ENVIRONMENTAL ASSESSMENT FOR THE USE OF RACTOPAMINE HYDROCHLORIDE PREMIX IN THE FEED OF CATTLE

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Environmental Assessment For the Use of Ractopamine Hydrochloride Premix In the Feed of Cattle

1. DATE November 1998

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 ractopamine hydrochloride premix in the feed of cattle in feedlot operations. Ractopamine hydrochloride is the active ingredient in the premix. Up to 30 ppm (30 g/ton) of ractopamine hydrochloride will be used continuously in the feed of feedlot cattle for up to 42 days prior to slaughter. The premix will be used to improve feed efficiency and promote an increase in weight gain and carcass leanness. Cattle will remain in the feedlot for a total of 180 days.

Based on the proposed action, ractopamine hydrochloride could potentially be introduced into the following environments from its use and disposal:

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

5. IDENTIFICATION OF THE CHEMICAL SUBSTANCE

A. RACTOPAMINE HYDROCHLORIDE PREMIX

Ractopamine hydrochloride premix will be incorporated into the complete feed used for cattle in feedlot operations. Ten percent of ractopamine hydrochloride premix will be the active ingredient, ractopamine hydrochloride. The premix also contains ground corn cobs and soybean oil. Soybean oil (1%) 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.

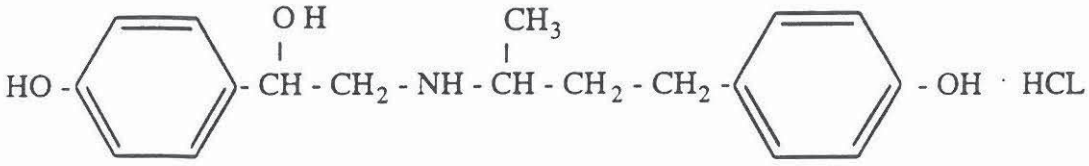
Chemical Name (ractopamine hydrochloride): Benzenemethanol, 4-hydroxy-α-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]methyl]-, hydrochloride

CAS Registry Number: 90274-24-1

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

Molecular Weight: 337.8

Structural Formula:



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.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

A. INTRODUCTION OF SUBSTANCE FROM FEED MIXING LOCATIONS

Feed mixing will be done by commercial feed vendors and by cattle feedlot 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 will be low.

An exposure monitoring study was conducted to determine the general airborne concentrations of ractopamine hydrochloride from the weighing operations in a feed mill (Appendix C). The weighing operation was chosen since it routinely yields the highest detectable levels of ractopamine in personal samplers. In this study, a 10% formulation of the ractopamine hydrochloride premix was weighed for 1 minute periods and 15 minute periods. Most weighing operations typically take only a few minutes. In 15, 1-minute weighing operations, analysis of respirator cartridges from samplers on individuals weighing the premix indicated an average airborne concentration of 23.4 $\mu g/m^3$. Sample monitoring of the area 3-4 feed from the weighing operation indicated average airborne ractopamine hydrochloride concentrations of 1.5 $\mu g/m^3$. These same measurements were made during 15-

minute continuous weighing operations and the levels were no higher. These levels are 10 and 160 times below the Lilly Short Term Exposure Guideline of 240 $\mu g/m^3$, respectively (Appendix D).

In a second study, personal monitoring for exposure to ractopamine hydrochloride during a full shift was performed (Appendix E). Personnel performing the weighing and bagging operations for 15 minutes were also assessed for potential short-term exposure to ractopamine hydrochloride. In this study, a premix containing 2% ractopamine hydrochloride was used. For the full shift exposures, the highest concentration of ractopamine hydrochloride detected in a personal sampler was 0.182 $\mu g/m^3$. During the short term weighing and bagging operations, the average exposures were 0.924 and 0.489 $\mu g/m^3$, respectively. These levels of ractopamine hydrochloride are substantially lower than concentrations determined to be safe for short term (240 $\mu g/m^3$) and full shift (17 $\mu g/m^3$) exposures (Appendix D). The premix with 10% ractopamine hydrochloride would, at most, provide exposures five times higher than those found with the 2% premix. Those high estimates, approximately 5 $\mu g/m^3$ for the short term and 0.9 $\mu g/m^3$ for the full shift, are still well below guidelines for acceptable exposure levels. Significant exposure to ractopamine hydrochloride while performing operations with the premix in a feed mill is not expected.

B. INTRODUCTION OF SUBSTANCE FROM THE USE SITE

Information from the United States Department of Agriculture indicates that there are about 25.7 million cattle slaughtered annually in the United States (National Agricultural Statistics Service 1988). Production is centered in the states of Arizona, California, Colorado, Illinois, Idaho, Iowa, Kansas, Minnesota, Nebraska, Oklahoma, South Dakota, Texas, and Washington.

There is substantial variation in the numbers of cattle finished in feedlots. A typical outdoor feedlot contains about 200 cattle with a pen space of 200 ft^2 per animal. Cattle

coming into a feedlot will stay a total of 180 days and receive ractopamine for up to the last 42 days. Cattle coming into the feedlot will have an average weight of 977 lb(440 kg) and during the last 42 days in the feedlot, an average weight of 518 kg. At this stage, cattle will consume food at a rate of 2.1% (dry matter basis) of their body weight per day. Thus, cattle will consume 10.9 kg of feed/day containing 327 mg of ractopamine. Over 42 days, cattle will consume 13.7 g of ractopamine per animal. A facility producing 200 head/cycle and 2 cycles a year could use as much as 5.5 kg of ractopamine hydrochloride or 54.8 kg of ractopamine hydrochloride premix in a year.

If ractopamine hydrochloride were used for all of the feedlot cattle produced in the United States, at most, 3.5×10^5 kg of ractopamine hydrochloride would be used each year (13.7 g ractopamine hydrochloride/animal x (25.7×10^6 animals)). This is equivalent to the use of 3.5×10^6 kg of ractoopamine hydrochloride premix each year. An optimistic market penetration of 35% could result at most, in an annual use of about 1.2×10^5 kg of ractopamine hydrochloride or 1.2×10^6 kg of ractopamine hydrochloride premix each year.

Airborne exposures of personnel handling ractopamine have been studied (Appendices C and E). The greatest potential for exposure to ractopamine hydrochloride will be in the feed mill. Personal air samplers worn during feed mill operations demonstrate that short term exposures and full shift exposures are considerably below the short term exposure guideline (240 $\mu g/m^3$) and the Lilly full-shift exposure guideline (17 $\mu g/m^3$) (Section 6A; Appendix D). Significant exposure while handling ractopamine hydrochloride premix in the feed mills is not expected. Handling the premix after it is diluted into cattle feed would yield even less exposure.

Ractopamine hydrochloride may be introduced into the environment via the waste products from cattle. The major metabolites excreted by cattle are ractopamine hydrochloride and glucuronide conjugates of ractopamine hydrochloride (Appendix F). Because the biological activity of these conjugates is unknown, it will be assumed that the metabolites have the same biological activity as ractopamine hydrochloride. For cattle fed a

diet with 30 ppm (highest recommended rate), essentially, all of the ^{14}C-ractopamine hydrochloride fed is excreted in the urine and feces, with an average of 45.6% in urine and 52.3% in the feces (Appendix G). Assuming that a 440 kg cow will produce about 30 kg of manure a day (Midwest Plan Service, 1985), the expected concentration of ractopamine equivalents in excreted manure would be about 11 ppm (13.7 g / (30 kg manure/day x 42 days)). Assuming that all of the excreta is collected for 1 cycle prior to application to land, the racopamine concentration in the manure will be 2.5 ppm (13.7g/(30 kg manure/day x 180 days)).

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 H and I). 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 J). 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 K). Ractopamine hydrochloride strongly adsorbs to soil with adsorption coefficients (K_d) 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 (K_{oc}) expressed on the basis of organic content of the soils are 2007, 2698, and 2090 for clay loam, loam, and sandy loam, respectively (Appendix L). Ractopamine hydrochloride is substantially biodegraded in soil resulting in evolution of CO_2 and volatile organic residues from the molecule (Appendix M). 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 N). 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 cattle 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 cattle excreta and in adjacent aquatic systems. It could also be possible to find measurable concentrations in the runoff from feedlot facilities for beef cattle.

A. POTENTIAL CONCENTRATIONS OF RACTOPAMINE HYDROCHLORIDE IN SOIL

1. POTENTIAL CONCENTRATION OF RACTOPAMINE HYDROCHLORIDE IN THE FEEDLOT

Cattle feedlot operations are open lots that typically have a dirt floor. Cattle excreta is collected from these facilities for later use as fertilizer on cropland. Cattle in these lots have approximately 200 ft^2/head. Each animal would be fed a total of 13.7 g of ractopamine hydrochloride (about 0.327 g/day for 42 days) and would excrete about the same amount of residue. The highest expected concentration of ractopamine hydrocholoride (11 ppm) at a feedlot would be found in cattle 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 N). Further dissipation would take several weeks. After initial dissipation, the concentration of

ractopamine hydrochloride in excreta would be 3.2 ppm (11 ppm x 0.29). The total amount of ractopamine hydrochloride remaining from each animal in a feedlot at the end of a finishing period would be at most 3.97 g (13.7 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 cattle excreta (2.5 ppm, Section 6C), and from the use rate of cattle excreta on cropland. A reasonable estimate of the application rate of cattle excreta as fertilizer is 1.8×10^4 kg/acre (Midwest Plan Service 1985). 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 acre weighs about 9.1×10^5 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 49.4 ppb ((2.5 mg/kg x 1.8×10^4 kg/acre) ÷ (9.1×10^5 kg of soil/acre)).

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. While movement from feedlots is less likely, because of containment systems, it is still possible that movement into aquatic systems could occur. 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 feedlot cattle was excreted as parent material, did not dissipate, and could be extracted from the excreta into the water of one two-inch runoff event, the highest theoretical concentration of ractopamine hydrochloride would be 14.5 ppm ((13.7 g/animal/200 ft^2) (28.32 L/ft^3 x 200 ft^2 x 2 in x 1 ft/12 in)). Assuming ractopamine hydrochloride does dissipate from excreta, the highest expected concentration in runoff from a feedlot would be 4.2 ppm (4 g ractopamine hydrochloride in a 200 ft^2 area). Well designed feedlots have catchment systems for this runoff. In some cases, however, 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 49.4 ppb. 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 cattle excreta to be extracted into runoff from one rainfall event, a two-inch runoff event would carry 45 g of ractopamine hydrochloride or 0.22 mg/L

((1.8 x 10^4 kg/acre x 2.5 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 (K_d) would limit the amount of residue extracted from the cropland soil. The lowest K_d found for ractopamine hydrochloride in soil was 14.5, which is the equilibrium ratio of the concentration of ractopamine in soil compared to the concentration in aqueous solution (Appendix L). 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 0.015 ppm (0.07 x 45 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.

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 J). Ractopamine hydrochloride does biodegrade (Appendix K) and has an initial dissipation half-life of 1.1 days in soil (Appendix N).

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 non-target 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 (0.015 ppm), the highest concentration of ractopamine hydrochloride in fish tissue would be 0.09 ppm (6.3 x 0.015 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-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.

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 Year Rat Oncogenic Study: Rats were administered ractopamine hydrochloride in the diet for 2 years. Male rats were administered daily dietary doses of approximately 0, 2, 60, or 200 mg/kg and females 0, 2, 60, 200, or 400 mg/kg. Survival was significantly increased at 200 mg/kg for males and 400 mg/kg for females. Survival in males was 23%, 30%, 18%, and 68% at doses of 0, 2, 60, and 200 mg/kg, respectively. For females,

survival frequencies were 52%, 63%, 75%, 63%, and 78% at doses of 0, 2, 60, 200,and 400 mg/kg. Increased survival was associated with decreased body weights at 1 year. Fewer deaths from chronic progressive nephropathy in males and from neoplasms in males and females accounted for most of the increased survival. No adverse compound-related clinical observations were noted at any dose. The only tumor with increased incidence was leiomyoma of the costo-uterine ligament in females. This was an expected pharmacologic effect associated with prolonged excessive stimulation by β-adrenergic agonists. Other β-adrenergic agonist effects included an increased incidence of slight to moderate cardiomyopathy with fibrosis and mucous cell hypertrophy of the sub-mandibular salivary gland at the higher doses. The no-observed-adverse effect dose for leiomyoma of the costo-uterine ligament was 60 mg/kg.

Two Year Mouse Oncogenic Study: Mice were administered ractopamine hydrochloride in the diet for 21 months. Male mice were administered daily dietary doses of approximately 0, 25, 132, or 843 mg/kg and females 0, 34, 175, or 1086 mg/kg. Survival frequencies in males were 62%, 75%, 63%, and 25% at doses of 0, 25, 132, and 843 mg/kg, respectively. For females, survival frequencies were 72%, 58%, 63%, and 32%, at doses of 0, 34, 175, and 1086 mg/kg, respectively. The highest dose for both males and females exceeded the maximum tolerated dose and mortality was attributed to cardiomyopathy. No adverse compound-related clinical observations were noted at any dose. The only tumor with increased incidence was leiomyoma of the uterus in females at the two upper doses. This was an expected pharmacologic effect associated with prolonged excessive stimulation by β-adrenergic agonists. The no-observed-adverse effect dose for leiomyoma of the uterus was 34 mg/kg.

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 ≤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 (night time 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 RACTOPAMINE HYDROCHLORIDE PREMIX

The ractopamine hydrochloride premix label will instruct people to routinely wear protective clothing, impervious gloves, protective eyewear, and a NIOSH-approved dust mask when mixing and handling the product. The time for greatest potential exposure to ractopamine hydrochloride would be during the weighing of the premix at feed mills, but this only requires a few minutes. An average concentration of 0.0234 mg/m^3 has 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 at or below 0.005 mg/m^3. Detectable concentrations would normally not be found around operations such as feed mixing and bagging. No special precautions will be recommended to handle treated feed in cattle feedlot operations. 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 in the feedmill and feedlot.

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 DRINKING WATER

Exposure of humans to biologically active amounts of ractopamine hydrochloride via the drinking water is not expected. As has already been shown (Section 7), surface water concentrations would be well below 15 ppb. This concentration is well below any level that would result in effects in people. The proposed action is not expected to adversely affect human health through the drinking water.

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 O): 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 ≥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 P): 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 Q): 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 R): 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 ≤48.2 ppm.

Bluegill 96-Hour Toxicity Study (Appendix S): 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 T): 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 U): 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 EC_{50} 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 V): 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 EC_{50} value of 1413 ppm was estimated based on linear regression analysis.

Terrestrial Species

Earthworm 28-Day Toxicity Studies (Appendix W): 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 X and Y): 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 ≤7.5 ppm.

Seedling Growth (Appendix Z): 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 ≤100 ppm.

Antimicrobial Activity (Appendix AA): 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 ≤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 cattle 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. The highest expected concentration of ractopamine hydrochloride extracted into runoff water from a feedlot is 4.2 ppm. The maximum expected concentration of ractopamine hydrochloride in runoff from cropland soil is 0.015 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 623 times higher than the maximum expected concentration of ractopamine hydrochloride in runoff water from cropland soil. The maximum expected concentration of ractopamine hydrochloride in runoff from a feedlot (4.2 ppm) is lower than the acute no-effect concentration for fish, daphnids, and algae. 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 cattle manure and cropland soil are 2.5 ppm and 49.4 ppb, respectively. Earthworm survival, appearance, and growth are not significantly affected when worms are exposed to a ractopamine hydrochloride concentration of 8.1 ppm in soil. Earthworm survival, appearance, and growth would not be affected if worms were exposed to cropland soil or piles of manure containing the highest expected concentration of ractopamine hydrochloride. The use of ractopamine hydrochloride premix in the feed of feedlot cattle 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 ≤44 ppm. The recommended use rates of ractopamine hydrochloride in cattle feed would result in a maximum dietary level of 30 ppm. Even if wild birds were allowed to forage in cattle 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 152 times higher than the highest expected concentration of ractopamine hydrochloride in cropland soil, 49.4 ppb. Seedlings of all species germinated and grew normally when irrigated with nutrient media with ≤100 ppm ractopamine hydrochloride. This concentration is 2,198 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 15 times higher than the highest expected concentration of ractopamine hydrochloride in runoff

None of the gram-negative or gram-positive microbes tested were inhibited by concentrations of ractopamine hydrochloride ≤64 ppm. This concentration is about 15 times higher than the highest expected concentration of ractopamine hydrochloride in runoff (4.2 ppm) from a feedlot and about 4,267 times higher than the maximum expected concentration of ractopamine hydrochloride in runoff (0.015 mg/L) from cropland soil. These aqueous concentrations are at least 69 times lower than 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 ppm and 49.4 ppb. These concentrations are at least six 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. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. Protective measures recommended for feedmill operators are described in Section 8B1. The premix label will instruct people to routinely wear protective clothing, impervious gloves, protective eyewear, and a NIOSH-approved dust mask when mixing and handling ractopamine hydrochloride premix, and to wash thoroughly after handling the product. A material safety data sheet for ractopamine hydrochloride is available.

10. 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.

11. LIST OF PREPARERS

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

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12. CERTIFICATION

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

Dennis M. Hoover, DVM, PhD
Director, Pathology & Toxicology Studies

2 December 1998
Date

13. 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: 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.

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 D: 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.

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.

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 $\mu g/m^3$. 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.

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 post exposure. 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 (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.

Conclusions: Exposure monitoring of the current formulation of 10% 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 μμg/m^3 and for the 15-minute exposure, 240 μg/m^3. The average breathing zone concentration of the balance operator during the weighing operation from a series of exposure monitoring studies was approximately 23.4μg/m^3 for a 1-minute period. The mean breathing zone concentration for the 15-minute weighing operation was 17.6 μg/m^3 (N=3).

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:

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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 β_1-Adrenergic Receptor Agonist. J. Lab. Clin. Med., June 1983.

Appendix E: 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. 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 µg/m^3 (the LSTEG) for a 15 minute sample at a sampling rate of 20 l/min., and approximately 80 µg/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 µg/m^3 to 0.155 µg/m^3), with the exception of one sample with a detectable concentration of 0.182 µg/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 µg/m^3, a maximum of 1.523 µg/m^3, a geometric mean of 0.924 µg/m^3, and a geometric SD of 1.953.

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 ($\mu g/m^3$)	Applicable Exposure Guideline ($\mu g/m^3$)
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 $\mu g/m^3$) were below the limit of detection (0.105 - 0.155$\mu g/m^3$).

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 $\mu g/m^3$). 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 $\mu g/m^3$ to 1.155 $\mu g/m^3$, with a geometric mean of 0.802 $\mu g/m^3$ 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 $\mu g/m^3$). Thirty area samples taken during medicated feed bagging yielded non-detectable concentrations (less than 0.433 $\mu g/m^3$).

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 $\mu g/m^3$. 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 $\mu g/m^3$.

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.

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/m³)**	**Exposure Guideline (µg/m³)**
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

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

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

Task	Location*	Result ($\mu g/m^3$)
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 F: Report Summary

Title: Comparative metabolism of ^{14}C Ractopamine HCl in Cattle, Dogs, and Rats

Study Number: ABC-0387

Study Dates: June 15, 1987 to December 6, 1989

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: Cattle

Summary of Experimental Design: Liver, kidneys, and excreta from cattle 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 was a 50:50 mixture of uniform ^{14}C-label on ring A and ring B. Parent material and metabolic products were identified and quantitated by high-performance liquid chromatography, radioactivity, thin-layer chromatography, and fast atom bombardment mass spectroscopy. .

Summary of Results:

Cattle urine was extracted with diethyl ether. 58% of the radioactivity was partitioned into this phase. HPLC demonstrated that 84.2% of this radioactivity was ractopamine. The aqueous phase was further processed. Three glucuronide metabolites (A, B, and C) were identified from 19.1% to 43.4% of the radioactivity in the aqueous phase. Fecal residues were subjected to the same extraction as above. Seventy three percent of the radioactivity was extracted into the ether phase. The majority of this was ractopamine. The aqueous phase was not further characterized.

Appendix G: Report Summary

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

Study Number: ABC-0422

Study Dates: August 3, 1988to May 19, 1989

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

Test Article: ^{14}C -Ractopamine hydrochloride

Test System: Cattle

Summary of Experimental Design: Two crossbred steers were each fed twice daily 6.5 lb of feed containing 30 ppm of unlabeled ractopamine hydrochloride for 8 days. At the end of the predosing period, each steer received a one-time dose of 0.67 mg/kg of ^{14}C -ractopamine hydrochloride by gavage. The steers then continued to receive 6.5 lb of ration containing 30 ppm of unlabeled ractopamine hydrochlordie twice daily until termination of the experiment. The entire urinary and fecal output of each animal was collected at 24-hour intervals for 10 days.

Summary of Results: During the 10-day collection period, the two animals excreted an average of 97.8% of the theoretical dose of ^{14}C -ractopamine hydrochloride administered. A total of 45.6% of the recovered ^{14}C -ractopamine hydrochloride was found in urine and 52.3% was in feces. The bulk of the ^{14}C -ractopamine dose (92.5%) was excreted in the first 4 days.

Appendix H: 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 I: 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 J: 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 K: 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 L: 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.

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].

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 M: 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 N: 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.

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 0: 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 ≥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.

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) Test Day			Mean Body Weight Gain ± SD (g) Treatment Phase (5 Days)	Basal Diet Phase (3 Days)
		Initial	5	8		
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 ±0.6	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 ($p \leq 0.05$).

[a] N = 9 birds

[b] N = 7 birds

Appendix P: 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).

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 Q: 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 ≥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.

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) Test Day			Mean Body Weight Gain ± SD (g) Treatment Phase (5 Days)	Basal Diet Phase (3 Days)
		Initial	5	8		
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 ($p \leq 0.05$).

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		Basal Diet Phase	
		(5 Days)	% of Control	(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 R: 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 ≥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 ≤48.2 ppm.

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/L)	Individual Fish Condition/Behavior Value at 24 Hr[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 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.

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/L)	Individual Fish Condition/Behavior Value at 48 Hr[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 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.

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/L)	Individual Fish Condition/Behavior Value at 72 Hr[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 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.

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/L)	Individual Fish Condition/Behavior Value at 96 Hr [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 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.

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 anayltical detection limit was 1.0 mg/L.

Appendix S: 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 ≥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 ≤191 ppm.

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/L)	Individual Fish Condition/Behavior Value at 24 Hr[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 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.

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/L)	Individual Fish Condition/Behavior Value at 48 Hr [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 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.

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/L)	Individual Fish Condition/Behavior Value at 72 Hr[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 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.

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/L)	Individual Fish Condition/Behavior Value at 96 Hr [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 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.

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 anayltical detection limit was 1.0 mg/L.

Appendix T: 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.

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				48 Hr			
		N	H	P	I	N	H	P	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.

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 U: 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), EC_{50} value for growth rate, and the EC_{50} 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 EC_{50} values for μ-reg and growth of cell populations (AUC) were greater than 101.2 mg/L.

Appendix V: 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 EC_{50} 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 EC_{50} for ractopamine hydrochloride was estimated by linear regression analysis to be 1413 mg/L.

Appendix W: 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.

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.

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

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		
	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.

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

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		
	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.

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.

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.

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, these 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.

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, these 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.

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.

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		Day28	
	Body Weight	Body Weight	% Gain	Body Weight	% Gain	Body Weight	% Gain	Body Weight	% Gain
0.0 (Control)	4.4115 ±0.0648	5.2675 ±0.2069	19.4 ±4.1	5.3928 ±0.2455	22.2 ±4.5	5.4865 ±0.2189	24.4 ±4.7	5.6419 ±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.

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%

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

Organic Matter	2.1%
Cation Exhange 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 X: 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 Y: 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 approimately 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 Z: 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 ≤100 ppm of ractopamine hydrochloride resulted in no effects on the growth of seedling barley, corn, cucumber, soybean, turnip, or wheat plants.

Appendix AA: 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 ≤64 ppm.

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

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