Experior™
(lubabegron Type A medicated article)

Environmental Assessment for the use of Experior™ (lubabegron Type A medicated article) for reduction of ammonia gas emissions per pound of live weight and hot carcass weight in beef steers and heifers fed in confinement for slaughter during the last 14 to 91 days on feed

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1.0 Purpose and Need for Action

Experior™ is a Type A medicated article. Lubabegron (as lubabegron fumarate) is the active ingredient in Experior™. The following environmental risk assessment is provided to support approval of a New Animal Drug Application for the use of Experior™ (lubabegron Type A medicated article) for reduction of ammonia gas emissions per pound of live weight and hot carcass weight in beef steers and heifers fed in confinement for slaughter during the last 14 to 91 days on feed. The maximum dose is 4.54 g/ton (90% dry matter basis) in complete Type C medicated feed for a maximum of 91 days. Livestock are the largest known source of ammonia gas emissions in the United States and use of Experior™ is intended to reduce these emissions.

2.0 Scope of the Environmental Assessment

Experior™ will be administered to beef steers and heifers fed in confinement for slaughter via complete Type C medicated feed at doses between 1.25 and 4.54 g/ton (90% dry matter basis) or 1.4 to 5 g lubabegron/ton on a 100% dry matter basis (1.5 mg lubabegron /kg feed to 5.5 mg lubabegron /kg feed; 100 % dry matter basis) for a minimum of 14 days up to a maximum of 91 days prior to slaughter.

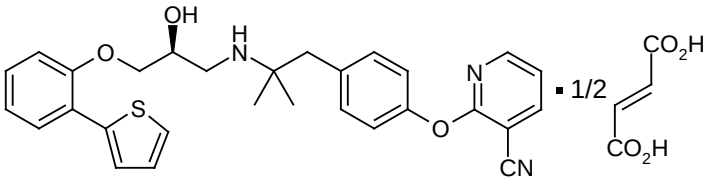
The environmental assessment includes two types of analysis for the use of Experior™. The first addresses the introduction and effects of lubabegron in terrestrial and aquatic systems. The second addresses the potential effects from the intended use of Experior™ to reduce ammonia gas emissions (see Sections 4.0 and 5.0). When applicable, this environmental risk assessment has been conducted based on the VICH guidelines for both Phase I (VICH GL6) and Phase II (VICH GL38) assessments.

The primary route of environmental exposure to lubabegron will be from cattle manure removed from animal pens with subsequent application to agricultural land as fertilizer. While unlikely, any possible spillage and breakage of bags of Experior™ or medicated feed might also be applied with manure to agricultural land. However, any additional environmental exposure as a result of breakage and/or spillage is expected to be insignificant compared to that following administration to cattle.

As a result of the use of Experior™, other environmental impacts may occur due to changes in ammonia and other nitrogen compound concentrations in both the air and in water [e.g, NH_4^+, NO_x, particulate matter ≤ 2.5 µm in diameter ($PM_{2.5}$)] (see Section 6.0).

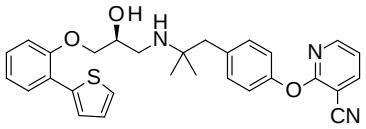
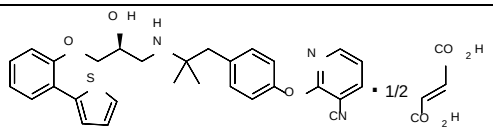
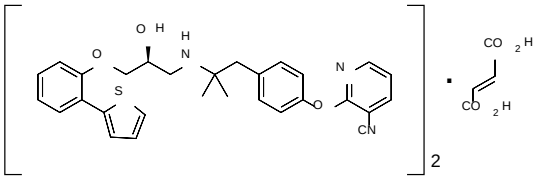
3.0 Description of the Product

The active ingredient in Experior™ is lubabegron (as lubabegron fumarate). Lubabegron is characterized as a beta-adrenergic agonist/antagonist. Lubabegron binds to all three beta-adrenergic receptors. Activity of lubabegron is through its beta-3 adrenergic receptor agonist activity *in vivo*, but blocks activity at the beta-1 and beta-2 receptors. The company identifiers of lubabegron (freebase) as LY488756 and lubabegron hemifumarate (salt) as LSN591281 were used to identify the compound in the final study reports and summaries provided in this environmental assessment.

International Non-proprietary Name (INN):	Lubabegron
Non-Proprietary Name (USAN):	Lubabegron fumarate
Chemical Name:	2-[4-[2-[[(2S)-2-hydroxy-3-[2-(2-thienyl)phenoxy]propyl]amino]-2-methylpropyl]phenoxy-3-pyridinecarbonitrile, (2E)-2-butenedioate (2:1) salt
Synonyms:	Lubabegron
Chemical Abstracts Service (CAS) Number:	391926-19-5 [as lubabegron fumarate or $(C_{29}H_{29}N_3O_3S)_2 \cdot C_4H_4O_4$]
Lilly Compound Number:	LY488756 hemifumarate
Lilly Serial Number:	591281 (as the hemifumarate salt)
Lilly Serial Number:	488756 (freebase)
Molecular Formula:	$(C_{29}H_{29}N_3O_3S)_2 \cdot C_4H_4O_4$
Molecular Weight:	1115.3 (as lubabegron fumarate) 557.7 (as lubabegron hemifumarate LSN591281) 499.6 (as lubabegron freebase LY488756)
Structural Formula (LSN591281):	

Note: In aqueous solutions containing lubabegron such as those encountered in the environment, the free base molecule $C_{29}H_{29}N_3O_3S$ would be the chemical entity dissolved in solution unassociated with the fumaric acid salt counterion. Although lubabegron is referred to as lubabegron hemifumarate, new CAS conventions no longer use the ½ designation for the hemifumarate and therefore express the salt as the fumarate equivalent molecule (i.e, not as X •½A but rather as X_2•A; where X is lubabegron and A is fumarate).

Table 3-1. Lubabegron Nomenclature, Structure, Chemical Formula, and Molecular Weight

Compound Name/ Number	Chemical Structure	Chemical Formula	MW (g/mol)
Lubabegron LY488756 (freebase)		$C_{29}H_{31}N_3O_4S$	499.6
Lubabegron hemifumarate LSN591281		$C_{29}H_{31}N_3O_4S \bullet ½(C_4H_4O_4)$	557.7
Lubabegron fumarate CAS 391926-19-5		$2(C_{29}H_{31}N_3O_4S) \bullet C_4H_4O_4$	1115.3

Lubabegron fumarate is a crystalline salt compound consisting of two equivalents of the active pharmaceutical ingredient freebase lubabegron and one equivalent of fumaric acid. The Chemical Abstracts Service (CAS) reference for lubabegron is as lubabegron fumarate with a chemical structure for the salt as $(C_{29}H_{29}N_3O_3S)_2 \cdot C_4H_4O_4$ and a molecular weight of 1115.32. Earlier in the development of this product, lubabegron was referenced as the hemifumarate salt or $C_{29}H_{29}N_3O_3S \cdot ½\, C_4H_4O_4$. In this environmental assessment, the name "lubabegron" refers to the freebase entity and where necessary as lubabegron fumarate or lubabegron hemifumarate to distinguish the chemical form as the salt. Lubabegron as LY488756 (freebase) is the active moiety in LSN591281 (the hemifumarate salt of LY488756).

Potency refers to the amount of freebase present in the material and reflects both the inactive salt (fumaric acid or fumarate) and impurities in the material. Purity refers to the chemical purity of the salt (LSN591281). For example, 100.00% pure LSN591281, with the assumption that it consists of exactly one equivalent of lubabegron and one half of an equivalent of fumaric acid (or two equivalents of lubabegron and one equivalent of fumaric acid), has a potency of 89.59% as lubabegron. Therefore, the GLP Test Article Characterized (TAC) material (lot 160SB1) used for selected physical/chemical properties and fate studies had a purity of 98.9% as lubabegron hemifumarate and a potency of 88.6% as lubabegron. Table 3-2 below indicates the chemical purity of lubabegron hemifumarate salt and the corresponding potency as lubabegron. The TAC material used for the GLP studies represents the product that will be administered to animals in the approved product.

Table 3-2. Purity and Potency Equivalency Table for Lubabegron

Purity as salt LSN591281	Potency as freebase lubabegron (LY488756)
96.8	86.7
98.9	88.6
99.0	88.7
100.0	89.6

MW of LSN591281 = 557.67 (formula: $C_{29}H_{29}N_3O_3S \cdot ½ (C_4H_4O_4)$).
MW of LY488756 = 499.63 (formula: $C_{29}H_{29}N_3O_3S$)

Lubabegron has a single chiral center. The absolute configuration of this center could not be established by single crystal X-ray diffraction structure determination; therefore, the assignment was based on synthesis from an intermediate with a known stereochemical configuration ((S) – isomer). The chiral center of the molecule is not prone to racemization and remains unchanged throughout the remainder of the synthesis. Therefore, lubabegron exists in a single stereochemical orientation as a single enantiomer.

4.0 Phase I Environmental Impact Assessment

Final Guidance for Industry #89 (CVM, 2001) published by the FDA, Center for Veterinary Medicine, and the VICH GL6 Phase I guidance for Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) were consulted to conduct a Phase I Environmental Impact Assessment for the use of Experior™ in beef steers and heifers fed in confinement for slaughter. In this Phase I assessment, the maximum concentration of lubabegron in the manure and the soil has been calculated. A total residue approach was taken for the Phase I assessment and no metabolism or degradation in manure is assumed. The initiation of a Phase II assessment is dependent upon the trigger value established in the VICH GL6 guidance: if the predicted environmental concentration of the total residue in soil is greater than 100 µg/kg, a Phase II assessment is warranted.

4.1 Calculation of Predicted Environmental Concentration

4.1.1 Calculation of concentration in manure

To estimate the concentration of lubabegron in cattle manure, it was assumed that cattle were fed 4.54 g/ton lubabegron (90% dry matter basis) continuously for 91 days prior to slaughter corresponding to the proposed label maximum use rate and duration. The maximum label dose is 90 mg lubabegron/hd/d, which is based on the expected daily feed intake of the highest weight animals expected to be fed the drug and the maximum concentration of lubabegron in feed. The concentration of lubabegron in manure was determined for both the 4.54 g/ton (90% dry matter basis) feed rate and the maximum label dose of 90 mg/hd/d.

Other assumptions used to calculate a manure concentration are listed in Table 4-1.

Table 4-1. Assumptions used to Calculate Lubabegron Concentration in Cattle Manure

Dosing Duration:	91 days
Days on Feedlot:	130 days[a]
Body Weight (BW):	603 kg[b]
Maximum Concentration in Feed (90% dry matter basis):	4.54 g/ton (5.0 mg/kg)
Maximum Concentration in Feed (100% dry matter basis):	5 g/ton (5.5 mg/kg)
Maximum Daily lubabegron dose (from label):	90 mg/hd/d
Average Daily Gain (ADG) :	1.46 kg/hd/d[c]
Dry matter Intake (DMI) :	10.4 kg/hd/d[d]
Daily Feed Intake:	14.4 kg_{feed}/hd[d]
Daily Manure Production:	27.3 kg_{manure}/hd[a]

[a] Value is consistent with values typically used in environmental risk assessments.

[b] Average body weight (BW) of finished beef cattle in the USA for 2014 (USDA Livestock Slaughter 2014 Summary April 2015, page 37).

[c] Value from Table 18 for 1300 lb finishing animal (NRC Update 2000, page 213).

[d] Calculated according to NRC equation 7-2 (NRC Update 2000, page 90) for predicting dry-matter intake of finishing beef cattle from initial body weight. Assumptions: initial weight = 470 kg/hd; dry-matter = 72%; iBW = 603 kg/hd – (91d X 1.46 kg/hd/d) = 470 kg/hd; DMI = 4.54 + 0.0125 X iBW (kg/hd) = 4.54 + (0.0125 X 470 kg/hd) = 10.4 kg/hd/d; at 72% DM, Daily Feed Intake = 10.4 kg/hd/d /0.72 = 14.4 kg/d.

Based on the 4.54 g/ton (90% dry matter basis) feed rate, the concentration of total lubabegron residues (e.g. lubabegron plus any metabolites) in manure was calculated as (PEC_{manure}):

$$[LY488756]_{manure} = \frac{Concentration\ in\ Feed \times Daily\ Feed\ Intake}{Daily\ Manure\ Production} \times \frac{Dosing\ Duration}{Days\ on\ Feedlot}$$

$$[LY488756]_{manure} = \frac{(5.5\,mg)/\ 〚kg〛\_feed\ \ X\ \ (14.4\ 〚kg〛\_feed)/hd}{\frac{27.3 kg_{manure}}{hd}} \times \frac{91d}{130d} = 2.0\,mg/kg$$

In contrast, when considering the maximum daily lubabegron dose fed on a mg/hd/d basis, the concentration of total lubabegron residues (e.g. lubabegron plus any metabolites) in manure was calculated as (PEC_{manure}):

$$[LY488756]_{manure} = \frac{Maximum\ Daily\ lubabegron\ Intake}{Daily\ Manure\ Production} \times \frac{Dosing\ Duration}{Days\ on\ Feedlot}$$

$$[LY488756]_{manure} = \frac{90\,mg/hd/d}{\frac{27.3 kg_{manure}}{hd}} \times \frac{91d}{130d} = 2.3\,mg/kg$$

The maximum calculated lubabegron concentration in manure from lubabegron fed animals is 2.3 mg/kg based on these two scenarios. Therefore, the larger value of 2.3 mg lubabegron/kg manure was carried forward throughout this assessment. This value includes further adjustment to reflect the concentration of lubabegron in the manure from cattle pens that would be land

applied by using the dosing duration and the manure production period (average days on the feedlot) for treated animals.

4.1.2 Calculation of concentration in soil

The maximum concentration of lubabegron in the soil has been calculated using commonly used agronomic and manure management practices for application of cattle manure to agricultural land as fertilizer for crops.

Manure from cattle in intensive confined feed systems is typically collected, stored and composted. It is eventually land applied as a solid and incorporated in soil using plowing or tilling methods at a maximum application rate of 27,200 kg manure/acre. Manure is incorporated into soil to reduce nutrient loss, control odor, and reduce runoff. An incorporation depth of 15 cm is appropriate for conventional methods. Assuming an incorporation depth of 15 cm and an average bulk density of soil of 1500 kg/m^3, the mass of soil in one acre into which the manure is mixed is approximately 910,500 kg:

$$Weight\ of\ Soil = 1\ acre\ \times 4047\frac{m^2}{acre} \times 0.15\ m \times 1500\ kg/m^3$$

$$\approx 910{,}500\ kg$$

Table 4-2. Assumptions used to Calculate Lubabegron Concentration in Soil

Application Rate of Cattle Manure to Soil:	27,200 kg/acre[a]
Plow Depth:	15 cm[a]
Average Bulk Density of Soil:	1500 kg/m^3

[a] These are traditionally used values in environmental assessments and reflect typical agricultural practices.

PEC_{soil} =

$$[lubabegron]_{Soil} = \frac{Concentration\ in\ Manure\ \times Application\ Rate\ of\ Manure\ to\ Soil}{Weight\ of\ Soil/acre}$$

$$= \frac{2.3\ mg/kg \times 27200 kg/acre}{910{,}500 kg/acre} = 0.069\frac{mg}{kg} = 69\ \mu g/kg$$

Assuming no degradation in manure or soil, the concentration of lubabegron in soil after application and incorporation of cattle manure from treated animals could be as high as 69 µg/kg. Although this value is less than the trigger value of 100 µg/kg, a Phase II environmental risk assessment was conducted to support Experior™ according to the VICH GL6 Final Guidance.

5.0 Phase II Environmental Impact Assessment

Final Guidance for Industry #166 (CVM, 2006) published by the FDA, Center for Veterinary Medicine, and the VICH GL38 Phase II guidance for Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) were consulted to conduct a Phase II

Environmental Impact Assessment for the use of Experior™ in beef steers and heifers fed in confinement for slaughter. In this Phase II assessment, data reflecting the physical/chemical properties, environmental fate and environmental effects of lubabegron were used to assess the environmental risk for the use of lubabegron. Phase II progresses as two tiers; in the first (Tier A), a basic set of less complex studies is evaluated and is used to prepare a conservative risk assessment. If that risk assessment cannot rule out the possibility of a risk to the environment, more complex studies are conducted and evaluated in Tier B. In the environmental risk assessment presented here, Tier A and select Tier B data (NOEC for *Daphnia magna* reproduction and NOEC for algal growth inhibition were determined) are provided and used in this assessment.

5.1 Summary of Available Data

This section reviews the environmental chemistry, fate, and effects data that have been collected for lubabegron to support this assessment.

5.1.1 Physical and Chemical Properties

Selected physical and chemical properties of lubabegron relevant to environmental exposure are listed in Table 5-1.

The dissociation constant (pK_a) was determined using potentiometric titration of lubabegron in aqueous media. The limited aqueous solubility of lubabegron necessitates the use of a co-solvent in order to provide enough material in solution and therefore provide an inflection point in the curve of the potentiometric titration and correspondingly a pK_a value.

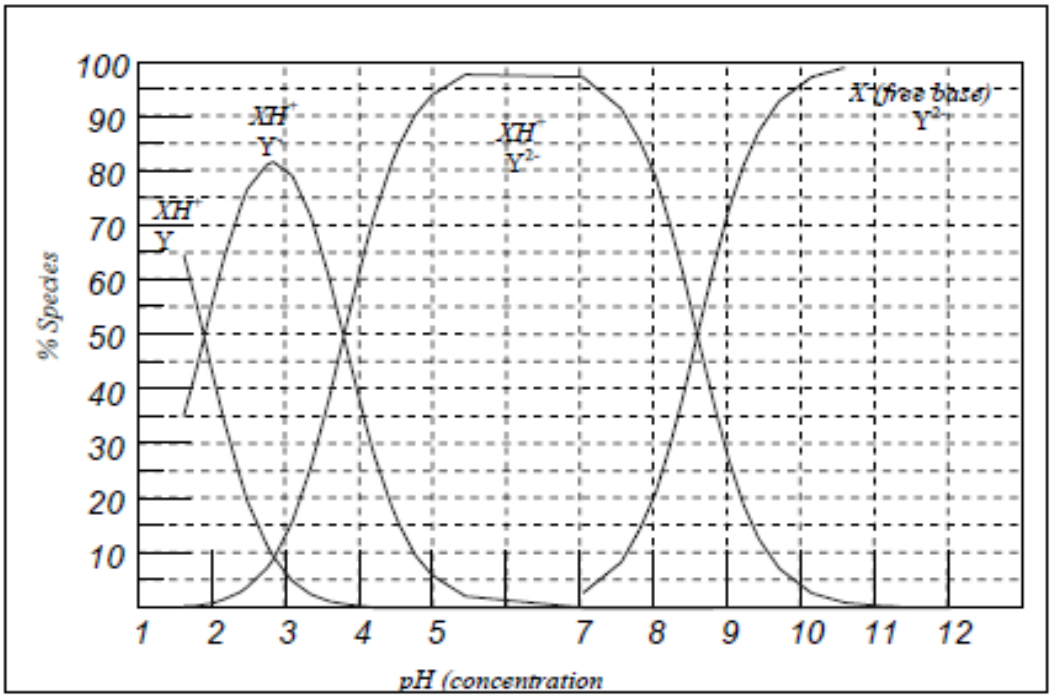
During the pilot work for the pK_a determination (Study No. 14028.6105, Appendix A), various solvents and concentrations were used to investigate appropriate titration conditions to perform the test. During the initial titration pilot study work, lubabegron precipitated from solution (formed a cloudy suspension) as the pH was increased from acidic to alkaline conditions in the titration vessel. Due to the limited water solubility of lubabegron throughout the titration range of pH, it was not possible to determine the pK_a in pure aqueous solution without using sufficient co-solvent to enhance the solubility of the test article in the test system. Sufficient test article must also be soluble in the aqueous medium to exhibit a potentiometric response using a pH probe and therefore determine a titration curve (observation of the inflection point of the curve and/or peak of the first derivative curve). The difficulty of determining pK_a values in titrations of aqueous solutions is a known problem for compounds with limited aqueous solubility under the test conditions. The use of DMF as a co-solvent for the pK_a determination of lubabegron (Study 14028.6105) was required to provide the necessary system conditions to perform the test. The pK_a for the secondary amine of lubabegron in this system was reported as 8.68.

Elanco also has product development chemistry data from a Confirmation of Structure of LY488756 Hemifumarate report (Eli Lilly and Company, 2002) which corroborates the secondary amine pK_a for lubabegron as 8.6. There are discrepancies for the reported pK_a's for the fumaric acid moiety (fumaric acid; a dicarboxylic acid) between the two systems (50:50 DMF:water in Study 14028.6105 and 26-45% dioxane in water in Eli Lilly and Company, 2002), presumably from cosolvent effects.

Based on the available data, the pK_a for lubabegron is appropriately referenced as approximately 8.6 to 8.7. A further description of the acid/base chemistry of lubabegron relative to solution pH, pK_a, and chemical species is presented below.

Based on the chemical moieties and acid/base chemistry of salts and ions, the transition of lubabegron hemifumarate in aqueous media expressed by chemical species as a function of solution pH is represented in the Figure 1 below. The figure is from the Confirmation of Structure of LY488756 Hemifumarate report (Eli Lilly and Company, 2002) and indicates that above the pK_a of the secondary amine (8.6) the proton (H^+) associated with the secondary amine of lubabegron is removed. In general, 2 pH units above or below the pK_a of an acid/base active moiety, the H^+ is considered either completely on or off the chemical moiety. For example, if the pK_a for the secondary amine of lubabegron is 8.6, at solution pH 6.6 the molecule would exist as an ion H^+[lubabegron] and at pH 10.6 it would be a neutral molecule as [lubabegron]. By definition, at the pH of the pK_a the chemical species exists 50:50 as H^+[lubabegron]: [lubabegron]. In dilute aqueous solution, such as those encountered in the natural environment; the association of the counter-ion (fumarate) on lubabegron solubility is negligible.

Figure 1. Distribution of Species for Lubabegron Hemifumarate (591281) where X = Lubabegron and Y = Fumaric Acid.



The aqueous solubility of lubabegron is pH-dependent with greater aqueous solubility at lower solution pH values. The aqueous solubility values for lubabegron were measured to be 56.2, 6.03, and 0.532 mg lubabegron/L at pH 4, 7, and 9, respectively (Study No. 14028.6104, 2014, Appendix B).

The n-octanol-water partition coefficient for lubabegron is also pH dependent. The logarithm of the n-octanol-water partition coefficient (Log K_{ow} or Log P_{ow}) was measured to be 1.38, 3.60, and 4.93 at pH 4, 7, and 9, respectively (Study No. 14028.6110, 2014, Appendix C).

The octanol-water partition coefficient for lubabegron is inversely proportional to its measured water solubility. As expected, the octanol-water partition coefficient is greatest at pH 9 where the compound exhibits the lowest aqueous solubility of the solution pH values investigated.

Table 5-1. Selected Physical and Chemical Properties of Lubabegron

Study	Results
Melting Point, Study No. 14028.6107 2014, (Appendix D)	175.44°C (standard deviation = 0.026°C)
Dissociation Constant, Study No. 14028.6105 2014, (Appendix A)	5.46 (acid titration) 7.58, 8.68 (base titration) The pK_a of the secondary amine is 8.68 in this system
Physical and Structural Characterization, Eli Lilly and Company, 2002	The pK_a of secondary amine of lubabegron is 8.6 in this system
Aqueous Solubility at 20°C, Study No 14028.6104 2014 (Appendix B)	pH: 4 — Mean Solubility (mg/l): 56.2 pH: 7 — Mean Solubility (mg/l): 6.03 pH: 9 — Mean Solubility (mg/l): 0.532
Vapor Pressure, Study No. 14028.6108 2014, (Appendix E)	at 20°C 10.1 Pa (7.59 X 10^{-2} mmHg) at 25°C 12.2 Pa (9.13 X 10^{-2} mmHg)
n-Octanol/Water Partition Coefficient at 25°C, Study No. 14028.6110 2014, (Appendix C)	pH: 4 — log P_{ow}: 1.38 pH: 7 — log P_{ow}: 3.60 pH: 9 — log P_{ow}: 4.93
UV-Vis Absorption, Study No. 14028.6106 2013, (Appendix F)	**Sample Solution** / **Concentration (Mole/L)** / **Mean Wavelength (nm)** / **Mean Extinction Coefficient (L/mol-cm)** Methanol / 0.0000179 / 203 / 62,216 Methanol / 0.0000179 / 285 / 17,009 10% (1.0 M) HCl in methanol / 0.0000179 / 203 / 59,948 10% (1.0 M) HCl in methanol / 0.0000179 / 285 / 17,362 10% (1.0 M) NaOH in methanol / 0.0000179 / 218 / 41,694 10% (1.0 M) NaOH in methanol / 0.0000179 / 285 / 18,087

All studies in Table 5-1, except for the Physical and Structural Characterization, Eli Lilly and Company 2012, used TAC material lot 160SB1 with a purity of 98.9% as lubabegron hemifumarate and a potency of 88.6% as lubabegron.

5.1.2 Fate

The fate and metabolism of lubabegron in cattle and in the environment is described below. The environmental fate data collected with lubabegron is summarized in Table 5-2.

5.1.2.1 Metabolism and Excretion of Lubabegron in Beef Cattle

Following multiple-dose oral administration of radiolabeled lubabegron in beef cattle (Study No. 286652, Report 33878, 2014), 80% to 106% of the dosed radioactivity was excreted in the feces. Minimal ^{14}C-residues were excreted via the urine and accounted for *ca* 2.2-3.1% of the administered dose. No apparent differences in metabolism were noted between the sexes.

Radioactivity in urine samples was very low and thus precluded metabolite profiling. Initial thin-layer chromatography (TLC) results suggested that the urine samples contained a high degree of very polar material residues. Follow up hydrolysis experiments and subsequent TLC of urine extracts proved inconclusive with the radioactivity remaining bound to the origin of the TLC plate or spreading across the majority of the plate. These results indicate that a negligible fraction of the material excreted in urine was as lubabegron.

In beef cattle fecal samples, 58.1% to 70.6% of the total radioactivity recovered (TRR) was extractable. Following concentration, the final extracts contained 42.0% to 43.6% of the TRR. The major peak detected in the feces was unchanged lubabegron accounting for *ca* 4-6% of the administered dose. The most abundant detected metabolite accounted for no greater than 1.3% of the total dose administered. Using the minimum value for percent recovery of TRR in the final feces extract (42%) and the maximum dose represented (of the total administered dose, 6%) yields a maximum of 14.3% of the applied dose as lubabegron excreted in feces.

Extractability of labelled residues from tissue samples was low in all liver and kidney samples suggesting a high degree of protein binding in these samples. Protease digestion experiments confirmed that a high proportion of the radioactivity could be liberated. However, the low level of tissue residues and low extractability yielded results below the limit of reliable radiochemical detection and thus precluded metabolite profiling.

In an *in vitro* comparative metabolism study of [^{14}C]-lubabegron in cattle, swine, rat, dog and human hepatocytes and microsomes (Study No. 196171, Report 34300, 2014), two polar metabolites were detected and/or quantified as common to all species tested. A total of six metabolites were detected for all species. All metabolites were derived from oxidative biotransformation and only quantitative differences in relative abundance of individual metabolites were noted across species. Significant lubabegron binding to hepatic proteins and exogenous protein (BSA) added to incubations with hepatocytes was noted.

Taken together, these results indicate that lubabegron undergoes exhaustive biotransformation *in vivo* and is also subject to significant binding to hepatic and other endogenous proteins. Therefore, very limited amounts of lubabegron residues would be expected to be excreted into the environment.

Although there is evidence of extensive metabolism in vivo, these data will not be used to refine the mass of lubabegron that will be excreted and introduced into the environment in this assessment. When considering the actual amount of lubabegron that would be excreted from treated animals and ultimately introduced to the environment, the levels will likely be well below those presented in this environmental assessment.

5.1.2.2 Degradation

5.1.2.2.1 Aerobic Degradation in Soil

A laboratory study was conducted to determine the aerobic degradation of lubabegron in soil (Study No. 14028.6112, 2014, Appendix G). The study was designed to evaluate the kinetics of degradation and identify major degradation products if found in four different soils. The four soils varied in pH, textural characteristics, organic matter content and microbial mass content. Average material balance ranged from 93.0% to 94.6% of the applied radioactivity (AR) for all soils tested over the course of the 122-day study. Evolved $^{14}CO_2$ was trapped using KOH and soils were extracted and the resulting extracts profiled using HPLC with radiometric detection.

Soil was dosed with [^{14}C]lubabegron at approximately 1.00 µg/g in glass metabolism vessels containing 50.0 g (dry weight equivalent) of soil. Soils were incubated under aerobic conditions at 20 ± 2 ºC in the dark with soils adjusted to a moisture level of pF 2.0. Duplicate vessels were processed and soils were extracted at various time intervals (0, 3, 7, 14, 31, 60 and 122 days). Extracts were analyzed by high performance liquid chromatography with radiochemical detection (HPLC/RAM). Evolved volatiles were analyzed by liquid scintillation counting (LSC) while non-extractable radioactivity was quantified by combustion followed by liquid scintillation counting (LSC).

Ultimate degradation (mineralization) is indicated by the observed liberation of $^{14}CO_2$ (originating from [^{14}C]lubabegron) from over the course of the 122-day study. The cumulative amount of evolved $^{14}CO_2$ was 0.9%, 1.5%, 2.1% and 1.5% of AR in the DU, MSL, RMN and PD aerobic soil test systems, respectively. Radioactivity was below detection (<0.1% AR) in the ethylene glycol volatile organic traps for the aerobic test system through Day 122 of the study indicating that other volatile compounds as intermediates in the degradation of [^{14}C]lubabegron were not found.

One degradation product was observed at >10% of AR during the study at a retention time of approximately 12 minutes in the DU soil. Several minor regions of radioactivity were observed in the chromatograms for the three remaining soils (MSL, RMN and PD) including peaks at retention times of approximately 12 and 20 minutes. In all cases, other than the single exception for the DU soil as indicated above, these individual peaks represented less than 10% of AR and were not evaluated further.

The half-lives (DT_{50}) for degradation of lubabegron in soils were 289, 365, 533 and 866 days for the DU, MSL, RMN and PD soils, respectively. The half-life calculated for the DU soil includes the mass from the degradation product that was greater than 10% of the AR as being parent lubabegron (included at each time-point regardless of the %AR at that time-point).

In this study (14028.6112), the pH of the test soils did not vary substantially (pH range = 5.6 to 6.9). However, variation in pH is unlikely to change the observed degradation characteristics markedly since at environmentally relevant pH, lubabegron will not ionize or change form (the pK_a is approximately 8.6-8.7 [Section 5.1.1; study 14028.6105]). In addition, the experimentally derived degradation rates do not correlate well with pH (linear regression analysis of soil pH versus k (degradation rate); $R^2 = 0.5470$). Instead, the apparent degradation rate of lubabegron observed in the soil transformation study was proportional to the soil biomass and the percent

organic carbon in the soil (i.e., the higher the soil biomass and/or percent organic carbon, the greater the rate of degradation in the soil; $R^2 = 0.8600$ and 0.7501 for linear regression analysis of biomass or %OC versus k (degradation rate), respectively). Therefore, in this EA it is assumed that the degradation rate is invariant with respect to soil pH.

5.1.2.2.2 Degradation in Excreta or Compost

No studies have been conducted to evaluate the degradation of lubabegron in cattle excreta or manure compost.

5.1.2.3 Soil Adsorption

The adsorption of lubabegron to soil and its potential mobility in soil has been evaluated.

A batch equilibrium soil adsorption study was conducted in which [^{14}C]lubabegron in 0.01 M $CaCl_2$ was equilibrated with five different soils (sandy loam, loam, sandy clay loam, and clay) at a ratio of soil to aqueous phase of 1:100 for 24 hours (Study 14028.6111, 2014, Appendix H). The resulting K_d values ranged from 3051 mL/g to 3887 mL/g and K_{oc} values ranged from 47,347 mL/g to 370,420 mL/g for the soils tested. These results indicate significant binding of lubabegron to soils and correspondingly, preclude transport of lubabegron through soil to groundwater. Similarly, surface water concentrations of lubabegron would be greatly reduced where soil and sediments are present in aqueous systems.

Table 5-2. Environmental Fate and Chemistry of Lubabegron

Study	Results

Soil Adsorption/Desorption Study No. 14028.6111, 2014, (Appendix H)

Soil	Soil Taxonomy	%Organic Matter	%Organic Carbon	% Adsorbed
MSL	Sandy Clay Loam[a]	4.2	2.5	93.3
DU	Loam	13.5	7.9	93.7
PD	Sandy Loam	1.4	0.82	93.1
MT	Clay	2.0	1.2	93.6
ROE	Sandy Clay Loam	5.7	3.4	93.6

Soil	Soil Taxonomy	K_d (mL/g)	K_{oc} (mL/g)
MSL	Sandy Clay Loam[a]	3318	134284
DU	Loam	3760	47347
PD	Sandy Loam	3051	370420
MT	Clay	3887	330433
ROE	Sandy Clay Loam	3771	112455

Table 5-2. Environmental Fate and Chemistry of Lubabegron (cont.)

Study	Results
Aerobic Degradation in Soil, Study No. 14028.6112 2014, (Appendix G)	

Soil	Soil Taxonomy	DT (days)	VOCs (%AR)	$^{14}CO_2$ (%AR)
MSL	Sandy Loam[a]	DT_{50}: 365 DT_{90}: 1212	ND	1.5
DU[b]	Loam	DT_{50}: 289 DT_{90}: 960	ND	0.9
RMN	Loamy Sand	DT_{50}: 533 DT_{90}: 1772	ND	2.1
PD	Sandy Loam	DT_{50}: 866 DT_{90}: 2879	ND	1.5

Studies 14028.6111 and 14028.6112 used [Nitrile-14C] lubabegron hemifumarate with a purity ≥ 98.0%.

[a] In Study 14028.6111 the MSL sample was classified by USDA textural standards as a sandy clay loam. The MSL sample for study 14028.6112 was classified as a sandy loam by the USDA textural standards. This was the result of slightly varying proportions of sand, silt, and clay in the media when tested.

[b] Values for DT_{50} and DT_{90} calculated including unidentified degradation product >10% of initial dose.

5.1.3 Toxicity

The toxic effects of lubabegron to terrestrial and aquatic organisms are described below and summarized in Table 5-3.

5.1.3.1 Terrestrial Organisms

The toxicity of lubabegron to soil microflora, plants, and earthworms has been evaluated.

The effects of lubabegron on soil microflora, specifically carbon transformation (respiration) and nitrate formation (nitrification), were evaluated in Study 14028.6113 (2014, Appendix I). The initial soil concentrations of lubabegron were 0.25 and 2.5 mg lubabegron/kg soil (dry weight). After 28 days, the carbon transformation rate in the highest treatment soil differed from the control rate by 6.21%. There were variable and transient effects on nitrification by lubabegron treatment. However, by Day 28, the nitrate formation rate was +19.8% and -5.62% different than the control soil for the 0.25 mg lubabegron/kg and 2.5 mg lubabegron /kg treated soils, respectively. The threshold criteria for effects were not achieved in this study and therefore, no long-term impacts from lubabegron are expected on the soil microflora population.

Six crop species were tested (three monocotyledons and three dicotyledons) in a seedling emergence and growth test for sensitivity to lubabegron (Study 14028.6114, 2014, Appendix J). The species tested were corn, radish, perennial ryegrass, soybean, tomato, and wheat. No effects on seedling emergence were found at the dose levels tested and the corresponding NOEC was 1000 mg lubabegron/kg for all species tested. For fresh shoot weight, The EC_{50} was 790 mg lubabegron/kg and 570 mg lubabegron/kg for perennial ryegrass and wheat, respectively. For all other species tested, the EC_{50} for fresh shoot weight was greater than 1000 mg lubabegron/kg. The NOEC for effects on fresh shoot weight were 250, 250, and 63 mg lubabegron/kg for radish, perennial ryegrass, and wheat, respectively. Based on a comparison of the EC_{25} values, the most sensitive monocotyledon species tested was wheat (*Triticum aestivum*), with an EC_{25} value of

290 mg lubabegron/kg for fresh shoot weight. The most sensitive dicotyledon species tested was radish (*Raphanus sativus*) with an EC_{25} of 550 mg lubabegron/kg for fresh shoot weight. In general, the monocotyledons tested were more sensitive to lubabegron than the dicotyledons tested. Additionally, fresh shoot weight was generally a more sensitive indication of exposure to lubabegron than percent emergence.

The effects of lubabegron on earthworms were evaluated in a 56-day chronic toxicity and reproduction test using *Eisenia fetida*. In Study 14028.6115 (2014, Appendix K) adult worms were exposed to lubabegron for 28 days with no adverse effects on survival, growth, or behavior observed at concentrations up to 1000 mg lubabegron/kg in soil (dry weight). Additionally, offspring (cocoons and juveniles) produced during the initial 28-day exposure of the adult worms remained in the test soils for an additional 28-days to assess effects on reproduction. No statistically significant reduction in reproduction among organisms exposed to lubabegron at any treatment level was observed. Therefore, the 56-day NOEC for reproduction was determined to be 1000 mg lubabegron/kg in soil.

5.1.3.2 Aquatic Organisms

The toxicity of lubabegron to algae, daphnia, and fish has been evaluated.

The effects of lubabegron on *Pseudokirchneriella subcapitata*, a freshwater green alga, have been evaluated in a 72-hour static exposure (Study 14028.6101, 2012, Appendix L). The nominal test concentrations were 0.010, 0.026, 0.064, 0.16, 0.40 and 1.0 mg lubabegron/L and the time weighted average concentrations were 0.0058, 0.015, 0.035, 0.10, 0.27 and 0.60 mg lubabegron/L, respectively.

The results are based on the time-weighted average concentrations of lubabegron and are reported as the 72-hour EC_{10}, EC_{20} and EC_{50} values for biomass expressed as yield and average growth rate data calculated from the 72-hour cell density counts. The No-Observed-Effect Concentration (NOEC) values for total yield and average growth rate were also determined. The EC_{50} values for growth rate and yield were determined to be 0.032 mg lubabegron/L and 0.025 mg lubabegron/L, respectively, based on the time-weighted average concentrations of lubabegron. The NOEC values for growth rate and yield were both 0.015 mg lubabegron/L.

To evaluate the toxicity of lubabegron to aquatic invertebrates, a full life-cycle study was conducted for 21-days with *Daphnia magna* (Study 14028.6103, 2012, Appendix M). Nominal concentrations of 0.0016, 0.0031, 0.0063, 0.013, 0.025 and 0.050 mg lubabegron/L were chosen for the definitive exposure. Solution renewals occurred daily in order to maximize exposure solution concentrations. Mean measured concentrations of lubabegron ranged from 87% to 96% and defined the treatment levels tested as 0.0014, 0.0029, 0.0059, 0.012, 0.024 and 0.048 mg lubabegron/L.

The number of immobilized adult daphnids and observations of abnormal behavior were recorded daily. Numbers of offspring were determined upon the first brood release in any vessel and daily throughout the remainder the test. Offspring were removed, counted and discarded at each observation interval. In addition, the number of immobilized offspring was recorded for each treatment level and the control. At test termination (Day 21), the total body length (from

the apex of the head to the base of the carapace spine) of each surviving adult daphnid was measured.

Based on survival as the most sensitive indicator of toxicity, the 21-day No-Observed-Effect Concentration (NOEC) was determined to be 0.012 mg lubabegron/L. The Lowest-Observed-Effect Concentration (LOEC) was determined to be 0.024 mg lubabegron/L.

The 21-day EC_{50} value for survival was determined by Spearman-Kärber Estimates to be 0.015 mg lubabegron/L, with 95% confidence limits of 0.012 to 0.018 mg lubabegron/L. No effects on behavior or mean body length were observed.

Following 21 days of exposure, the organisms exposed to the 0.0014, 0.0029, 0.0059 and 0.012 mg lubabegron/L treatment levels had released a mean cumulative offspring per female of 124, 130, 134 and 136, respectively. Statistical analysis (Wilcoxon's Test with Bonferroni's Adjustment) determined no significant reduction in offspring per female in any of the treatment levels statistically analyzed compared to the pooled control (136 offspring per female). Since no concentration resulted in ≥ 50% reduction in reproductive output, the 21-day EC_{50} for *Daphnia magna* reproduction was empirically estimated to be > 0.012 mg lubabegron/L, the highest mean measured lubabegron concentration level statistically analyzed. Correspondingly, the 21-day NOEC for reproduction effects was 0.012 mg lubabegron/L.

A 96-hour acute toxicity study in rainbow trout (*Oncorhynchus mykiss)*, using lubabegron was conducted at 0.063, 0.13, 0.25, 0.50 and 1.0 mg lubabegron/L (Study 14028.6102, 2012, Appendix N). The resulting geometric mean measured concentrations of lubabegron in the test solutions were 0.050, 0.10, 0.20, 0.41 and 0.93 mg lubabegron/L, respectively.

At test termination, 100% mortality was observed at the 0.93 mg lubabegron/L treatment level. No mortality or sublethal effects were observed among fish exposed to any of the remaining treatment levels tested (0.050, 0.10, 0.20 and 0.41 mg lubabegron/L) or the controls.

Based on the geometric mean measured concentrations, the 96-hour LC_{50} value for *Oncorhynchus mykiss* exposed to lubabegron was determined by binomial probability to be 0.62 mg lubabegron/L with 95% confidence interval of 0.48 to 0.80 mg lubabegron/L. The No-Observed-Effect Concentration (NOEC) was determined to be 0.41 mg lubabegron/L.

Table 5-3. Environmental Effects of Lubabegron

Terrestrial Effects-Tier A

Soil Microflora Respiration and Nitrogen Transformation Tests (28 days), Study No. 14028.6113 2014. (Appendix I)

Lubabegron concentration as high as 2,500 µg lubabegron/kg soil resulted in less than 25% difference from control after 28 days.

Seedling Emergence and Growth, Study No. 14028.6114 2014. (Appendix J)

Species	Emergence (mg lubabegron/kg)		Fresh Shoot Weight (mg lubabegron/kg)	
	EC_{50}	NOEC	EC_{50}	NOEC
Corn	>1000	1000	>1000	1000
Radish	>1000	1000	>1000	250
Perennial Ryegrass	>1000	1000	790	250
Soybean	>1000	1000	>1000	1000
Tomato	>1000	1000	>1000	1000
Wheat	>1000	1000	570	63

Earthworm, Study No. 14028.6115 2014. (Appendix K)

Results:
Results based on nominal concentrations are presented in the following table:

Toxicity Endpoint	Toxicity Value
28-day F_0 LC_{50} (95% confidence limits)	> 1000 mg lubabegron/kg (NA)
28-day F_0 $NOEC_{survival}$	1000 mg lubabegron/kg
28-day F_0 $NOEC_{weight\ change}$	1000 mg lubabegron/kg
56-day F_1 $EC_{50\ reproduction}$ (95% confidence limits)	> 1000 mg lubabegron/kg (NA)
56-day F_1 $NOEC_{reproduction}$	1000 mg lubabegron/kg

NA = Not Applicable. LC/EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

Based on F_0 earthworm percent survival and weight change and F_1 reproduction, NOEC values were determined to be 1000 mg lubabegron/kg and the LC_{50} or EC_{50} values were > 1000 mg lubabegron/kg, indicating that lubabegron was not toxic to earthworms at soil concentrations ≤ 1000 mg lubabegron/kg.

All studies in Table 5-3 used TAC material lot 160SB1 with a purity of 98.9% as lubabegron hemifumarate and a potency of 88.6% as lubabegron.

Table continued on next page.

Table 5-3. Environmental Effects of Lubabegron (cont.)

<table>
<tr><th colspan="2">Aquatic Effects- Tier A</th></tr>
<tr><td>Algae
Study No. 14028.6101
2012. (Appendix L)</td><td>Results:
Based on Time –Weighted Average Concentrations
(mg lubabegron/L)
<table>
<tr><th>Biological Parameter</th><th>EC10 (95% Confidence Intervals)</th><th>EC20 (95% Confidence Intervals)</th><th>EC50 (95% Confidence Intervals)</th></tr>
<tr><td>0 - 72-Hour Yield</td><td>0.014
(0.0053 – 0.020)</td><td>0.017
(0.012 – 0.022)</td><td>0.025
(0.021 – 0.027)</td></tr>
<tr><td>0 - 72-Hour Average Growth Rate</td><td>0.018
(0.015 – 0.020)</td><td>0.021
(0.019 – 0.024)</td><td>0.032
(0.028 – 0.066)</td></tr>
</table></td></tr>
<tr><td>Fish Acute
Study No. 14028.6102
2012. (Appendix N)</td><td>Results:
100% mortality at 0.93 mg lubabegron/L
No mortality or sublethal effects observed at 0.050, 0.10, 0.20 and 0.41 mg lubabegron/L or in the controls.

Based on geometric mean measured concentrations:
96-hour LC_{50} = 0.62 mg lubabegron/L
95% confidence interval of 0.48 to 0.80 mg lubabegron/L
NOEC = 0.41 mg lubabegron/L</td></tr>
<tr><th colspan="2">Aquatic Effects- Tier B</th></tr>
<tr><td>Daphnia,
Study No. 14028.6103
2012. (Appendix M)</td><td>Results:
Based on survival as the most sensitive indicator of toxicity,
21-day NOEC = 0.012 mg lubabegron/L
LOEC = 0.024 mg lubabegron/L

The 21-day EC_{50} value (survival) = 0.015 mg lubabegron/L
95% confidence limits of 0.012 to 0.018 mg lubabegron/L

The 21-day EC_{50} value for reproduction and growth was empirically estimated to be > 0.012 mg lubabegron/L, the highest mean measured concentration statistically evaluated.

21-Day NOEC (reproduction) = 0.012 mg lubabegron/L</td></tr>
<tr><td>Algae,
Study No. 14028.6101
2012. (Appendix L)</td><td>Results:
Based on Time –Weighted Average Concentrations (mg lubabegron/L)
<table>
<tr><th>Biological Parameter</th><th>NOEC</th></tr>
<tr><td>0 - 72-Hour Yield</td><td>0.015</td></tr>
<tr><td>0 - 72-Hour Average Growth Rate</td><td>0.015</td></tr>
</table></td></tr>
</table>

All studies in Table 5-3 used TAC material lot 160SB1 with a purity of 98.9% as lubabegron hemifumarate and a potency of 88.6% as lubabegron.

5.2 PEC Calculations and Refinements (Exposure Assessment)

5.2.1 Soil

The initial PEC_{soil} was calculated in Section 4.0, Phase I Environmental Impact Assessment as 69 µg/kg.

The PEC_{manure} concentration was first calculated using the following assumptions: all cattle in a pen will be treated for 91 days and all residues eliminated as lubabegron or metabolites as active as lubabegron. The PEC calculation also assumes that the manure is applied to soil without dilution by manure from non-treated cattle (cattle from untreated pens). The PEC_{manure} of total lubabegron residues using these assumptions is 2.3 mg/kg.

Per the VICH guideline (GL38), the PEC_{manure} may be refined based on the actual composition of the dose excreted by treated animals by adding the active substance (lubabegron) and the relevant metabolites (those that are 10% or more of the administered dose). Data from the metabolism study (Study No. 286652, Report 33878, 2014) suggests that very low levels of lubabegron and/or metabolites are excreted from beef cattle. However, those data were not used to refine the exposure concentrations in this environmental assessment.

For slowly degrading compounds, the possibility of accumulation in soil from repeated yearly application of manure containing lubabegron residues to the same field was evaluated. To model the potential impact of yearly application of cattle manure to agricultural soils, the EPA 90th percentile confidence-interval upper bound (USEPA, 2009) value for the soil degradation half-life from the aerobic soil degradation study was calculated. This value was used (723 days or 1.98 years with a corresponding rate constant of 0.35 year^{-1}) to determine calculated soil concentrations over time. Using the rate equation below, soil lubabegron concentrations can be calculated for any time (t).

$$\frac{C}{C_0} = e^{-kt}$$

Where C is the concentration at time t, C_0 is the original concentration at time-zero (0) such that $\frac{C}{C_0}$ is the fraction remaining at time t, and the rate constant is k.

Assuming that cattle manure containing lubabegron is applied annually to the same field and that lubabegron degrades in soil at a rate of 0.35 year^{-1}, then each year 69 µg lubabegron/kg is added and 70.5% ($\frac{C}{C_0} = e^{-kt}$ or $e^{-0.35*1}$ or 0.705) remains from the previous year. Table 5-4 shows the calculated soil concentrations based on the EPA 90th percentile confidence-interval upper bound half-life measured in the soils tested.

Based on these calculations the lubabegron soil concentration plateaus at approximately 234 µg lubabegron/kg.

Table 5-4. Calculated Lubabegron Soil Concentrations

Year	Calculated Soil Concentration (potential accumulation) µg lubabegron/kg[a]
1	69
2	118
3	152
4	176
5	193
10	227
15	233
20	234
25	234

[a] Calculated for year 2 as (69 µg/kg X 0.705) + 69 µg/kg = 118 µg/kg; year 3 (118 µg/kg X 0.705) + 69 µg/kg = 152 µg/kg, and so on. Values plateau at 234 µg/kg after approximately 20 years. Values may vary due to rounding.

5.2.2 Groundwater

Lubabegron and its residues are strongly adsorbed to soil, with K_d values ranging from 3051 mL/g to 3887 mL/g and K_{oc} values ranging from 47,347 mL/g to 370,420 mL/g (Study 14028. 6111, 2014, Appendix H) in the soils tested. It is extremely unlikely that significant levels of lubabegron or active residues would be found in groundwater.

5.2.3 Surface Water

Movement of lubabegron from the soil to surface water may occur through runoff following rainfall events. A scenario of 1% runoff of lubabegron from 10 acres of soil into a one-acre pond which is 2 m deep was considered in this assessment. A one-acre pond that is 2 m deep contains a volume of 8,100,000 L. Using the unrefined total residue concentration of lubabegron residues in manure (2.3 mg lubabegron/kg) and the application rate of cattle manure per acre (27,200 kg manure/acre) results in a $PEC_{soil\text{-}initial}$ or $[lubabegron\ residues]_{soil}$ of 69 µg lubabegron/kg soil. The following calculation was performed to estimate the maximum concentration of lubabegron residues in a pond (or surface water):

$$[lubabegron\ residues]_{pond} = \frac{[lubabegron\ \ residues]_{soil} \times Soil\ Mass\ kg/Acre\ \times 10\ acres\ \times 0.01}{8{,}100{,}000\ L}$$

$$[\text{lubabegron}\ \ residues]_{pond} = \frac{69\,\frac{\mu g}{kg} \times 910{,}500\,\frac{kg}{acre}\ \times 10\ acres\ \times 0.01}{8{,}100{,}000\ L} = 0.776\ \mu g/L$$

The concentration of total residues of lubabegron in soil following application of manure is 69 µg lubabegron/kg ($PEC_{soil,\ total\ residues}$). An acre of soil 15 cm deep (plow depth) contains 910,500

kg of soil. A one percent (1%) run-off event corresponds to 91,050 kg of soil from the ten acres. The mass of lubabegron and residues from this soil in the run-off is 6,282 mg. This mass of lubabegron and residues in 8,100,000 L of pond water corresponds to a concentration of 0.776 µg/L. Therefore, the concentration of total lubabegron residues in the pond or $PEC_{surface\ water}$, would be 0.776 µg/L. A similar calculation can be made for the case where the soil concentration is refined for accumulation (234 µg lubabegron/kg; $PEC_{soil,\ refined\ for\ potential\ accumulation}$) and results in a $PEC_{surface\ water\text{-}refined\ for\ accumulation}$ of 2.63 µg/L.

This value can be refined to account for the adsorption of lubabegron to sediment in the pond (or surface water). For this calculation, values traditionally used in environmental assessments were used to refine for sediment adsorption as follows. Assuming 5% organic matter (%OM) the corresponding percent of organic carbon (%OC) is approximately 2.9% (since %OC = %OM / 1.724; Hamaker, 1975). Assuming mixing of lubabegron in the top 5 cm of sediment and using a conservative approach for soil adsorption by determining the lowest 10% confidence-interval bound value (same methodology as in USEPA 2009, but for lowest bound value of the confidence-interval) for K_{oc} and thusly K_d. For the soils tested, the lower bound 10% value for the K_{oc} is 101,223 mL/g with a corresponding K_d of 2935 mL/g (calculated as $K_d = 0.029 \times K_{oc}$).

Using the following equation:

$$PEC_{surface\ water,refined\ for\ adsorption} = \frac{mass_{LY488756}}{mass_{water} + (mass_{sediment} \times K_d)}$$

The mass of soil as part of the run-off event is 91,050 kg and the mass of lubabegron residues that enter the pond is 6,282 mg (91,050 kg X 69 µg lubabegron/kg = 6,282 mg lubabegron). The mass of water in the pond is 8,100,000 kg, the mass of sediment 5 cm deep is 300,000 kg and the resulting PEC for surface water refined for adsorption is 0.007 µg lubabegron/L. Similarly, the PEC surface water, refined for potential accumulation and adsorption can be calculated using the PEC soil, refined for potential accumulation value of 234 µg lubabegron/kg resulting in an aqueous concentration of 0.024 µg lubabegron/L.

Table 5-5 provides a summary of values for the predicted environmental concentrations for lubabegron residues in the terrestrial and the aquatic compartments based on a total residue approach and various refinements.

Table 5-5. Summary of PEC Calculations for Residues of Lubabegron

Compartment	Scenario	Concentration
Terrestrial	$PEC_{soil,\ total\ residues}$	69 µg lubabegron/kg
	$PEC_{soil,\ refined\ for\ potential\ accumulation}$	234 µg lubabegron/kg
Aquatic	$PEC_{surface\ water,\ total\ residues}$	0.776 µg lubabegron/L
	$PEC_{surface\ water,\ refined\ for\ potential\ accumulation}$	2.63 µg lubabegron/L
	$PEC_{surface\ water,\ total\ residues,\ refined\ for\ adsorption}$	0.007 µg lubabegron/L
	$PEC_{surface\ water,\ refined\ for\ potential\ accumulation/adsorption}$	0.024 µg lubabegron/L

5.3 PNEC Calculations (Effect Assessment)

In accordance with VICH GL38 Phase II guidance for Environmental Impact Assessments (EIA's), predicted no-effect concentrations (PNECs) were calculated using the recommended data set and the appropriate assessment factors for a particular study endpoint (i.e., EC_{50}, NOEC).

5.3.1 Terrestrial

The calculated PNECs and assessment factors used for the terrestrial species tested are provided in Table 5-6. The terrestrial species tested are not particularly sensitive to lubabegron. The lowest PNEC value determined was in soil microflora study and reflects the highest level tested in that study: 2,500 µg lubabegron/kg soil.

Table 5-6. Tier A - Terrestrial PNEC Values

Study	Toxicity endpoint	Assessment Factor	PNEC
Soil Microflora (28-day)	≤ 25% change from control = 2,500 µg/kg	1	2,500 µg lubabegron/kg
Terrestrial Plants (Seedling emergence and growth)	EC_{50} = 570 mg/kg	100	5,700 µg lubabegron/kg
Earthworm (Chronic Toxicity/ Reproduction)	NOEC = 1,000 mg/kg	10	100,000 µg lubabegron/kg

5.3.2 Aquatic

The assessment factors and calculated PNECs used for aquatic species tested for Tier A and Tier B are provided in Table 5-7 and Table 5-8, respectively. Daphnids appear to be more susceptible to toxicity from lubabegron than fish or algae based on the calculated PNEC for each species. The Tier-B PNEC for *D. magna* was calculated using the NOEC and an assessment factor of 10 applied yielding a PNEC of 1.2 µg/L.

Table 5-7. Tier A- Aquatic PNEC Values

Study	Toxicity endpoint	Assessment Factor	PNEC
Algal Growth Inhibition	72 hr EC_{50} = 25 µg/L	100	0.25 µg lubabegron/L
Fish Acute Toxicity	96 hr LC_{50} = 620 µg/L	1000	0.62 µg lubabegron/L

Table 5-8. Tier B- Aquatic PNEC Values

Study	Toxicity endpoint	Assessment Factor	PNEC
Algal Growth Inhibition	72 hr NOEC = 15 µg/L	10	1.5 µg lubabegron/L
D. magna Reproduction	21-day NOEC = 12 µg/L	10	1.2 µg lubabegron/L

5.4 Risk Characterization

5.4.1 Terrestrial Compartment

The predicted maximum concentration of total residues of lubabegron in soil ($PEC_{soil\ initial}$) after a single manure application to cropland soil is 69 µg lubabegron/kg. The lowest terrestrial PNEC value for lubabegron is 2,500 µg lubabegron/kg and is for soil microflora.

The PEC/PNEC ratio (or risk quotient, RQ) for the terrestrial compartment is 0.03 (Table 5-9). The ratio is less than one indicating that there is no significant risk to terrestrial species (soil microflora, plants, or other soil-dwelling species).

If accumulation in soil without refinement for metabolism *in vivo* (using the total residue approach) is considered as the worst case scenario, the $PEC_{soil\ refined\ for\ accumulation}$ is 234 µg lubabegron/kg and the resulting RQ is 0.09. This RQ also indicates that there is no significant risk to soil organisms even under the worst case scenario of accumulation of lubabegron in soils following repeated applications of manure to soils without benefit of refinement for *in vivo* metabolism.

Table 5-9. Tier A- PEC/PNEC Ratio (RQ) for Terrestrial Compartment

Species	PECsoil	PNEC	PEC/PNEC Ratio (RQ)
Soil Microflora	Total lubabegron residues: 69 µg lubabegron/kg	2,500 µg/kg	0.03
	Total lubabegron residues refined for potential accumulation: 234 µg lubabegron/kg		0.09

Values may vary due to rounding.

5.4.2 Aquatic Compartment

To evaluate the impacts of lubabegron in the aquatic environment, known fate and environmental chemistry properties were used to refine the calculations of the aquatic PECs. In order to calculate refined surface water PECs, calculation of refined PEC_{soil} values was performed (Table 5-5). The resulting refinements to the $PEC_{surface\ water}$ were used to determine the RQs listed in Table 5-10 and Table 5-11.

The maximum predicted concentration of lubabegron residues in surface water ($PEC_{surface\ water,\ total\ residues}$) following a single manure application to cropland as fertilizer is 0.776 µg lubabegron/L. When considering the potential for accumulation in soil from repeated application of manure from treated animals, the maximum concentration is surface water ($PEC_{surface\ water,\ refined\ for\ potential\ accumulation}$) as a result of run-off is 2.63 µg lubabegron/L. When these values are refined to account for adsorption of lubabegron to sediment, the values are 0.007 µg lubabegron/L and 0.024 µg lubabegron/L, respectively.

The most sensitive aquatic species tested in Tier-A is algae with a PNEC of 0.25 µg lubabegron/L. The Tier-A RQs based on the $PEC_{surface\ water,\ refined\ for\ potential\ accumulation/adsorption}$ are 0.10 and 0.04 for algae and fish, respectively. These RQ values are <1 indicating that there is no significant risk to algae and fish.

The lowest Tier-B aquatic PNEC determined for lubabegron was the 21-day NOEC for reproduction effects for *D magna* (1.2 µg lubabegron/L). The RQs based on the Tier-B PNEC for *D magna* for a single manure application and considering the potential for accumulation in soil from repeated application of manure, are 0.01 and 0.02, respectively (Table 5-11).

For daphnia, the assessment went directly to the Tier-B endpoints since the 21-day reproduction study was performed and the 21-day NOEC was determined. When considering the case of $PEC_{surface\ water,\ refined\ for\ potential\ accumulation/adsorption}$, where the $PEC_{surface\ water}$ is refined to account for potential accumulation in soils after repeated manure applications, and adsorption to sediments in surface waters, the Tier-B RQ for *Daphnia magna* is 0.02. This RQ value is well below 1.0 indicating that there is no risk to daphnia.

Table 5-10. Tier A- PEC/PNEC Ratios (RQ) for the Surface Water Compartment

$PEC_{surface\ water}$	PEC/PNEC Ratio (RQ)	
	Algae	**Fish**
Total lubabegron residues: 0.776 µg/L	3.1	1.3
Total lubabegron residues refined for potential accumulation: 2.63 µg/L	11	4.2
Total lubabegron residues refined for adsorption: 0.007 µg/L	0.03	0.01
Total lubabegron residues refined for potential accumulation and adsorption: 0.024 µg/L	0.10	0.04

Values may vary due to rounding.

Table 5-11. Tier B- PEC/PNEC Ratios (RQ) for the Surface Water Compartment

$PEC_{surface\ water}$	PEC/PNEC Ratio (RQ)
	Daphnia
Total lubabegron residues: 0.776 µg/L	0.65
Total lubabegron residues refined for potential accumulation: 2.63 µg/L	2.2
Total lubabegron residues refined for adsorption: 0.007 µg/L	0.01
Total lubabegron residues refined for potential accumulation and adsorption: 0.024 µg/L	0.02

Values may vary due to rounding.

5.5 Bioconcentration

One approach to estimate the bioconcentration potential of a chemical is to consider its lipophilicity using a laboratory generated surrogate parameter such as a compound's n-octanol-water partition coefficient (log K_{ow} or log P_{ow}). The experimentally derived log P_{ow} values for lubabegron range from 1.38 (at pH 4) to 4.93 (at pH 9). Veith and Kosian (1983) generated a linear model using a training set of 122 molecules to predict the bioconcentration factor for chemicals in fathead minnows:

$$\log BCF = (0.79 \times \log K_{ow}) - 0.40$$

A conservative approach to address the potential impacts from the bioconcentration of lubabegron is to consider the potential residue levels in fish resulting from exposure to aqueous concentrations of lubabegron followed by an assessment of exposure and potential toxic effects to wildlife predators that consume the fish (prey) as part of their diet. Considering the bioconcentration factor (BCF) in pH 9 waters as representing the worst-case scenario for fish exposed to lubabegron residues through surface water (highest BCF); the calculated log BCF for lubabegron as determined by the Veith and Kosian equation is 3.4947.

$$\log BCF = (0.70 \times 4.93) - 0.40 = 3.4947$$

and therefore at pH 9,

$$BCF = 10^{3.4947} = 3124$$

Using the PEC for surface water refined for potential accumulation and adsorption to represent the maximum predicted lubabegron concentration in water (0.024 µg/L as in Section 5.2.3.) results in a calculated fish tissue concentration of 75 µg/kg based on the equation for BCF below.

$$BCF_{fish} = \frac{[concentration\ of\ lubabegron\ in\ fish\ tissues]}{[concentration\ of\ lubabegron\ in\ water\ at\ pH\ 9]}$$

A biomagnification factor (BMF) was used in calculating the maximum dose in the wildlife species presented in Table 5-12. The BMF was used to adjust for the differential uptake of lubabegron in the predator versus the prey species and represents the potential for magnification of residues in the food chain for molecules based on their respective log P_{ow} value. The BMF for a compound with a log P_{ow} of 4.93 is 2 (EMEA 2008, page 27, Table 7).

Several acute and chronic (repeat dose up to 1-year duration) toxicology studies were conducted in the rat, monkey, rabbit, and human to support the Toxicology component of the Human Safety Technical Section of the lubabegron application. These studies consistently demonstrated that at doses equal to or below 15 mg/kg body weight only non- adverse pharmacological effects are observed following daily oral exposure to lubabegron. In studies conducted in human and monkey, effects level values were based solely on non-adverse acute cardiac effects that do not change with chronic dosing and therefore are not a suitable model for chronic wildlife exposures. The most appropriate model for a chronic wildlife oral exposure to evaluate potential adverse effects resulting from potential bioaccumulation is the 1-year chronic rat study (Study No. 130-189). In this study the biologically relevant NOEL established was 5 mg/kg body weight. The sole equivocal effect noted at this dose in rats was an increase in food consumption without a change in body weight relative to control animals.

Table 5-12. Typical Body Weight Range, Fish Consumption Rates, Calculated Maximum Dose of Lubabegron assuming Bioconcentration in Fish, and Toxicology Safety Margin for Selected Wildlife Species

Species (Common Name)	Typical Body Weight Range (kg)[a]	Fish Consumption[a]	Calculated Maximum Dose (mg/kg)[b]	Toxicology Safety Margin[c]
Osprey	1.2 to 1.9	0.21 kg/d	0.026	6.4
Belted Kingfisher	0.125 to 0.215	0.5 g/g BW/d	0.129	1.3
River Otter	5.0 to 15	[d]0.636 kg/d	0.019	8.8

[a] Values from Wildlife Exposure Factors Handbook (USEPA Dec 1993).

[b] Maximum Dose calculated as the lubabegron residue consumed based on the consumption of fish for a maximum BW animal from the range provided and using the minimum animal BW for animal dose from the BW range provided. Resultant values were multiplied by a BMF of 2 to reflect the potential magnification of lubabegron in predator versus prey.

[c] Toxicology Safety Margin calculated as toxicology NOEL for chronic rat 1-year study (5 mg/kg) divided by the Calculated Maximum Dose from consumption of fish and dividing by an assessment factor of 30.

[d] Calculated from equation [3-7] in Wildlife Exposure Factors Handbook (USEPA Dec 1993).

Comparison of calculated maximum doses for selected predator wildlife species which feed on fish versus toxicology endpoints indicates that there are considerable safety margins when evaluated using the most conservative assumptions as indicated by the Toxicology Safety Margins listed in Table 5-12. The results provided indicate that under the conservative assumptions used to determine bioconcentration effects in this risk assessment, there is a 6.4, 1.3, and 8.8-fold margin to toxicity for the osprey, belted kingfisher, and river otter, respectively. These results indicate the risk for toxicity from potential bioconcentration of lubabegron in fish to wildlife predator species which feed primarily on fish is low.

6.0 Environmental Impacts Analysis of Ammonia Reductions

Experior™ (lubabegron Type A medicated article) is indicated for reduction of ammonia gas emissions per pound of live weight and hot carcass weight in beef steers and heifers fed in confinement for slaughter during the last 14 to 91 days on feed. Livestock are the largest known source of ammonia gas emissions in the United States (U.S.) and use of Experior™ is intended to reduce these emissions from beef steers and heifers fed in confinement for slaughter (i.e., in feedlots), which may have effects (including beneficial effects) on the U.S. environment. Therefore, the potential effects arising from the intended use of lubabegron in beef steers and heifers fed in confinement for slaughter (i.e., for reduction of ammonia gas emissions) on the U.S. environment were also evaluated in this assessment.

Although ammonia (NH_3) has many beneficial industrial and agricultural uses, ammonia emissions can have detrimental effects on the environment and human health, either directly or secondarily through formation of other nitrogen containing compounds. In the last century, production of reactive nitrogen from human sources has increased drastically and agriculture is the greatest source of anthropogenic ammonia, accounting for 82% of total U.S. emissions to air (USEPA, 2014). Ammonia emissions due to the agriculture sector occur in three areas: 1) crops and livestock dust, 2) fertilizer application, and 3) livestock waste. Waste from livestock (i.e, poultry, swine, and cattle) contribute the greatest proportion of ammonia emissions, accounting for 68% of total U.S. agricultural emissions, with beef cattle producing the highest proportion of these emissions at 27% of the total agriculture ammonia emissions (USEPA, 2004, 2014).

Although ammonia is the primary chemical of concern, ammonia can convert to many other forms of nitrogen in the soil, water, or atmosphere (see Section 6.1 below) depending on local environmental conditions. These compounds can have effects on the environment and human health, including eutrophication of water bodies, atmospheric haze and deposition, climate change, and quality of human life (e.g., noxious odor, asthma). These effects can occur at various scales in the U.S., including local, regional, and national.

Therefore, this assessment evaluates whether there are adverse and/or beneficial effects to air and/or water as a result of a reduction in ammonia gas emissions due to the use of lubabegron in beef steers and heifers fed in confinement for slaughter.

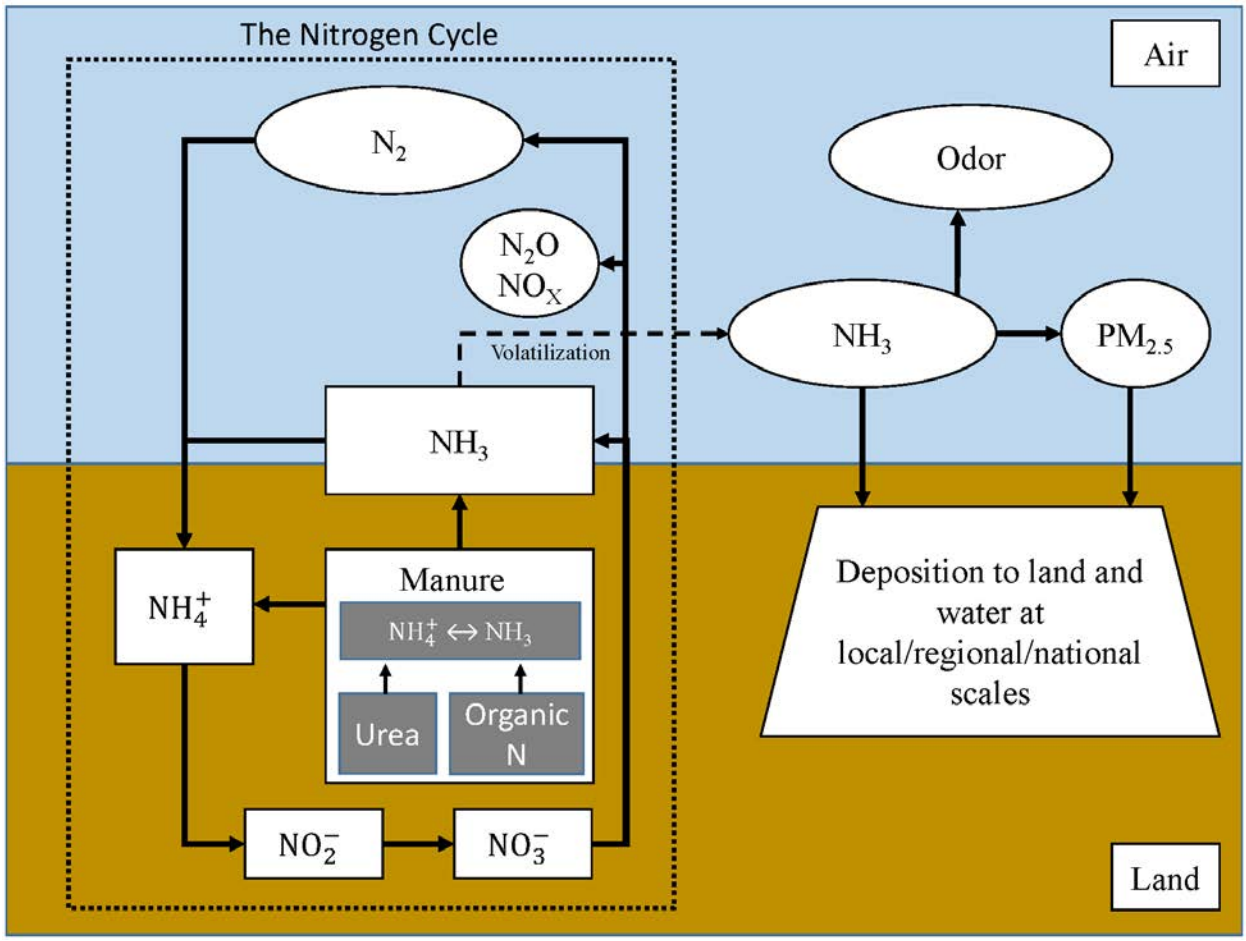
6.1 Scope of Assessment

Beef cattle utilize nitrogen to build proteins (e.g., muscle) and other compounds necessary for life. Nitrogen in excess of these needs is excreted. The typical nitrogen balance for beef cattle fed in confinement for slaughter estimates that 15% of all nitrogen intake is retained in the carcass and the remaining is excreted; approximately 31% as organic-N in feces and 54% as urea in urine (Cole and Todd 2009). Studies conducted *in vivo* in beef cattle support that Experior™ acts by decreasing the amount of urea excreted by cattle, which results in less ammonia volatilized from manure (approximately 14-18% reduction per pound of live weight and hot carcass weight in beef steers and heifers fed in confinement for slaughter). This reduction in excreted urea is thought to occur because more nitrogen is retained in the carcass in the form of muscle protein. An *in vivo* metabolic profile study supports that feeding of lubabegron results in increased uptake

of nitrogen as branched chain amino acids that are subsequently used in the synthesis of skeletal muscle protein (see Appendix R).

Urea is the primary source of volatilized ammonia. Organic nitrogen serves as another source (Hristov et al (2011)). Following excretion, urea is rapidly (<3 d) converted to ammonia or the ammonium ion (NH_4^+) and bicarbonate ion (HCO_3^-) on the feedlot surface, including manure, due to the action of urease, a common enzyme produced by soil bacteria. Ammonia and ammonium then readily volatilize from the feedlot surface, and can be transformed in the atmosphere to nitric oxide compounds (collectively known as NO_x), nitrous oxide (N_2O), particulate matter ≤ 2.5 µm in diameter ($PM_{2.5}$), nitrite (NO_2^-), nitrate (NO_3^-), and nitrogen gas (N_2). Transport of these compounds may also occur to water bodies via deposition of nitrogenous particulate matter ($PM_{2.5}$), ammonia, or ammonium. Runoff can also transport soluble forms of nitrogen (e.g., NH_3, NH_4^+, NO_2^-, and NO_3^-). The nitrogen cycle is illustrated in Figure 2. All of these compounds are of interest in this evaluation.

Figure 2. Transformation and Mobilization of Nitrogen Compounds Related to Cattle Manure



In Figure 2, boxes depict nitrogen compounds in soil and manure environments. Ovals depict nitrogen compounds in the atmosphere. Deposition to land and water may occur at a great distance from the source (e.g., tens to hundreds of kilometers).

The spatial scale of effects is an important factor that must be considered in this assessment. Ammonia and other nitrogen compounds can occur in gaseous form, and therefore, can be transported over long distances and deposited in waterbodies far from the feedlot. Therefore, adverse or beneficial effects due to these compounds could potentially occur at the local (e.g., farm), regional (e.g., watershed), or national scale.

6.2 Assessment of Potential Effects

As described above (Section 6.1), Experior™ is thought to act by increasing nitrogen (amino acid) uptake and increasing the amount of nitrogen retained in the carcass as muscle protein, thereby reducing the amount of urea excreted in manure (manure is considered the urine and feces combined). The increase in nitrogen uptake and retention is supported by data from pilot studies (D5CUS140021 Appendix O and D5CUS130020 Appendix P) which show an increase in amino acid uptake and insulin responsiveness. Therefore, based on this data and an understanding of how nitrogen is utilized and cycles through cattle, less urea is expected to be produced and excreted resulting in less ammonia being formed and volatilized to the air.

The distribution of nitrogen compounds (i.e., the concentration and form) in manure is not expected to change as a result of the increase in nitrogen retention. This is supported by a pilot study (D5CUS110003, Appendix Q), which showed that total nitrogen, organic nitrogen, and ammonia nitrogen concentrations in manure were not affected from the use of lubabegron at doses of up to 20 g/ton of feed (100% dry matter basis). The lack of a change to the ammonia nitrogen concentrations in comparison to control concentrations can be explained by manure samples being collected several days after excretion allowing for all volatile ammonia to be emitted prior to collection. Therefore, based on Experior™'s expected reduction to urea production and this excretion data, the distribution of other forms of nitrogen in manure is not expected to change and the use of Experior™ is not expected to result in adverse effects on air or water.

Because Experior™ is expected to reduce ammonia gas emissions from beef cattle feedlots where the drug is used, it is expected to have some beneficial effects on the environment and human health; however, the magnitude and scale at which the benefits could occur cannot be easily or reliably quantified.

A predictive evaluation of the magnitude of benefits is not easy or reliable for several reasons. Although there is extensive existing information on ammonia and the other nitrogen compounds that can be formed from ammonia (e.g. including information on their chemistry, formation, sources, transport, and effects), many environmental factors and animal production conditions are needed for this evaluation that are spatially and temporally specific and/or prone to high variability. Environmental factors that need to be accounted for when quantifying ammonia gas emissions (or a reduction thereof) include: temperature, type and abundance of microbial communities, pH, moisture content in the affected environment (manure, soil and air), wind speed and direction, climate, meteorological conditions, inorganic aerosol concentrations, and input from other nitrogen sources. All of these factors can vary depending on geographic area of the U.S. and time of year. In addition, there are animal production conditions that need to be accounted for in order to quantify ammonia gas emissions from a feedlot. These conditions will vary from feedlot to feedlot and include: nutrition of the animals, manure storage and handling,

and administration of Experior™ (e.g., number of animals, dose, duration). A prediction of the beneficial effects is further complicated because environmental factor and animal production condition data are scarce and extremely variable (Hristov 2011, Pinder 2003). Most published literature agrees that quantitatively assessing ammonia gas emissions is not currently possible due to the variety of environmental factors that affect ammonia gas emissions, as well as the lack of data (Pinder 2003, Roe 2004, NRC 2003, USDA 2014, Herbert et al).

Thus, based on available information, it can be concluded that by reducing ammonia gas emissions, Experior™ will likely only have beneficial effects on the environment and human health. The magnitude and scale of the beneficial effects cannot be reliably predicted due to the number of, and variability in, the factors influencing emissions and transport of ammonia and other nitrogen compounds.

7.0 Alternatives to Proposed Action

Experior™ is proposed for reduction of ammonia gas emissions per pound of live weight and hot carcass weight in beef steers and heifers fed in confinement for slaughter during the last 14 to 91 days on feed. The only alternative to the proposed action is the 'no action' alternative, which would be the failure to approve the NADA for Experior™. However, based on our analysis in this EA, we do not think that significant adverse environmental impacts will occur from this action; therefore, the preferred alternative is the approval of the NADA and the no action alternative was eliminated from consideration."

8.0 Summary and Conclusions

The environmental impact from the administration of lubabegron (as Experior™) via Type C medicated feed at a concentration of 4.54 g/ton of complete feed (90% dry matter basis) for 91 days for the reduction of ammonia gas emissions from beef steers and heifers fed in confinement for slaughter has been evaluated. The evaluation included a review of a base set of data collected describing the physical/chemical properties, environmental fate, and environmental effects of lubabegron and its residues. The pathway for introduction of lubabegron into the environment is via the application of cattle manure as fertilizer to agricultural soil. Runoff to surface water from soil fertilized with cattle manure containing lubabegron residues was also considered. The risk assessment evaluated worst case scenarios as well as a total residue approach with appropriate refinement for potential accumulation in soil, and adsorption to soils/sediments in surface waters.

The maximum predicted soil concentration of total lubabegron residues is 69 µg lubabegron/kg when considering a single annual application of manure to cropland from lubabegron treated animals. The maximum soil concentration when considering the total residue approach and also refining for potential accumulation in soils yields a soil concentration of 234 µg lubabegron/kg. The lowest predicted no effect concentration (terrestrial PNEC) for the terrestrial compartment is for soil microflora and is 2,500 µg lubabegron/kg soil. The corresponding RQ for the terrestrial compartment was determined to be a maximum of 0.09.

The maximum predicted surface water concentration of lubabegron from a runoff event from a single annual application of manure to cropland from lubabegron treated animals when refined for adsorption is 0.007 µg lubabegron/L. The maximum predicted surface water concentration of

lubabegron from a runoff event when refined for potential accumulation in soil, and adsorption is 0.024 µg lubabegron/L. The lowest predicted no effect concentration (aquatic PNEC) for species tested in Tier-A is 0.25 µg lubabegron/L based on the EC_{50} for algal growth. The corresponding aquatic RQ for the most sensitive species tested in Tier-A is 0.09. The lowest predicted no effect concentration (aquatic PNEC) for species tested in Tier-B is 1.2 µg lubabegron/L based on the 21-day NOEC for *D magna* reproduction. The corresponding aquatic RQ for the most sensitive species tested in Tier-B is 0.02.

Based on the conclusions in this risk assessment, the lubabegron RQs are less than one for both the terrestrial and aquatic compartments. Therefore, the use of Experior™ in beef steers and heifers fed in confinement for slaughter is not expected to result in environmental impact through the application of cattle manure to cropland soil. The PEC/PNEC ratios are sufficiently low such that even if there were some spillage of the product which was then disposed of into the manure containment system and applied to land with the manure, adverse effects would be unlikely.

Secondary effects due to the use of Experior™ to reduce ammonia gas emissions in beef steers and heifers fed in confinement for slaughter are expected to only be beneficial to the environment and human health. The magnitude and scale of the beneficial effects cannot be reliably predicted due to the number of, and variability in, the factors influencing emissions and transport of ammonia and other nitrogen compounds.

Because this conservative risk assessment has concluded that there is no expected harm to the environment from the use of Experior™, further data collection beyond what has been provided here is not warranted to support the intended use.

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10.0 Persons and Agencies Consulted

This EA was prepared with input and assistance from members of the Environmental Safety Team in the Office of New Animal Drug Evaluation in FDA's Center for Veterinary Medicine.

11.0 References

Center for Veterinary Medicine, US Food and Drug Administration. 2001. Guidance for Industry: Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's)-Phase I. 89.

Center for Veterinary Medicine, US Food and Drug Administration. 2006. Guidance for Industry: Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's)-Phase II. 166.

Cole, N.A., and R.W. Todd. 2009. Nitrogen and phosphorus balance of beef cattle feedyards. Proc Texas Animal Manure Management Issues Conference.

Eli Lilly and Company. Confirmation of Structure of LY488756 hemifumarate (591281, SAM II) Amended. Steven W. Baertschi, Bradley M. Campbell, Matthew Clemens, Linda K. Dow, David E. Reed, Sharon E. Semones, W. Kimmer Smith, Tim A. Smitka, Gregory A. Stephenson, Brian E. Winger and Larry L. Zornes. July 11, 2002.

European Medicines Agency (EMEA), Committee for Medicinal Products for Veterinary Uses (CVMP). Revised Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 and GL38. Doc. Ref. EMEA/CVMP/ERA/41828/2005-Rev.1. London 17 November 2008.

Gentile, D.M. (2014). The Comparative Metabolism of [^{14}C]-LY488756 in Cattle, Landrace Pig, Rat, Dog and Human Hepatocytes and Microsomes. Study No. 196171, Report No. 34300.

Hamaker, J.W. The interpretation of soil leaching experiments. In: Haque, R. and V.H. Freed (eds) Environmental Dynamics of Pesticides. Plenum Press, New York, pp115-133. 1975.

Herbert, S., Hashemi, M., Chickering-Sears, C., and Weis, S. in collaboration with Miller, K, Carlevale, J., Campbell-Nelson, K, and Zenk, Z. Conserving Ammonia in Manure. University of Massachusetts Amherst Extension: Crops, Dairy, Livestock and Equine Program, https://ag.umass.edu/crops-dairy-livestock-equine/fact-sheets/conserving-ammonia-in-manure, accessed March 16, 2018.

Hristov, A. N., et al. (2011). "Review: Ammonia emissions from dairy farms and beef feedlots." Canadian Journal of Animal Science 91(1): 1-35.

National Research Council (NRC). Nutrient Requirements of Beef Cattle (Update 2000) Seventh Revised Edition, 1996. National Academy Press. Washington, D. C.

National Research Council. 2003. Air Emissions from Animal Feeding Operations: Current Knowledge, Future Needs. Washington, DC: The National Academies Press. https://doi.org/10.17226/10586.

Pinder, R.W., N.J. Anderson, R. Strader, C.I. Davidson, and P.J. Adams. 2003. Ammonia emissions from dairy farms: Development of a farm model and estimation of emissions from the United States. In Proc. 12th Int. San Diego, CA.

Roe, S.M., Spivey, M.D., Lindquist, H.C., Thesing, K.B., and R.P. Strait, Estimating ammonia emissions from anthropogenic nonagricultural sources – Draft final report, Emission Inventory Improvement Program, 2004.

USDA. Agricultural Air Quality Task Force White Paper. Ammonia emissions: what to know before you regulate. 2014. http://www.soils.usda.gov/wps/portal/nrcs/detail/national/air/taskforce/?cid=stelprdb1268645. Accessed on March 15, 2018.

United States Department of Agriculture (USDA). Livestock Slaughter 2014 Summary (April 2015), National Agricultural Statistics Service. ISSN: 0499-0544.

USEPA. 2004. National Emission Inventory - Ammonia Emissions from Animal Husbandry Operations. Draft Report.
https://www3.epa.gov/ttnchie1/ap42/ch09/related/nh3inventorydraft_jan2004.pdf

United States Environmental Protection Agency (USEPA). Guidance for selecting input parameters in modeling the environmental fate and transport of pesticides. Appendix A. 2009.

USEPA. 2014. 2014 National Emissions Inventory (NEI) Data.

United States Environmental Protection Agency (USEPA). Wildlife Exposure Factors Handbook USEPA December 1993 (EPA/600/R-93/187 December 1993).

Veith GD, Kosian P. 1983. Estimating bioconcentration potential from octanol/water partition coefficients. In: Mackay D, Paterson S, Eisenreich SJ, Simons MS eds. Physical Behavior of PCBs in the Great Lakes, Ann Arbor Sciences Publishers, Ann Arbor, pp 269-282.

VICH 2000, Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) – Phase I, VICH GL6 Final Guidance.

VICH 2006, Environmental Impact Assessments (EIAs) for Veterinary Medicinal Products (VMPs) – Phase II, VICH GL38 Final Guidance.

Wicksted, G. (2014). Pivotal Total Radioactive Residue Depletion Study in Beef Cattle Treated with [^{14}C]-LY488756 Orally at a Dose rate of 0.4 mg LY488756/kg Body Weight for Five Consecutive Days. Study No. 286652, Report No. 33878.

Appendices

Appendix A – Determination of the Dissociation Constant for LY488756 Following OECD Guideline 112; Study 14028.6105, Report Date: 28 April 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 112, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Potentiometric titration with a glass, hydrogen selective electrode was used for the determination of the acid dissociation constant (pK_a) of LY488756 at 20 ± 1 ºC. Test solutions of approximately 0.100 and 1.00 mg/mL were prepared in 50:50 dimethylformamide:purified reagent water. The solutions were titrated with 0.01 N sodium hydroxide to a pH value greater than 11.0. Samples were also titrated with 0.1 N hydrochloric acid to a pH value less than 1.5.

Results:

Testing was conducted at 20 ± 1 ºC with LY488756 at concentrations of 0.100 and 1.00 g/L. Triplicate samples were titrated with 0.01 N sodium hydroxide as well as 0.1 N hydrochloric acid. LY488756 produced titration curves with mean pKa values of 7.58 and 8.68 for base titration and a mean pKa value of 5.46 for acid titration. The pK_a of the secondary amine is 8.68 in this system.

Appendix B – Determination of the Water Solubility of LY488756 Following OECD Guideline 105; Study 14028.6104, Report Date: 25 February 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 105, GLP.

Test Articles: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Solubility at pH 4 was determined using the shake-flask method. The solutions were shaken at 20°C and aliquots were removed for analysis periodically. Solubility at pH 7 and 9 was determined using the column elution method. All samples were submitted for analysis by HPLC/UV.

Results:

The mean water solubility of LY488756 at pH 4 was determined at approximately 20 ºC using the shake-flask method. The results are summarized below:

pH	Mean Water Solubility (g LY488756/L)
4	0.0562

The mean water solubility of LY488756 at pH 7 and pH 9 was determined at approximately 20 ºC using the column elution method at flow rates of 25 and 12.5 mL/hour. The results are summarized below:

pH	Mean Water Solubility (g LY488756/L)
7	0.00603
9	0.000532

Appendix C – LY488756 - Determining the Partitioning Coefficient (n-Octanol/Water) by the Slow-Stirring Method Following OECD Guideline 123; Study 14028.6110, Report Date: 26 February 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 123, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, potency of 88.6% (as LY488756).

Methods:

For the definitive test, the *n*-octanol-saturated pH 4, 7 and 9 buffer quality control samples were prepared at concentrations of 2.50, 0.0313 and 0.00100 mg LY488756/L, respectively, by fortification with the 100, 10.0 and 0.100 mg LY488756/L, respectively, secondary stock solution. For the definitive test, the pH 4, 7 and 9 buffer-saturated *n*-octanol quality control samples were prepared at concentrations of 20.0, 30.0 and 50.0 mg LY488756/L. The test vessels containing the test solutions with Teflon®-coated stir bars were placed in a dark chamber on nine separate stir plates. The stirring rate was adjusted so that a vortex between both phases was created to a height of approximately 0.5 cm to a maximum of 2.5 cm. The vessels were slow-stirred continuously for a period of three days to establish equilibrium prior to sampling. The first sampling interval was day 3 of the test. Sampling continued daily thereafter (n=4) to corroborate that equilibrium was achieved. For each test sample, a subsample was removed from the collected buffer phase and from the collected *n*-octanol phase. All *n*-octanol test and QC samples were analyzed for LY488756 concentration by high performance liquid chromatography with ultraviolet detection (HPLC/UV). All aqueous buffer solutions and QC samples were analyzed for lubabegron (LY488756) using liquid chromatography/mass spectrometry (LC/MS/MS).

Results:

Analyses of the test samples determined that the mean log of the partition coefficient (log $P_{ow,AV}$) values of the test substance for pH 4, 7 and 9 were 1.38, 3.60 and 4.93, respectively (at 25 to 26 °C).

Appendix D – LY488756 - Determination of the Melting Point/Melting Range Following OECD Guideline 102; Study 14028.6107, Report Date: 21 January 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 102, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756), tested as 100%.

Methods:

A differential scanning calorimeter (TA Instruments DSC Q200) was used to determine the melting point. Indium was used as the reference material to verify the DSC calibration. For the definitive analysis, triplicate samples (7.216, 6.907 and 6.061 mg) were prepared and analyzed as described using an analytical program based on range analysis data obtained. The temperature program was started approximately 20 ºC below the onset of melting with ramps set at 2 ºC/minute through the peak and tail and on for an additional 10 ºC.

Results:

The melting point was determined in triplicate to be 175.46°C (448.61 K), 175.41 (448.56 K) and 175.45 ºC (448.60 K), respectively. The mean melting point of LY488756 was 175.44 ºC (448.59 K) with a standard deviation (SD) of 0.026 ºC and a relative standard deviation of 0.015%.

Appendix E – LY488756 - Determination of the Vapor Pressure by the Static Procedure Following OECD Guideline 104; Study 14028.6108, Report Date: 21 January 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 104, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756), tested as 100%.

Methods:

The test apparatus consisted of a sample chamber connected to a manometer and pressure regulation system and a detection zone. On the measuring side of the test system, valves were provided for the vacuum pump, the nitrogen balancing gas and the pressure measurement device (e.g., pressure gauge or manometer). The vapor pressure determination was performed at increasing temperatures (approximately 4 to 6 ºC higher), up to 51.1 ºC. This procedure was repeated until a minimum of three vapor pressure readings were recorded at each temperature.

Results:

The following table summarizes the results of the static vapor pressure experiment with LY488756:

Temp. (°C)	Temp. (K)	Temp. (1/K)	Vapor Pressure (Pa)	Ln (Pa)
29.9	303.05	0.00330	13.4	2.59
35.7	308.85	0.00324	17.4	2.86
40.5	313.65	0.00319	21.9	3.09
45.3	318.45	0.00314	24.9	3.22
51.1	324.25	0.00308	27.9	3.33
29.8	302.95	0.00330	15.3	2.73

When this data is plotted and linear regression is performed, the vapor pressure at 20 and 25 ºC is extrapolated to be 10.1 (7.59×10^{-2} mmHg) and 12.2 Pa (9.13×10^{-2}), respectively.

Appendix F – LY488756 - Determination of the Ultraviolet-Visible Absorption Spectrum Following OECD Guideline 101; Study 14028.6106, Report Date: 23 July 2013

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 101, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

The absorption spectra of the test solutions were measured using a Beckman Coulter A23615 Du 720 general purpose spectrophotometer, scanning single-beam, microprocessor-controlled, UV-Vis spectrophotometer. The UV-Vis spectrum was evaluated from 200 through 800 nm. A scan was performed of the test substance in the appropriate solution (i.e., 10% (1.0 M) HCl in methanol, 100% methanol or 10% (1.0 M) NaOH in methanol). This procedure was performed in triplicate for each matrix.

Results:

Absorption maxima were observed in the region of 203 and 285 nm for the LY488756 in pure methanol sample and the LY488756 in 10% (1.0 M) hydrochloric acid in methanol sample. Absorption maxima were observed in the region of 218 and 285 nm for the LY488756 in 10% (1.0 M) sodium hydroxide in methanol sample. Absorption maxima and corresponding molar extinction coefficients are summarized below:

LY488756 Sample	Concentration (Mole/L)	Mean Wavelength (nm)	Mean Extinction Coefficient (L/mol-cm)
Methanol	0.0000179	203	62,216
	0.0000179	285	17,009
10% (1.0 M) HCl in methanol	0.0000179	203	59,948
	0.0000179	285	17,362
10% (1.0 M) NaOH in methanol	0.0000179	218	41,694
	0.0000179	285	18,087

Appendix G – [14C]LY488756 - Soil Transformation Study under Aerobic Conditions Following OECD Guideline 307; Study 14028.6112, Report Date: 04 June 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 307, GLP.

Test Article: [Nitrile-^{14}C]LY488756 hemifumarate (lubabegron), Lot No. 09-096-100-15, reported to have a radiochemical purity of ≥ 98.0%.

Methods:

Soil	Soil Taxonomy	Location Source
DU	Loam	Northwood, North Dakota
MSL	Sandy Loam	Northwood, North Dakota
RMN	Loamy Sand	Northwood, North Dakota
PD	Sandy Loam	Northwood, North Dakota

Soil was dosed with [^{14}C]LY488756 at approximately 1.00 µg/g in glass metabolism vessels containing 50.0 g (dry weight equivalent) of soil.

Soils were incubated under aerobic conditions at 20 ± 2 ºC in the dark with soils adjusted to a moisture level of pF 2.0.

Analysis:

Duplicate vessels were processed and soils were extracted at various time intervals (0, 3, 7, 14, 31, 60 and 122 days). Extracts were analyzed by high performance liquid chromatography with radiochemical detection (HPLC/RAM). Evolved volatiles were analyzed by liquid scintillation counting (LSC) while non-extractable radioactivity was quantified by combustion followed by LSC.

Results:

The average material balance ranged from 93.0% to 94.6% of the applied radioactivity (% AR) for all soil-types tested over the course of the 122-day study.

The overall study results are summarized in the following table.

Soil Name	DU Soil	MSL Soil	RMN Soil	PD Soil
Texture	**Loam**	**Sandy Loam**	**Loamy Sand**	**Sandy Loam**
Mean material balance (% AR)	94.6	93.9	93.3	93.0
[^{14}C]LY488756 by HPLC/RAM at Day 0 in aerobic soil test systems (% AR)	90.2	90.0	88.7	92.5
[^{14}C]12-minute max peak by HPLC/RAM (% AR)	18.1 (Day 122)	4.5 (Day 122)	1.8 (Day 122)	1.1 (Day 122)
[^{14}C]20-minute max peak by HPLC/RAM (% AR)	ND	ND	2.8 (Day 0)	ND
$^{14}CO_2$ at Day 122 (cumulative % AR)	0.9	1.5	2.1	1.5
Volatile organic compounds at Day 122 (cumulative % AR)	ND	ND	ND	ND
Extractable radioactive residues (total) from soil at Day 122 (% AR)	65.8	76.5	79.7	83.2
[^{14}C]LY488756 by HPLC/RAM at Day 122 in test system (% AR)	47.7	71.9	77.4	82.0
% Radioactive residues bound to soil at Day 122 (% AR)	27.8	16.4	11.4	6.2
Disappearance of LY488756 in the aerobic soil test system (days)	DT_{50}: 141 DT_{75}: 283 DT_{90}: 470	DT_{50}: 365 DT_{75}: 729 DT_{90}: 1212	DT_{50}: 533 DT_{75}: 1066 DT_{90}: 1772	DT_{50}: 866 DT_{75}: 1733 DT_{90}: 2879
Disappearance of LY488756 in the aerobic soil test system including the degradate >10% AR (days)	DT_{50}: 289 DT_{75}: 578 DT_{90}: 960	Not applicable	Not applicable	Not applicable

ND = Not Detected; AR = Applied Radioactivity

The transformation of [^{14}C]LY488756 in soil was assessed in four different aerobic soil test systems incubated at a temperature of 20 ± 2 ºC and continuously in the dark. The four soils varied in pH, textural characteristics, organic matter content and microbial content.

Ultimate degradation is indicated by the observed accumulation of $^{14}CO_2$ over the course of the 122-day study. The cumulative amount of evolved $^{14}CO_2$ was 0.9%, 1.5%, 2.1% and 1.5% AR in the DU, MSL, RMN and PD soil aerobic test systems, respectively. Radioactivity was below detection (<0.1% AR) in the ethylene glycol volatile organic traps for the aerobic test system through Day 122 of the study.

One degradate was observed at >10% AR during the study at a retention time of approximately 12 minutes in the DU soil. Several minor regions of radioactivity were observed in the chromatograms for the three remaining soils (MSL, RMN and PD) including peaks at retention times of approximately 12 and 20 minutes. In all cases, these individual peaks represented less than 10% AR and were not considered further.

Based on the analytical concentration of LY488756 alone, the half-lives were 141, 365, 533 and 866 days for DU, MSL, RMN and PD soils, respectively. The DT90 values in the DU, MSL, RMN and PD soils were 470, 1212, 1772 and 2879 days, respectively. When including the degradate which comprised >10% AR in the DU soil the resulting half-life is 289 days for the DU soil.

Appendix H - LY488756 - Determination of Adsorption Coefficient, Koc, Following OECD Guideline 106; Study 14028.6111, Report Date: 27 January 2014 (Amended Final Completion Date: 10 July 2015)

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 106, GLP.

Test Article: [Nitrile-^{14}C]LY488756 hemifumarate (lubabegron), Lot No. 09-096-100-15, reported to have a radiochemical purity of ≥ 98.0%.

Methods:

Tier 1 (Preliminary): The following parameters were established: optimum soil to solution ratio, equilibration time, adsorption to containers and the stability of test substance.

Tier 2 (Adsorption Kinetics): The adsorption/desorption of [^{14}C]LY488756 at a nominal concentration of 1.02 mg/L, was performed in five soils (MSL, DU, PD, MT and ROE) using 0.01 M $CaCl_2$ over 24 hours. The soil to solution ratio was 1:100 for all five soils.

Tier 3 (Adsorption and Desorption Isotherms): Adsorption and desorption isotherms were constructed for five soils using five concentrations of the test substance for 24 hours.

Results:

K_{oc} Definitive Evaluation − Tier 1

[^{14}C]LY488756 was stable over 48 hours in $CaCl_2$. The equilibration time was established as 24 hours and there was less than 3% binding to test vessels. Based on the results of the Tier 1 test, Tier 2 and Tier 3 testing was conducted at a soil to solution ratio of 1:100 for 24 hours.

K_{oc} Definitive Evaluation − Tier 2

Adsorption and Desorption						
Soil to Solution Ratio	**Hour**	**Mean % Adsorption**	**K_d (mL/g)**	**K_{oc} (mL/g)**	**Mean % Desorption**	**K_{des} (mL/g)**
MSL Soil						
1:100	24	93.3	3318	134284	1.37	7019
DU Soil						
1:100	24	93.7	3760	47347	1.14	8354
PD Soil						
1:100	24	93.1	3051	370420	3.19	3941
MT Soil						
1:100	24	93.6	3887	330433	1.04	9187
ROE Soil						
1:100	24	93.6	3771	112455	1.16	8358

Isotherms − Tier 3

Soil Type	**1/n (Freundlich exponent)**	**Mean % Adsorption**	**K_F^{ads} (mL/g)**	**K_F^{oc} (mL/g)**	**Mean % Desorption**	**K_F^{des} (mL/g)**
MSL Soil	1.13	97.1	7678	310775	1.04	19079
DU Soil	0.907	97.5	2367	29802	0.94	8589
PD Soil	1.05	96.5	3642	442301	1.81	3743
MT Soil	1.18	97.5	11567	983177	2.16	16552
ROE Soil	0.982	97.4	3411	101744	2.09	21458

Appendix I – LY488756 – Determination of the Effects on Soil Microflora Activity Following OECD Guidelines 216 and 217; Study 14028.6113, Report Date: 18 June 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 216 & 217, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Test Soil:	Speyer 2.3 Soil: sandy loam, Germany; organic carbon content: 0.94%. Maximum water holding capacity: 37.3%. Microbial biomass prior to test start: 1.6% of total organic carbon. Microbial biomass at test termination: 1.1% of total organic carbon.
Test Conditions:	20 ± 2 °C
Nominal Test Concentrations:	Control, 0.25 and 2.5 mg LY488756/kg dry soil
Parameters Measured:	Nitrogen transformation (nitrification) Carbon transformation (respiration)
Sampling Intervals:	Days 0, 7, 14 and 28

Results:

Results, Nitrate Transformation Rate:	**% Difference (+/-) from Control**			
	Day	**0.25 mg/kg**	**2.5 mg/kg**	**Dinoseb Acetate**
	7	-78.8	409	555
	14	58.1	-139	-304
	28	16.2	-8.1	46.2
Results, Respiration:	**% Difference (+/-) from Control**			
	Day	**0.25 mg/kg**	**2.5 mg/kg**	**Dinoseb Acetate**
	0	20.7	24.9	-20.9
	28	4.13	6.21	87.5

The results of this study showed that, at both test concentrations, the differences in the nitrate formation rates and in the respiration rates were below the guideline trigger value of 25% difference from the control after 28 days of exposure.

Based on the differences in nitrate formation rates and soil microbial respiration rates for 28 days of exposure, it can be concluded that LY488756 has no long-term effects on soil microflora at the concentrations tested.

Appendix J – LY488756 – Seedling Emergence and Seedling Growth Test Following OECD Guideline 208; Study 14028.6114, Report Date: 23 January 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 208, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Application of Test Substance:	Mixed into sandy loam
Test Species:	Corn (*Zea mays*) Radish (*Raphanus sativus*) Perennial Ryegrass (*Lolium perenne*) Soybean (*Glycine max*) Tomato (*Lycopersicon esculentum*) Wheat (*Triticum aestivum*)
Effect Criteria:	14-Day percent emergence, fresh shoot weight and treatment-related morphological abnormalities were determined for each species.
Nominal Test Concentrations:	Corn and Soybean: 63, 130, 250, 500 and 1000 mg LY488756/kg soil (dry weight) Radish, Perennial ryegrass, Tomato and Wheat: 3.9, 16, 63, 250 and 1000 mg/kg soil (dry weight)
Measured Concentrations:	The measured concentrations of the stock solutions applied to the soil, 0.14, 0.48, 2.0, 3.3, 6.3, 13 and 30 mg LY488756/mL, indicated that the stock solutions closely approximated the desired nominal concentrations of 0.12, 0.48, 1.9, 3.9, 7.5, 15 and 30 mg LY488756/mL, respectively.

Results:

Since the measured concentrations in the stock solutions closely approximated the desired nominal stock concentrations, the results of this study are based on nominal test concentrations (mg LY488756/kg).

The results of this study are summarized in the following tables:

	Based on Nominal Application Rate (mg LY488756/kg)		
Corn	**EC25[a]**	**EC50[a]**	**NOEC**
Percent Emergence	> 1000 (NA[b])	> 1000 (NA)	1000
Fresh Shoot Weight	> 1000 (NA)	> 1000 (NA)	1000

[a] 95% confidence limits are presented in parentheses.

[b] NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

	Based on Nominal Application Rate (mg LY488756/kg)		
Perennial Ryegrass	**EC25[a]**	**EC50[a]**	**NOEC**
Percent Emergence	> 1000 (NA[b])	> 1000 (NA)	1000
Fresh Shoot Weight	440 (330 - 530)	790 (560 - ND[c])	250

[a] 95% confidence limits are presented in parentheses.

[b] NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

[c] ND = Not Determined. Corresponding upper 95% confidence limit could not be determined.

	Based on Nominal Application Rate (mg LY488756/kg)		
Radish	**EC25[a]**	**EC50[a]**	**NOEC**
Percent Emergence	> 1000 (NA[b])	> 1000 (NA)	1000
Fresh Shoot Weight	550 (300 - 920)	> 1000 (NA)	250

[a] 95% confidence limits are presented in parentheses.

[b] NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

	Based on Nominal Application Rate (mg LY488756/kg)		
Soybean	**EC25[a]**	**EC50[a]**	**NOEC**
Percent Emergence	> 1000 (NA[b])	> 1000 (NA)	1000
Fresh Shoot Weight	> 1000 (NA)	> 1000 (NA)	1000

[a] 95% confidence limits are presented in parentheses.

[b] NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

Tomato	Based on Nominal Application Rate (mg LY488756/kg)		
	EC25[a]	**EC50[a]**	**NOEC**
Percent Emergence	> 1000 (NA[b])	> 1000 (NA)	1000
Fresh Shoot Weight	> 1000 (NA)	> 1000 (NA)	1000

[a] 95% confidence limits are presented in parentheses.

[b] NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

Wheat	Based on Nominal Application Rate (mg LY488756/kg)		
	EC25[a]	**EC50[a]**	**NOEC**
Percent Emergence	> 1000 (NA[b])	> 1000 (NA)	1000
Fresh Shoot Weight	290 (98 - 380)	570 (440 - 690)	63

[a] 95% confidence limits are presented in parentheses.

[b] NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

Six crop species were tested (three monocotyledons and three dicotyledons) for sensitivity to LY488756. Based on a comparison of the EC25 values, the most sensitive monocotyledon species tested was wheat (*Triticum aestivum*), with an EC25 value of 290 mg LY488756/kg for fresh shoot weight. The most sensitive dicotyledon species tested was radish (*Raphanus sativus*), with an EC25 of 550 mg LY488756/kg for fresh shoot weight. In general, the monocotyledons tested were more sensitive to LY488756 than the dicotyledons tested. Additionally, fresh shoot weight was generally a more sensitive indication of exposure to LY488756 than percent emergence.

Appendix K – LY488756 - Chronic Toxicity and Reproduction Test Exposing the Earthworm *Eisenia fetida* in Artificial Soil, Based on OECD Guideline 222; Study 14028.6115, Report Date: 21 January 2014

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 222, GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Test Organism:	*Eisenia fetida*, SMV Lot No. 12A176 F_0 Generation age: 10 months old Original Source of Culture: Happy D Ranch, Visalia, California
Test Conditions:	Temperature range of 20 ± 2 °C (continuous soil temperature monitoring), a 16 hours of light at an intensity range of 400 to 720 lux and 8-hour dark photoperiod
Test Substrate:	Artificial soil substrate
Nominal Test Concentrations:	63, 130, 250, 500 and 1000 mg LY488756/kg
Nominal Stock Solution Concentrations:	1.9, 3.9, 7.5, 15 and 30 mg LY488756/mL
Day 0 Measured Stock Solution Concentrations:	1.6, 3.6, 6.7, 16 and 32 mg LY488756/mL

Results:

Results based on nominal concentrations are presented in the following table:

Toxicity Endpoint	Toxicity Value
28-day F_0 LC_{50} (95% confidence limits)	> 1000 mg LY488756/kg (NA)
28-day F_0 $NOEC_{survival}$	1000 mg LY488756/kg
28-day F_0 $NOEC_{weight\ change}$	1000 mg LY488756/kg
56-day F_1 $EC_{50\ reproduction}$ (95% confidence limits)	> 1000 mg LY488756/kg (NA)
56-day F_1 $NOEC_{reproduction}$	1000 mg LY488756/kg

NA = Not Applicable. LC/EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

Based on F_0 earthworm percent survival and weight change and F_1 reproduction, NOEC values were determined to be 1000 mg LY488756/kg and the LC/EC_{50} values were > 1000 mg LY488756/kg, indicating that LY488756 was not toxic to earthworms at concentrations ≤ 1000 mg LY488756/kg.

Table 1: Mean percent survival, mean weight of live organisms and percent weight change for the F_0 generation during the 28-day exposure of F_0 earthworms (*Eisenia fetida*) to LY488756

Nominal Concentration (mg LY488756/kg)	Replicate	Day 28 Survival (%)	Mean Individual Earthworm Weight[a] (g)		Mean Percent Weight Change (SD)[bc]
			Day 0	Day 28	
Control	1	100	0.3440	0.3131	
	2	100	0.3878	0.3663	
	3	100	0.3733	0.3366	
	4	100	0.3625	0.3060	
	Mean	**100**	**0.3669**	**0.3305**	**-10 (4.2)**
Solvent Control	1	100	0.3644	0.3349	
	2	100	0.3574	0.3142	
	3	100	0.3616	0.3051	
	4	100	0.3709	0.2988	
	5	100	0.3726	0.3438	
	6	100	0.3523	0.3097	
	7	100	0.3484	0.3349	
	8	100	0.3914	0.3681	
	Mean	**100**	**0.3649**	**0.3262**	**-11 (5.2)**
63	1	100	0.3592	0.3228	
	2	100	0.3520	0.3307	
	3	100	0.3622	0.3582	
	4	100	0.3668	0.3540	
	Mean	**100**	**0.3600**	**0.3414**	**-5.2 (3.9)**
130	1	100	0.3672	0.2829	
	2	100	0.3692	0.3477	
	3	100	0.3676	0.3725	
	4	100	0.3541	0.3171	
	Mean	**100**	**0.3645**	**0.3301**	**-9.5 (10)**
250	1	100	0.3471	0.3604	
	2	100	0.3593	0.3482	
	3	100	0.3907	0.3529	
	4	100	0.3736	0.3596	
	Mean	**100**	**0.3677**	**0.3553**	**-3.2 (5.5)**
500	1	100	0.3482	0.3601	
	2	100	0.3395	0.3712	
	3	100	0.3431	0.3597	
	4	100	0.3424	0.3651	
	Mean	**100**	**0.3433**	**0.3640**	**6.1 (2.6)**
1000	1	100	0.3387	0.3345	
	2	100	0.3749	0.3564	
	3	100	0.3672	0.3518	
	4	100	0.3738	0.3627	
	Mean	**100**	**0.3637**	**0.3514**	**-3.3 (1.6)**

[a] Mean individual weights were determined by dividing the group weight for each replicate by the number of surviving adults for that replicate.

[b] ((mean replicate weight, day 28 - mean replicate weight, day 0)/mean replicate weight, day 0) x 100.

[c] Values based on unrounded data and not on the rounded values (two significant figures) presented in this table.

SD = Standard Deviation.

Table 2: Number of offspring produced during the exposure of earthworms (*Eisenia fetida*) to LY488756

Nominal Concentration (mg LY488756/kg)	Replicate	Total Number of Surviving Offspring Per Vessel			Number of Offspring (Day 56) per Surviving Adult (Day 28)[a]
		Day 56	Day 57	Total	
Control	1	52	23	75	7.5
	2	64	23	87	8.9
	3	40	46	86	8.6
	4	0	59	59	5.9
	Mean (SD)[b]			**77 (13)**	**7.7 (1.3)**
Solvent Control	1	38	49	87	8.7
	2	60	24	84	8.4
	3	46	26	72	7.2
	4	4	54	58	5.8
	5	51	47	98	9.8
	6	14	32	46	4.6
	7	0	39	39	3.9
	8	0	78	78	7.8
	Mean (SD)			**70 (21)**	**7.0 (2.1)**
63	1	0	102	102	10
	2	9	52	61	6.1
	3	0	71	71	7.1
	4	0	100	100	10
	Mean (SD)			**84 (21)**	**8.4 (2.1)**
130	1	0	69	69	6.9
	2	0	60	60	6.0
	3	0	59	59	5.9
	4	0	43	43	4.3
	Mean (SD)			**58 (11)**	**5.8 (1.1)**
250	1	0	78	78	7.8
	2	0	53	53	5.3
	3	0	87	87	8.7
	4	0	54	54	5.4
	Mean (SD)			**68 (17)**	**6.8 (1.7)**
500	1	0	64	64	6.4
	2	0	63	63	6.3
	3	0	53	53	5.3
	4	0	64	64	6.4
	Mean (SD)			**61 (5)**	**6.1 (0.54)**
1000	1	0	52	52	5.2
	2	0	63	63	6.3
	3	0	77	77	7.7
	4	0	38	38	3.8
	Mean (SD)			**58 (17)**	**5.8 (1.7)**

[a] Adults (F_0) were removed on day 28 and cocoons present in the soil were allowed to hatch and young mature until exposure day 56.

[b] SD = Standard Deviation.

Appendix L - Study LY488756 (LSN 591281) – 72-Hour Acute Toxicity Test with Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following OECD Guideline 201; Study 14028.6101, Report Date: 07 May 2012

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 201, GLP.

Test Articles: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Test Organism:	*Pseudokirchneriella subcapitata*, inoculum - three days since previous transfer, source – Smithers Viscient culture
Dilution Water:	Algal Assay Procedure (AAP) medium
Test Conditions:	72-hour duration, temperature of 24 ºC, continuous illumination at 450 to 530 footcandles (4900 to 5700 lux), shaking rate of 100 rpm
Nominal Test Concentrations:	0.010, 0.026, 0.064, 0.16, 0.40 and 1.0 mg LY488756/L
Time-Weighted Average Concentrations:	0.0058, 0.015, 0.035, 0.10, 0.27 and 0.60 mg LY488756/L

Results:

	Based on Time –Weighted Average Concentrations (mg LY488756/L)			
Biological Parameter	**EC10 (95% Confidence Intervals)**	**EC20 (95% Confidence Intervals)**	**EC50 (95% Confidence Intervals)**	**NOEC**
0 - 72-Hour Yield	0.014 (0.0053 – 0.020)	0.017 (0.012 – 0.022)	0.025 (0.021 – 0.027)	0.015
0 - 72-Hour Average Growth Rate	0.018 (0.015 – 0.020)	0.021 (0.019 – 0.024)	0.032 (0.028 – 0.066)	0.015

The results are based on the time-weighted average concentrations of LY488756 and are reported as the 72-hour EC10, EC20 and EC50 values for biomass expressed as yield and

average growth rate data calculated from the 72-hour cell density counts. The No-Observed-Effect Concentration (NOEC) values for total yield and average growth rate were also determined. The EC50 values for growth rate and yield were determined to be 0.32 mg/L and 0.25 mg/L, respectively, based on nominal concentrations. The NOEC values for growth rate and yield were both 0.015 mg/L.

Appendix M – LY488756 (LSN 591281) - Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static Renewal Conditions, Following OECD Guideline 211; Study 14028.6103, Report Date: 24 May 2012

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 211**,** GLP.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Nominal concentrations of 0.0016, 0.0031, 0.0063, 0.013, 0.025 and 0.050 mg LY488756/L were chosen for the definitive exposure. During the definitive exposure, renewals took place daily in order to maximize exposure solution concentrations. Control and solvent control vessels were maintained under the same conditions as the treatment solutions, but contained no LY488756. The number of immobilized adult daphnids and observations of abnormal behavior were recorded daily. Numbers of offspring were determined upon the first brood release in any vessel and daily throughout the remainder the test. Offspring were removed, counted and discarded at each observation interval. In addition, the number of immobilized offspring was recorded for each treatment level and the control. At test termination (day 21), the total body length (from the apex of the head to the base of the carapace spine) of each surviving adult daphnid was measured. Daphnids were measured (to the nearest 0.01) mm using an Olympus SZ40/SZ-STS dissecting scope. Dissolved oxygen, temperature and pH were measured in each test and control solution at the beginning (new solutions) and end (aged solutions) of each renewal period. Total hardness, alkalinity and specific conductance were measured and recorded in solutions of either the 0.050 mg LY488756/L (days 0 and 7), the 0.025 mg LY488756/L (day 14) or 0.013 mg LY488756/L (day 21) nominal test concentration and the dilution water control at test initiation and weekly thereafter. On days 0, 7 and 14, all measurements were taken from freshly prepared solutions. On day 21 (test termination), all measurements were taken from aged solutions. The dissolved oxygen concentration and daily temperature were measured using a Yellow Springs Instrument (YSI) Model No. 550A or Model No. Pro20 dissolved oxygen meter/temperature probe. The pH was measured with a YSI Model pH100 pH meter. Total hardness and alkalinity of the test solutions were determined according to APHA et al., (1995). Specific conductance was monitored with a YSI Model No. 3100-115V salinity-conductivity-temperature (SCT) meter.

Results:

Mean measured concentrations of LY488756 ranged from 87% to 96% and defined the treatment levels tested as 0.0014, 0.0029, 0.0059, 0.012, 0.024 and 0.048 mg LY488756/L

Mean total body length at test termination among daphnids exposed to the control and solvent control was 4.70 and 4.69 mm per daphnid, respectively. Mean total body length among daphnids exposed to the 0.0014, 0.0029, 0.0059 and 0.012 mg LY488756/L treatment levels was 4.65, 4.64, 4.67 and 4.67 mm, respectively. Statistical analysis (Bonferroni's Adjusted t-Test) determined no significant reduction in mean total body length in any of the treatment levels statistically analyzed compared to the pooled control (4.69 mm per daphnid).

Based on survival as the most sensitive indicator of toxicity, the 21-day No-Observed-Effect Concentration (NOEC) was determined to be 0.012 mg LY488756/L. The Lowest-Observed-Effect Concentration (LOEC) was determined to be 0.024 mg LY488756/L.

The 21-day EC50 value for survival was determined by Spearman-Kärber Estimates to be 0.015 mg LY488756/L, with 95% confidence limits of 0.012 to 0.018 mg LY488756/L.

The 21-day EC50 value for reproduction and growth was empirically estimated to be > 0.012 mg LY488756/L, the highest mean measured concentration statistically analyzed.

The water quality characteristics in the control samples during definitive testing were as follows: pH: 7.8 to 8.4; dissolved oxygen: 7.8 to 10; specific conductance: 730 to 760 µS/cm; total hardness as $CaCO_3$: 190 mg/L; and total alkalinity as $CaCO_3$: 90 to 100 mg/L.

Table 1: Mean survival, reproduction and total body lengths of parental daphnids at termination of the 21-day static renewal toxicity test exposing daphnids (*Daphnia magna*) to LY488756

Mean Measured Concentration (mg LY488756/L)	Measured Endpoint		
	Mean Percent Survival	Mean Reproduction[a] (SD[b])	Mean Total Body Length in mm (SD)
Control	100	135 (8)	4.70 (0.08)
Solvent Control	100	138 (10)	4.69 (0.11)
Pooled Control	100	136 (9)	4.69 (0.09)
0.0014	100	124 (28)	4.65 (0.11)
0.0029	90	130 (11)	4.64 (0.06)
0.0059	100	134 (8)	4.67 (0.10)
0.012	90	136 (15)	4.67 (0.11)
0.024	0[c]	NA[d] (NA)	NA (NA)
0.048	0[c]	NA (NA)	NA (NA)

[a] Mean reproduction is based on the number of offspring produced from adults that survived through 21 days of exposure.

[b] SD = Standard Deviation.

[c] Significantly reduced compared to the pooled control, based on Fisher's Exact Test with Bonferroni-Holm's Adjustment. This treatment level was excluded from further statistical analysis due to the survival effect observed.

[d] NA = Not Applicable. All daphnids exposed to this treatment level were immobilized.

Appendix N - LY488756 (LSN591281) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline 203; Study 14028.6102, Report Date: 14 June 2012

Performing Laboratory: Smithers Viscient, 790 Main Street, Wareham, Massachusetts, 02571-1037.

Guidelines: OECD Guideline 203.

Test Article: LY488756 (lubabegron), Lot No. 160SB1, CAS No. 391926-19-5, reported to have a potency of 88.6% (as LY488756).

Methods:

Nominal Test Concentrations:	0.063, 0.13, 0.25, 0.50 and 1.0 mg LY488756/L
Geometric Mean Measured Concentrations:	0.050, 0.10, 0.20, 0.41 and 0.93 mg LY488756/L

The toxicity test was conducted in glass aquaria (length, width and height of 39 x 20 x 25 cm), each containing 15 L of test solution. One aquarium was established for each treatment level and each of the controls. The control was prepared containing 15 L dilution water only. The solvent control was also prepared containing 1.5 mL DMF in 15 L dilution water. All test vessels were examined at 0, 24, 48, 72 and 96 hours of exposure as follows: mortalities were recorded and removed, biological observations, including stress, abnormal behavior and adverse effects (e.g., darkened pigmentation), of the exposed rainbow trout and observations of the physical characteristics of the test solutions (e.g., presence of precipitate, cloudiness) were made and recorded. Daily pH was measured with a Yellow Springs Instrument (YSI) Model pH100 pH meter. Daily dissolved oxygen concentration and daily temperature were measured with a YSI Model No. 550A or a YSI Model No. pro20 dissolved oxygen meter/temperature probe. Temperature was continuously monitored throughout this study in the 0.50 mg LY488756/L (nominal) treatment level using a VWR Minimum-Maximum thermometer. All exposure solutions and QC samples were analyzed for LY488756 using liquid chromatography/mass spectrometry (LC/MS/MS) based on methodology validated at Smithers Viscient.

Results:

During preliminary testing, one exposure vessel containing ten fish was established for each treatment level and the controls. Following 96 hours of exposure, 100% mortality was observed among fish exposed to the 1.0 mg LY488756/L treatment level. No mortality or adverse effects were observed among fish exposed to any of the remaining treatment levels tested (0.00011, 0.0011, 0.011 and 0.11 mg LY488756/L) or the controls. Nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg LY488756/L were selected for the definitive study using a 1.0 mg LY488756/L stock solution.

At test termination, 100% mortality was observed in the 0.93 mg LY488756/L treatment level. No mortality or sublethal effects were observed among fish exposed to any of the remaining treatment levels tested (0.050, 0.10, 0.20 and 0.41) or the controls.

Based on geometric mean measured concentrations, the 96-hour LC50 value for *Oncorhynchus mykiss* exposed to LY488756 was determined by binomial probability to be 0.62 mg LY488756/L with 95% confidence interval of 0.48 to 0.80 mg LY488756/L. The No-Observed-Effect Concentration (NOEC) was determined to be 0.41 mg LY488756/L.

Appendix O - Pilot Efficacy Study: The Effect of Feeding Optaflexx or LY488756 During the Last 28 or 56 Days Before Slaughter on Beta-Receptor Dynamics and Metabolic Profile in Finishing Beef Cattle. Study Number D5CUS140021

Objective/Purpose

This study was conducted to determine tissue specific distribution of beta-receptor subtypes, metabolic profiles and glucose and insulin response in cattle fed Optaflexx or LY488756 during the last 28 or 56 days before slaughter.

Study Overview

Live Phase:

Thirty-five calves [23 steers and 12 heifers were used in this study. Three diets were fed: 1) control diet, 2) 5 g/ton LY488756 (100% dry matter; Elanco; Greenfield, IN), and 3) 27.3 g/ton ractopamine HCl (100% dry matter; Optaflexx; Elanco, Greenfield, IN). Treated cattle received medicated diets for either 28 or 56 days.

Sample Collection:

Plasma samples were collected on all animals at the initiation of the trial and then at d 10, 28, 38 and 56. At d 21 and 22 and again at d 49 and 50 of the study, glucose tolerance tests were conducted on all animals. Tissue samples collected included heart, lung, muscle (longissimus dorsi, psoas major, and semitendinosus) and adipose tissue for receptor subtype determination.

Analyses:

Plasma samples were analyzed for concentrations of glucose and insulin. Metabolic profile was analyzed using plasma samples. Tissue samples were analyzed for beta-receptor subtype (beta-1, beta-2, and beta-3) protein abundance with Western blotting techniques.

Results and Discussion:

Glucose and Insulin Response: For baseline, non-fasted glucose, there was not an effect of treatment ($P \geq 0.13$) at any time point in steers or heifers, however there was a treatment effect for insulin concentrations at d 38 in steers and d 28 in heifers. On d 38, insulin concentrations were less ($P \leq 0.05$) in steers fed RAC and LY488756 treatments compared with control steers. On d 28, insulin concentrations were less ($P \leq 0.05$) for heifers fed LY488756 and RAC compared with control heifers.

The first glucose tolerance test was conducted on d 21 and d 22 of the study. Insulin AUC was less ($P < 0.001$) in steers fed RAC and LY488756 compared with steers fed control diets, with no differences in glucose parameters across treatments. There were no differences ($P \geq 0.10$) for glucose tolerance test parameters in heifers.

The second glucose tolerance test was conducted on d 49 and d 50 of the study. The effect of treatment was not statically significant different ($P \geq 0.08$) for test parameters. However, the same trending differences were present as in the first glucose tolerance test. Insulin AUC was numerically reduced 16-30% in RAC fed for 49 d and LY488756 treated animals fed for 21 and 49 d compared to control cattle. However, insulin AUC was not different in cattle fed RAC for 21.

Metabolic Profiling: Metabolic profiling data revealed reduced creatine and increased creatinine in both LY488756 and Optaflexx treated cattle. This suggests an increase in protein synthesis and muscle hypertrophy with both compounds.

Receptor Subtype Determination: Due to the nature of sample analysis undertaken in the samples collected at slaughter, a true comparison of the abundance of beta-receptor subtypes in various tissues of cattle cannot be determined. However, from these results, it can be concluded the all three receptor subtypes are present in heart and lung tissue, in subcutaneous adipose tissue and in all three muscle tissues analyzed (longissimus dorsi, semitendinosus, and psoas major).

Conclusions

Based on the results of this study, insulin responsiveness was improved by feeding both RAC and LY488756 although the improvement was less consistent with RAC. Increased protein synthesis without alterations in protein degradation were also evident in metabolic profiling from both LY488756 and RAC fed cattle. All three beta receptor subtypes are present in heart, lung, subcutaneous adipose tissue, and in all three muscle tissues analyzed (longissimus dorsi, semitendinosus, and psoas major).

Appendix P - Metabolic Profiling of Beef Cattle Receiving LY488756. Study Number D5CUS130020

Objective

To determine changes in metabolic profile attributable to LY488756 from serum samples of steers fed 0 or 20 g/ton LY488756.

Methods

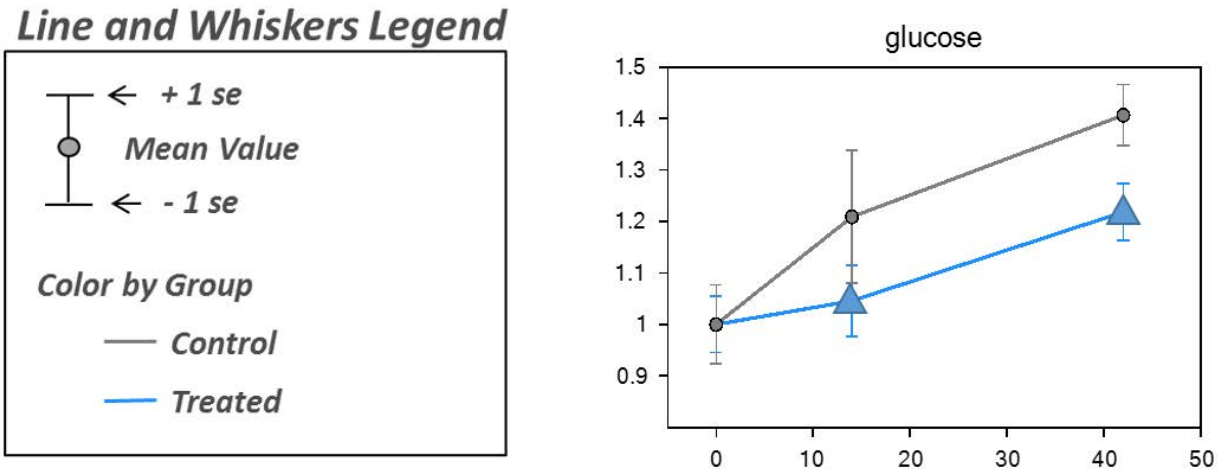
Serum samples were collected for metabolic profiling from finishing steers on study D5CUS110003. Steers were fed either 0 or 20 g /ton LY488756 (100% dry matter). Blood was collected prior to daily feeding before treatment initiation (day 0) and then 14 and 42 days after treatment initiation. At the conclusion of the trial, serum samples were selected from 2 steers closest to the treatment mean live weight from each pen for metabolic profiling.

Results

Glucose and Energy Metabolism

Cattle fed LY488756 had alterations in pathways used to maintain blood glucose and produce energy, namely gluconeogenesis and ketogenesis. This data demonstrated a reduction in blood glucose of cattle fed with LY488756. The reduced glucose levels of treated cattle compared with control at d 42 may be indicative of an improvement in insulin sensitivity. However, in both treated and control cattle, glucose tended to increase with increasing time on feed (Figure 1).

Figure 1. Legend and Cattle Blood Glucose



Units for figure are as follows: y-axis represents fold change differences between control and treated cattle; x-axis represents days on treatment.

The branched chain amino acids (Figure 2) valine, isoleucine, and leucine account for 14-18% of the amino acids in skeletal muscle protein. These amino acids are not used by the enterocytes; therefore, their concentration in the blood largely should reflect the balance between consumption from the diet and use by skeletal muscle for protein synthesis. Valine tended to be reduced and isoleucine was reduced at d14 in treated cattle compared to control. Leucine concentrations were unchanged. However, the dipeptides glycylvaline and leucylleucine were consistently reduced at d 14 and d 42 in treated cattle. Branched chain amino acids not used for skeletal muscle protein synthesis are oxidized resulting in a host of catabolites. Several of these catabolites (3-methyl-2-oxovalerate, 4-methyl-2-oxopentanoate, alpha-hydroxyisocaproate and isovalerylcarnitine) were all consistently ($P < 0.10$) reduced in cattle treated with LY488756 compared with control cattle (Figure 3). Taken together, these data suggest increased use of branched chain amino acids for muscle protein synthesis and therefore, reduced oxidation of excess branched chain amino acids.

Figure 2. Branched chain amino acids in blood

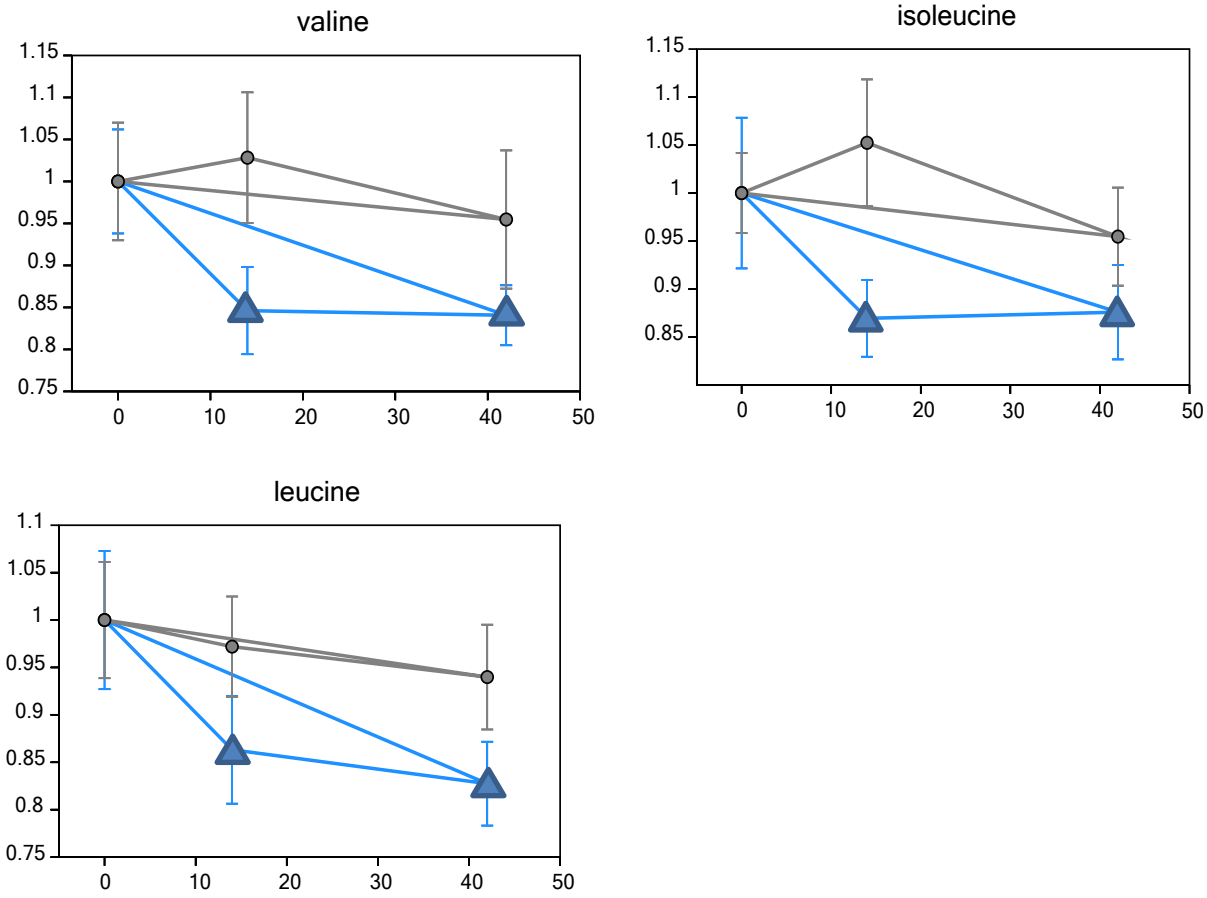
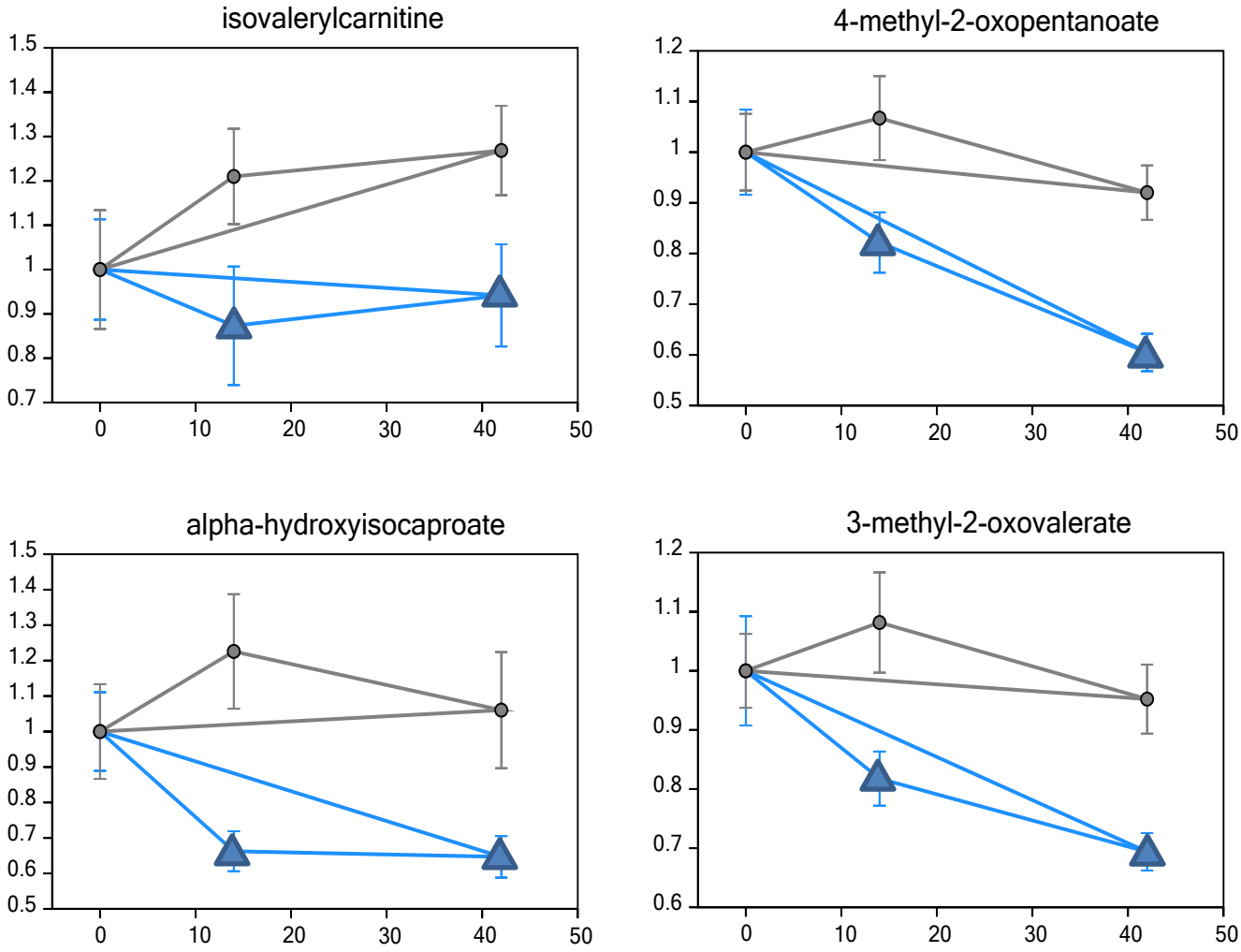


Figure 3. Catabolites of branched chain amino acid oxidation in blood



Conclusions

From these metabolite data, two main conclusions regarding the outcomes of LY488756 treatment in cattle can be drawn. Reductions in circulating branched chain amino acids along with reductions in catabolites of branched chain amino acids are observed. This suggests increased use of branched chain amino acids in muscle protein synthesis.

Appendix Q - Pilot Efficacy Study: Effect of Feeding LY488756 to Beef Cattle for the Last 42 Days of a Finishing Period on Environmental Measures and Animal Performance – Comparison of Response in Steers and Heifers. Study Number D5CUS110003

Objective/Purpose

This study was conducted to evaluate the 42 day animal performance of heifers compared to steers fed LY488756 at target doses of 0, 5, 10 or 20 g/ton (DM), and evaluate the effect of feeding LY488756 on nitrogen dynamics in cattle.

Study Overview

One hundred twenty healthy calves (72 heifers and 48 steers) were selected and randomly assigned to treatment groups and pens in the facility. During the 42 day treatment period animals were fed 0, 5, 10 or 20 g/ton of LY488756.

Results

Feeding LY488756 generally increased live body weight and had no impact ($P > 0.4321$) on feed intake. At the conclusion of the 42 d study period, mean treatment differences for BW between treated and control cattle were 14, 17, and 28 lbs for the 5, 10, and 20 g/ton groups, respectively. Compared to control cattle, cattle that received 5, 10, and 20 g/ton of LY488756 produced carcasses that were 20, 19, and 22 lbs heavier ($P < 0.05$), respectively..

In general, treatment of steers and heifers with LY488756 resulted in lower serum glucose values throughout the 42 d study period. Comparison of serum urea nitrogen changes from study Day 0 to 14 showed that cattle which received LY488756 had a 5 to 15% reduction compared to an 18% increase for control animals. No treatment effects were detected for serum urea nitrogen at completion of the study (day 42), however, there was a quadratic treatment effect ($P = 0.0210$) over the entire study (average of Day 14, 28, and 42) with a significant serum urea nitrogen reduction for the 10 g/ton dose (i.e., 4.4% reduction compared to a 24.6% increase for control animals). LY488756 did not affect serum creatinine concentrations. Feed intake, excretion, and apparent retention of ammonia N, total N, phosphorus, and potassium were not impacted by LY488756. Apparent retention efficiency for dry matter, ammonia N, nitrogen, phosphorus, and potassium when expressed as a ratio with calculated hot carcass weight gain were improved ($P < 0.05$) approximately 20 to 42% when cattle were supplemented with LY488756.

Feeding cattle LY488756 did not increase organic-N (i.e., non-ammonia-N) or total-N excretion (Table 1). Similarly, there was no effect on ammonia-N in manure.

Table 1. Least squares means (lb/hd/d) for average daily nitrogen in excreta (feces and urine mixed) during entire treatment duration (Day 0 through 42) pooled for steers and heifers[1]

Variable	P-value$_{(Dose)}$	Dosage level, g LY488756/ton			
		0	5	10	20
Ammonia-N	0.3570	0.0150	0.0166	0.0185	0.0145
Organic-N	0.7598	0.0828	0.0884	0.0908	0.0824
Total-N	0.6667	0.0977	0.1050	0.1093	0.0968

[1]Dosage by Sex interaction: $P \geq 0.4579$

Conclusion

Feeding LY488756 improved production measurements similarly in both steers and heifers. Directionally, increasing the dose of LY488756 resulted in increased live body weight and did not affect feed intake. Hot carcass weight increased as a result of dosing with LY488756 but the impact of an increased dose on carcass weight was not evident. Overall serum glucose levels were lower with LY488756 administration. Serum urea nitrogen levels were transiently decreased after 14 days of LY488756 administration contributing to reduction over the entire 42-day treatment period. LY488756 did not impact apparent retention of ammonia N, total N, phosphorus, or potassium, however, the apparent efficiency of dry matter, ammonia N, nitrogen, phosphorus, and potassium retention expressed in a ratio with calculated hot carcass weight gain did improve with feeding LY488756. Additionally, feeding cattle LY488756 did not increase organic-N (i.e., non-ammonia-N) or total-N excretion. Similarly, there was no effect on ammonia-N in manure.

Appendix R – Proposed Mode of Action of Experior™ (lubabegron)

1. Mode of Action

Elanco has conducted *in vivo* studies in cattle and observed three physiological responses of lubabegron that provide information about the fate of nitrogen post-absorption from the diet. These are (1) improved responsiveness to insulin, (2) reduced blood urea nitrogen, and (3) increased use of amino acids for muscle protein synthesis. A summary of these observed mechanisms of action in cattle fed lubabegron is included in Section 1.1, 1.2, and 1.3.

1.1 Insulin Responsiveness

Lubabegron improves the responsiveness to insulin: Cattle in study D5CUS140021 were administered a glucose challenge. Glucose area under curve (AUC) was relatively unchanged (Figure 1) following an intravenous infusion with 0.5 mL of a 50% glucose solution/kg of bodyweight while insulin AUC (Figure 2) was 27% less (Table 1) in cattle fed lubabegron at 5 g/ton (100% DM basis) for 21 days compared to controls. These data indicate that less insulin was produced as a response to clear the increased glucose blood levels after the glucose challenge. Additionally, in study D5CUS110003, significant reductions in circulating blood glucose concentrations were observed in cattle fed 5, 10, and 20 g/ton (100% DM basis) lubabegron (Table 2), which could also be an indication that insulin is more effective in driving glucose into the cell in lubabegron-treated cattle resulting in lower serum glucose. In addition to insulin's effect on entry of glucose into cells, insulin also promotes decreased plasma concentrations of branched chain amino acids either by promoting uptake of amino acids into tissues and subsequently increasing the rate of protein synthesis or decreasing proteolysis and branched chain amino acid release (Prior and Smith. J of Nutrition, 1983). In either case, the net effect of an improvement in insulin responsiveness is an increase in muscle protein in the carcass. Evidence that lubabegron increases the use of branched chain amino acids for muscle protein synthesis in cattle is provided in Section 1.3.

Figure 1. Blood Glucose Concentration

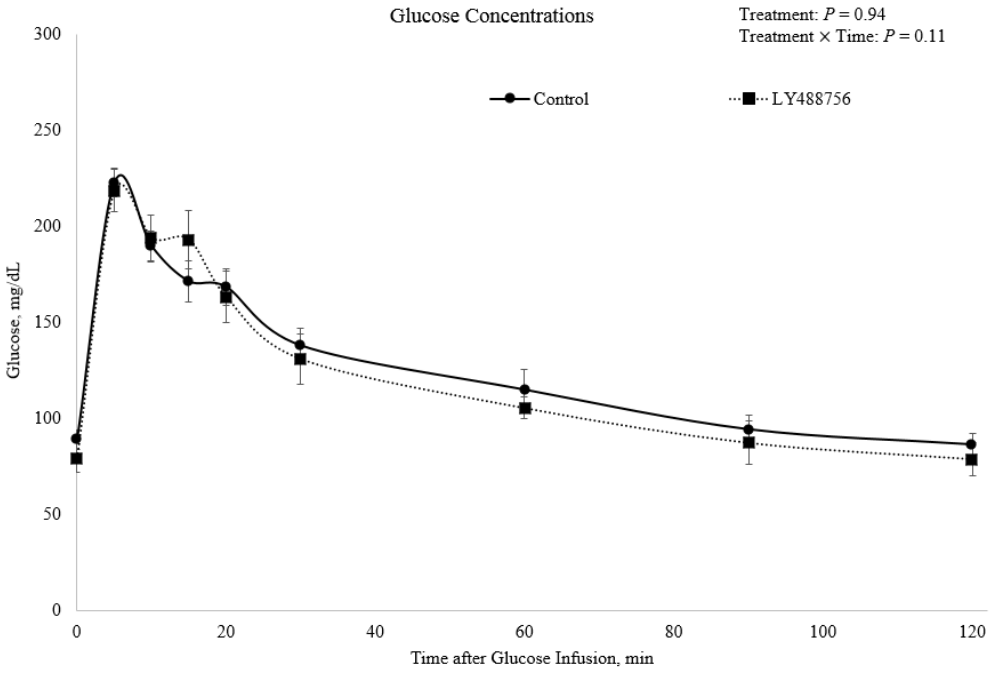


Figure 2. Blood Insulin Concentration in Cattle

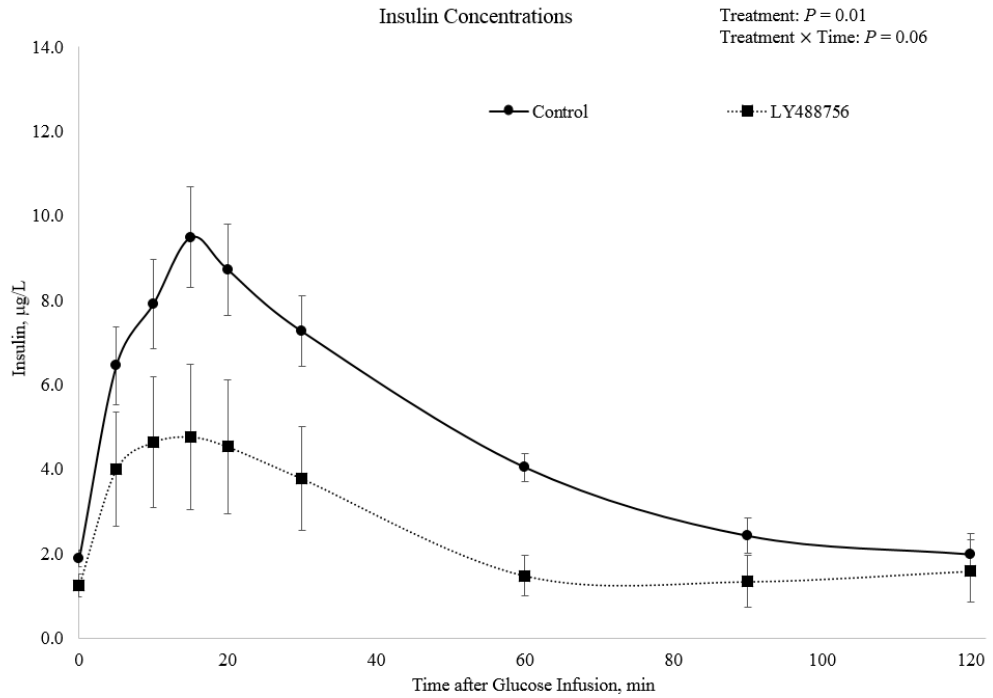


Table 1. Glucose tolerance test parameters of cattle fed lubabegron or a control diet sampled at d 21 and d 22 of study.

Parameter		Control	LY488756 [1]	*P*-value
Glucose				
	Fasted[2], mg/dL	86.09	81.25	0.87
	AUC[3]	178.75	210.22	0.55
	CR[4], $mg \cdot dL^{-1} \cdot min^{-1}$	0.75	0.69	0.17
Insulin				
	Fasted[2], µg/L	1.83	1.34	0.15
	AUC[3]	234.53[a]	170.48[b]	<0.001
	IIR[5], µg/L	12.23	7.26	0.34

Study: D5CUS140021. Supplementary Table 14.
[a-b]Means within row without a common superscript differ (P ≤ 0.05).
[1]LY488756 = 5 g/ton LY488756.
[2]Fasted: the average of the plasma concentrations collected 5 and 2 min prior to glucose infusion.
[3]AUC = area under the curve using a square-root adjustment after glucose infusion.
[4]CR = clearance rate; calculated as the slope of the regression line after glucose infusion.
[5]IIR = initial insulin response; calculated as the sum of concentrations 5 and 10 min after glucose minus the fasted concentration.

Table 2. Covariately adjusted[1] least squares means for glucose (mg/dl) during entire treatment duration (sample days 14, 28, and 42) pooled for Steers and Heifers[2].

Variable	P-value(Dose)	Dosage level, g lubabegron/ton			
		0	5	10	20
Serum glucose	0.0002[3]	63.3	54.2*	55.3*	54.6*

Study: D5CUS110003. Tables 18 and 24.
*Significantly different from 0 dose group, P < 0.05.
[1]Covariate = baseline value (Day 0) for serum glucose. Baseline for covariate was significant: P = 0.004.
[2]Dosage by Sex interaction: P = 0.7246
[3]Linear contrast P = 0.0017; quadratic contrast P = 0.0027

1.2 Blood Urea Nitrogen (BUN)

Serum or blood urea nitrogen (BUN) is a waste product of protein metabolism. BUN is formed by the liver from excess peptides and amino acids absorbed from the gastrointestinal tract that are not used for production (e.g., maintenance and growth). BUN is carried by the blood to the kidneys for excretion. In animals with normally functioning kidneys, BUN is cleared from the bloodstream by the kidneys and excreted as a waste product in the urine. There are many factors besides renal disease that can cause BUN alterations, including muscle protein breakdown, hydration status, and liver failure. In normal healthy animals, reductions in BUN from baseline can be used as an indicator for net protein accretion into the muscle. If the BUN concentration decreases from baseline after a compound is administered to an animal, BUN is either being utilized for muscle growth or less muscle is catabolized to BUN, thereby

decreasing the amount of urea excreted as a waste product in the urine. Therefore, any significant change in BUN observed after dosing of lubabegron can be assumed to be attributed to the protein accretion effects of lubabegron in cattle.

BUN was reduced in steers and heifers fed lubabegron at 5, 10, and 20 g/ton in study D5CUS110003. Kohn et al (2005) demonstrated that BUN is highly correlated with urinary nitrogen excretion rate. Since nitrogen in urine is largely in the form of urea, a reduction in BUN results in a reduction of urinary-urea excretion.

1.3 Muscle Protein Synthesis

A metabolic profile study (Study D5CUS130020; Appendix P) suggests feeding lubabegron to cattle increases the use of amino acids for muscle protein synthesis.

The branched chain amino acids (BCAA) valine, isoleucine, and leucine account for 14-18% of the amino acids in skeletal muscle protein. These BCAA largely escape catabolism by splanchnic tissue; therefore, their concentration in the blood largely reflects the balance between consumption from the diet and use by skeletal muscle for protein synthesis (Brosnan and Brosnan. J. of Nutrition, 2006). Valine tended to be reduced and isoleucine was reduced after feeding cattle lubabegron at 20 g/ton (100% DM basis) for 14 days compared to control. Leucine concentrations were statistically unchanged although numerically lower. Overall, BCAA in blood tended to be lower in lubabegron fed cattle (Figure 3).

Branched chain amino acids not used for skeletal muscle protein synthesis are oxidized resulting in a host of catabolites. Several of these catabolites (3-methyl-2-oxovalerate, 4-methyl-2-oxopentanoate, alpha-hydroxyisocaproate and isovalerylcarnitine) were all consistently ($P < 0.10$) reduced in cattle fed lubabegron compared to control cattle (Figure 4).

In summary, lubabegron increases the use of BCAA for skeletal muscle protein. As a result of reducing BCAA in blood, less is oxidized. These shifts in metabolic indicators suggest lubabegron increases muscle protein in cattle.

Legend for Figures 3 and 4.

Units for all figures are as follows: y-axis represents fold change differences between control and treated cattle; x-axis represents days on treatment.

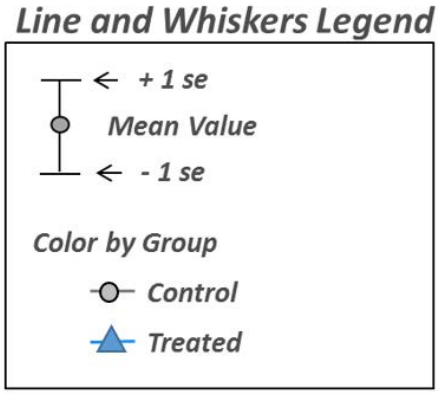
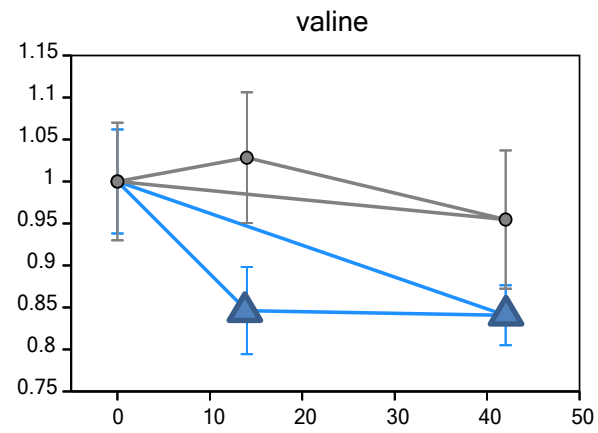
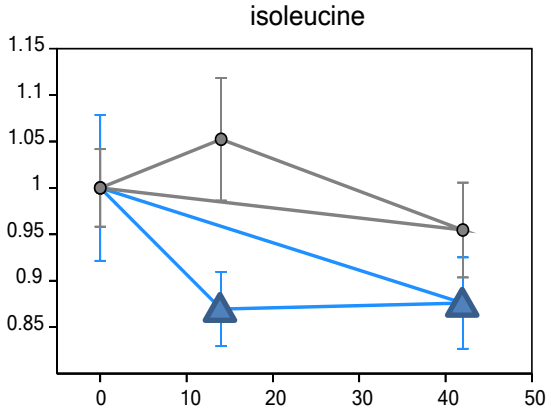


Figure 3. Branched chain amino acids in blood





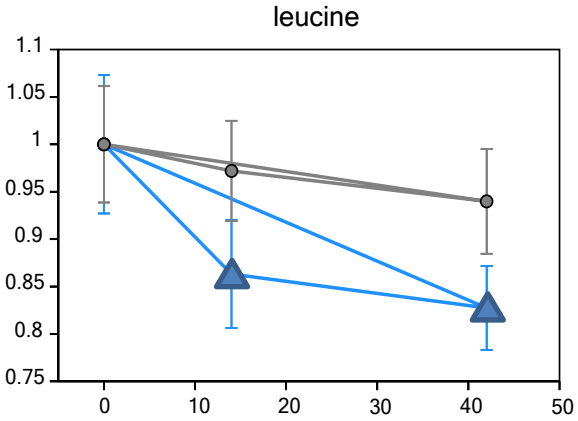
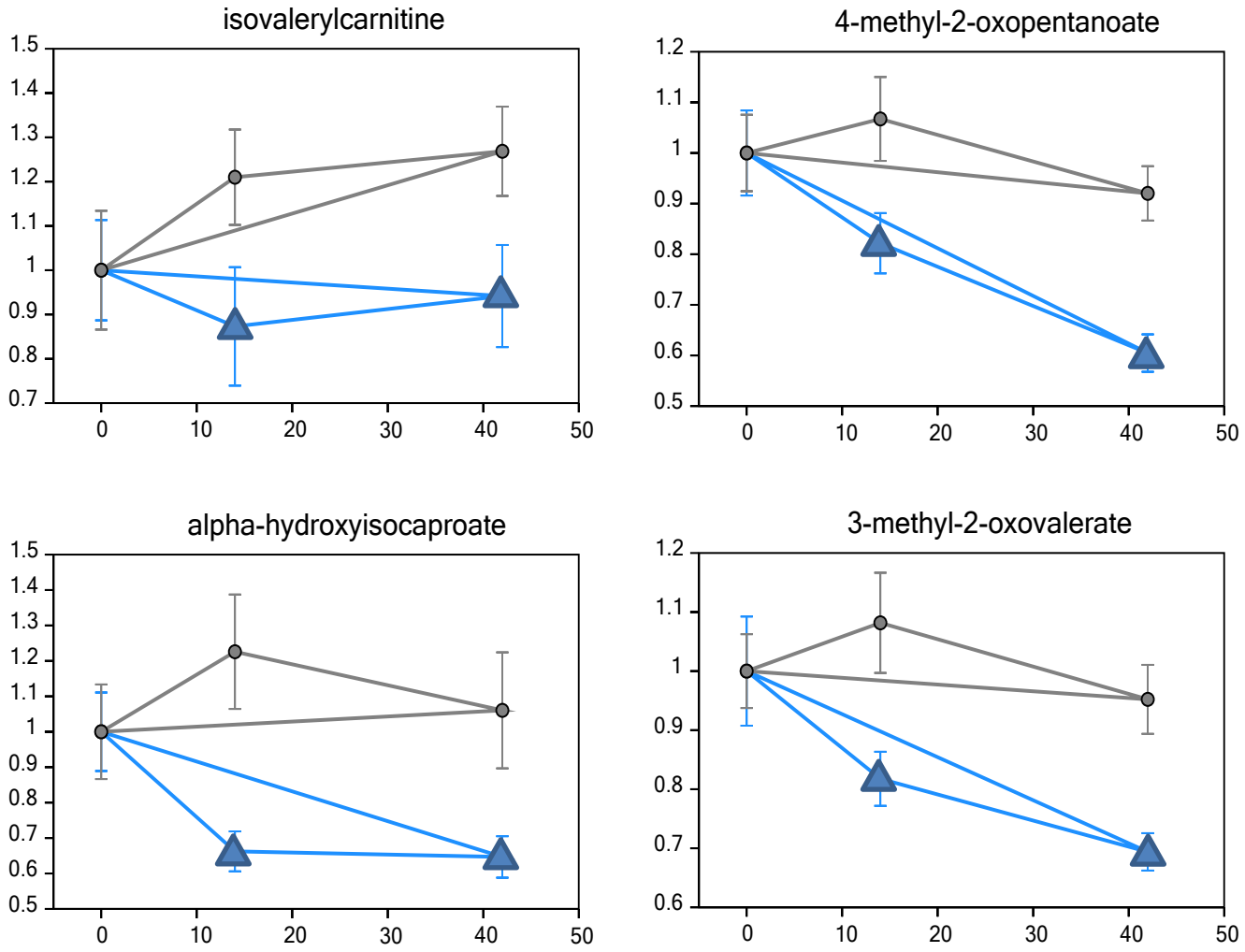


Figure 4. Catabolites of branched chain amino acid oxidation in blood



2. Manure Nitrogen Excretion

A study was conducted to measure the effect of lubabegron on nitrogen excretion (Study Number D5CUS110003; Appendix Q). Ammonia gas emissions were not monitored in this study. In this study, the manure was a mixture of feces and urine as accumulated on the pen floor. Feeding cattle lubabegron did not increase organic-N (i.e., non-ammonia-N) or total-N excretion (Table 3). There was no effect on ammonia-N in manure, presumably because the manure was allowed to accumulate, thereby permitting the loss of volatile ammonia prior to sampling.

Table 3. Least squares means (lb/hd/d) for average daily nitrogen in excreta (feces and urine mixed) during entire treatment duration (Day 0 through 42) pooled for steers and heifers[1]

Variable	P-value$_{(Dose)}$	Dosage level, g lubabegron/ton			
		0	5	10	20
Ammonia-N	0.3570	0.0150	0.0166	0.0185	0.0145
Organic-N	0.7598	0.0828	0.0884	0.0908	0.0824
Total-N	0.6667	0.0977	0.1050	0.1093	0.0968

Study: D5CUS110003. Tables 34 and 35.
[1]Dosage by Sex interaction: $P \geq 0.4579$

3. Stoichiometric Nitrogen Calculation

Based on the proposed mode of action, and the rapid liberation of ammonia gas from excreted urea, the reduced ammonia gas emissions in lubabegron fed cattle can be explained.
Formula 1 provides the stoichiometric calculation for the nitrogen conserved through reduced ammonia gas emissions (assuming 1000 g of reduced ammonia) and the nitrogen utilized for muscle protein in beef carcass.

Formula 1:
1000 g of ammonia is equivalent to 11.33 lb[1] of protein or 68.7 lb[2] of carcass.

[1]1000 g NH_3 x 0.8224 = 822.4 g N (ammonia is 82.24% N) / 0.16 (protein is 16% N; FAO 2003 – Section 2.1) = 5,140 g protein or 11.33 lb protein.

[2]11.33 lb protein / 0.165 (beef carcass is 16.5% protein; Heinz & Hautzinger, 2007- Table 1 on page 2) = 68.7 lb carcass.

3.1 Nitrogen Conversion Efficiency

Conversion efficiency is the ratio of actual hot carcass weight increase over the stoichiometrically calculated carcass weight increase. Using the stoichiometric calculations and the results from the 14-day (D5CUS130028) and 91-day (D5CUS130029) clinical effectiveness studies, approximately 57-100% of the nitrogen conserved, as manifested as reduced ammonia gas emission, can be accounted for by an increase in the carcass weight of lubabegron treated cattle (Formula 2).

Formula 2:

- 14-day study (D5CUS130028): The nitrogen conserved (i.e., 60 and 79 g reduced ammonia gas per animal for 1.25 and 5 g/ton dose levels, respectively) could result in 4.1 and 5.4 lb of additional carcass based on the stoichiometric calculation. Actual increase in hot carcass weight was 9 and 4 lb, respectively (i.e., 220 and 74% conversion efficiency, respectively).
- 91-day study (D5CUS130029): The nitrogen conserved (i.e., 690 and 923 g reduced ammonia gas per animal for 1.25 and 5 g/ton dose levels, respectively) could result in 47.4 and 63.4 lb of additional carcass based on the stoichiometric calculation. Actual increase in hot carcass weight was 34 and 36 lb (i.e., 72 and 57% conversion efficiency, respectively).

4. Conclusions

The data from these studies demonstrate the proposed mode of action of lubabegron. The evidence supports a conclusion that lubabegron directs nitrogen into carcass tissues. A commensurate reduction in blood urea nitrogen levels (BUN) occurs as a result of the increased nitrogen utilization in the animal. Urea excretion is reduced, which ultimately results in reduced ammonia gas emissions from lubabegron fed animals.

5. References

Brosnan, J.T., and M.E.Brosnan. 2006. Branched-Chain Amino Acids: Enzyme and Substrate Regulation. Published in a supplement to The Journal of Nutrition. Presented at the conference ‘‘Symposium on Branched-Chain Amino Acids,’’ held May 23–24, 2005 in Versailles, France.

Elanco Animal Health. Clinical Study (GCP): Effect of LY488756 (provided as LSN591281) on Environmental Emissions when fed to Beef Cattle for the Last 14 Days of Finishing. Study Number D5CUS130028. 2015.

Elanco Animal Health. Clinical Study (GCP): Effect of Feeding LY488756 (provided as LSN591281) on Environmental Emissions when fed to Beef Cattle for the Last 91 Days of Finishing. Study Number D5CUS130029. 2015.

FAO 2003. Food energy – methods of analysis and conversion factors. Report of a Technical Workshop, Rome, 3-6 December, 2002. FAO-UN. FAO Food and Nutrition Paper 77.

Heinz, G., and P. Hautzinger. RAP Publication 2007/20. Meat Processing Technology for Small to Medium Scale Producers. FAO-UN, 2007. ISBN: 978-974-7946-99-4.

Kohn, R.A., Dinneen, M.M., and E. Russek-Cohen Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim. Sci. 2005. 83:879–889.

Prior, R.L, and S.B. Smith. 1983. Role of Insulin in Regulating Amino Acid Metabolism in Normal and Alloxan-Diabetic Cattle. J of Nutrition, 113: 1016-1031.