	955	562	950	506	883	656 +/- 24 (621-677)	921 +/- 37	573 +/- 62	747 +/- 192

[a] Mean +/- SD for the four replicates; the range is given in parenthesis.

[b] ND = None detected. The detection limit for ractopamine hydrochloride in the test soil was 1.0 mg/kg.

Appendix Y: Continued

Table 2. Analyzed Concentrations of Ractopamine Hydrochloride in the Test Soil on a Dry Weight Basis. Study W00986.

Nominal Ractopamine Hydrochloride Concentration	Analyzed Ractopamine Hydrochloride Concentration (mg/kg)										
	Day 0 [a]	Day 7		Day 14		Day 21		Day 28 [a]	Mean +/- SD		
(mg/kg)	New	Old	New	Old	New	Old	New	Old	New	Old	Overall
0.0 (control)	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
50	54.0 +/- 1.7 (52.3–55.9)	18.6	51.0	19.2	52.9	22.1	58.1	33.4 +/- 5.2 (26.0–38.0)	54.0 +/- 3.0	23.3 +/- 6.9	38.7 +/- 17.1
100	113 +/- 15 (100-126)	45.2	107	48.0	108	52.7	105	54.4 +/- 10.9 (47.3-70.3)	108 +/- 3	50.1 +/- 4.2	79.2 +/- 31.3
500	559 +/- 42 (505-595)	279	554	306	557	271	556	333 +/- 32 (300-375)	556 +/- 2	297 +/- 28	427 +/- 140
1000	1127 +/- 100 (991-1222)	700	1192	686	1195	628	1100	799 +/- 32 (755-830)	1154 +/- 48	703 +/- 71	928 +/- 247

[a] Mean +/- SD for the four replicates; the range is given in parenthesis.

[b] ND = None detected. The detection limit for ractopamine hydrochloride in the test soil was 1.0 mg/kg.

Appendix Y: Continued

Table 3. Physical Condition and Survival of Earthworms Exposed to Ractopamine Hydrochloride for 7, 14, 21, and 28 days. Study W00986.

Analyzed Ractopamine Hydrochloride Concentration (mg/kg)	Physical Condition [a]																			
	Day 7					Day 14					Day 21					Day 28				
	1	2	3	4	% Surv	1	2	3	4	% Surv	1	2	3	4	% Surv	1	2	3	4	% Surv
0.0 (control)	39	0	0	1	97.5	39	0	0	0	97.5	39	0	0	1	97.5	39	0	0	1	97.5
39.9	40	0	0	0	100	39	1	0	0	100	39	0	0	1	97.5	39	0	0	1	97.5
63.1	40	0	0	0	100	39	1	0	0	100	39	0	0	1	97.5	39	0	0	1	97.5
341	37[b]	0	1	1	97.4	36	1	0	2	94.9	34	0	0	5	87.2	34	0	0	5	87.2
747	39	0	0	1	97.5	36	2	1	1	97.5	34	0	1	5	87.5	30	1	0	9	77.5

[a] Physical condition expressed as the number of earthworms that exhibited one of the following conditions:

1 – Normal
2 – Flaccid
3 – Prostrate
4 – Dead

[b] One earthworm died from mechanical injury.

Appendix Y: Continued

Table 4. Body Weight of Earthworms Exposed to Ractopamine Hydrochloride for 7, 14, 21, and 28 days. Study W00986.

Analyzed Ractopamine Hydrochloride Concentration (mg/kg)	Body Weight (g) and Percent Gain in Body Weight [a]								
	Day 0	Day 7		Day 14		Day 21		Day 28	
	Body Weight	Body Weight	% Gain	Body Weight	% Gain	Body Weight	% Gain	Body Weight	% Gain
0.0 (control)	4.3516 +/- 0.1077	5.5950 +/- 0.1477	28.6 +/- 3.2	5.7195 +/- 0.1126	31.5 +/- 2.8	5.9288 +/- 0.1870	36.3 +/- 3.4	5.9538 +/- 0.2352	36.8 +/- 2.0
30.9	4.3736 +/- 0.1359	5.2167 +/- 0.1317	19.3 +/- 3.0	5.3508 +/- 0.1391	22.4 +/- 5.3	5.6149 +/- 0.1384	28.4 +/- 4.1	5.6140 +/- 0.0085	28.4 +/- 3.8
63.1	4.3622 +/- 0.0541	4.8888 +/- 0.1229	12.1 +/- 1.9	5.0175 +/- 0.0888	15.0 +/- 2.6	5.2166 +/- 0.1621	19.6 +/ 2.3	5.1364 +/- 0.0440	17.8 +/- 1.1
341	4.4445 +/- 0.0904	4.4385 +/- 0.1421	-0.1 +/- 2.9	4.6813 +/- 0.0777	5.3 +/- 1.8	4.7421 +/- 0.1536	6.7 +/- 4.7	4.4813 +/- 0.2345	0.9 +/- 5.7
747	4.4620 +/- 0.0379	4.2872 +/- 0.1321	-3.9 +/- 2.3	4.2865 +/- 0.1354	-3.9 +/- 3.0	4.1116 +/- 0.0788	-7.9 +/- 1.7	3.5815 +/- 0.0766	-19.7 +/- 1.5

[a] Mean +/- SD for four replicates. Percent gain is based on initial (Day 0) body weight. Each replicate initially contained 10 earthworms. All treatment means for Days 7, 14, 21, and 28 are significantly ($P \leq 0.05$) lower than the respective control value.

Appendix Y: Continued

Table 5. Physical-Chemical Characteristics of the Sandy Loam Soil Used in Study W00986.

Organic Matter	2.1%
Cation Exchange Capacity	14.4 meq/100 g
Potassium	126 mg/kg
Magnesium	255 mg/kg
Calcium	2300 mg/kg
Sand	71%
Silt	18%
Clay	11%

Appendix Y: Continued

Table 6. Analyzed Concentrations of Ractopamine Hydrochloride in the Test Soil. Study W01186.

Nominal Ractopamine Hydrochloride Concentration (mg/kg)	Analyzed Ractopamine Hydrochloride Concentration (mg/kg)										
	Day 0 [a]	Day 7		Day 14		Day 21		Day 28 [a]	Mean +/- SD (n = 3)		
	New	Old	New	Old	New	Old	New	Old	New	Old	Overall
0.0 (control)	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2.0	1.34 +/- 0.13[c] (1.21 – 1.51)	1.58[c]	1.59	1.17	1.77	1.38	1.20	0.97 +/- 0.42 (0.67 – 1.56)	1.52 +/- 0.29	1.17 +/- 0.21	1.35 +/- 0.30
10	5.8 +/- 0.36[c] (5.60-6.38)	6.75[c]	8.15	7.12	10.03	6.87	8.92	7.55 +/- 0.26 (7.37-7.93)	9.03 +/- 0.95	7.18 +/- 0.34	8.11 +/- 1.20

[a] Mean +/- SD for the four replicates; the range is given in parenthesis.

[b] ND = None detected. The detection limit for ractopamine hydrochloride in the test soil was 0.2 mg/kg.

[c] The analytical data for the Day 0 samples apparently are in error, as they are 13 to 15% lower than the corresponding "old" values for Day 7. This problem may have resulted from the unusually long time (5 days) between submission of the samples for analysis and extraction of the ractopamine hydrochloride. Therefore, to keep the average exposure concentrations as accurate as possible, the data were not included in the means presented on the right side of this table. Omission of these values diminished the overall mean at 2.0 mg/kg by only 1.5% and increased the overall mean by just 5.9% at 10 mg/kg.

Appendix Y: Continued

Table 7. Analyzed Concentrations of Ractopamine Hydrochloride in the Test Soil on a Dry Weight Basis. Study W01186.

Nominal Ractopamine Hydrochloride Concentration (mg/kg)	Analyzed Ractopamine Hydrochloride Concentration (mg/kg)										
	Day 0 [a]	Day 7		Day 14		Day 21		Day 28 [a]	Mean +/- SD (n = 3)		
	New	Old	New	Old	New	Old	New	Old	New	Old	Overall
0.0 (control)	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2.0	1.66 +/- 0.15[c] (1.49–1.85)	1.94[c]	1.97	1.42	2.19	1.65	1.46	1.16 +/- 0.50 (0.81-1.88)	1.87 +/- 0.37	1.41 +/- 0.24	1.64 +/- 0.38
10	7.28 +/- 0.48[c] (6.91-7.98)	7.88[c]	10.09	8.66	12.48	8.24	10.85	9.07 +/- 0.35 (8.74-9.57)	11.14 +/- 1.22	8.66 +/- 0.42	9.90 +/- 1.58

[a] Mean +/- SD for the four replicates; the range is given in parenthesis.

[b] ND = None detected. The detection limit for ractopamine hydrochloride in the test soil was 0.2 mg/kg.

[c] The analytical data for the Day 0 samples apparently are in error, as they are 13 to 15% lower than the corresponding "old" values for Day 7. This problem may have resulted from the unusually long time (5 days) between submission of the samples for analysis and extraction of the ractopamine hydrochloride. Therefore, to keep the average exposure concentrations as accurate as possible, the data were not included in the means presented on the right side of this table. Omission of these values diminished the overall mean at 2.0 mg/kg by only 1.5% and increased the overall mean by just 5.9% at 10 mg/kg.

Appendix Y: Continued

Table 8. Physical Condition and Survival of Earthworms Exposed to Ractopamine Hydrochloride for 7, 14, 21, and 28 days. Study W01186.

Analyzed Ractopamine Hydrochloride Concentration (mg/kg)	Physical Condition [a]																			
	Day 7					Day 14					Day 21					Day 28				
	1	2	3	4	% Surv	1	2	3	4	% Surv	1	2	3	4	% Surv	1	2	3	4	% Surv
0.0 (control)	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100
1.35	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100
8.11	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100

[a] Physical condition expressed as the number of earthworms that exhibited one of the following conditions:

1 – Normal
2 – Flaccid
3 – Prostrate
4 – Dead

Appendix Y: Continued

Table 9. Body Weight of Earthworms Exposed to Ractopamine Hydrochloride for 7, 14, 21, and 28 days. Study W01186.

Analyzed Ractopamine Hydrochloride Concentration (mg/kg)	Body Weight (g) and Percent Gain in Body Weight [a]								
	Day 0	Day 7		Day 14		Day 21		Day 28	
	Body Weight	Body Weight	% Gain	Body Weight	% Gain	Body Weight	% Gain	Body Weight	% Gain
0.0 (control)	4.4115 +/- 0.0648	5.2675 +/- 0.0648	19.4 +/- 4.1	5.3928 +/- 0.2455	22.2 +/- 4.5	5.4865 +/- 0.2189	24.4 +/- 4.7	5.6519 +/- 0.2317	27.9 +/- 5.6
1.35	4.4804 +/- 0.1387	5.3986 +/- 0.2437	20.5 +/- 3.2	5.6609 +/- 0.2938	26.3 +/- 3.0	5.6929 +/- 0.2773	27.0 +/- 3.2	5.7507 +/- 0.2839	28.3 +/- 3.6
8.11	4.4214 +/- 0.0893	5.3811 +/- 0.3051	21.7 +/- 5.4	5.6278 +/- 0.2773	27.3 +/- 4.9	5.6914 +/- 0.2540	28.7 +/- 4.9	5.8242 +/- 0.3074	31.7 +/- 5.8

[a] Mean +/- SD for four replicates. Percent gain is based on initial (Day 0) body weight. Each replicate contained 10 earthworms.

Appendix Y: Continued

Table 10. Physical-Chemical Characteristics of the Sandy Loam Soil Used in Study W01186.

Organic Matter	2.1%
Cation Exchange Capacity	14.4 meq/100 g
Potassium	126 mg/kg
Magnesium	255 mg/kg
Calcium	2300 mg/kg
Sand	71%
Silt	18%
Clay	11%

Appendix Z: Report Summary

Title: A Test for Seed Germination and Radicle Development in Four Common Cultivars in the Presence of Ractopamine Hydrochloride

Study Number: ABC-0359

Study Dates: July 29, 1986 to August 6, 1986 (corn and cucumber)
August 19, 1986 to August 25, 1986 (radish)
February 26, 1987 to March 3, 1987 (wheat)

Name and Address of Investigators: J. A. Manthey and J. E. Dalidowicz, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Ractopamine hydrochloride

Test System: Seeds germinated in the dark in Petri dishes.

Summary of Experimental Design: Seeds of corn (*Zea mays*), cucumber (*Cucumis sativus*), turnip (*Brassica rapa*), and wheat (*Triticum aestivum*) were pretreated at room temperature for 24 hours in aqueous solutions which contained 0, 1, 10, and 100 ppm ractopamine hydrochloride. The seeds were then washed with successive portions of distilled water. Immediately, a predetermined number of seeds of each cultivar was placed between layers of moist (plain water) filter paper in Petri dishes and allowed to germinate in the dark at 30°C for 3 to 5 days depending upon the particular test cultivar.

Summary of Results:

The results show that the seeds of wheat and corn at all treatment levels of ractopamine hydrochloride had the same extent of germination as controls. Reduced germination occurred in turnips and cucumbers at 100 ppm. The development of the radicle in corn and wheat was not affected by ractopamine hydrochloride at all levels tested. Reduced radicle length occurred in turnip at treatment levels of 10 and 100 ppm and in cucumber at 100 ppm.

The four test cultivars were not affected by ractopamine hydrochloride at a treatment level of 1 ppm. It is unlikely that soil residue levels >1 ppm ractopamine hydrochloride could result from the agricultural use of the compound. Therefore, no phytotoxic problem is anticipated by the use of ractopamine hydrochloride.

Appendix AA: Report Summary

Title: Ractopamine Hydrochloride Seed Germination and Root Elongation Test

Study Number: ABC-0421

Study Dates: July 11, 1988 to July 16, 1988 (cucumber and soybean)
July 21, 1988 to July 25, 1988 (turnip)
July 22, 1988 to July 26, 1988 (barley)

Name and Address of Investigators: J. E. Dalidowicz, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Ractopamine hydrochloride

Test System: Seeds germinated in the dark in Petri dishes.

Summary of Experimental Design: The seeds of cucumber (*Cucumis sativus*), barley (*Hordeum valgare*), and soybean (*Glycine max*) were soaked for one hour, and turnip (*Brassica rapa*) seeds were soaked overnight in distilled water. A pre-determined number of seeds of each cultivar was placed between layers of filter paper saturated with water or ractopamine hydrochloride test solutions. The concentrations of the ractopamine hydrochloride solutions were 1, 10, and 100 ppm for barley and soybeans; 50, 75, and 100 ppm for cucumbers; and 5, 7.5, and 10 ppm for turnips. These seeds were allowed to germinate in the dark at approximately 30°C for 4 to 5 days, depending on the particular test cultivar.

Summary of Results: The results show that ractopamine hydrochloride at all treatment levels had no effect on seed germination of the four cultivars tested and no effect on the development of the radicle of cucumber, barley, and soybean. Reduced radicle length was found for turnip exposed to a ractopamine hydrochloride treatment level of 10 ppm. The development of the turnip radicle was not affected by ractopamine hydrochloride at treatment levels up to 7.5 ppm.

Appendix BB: Report Summary

Title: Ractopamine Hydrochloride Seedling Growth Test

Study Number: ABC-0432

Study Dates: November 22, 1988 to December 14, 1988 (barley, corn, cucumber, wheat); November 28, 1988 to December 19, 1988 (soybean); January 3, 1989 to January 24, 1989 (turnip)

Name and Address of Investigators: J. E. Dalidowicz and T. W. Waldrep, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Ractopamine hydrochloride

Test System: Seeds germinated and plants grown in sand.

Summary of Experimental Design: Seeds of barley (*Hordeum valgare*), corn (*Zea mays*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), turnip (*Brassica rapa*), and wheat (*Triticum aestivum*) were germinated in quartz sand medium. After germination, the plants were subirrigated once or twice a day for 21 days with nutrient solutions containing 0, 1, 10, 50, and 100 ppm ractopamine hydrochloride. The shoot lengths of each plant at each treatment level were measured after 7, 14, and 21 days of treatment with ractopamine hydrochloride. At the end of 21 days, the shoots were separated from the roots and both were dried and weighed for each replicate.

Summary of Results: No significant effect at any exposure level was found for the shoot lengths of any species at any time during the study. Shoot and root weights of all species exposed to ractopamine hydrochloride were not significantly different than those weights of control plants. Exposure to nutrient solutions containing $\leq$100 ppm of ractopamine hydrochloride resulted in no effects on the growth of seedling barley, corn, cucumber, soybean, turnip, or wheat plants.

Appendix CC: Report Summary

Title: Antimicrobial Activity of Ractopamine Hydrochloride in Aqueous Buffers

Study Number: JJL8603

Study Dates: January 23 to January 30, 1985

Name and Address of Investigator: J. J. Lewis, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test Article: Crystalline Ractopamine Hydrochloride

Test System: In vitro, agar plate test with gram-positive and gram-negative aerobic animal pathogens and anaerobic bacteria.

Summary of Experimental Design: Ractopamine hydrochloride was incorporated into agar plates at concentrations ranging from 0.008 to 256 ppm. A group of 36 gram-positive and gram-negative animal pathogens and 19 gram-positive and gram-negative anaerobes were inoculated onto the surface of the plates and incubated 16 to 24 hours.

Summary of Results: The minimum inhibitory concentrations (MIC) were greater than 128 ppm for all aerobic pathogens and greater than 256 ppm for all anaerobes except for two species, where the MIC values were equal to 256 or 128 ppm (Tables 1 and 2). Antimicrobial activity was not found for any of the microbes tested at ractopamine hydrochloride concentrations $\leq$64 ppm.

Appendix CC: Continued

Table 1

Antimicrobial Activity of Ractopamine Hydrochloride to Pathogens

Microorganism (strain)	Minimum Inhibitory Concentration (ppm)
Staphylococcus aureus (X1.1)	>128
" " (V41)	>128
" " (X400)	>128
" " (S13E)	>128
Staphylococcus epidermidis (Epil)	>128
" " (222)	>128
Streptococcus agalactiae (C203)	>128
" *pneumoniae* (PARK)	>128
" group d (X66)	>128
" group d (2041)	>128
Haemophilus influenzae (sens)	>128
" " (res)	>128
Escherichia coli (N10)	>128
" " (EC14)	>128
" " (TEAM)	>128
Klebsiella (X26)	>128
" (KAE)	>128
" (X68)	>128
Enterobacter aerogenes (C32)	>128
" " (EB17)	>128
" *cloacae* (EB5)	>128
" " (265A)	>128
Salmonella (X514)	>128
" (1335)	>128
Pseudomonas (X528)	>128
" (X239)	>128
" (PS18)	>128
" (PS72)	>128
Serratia (X99)	>128
" (SE3)	>128
Shigella sonnei (N9)	>128
Proteus morganii (PR15)	>128
" *inconstans* (PR33)	>128
" *rettgeri* (C24)	>128
Citrobacter (CF17)	>128
Acinetobacter (AC12)	>128

Appendix CC: Continued

Table 2

Antimicrobial Activity of Ractopamine Hydrochloride
to Anaerobic Microorganisms

Microorganism (strain)	Minimum Inhibitory Concentration (ppm)
Clostridium difficile (2994)	>256
" *perfringens* (81)	>256
" *septicum* (1128)	>256
Eubacterium aerofaciens (1235)	>256
Peptococcus asaccharolyticus (1302)	>256
" *prevoti* (1281)	>256
Peptostreptococcus anaerobius (1428)	>256
" *intermedius* (1264)	>256
Propionibacterium acnes (79)	>256
Bacteroides fragilis (111)	>256
" " (1877)	>256
" " (1936B)	256
" *thetaiotaomicron* (1438)	>256
" *melaninogenicus* (1856/28)	>256
" " (2736)	>256
" *vulgatus* (1211)	128
" *corrodens* (1874)	>256
Fusobacterium symbiosum (1470)	>256
" *necrophorum* (6054A)	>256

Appendix DD: Material Safety Data Sheet for Paylean Premix

The MSDS in this EA is an example of the MSDS that Elanco has, and will, provide for the use of those who handle this product. The MSDS is continually updated and the reader should contact Elanco for the most current MSDS.

For additional information contact:
ELANCO Animal Health
1-800-428-4441

ELANCO

Material Safety Data Sheet

PAYLEAN® Premix AF0602

Paylean® Premix (Type A Medicated Article) is used in swine finisher feeds for increased carcass leanness, increased carcass dressing percent, improved rate of weight gain, and improved feed efficiency.

I. MANUFACTURER / EMERGENCY INFORMATION
- A. Manufacturer
  Elanco Animal Health
  Division of Eli Lilly and Company
  Lilly Corporate Center
  Indianapolis, IN 46285
- B. Emergency Telephone Numbers
  Eli Lilly and Company (317) 276-2000
  CHEMTREC: 1-800-424-9300 (North America)
- C. General Information Telephone Number
  Elanco Animal Health: 1-800-428-4441
- D. Issued: 2/89; Revised: 1/95; 4/95

II. MATERIAL IDENTIFICATION
- A. Generic Name
  Ractopamine hydrochloride 2%
  1. Chemical Abstract Registry Number (CAS#): 99095-19-9
- B. Other Ingredients
  Ground con cobs (inert ingredient) 97%
  Antidusting oil 1%

III. PHYSICAL AND CHEMICAL PROPERTIES
- A. Chemical Name: Benzenemethanol, 4-hydroxy-alpha-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]methyl]-, hydrochloride, (R*,R*)-(+-)-
- B. Normal Physical State, Odor, Appearance: Yellowish-tan to reddish-tan free-flowing granular material
- C. pH (aqueous 50/50): 6-7
- D. Solubility in Water: Ractopamine hydrochloride is slightly water soluble. The ground corn cobs (inert ingredients) are insoluble in water.
- E. Melting point; Not applicable.

IV. FIRE AND EXPLOSION HAZARD DATA
- A. Auto-Ignition Temperature (Dust Layer): No information found.
- B. Flash Point: Not Applicable
- C. Flammable Limit
  1. Lower Explosive Limit (LEL): No information found.
  2. Upper Explosive Limit (UEL): Not Applicable

D. Unusual Fire and Explosion Hazard: As a finely divided material, may form dust mixtures in air which could explode if subjected to an ignition source.

E. Fire Fighting Information: Use water, carbon dioxide, dry chemical, foam, or Halon to extinguish. Wear full protective clothing and use self-contained breathing apparatus. Nonessential personnel should be restricted from area. Do not allow water runoff from fire site to enter nearby streams, ponds, or lakes. Keep containers cooled with water spray.

V. NATIONAL FIRE PROTECTION ASSOCIATION (NFPA 704)
(4=Extreme; 3=High; 2=Moderate; 1-Slight; 0=Insignificant)

A. Health: 2

B. Flammability: 1

C. Reactivity: 0

VI. A. Proper shipping Name / Hazard Class / UN Number:
DOT: not regulated for surface transport.
IMO: Not regulated for air transport.
ICAO: Not regulated for water transport.

B. Reportable Quantity (RQ): Not established.

VII. REACTIVITY DATA

A. Stability: Stable at normal temperatures and pressures.

B. Incompatibility: None known.

C. Hazardous Decomposition: May emit toxic fumes when heated to decomposition.

D. Hazardous Polymerization: None known.

VIII. HEALTH HAZARD DATA

A. Toxicology (Animal Toxicology Data)

1. Acute Exposure (PAYLEAN 2% GRANULAR)
There are no acute data for PAYLEAN 2%. Acute hazards for that formulation are expected to be no greater than those reported for PAYLEAN 10%.

a. Eyes - Rabbit, irritant

b. Skin - Rabbit, slight irritant

c. Inhalation - PAYLEAN 2% granules pose a low dust potential. When the PAYLEAN 10% formulation was tested as a powder, a four-hour exposure in rats at a level of 3.34 mg total formulation/L caused deaths, labored breathing, reduced activity, poor grooming, and dry nasal discharge.

d. Ingestion - Rat, 2000 mg/kg, no deaths, leg weakness, reduced activity.

e. Sensitization - PAYLEAN 10% - No applicable information.
Ractopamine hydrochloride – Guinea pig, positive contact sensitizer.

2. Chronic Exposure (ractopamine hydrochloride)
The following effects were reported in chronic, teratogenic, and reproductive toxicity studies in laboratory animals where ractopamine hydrochloride was tested at dosage levels in excess of those anticipated to occur in humans with the 2% formulation.

a. Chronic Toxicity - Ingestion of ractopamine hydrochloride produces effects consistent with its pharmacology as a partial beta adrenergic agonist. Principal effects in animals included cardiovascular effects characterized by increased heart rate and blood pressure.

b. Special Studies

1.) Intravenous infusion - Intravenous doses produced increases in heart rate, increased blood and pulse pressure, peripheral dilation of blood vessels, and increased cardiac output.

2.) Inhalation - Increased heart rates occurred in monkeys exposed for four hours per day for 8 days to airborne levels of ractopamine hydrochloride of 0.38 mg/m^3 or greater. Fifteen-minute inhalation exposures resulted in increased heart rate at concentrations of 13.9 mg/m^3 and greater (2.4 mg/m^3 was a no-effect level).

c. Teratology & Reproduction: There were no effects on mating performance or fertility, but increased mortality, growth retardation, and structural abnormalities were seen in offspring where doses were maternally toxic.

d. Mutagenicity: Ractopamine hydrochloride - Not mutagenic in a battery of tests using both bacterial and mammalian cell assays, with the exception of an in vitro assay for induction of chromosome aberrations in human lymphocytes. Since two in vivo cytogenetic tests demonstrated no mutagenicity, it can be concluded that ractopamine hydrochloride does not present a genotoxic hazard in man.

e. Carcinogenicity: Not listed as a carcinogen or potential carcinogen by ACGIH, NCI/NTP, IARC, or OSHA.

B. Effects of Exposure

Based on laboratory animal studies that have been conducted with Paylean, Elanco Animal Health has concluded that the use of Paylean does not present a hazard when recommended handling procedures are followed.

1. Signs and Symptoms of Exposure:
On the basis of animal studies and the known pharmacology of ractopamine hydrochloride (a partial beta adrenergic agonist), anticipated effects from accidental exposure to ractopamine would principally include cardiovascular effects characterized by increased heart rate and cardiac output. Manufacturing personnel wearing appropriate protective clothing have not reported ill effects. Based on animal data, may be irritating to the eyes.

2. Medical Conditions Aggravated by Exposure:
Individuals with cardiovascular disease should exercise special caution to avoid exposure. The premix granular formulation poses a low dust potential under usual conditions of handling and mixing.

C. Exposure Guidelines

1. Permissible Exposure Limit (PEL) (Paylean Premix and ractopamine hydrochloride): Not established.

2. Threshold Limit Value (TLV) (Paylean Premix and ractopamine hydrochloride): Not established.

3. Lilly Exposure Guideline (LEG) (ractopamine hydrochloride): 0.017 mg/m^3 TWA for 12 hours

4. Lilly Short-Term Exposure Guideline (STEG) (ractopamine hydrochloride): 0.24 mg/m^3 for 15 minutes

D. Primary Route of Entry: Inhalation and skin contact.

X. FIRST AID (Statement of Practical Treatment)

A. Eyes

Immediately flush eyes with plenty of water. Call a physician if irritation develops.

B. Skin

Remove contaminated clothing and clean before reuse. Wash all exposed areas of skin with plenty of soap and water. Get medical attention if irritation develops.

C. Inhalation

Move individual to fresh air. Get medical attention if breathing difficulty occurs. If not breathing, provide artificial respiration assistance (mouth-to-mouth) and call a physician immediately.

D. Ingestion

Do not induce vomiting. Call a physician or poison control center. If available, administer activated charcoal (6-8 heaping teaspoons) with two to three glasses of water. Do not give anything by mouth to an unconscious person. Immediately transport to a medical care facility and see a physician.

Notes to Physician: Treat as for other beta adrenergic agonists.

X. PRECAUTIONS FOR SAFE HANDLING AND USE

A. Spill Handling Information

Prevent spilled material from flowing onto adjacent land or into streams, ponds, or lakes. In case of leak or spill, sweep up material and dispose of as waste. Place cleanup material and unusable containers in an approved landfill in accordance with applicable state regulations. Wear protective clothing and appropriate protection during cleanup.

B. Disposal

Dispose of any cleanup materials and waste residue according to all applicable federal, state, and local regulations.

C. Container Disposal

Bags may be burned or buried in accordance with approved safety and environmental standards.

D. Warning

When mixing and handling PAYLEAN Premix, use protective clothing, impervious gloves, protective eyewear, and an approved dust mask. Operators should wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse thoroughly with water. If irritation persists, seek medical attention.

E. CAUTION

Avoid inhalation and direct contact. Avoid contact with eyes.

F. Storage

Store at room temperature. Product should not be used after the date printed on the bag. A dating of 24 months is noted on the bag.

XI. PROTECTIVE EQUIPMENT REQUIREMENTS

During manufacture, wear goggles to protect eyes, wear impermeable gloves and protective equipment to avoid direct contact with skin. Use approved respirator.

XII. OTHER INFORMATION

A. NADA Number: 140-863

B. PAYLEAN® (ractopamine hydrochloride, Elanco)

C. NOTE: This information applies only to Paylean which is sold in the U.S.

All information contained herein is offered with the good faith belief that it is accurate. As of the date of issuance or revision, we are providing all information that we have or are aware of relevant t o the anticipated use or handling of the material. However, in the event of an adverse incident associated with this material, this material safety data sheet is not intended to be a substitute for consultation with appropriately trained personnel.

Appendix EE: Report Summary

Title: Exposure Monitoring Study Comparing Four Formulations of Ractopamine Hydrochloride in a Feed Mill at Greenfield, Indiana

Study Number: T4V929201

Study Dates: August 27, November 4, and November 5, 1992

Names of Investigators: J. S. Rybka and M. A. Moreman

Test Articles: 2 percent ractopamine hydrochloride corn cob grit premix without oil
2 percent ractopamine hydrochloride corn cob grit premix with 1 percent soybean oil
5 percent ractopamine hydrochloride corn cob grit premix with 1 percent soybean oil
10 percent ractopamine hydrochloride corn cob grit premix with 1 percent soybean oil

Summary of Experimental Design: This study was designed to determine if a decrease in operator exposure could be achieved by adding soybean oil to the formulation of 2% ractopamine hydrochloride premix. Another purpose was to determine the effects of adding soybean oil to the formulation of 2%, 5%, and 10% ractopamine hydrochloride premix. Of the tasks in a feed-mill operation, weighing typically results in the highest exposure to workers, thus that task was the subject of this study. The task was conducted so as to produce detectable airborne concentrations of ractopamine by disarming all engineering exposure control measures, including local exhaust ventilation and weighing hoods. This study was not designed to assess compliance with recommended exposure guidelines.

Monitoring was conducted for one minute periods of time, the approximate time required to weigh enough premix for one lot of feed. In addition to this one minute weighing task, a 15 minute weighing task was conducted using each formulation. Replicate weighing operations were conducted on four ractopamine hydrochloride premix formulations. Weighing of the premix was conducted on an electronic platform scale. The scale was housed in a cement-block room (7 ft X 7 ft) situated in a feed storage warehouse. Monitoring was conducted at the Eli Lilly and Company Greenfield Feed Mill. Samples were collected on glass fiber filters fitted in 37 mm closed face dust sampling cassettes attached by tygon tubing to GAST Model DOA-104-AA high volume air sampling pumps for short-term monitoring (<30 minutes). For personal monitoring, the filter cassettes were placed in the worker's breathing zone. The pumps were calibrated with a Kurz electronic flowmeter and had an average air flow rate of approximately 20.0 liters per minute.

A 3M9920 dust, fume, and mist respirator and latex gloves were worn while the premix was weighed.

Spiked samples were used to assure the recovery and stability of ractopamine hydrochloride during the handling operations. Blank samples were used to assure that accidental contamination did not occur. Four types of controls were used: field, remote, shipping, and retained. The spiking level for the spiked control samples was approximately 100 µg/pad for all of the recovery studies.

Appendix EE: Continued

Summary of Results: The data collected were organized by homogenous exposure groups (HEG), one for each formulation, and statistically analyzed assuming a log-normal distribution to calculate the geometric mean, geometric standard deviation (SD), and 95% upper confidence limit (UCL) for each HEG.

The personal monitoring results from the task of weighing premix for one minute indicated exposure was higher when no soybean oil was added to the premix. One minute weighing of the formulation containing 2% ractopamine with no added oil yielded a mean breathing zone concentration of 68.9 $\mu g/m^3$ with a SD of 1.9 and a 95% UCL of 92 $\mu g/m^3$. The formulations with soybean oil yielded lower mean breathing zone concentrations of 9.2 $\mu g/m^3$ with a SD of 1.9 and a 95% UCL of 12.3 $\mu g/m^3$ - formulation containing 2% ractopamine with 1% added oil; 11.6 $\mu g/m^3$ with a SD of 1.9 and a 95% UCL of 15.4 $\mu g/m^3$ - formulation containing 5% ractopamine with 1% added oil; and 23.4 $\mu g/m^3$ with a SD of 2.1 and a 95% UCL of 32.7 $\mu g/m^3$ - formulation containing 10% ractopamine with 1% added oil.

The personal monitoring results of weighing premix for 15 minutes confirm that higher exposure was found for the formulation without soybean oil. Personal monitoring of this 15 minute weighing operation yielded a mean breathing zone concentration of 91.2 $\mu g/m^3$ while weighing the formulation with no added oil. Lower breathing zone concentrations resulted from the formulations containing added oil: 6.9 $\mu g/m^3$ for the formulation containing 2% ractopamine with 1% added oil; 17.3 $\mu g/m^3$ for the formulation containing 5% ractopamine with 1% added oil; and 17.6 $\mu g/m^3$ for the formulation containing 10% ractopamine with 1% added oil.

Area monitoring was conducted to determine the ambient concentration of ractopamine while weighing was occurring. These samples were located approximately 3 to 4 feet from the weighing operation. The ambient concentration of ractopamine while weighing the formulation with no added oil was 2.6 $\mu g/m^3$, while that for the formulation containing 2% ractopamine with 1% added oil was 0.8 $\mu g/m^3$; 1.0 $\mu g/m^3$ for the formulation containing 5% ractopamine with 1% added oil; and 1.5 $\mu g/m^3$ for the formulation containing 10% ractopamine with 1% added oil.

Area monitoring was also conducted to determine the ambient concentration of ractopamine during the 15-minute weighing operation. These samples were located approximately 3 to 4 feet from the weighing operation. The ambient concentration of ractopamine while weighing the formulation with no added oil was 6.3 $\mu g/m^3$; while that for the formulation containing 2% ractopamine with 1% added oil was 0.8 $\mu g/m^3$; 1.6 $\mu g/m^3$ for the formulation containing 5% ractopamine with 1% added oil; and 1.5 $\mu g/m^3$ for the formulation containing 10% ractopamine with 1% added oil.

These data support incorporating soybean oil into the premix as an effective means of limiting exposure to workers.

Appendix FF: Report Summary

Title: Exposure Monitoring Study Comparing Two Formulations of Ractopamine Hydrochloride in a Feed Mill at Greenfield, Indiana

Study Number: T4V759303

Study Date: April 8, 1993

Names of Investigator: M. A. Moreman

Test Articles: 2 percent ractopamine hydrochloride corn cob grit premix without oil
2 percent ractopamine hydrochloride corn cob grit premix with 1 percent soybean oil

Summary of Experimental Design: This study was designed to determine if a decrease in operator exposure could be achieved by adding soybean oil to the formulation of 2% ractopamine hydrochloride premix. Of the tasks in a feedmill operation, weighing typically results in the highest exposure to workers, thus that task was the subject of this study. The task was conducted so as to produce detectable airborne concentrations of ractopamine hydrochloride by disarming all engineering exposure control measures including local exhaust ventilation and weighing hoods. This study was not designed to assess compliance with recommended exposure guidelines. Monitoring was conducted for one minute periods of time, the approximate time required to weigh enough premix for one lot of feed, and 15 minute periods of time, the approximate time required to weigh enough for 15 lots of feed. Duplicate weighing operations were conducted on two ractopamine hydrochloride premix formulations: one containing 2% ractopamine hydrochloride with no soybean oil; and one containing 2% ractopamine hydrochloride with 1% soybean oil.

Weighing of the premix was conducted on an electronic platform scale. The scale was housed in a cement-block room (7 ft X 7 ft) situated in a feed storage warehouse.

Monitoring was conducted at the Eli Lilly and Company Greenfield Feed Mill. Samples were collected on glass fiber filters fitted in 37 mm closed face dust sampling cassettes attached by tygon tubing to GAST Model DOA-104-AA high volume air sampling pumps for short-term monitoring (<30 minutes). For personal monitoring, the filter cassettes were placed in the worker's breathing zone. The pumps were calibrated with a Kurz electronic flowmeter and had an average air flow rate of approximately 20.0 liters per minute (l/min).

A 3M9920 dust, fume, and mist respirator and latex gloves were worn while weighing the premix.

Spiked samples were used to assure the recovery and stability of ractopamine hydrochloride during the handling operations. Blank samples were used to assure that accidental contamination did not occur. Four types of controls were used: field, remote, shipping, and retained. The spiking level for the spiked control samples was 5.06 µg/pad for all of the recovery studies.

Appendix FF: Continued

Summary of Results: The data collected were organized by homogenous exposure groups (HEG), one for the formulation with no added soybean oil, one for the formulation with 1% added soybean oil, and statistically analyzed assuming a log-normal distribution to calculate a geometric mean and geometric standard deviation (SD). One minute weighing of the formulation with no added oil yielded a mean breathing zone concentration of 1575 $\mu g/m^3$ with a SD of 1.6. One minute weighing of the formulation with 1% added oil yielded a mean breathing zone concentration of 15 $\mu g/m^3$ with a SD of 2.3.

Personal monitoring of the 15 minute weighing operation yielded a breathing zone concentration of 5123 $\mu g/m^3$ while weighing the formulation with no added soybean oil and 14 $\mu g/m^3$ while weighing the formulation with 1% added soybean oil.

Area monitoring was conducted to determine the ambient concentration of ractopamine hydrochloride while weighing was occurring. These samples were located approximately 3 to 4 feet from the weighing operation. The ambient concentration of ractopamine hydrochloride while weighing the formulation with no added oil was 8.191 $\mu g/m^3$, while that for the formulation with 1% added oil was 3.054 $\mu g/m^3$.

These data support incorporating soybean oil into the premix as an effective means of limiting exposure to workers.

Appendix FF: Continued

Results of Personal Monitoring for Exposure Monitoring Study Comparing Two Formulations of Ractopamine Hydrochloride in a Feed Mill at Greenfield, Indiana - Study Number T4V759303

Formulation	Sampling Time (min).	Result (µg/m^3)
2% without oil	1	1155.893
2% without oil	1	1097.038
2% without oil	1	1203.362
2% without oil	1	2970.385
2% without oil	1	2138.407
2% with oil	1	22.548
2% with oil	1	11.992
2% with oil	1	22.749
2% with oil	1	<4.247
2% with oil	1	31.532
2% without oil	15	5123.279
2% with oil	15	13.865

Results of Area Monitoring for Exposure Monitoring Study Comparing Two Formulations of Ractopamine Hydrochloride in a Feed Mill at Greenfield, Indiana - Study Number T4V759303

Formulation	Sample Description	Sampling Time (min)	Result (µg/m^3)
2% without oil	background sample prior to weighing	15	<0.375
2% without oil	sample during weighing (5 one-min. replicates and 15-min. weighing)	27	8.191
2% without oil	background sample between weighing formulations A & B	16	5.318
2% with oil	sample during 5 one-min. weighing replicates	9	3.054
2% with oil	sample during 15-minute weighing	15	0.270
2% with oil	background after weighing	15	<0.375

Appendix GG: Report Summary

Title: Exposure Monitoring Study for Ractopamine Hydrochloride in a Feed Mill at Greenfield, Indiana

Study Number: T4V759304

Study Date: July 7, 8, and 9, 1993

Names of Investigator: M. A. Moreman

Test Articles: 2 percent ractopamine hydrochloride corn cob grit premix with soybean oil

Summary of Experimental Design: This study was designed to assess the worker exposure to a formulation containing 2% ractopamine hydrochloride with 1% added soybean oil in a feed mill operation. Three types of monitoring were conducted: full shift (8-hr) personal monitoring for the purpose of comparison with the Lilly Exposure Guideline (LEG) of 17 $\mu g/m^3$; short term (15-min) personal monitoring for comparison with the Lilly Short Term Exposure Guideline (LSTEG) of 240 $\mu g/m^3$; and area monitoring to assess the concentration of ractopamine hydrochloride in ambient air in various locations. The feed mill at the Eli Lilly Greenfield Laboratories was the site of the study. To simulate an exposure environment to the worker employed in a commercial feed mill with minimal or no engineering controls, all engineering exposure control measures were disarmed, including local exhaust ventilation and weighing hoods. A working regimen that simulates commercial feed mill practice was followed: weighing five lots of ractopamine hydrochloride premix followed by mixing and bagging of each individual lot. Full-shift personal monitoring encompassed all tasks, while short-term personal monitoring was conducted on the tasks of premix weighing and medicated feed bagging. Mixing was not assessed with short-term personal monitoring due to the short duration of the task (dumping the premix into the mixer lasted approximately 5 seconds).

Weighing of the premix was conducted on an electronic platform scale. The scale was housed in a cement-block room (7 ft X 7 ft) situated in a feed storage warehouse. After weighing, the premix was poured through a small door at the top of the mixer for blending. The mixer is a 1.5 ton capacity, horizontal double-ribbon, tilt-tub device made by Wenger. The mixer is totally enclosed (one cloth dust bag for air displacement) with dust exhaust lines attached. The mixer is situated in an area that is about 35 ft X 55 ft. Once the mixing cycle was completed, the blended feed was dumped into a surge bin directly below the mixer and transported pneumatically to the finished feed bin for bagging. The feed was discharged from the finished feed bin down a metal tube to the hopper on the baghouse scale. Finished feed (50 pounds) was released from the scale into a bag. The top of the bag was stitched closed and placed on a pallet for transport to a warehouse.

The premix was mixed into fifteen separate 750-pound batches of a 16% crude protein corn-soy swine grower diet (ration no. 31) with no liquid additions. Each batch of feed was mixed to contain about 20 ppm (18.14 g/ton) of ractopamine hydrochloride.

Appendix GG: Continued

Each batch of feed was mixed, bagged, and stacked before the next batch was started. Exposure monitoring was conducted during premix weighing, and medicated feed mixing and bagging operations by pumping ambient air through glass fiber filters and analyzing them for their ractopamine hydrochloride content. The actual ambient levels of ractopamine hydrochloride in air were calculated based on the amount found on the filter, the volume of air pumped through the filter, and the survey recovery value.

Monitoring was conducted at the Eli Lilly and Company Greenfield Feed Mill. Samples were collected on glass fiber filters fitted in 37 mm open face dust sampling cassettes attached by tygon tubing to GAST Model DOA-104-AA high volume air sampling pumps for short-term monitoring (<30 minutes). For personal monitoring, the filter cassettes were located in the worker's breathing zone. The pumps were calibrated with a Kurz electronic flowmeter and had an average air flow rate of approximately 20.0 liters per minute (l/min.). Glass fiber filters in 37 mm closed face dust sampling cassettes were attached by tygon tubing to DuPont P2500B air sampling pumps for full-shift monitoring (approximately 8 hours). The pumps were calibrated with a Kurz electronic flowmeter and had an average air flow rate of approximately 2.4 l/min.

A 3M9920 dust, fume, mist respirator and latex gloves were worn while weighing the premix.

Spiked samples were used to assure the recovery and stability of ractopamine hydrochloride during the handling operations. Blank samples were used to assure that accidental contamination did not occur. Four types of controls were used: field, remote, shipping, and retained. The spiking level for the spiked control samples was approximately 75 µg/pad for all of the recovery studies. This is approximately equivalent to 240 $\mu g/m^3$ (the LSTEG) for a 15 minute sample at a sampling rate of 20 l/min., and approximately 80 $\mu g/m^3$ for the full-shift samples at 2.4 l/min.

Summary of Results: The data collected were organized by homogenous exposure groups (HEG) and statistically analyzed assuming a log-normal distribution. The data were organized into three HEGs: one for full-shift personal monitoring; one for short-term personal monitoring assessing the weighing task; and one for short-term personal monitoring assessing the bagging task. Where applicable, a geometric mean and geometric standard deviation (SD) were calculated for each HEG. Full-shift personal monitoring was conducted for approximately 6.5 to 7.5 hours at a flow rate ranging from 2.10 l/min. to 2.55 l/min. Nine samples were taken yielding breathing zone concentrations below the limit of detection (LOD) (sample LOD ranged from 0.105 $\mu g/m^3$ to 0.155 $\mu g/m^3$), with the exception of one sample with a detectable concentration of 0.182 $\mu g/m^3$.

Short-term personal monitoring for the premix weighing operation was conducted for approximately 15 minutes at a flow rate ranging from 19.15 l/min. to 20.35 l/min. Three samples were taken with a minimum of 0.432 $\mu g/m^3$, a maximum of 1.523 $\mu g/m^3$, a geometric mean of 0.924 $\mu g/m^3$, and a geometric SD of 1.953.

Appendix GG: Continued

Short-term personal monitoring for the medicated feed bagging operation was conducted for approximately 15 minutes at a flow rate ranging from 19.35 l/min. to 20.30 l/min. Thirty samples were taken, most yielding breathing zone concentrations below the LOD (0.411 µg/m^3 to 0.436 µg/m^3), with the exception of four with detectable concentrations ranging from 0.428 µg/m^3 to 0.600 µg/m^3. The four detectable results had a geometric mean of 0.489 µg/m^3 and a geometric SD of 1.160.

All personal monitoring yielded concentrations below exposure guidelines as shown in the following table:

Summary of Personal Monitoring Results

Type of Personal Monitoring	Mean Result (µg/m^3)	Applicable Exposure Guideline (µg/m^3)
full shift	BLD	17
short-term - weighing	0.924	240
short-term - bagging	0.489	240

BLD - below limit of detection - all samples except one (0.182 µg/m^3) were below the limit of detection (0.105 - 0.155 µg/m^3)

Area monitoring was conducted to determine the ambient concentration of ractopamine hydrochloride while the various tasks (weighing, mixing, and bagging) were occurring. Samples were taken from areas contiguous to the task and approximately 15 feet from the task. All area samples were collected for approximately 15 minutes at a flow rate ranging from 19.00 l/min. to 21.80 l/min. The six area samples taken during the premix weighing operation yielding non-detectable concentrations (less than 0.436 µg/m^3). Fifteen area samples were taken near the mixing operation (pumps were located on top of the mixer). Eleven of those samples yielded detectable concentrations ranging from 0.536 µg/m^3 to 1.155 µg/m^3, with a geometric mean of 0.802 µg/m^3 and a geometric SD of 1.263,. Fifteen samples collected at a site approximately 15 feet from the feed mixing operation were below the LOD (<0.410 µg/m^3). Thirty area samples taken during medicated feed bagging yielded non-detectable concentrations (less than 0.433 µg/m^3).

This study was designed to assess the worker exposure to ractopamine hydrochloride while weighing premix and mixing and bagging medicated feed in a feed mill. Full-shift monitoring demonstrated exposure levels of ractopamine hydrochloride to be less than 1% of the exposure guideline of 17 µg/m^3. The mean breathing zone air concentrations of ractopamine hydrochloride at the operations judged to present the highest potential for short term exposure were 0.2% to 0.3% of the short-term exposure guideline of 240 µg/m^3.

Appendix GG: Continued

These data demonstrate that atmospheric ractopamine hydrochloride exposure to workers weighing ractopamine hydrochloride premix, or mixing or bagging medicated feed were below recommended exposure guidelines.

Appendix GG: Continued

Results of Personal Monitoring for Feedmill Exposure Study - 2% ractopamine hydrochloride formulation with 1% added oil- Study Number T4V759304

Full-Shift Personal Results		
Sample Duration (min).	**Result (µg/m^3)**	**Exposure Guideline (µg/m^3)**
451	<0.118	17
369	<0.139	17
445	<0.117	17
462	<0.115	17
466	<0.105	17
386	0.182	17
384	<0.155	17
454	<0.120	17
455	<0.117	17
Short-Term Personal Results - Weighing		
15	1.200	240
15	1.523	240
15	0.432	240
Short-Term Personal Results - Bagging		
15	<0.428	240
15	<0.436	240
15	<0.436	240
15	0.428	240
15	<0.436	240
15	0.600	240
15	<0.436	240
15	<0.428	240
15	<0.428	240
15	<0.436	240
15	<0.412	240
15	<0.422	240
15	<0.412	240
15	<0.422	240
15	<0.412	240
15	<0.422	240
15	<0.412	240
15	<0.411	240
15	<0.412	240
15	<0.422	240
15	<0.411	240
15	<0.425	240
15	<0.411	240
15	<0.425	240
15	0.493	240
15	<0.425	240
15	0.452	240
15	<0.432	240
15	<0.411	240
15	<0.425	240

Appendix GG: Continued

Results of Area Monitoring for Feedmill Exposure Study - 2% ractopamine hydrochloride formulation with 1% added oil- Study Number T4V759304

Task	Location *	Result ($\mu g/m^3$)
Weighing	near	<0.436
Weighing	near	<0.409
Weighing	near	<0.411
Weighing	far	<0.435
Weighing	far	<0.413
Weighing	far	<0.414
Mixing	near	<0.440
Mixing	near	<0.440
Mixing	near	0.747
Mixing	near	0.791
Mixing	near	<0.440
Mixing	near	<0.413
Mixing	near	0.536
Mixing	near	0.701
Mixing	near	1.155
Mixing	near	0.866
Mixing	near	1.092
Mixing	near	0.798
Mixing	near	0.630
Mixing	near	1.008
Mixing	near	0.714
Mixing	far	<0.383
Mixing	far	<0.383
Mixing	far	<0.383
Mixing	far	<0.383
Mixing	far	<0.383
Mixing	far	<0.435
Mixing	far	<0.411
Mixing	far	<0.411
Mixing	far	<0.411
Mixing	far	<0.411
Mixing	far	<0.414
Mixing	far	<0.414
Mixing	far	<0.414
Mixing	far	<0.414
Mixing	far	<0.414

*'near' samples were located contiguous to the task; 'far' samples were located approximately 15 feet away

Appendix GG: Continued

Results of Area Monitoring for Feedmill Exposure Study - 2% ractopamine hydrochloride formulation with 1% added oil- Study Number T4V759304

Task	Location*	Result (µg/m^3)
Bagging	near	<0.433
Bagging	near	<0.433
Bagging	near	<0.433
Bagging	near	<0.433
Bagging	near	<0.433
Bagging	near	<0.418
Bagging	near	<0.418
Bagging	near	<0.418
Bagging	near	<0.418
Bagging	near	<0.418
Bagging	near	<0.430
Bagging	near	<0.430
Bagging	near	<0.430
Bagging	near	<0.430
Bagging	near	<0.430
Bagging	far	<0.383
Bagging	far	<0.383
Bagging	far	<0.383
Bagging	far	<0.383
Bagging	far	<0.383
Bagging	far	<0.411
Bagging	far	<0.411
Bagging	far	<0.411
Bagging	far	<0.422
Bagging	far	<0.411
Bagging	far	<0.414
Bagging	far	<0.414
Bagging	far	<0.414
Bagging	far	<0.414
Bagging	far	<0.414

*'near' samples were located contiguous to the task; 'far' samples were located approximately 15 feet away

Appendix HH: Report Summary

Title: Overview of Ractopamine Hydrochloride Occupational Exposure

Authors: M. A. Moreman, Industrial Hygienist, Occupational Hygiene and Safety, and R. K. Wolff, Research Scientist, Inhalation Toxicology, Eli Lilly and Company

Summary: Two exposure guidelines have been established for ractopamine hydrochloride:

- 17 $\mu g/m^3$ time weighted average 12-hour exposure (Lilly Exposure Guideline, LEG)
- 240 $\mu g/m^3$ short term exposure guideline 15-minute exposure (Lilly Short Term Exposure Guideline, LSTEG)

The following information was considered in the development of the exposure guidelines:

The acute oral median lethal dose of ractopamine in male rats was 474 mg/kg and in female rats, 367 mg/kg. In mice the median lethal oral dose was greater than 2500 mg/kg. Signs of toxicity included, but were not limited to, hypoactivity, tremors, coma, nasal and ocular discharge, and hyperemia. In dogs a single oral dose of 0.05 mg/kg or greater produced increased heart rate and cutaneous erythema. Intravenous infusion of 0.0035 mg of ractopamine/kg/minute for ten minutes resulted in increased heart rate in both dogs and monkeys. There were no changes in electrocardiogram wave forms in either species. Dogs exhibited a decrease in arterial blood pressure that was not seen in monkeys.

The median lethal aerosol concentration of ractopamine in rats exposed for four hours was 2,800,000 $\mu g/m^3$.

Ractopamine was moderately irritating to the eyes of rabbits, but did not produce dermal irritation or evidence of systemic toxicity when placed on the skin.

Ractopamine was not mutagenic in a battery of tests in both bacterial and mammalian cell systems.

Three-month dietary administration of ractopamine to rats and mice at oral dose equivalents as high as 150 and 1250 mg/kg, respectively, was not lethal and resulted in only minor toxicologic effects. Metabolic activation of brown fat was detected in both species at the higher dose levels, an effect consistent with the pharmacologic activity of the chemical.

Appendix HH: Continued

Beagle dogs were given oral doses of 0.112, 0.224, or 5.68 mg/kg ractopamine daily in three equally divided portions six hours apart for one year. Treatment-related effects were minimal and were usually seen only at the high dose. Effects included transient peripheral redness of the skin; slight decrease in some red blood cell parameters; decrease in glucose, cholesterol, and triglyceride concentrations; and increases in serum potassium and urea nitrogen levels. Metabolic activation of brown fat was also detected in the high-dose group at the conclusion of the study. Effects on the heart were limited to a small decrease in resting heart rate at all dose levels during the first six months of treatment. The no-effect dose excluding the cardiovascular effects was 0.224 mg/kg ractopamine/day. In a special 90-day cardiovascular study in monkeys, there were no clinical signs of toxicity or effects on heart rate at a daily oral dose of 0.125 mg/kg.

Neither mating performance nor fertility were affected in rats treated with ractopamine via the diet for two successive generations. Reduced litter size, gestational survival, progeny size and growth, and the occurrence of developmental abnormalities were seen only at the dose level that was clearly maternally toxic (approximately 160 mg/kg/day).

Inhalation studies were conducted because the inhalation route of exposure is most relevant to workplace exposures. Two types of studies were conducted to provide information on: 1) repeated exposures that might take place over several days that could be related to average concentrations sustained over a workshift; and, 2) exposure for brief periods of time. Fifteen-minute exposures were studied because The American Conference of Governmental Industrial Hygienists defines 15 minutes as short-term exposure. Four-hour exposures were considered sufficiently long to assess the effects of full shift exposures of up to 12 hours duration. In a preliminary study, heart rates increased with exposure time until they maximized after about 1/2 to 2 hours of inhalation exposure. Therefore, increasing exposure time beyond 4 hours would not produce greater heart rate responses.

Appendix HH: Continued

Heart rate was of primary interest because the studies previously cited had identified it as the most sensitive endpoint. In order to examine effects related to repeated exposures in the workplace, rhesus monkeys were exposed to ractopamine aerosols (whole body exposure) four hours per day for up to eight days at concentrations ranging from 50 to 23,800 μg/m3. Real time heart rates in unrestrained monkeys were measured during whole body exposures using a remote computer-based data acquisition system and implanted heart rate transmitters. An initial study was conducted with aerosol concentrations of 380, 1690, 6420, and 23,800 $\mu g/m^3$. Heart rate responses were concentration related. Exposure to aerosol concentrations of 23,800 $\mu g/m^3$ produced heart rate increases of approximately 100 beats per min (bpm). Heart rates did not return to control levels after exposure. Exposures were stopped after two days, and heart rates returned to normal ranges after about two weeks. Seven days of exposure to aerosol concentrations of 1690 and 6420 $\mu g/m^3$ produced heart rates 38 and 48 bpm higher than controls, respectively. The heart rates returned to control ranges in about one week. Eight days of exposure at 380 $\mu g/m^3$ produced significant increases compared to controls (about 24 bpm) during daytime exposure. The heart rate remained significantly elevated above controls (about 30 bpm) during the nighttime nonexposure period.

Because a no-effect level was not achieved, a second 4-hour inhalation study was conducted in another group of rhesus monkeys with ractopamine aerosol concentrations of 50, 170, and 440 $\mu g/m^3$ for eight exposures over a ten-day period. Exposure to aerosol concentrations of 440 $\mu g/m^3$ produced small changes in heart rate, similar to the previous study at 380 $\mu g/m^3$. During daytime exposure to 440 $\mu g/m^3$, heart rates were not significantly different than controls (about 9 bpm greater), but during the nighttime nonexposure period, heart rates were significantly greater than controls (about 17 bpm). Exposure to aerosol concentrations of 50 and 170 $\mu g/m^3$ produced no significant change in heart rate. The statistical analysis of these studies had the power to detect heart rate changes of about 15 to 17 bpm ($p=0.05$). The no-observed-effect level (NOEL) was determined to be 170 $\mu g/m^3$. This determination can be made with confidence because of the well defined dose response relationship established from the two studies in combination. The effects observed at the next highest concentrations of 380 and 440 $\mu g/m^3$ were small, reinforcing the view that 170 $\mu g/m^3$ is clearly a NOEL.

Appendix HH: Continued

Rhesus monkeys were also exposed to ractopamine hydrochloride via inhalation (head only) to evaluate the effects of short term exposure. Monkeys were placed in a restraint chair with their head in a small volume dome to allow prompt onset and termination of aerosol exposures. Each animal was exposed for 15 minutes to average ractopamine activity concentrations of 0; 2400; 13,900; and 27,400 $\mu g/m^3$. Heart rates were monitored in real time using a computerized data acquisition of heart rate from standard patch electrodes for 15 minutes prior to exposure, during the 15-minute exposure period, and for 30 or 60 minutes postexposure. No statistically significant increase in heart rate occurred as the result of exposure to 2400 $\mu g/m^3$ for 15 minutes, either during exposure or for the half hour of heart rate monitoring after the end of exposure. Exposure to 13,900 and 27,400 $\mu g/m^3$ resulted in significant increases in mean heart rates of 25 and 47.5 beats per minute, respectively, at the end of the 15-minute aerosol exposure. Heart rates decreased after the end of exposure, returning to normal in the 13,900 $\mu g/m^3$ exposure group 30 minutes postexposure, but remaining significantly elevated in the 27,400 $\mu g/m^3$ exposure group. As in the 4-hour exposure studies, there was a clearly defined dose response with a direct relationship between exposure concentration and increased heart rate. The statistical analysis of the short term studies had the power to detect heart rate changes of approximately 10 bpm ($p = 0.05$). The NOEL was determined to be 2400 $\mu g/m^3$.

The results from the 15-minute and 4-hour exposure studies were complementary and consistent. Both studies showed increases in heart rate that were dependent on aerosol concentration; however, at a given concentration there was a greater heart rate response in the 4-hour exposures than in the 15-minute exposures. This occurred because it took time for heart rates to increase from baseline after the onset of inhalation exposure, and maximum heart rates were not usually achieved until after 1/2 hr to 2 hours of inhalation exposure. Thus, the NOEL determined for the 15-minute exposure is higher than that for the 4-hour exposures.

The NOEL for monkeys given a repeated 4-hour inhalation exposure to ractopamine hydrochloride aerosol was 170 $\mu g/m^3$. The lowest aerosol concentration level that produced a significant increase in heart rate was 380 $\mu g/m^3$ for 4 hours. A ten-fold uncertainty factor (see below) was applied to the NOEL of 170 $\mu g/m^3$ in monkeys, resulting in a Lilly Exposure Guideline of 17 $\mu g/m^3$/12 hours. The exposure guideline is more than 20 times lower than the lowest airborne concentration

Appendix HH: Continued

(380 $\mu g/m^3$) that caused detectable heart rate effects in monkeys.

The NOEL for monkeys exposed to an aerosol of ractopamine hydrochloride for 15 minutes was 2400 $\mu g/m^3$. A 10-fold uncertainty factor (consistent with the uncertainty factor used for the ractopamine LEG) was applied to the NOEL of 2400 $\mu g/m^3$, resulting in a LSTEG of 240 $\mu g/m^3$.

The Lilly Exposure Guideline Committee, representing the disciplines of industrial hygiene, toxicology, pharmacology, and occupational medicine, used an uncertainty factor of 10-fold to extrapolate from the inhalation toxicology studies in monkeys to exposure guidelines for man. From a review of extensive oral toxicology testing, the committee determined that heart rate increase was the most sensitive indicator of exposure. It was also felt that the heart rate endpoint was a transient physiological event that had a high likelihood of being noted by the subject if it occurred in an occupational setting. The monkey was chosen as the inhalation toxicology animal model because aerosol deposition in the lungs of monkeys is similar to man. The similarity of response of monkeys to beta agonists (the chemical class to which ractopamine belongs) compared to that of humans was an important factor used in supporting the 10-fold factor. Carlson *et al.* (1993) compared the responses of monkeys to inhaled isoproterenol (a classic beta agonist) to that previously demonstrated in humans by Patel *et al.* (1990) and found them to be similar. Also, the response of monkeys to intravenous ractopamine was similar to the response in man (Leier *et al*., 1983) to intravenous butopamine (the cardioactive RR isomer of ractopamine). Therefore, it was felt that responses in monkeys to inhaled ractopamine would be predictive of those in man and a 10-fold uncertainty factor was appropriate. The Committee felt that if workers were exposed to the LEG for full shifts, or to the LSTEG for isolated instances, no adverse health effects would result.

Both the short-term exposure guideline and the full shift LEG relate to distinct and important exposure events (with respect to exposure concentration and time). Exposure to the LSTEG concentrations of ractopamine for four 15-minute periods in one work shift will slightly exceed the full shift LEG exposure. (As applied to the ACGIH definition of a STEL). Therefore, it is important that both limits be complied with in assessing and controlling worker exposure. In cases where exposure at or below the LSTEG does not ensure compliance or conflicts with compliance to the full shift LEG, appropriate additional control measures should be used to lower the full shift exposure.

Appendix HH: Continued

Conclusions: Exposure monitoring of the current formulation of 2% ractopamine hydrochloride (on corn cob grits with 1% added soybean oil) in a feed mill setting monitored in the absence of local exhaust ventilation or other exposure control measures resulted in exposure concentrations to workers substantially less than the exposure guidelines established by the Lilly Exposure Guideline Committee. The exposure guidelines were derived from inhalation toxicology studies with a 10-fold uncertainty factor applied to the NOELs determined in repeated 4-hour exposure and single 15-minute exposure inhalation toxicology studies. The respective exposure guideline values for the time weighted average 12-hour exposure was determined to be 17 $\mu g/m^3$ and for the 15-minute exposure, 240 $\mu g/m^3$. The maximum breathing zone concentration during the weighing operation from a series of exposure monitoring studies was approximately 32 $\mu g/m^3$ for a one-minute period. The mean breathing zone concentrations for the 15-minute weighing operation ranged from approximately 0.9 to 7 $\mu g/m^3$ for the two studies in which there were at least duplicate weighing operations.

Dustiness of the new formulation of ractopamine on corn cob grits was controlled by adding 1% soybean oil to the final product. Workplace safety has been established for the feed mill operator on the basis of inhalation toxicology studies with 10-fold uncertainty factors and feed mill exposure data collected without the benefit of local exhaust ventilation or other exposure control measures. No adverse health effects in the workplace are expected when exposure levels are maintained at or below the LEG and LSTEG.

References:

Carlson, K. H., Wolff, R. K., Tielking R. L., Franklin P. A., Yeager, H. K., and Dorato, M. A. Real-Time Measurements of Cardiac Effects During Inhalation Exposures of Monkeys or Dogs. Inhalation Toxicol. 5: 291-301, 1993.

Patel, P., Mukai, D., and Wilson, A. F. Dose-Response Effects of Two Sizes of Monodisperse Isoproterenol in Mild Asthma. Am. Rev. Resp. Dis 141: 357-360, 1990.

Leier, C. V., Nelson, S., Magorien, R. D., Boudoulas, H., Gibb, L., and Unverferth, D. V. Heart Rate Responsiveness After Sustained Chronotropic Stimulation with a ß$_1$-Adrenergic Receptor Agonist. J. Lab. Clin. Med., June 1983.

Appendix II: Proposed Label for Use of Paylean in Finishing Swine Feeds

The label included in this EA is an example of the labeling that Elanco has proposed for this product. It is not finalized by the CVM at the time of the preparation of this document. The reader should contact Elanco or the CVM for a copy of the approved labeling.

ELANCO

AF0602-25B

For Use in Finishing Swine Feeds Only

Paylean® 9

Ractopamine Hydrochloride

Net Weight 25 lbs
(11.34 kg)

Type A Medicated Article

Do not feed undiluted.

Active Drug Ingredient: ractopamine hydrochloride - 9 g per lb (20 g per kg)

Important: Must be thoroughly mixed into feeds before use. Follow label directions.

Indication: Increased rate of weight gain, improved feed efficiency, and increased carcass leanness in finishing swine fed a complete ration containing at least 16% crude protein from 150 lb (68 kg) body weight to 240 lb (108 kg).

Carcass Measurements	Effect of Paylean	
	4.5 grams/ton	9 - 18 grams/ton
Carcass Fat	NC	↓
10th Rib Backfat (3/4 location)	NC	↓
Last Rib Backfat (midline)	NC	NC
Loineye Area (10th rib)	NC	↑
Rate of Lean Gain	NC	↑
Efficiency of Lean Gain	NC	↑
Dressing Percentage	NC	↑

NC= No Change, ↑ = increased, ↓ = decreased

Inert Ingredients: Ground corncobs.

Indications	Appropriate Concentration of Ractopamine in Type C Medicated Feed
Increased Rate of Weight Gain	4.5 grams/ton (5 ppm)
Improved Feed Efficiency and Increased Carcass Leanness	4.5 to 18 grams/ton (5 ppm to 20 ppm)

Mixing Directions: Thoroughly mix Paylean® Type A Medicated Article into one ton of appropriate feed ingredients or diluents according to the table below to obtain the proper concentration in the Type B Medicated Feed (maximum 3600 g/ton). The following table gives examples of how some Type B Medicated Feed concentrations can be prepared:

Pounds of Paylean® 9 To Add Per Ton To Make a Type B Medicated Feed	Resulting Ractopamine Concentration in Type B Medicated Feed	
	grams/ton	grams/pound
100	900	0.45
200	1,800	0.90
400	3,600	1.80

Thoroughly mix Paylean® Type A Medicated Article into one ton of complete swine feed according to the table below to obtain the proper concentration in the Type C Medicated Feed. Prepare an intermediate pre-blend of the premix prior to mixing in a complete feed. Thoroughly mix the required amount in a convenient quantity of feed ingredients then add to the remaining feed ingredients to make a ton of complete feed.

Pounds Paylean® 9 To Add Per Ton of Type C Medicated Feed	Resulting Ractopamine Concentration in Type C Medicated Feed
0.5	4.5 grams/ton (5 ppm)
1.0	9 grams/ton (10 ppm)
1.5	13.5 grams/ton (15 ppm)
2.0	18 grams/ton (20 ppm)

Feeding Directions: Feed continuously to finishing swine as the sole ration from 150 lb (68 kg) body weight to 240 lb (108 kg).

CAUTION: The impact of ractopamine on reproductive performance has not been tested in breeding animals.

WARNING: The active ingredient in Paylean®, ractopamine hydrochloride, is a beta-adrenergic agonist. Individuals with cardiovascular disease should exercise special caution to avoid exposure. Not for use in humans. Keep out of the reach of children. The Paylean® formulation (Type A Medicated Article) poses a low dust potential under usual conditions of handling and mixing. When mixing and handling Paylean®, use protective clothing, impervious gloves, protective eye wear, and a NIOSH-approved dust mask. Operators should wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse thoroughly with water. If irritation persists, seek medical attention. The material safety data sheet contains more detailed occupational safety information. To report adverse effects, access medical information, or obtain additional product information, call 1-800-428-4441.

Avoid moisture and excessive heat.
Expiration Date and Lot Number are printed on the bag. Not to be used after the expiry date.

Paylean® 9

Elanco Animal Health
A Division of Eli Lilly and Company
Indianapolis, IN 46285, U.S.A.

Questions or Comments: Call 1-800-428-4441 or Visit
www.elanco.com
3/25/99D

Appendix JJ: Report Summary

Title: Ractopamine HCl, Ready Biodegradability (Closed Bottle Test)

Study Number: DTA 3/931783

Study Dates: September 23 to October 21, 1993

Name and Address of Investigators: R. W. S. Halls and C. M. King, Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England

Test Article: Ractopamine hydrochloride

Test System: Sealed bottles containing ractopamine hydrochloride, inorganic nutrient media and activated sewage sludge bacteria.

Summary of Experimental Design: Sealed bottles containing ractopamine hydrochloride (2 mg/L) and inorganic nutrient medium were inoculated with activated sewage sludge bacteria and incubated for up to 28 days at 20±1°C. On days 0, 4, 7, 11, 14, 18, 21, 25, and 28 duplicate bottles were taken and dissolved oxygen measurements were performed electrochemically. Percentage biodegradation values were calculated by comparing the extent of oxygen depletion with the Theoretical Oxygen Demand (2.04 mgO_2/mg). Additional bottles, containing both the test substance and a readily biodegradable standard substance were prepared in order to provide additional information on the inhibitory effect of the test substance.

Summary of Results: Ractopamine hydrochloride attained 66% biodegradation after 28 days. Ractopamine hydrochloride can be considered to be ultimately biodegradable by the standards of this study. Sodium benzoate, the positive control, attained 72% biodegradation within 28 days. Ractopamine hydrochloride was considered to have a slight inhibitory effect on sewage bacteria respiration based on the conditions of this test. Inhibition of respiration was not found in a separate study (Study N00595) until ractopamine hydrochloride concentrations reached 1000 mg/L.

Appendix KK: Report Summary

Title: The 72-hour acute toxicity of ractopamine hydrochloride to the freshwater green alga *Selenastrum capricornutum* in a static test system.

Study Number: J00295

Study Dates: February 7 to February 9, 1995

Name and Address of Investigators: D. W. Poage, Lilly Research Laboratories, P.O. Box 708, Greenfield, Indiana 46140

Test Article: Ractopamine hydrochloride

Test System: Nutrient medium with ractopamine hydrochloride in flasks inoculated with the test species *Selenastrum capricornutum* .

Summary of Experimental Design: A static toxicity test was conducted to evaluate the effects of ractopamine hydrochloride on the green alga, Selenastrum capricornutum. Algal cells were cultured for 72 hours in a liquid nutrient medium that contained ractopamine hydrochloride at mean assayed concentrations of 0.0, 25.4, 51.0, and 101.2 mg/L. Each treatment consisted of three replicate 500-ml Erlenmeyer flasks containing 100 ml of nutrient medium with an algal density of 10,000 cells/ml. The algal population of each flask was quantified on Days 1, 2, and 3 using a compound microscope and hemacytometer, and algal biomass was measured on Day 3. These measurements were used to determine the no-observed-effect concentration (NOEC), EC50 value for growth rate, and the EC50 value for the growth of cell populations.

Summary of Results: Terminal cell count, maximum cell count, specific growth rate (µ-max), and area under the curve (AUC) were significantly reduced relative to water control cultures at the highest treatment concentration, 101.2 mg/L. The average specific growth rate (µ-reg) and terminal biomass were not significantly affected at any concentration tested. Based upon these results, the NOEC for reduced population growth of this green alga was 51.0 mg/L. The EC50 values for µ-reg and growth of cell populations (AUC) were greater than 101.2 mg/L.

Appendix LL: Report Summary

Title: An activated sludge respiration inhibition study conducted with ractopamine hydrochloride

Study Number: N00595

Study Dates: February 6, 1995

Name and Address of Investigators: W. A. Althaus and M. D. Gunnoe, Lilly Research Laboratories, P.O. Box 708, Greenfield, IN 46140

Test Article: Ractopamine hydrochloride

Test System: Nutrient medium with ractopamine hydrochloride in flasks inoculated with activated sludge.

Summary of Experimental Design: The respiration rate of activated sludge was measured after a contact time of 3 hours using control samples, five concentrations of ractopamine hydrochloride (10, 30, 100, 300, and 1000 mg/L), and three concentrations of a positive control for inhibition (3, 5-dichlorophenol). Each Eylenmeyer flask contained a total solution volume of 500 ml and activated sludge equivalent to 1200 mg/L. At the end of the 3-hour incubation period, the rate of dissolved oxygen utilization was measured in 10-second intervals over 10- to 14-minute periods. Inhibitory effects of the test substance and the positive control were calculated as a percent of untreated control samples.

Summary of Results: The EC50 for 3, 5 dichlorophenol, the positive control, was 28.2 mg/L. This level was consistent with inhibition information available for this compound. No inhibition was found for ractopamine concentrations up to 300 mg/L. At the highest test concentration, 1000 mg/L, respiration was inhibited (39%) by ractopamine hydrochloride. The EC50 for ractopamine hydrochloride was estimated by linear regression analysis to be 1413 mg/L.

LAST PAGE

ENVIRONMENTAL ASSESSMENT
FOR THE USE OF
PAYLEAN® TYPE A MEDICATED ARTICLE
(RACTOPAMINE HYDROCHLORIDE)
IN THE FEED OF SWINE

NADA 140-863

ELANCO ANIMAL HEALTH
A DIVISION OF ELI LILLY AND COMPANY
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INDIANAPOLIS, IN 46285