Date of Approval: November 6, 2018

# FREEDOM OF INFORMATION SUMMARY

## ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-508

Experior™

lubabegron Type A medicated article

Type A medicated article to be used in the manufacture of Type B and Type C medicated feeds

beef steers and heifers fed in confinement for slaughter

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.

Sponsored by:

Elanco US Inc.

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# I. GENERAL INFORMATION

## A. File Number

NADA 141-508

## B. Sponsor

Elanco US Inc.
2500 Innovation Way
Greenfield, IN 46140

Drug Labeler Code: 058198

## C. Proprietary Name

Experior™

## D. Product Established Name

lubabegron Type A medicated article

## E. Pharmacological Category

Beta-adrenergic agonist/antagonist

## F. Dosage Form

Type A medicated article to be used in the manufacture of Type B and Type C medicated feeds

## G. Amount of Active Ingredient

Lubabegron (as lubabegron fumarate) - 10 g per kg (4.54 g per lb)

## H. How Supplied

10 kg bags

## I. Dispensing Status

OTC

## J. Dosage Regimen

Feed 1.25 to 4.54 g/ton (1.39 to 5 ppm) of complete feed (90% dry matter basis) to provide 13 – 90 mg lubabegron/head/day continuously to beef steers and heifers fed in confinement for slaughter as the sole ration during the last 14 to 91 days on feed.

## K. Route of Administration

Oral

**L. Species/Class**

Beef steers and heifers fed in confinement for slaughter

**M. Indication**

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.

## II. EFFECTIVENESS

The effectiveness of lubabegron Type A medicated article for the reduction of ammonia ($NH_3$) gas emissions from beef cattle manure was tested in five studies on a total of 536 animals. Three preliminary studies showed that lubabegron reduced $NH_3$ gas emissions at doses of 1.25 to 20 g/ton (100% dry matter basis) of medicated feed for different durations. Results from these studies supported testing lubabegron at a dose range of 1.25 to 20 g lubabegron/ton of Type C medicated feed on a 100% dry matter basis fed for 14 to 91 days in the clinical effectiveness program.

In the largest study, 336 animals were dosed for 91 days (D5CUS130029) and demonstrated that cumulative $NH_3$ gas emissions were reduced by 14 – 18%. This study also showed that the reduction in $NH_3$ gas emissions at the highest dose tested, 20 g/ton, was not different from that seen in the next highest dose, 5 g/ton. Based on this result, and to achieve a zero-day withdrawal period, the sponsor chose 5 g/ton of Type C medicated feed (on a 100% dry matter basis) as the top dose for the indication. To establish the minimum treatment duration of 14 days, a fifth study (D5CUS130028) was conducted, which showed evidence of reduction in $NH_3$ gas emissions during days 7-14. This evidence, along with Day 0-14 data from previous studies, supported the drug's effectiveness at the minimum duration of 14 days.

The results summarized in this section measure the primary effectiveness outcome as $NH_3$ gas expressed in grams per pound (g/lb) of live weight (LW) and hot carcass weight (HCW). Ammonia from animals derives primarily from the breakdown of urea in their manure (urine and feces). Ammonia gas emissions vary depending on the size of the animal, the quantity of feed consumed, and other factors. It was important to standardize emissions based on animal size to enable interpretation and comparability across studies. Live weight and HCW are common measurements used in beef cattle production, which beef cattle producers can readily recognize and interpret.

In addition to the five effectiveness studies, four field studies were conducted to examine the safety of lubabegron under expected conditions of use, as well as any impact on animal performance. The safety evaluation from the field studies is presented in the Safety section (III.B.3), and average daily gain (ADG), feed efficiency (FE), carcass characteristics, and meat quality are summarized in this section. Measurements of thriftiness, such as feed intake, growth rate, and FE are important indicators of overall animal health and the animals' ability to represent the population of feedlot cattle.

Lubabegron did not negatively affect most variables relevant to beef production (FE, ADG, carcass characteristics) or consumers (meat quality). Meat tenderness and chewiness were reduced slightly in cattle that received lubabegron compared to the

controls, but these differences were minor and unlikely to be noticed by the average consumer.

## A. Dosage Characterization

To support the proposed dose range, Elanco provided the reports for three studies. Two were conducted in Michigan in single-animal chambers, and the third was conducted in California in cattle pen enclosures (CPE). These were the same facilities used for the emissions effectiveness studies described in Section II.B.

1. Study D5CUS110009

   This 28–day study was carried out in Michigan in single animal chambers to evaluate the potential for lubabegron to reduce gas emissions from beef steers. Doses were 0 or 200 mg/hd/day (approximately 20 g/ton). Twelve steers were selected for the study, with six per treatment group. Control and medicated feeds were top-dressed onto the basal ration and lightly incorporated into the top layer of feed. Cumulative $NH_3$ gas emissions standardized for LW were observed to be reduced for the 14 – 27-day period in the treatment group compared to controls.

2. Study D5CUS120004

   This 42–day study was also carried out at the Michigan site to evaluate the potential for lubabegron to reduce gas emissions from beef steers. Doses were 0, 12.5, or 50 mg/hd/day (approximately 0, 1.25, or 5 g/ton). Twelve steers, four per treatment, were housed in individual animal chambers. Cumulative $NH_3$ gas emissions were observed to be reduced for the 50 mg/hd/day dose groups compared to controls for the 1 – 7, 8 – 14, 1 – 14, 29 – 41, and 1 – 41-day periods. Cumulative $NH_3$ gas emissions standardized for LW were observed to be lower for both non-zero dose groups compared to control for the 8 – 14 and 1 – 14-day periods.

3. Study D5CUS120010

   This 93–day study was conducted at the California site in CPEs to evaluate the potential for lubabegron to reduce gas emissions from beef steers. Fifty-six steers were blocked by weight and assigned to CPEs (14 steers per CPE). Each CPE received either 0 or 1.25 g/ton lubabegron (two CPE per treatment) in a medicated feed which was top-dressed and hand mixed into the top layer of a total mixed ration. Cumulative $NH_3$ gas emissions standardized for LW and HCW were significantly lower for the 1.25 g/ton treatment group compared to controls.

Taken together, these studies supported testing lubabegron Type A medicated article at a dose range of 1.25 to 20 g lubabegron/ton of Type C medicated feed on a 100% dry matter (DM) basis fed for 14 to 91 days in the clinical effectiveness program.

## B. Substantial Evidence

To support the indication for reduction of $NH_3$ gas emissions per pound of LW and HCW, two emissions effectiveness studies were conducted in environmental

chambers designed to measure gas emissions from cattle at the same California and Michigan locations. As described in Section II.A above, the California site employed CPEs designed to mimic feedlot conditions by housing groups of feedlot cattle together, while the Michigan site used individual animal chambers. In addition, to determine if there were any negative effects on growth, FE, carcass characteristics, and animal health under expected conditions of use, four field studies were conducted at two locations and at each proposed duration (14 and 91 days).

1. Gas Emissions Effectiveness Study: CPE

   a. Study Number: D5CUS130029

   b. Study Standard: Clinical Study (Good Clinical Practices[GCP])[1]

   c. Study Objective: To provide data to demonstrate the effectiveness of lubabegron Type A medicated article to reduce emissions of specific gases produced by beef steers and heifers fed in confinement for slaughter.

   d. Study Design: Within each of three study cycles, a completely randomized 4 x 2 factorial design with four treatment groups (0, 1.25, 5, and 20 g/ton of Type C medicated feed on a 100% DM basis) and two genders (heifer or steer) was conducted. Two frame sizes of cattle were utilized (medium and large). All cattle within a cycle were of a single frame size.

   e. Study Location: California

   f. Study Duration: 91 days

   g. Study Animals, Management and Housing: Heifers and steers were purchased from commercial suppliers. For Cycles 1 and 3, large-frame, Continental-type crossbred cattle of predominantly Charolais breeding with Limousin and Simmental influence were used. Heifers weighed on average 978 lb and steers weighed 1043 lb at study start (Day 0). For Cycle 2, medium-frame British-type crossbred cattle of predominantly Angus breeding with Hereford influence were used. Heifers weighed on average 899 lb, and steers weighed 1052 lb. Each CPE housed 14 animals in one pen. In each cycle, four CPEs held heifers and four held steers, with treatments randomized within gender to CPE, and CPE was the experimental unit. Animals were shipped to the facility approximately four weeks prior to the start of each cycle. Cattle were provided feed and water *ad libitum*.

[1] Guidance for Industry #85: Good Clinical Practice (VICH GL9)
https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052417.pdf

h. Study Dates: Study start and end dates per cycle are listed in Table II.1.

**Table II.1. Study start and end dates by cycle**

| Study Cycle | Start of Acclimation | Start of Treatment | End of Treatment |
|---|---|---|---|
| 1 | 4/20/2014 | 4/27/2014 | 7/27/2014 |
| 2 | 8/10/2014 | 8/17/2014 | 11/16/2014 |
| 3 | 11/30/2014 | 12/7/2014 | 3/8/2015 |

i. Facility Description: The facility consisted of eight totally enclosed 185 $m^2$ dirt-floored pens, each with a 13 $m^2$ concrete feed bunk located on the west side and a float-activated water trough on the east side of each pen. A small concrete pad was located under and adjacent to the water trough and also inside the animal pen adjacent to the feed bunk. Each CPE had two doors: a large roll-up door provided access for animal movement in and out of the CPE, and a small door provided personnel access in and out of the CPE without the need to open the large door. Doors and bunk flaps to the CPE were kept closed and secure when not in use.

j. Measurements: The following gas emissions from each chamber were monitored using gas analyzers and automatic data capture: $NH_3$, nitrous oxide ($N_2O$), hydrogen sulfide ($H_2S$), methane ($CH_4$), and carbon dioxide ($CO_2$).

**Table II.2. Gas emission measurements**

| Gas Concentrations Monitored | Analyzer |
|---|---|
| $NH_3$ | TEI 17i $NH_3$ Analyzer |
| $N_2O$ | TEI 46i Analyzer |
| $H_2S$ | TEI 450i Analyzer |
| $CH_4$ | TEI 55c Direct Methane Non-Methane Hydrocarbon Analyzer |
| $CO_2$ | TEI 410i Analyzer |

LabVIEW Ver. 2011 (National Instruments, Austin, TX, USA) software was used to record instrument readings every fifteen seconds during the sampling period. Emissions readings (ppm or ppb) from the gas analyzers and various system monitors (i.e., fan sensors, temperature, relative humidity) were interfaced on an acquisition computer and recorded as emissions data files using the LabVIEW software. The air sampling equipment and data logging computers were located in an air-conditioned modular building (emissions trailer) adjacent to CPE 1. For each CPE, a 103 m length of Teflon tubing was used to connect the CPE sampling location to the gas analyzers via a valve system. Gas measurements, for 15-minute periods, were obtained in sequential order starting with the inlet air (ambient air reference) and followed by the eight outlet locations of the CPE units. Net emissions were calculated as the concentrations difference between the air outlet and inlet (ambient air) multiplied by the ventilation rate (airflow rate).

Cattle were weighed on Days 0, 7, 14, 28, 56, and 91, and carcasses were weighed at slaughter. Additional variables were measured to determine if there were any negative effects on ADG, FE, and carcass characteristics. Carcass measurements included liver abscess score, dressing percentage, fat thickness, adjusted fat thickness, ribeye area, kidney, pelvic, heart fat (KPH), yield grade, marbling score, skeletal maturity, lean maturity, overall maturity, quality grade, and Warner-Bratzler Shear Force (WBSF).

Animal health was evaluated as described in Section III.

k. Statistical Analysis: Analyses of effectiveness variables (cumulative $H_2S$, $N_2O$, $NH_3$, $CH_4$, and $CO_2$ g per lb of LW or HCW) were conducted using a linear mixed model (PROC MIXED SAS version 9.2). The statistical model included fixed effects of lubabegron dose (Dose), Gender, and Dose × Gender; study cycle was included as a random effect. All tests were conducted at alpha=0.05. If Dose × Gender was not significant and the main effect of Dose was significant, each non-zero dose group was compared to the control group in a pair-wise fashion. CPE was the experimental unit.

l. Results: For the effectiveness variables, only mean cumulative $NH_3$ for the treated groups was significantly different from and numerically lower than the mean of the control in the 91-day study (Table II.3). There was no Dose x Gender interaction and the main effect of Dose was significant. Cumulative $NH_3$ gas emissions/lb LW were different from and lower for all dose groups ($P \leq 0.009$) compared to control. The lowest effective dose was 1.25 g/ton. No evidence was found that the mean at 5 g/ton was different from that at 20 g/ton. Similarly, for the effectiveness variable cumulative $NH_3$ gas emissions/lb HCW, all non-zero dose groups were significantly different from control, with response peaking with the 5 g/ton dose. These results indicate that there is no added benefit of doses higher than 5 g/ton.

Treatment did not affect ADG, FE, liver abscess score, or carcass characteristics. Treatment resulted in a slight increase in WBSF, but at a level unlikely to be distinguishable by consumers. For additional information, see Section II.B.3.l.2 below.

Animal health observations are discussed in Section III.

**Table II.3. Summary of effects on total $NH_3$ gas emissions, $NH_3$/lb LW and HCW, and initial and final LW for 0 to 91 days (LSMean) – D5CUS130029**

| Variables | 0 g/ton | 1.25 g/ton | 5 g/ton | 20 g/ton | P-value for overall Dose Effect |
|---|---|---|---|---|---|
| $NH_3$ per Pound of LW, g/lb | 6.18 | 5.51 (P=0.009)[a] | 5.32 (p=0.002) | 5.26 (p<0.001) | 0.004 |
| $NH_3$ per Pound of HCW, g/lb | 10.1 | 8.83 (p=0.004) | 8.49 (p<0.001) | 8.40 (p<0.001) | 0.001 |
| $NH_3$ Emissions, g/hd | 7783 | 7093 (p=0.076) | 6860 (p=0.023) | 6751 (p=0.013) | 0.052 |
| Initial LW, lb | 994 | 1001 | 1004 | 996 | 0.937 |
| Final LW, lb | 1250 | 1286 | 1283 | 1282 | 0.257 |

[a]P-value for the contrast between 0 g/ton and each dose level

2. Gas Emissions Effectiveness Study: Individual Animal Chambers

   a. Study Number: D5CUS130028

   b. Study Standard: Clinical Study (GCP)

   c. Study Objective: To provide data to demonstrate the effectiveness of lubabegron Type A medicated article to reduce emissions of specific gases produced by feedlot cattle, i.e., beef steers and heifers fed in confinement for slaughter.

   d. Experimental Design: Completely randomized 4 x 2 factorial design with four treatment groups (0, 1.25, 5, and 20 g/ton of Type C medicated feed on a 100% DM basis) and two genders (heifer or steer), and 15 animals per gender and treatment.

   e. Study Location: Michigan

   f. Study Duration: 14 days

   g. Study Animals, Management and Housing: Heifers and steers were purchased from a commercial supplier and selected for the study based on overall health and ease of management. Cattle were British crossbreds of predominantly Angus breeding. Heifers averaged 1204 lb and steers 1262 lb at the start of study. Due to the limited number of chambers available (12), the study used ten 14–day cycles. Each cycle included eight heifers and four steers (or four heifers and eight steers) housed in individual chambers and assigned to treatment balanced by gender. Cattle were provided feed and water *ad libitum*. Cattle were removed from the chambers and walked at least weekly during the study.

h. Study Dates: Relevant study dates by cycle are listed in Table II.4.

**Table II.4. Study cycle dates**

| Cycle No. | Acclimation Start | Treatment Start | Treatment End |
|---|---|---|---|
| 1 | 3/03/2014 | 3/10/2014 | 3/24/2014 |
| 2 | 3/31/2014 | 4/07/2014 | 4/21/2014 |
| 3 | 4/28/2014 | 5/05/2014 | 5/19/2014 |
| 4 | 9/01/2014 | 9/08/2014 | 9/22/2014 |
| 5 | 9/29/2014 | 10/06/2014 | 10/20/2014 |
| 6 | 10/27/2014 | 11/03/2014 | 11/17/2014 |
| 7 | 11/24/2014 | 12/01/2014 | 12/15/2014 |
| 8 | 1/05/2015 | 1/12/2015 | 1/26/2015 |
| 9 | 2/01/2015 | 2/09/2015 | 2/23/2015 |
| 10 | 3/02/2015 | 3/09/2015 | 3/23/2015 |

i. Facility Description: The facility consisted of 12 animal rooms (chambers; each 7 ft wide x 13 ft long x 8.5 ft high) with interchangeable penning and watering systems for different animal species. Each chamber accommodated one animal. Each chamber had two doors, a large door and a small door. The large door was used for animal loading/unloading, and allowed personnel access to the rear of the animal, the manure pan, and access for cleaning activities. The small door permitted access to the head of the animal, the feed bin, and water cup.

j. Measurements: The following gas emissions from each chamber were monitored using gas analyzers and automatic data capture software: $NH_3$, $N_2O$, $H_2S$, $CH_4$, and $CO_2$.

**Table II.5. Gas emission measurements**

| Gas Concentrations Monitored | Analyzer |
|---|---|
| $NH_3$ | TEI 17i $NH_3$ Analyzer |
| $N_2O$ | TEI 46i Analyzer |
| $H_2S$ | TEI 450i Analyzer |
| $CH_4$ | TEI Model 55i (or Model 55c) Analyzer |
| $CO_2$ | Xstream 200M Analyzer |

LabVIEW Ver 8.3 (National Instruments; Austin, TX, USA) software was used to monitor gas concentrations, which began with incoming, outside ambient air for 15 minutes, then sequentially through each of the 12 chambers' exhaust air for 15 minutes per chamber. During each 15-minute period, the tubing was purged for the first 9.5 minutes and data collection occurred during the last 5.5 minutes. All gases were measured simultaneously within a sample stream. Samples from the chambers were pulled to a sampling manifold using a Cole-Parmer vacuum pump (Cole-Parmer Instrument Company, Vernon Hills, IL, USA) at a rate of approximately 8 to 9 L/min through Teflon tubing placed 12.7 cm into the exhaust duct of each individual chamber. Chamber air

and ambient air gas concentrations (background ambient sample) were sampled using the same methodologies. From the sampling manifold, the air stream was diverted into the gas analyzers.

Gas concentrations were recorded every 30 seconds during the last 5.5 minutes of sampling in each chamber (or ambient air) for a total of approximately 11 observations per sampling period, with approximately 6 to 8 sampling periods per day for each chamber. The concentration of each analyte gas in the incoming ambient air was subtracted from the average chamber gas concentrations for each period to provide the net concentration from the chamber. The average chamber airflow rate measured during the period was used to calculate the emission rate of analyte gas for that period. Daily average gas emissions were calculated from data available on a given day (24-hour period). Treatment phase emissions data collection began at 8:00 am on Day 0 and ended at 5:00 am on Day 14 for each cycle.

Cattle were weighed on Day 0, 7, and 14, and carcasses were weighed at slaughter. Additional variables were measured to determine if there were any negative effects on ADG, FE, and carcass characteristics. Carcass measurements included liver abscess score, dressing percentage, fat thickness, adjusted fat thickness, ribeye area, KPH, yield grade, marbling score, skeletal maturity, lean maturity, overall maturity, quality grade, and WBSF.

Animal health was evaluated as described in Section III.

k. Statistical Analysis: Analyses of effectiveness variables (cumulative $H_2S$, $N_2O$, $NH_3$, $CH_4$, and $CO_2$ (g) per lb of LW or HCW) were conducted using a linear mixed model (PROC MIXED, SAS version 9.2). The statistical model included fixed effects of lubabegron dose (Dose), Gender, and Dose × Gender; study cycle was included as a random effect. All tests were conducted at alpha=0.05. If Dose × Gender was not significant and the main effect of Dose was significant, each non-zero dose group was compared to the control group in a pair-wise fashion using linear contrasts. Chamber was the experimental unit.

l. Results: None of the gases, when cumulative mass was adjusted for LW, were statistically significantly affected by dose when measured for days 0 – 7 or 0 – 14 ($P > 0.05$). Similarly, no gases, when cumulative mass was adjusted for HCW, were significantly affected by dose for the 0 – 14-day period. Results for $NH_3$ are provided in Table II.6.

**Table II.6. Summary of effects on total $NH_3$ gas emissions, $NH_3$/lb LW and HCW, and initial and final LW, and HCW for 0 to 14 days (LSMean) – D5CUS130028**

| Variables | 0 g/ton | 1.25 g/ton | 5 g/ton | 20 g/ton | P-value Dose |
|---|---|---|---|---|---|
| $NH_3$ per lb of LW, g/lb | 0.642 | 0.594 | 0.582 | 0.551 | 0.130 |
| $NH_3$ per lb of HCW, g/lb | 1.00 | 0.917 | 0.900 | 0.852 | 0.093 |
| $NH_3$ Emissions, g | 823 | 763 | 744 | 701 | 0.113 |
| Initial LW, lb | 1237 | 1238 | 1226 | 1232 | 0.669 |
| Final LW, lb | 1277 | 1273 | 1268 | 1267 | 0.843 |
| HCW, lb | 819 | 828 | 823 | 821 | 0.798 |

The sponsor conducted additional analyses of the 7 – 14-day period of treatment with appropriate adjustment (Bonferroni correction) for multiple simultaneous statistical tests. The analysis showed a significant association between treatment and the mean cumulative $NH_3$ gas emissions per lb of LW and HCW during the last 7–14 days of treatment. Pairwise comparisons for $NH_3$ gas emissions standardized to HCW showed significantly different and numerically reduced emissions for all dose groups compared to controls. Pairwise comparisons for $NH_3$ gas emissions standardized to LW indicated the 5 and 20 g/ton dose groups, but not the 1.25 g/ton dose group, were significantly different and numerically reduced compared to controls. However, additional analysis (see below) and the weight of evidence consideration in the next section indicate that the 1.25 g/ton dose is also effective in reducing $NH_3$ gas emissions standardized to LW.

The sponsor also conducted an outlier analysis to determine if any animals or cycles presented anomalous data. The analysis indicated that Cycles 6 and 10 had average daily DM intake (ADDMI), LW, and HCW that were very different from the other eight cycles, outside of what would normally be expected from 99.7% of this population of study animals, although no obvious reason for these deviations could be determined. Analyses excluding the data from Cycles 6 and 10 indicated that $NH_3$ gas emissions standardized to LW and HCW were significantly different and numerically reduced for all non-zero treatments compared to controls in the 0-14-day period.

Treatment did not have any negative effects on ADG, FE, or carcass characteristics. There was a slight increase in WBSF, but this is unlikely to be noticed by consumers.[2] For additional information on carcass effects, see the discussion in section II.B.4.l below.

Animal health observations are discussed in Section III.

[2] Guelker, M.R, A.N. Haneklaus, J.C. Brooks, C.C. Carr, R.J. Delmore, Jr., D.B. Griffin, D.S. Hale, K.B. Harris, G.G. Mafi, D.D. Johnson, C.L. Lorenzen, R.J. Maddock, J.N. Martin, R.K. Miller, C.R. Raines, D.L. VanOverbeke, L.L. Vedral, B.E. Wasser, and J.W. Savell. 2013. National Beef Tenderness Survey – 2010: Warner-Bratzler shear force values and sensory panel ratings for beef steaks from United States retail and food service establishments. J. Anim. Sci. 91: 1005 – 1014.

3. Weight of Evidence of Effectiveness at 14 Days of Treatment

While a maximum duration of 91 days of treatment was established during study D5CUS130029, a minimum duration of treatment needed to be determined. The weight of evidence evaluation of effectiveness at 14 days of treatment considered evidence from multiple studies where data on gas emissions during the first 14 days were available. These studies are listed in Table II.7.

The additional analyses conducted for the 14-day study above (D5CUS130028) showed evidence of reduction in mean $NH_3$ gas emissions standardized to LW and HCW for the 1.25, 5, and 20 g/ton dose groups. In addition, the sponsor examined results from study D5CUS130029, discussed in the previous section, as well as three additional studies (D5CUS110009, D5CUS120004, and D5CUS120010) where gas emissions and LW were measured during the first 14 days. These studies were previously discussed in Section II.A.

**Table II.7. Studies to support effectiveness of lubabegron for 14-day duration**

| Study No. | Location | Animals | Gender | Doses | Exp. Unit | Duration (days) |
|---|---|---|---|---|---|---|
| D5CUS130028 | Michigan | 120 | steers & heifers | 0, 1.25, 5, 20 g/ton | chamber | 14 |
| D5CUS130029 | California | 336 | steers & heifers | 0, 1.25, 5, 20 g/ton | CPE[a] | 91 |
| D5CUS110009 | Michigan | 12 | steers | 0, 200 mg/hd/d | chamber | 28 |
| D5CUS120004 | Michigan | 12 | steers | 0, 12.5, 50 mg/hd/d | chamber | 42 |
| D5CUS120010 | California | 56 | steers | 0, 1.25 g/ton | CPE | 93 |

[a] There were 14 animals per pen, one pen per CPE (experimental unit)

Results for cumulative $NH_3$ emissions and $NH_3$ emissions per lb of LW at day 14 for each study, as well as cumulative $NH_3$ emissions per lb of HCW at the end of each study, are presented in Table II.8. The results from the three studies were not statistically analyzed and not all doses were used in each study. Rather, these results were used to look for trends.

**Table II.8. Comparison of 14-day cumulative absolute $NH_3$ gas emissions and emissions per lb LW and HCW across five studies[a]**

| Study No. | Measure | 0 g/ton | 1.25 g/ton | 5 g/ton | 20 g/ton |
|---|---|---|---|---|---|
| D5CUS130028 | 14-d $NH_3$ (g/hd) | 823 | 763 | 744 | 701 |
| D5CUS130028 | 14-d $NH_3$ (g/lb LW) | 0.64 | 0.59 | 0.58 | 0.55 |
| D5CUS130028 | 14-d $NH_3$ (g/lb HCW) | 1.00 | 0.92 | 0.90 | 0.85 |
| D5CUS110009 | 14-d $NH_3$ (g/hd) | 171 | NA | NA | 150 |
| D5CUS110009 | 14-d $NH_3$ (g/lb LW) | 0.16 | NA | NA | 0.14 |
| D5CUS110009 | 28-d $NH_3$ (g/lb HCW) | 0.53 | NA | NA | 0.41 |
| D5CUS120004 | 14-d $NH_3$ (g/hd) | 494 | 397 | 355 | NA |
| D5CUS120004 | 14-d $NH_3$ (g/lb LW) | 0.40 | 0.32 | 0.30 | NA |
| D5CUS120004 | 41-d $NH_3$ (g/lb HCW) | 1.98 | 1.74 | 1.47 | NA |
| D5CUS130029 | 14-d $NH_3$ (g/hd) | 953 | 835 | 801 | 699 |
| D5CUS130029 | 14-d $NH_3$ (g/lb LW) | 0.91 | 0.79 | 0.75 | 0.67 |
| D5CUS130029 | 91-d $NH_3$ (g/lb HCW) | 10.10 | 8.83 | 8.49 | 8.40 |
| D5CUS120010 | 14-d $NH_3$ (g/hd) | 1301 | 1177 | NA | NA |
| D5CUS120010 | 14-d $NH_3$ (g/lb LW) | 1.08 | 0.98 | NA | NA |
| D5CUS120010 | 93-d $NH_3$ (g/lb HCW) | 10.61 | 9.12 | NA | NA |

[a] NA – not applicable – dose was not tested

For all studies, mean cumulative $NH_3$ gas emissions and emissions per lb of LW were numerically lower at 14 days of treatment in response to lubabegron. Also, mean cumulative $NH_3$ gas emissions were numerically reduced per lb of HCW, regardless of the length of the study. For study D5CUS130029, conducted for substantial evidence of effectiveness, these results for HCW were statistically significant (See Section II.B.2).

Given the consistency of response to lubabegron across all five studies, FDA concluded that effectiveness at the minimum duration of 14 days is supported. Furthermore, the following statement is provided on approved labeling in the Indications for Use section "Effectiveness has not been demonstrated when fed for less than 14 days."

4. Field Studies:

   a. Study Numbers: D5CUS130044, D5CUS140001, D5CUS130043, D5CUS140002.

   b. Study Standard: Clinical Study (GCP)

   c. Study Objective: To provide data to demonstrate the safety, and impact on production and meat quality, of lubabegron Type A medicated article under expected conditions of use when fed to beef steers and heifers fed in confinement for slaughter at the proposed doses during the finishing period.

   d. Experimental Design: Randomized complete block design with an unbalanced factorial design of four dose levels (0, 1.25, 5, and 20 g lubabegron/ton of Type C medicated feed on a 100% DM basis), two genders (heifer and steer), and two frame sizes of cattle (medium and

large). Within each site and duration, there were 5 blocks of cattle per gender (four pens per block); one study site used 3 blocks of large frame cattle and 2 blocks of medium-frame cattle and vice versa for the other site. Within each block, pens were randomly assigned to one of the 4 lubabegron doses.

e. Study Locations: Idaho, Nebraska

f. Study Duration: 14 or 91 days

g. Study Animals, Management and Housing: For each location and duration, 480 animals (240 heifers and 240 steers) were obtained and were on site by Day -56 and began acclimation to basal finishing diet. Large frame cattle were represented by Continental crossbreds, and medium-frame cattle were represented by British crossbreds. Cattle were managed initially in large pens. On or prior to Day -29, cattle were examined for implants and general health, and heifers were examined for pregnancy. Implants, if found, were removed, and heifers diagnosed pregnant were excluded from study. Eligible animals were moved to study pens (eight head per pen) on Day -20 to Day -16. Cattle had *ad libitum* access to feed and water throughout.

h. Study Dates: Study number and relevant dates are listed in Table II.9.

**Table II.9. Study number, location, duration, and dates for field safety studies**

| Study No. | Location | Duration (days) | Start Date | End Date |
|---|---|---|---|---|
| D5CUS140001 | Nebraska | 14 | 9/10/2014 | 9/24/2014 |
| D5CUS130044 | Nebraska | 91 | 7/16/2014 | 10/15/2014 |
| D5CUS140002 | Idaho | 14 | 10/21/2014 | 11/04/2014 |
| D5CUS130043 | Idaho | 91 | 9/02/2014 | 12/02/2014 |

i. Facility Description: The Nebraska site utilized cement-floored pens that were 48 x 12.5 feet, with 125 $ft^2$ of shade, 1.56 linear feet of bunk space, and 0.5 linear feet of water accessibility per animal. In Idaho, pens were dirt-floored, with a total of 1500 $ft^2$ of floor space, with a concrete apron at the feed bunk (250 $ft^2$), 3.13 linear feet of bunk space, and 0.5 linear feet of water accessibility per animal. Study pens at both sites housed eight animals each.

j. Measurements: Animal health observations were conducted at least once daily. Cattle were weighed on Day -1 and 14 or 91 (depending on the duration of the study), feed consumption was measured, and carcasses were weighed at slaughter.

Additional measurements were also obtained on the following carcass measurements: liver abscess score, dressing percentage, fat thickness, adjusted fat thickness, ribeye area, KPH, yield grade, marbling score, skeletal maturity, lean maturity, overall maturity, quality grade, and WBSF. In addition, trained sensory panelists evaluated steaks for tenderness, chewiness, juiciness, beef flavor, and off-flavor attributes using a 15-point

continuous line scale. Animal health was evaluated as described in Section III.

k. Statistical Analysis: Continuous variables were analyzed by study using a linear mixed model (PROC MIXED, SAS version 9.2); pen was the experimental unit. Initial body weight (BW) was used as a covariate for the analysis of final BW, HCW, and daily DM intake (DMI). For discrete carcass variables (e.g., quality grade) a binomial distribution was assumed and a logit link function was used in a generalized linear mixed model (PROC GLIMMIX). Statistical models included fixed effects of lubabegron dose (Dose), Gender, breed type (Type), Dose x Gender x Type, and all possible two-way interactions; block nested within Gender and Type was included in the model as a random effect. If the main effect of Dose or a Dose-related interaction was significant, each non-zero dose group was compared to the control group in a pair-wise fashion using linear contrasts; contrasts were performed within the appropriate subclass for dose-related interactions (i.e., within Gender, within Type, within Gender x Type). Differences were deemed significant at alpha=0.10 for the variables LW, HCW, and daily DMI; for all other variables differences were deemed significant at alpha=0.05.

l. Results:

(1) Animal health observations are discussed in Section III.

(2) ADG, FE, and HCW were not negatively affected by lubabegron at any dose.

(3) Carcass and Sensory Characteristics: Data from these four studies indicated that lubabegron did not negatively affect dressing percent, fat thickness, adjusted fat thickness, ribeye area, KPH, or yield grade.

No consistent negative effects of lubabegron were detected for skeletal maturity, lean maturity, and overall maturity. No consistent dose-related effects were observed for marbling score or WBSF across the four field studies. No evidence of a difference in dark cutters[3] was observed as the distribution of dark cutters by dose across all four studies was 6, 5, 4, and 3 for 0, 1.25, 5, and 20 g/ton lubabegron, respectively.

Of the sensory traits evaluated, tenderness and chewiness were most frequently impacted by Dose. For a summarization of the sensory variables for each individual study site, see Tables II.10 through II.13. Dose-effects on tenderness scores were detected for all four studies ($P \leq 0.034$), but in general, the impact of lubabegron on tenderness was more consistent in the 91-day studies compared to the 14-day

[3] Beef with lean tissue that is dark in color. It is the result of long term stress that has reduced the glycogen content in muscle prior to slaughter. The muscle pH of a dark cutter is generally high (approx. 6.5) which results in higher water-holding capacity and more light absorbency than normal thus causing a dark lean color.
https://animalscience.unl.edu/meats-terms-glossary

studies. Evaluations of beef chewiness generally paralleled that of tenderness. In the Nebraska 14-day study, only a single dose of lubabegron was different from that of control; regardless of gender and breed type, cattle that received 20 g/ton lubabegron produced steaks that were scored less tender ($P = 0.005$) and chewier ($P = 0.006$) than steaks from control animals.[4] In the 14-day study conducted in Idaho, the impact of lubabegron on tenderness and chewiness scores was not the same for all breed types and genders. The only lubabegron effect for tenderness and chewiness was detected in British crossbred heifers. British crossbred heifers that received 5 g/ton produced steaks that were scored as less tender ($P = 0.009$) and chewier ($P = 0.006$) than steaks from control British crossbred heifers. In the 91-day studies, steaks from cattle that received lubabegron were marked as less tender ($P \leq 0.05$) than steaks from control cattle regardless of study, breed type, gender, or dose.

To aid in the interpretation of how consumers would respond to differences observed by trained panelists, the Sensory Laboratory in the Food Science and Human Nutrition Department at Iowa State University previously conducted a study with pork loin chops, where trained and consumer panelists both evaluated samples from the same meat cut.[5] The trained panelists evaluated intensity differences, whereas consumers provided their opinions on how much they "liked" something. Although several sensory factors were evaluated for the pork loin chop study, differences in tenderness led to a lower overall opinion and less purchase intent for consumers. The relationship between trained panelist tenderness scores and consumer tenderness scores indicated that, when trained panelist scores differed by approximately 20% of the scoring system, consumers were able to discern a difference in how well they "liked" tenderness. Therefore, differences in scores from a trained sensory panel of approximately 20% or greater may relate to significant differences in consumer acceptability.

In the current field studies, when statistically significant differences in tenderness scores between control steaks and steaks from lubabegron-treated animals were observed, differences ranged from 6.5% (Nebraska – 91 days) to 16.1% (Idaho - 14 days) of the scoring system. In general, trained sensory panelists identified treatment differences for tenderness within more studies than did the instrumental measure of tenderness (WBSF); however, the magnitude of the difference in trained sensory panel tenderness scores approached, but did not exceed, the 20% guideline used as an indication of changes that impact consumer acceptance. Therefore, these changes in sensory variables were deemed acceptable.

[4] The sponsor subsequently decided to reduce the upper dose to 5 g/ton of Type C medicated feed on a 100% DM basis, which also mitigates concerns related to beef tenderness and chewiness in this study.
[5] Unpublished data; Prusa, 2014

m. Conclusions: Treatment did not negatively affect ADG, FE, or carcass characteristics. There was a slight increase in WBSF and a decrease in tenderness in some classes of animals, but this is unlikely to be noticed by consumers.

## C. Additional Effectiveness Information

The sponsor conducted an additional study (Study No. D5CUS120019) to investigate feeding 1.25 g/ton lubabegron in combination with 27.3 g/ton ractopamine (Optaflexx™, NADA 141-221)[6] compared to a control diet without any drug during the last 28 days on feed to study the effects on ADG, FE, and carcass characteristics of steers (N = 24 steers per treatment group). Steers receiving the combined treatment (1.25 g/ton lubabegron and 27.3 g/ton ractopamine) had similar ADG and FE compared to the control group. There was no effect of treatment on feed intake or HCW. Marbling was reduced in the combined treatment group compared to control. The combined treatment group had lower frequencies of prime and choice or better quality grades compared to control. WBSF was increased in treated animals compared to control regardless of aging time; however, there was no treatment effect on WBSF score < 4.5 kg. Treatment also had no effect on ultrasound fat thickness.

## D. Conclusions for Effectiveness

Taken together, these studies support the use of lubabegron Type A medicated article for reduction of $NH_3$ gas emissions per pound of LW and HCW in beef steers and heifers fed in confinement for slaughter during the last 14 to 91 days on feed at the doses evaluated. The sponsor subsequently decided to reduce the upper dose of lubabegron to 5 g/ton of medicated feed on a 100% DM basis to achieve a zero-day withdrawal period (see Section IV). Feed for beef steers and heifers is typically formulated on a 90% DM basis. Therefore, the approved dose range is expressed on labeling as 1.25 to 4.54 g/ton, which equates to 1.39 to 5 ppm on a 90% DM basis in Type C medicated feed.

Beef cattle approximately 91 days prior to slaughter weigh on average 1000 lb (900 – 1100 lb) and consume approximately 20 lb DM per day. At the minimum approved dose (1.25 g/ton, 0.0625 g lubabegron/lb Type C medicated feed on a 90% DM basis), these animals would consume on average 13 mg/head/day. At slaughter, beef steers and heifers weigh approximately 1400 lb (range between 1300 – 1500 lb) and consume approximately 40 lb DM per day. Given the maximum approved concentration of 2.27 mg lubabegron/lb of Type C medicated feed (4.54 g/ton 90% DM basis), animals at slaughter would then consume a maximum dose of 90 mg/head/day. Thus, as identified on the labeling, the approved range in lubabegron consumption on a per head per day basis is 13 – 90 mg lubabegron/head/day.

To clarify the basis for the finding of reduced $NH_3$ gas emissions per lb of LW and HCW, the Indications for Use section of labeling includes the following statement: "Ammonia gas emissions were measured for individual animals or small groups of animals held in environmentally controlled facilities. Based on existing information, reliable predictions of the reduction of ammonia gas emissions cannot be made on a herd, farm, or larger scale."

[6] This combination is not approved; it is provided as additional information only.

In addition, the Indications for Use section of labeling includes the following information to clearly differentiate its effects from other Type A medicated articles with beta-adrenergic activity approved for use in medicated feeds for beef steers and heifers fed in confinement for slaughter: "Increased rate of weight gain, improved feed efficiency, and increased carcass leanness have not been demonstrated with this product."

## III. TARGET ANIMAL SAFETY

Lubabegron Type A medicated article was found to be safe to the target animal when administered for reduction of $NH_3$ gas emissions per pound of LW and HCW in beef steers and heifers fed in confinement for slaughter during the last 14 to 91 days on feed.

FDA evaluated target animal safety for lubabegron in non-clinical and clinical studies. Other sources of information, such as preliminary studies conducted by the sponsor, were also reviewed to evaluate its impact on animal health. In feedlot beef cattle, health was evaluated through physical exams, blood chemistry and hematology, animal health observations, and necropsy/histopathology evaluations. In addition, feed intake, weight gain, and FE are assessed to ensure animals are normal and representative of their class. Often the first sign that beef cattle may be unhealthy is poor performance, demonstrated by reduced appetite, growth rate, and FE, sometimes referred to as "unthriftiness".

In a non-clinical laboratory study to test the margin of safety for lubabegron, beef steers and heifers were fed elevated doses of up to 100 g lubabegron/ton of feed DM. The margin of safety study indicated that increasing concentrations of lubabegron led to decreased feed intake; however, animals in the treatment groups continued to gain weight comparable to control animals. Clinical chemistry and necropsy findings indicated minor changes in renal values and kidney size, but these values were within the normal range for feedlot beef cattle, were not dose-dependent, and changes in renal chemistry values did not progress over time.

Daily animal health observations were also conducted during the emissions effectiveness and field studies. Respiratory and digestive issues were the most common abnormal health effects noted, but these occurred at rates similar to what is seen in commercial feedlots (USDA NAHMS, 2011)[7]. Incidence of adverse health events were too low for statistical analysis. Animals in all groups grew and performed normally during the studies.

Because of concerns about the relationship of administration of drugs with beta-adrenergic activity and the incidence of lameness in livestock, particular attention was focused on lameness evaluation during the margin of safety, emissions, and field studies. Section III.B.4 discusses lameness in detail. Overall, lameness in feedlot beef cattle in these studies was associated primarily with animal management issues (i.e., nutrition, pen conditions, injuries). Most lameness resolved during the treatment period. When considering lameness that did not resolve, lameness incidence was

[7] Feedlot 2011 Part IV: Health and Health Management on U.S. Feedlots with a Capacity of 1,000 or More Head. USDA APHIS.
https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf

similar to or lower in lubabegron-treated animals when compared to control animals, and was similar to commercial feedlot incidence (USDA NAHMS, 2011).

**A. Margin of Safety Study**

1. Study Number: D5CUS120020
2. Study Standard: Non-Clinical Laboratory Study (Good Laboratory Practices [GLP])
3. Objective: To evaluate the safety of feeding lubabegron Type A medicated article at 0, 20, 60, and 100 g/ton of Type C medicated feed on a 100% DM basis to beef steers and heifers fed in confinement for slaughter for 95 – 99 days.
4. Study Location: Idaho
5. Study Duration: 99 days
6. Experimental Design and Study Management: The study was designed as a randomized complete block with four top-dress treatments of 0, 260, 780, and 1300 mg lubabegron per head per day intended to represent 0, 20, 60, or 100 grams lubabegron per ton of Type C medicated feed on a 100% DM basis and 0, 1X, 3X, and 5X the proposed maximum dose[8], respectively. Forty British and Continental crossbred steers and heifers (n = 5 per gender per dose group) were used in this study. Treatments were provided in 590 g Type C top-dress medicated feed per head per day. Cattle were maintained in individual pens with *ad libitum* access to clean water. The basal diet contained earlage, wheat, rolled corn, water, alfalfa hay, a proprietary supplement, and canola meal and was offered for *ad libitum* consumption. Treatments were initiated on study day 0, following a 34 to 38-day acclimation phase. Animals were observed daily for adverse reactions. Body weight and blood samples were collected on study days 0, 14, 60, and 92 relative to the start of feeding lubabegron. Physical examinations were conducted on study days -15 and 92. Feed refusals were weighed weekly. On days 95 to 99, cattle were weighed and sacrificed for necropsy which included evaluation of organs, collection of tissue samples, and collection of urine samples.
7. Statistical Analysis: Performance and safety (organ weights and organ weights as percent of final BW) variables measured only once were analyzed using a linear mixed model, Proc Mixed SAS. ANOVA was used to evaluate a model containing Dose, Gender, and Gender x Dose interactions as fixed effects. Block (location) was included as a random effect. If the Dose x Gender interaction was significant at the 10% level, within-Gender Dose effects were evaluated using pair-wise comparisons of each dose group against control using linear contrasts at an unadjusted alpha=0.10. If the Dose x Gender interaction was not significant, pair-wise comparisons of each dose group against control using linear contrasts at an unadjusted alpha=0.10 were performed. The Kenward-Rogers' adjustment was used to adjust the degrees

[8] The sponsor subsequently decided to reduce the upper dose of lubabegron to 5 g/ton of Type C medicated feed on a 100% DM basis.

of freedom. Least squares means and standard errors were used to summarize the results.

Safety variables measured more than once after Day 0 (physical examination outcomes, hematology, coagulation, and serum chemistry analysis) were analyzed by a repeated measures analysis of covariance with Dose, Gender, Day, Dose x Gender, Gender x Day, Dose x Day, and Dose x Gender x Day terms in the model as fixed effects; animal was identified as the subject in the repeated statement (Proc Mixed SAS). Contrasts, as indicated by the fixed effect tests that were significant, were used to compare treatment groups to control. Pre-dose values were used as a covariate and remained in the model regardless of statistical significance. Where multiple pre-dose values were provided, the values nearest to the first day of dose were used as the covariate.

8. Results:

   a. Feed Intake and Weight Gain: Results for average daily DM intake (ADDMI) and ADG by period are in Table III.1, below. Acceptance of the test article was diminished at increased levels of inclusion in the Type C top-dress medicated feed, but this delay in consumption did not affect overall feed consumption by the animals, and thus does not introduce a bias in performance due to decreased feed or drug consumption.

Table III.1. Animal numbers, BW, ADG, ADDMI, and FE per dose group for study D5CUS120020

| **Item** | **0 mg/hd/d** | **1X - 260 mg/hd/d** | **3X - 780 mg/hd/d** | **5X - 1,300 mg/hd/d** |
|---|---|---|---|---|
| Total animals starting study | 10 | 10 | 10 | 10 |
| Total animals completing study | 10 | 10 | 10 | 10 |
| Initial weight, lb | 1010.5 | 979.8 | 952.6 | 980.8 |
| Final weight, lb | 1306.4 | 1304.4 | 1265.2 | 1273.1 |
| ADG, lb/day | 3.2 | 3.5 | 3.4 | 3.2 |
| FE, lb gain/lb DMI | 0.14 | 0.17 | 0.16 | 0.16 |
| ADDMI, lb/day | 22.7 | 20.9 | 21.7 | 19.8 |

   b. Animal observations, adverse events (AE) and animal removals:

      No animals were removed from the study, although one control animal was depressed, anorexic, and febrile at various points throughout the study. During the scheduled post-study necropsy, it was determined that the animal had traumatic abomasitis. One control heifer was found to be pregnant during necropsy, but it was determined that her reproductive status did not alter the evaluation of her performance in the study, thus she was not excluded from the final study report. One 1X steer had curvature of both front hooves consistent with laminitis on the Day 92 physical examination; however, the animal did not exhibit signs of lameness. No adverse treatment-related events were reported during the live phase of this study.

c. Blood clinical chemistry and necropsy findings:

When comparing the treated groups to the control group, there were no clinically or biologically significant differences in hematology values, clinical pathology values, coagulation values, urinalysis, or physical examination findings. Statistically significant changes in renal clinical chemistry values were noted in the treated groups as compared to the control group. Serum urea nitrogen was decreased and creatinine and phosphorous were both increased when compared to controls in most animals receiving the test article. However, these changes were minor, did not progress over time, were not dose-dependent, and were within the laboratory reference range. Therefore, these changes were not considered treatment related and not a safety concern. At necropsy, heifers in the 1X, 3X, and 5X dose groups had kidney weights lighter than the control heifers. Steers in the 1X group also demonstrated lighter kidney weights as compared to controls. However, these changes were considered minor and most likely due to normal biological variability, as no clinical signs were noted and no histopathological evidence of renal damage was found. No treatment-related systemic lesions were observed in animals administered lubabegron.

9. Conclusions:

This study demonstrated that lubabegron when fed continuously for up to 99 days as a Type C top-dress medicated feed at up to 5X the proposed maximum dose level of 20 g/ton[9] was found to be safe in beef steers and heifers fed in confinement for slaughter.

**B. Field Safety Evaluations**

Safety to the treated animals under expected conditions of use was evaluated as part of the four field studies, as well as in both gas emissions effectiveness studies described in Section II. Safety variables measured during all six studies included: BW, ADG, ADDMI, and animal health observations. Initial (Day 0) BW was used as a covariate in analyses of final weight. These variables were analyzed only to determine whether lubabegron had any negative effect on animal performance. These studies were not designed or powered to detect improvements in animal performance.

Health observations were also recorded during loading for transport, unloading after transport to the slaughter facility, and during the ante-mortem period prior to slaughter. Other than first aid, no concomitant medications were allowed. Differences were deemed significant at alpha=0.10. Table III.2 lists the study locations, durations, and numbers of animals for these six studies.

During the treatment phase and up until slaughter, necropsies were performed on any enrolled animals that were removed or found dead. Necropsies included a systematic gross exam of each animal's general physical condition, and external and internal organs and tissues. For animals that were found dead or removed which had any prior observation of lameness, the feet were collected as

[9] The sponsor subsequently decided to reduce the upper dose of lubabegron to 5 g/ton of Type C medicated feed on a 100% DM basis.

appropriate and additional histopathological evaluation was performed by a board-certified pathologist.

**Table III.2: Two gas emissions effectiveness studies and four field safety studies**

| Study No. | Study Location | Study Duration (days) | Total Animals (N) | Animals per Treatment Group |
|---|---|---|---|---|
| D5CUS130028 | Michigan | 14 | 120 | 30 |
| D5CUS140002 | Idaho | 14 | 320 | 80 |
| D5CUS140001 | Nebraska | 14 | 320 | 80 |
| D5CUS130029 | California | 91 | 336 | 84 |
| D5CUS130043 | Idaho | 91 | 320 | 80 |
| D5CUS130044 | Nebraska | 91 | 320 | 80 |

1. Study Number: D5CUS130028

   Gas emissions were measured during a 14-day study in individual animal chambers at Michigan (see further description of study design in Section II.B.2).

   a. Mortality: Out of the 120 animals in this study, two animals were removed during the treatment phase and euthanized. One animal was from the 5 g/ton dose group during Cycle 3. This animal was removed and euthanized due to low feed intake and suspected acidosis. Necropsy findings were inconclusive. Weight loss in this animal prior to test article administration led to the conclusion that the decreased feed intake and subsequent removal was not test article related. The second animal was from the 20 g/ton dose group during Cycle 5 and euthanized because it was found non-ambulatory after several days of a swollen hock. Chronic synovitis of the right hock was found on necropsy. It was concluded this was not test article related.

   b. Abnormal Health Observations: Health observations were conducted at least twice daily throughout the study. The majority of abnormal health observations occurred similarly across all treated and control groups and were minor and common to the feedlot industry, such as lice, corneal scars, self-limiting diarrhea, and skin abrasions. Four animals were observed lame after transport to the slaughter facility: one in the 1.25 g/ton group; two in the 5 g/ton group; and one in the 20 g/ton group. However, lameness in all four animals resolved by the ante-mortem observation period. Therefore, it was concluded these cases of lameness were not clinically relevant. A more detailed discussion of lameness is in Section III.4 below.

   c. BW and ADG: Animal weights were collected on Days -9, 0, 7, and 14. There were no statistically significant differences in final BW or ADG ($P \geq 0.625$) of animals across all dose groups compared to the control group.

   d. ADDMI: Dose had a statistically significant impact on ADDMI. Least squares means for ADDMI were 17.7, 16.7, 15.3, and 14.8 lb/day for the

control, 1.25, 5, and 20 g/ton dose groups, respectively. Lower ADDMI ($P \leq 0.015$) was observed in the two highest dose groups compared to the control. Lower ADDMI was also reported in cattle fed lubabegron in the margin of safety study. However, this was concluded not to be a safety issue, as BW and ADG were not affected.

2. Study Number: D5CUS130029

Gas emissions were measured during a 91-day study in CPE with 14 animals per pen at the California site (see further description of study design in Section II.B.1).

a. Mortality: Out of 336 animals, 10 animals died or were euthanized during the study.

(1) In Cycle 1, one animal from the 1.25 g/ton dose group was found dead, and cause of death was determined to be rumen acidosis and bloat. Two animals from the 20 g/ton dose group were removed: one was euthanized due to recurrent bloat. The other was found dead, and necropsy revealed rumen acidosis and bronchopneumonia. It was concluded that the frequency of these conditions is consistent with that likely to be observed among feedlot cattle.

(2) In Cycle 2, two control animals were found dead; one death was from interstitial pneumonia and the other animal had a presumptive cause of death as rumen acidosis based on a rumen pH of 4.9. Two animals from the 20 g/ton dose group were removed; one was found dead and the presumptive cause of death was rumen acidosis based on a rumen pH of 5.23. The other animal was euthanized due to an injury that occurred when the animal was being moved for examination of a hoof abscess.

(3) In Cycle 3, three animals from the control group were removed. One animal was removed and euthanized due to a non-healing laceration and severe bacterial infection in the leg. This animal had evidence of laminitis, and it was concluded from the necropsy that the laminitis was a result of the bacterial infection. One control animal was euthanized due to marked facial swelling and difficulty breathing due to an injury. One control animal was found dead. This animal had a history of bloat during the study, but no cause of death was determined at necropsy.

b. Abnormal health observations: Animal health observations were conducted at least once daily and at approximately the same time each day.

(1) During Cycle 1, there were 26 animals reported as lame at some point during the treatment phase with the following frequency: 2, 6, 11, and 7 animals in the control, 1.25, 5, and 20 g/ton dose groups, respectively. Four animals, one in each treatment group, were diagnosed with chronic laminitis during this cycle; however, the lameness observed in these animals resolved prior to slaughter. During unloading, the following incidence of lameness was observed: 1, 6, 7, and 4 animals in the control, 1.25, 5, and 20 g/ton dose groups, respectively. Clinical signs associated with respiratory disease and gastrointestinal disturbances (e.g., bloat or diarrhea) were also

observed; however, cases were represented across all treatment groups, did not appear to be dose-related, and occurred at a frequency commonly seen in feedlot cattle.

(2) Lameness during the treatment phase of Cycle 2 occurred at the following frequency: 1, 2, 4, and 5 cases in the control, 1.25, 5, and 20 g/ton dose groups, respectively. During unloading, two animals were observed lame, one in the 5 g/ton group and one in the 20 g/ton group. Clinical signs associated with respiratory disease were also observed; however, cases were represented throughout all groups, did not appear to be dose-related, and occurred at a frequency commonly seen in feedlot cattle.

(3) Three animals were reported as lame during the treatment phase of Cycle 3: two animals in the control group and one animal in the 5 g/ton group. No animals were found lame during unloading at the slaughter facility. Clinical signs associated with respiratory issues and gastrointestinal disturbances (e.g., bloat) were also observed; however, cases were represented throughout all groups, did not appear to be dose-related, and occurred at a frequency commonly seen in feedlot cattle.

A more detailed discussion of lameness is in Section III.4

c. BW and ADG: Across all three cycles, animal weights were measured on Days -8, 0, 7, 14, 28, 56, and 91. There were no statistically significant differences in final BW or weight gain ($P \geq 0.075$) of animals across all dose groups compared to the control group.

d. ADDMI: Across all three cycles, dose did not impact ADDMI during this study ($P = 0.585$). The Least Squares Means for ADDMI for the control, 1.25, 5, and 20 g/ton dose groups were 19.4, 20.2, 19.4, and 19.3 lb/animal/day, respectively.

3. Field Safety Studies

Four field safety studies were conducted at two different sites, Idaho and Nebraska (see further description of study design in Section II.B.3). At each site, two different treatment durations were employed: 14 days and 91 days, according to the following study numbers:

**Table III.3. Study number, location, and duration of field studies**

| Study No. | Study Location | Study Length | Flooring Type | No. of Animals/Pen |
|---|---|---|---|---|
| D5CUS140002 | Idaho | 14 days | Dirt Lot | 8 |
| D5CUS140001 | Nebraska | 14 days | Concrete Lot | 8 |
| D5CUS130043 | Idaho | 91 days | Dirt Lot | 8 |
| D5CUS130044 | Nebraska | 91 days | Concrete Lot | 8 |

a. Study Number: D5CUS140002 (Idaho, 14 days)

(1) Mortality: Out of 320 animals, two were removed during this study. One animal from the 5 g/ton dose group was euthanized for rumen acidosis and persistent bloat lasting two weeks. Another animal from the 5 g/ton dose group was found dead. Findings at necropsy were consistent with bloat. These findings were considered incidental to feedlots and not considered test article related.

(2) Abnormal health observations: Five animals were reported as lame during the treatment phase of this study at the following frequency: 1, 0, 1, and 3 in the control, 1.25, 5, and 20 g/ton dose groups, respectively. Bloat was also seen in five animals during the treatment phase: 0, 1, 1, and 3 in the control, 1.25, 5, and 20 g/ton dose groups, respectively. The bloat and lameness observations were within expected limits in this population of feedlot animals and were, therefore, deemed to not be test article related. Two animals (one in 1.25 g/ton group; one in 5 g/ton group) were observed as lame during the ante-mortem phase. Neither animal had previous signs of lameness during the treatment phase of the study or off the truck after transport, and the observations were, therefore, not considered treatment related. Statistical analysis was not performed due to the low incidence rate of AE.

(3) BW and ADG: There were no statistically significant differences in final BW or ADG ($P > 0.137$) of animals across all dose groups compared to the control group.

(4) ADDMI: There were no statistically significant differences between treated and control groups for ADDMI ($P = 0.385$).

b. Study Number: D5CUS140001 (Nebraska, 14 days)

(1) Mortality: Out of 320 animals, one animal in the 20 g/ton group was found dead during this study. On necropsy, death was attributed to a large ruptured pulmonary abscess. This was not considered test article related.

(2) Abnormal health observations: The most frequent abnormal health observation was lameness. During the treatment phase, 3, 0, 2, and 2 animals from the control, 1.25, 5, and 20 g/ton dose groups were observed with lameness, respectively. No other abnormal health observations were noted during the treatment phase for this study. Thirteen animals were observed as lame during loading (5, 2, 3, and 3 animals in the control, 1.25, 5, and 20 g/ton dose groups, respectively), and these same 13 animals were lame during unloading and ante-mortem. The lameness was not considered treatment related, as it was observed across all treatment groups, with a preponderance observed among control animals.

(3) BW and ADG: There were no statistically significant differences in final BW or ADG ($P > 0.183$) of animals across all dose groups compared to the control group.

(4) ADDMI: A statistically significant Dose x Gender interaction (P = 0.067) was observed due to lower ADDMI in the steer 20 g/ton group compared to control steers. The control steers consumed 25.0 lb/day of DM compared to 21.4 lb/day for the 20 g/ton steer group.

c. Study Number: D5CUS130043 (Idaho, 91 days)

(1) Mortality: Out of 320 animals, five animals were removed from this study. One animal in the 1.25 g/ton dose group was euthanized due to severe otitis; one animal in the 20 g/ton dose group was euthanized for pneumonia. Three animals were removed and euthanized due to laminitis and unsuitability for shipment: one in the 1.25 g/ton group, one in the 5 g/ton group, and one in the 20 g/ton group. The animal in the 5 g/ton dose group was observed with lameness prior to initiation of treatment. On histopathology, laminitis was not confirmed and it was determined that the changes observed in the hooves were mild and expected for the age and conditions in which the animals were housed.

(2) Abnormal health observations: Lameness occurred similarly and in similar severity across all groups during the treatment phase of this study: 9, 8, 10, and 8 animals in the control, 1.25, 5, and 20 g/ton dose groups, respectively. Gastrointestinal observations were the second most common abnormal health observation, with reports of bloat seen in 1, 4, 6, and 6 animals in the control, 1.25, 5, and 20 g/ton dose groups, respectively, and three reports of diarrhea in the 1.25 g/ton dose group. It was concluded that the highly fermentable diet provided during this study contributed to the high frequency of cases of bloat seen. Ten animals were found to be lame after transport to the slaughter facility: 2, 0, 5, and 3 in the control, 1.25, 5, and 20 g/ton dose groups, respectively. Due to the low overall incidence of AE, and the fact that most cases resolved prior to the end of the treatment period, these AE were not considered test article related.

(3) BW and ADG: There were no statistically significant differences in final BW or ADG ($P > 0.303$) of animals across all dose groups compared to the control group.

(4) ADDMI: A statistically significant difference between treated and control groups was seen with ADDMI. ADDMI was 0.94, 0.78, and 1.55 lb lower for the 1.25, 5, and 20 g/ton dose groups, respectively, as compared to control ($P \leq 0.050$).

d. Study Number: D5CUS130044 (Nebraska, 91 days)

(1) Mortality: Out of 320 animals, four were removed during this study. One control animal was euthanized due to an injury. The remaining three animals, two in the 1.25 g/ton group and one in the 20 g/ton group, were found dead, and presumptive diagnosis on necropsy was bloat.

(2) Abnormal health observations: Lameness was the most common abnormal health observation reported during this study. The following numbers of animals were reported as lame during the treatment phase in each group: 12, 2, 7, and 7 in the control, 1.25, 5, and 20 g/ton dose groups, respectively. Eighteen animals were found to be lame after transport to the slaughter facility: 7, 0, 7, and 4 in the control, 1.25, 5, and 20 g/ton dose groups, respectively.

(3) BW and ADG: There were no statistically significant differences in final BW or ADG ($P > 0.443$) of animals across all dose groups compared to the control group.

(4) ADDMI: A statistically significant difference between treated and control groups was observed with ADDMI ($P = 0.0007$). Lower ADDMI was observed in the 5 g/ton and 20 g/ton dose groups (0.76 and 1.00 less lb/day for 5 and 20 g/ton, respectively) compared to control.

4. Further evaluation of lameness observed in these studies:

There are multiple reasons why cattle may go lame, including laminitis, heel warts, hoof abscesses, foot rot, and injuries. These causes are not always obvious without close examination. In the current studies, lameness was generally characterized as mild or moderate.

Because some lameness was observed in the effectiveness and field safety studies, a more focused evaluation of lameness was conducted to determine if observations were related to treatment. Table III.4 below demonstrates that the 5 g/ton and 20 g/ton dose groups had a numerically higher incidence of lameness compared to the control group.

**Table III.4: Number of animals observed with lameness across all phases of the six studies**

| Study | N | N per Treatment Group | Control | 1.25 g/ton | 5 g/ton | 20 g/ton | Total Lame per Study | % Lame per Study |
|---|---|---|---|---|---|---|---|---|
| **14 day studies** | | | | | | | | |
| MSU | 120 | 30 | 0 | 1 | 2 | 1 | 4 | 3.3 |
| ID 14 | 320 | 80 | 1 | 1 | 2 | 3 | 7 | 2.2 |
| NE 14 | 320 | 80 | 5 | 2 | 3 | 3 | 13 | 4.1 |
| **91 day studies** | | | | | | | | |
| UCD | 336 | 84 | 6 | 13 | 23[a] | 17 | 59 | 17.6 |
| ID 91 | 320 | 80 | 11 | 9 | 15 | 11 | 46 | 14.4 |
| NE 91 | 320 | 80 | 12 | 2[b] | 8 | 7 | 29 | 9.1 |
| **Total N** | **1736** | **434** | | | | | | |
| **Lame/treatment (N)** | | | **35** | **28** | **53** | **42** | | |
| **Lame/treatment (%)** | | | **8.1** | **6.5** | **12.2** | **9.7** | | |

[a] Difference from control was significant during the treatment phase (P = 0.018) and unloading phase (P = 0.035).
[b] Difference from control was significant for the treatment period (P = 0.009), loading, unloading and ante-mortem periods (P = 0.014).

Lameness incidence for cattle treated with lubabegron was highest at the California 91-day study, particularly during the first cycle. In addition to observations of laminitis, several animals in California were diagnosed with interdigital dermatitis (foot rot), likely due to muddy conditions inside the CPE[10] and diets that were highly fermentable.[11] Following the first cycle, medicated foot baths were instituted which resulted in reduced lameness incidence in the subsequent two cycles. In contrast, the Idaho and Nebraska 91-day studies (ID 91 and NE 91), which are more typical of U.S. feedlots, had a lower incidence of lameness in lubabegron-treated cattle compared to the California location.

Most cases of lameness resolved during the study. If lameness were treatment related, it is unlikely to resolve during treatment, as the drug was administered continuously over that time period. Thus, to further describe lameness and whether there was a drug effect, the duration of lameness and whether it resolved during the treatment period was evaluated. Animals with unresolved lameness during the treatment period were evaluated separately. It was observed that the control group, overall, had more cases of unresolved lameness as compared to any treated group (see Table III.5).

**Table III.5: Number of animals with unresolved lameness[a]**

[10] Stokka, G.L, Lechtenberg, K., Edwards, T., MacGreggor, S., Voss, K., Griffin, D., Grotelueschen, D.M., Smith, R.A., Perino, L.J. 2001. Lameness in feedlot cattle. Veterinary Clinics in North America: Food Animal Practice. 17: 189 – 207.
[11] Nocek, J.E. 1997. Bovine acidosis: implications on laminitis. J. Dairy Sci. 80: 1005 – 1028.

| Study | N per Treatment Group | Control | 1.25 g/ton | 5 g/ton | 20 g/ton |
|---|---|---|---|---|---|
| MSU | 30 | 0 | 0 | 0 | 0 |
| ID 14 | 80 | 0 | 0 | 0 | 0 |
| NE 14 | 80 | 3 | 0 | 2 | 2 |
| UCD[b] | 84 | 0 | 0 | 0 | 0 |
| ID 91[c] | 80 | 0 | 1 | 1 | 1 |
| NE 91 | 80 | 7 | 0 | 6 | 4 |
| **Total # animals with unresolved lameness** | | **10** | **1** | **9** | **7** |

[a] Unresolved lameness is defined as an animal observed lame during the treatment period that continued to be lame through the ante-mortem observation period.
[b] Three animals (1 in control, 1 in 1.25 g/ton group, 1 in 20 g/ton group) in Cycle 1 at UCD were examined at the veterinary school for chronic lameness; chronic laminitis was diagnosed, but animals were kept in the study and lameness was not reported in later phases (loading, unloading, or ante-mortem).
[c] All three animals had chronic lameness during the treatment phase. They were determined not to be suitable candidates for shipping at end of study and therefore were euthanized; laminitis was not diagnosed on histopathology.

It was decided that unresolved lameness should be considered an indicator of an adverse drug effect because, if the drug caused lameness, lameness was unlikely to resolve while the drug was being administered. Based on the data from these six studies, unresolved lameness was observed at similar levels across all treated groups and at a smaller incidence rate as the control group. Therefore, lameness was not considered to be test article related. Additionally, no animals were non-ambulatory due to lameness following transportation to slaughter.

## C. Additional Animal Safety Information

Twelve studies were conducted to investigate the impact of lubabegron on other variables, including ADG and FE, carcass characteristics, impact on hematology and immunology, and responses of β-receptors. Although these studies were not intended specifically to examine lubabegron's effect on animal health, health observations were documented in eight of these studies: D5CUS110007, D5CUS110008, D5CMX120003, D5CUS120007, D5CUS120008, D5CUS120018, D5CUS120019, and D5CUS140021. Adverse events recorded from each study are summarized in Table III.6. Note that not all studies included all doses. Treatment periods ranged from 14 to 98 days among studies. Only one of the eight studies, D5CUS110008, reported observations of lameness.

**Table III.6. Adverse event data for feedlot studies: number of animals affected by dose per study[a,b]**

| Study No. | N | System[c] | Clinical Sign | 0 g/ton | 1.25 g/ton | 2.5 g/ton | 5 g/ton | 10 g/ton | 20 g/ton |
|---|---|---|---|---|---|---|---|---|---|
| D5CUS110007 | 120 | GI | bloat | 0 | 0 | 0 | 2 | NA | NA |
| | | INTG | swelling | 1 | 0 | 0 | 0 | NA | NA |
| D5CUS110008 | 555 | MS | lame | 2 | 0 | 0 | 1 | NA | NA |
| | | MS | laceration | 0 | 1 | 0 | 0 | NA | NA |
| | | GI | bloat | 1 | 0 | 0 | 1 | NA | NA |
| D5CUS120007 | 120 | INTG | swelling | 1 | 2 | NA | 1 | NA | 1 |
| D5CUS120008 | 896 | HEP | abscess | 0 | 0 | 0 | NA | NA | 1 |
| | | RESP | pneumonia/ diphtheria | 0 | 2 | 0 | NA | NA | 1 |
| | | GI | bloat | 0 | 0 | 0 | NA | NA | 1 |
| D5CUS120018 | 192 | MS | foot rot | 1 | 0 | NA | NA | NA | NA |
| | | RESP | pneumonia | 1 | 0 | NA | NA | NA | NA |
| | | GI | other | 2 | 0 | NA | NA | NA | NA |
| D5CUS120019 | 120 | GEN | lethargic | 0 | 1 | NA | NA | NA | NA |
| | | INTG | laceration | 0 | 1 | NA | NA | NA | NA |
| | | GI | bloat | 0 | 1 | NA | NA | NA | NA |
| | | INTG | swelling | 0 | 1 | NA | NA | NA | NA |
| | | GEN | found dead | 0 | 1 | NA | NA | NA | NA |
| D5CUS140021 | 21 | GI | diarrhea | 0 | NA | NA | 2 | NA | NA |
| | | INTG | laceration | 0 | NA | NA | 2 | NA | NA |
| | | EYE | discharge | 0 | NA | NA | 3 | NA | NA |
| | | INTG | swelling | 0 | NA | NA | 1 | NA | NA |
| | | INTG | abscess | 1 | NA | NA | 0 | NA | NA |
| D5CMX120003 | 480 | MS | broken bone | 0 | NA | NA | 0 | 1 | 1 |
| Total N | 2504 | | | 665 | 641 | 318 | 354 | 120 | 406 |
| Total AE | 39 | | | 10 | 10 | 0 | 13 | 1 | 5 |
| Total injuries (e.g., lacerations, swellings, external abscesses, broken bones) | 15 | | | 3 | 5 | 0 | 4 | 1 | 2 |
| Total lameness/foot rot | 4 | | | 3 | 0 | 0 | 1 | 0 | 0 |
| Total GI | 10 | | | 3 | 1 | 0 | 5 | 0 | 1 |
| Total RESP | 4 | | | 1 | 2 | 0 | 0 | 0 | 1 |

[a] Data from animals receiving lubabegron alone
[b] NA – not applicable – the dose was not used
[c] GI = gastrointestinal, INTG = integumentary, MS = musculoskeletal, HEP = hepatic, RESP = respiratory

The incidence of AE across lubabegron dose levels was low (1.6%) and not greater than would be expected in commercial beef cattle feeding operations

(USDA NAHMS, 2011)[12]. The most common AE reported were related to injuries (N=15), followed by gastrointestinal issues (bloat, diarrhea; N = 10), lameness not associated with injury (N = 4), and respiratory (N = 4). No pattern could be discerned across dose levels; increasing dose of lubabegron did not appear to increase the incidence of AE in the studies where multiple dose levels were tested.

### D. Conclusions for Target Animal Safety

The combined animal safety information represents 4240 animals across 15 studies. These studies indicate a low incidence of health issues overall, and no difference in incidence between control animals and those receiving lubabegron at the approved dose levels for beef steers and heifers fed in confinement for slaughter. Lameness incidence, which was highest during two of the 91-day studies, California (D5CUS130029) and ID 91 (D5CUS130043), were primarily attributed to nutritional management and conditions in the pens, and most cases resolved while the animals were still receiving lubabegron, indicating that the lameness was not test article related.

There were no other clinical signs observed or variables measured in these studies which indicated any adverse drug effects from feeding up to 20 g/ton of lubabegron continuously for up to 91 days. ADDMI was significantly reduced in treated animals compared to controls in some studies. However, ADG and BW were not affected, and it was concluded not to be a safety issue. The approved labeling includes the following Caution statement so that users are aware of the potential effect on DMI: "A decrease in dry matter intake may be noticed in some animals."

The additional information strengthens the conclusion that lubabegron is safe to the target animal when fed the approved dose range (1.25 – 4.54 g/ton on a 90% DM basis) for the approved duration (during the last 14 – 91 days on feed). The animal health observations across eight studies (N = 2504) revealed no novel animal safety issues when lubabegron was fed. The incidence of common feedlot health issues was similar to commercial conditions (1.6%) and did not appear to differ for lubabegron-treated cattle when compared to control.

## IV. HUMAN FOOD SAFETY

Because lubabegron is a beta-adrenergic agonist/antagonist, not an antibacterial, human food safety was assessed from the perspectives of toxicology (Sections C, D, and E) and residue chemistry (Sections F and G). The toxicology assessment determined an Acceptable Daily Intake (ADI) and safe concentrations for lubabegron residues in foods. To determine the ADI, a series of *in vitro* and *in vivo* pharmacology studies were performed to characterize the beta-adrenergic agonist/antagonist properties, and toxicity studies were conducted to assess acute toxicity, subchronic and chronic toxicity, reproductive and developmental toxicity, and genotoxicity.

[12] Feedlot 2011 Part IV: Health and Health Management on U.S. Feedlots with a Capacity of 1,000 or More Head. USDA APHIS.
https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf

*In vitro* and *in vivo* pharmacology studies demonstrated that lubabegron has (1) agonistic activity to the $\beta_3$-adrenergic receptor; (2) high binding affinity with the $\beta_1$- and $\beta_2$-adrenoceptors and low binding affinity with non-β-adrenoceptors; and (3) antagonistic activity to the $\beta_1$- and $\beta_2$-adrenergic receptors.

The toxicity data showed that lubabegron does not cause developmental or reproductive effects and is not genotoxic. The no-observed-effect-level (NOEL)/no-observed-adverse-effect level (NOAEL) of 0.16 mg/kg BW/day from the single dose human study was determined to be most appropriate for the calculation of the ADI and is predictive of the chronic effects. Applying an overall safety factor of at least 50 to the NOEL/NOAEL, the ADI for lubabegron is established at 3 µg/kg BW/day (see Section C, 2-3 and Section D). Based on the ADI, the safe concentrations for total residues of lubabegron in the edible tissues of beef cattle are 0.6 ppm for muscle, 1.8 ppm for liver, 3.6 ppm for kidney, and 3.6 ppm for fat (see Section E).

For residue chemistry, the sponsor performed a comparative metabolism study, a total radiolabeled residue study, and a marker residue depletion study. The comparative metabolism study demonstrated that lubabegron is metabolized similarly in liver cells from the target species, cattle, and the species used for the toxicology assessment, humans and rats. This showed that the laboratory animals used for toxicological testing were exposed to the metabolites that humans can be exposed to as residues in edible tissues from treated cattle. The total radioactive residue study demonstrated that lubabegron is the marker residue, liver is the target tissue, and that a 10 ppb tolerance for lubabegron in cattle liver is appropriate to protect the public health. Data from the marker residue depletion study supported a 0-day withdrawal period. An analytical method is available for monitoring residues of lubabegron in cattle liver.

## A. Antimicrobial Resistance

This product is not an antibacterial.

## B. Impact of Residues on Human Intestinal Flora

This product is not an antibacterial.

## C. Toxicology

1. Summary of Toxicology Studies

   Toxicity studies used to determine the human food safety of lubabegron are summarized below:

   a. Subchronic Oral Toxicity Study in Rodents

      (1) Report Numbers: R03102, R03202

      (2) Report Date: March 7, 2009

      (3) Study Location (in-life): Greenfield, Indiana, USA

      (4) Study Design: This GLP-compliant study was conducted generally according to the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products

(VICH) Guideline (GL) 31. Fischer 344 rats (20/sex/group) were administered lubabegron by oral gavage for 6 months at a dose of 0, 30, 150, or 300 mg/kg BW/day. At the end of the 6 months, 15 rats/sex/group were sacrificed, and the remaining 5 animals/sex in control and mid- and high-dose groups were observed for one month without the administration of the drug for the reversibility of toxicity. In the toxicokinetic phase, blood samples were taken on Days 13, 90, and 180 in a separate group of animals of 5 rats/sex/group. Survival, clinical observations, BW, food consumption, efficiency of food utilization, ophthalmology, enzyme induction, clinical pathology, toxicokinetic, gross pathology, urinalysis, organ weights, and histopathology parameters were evaluated.

(5) Results and Conclusion: No mortality or abnormal clinical observations were observed at any dose tested. At 150 and 300 mg/kg BW/day, decreases in BW and food consumption, increases in alkaline phosphatase and gamma-glutamyltransferase (reversible), increased liver weight with granulomatous inflammation, increased incidences of granulomatous inflammation in the mesenteric lymph node and spleen, and increased kidney weight were observed. Increases in alanine transaminase and aspartate transaminase, increased incidences of granulomatous inflammation in brown fat, and increased spleen and uterine weights were observed in all treated groups. At 30 mg/kg BW/day, minor increases in BW as well as mild granulomatous inflammation in the liver and mesenteric lymph node were observed. The time to peak plasma concentrations ($T_{max}$) was achieved in 1 to 8 hours, with the longer times generally occurring at higher doses. The half-life for elimination ranged from 1.8 - 4.7 hours. The exposure (AUC and $C_{max}$) to lubabegron increased with administered doses, but the increases were not always dose proportional. No marked sex-related differences were observed on Day 13. However, sex-related differences were observed on Days 90 and 180, with higher exposure in female rats. Accumulation occurred after multiple dosing, and steady state was reached by three months. The lowest-observed-effect level (LOEL)/lowest-observed-adverse-effect level (LOAEL) was established at 30 mg/kg BW/day, the lowest dose tested, based on the findings of granulomatous inflammation in the liver and increased incidence and magnitude of granulomatous inflammation of the mesenteric lymph nodes noted during the treatment period. These findings were used to set the dose levels in the chronic oral toxicity study in rodents discussed under item b below.

b. Subchronic Oral Toxicity Study in Non-Rodents

(1) Report Numbers: 6180-463

(2) Report Date: April 20, 2008

(3) Study Location (in-life): Greenfield, Indiana, USA

(4) Study Design: This GLP-compliant study was generally conducted according to VICH GL31. Cynomologus monkeys (4/sex/group) were administered lubabegron by oral gavage at a dose of 0, 15, 40, or

100 mg/kg BW/day for six months. In addition, to assess the reversibility or persistence of any potential toxic effects, an additional four animals were given 40 mg/kg BW/day for six months, followed by four weeks without dosing. Plasma concentrations of lubabegron were determined on Days 14, 23, and 27 and during Weeks 13 and 26. Plasma concentrations of an amine metabolite (Compound 450806) were determined on Days 14 and 27. Survival, clinical observations, BW, body temperature, electrocardiogram, food consumption, efficiency of food utilization, ophthalmology, enzyme induction, clinical pathology, toxicokinetics, gross pathology, urinalysis, organ weights, and histopathology parameters were evaluated.

(5) Results and Conclusion: At 100 mg/kg BW/day, mortalities (1 male, 3 females) occurred from Days 20-27. Compound related morphological changes were observed in heart (focal myocardial necrosis, myocardial vacuolar degeneration, and diffuse interstitial and focal inflammation), liver (hepatocellular degeneration and increased glycogen, parasitic granuloma, lymphohistiocytic infiltrates, focal subcapsular coagulative necrosis, hepatocellular vacuolation), bone marrow (hypocellular), and kidney (congestion, vacuolar tubular degeneration and necrosis, lymphohistiocytic infiltration, vascular and tubular mineralization). At 40 or 100 mg/kg BW/day, a dose-dependent increase in QTc interval was observed. At 15 and 40 mg/kg BW/day, there were slight decreases in heart weight; no mortalities, clinical observations, abnormal ophthalmic observations, clinical chemistry, or histopathology findings were observed. Heart rate was decreased at all doses tested. Plasma lubabegron exposure after oral administration of lubabegron generally increased with dose, although the increases were not always dose-proportional. No marked sex-related differences were observed. Prolonged absorption was observed, especially at the highest dose tested. Exposure to Compound 450806, an amine metabolite, was significantly (3X to 10X) higher than exposure to the parent compound. The LOEL/LOAEL was 15 mg/kg BW/day, the lowest dose tested, based on slight decreases in heart weight and slight decreases in heart rate observed during the treatment period.

c. Chronic Oral Toxicity Study in Rodents

(1) Report Number: 130-189

(2) Report Date: July 27, 2010

(3) Study Location (in-life): Mattawan, Michigan, USA

(4) Study Design: This GLP-compliant study was conducted based on VICH GL37. Fischer 344/NHsd rats (20/sex/dose) were administered lubabegron orally by gavage at a dose of 0.05, 0.5, 5.0, or 15.0 mg/kg BW/day for one year. One additional group of 20 animals/sex served as the control and received the vehicle (10% Acacia and 0.05% DOW Antifoam® 1510-US in distilled water). Observations for morbidity, death, injury, and the availability of food and water were conducted twice daily for all animals. Toxicity was assessed by detailed clinical and cageside observations, food consumption and BW

measurements, ophthalmoscopic examinations, and clinical pathology evaluations. At study termination, necropsy examinations were performed and organ weights were recorded. All tissues were microscopically examined for animals in control and high-dose (15.0 mg/kg BW/day) groups.

(5) Results and Conclusion: Lubabegron was well-tolerated following one-year of oral administration in rats at doses of 0.05 to 15 mg/kg BW/day. No treatment related clinical signs of toxicity, ophthalmoscopic effects, or changes in clinical pathology were observed in males and females at any doses tested. An increase in BW was observed in males in the 15 mg/kg BW/day group. Dose-related increases in food consumption were seen in both sexes at all doses and the effect occurred in more males than females. There were no test article-related organ weight changes, macroscopic observations, or microscopic changes. The no-observed-effect level (NOEL)/no-observed-adverse-effect level (NOAEL) was 5 mg/kg BW/day based on the greater than 5% treatment-related increase in BW in males and decreased food consumption in both sexes at 15 mg/kg BW/day.

d. Oral Developmental Toxicity Study in Rodents

(1) Report Number: WIL 668012

(2) Report Date: April 11, 2011

(3) Study Location (in-life): Ashland, Ohio, USA

(4) Study Design: This GLP-compliant study was conducted mostly according to VICH GL32. Crl:CD(SD) pregnant female rats (22/group) were administered lubabegron dissolved in the vehicle (10% Acacia, 0.05% DOW antifoam®1510-US and deionized water) at 0, 30, 100 and 300 mg/kg BW/day *via* daily gavage in 10 mL/kg BW dose volume from gestation days (GDs) 6-19. In addition, a toxicokinetic phase was conducted with four female rats/group administered lubabegron at 0, 30, 65 and 150 mg/kg BW/day from GDs 6-19 with blood samples taken on GD 19. Animals were euthanized at GD 20. Clinical signs of toxicity, mortality, survival; the BW and food consumption were recorded for dams. At necropsy, standard parameters assessing maternal and fetal toxicities were examined.

(5) Results and Conclusion: The major signs of maternal toxicities at 300 mg/kg BW/day were alopecia, red mucous discharge around the mouth and nose, salivation, and reduced BW and food consumption. None of the other doses produced any signs of toxicity. No fetal toxicities were noted at any of the doses tested. Detectable plasma concentrations of lubabegron were found in all dose groups. The $AUC_{0-24\ hr}$ and $C_{max}$ values revealed that plasma exposure decreased with increasing dose on GD 19. $T_{max}$ was between three and seven hours. A maternal toxicity NOEL/NOAEL of 100 mg/kg BW/day was established based on reduced mean BW with corresponding reductions in mean food consumption and adverse clinical findings observed in the 300 mg/kg BW/day dose group throughout gestation. A

fetal/developmental NOEL/NOAEL of 300 mg/kg BW/day (the highest dose tested) was established. Lubabegron was not considered teratogenic in rats under the conditions of this study.

e. Oral Developmental Toxicity Study in Non-Rodents

(1) Report Number: WIL-353025

(2) Report Date: May 20, 2008

(3) Study Location (in-life): Ashland, Ohio, USA

(4) Study Design: This GLP-compliant study was conducted mostly according to VICH GL32. Lubabegron was orally administered to pregnant New Zealand White rabbits (22/group) on GDs 7-19 at doses of 0, 30, 65, or 150 mg/kg BW/day in 10 mL/kg BW dose volume. In addition, a toxicokinetic phase was conducted with four female rabbits/group administered lubabegron at 0, 30, 65, and 150 mg/kg BW/day from GDs 7 to 19, with blood samples taken on GD 19. The rabbits were euthanized on GD 28 for evaluation of maternal reproductive parameters, and the fetuses were assessed for viability, weight, gender, and morphology.

(5) Results and Conclusion: Marked mean maternal BW losses and reductions in food consumption were noted in the 150 mg/kg BW/day group throughout GDs 7-20. Because of the continued decrease in mean BW gain and food consumption observed in this group from GDs 20-23, the remaining animals in this group were euthanized with no further examination. Mean maternal BW, BW gains, gravid uterine weights, net BW, net BW gains, and food consumption in the 30 and 65 mg/kg BW/day groups were unaffected by treatment with lubabegron. No test article-related findings were observed in females that aborted, died, were euthanized *in extremis,* or that survived to GD 29 laparohysterectomy. Intrauterine growth and survival and external, visceral and skeletal malformations in the 30 and 65 mg/kg BW/day groups were unaffected by treatment. Detectable plasma concentrations of lubabegron were found in all dose groups. The $AUC_{0\text{-}24\,hr}$ and $C_{max}$ values revealed that plasma exposure decreased with increasing dose on GD 19. $T_{max}$ was between three and seven hours. Based on the results of this study, no effect for maternal and developmental toxicity in rabbits was observed up to the highest dose administered (65 mg/kg BW/day), excluding the 150 mg/kg BW/day group. Evaluation of intrauterine parameters and fetal morphological data in the 150 mg/kg BW/day group was precluded by maternal mortality. A second study in rabbits, described in item f below, allowed the determination of a definitive NOEL/NOAEL.

f. Oral Developmental Toxicity Study in Non-Rodents

(1) Report Number: B010602 and B01702

(2) Report Date: September 18, 2001

(3) Study Location (in-life): Greenfield, Indiana, USA

(4) Study Design: This GLP-compliant study was conducted mostly according to VICH GL32. Lubabegron was orally administered to pregnant New Zealand White rabbits (20/group) on GDs 7 - 19 at doses of 0, 3, 10, or 100 mg/kg BW/day in 10 mL/kg BW dose volume. The rabbits were euthanized on GD 28 for evaluation of maternal reproductive parameters, and the fetuses were assessed for viability, weight, gender, and morphology. Additional mated rabbits received lubabegron at doses of 3, 10, or 100 mg/kg BW/day on GDs 7 - 19 and bloods were sampled for toxicokinetic evaluation on GD 19.

(5) Results and Conclusion: One and two rabbits in the 3 and 100 mg/kg BW/day groups, respectively, aborted following periods of decreased food consumption and were euthanized. During GDs 14 - 24, BW were decreased in the 100 mg/kg BW/day group. In the 100 mg/kg BW/day group, decreased or absent feces or absence of urine were observed and considered treatment-related. Mean absolute and/or relative food consumption was decreased, compared to the control, from GDs 14 through 20 and throughout the treatment period for rabbits in 10 and 100 mg/kg BW/day groups, respectively. There were no treatment-related differences in maternal reproductive parameters. No fetal toxicities were noted at any of the doses tested. The plasma concentrations of lubabegron were highly variable between animals, especially in the 100 mg/kg BW/day group. The mean AUC and $C_{max}$ values increased with increasing dose, but were less than dose proportional. The NOEL/NOAEL for maternal toxicity was 10 mg/kg BW/day based on decreases in BW and food consumption observed at the next higher dose tested. The NOEL/NOAEL for developmental toxicity was 100 mg/kg BW/day, the highest dose tested. Lubabegron was not considered teratogenic in rabbits under the conditions of this study.

g. Two-Generation Oral Reproductive Toxicity Study in Rats

(1) Report Number: WIL 668018

(2) Report Date: April 11, 2011

(3) Study Location (in-life): Ashland, Ohio, USA

(4) Study Design: This GLP-compliant study was conducted according to VICH GL22. Lubabegron was administered to post-weaning male and female Sprague-Dawley rats (F0 generation, 30/sex/group), and their male and female offspring (F1 generation) starting on postnatal day (PND) 21, through gavage at doses of 0, 5, 15, and 30 mg/kg BW/day. The male and female rats in the F0 and F1 generations were continuously dosed for at least 70 days before mating, and the treatments continued during mating, pregnancy, lactation, until weaning of the offspring on PND 21. BW, food consumption, reproductive performance, litter data, sexual maturation, organ weight, sperm parameters, and macroscopic and microscopic examinations were evaluated.

(5) Results and Conclusion: There were no treatment-related effects on F0 or F1 parental survival and food consumption at any of the doses tested. Lower mean BW of F1 males in the 30 mg/kg BW/day group were noted for about three weeks. No effects on BW were seen in the F0 generation, F1 females, or pre-weaning F2 pups at any dose levels. Dilatations of the reproductive organs in F0 females were observed at 30 mg/kg BW/day, but not in the lower doses; no functional deficiencies were associated with this finding. Reduced thyroid gland weights in the F1 females and increased incidence of chronic progressive nephropathy in the F1 males were seen at 30 mg/kg BW/day. There were no other adverse effects identified through macroscopic or microscopic evaluations in the F0 and F1 males and females. Mean organ weights in the F0 and F1 adults were not affected by the treatments. The mean numbers of F1 and F2 pups born, percentage of males at birth, live litter size on PND 0, the number of implantation sites, and postnatal survival in any of the treatment groups were unaffected by the treatment of lubabegron to F0 and F1 parental rats. No treatment-related effects were noted for F1 and F2 pup organ weights on PND 21 at any doses. Based on the lack of effects on reproductive processes of F0 and F1 rats at any of the tested doses, 30 mg/kg BW/day (the highest dose tested in this study) was the NOEL/NOAEL for reproductive toxicity. The NOEL/NOAEL of systemic toxicity was 15 mg/kg BW/day based on multiple treatment-related findings in rats dosed at 30 mg/kg BW/day.

h. Single Oral Dose Safety and Pharmacokinetic Study in Human Subjects

(1) Report Number: H7v-BD-GPAA

(2) Report Date: November 2, 2008

(3) Study Location (in-life): Zuidlaren, The Netherlands

(4) Study Design: The purpose of the study was to examine the safety and tolerability, evaluate the β1 adrenoceptor blocking properties of lubabegron using the isoproterenol test, and compare lubabegron pharmacokinetics in healthy human subjects of both sexes (males and non-fertile females) in fasted and fed conditions, following a single rising dose administration in a capsule. The study was conducted in two phases according to Good Clinical Practices. In the first phase (dose escalation), a subject and investigator-masked, randomized and placebo-controlled single oral dose study was conducted in three alternate panels of nine healthy overweight males and non-fertile females; nine rising doses administered were 15, 45, 90, 135, 200, 250, 300, 250, and 400 mg lubabegron. The second phase study (fed/fasted condition) was an open label, randomized, balanced, two-period cross-over study in which eight subjects (four males and four non-fertile females who did not participate in the first phase) received the same oral dose of lubabegron (135 mg) once in the fasted state and once in the fed state. For both phases, the following information was collected: blood pressure and heart rate, standard 12-lead electrocardiograms (ECGs), body temperature, BW, a full physical examination, a post-study follow-up occurred within 3 to 7 days after completion of the last

treatment period, and routine hematology, clinical chemistry and urinalysis.

(5) Results and Conclusion: Lubabegron was well-tolerated by the human subjects when administered as single oral doses between 15 and 400 mg. Similarly, the 135 mg dose administered following a fasting period or a fasting period then a high fat breakfast was well-tolerated. There were no severe adverse effects observed. There was no apparent relationship between dose level of lubabegron and the number of AE reported. No clinically significant changes in hematology, clinical chemistry and urinalysis were observed during the conduct of the study. There were no clinically important findings in the morphology of the 12-lead ECG for individual subjects at each dose level or in each dietary state. All subjects (control and treated groups) experienced decreases in systolic and diastolic blood pressure of greater than 20 mmHg and/or greater than 10 mmHg, respectively. Similarly, all subjects had increases in heart rate of greater than 20 bpm on standing on at least one time point during the study following both lubabegron, irrespective of dietary state, and placebo. None of these changes were associated with hypotensive symptoms and none were considered to be dose-related or of clinical significance. The result of fitting the linear mixed model indicates a consistent decrease in blood pressure (approximately 4 to 6 mmHg; both supine and standing) at 6 hours post-dose for the lubabegron treatment compared to placebo. It also suggested a small decrease in heart rate at 10 hours post-dose in supine position and at 6 and 10 hours post dose in standing position for the lubabegron treatment. Orthostatic changes in heart rate also seem to differ between placebo and the 400 mg dose of lubabegron, with a smaller increase in standing position from 6 to 48 hours post-dose for the lubabegron treatment. Analysis of data by a linear mixed model showed a statistically significant increase in QTc (+7.7 msec) after lubabegron 400 mg treatment. A physiological increase in heart rate induced by standing between 6 and 48 hours post dose observed in the placebo group was not seen in the treated group. Treatment with lubabegron was also characterized by a significant blockade of the physiological increase in heart rate induced by standing between 6 and 48 hours post-dose.

Oral administration of lubabegron showed a high degree of inter-subject variability in pharmacokinetic parameters ($C_{max}$ and $AUC_{0-\infty}$). Blood lubabegron concentration measurements indicated that $T_{max}$ occurred between 0.5-6 hours post-dose, with a secondary peak appearing between 12-24 hours post-dose under the fasting condition. Body weight, BMI, and gender had no effect on $C_{max}$ and $AUC_{0-\infty}$. A dose proportional increase in $AUC_{0-\infty}$ was observed across all doses tested in this study (15 to 400 mg), whereas the increase in $C_{max}$ was less than dose proportional. Administration of lubabegron with food dramatically increased the maximal lubabegron plasma concentrations ($C_{max}$) by nearly 300% and total exposure (AUC) by 69%.

As conducted as part of the study, when the $\beta_1$ antagonism of lubabegron was characterized with an $E_{max}$ model, the minimal $\beta_1$

activity ($EC_{20}$) was seen at 5.7 ng/mL of lubabegron plasma concentration. Isoproterenol induced a 2-fold shift for the lubabegron dose-response curve, similar to that associated with other beta-blockers reported in published studies.

A NOEL/NOAEL was established at the 15 mg single dose (equivalent to 0.16 mg/kg BW using the mean BW of 93.9 kg of the subjects in the study), based on changes in cardiovascular parameters (decrease in blood pressure and heart rate in the supine and standing position).

i. Multiple Oral Dose Safety and Pharmacokinetics Study in Human Subjects

(1) Report Number: H7V-BD-GPAB

(2) Report Date: November 2, 2008

(3) Study Location (in-life): Zuidlaren, The Netherlands

(4) Study Design: The purpose of the human multiple oral dosing study was to examine the safety, tolerability, and the β1-adrenoceptor blocking properties of lubabegron in comparison to the single dose study, and to evaluate the effects on energy expenditure with indirect calorimetry after multiple-dose administration of lubabegron. The study was conducted in overtly overweight healthy male and female human subjects (8/group) according to Good Clinical Practices. Human volunteers were given lubabegron at doses at 50 and 125 mg, once daily on Days 1 to 14, in a sequential fashion. For each dose level, 6 subjects received lubabegron and 2 subjects received placebo. Blood samples were taken for pharmacokinetic analysis at regular intervals following dose administration on Day 1 (up to 24 hours post-dose) and following the final dose on Day 14 (up to 120 hours post-dose). On Days 6, 7, 8, 12, and 13, blood samples were obtained at pre-dose and 2 hours post-dose. The following pharmacodynamic tests were performed at pre-determined time points: isoproterenol test, measurement of energy expenditure by indirect hood calorimetry, oral body temperature, and biochemical markers (plasma potassium, serum leptin, serum non-esterified fatty acid and glycerol).

(5) Results and Conclusion: One subject withdrew from the study for personal reasons after receiving eight doses of 125 mg lubabegron. All subjects experienced mild somnolence, and flatulence. Overall, daily dosing of 50 and 125 mg lubabegron for 14 days was considered to be well-tolerated by the study subjects. Although standing heart rate decreased at 6 hours post-dose for both dose groups compared to that of placebo-treated subjects, this effect was not considered to be of clinical concern and not to be biologically significant. Lubabegron treatment did not cause clinical changes in vital signs, 12-lead ECG, clinical laboratory evaluations, or physical examination during the study.

With multiple dosing, peak lubabegron concentrations occurred typically at 4 hours and were similar to that observed in the single-dose, food-effect study. Post-dose plasma lubabegron concentrations declined in a biexponential manner with a mean terminal half-life of 12.4 hours.

Based on the terminal half-life, the steady state was expected by Day 3, whereas the first trough concentrations were measured on Day 6, indicating that the steady state was reached on Day 6. The mean accumulation ratio was 1.56, which was consistent with the terminal half-life and the dosing interval. The pharmacokinetics of lubabegron in the multiple dose study were similar to the single-dose, food effect study.

Measurement of Compound 450806, a metabolite of lubabegron, indicated a longer terminal half-life (43.7 hours) and higher $C_{max}$ (52% greater than that of lubabegron) for this metabolite compared to the parent drug.

Lubabegron-associated $\beta_1$ antagonism was demonstrated through the isoproterenol test, as the isoproterenol dose required to cause an increase of the heart rate by 25 bpm was significantly higher in the lubabegron-treated subjects (15 and 16 folds of increase compared to the baseline dose, for the 50 and 125 mg lubabegron groups, respectively). No increase in thermogenesis or lipolysis was detected in lubabegron-treated subjects.

A NOEL/NOAEL was not established in this study, because it was designed to address the safety, tolerability, and the $\beta_1$-adrenoceptor blocking properties of lubabegron in comparison to the single dose study, and to evaluate the effects on energy expenditure with indirect calorimetry after multiple-dose administration of lubabegron.

In conclusion, lubabegron-associated $\beta_1$ antagonism was demonstrated through the isoproterenol test and no increase in thermogenesis or lipolysis was detected in lubabegron-treated subjects.

j. Genetic Toxicity studies

(1) Bacterial Reverse Mutation Assay (Ames Test)

(a) Report Numbers: 010508AMT4969; 015016AMT4969; 015023AMS4969

(b) Report Date: July 2001

(c) Study Location (in-life): Greenfield, Indiana, USA

(d) Study Design: This GLP-compliant study was conducted according to VICH GL23. The purpose of this study was to evaluate the potential of the test substance, lubabegron, to induce a reverse mutation in specific bacterial tester strains of *Salmonella typhimurium* and *Escherichia coli* strain WP2uvrA. The procedure used was based on the direct plate method of Ames *et al.* (Mutation Research 31, 347-364. 1975). Four strains of *S. typhimurium* (TA 1535, TA 1537, TA 98, TA100) and one strain of *E. coli* (WP2*uvr*A), were used in the assay. The metabolic activation system (S9 mix) consisted of liver homogenate (S9) from Aroclor-1254 induced male Sprague-Dawley rat liver and the necessary co-

factors. The test substance was dissolved in dimethylsulfoxide (DMSO). Positive control substances used in the assay included 2-aminoanthracene, 9-aminoacridine, 2-nitrofluorene, and *N*-methyl-*N*-nitrosoguanidine. Toxicity of the test substance was assessed in a preliminary toxicity-mutation assay using the four *S. typhimurium* strains and the *E. coli* strain (+/-S9). Five concentrations of the test article ranged from 312.5 to 5000 µg/plate in the first preliminary range-finding/mutagenicity assay (+/-S9). Precipitate was observed at 5000 µg/plate and the test substance was toxic to all the tester strains at 625 µg/plate and higher doses. Based on the combined results from the two range-finding/mutagenicity tests, the concentrations for the definitive mutagenicity assay were determined. For TA1535 and WP2uvrA, both with and without activation, the concentrations were 40, 80, 160, 320, and 640 µg/plate. Concentrations of 20, 40, 80, 160, and 320 µg/plate were used for TA1537 and TA98 with activation. For TA100 with activation, the top concentration used was 160 µg/plate. Without activation, the top concentration used for TA1537, TA98, and TA100 was 80 µg/plate.

(e) Results and Conclusion: In the definitive mutation assay, there was some toxicity to the tester strains at the top concentration of the test article, but chemical precipitate was not observed. Mean values from the triplicate plates in the non-activation and activated assays showed that the incidence of revertant colonies for each tester strain treated with lubabegron was not significantly different from the corresponding DMSO-treated controls. The numbers of *S. typhimurium* and *E. coli* revertants observed following treatment with lubabegron, with or without metabolic activation, were not significantly different from vehicle control values. It was concluded that lubabegron was not mutagenic in the *S. typhimurium* and *E. coli* bacterial strains.

(1) *In Vitro* Mammalian Cell Gene Mutation Test

(a) Report Number: 21513-0-437

(b) Report Date: August 2001

(c) Study Location (in-life): Chantilly, Virginia, USA

(d) Study Design: This GLP-compliant study was conducted according to VICH GL23. The potential of lubabegron to induce chromosomal aberrations in Chinese Hamster Ovary (CHO) cells was investigated in the *in vitro* chromosome aberration assay. The test was conducted both with and without metabolic activation using an S9 fraction prepared from the livers of Aroclor 1254 induced rats. Chromosomal aberrations were evaluated in CHO cells exposed for 4 hours to lubabegron at concentrations of 2.00, 2.50, 3.00 µg/mL without metabolic activation, 2.00, 2.25, and 2.50 µg/mL for 17.7 hours without metabolic activation, and 6.5, 7.50, and 8.00 µg/mL with metabolic activation.

Results and Conclusion: No significant increase in cells with chromosomal aberrations was observed in the cultures analyzed. Lubabegron was considered negative for inducing chromosomal aberrations in CHO cells with and without metabolic activation.

(2) Mammalian Erythrocyte Micronucleus Test

(e) Report Number: 010523MNT4969

(f) Report Date: September 2000

(g) Study Location (in-life): Greenfield, Indiana, USA

(h) Study Design: This GLP-compliant study was conducted according to VICH GL23. The potential of lubabegron to induce micronuclei *in vivo* was investigated in bone marrow of male and female ICR mice. Mice (5/sex/dose) were given orally by gavage on 2 consecutive days either 20 mL/kg BW of the vehicle control (10% acacia), or 500, 1000, or 2000 mg/kg BW of lubabegron. Approximately 24 hours after the second treatment, bone marrow was collected and the frequency of micronucleated polychromatic erythrocytes (MPCE) was determined microscopically.

(i) Results and Conclusion: The incidence of MPCE in mice treated with lubabegron was not elevated over animals receiving the vehicle control. Lubabegron did not induce micronuclei *in vivo* in bone marrow of ICR mice.

(1) Summary of genotoxicity studies

The findings from the genotoxicity testing are presented below in Table IV.C.1.

**Table IV.C.1. Summary of genotoxicity studies**

| Study Type | Study Number | Results |
|---|---|---|
| Bacterial Reverse Mutation Assay (Ames Test) | 010508AMT4969<br>015016AMT4969<br>015023AMS4969 | Negative |
| *In vitro* Mammalian Cell Gene Mutation Test | 21513-0-437 | Negative |
| Mammalian Erythrocyte Micronucleus Test | 010523MNT4969 | Negative |

These genotoxicity study data indicated that lubabegron does not have the potential to cause genetic toxicity in the tested systems.

k. Pharmacology Studies

*In vitro* and *in vivo* pharmacology studies as summarized below indicated that lubabegron has (1) agonistic activity to the $\beta_3$-adrenergic receptor; (2) high binding affinity with the $\beta_1$- and $\beta_2$-adrenoceptors and low binding affinity with non-β-adrenoceptors; and (3) antagonistic activity to the $\beta_1$- and $\beta_2$-adrenergic receptors.

(1) *In Vitro* Pharmacology Studies

A series of *in vitro* receptor binding assays were conducted to examine the binding of lubabegron to various receptors: cholinergic receptor (muscarinic), adrenergic receptor ($\alpha_1$-adrenergic, $\alpha_2$-adrenergic, $\beta_1$ adrenergic, $\beta_2$-adrenergic), dopaminergic receptor (dopamine $D_1$, dopamine $D_2$), serotonergic receptor (5-$HT_2$), histaminergic receptor (histamine H1), GABA receptor-mediated ionophore ($GABA_A$), and benzodiazepine receptor. In receptor binding assays, the receptor binding affinities were measured by the competitive displacement of radiolabeled receptor specific ligands with the unlabeled lubabegron in transfected Sf9 cells (for $\beta_1$- and $\beta_2$-adrenergic binding studies), or frozen brain tissues from rats (for muscarinic, 5-$HT_2$, dopamine $D_1$ and $D_2$, $\alpha_1$- and $\alpha_2$-adrenergic, benzodiazepine, and $GABA_A$ binding studies) or guinea pigs (for histamine $H_1$ binding study). Lubabegron had binding affinity (Ki) of 0.5 ± 0.01 and 0.3 ± 0.05 nM for human $\beta_1$- and $\beta_2$-adrenergic receptors, respectively. No affinity was observed at > 300 nM for muscarinic, 5-$HT_2$, dopamine $D_1$ and $D_2$, $\alpha_1$- and $\alpha_2$-adrenergic, benzodiazepine, histamine $H_1$, or $GABA_A$ receptors. This study suggested that lubabegron can be characterized as a $\beta_1$- and $\beta_2$-adrenergic receptors ligand.

In the cAMP accumulation assays, the $\beta_1$-, $\beta_2$-, or $\beta_3$-adrenergic receptor activation was assessed through measuring the cAMP accumulation in CHO cells transfected with human, rat, dog, or monkey $\beta_3$-adrenergic receptor upon stimulation with lubabegron or isoproterenol. Lubabegron was determined as a $\beta_3$-adrenergic receptor agonist, but demonstrated minimal agonistic activity (< 10% isoproterenol maximum) for human $\beta_1$- and $\beta_2$-adrenergic receptors.

In the atria contraction experiments at the concentrations tested, lubabegron did not show any $\beta_1$ agonist activity, nor did it block isoproterenol-induced contractions. Although lubabegron may have affinity for these receptors, the tissue assay suggested that lubabegron may not have a functional effect on $\beta_1$-, $\beta_2$-, and $\beta_3$-adrenergic receptors. In addition, it may also lack a lipolytic activity that is characteristic of other $\beta_3$-adrenergic receptor agonists.

Lubabegron inhibited basal glycerol production, a measure of lipolysis, in human omental adipocytes only at the highest compound concentration in the presence of 600 nM propranolol. In comparison, the lipolytic effect in isolated rat epididymal adipocytes was more robust.

An *in vitro* test was conducted to determine the $I_{Kr}$ (delayed inward rectifying potassium current) ion channel blocking profile for lubabegron in a stably transfected human embryonic kidney (HEK 293) expressing human $I_{Kr}$ (HERG) mRNA. Blockade of the $I_{Kr}$ channel could lead to prolonged electrocardiographic QT interval. The $IC_{50}$ for HERG activity for lubabegron blocking HERG current was determined to be 0.799 µM, which is less than a plasma value of 81.3 µM, a predicting value causing a risk of prolonged QT interval (20% HERG blockade). Thus, the blocking response in humans was predicted to be too weak to cause cardiovascular effects.

In a $\beta_3$-adrenergic receptor activation assay, the $\beta_3$-adrenergic receptor activation was assessed through measuring the cAMP accumulation in CHO cells transfected with human $\beta_3$-adrenergic receptor upon stimulation with lubabegron or isoproterenol (a classic nonselective β-adrenoceptor agonist). Lubabegron was determined to be a $\beta_3$-adrenergic receptor agonist.

A cAMP accumulation assay was conducted using CHO cells transfected with rat, dog, or monkey $\beta_3$-adrenergic receptor to evaluate the $\beta_3$-agonistic effect of lubabegron across these three species. The results suggested that lubabegron was a $\beta_3$-adrenergic receptor agonist in all three species.

(2) *In Vivo* Pharmacology Studies

In a single-dose monkey study, monkeys were administered nasogastrically lubabegron at 0, 10, 50, or 300 mg/kg BW and cardiovascular effects were measured. There were no changes in systolic, diastolic, mean arterial, or left ventricular end-diastolic pressures. However, decreased heart rate and lower left ventricular inotropic state were observed at all doses. These results appear to demonstrate a reduction in sympathetic tone and are consistent with antagonism of the $\beta_1$- or $\beta_2$-adrenergic receptors in the heart and systemic vasculature.

In a single-dose rat study, rats were administered lubabegron at 0, 30, 70, and 150 mg/kg BW and respiratory functions were studied. Elevated respiratory rates and minute volumes in a dose-dependent fashion were observed, but there were no significant changes in tidal volume. These changes are consistent with $\beta_2$-adrenergic antagonist activity.

A single-dose mouse study was conducted to examine effects on the central nervous system (CNS) and behavioral parameters. CD-1 mice were administered lubabegron at 0, 30, 70, and 150 mg/kg BW. Clinical signs, spontaneous ambulatory and non-ambulatory activity levels, convulsion threshold, hexobarbital-induced sleep time, body temperature, grip strength, auditory startle, and acid-induced writhing were examined. No effects were observed up to the highest dose tested.

2. Determination of Toxicological NOEL/NOAEL for Chronic Exposure.

Studies for total residues of lubabegron considered for determination of the toxicological NOEL/NOAEL or LOEL/LOAEL for chronic exposure are summarized in Table IV.C.2.

**Table IV.C.2: NOEL/NOAEL or LOEL/LOAEL in toxicology studies for lubabegron**

| Study Type | Report Number | NOEL/NOAEL or LOEL/LOAEL (mg/kg BW/day)* |
|---|---|---|
| Subchronic Oral Toxicity Study in Rats | R03102 and R03202 | 30 (LOEL/LOAEL) |
| Subchronic Oral Toxicity Study in Monkeys | 6180-463 | 15 (LOEL/LOAEL) |
| Chronic Oral Toxicity Study in Rats | 130-189 | 5 (NOEL/NOAEL) |
| Oral Developmental Toxicity Study in Rabbits | B010602 and B01702 | 10 (NOEL/NOAEL for maternal toxicity)<br><br>100 (NOEL/NOAEL for developmental toxicity) |
| Oral Developmental Toxicities Study in Rats | 668012 | 100 (NOEL/NOAEL for maternal toxicity)<br><br>300 (NOEL/NOAEL for developmental toxicity) |
| Single-Dose Oral Study in Human Volunteers | H7V-BD-GPAA | 0.16 (NOEL/NOAEL) |
| Multiple Dose Oral Study in Human Volunteers | H7V-BD-GPAB | NOEL/NOAEL was not established |

*The NOEL/NOAEL or LOEL/LOAEL was not corrected for the purity of the test substance (88.9%) in all the toxicology studies.

Based on the totality of the available toxicological data, the NOEL/NOAEL of 0.16 mg/kg BW (15 mg/person) from the single oral dose escalation safety and pharmacokinetics study in healthy overweight subjects is selected to be the most appropriate for the determination of the toxicological acceptable daily intake (ADI) for chronic exposure of total residues of lubabegron to human consumers. The selection of the lowest acute dose from the human study is protective of the effects from chronic exposure seen at higher doses and, thus, is protective to humans consuming edible tissues from treated animals which contain the residues.

3. Toxicological Acceptable Daily Intake (ADI)

   The toxicological ADI for total residues of lubabegron is calculated using the following formula based on the NOEL/NOAEL of 0.16 mg/kg BW/day and an overall safety factor of 50. This overall safety factor includes a factor of 10 to account for expected variation in the human population's responses to the drug residues, and a factor of 5 to account for subpopulations that may be more sensitive to the effects of the drug residues than the overweight volunteers and to account for the small number of test subjects in the study (8 male and 8 female subjects). The toxicological ADI is calculated using the following formula:

$$\text{Toxicological ADI} = \frac{\text{NOEL/NOAEL}}{\text{Safety Factor}} = \frac{0.16 \text{ mg/kg bw/day}}{50}$$
$$= 0.0032 \text{ mg/kg bw/day} = 3.2 \text{ µg/kg bw/day}$$

   The toxicological ADI for total residues of lubabegron is 3.2 µg/kg BW/day. Because the toxicological ADI is based on the NOEL/NOAEL from a single dose study, it can be considered to be an acute reference dose in this situation.

## D. Establishment of the Final ADI

Because lubabegron is not an antibacterial, a microbiological ADI was not needed. The toxicological ADI (3.2 µg/kg BW/day) is established as the final ADI for total residues of lubabegron. Rounding down to one significant figure, the final ADI for total residues of lubabegron is 3 µg/kg BW/day.

## E. Safe Concentrations for Total Residues in Edible Tissues

The calculation of the tissue safe concentrations is based on the General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals (FDA/CVM, Guidance for Industry #3, revised July 2016). The safe concentration for total residues of lubabegron (ppm) in each edible tissue of beef cattle is calculated using the following formula:

$$\text{Safe Concentration} = \frac{\text{ADI} \times \text{Human Body Weight}}{\text{Food Consumption Value}}$$

The average human BW is approximated at 60 kg. The daily food consumption values for each edible tissue of beef cattle are 300 g for muscle, 100 g for liver, 50 g for kidney, and 50 g for fat.

Therefore, the safe concentration for total residues of lubabegron in each edible tissue of beef cattle is 0.6 ppm for muscle, 1.8 ppm for liver, 3.6 ppm for kidney, and 3.6 ppm for fat (see Table IV.E.1).

**Table IV.E.1. Summary table of safe concentrations for total residues**

| Edible Tissue | Amount Consumed Per Day | Safe Concentration |
|---|---|---|
| Muscle | 300 g | 0.6 ppm |
| Liver | 100 g | 1.8 ppm |
| Kidney | 50 g | 3.6 ppm |
| Fat | 50 g | 3.6 ppm |

## F. Residue Chemistry

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Studies

(1) Study Number: 286652

(2) Study Dates: October 2012 to April 2014

(3) Study Location: Edinburgh, Scotland, United Kingdom

(4) Objective: The aim of this GLP study was to determine the concentrations of total residues in tissues of cattle following a five-day treatment with [$^{14}$C]-lubabegron administered orally. The dosing rate in the study was 0.4 mg/kg BW/day, which is approximately four times the drug intake expected with the 5 g/ton maximum dosing level in feed for cattle in this application.

(5) Experimental Design: A [$^{14}$C]-lubabegron metabolism and residue study was conducted using 18 Aberdeen Angus-cross beef cattle (9 male and 9 female), aged between 4 and 9 months and weighing 174-244 kg. Sixteen animals were randomly assigned to four groups each comprised of two males and two females. The two remaining animals served as controls and were slaughtered prior to the first group of treated animals. The tissues from the control animals were used as control matrices. The radiolabeled lubabegron was administered in two daily doses 12 hours apart *via* oral capsule for five consecutive days at a concentration equivalent to 0.4 mg/kg BW/day to the animals in the treated groups.

Four groups of 4 cattle (2 male, 2 female) were slaughtered at 12, 24, 48, and 72 hours after the last treatment dose. Urine and feces were collected from 4 cattle for 72 hours after the last treatment. The following tissues were collected: kidneys, liver, muscle, and fat. Control tissue samples were collected from the two untreated animals prior to slaughter of the Group 1 animals at 12 hours post-last dose.

Samples of urine, feces, and tissues were analyzed for total radioactivity by either direct liquid scintillation counting (LSC) or LSC following oxidation. Metabolites were characterized by thin-layer chromatography (TLC) analysis for urine samples. Liver and kidney samples were

extracted for metabolite profiling. The marker residue concentration was determined using an LC-MS/MS method.

(6) Results: The administered dose was primarily (80-106%) excreted in the feces, and reached a relatively steady rate of excretion by 36-60 hours post first dose. Recoveries in urine and cage-wash were very low, accounting for 2.2-3.1% and 1.1-1.7%, respectively, of the administered dose. Levels attained relative stability by 36-60 hours post-first dose. No apparent differences were noted by gender. The low radioactivity in urine samples precluded metabolite profiling.

Initial TLC results suggested that urine samples contained a high degree of highly polar material. The major peak detected in the 156 hour fecal samples was unchanged lubabegron accounting for ~4-6% of the dose in male and female samples. A minor polar metabolite detected in feces and accounted for ≤1.3% of the dose.

The highest mean concentrations of radioactivity in tissues were in liver at 12 hours post-last dose (1,088 ppb). The mean radioactivity in liver at 24, 48, and 72 hours post-last dose were 1,011 ppb, 1,014 ppb, and 890 ppb, respectively. The mean radioactivity in muscle was below background at 12, 24, 48, and 72 hours post-last dose. The mean radioactivity in kidney was 370 ppb, 400 ppb, 479 ppb, and 459 ppb at 12, 24, 48, and 72 hours post-last dose. The mean radioactivity in fat was 5 ppb, 5 ppb, 145 ppb, and 102 ppb at 12, 24, 48, and 72 hours post-last dose. The total radioactive concentrations in the tissues are shown below in Table IV.F.1.

**Table IV.F.1. Mean total radioactive residues in tissues as µg equiv./kg (ppb)**

| Withdrawal Time (hours) | Mean Total Lubabegron Residues in Muscle (ppb) ± S.D. | Mean Total Lubabegron Residue in Liver (ppb) ± S.D. | Mean Total Lubabegron Residue in Kidney (ppb) ± S.D. | Mean Total Lubabegron Residue in Fat (ppb) ± S.D. |
|---|---|---|---|---|
| 12 | 0 ± 1* | 1,088 ± 219 | 374 ± 50 | 5 ± 4* |
| 24 | 2 ± 3* | 1,011 ± 202 | 401 ± 79 | 5 ± 7* |
| 48 | 2 ± 2* | 1,014 ± 108 | 479 ± 111 | 145 ± 277* |
| 72 | 10 ± 7* | 890 ± 183 | 459 ± 83 | 102 ± 173* |

* indicates mean calculated from data less than 30 disintegrations per minute above background

The corresponding results for parent lubabegron are outlined in Table IV.F.2 below. The highest mean concentrations were in liver at 12 hours post-last dose and 24 hours post-last dose (6.16 ppb and 6.61 ppb, respectively). The mean radioactive lubabegron equivalents in liver at 48 and 72 hours post-last dose were 1.55 ppb and <LLOQ, respectively.

**Table IV.F.2. Mean parent lubabegron residues in tissues as µg equiv./kg (ppb)**

| Withdrawal Time (hours) | Mean Lubabegron Residue in Muscle (ppb) ± S.D. | Mean Lubabegron Residue in Liver (ppb) ± S.D. | Mean Lubabegron Residue in Kidney (ppb) ± S.D. | Mean Lubabegron Residue in Fat (ppb) ± S.D. |
|---|---|---|---|---|
| 12 | 1.51 ±0.19 | 6.16 ± 1.78 | 4.40 ± 0.65 | 1.30 ± 0.13 |
| 24 | 1.38 ± 0.13 | 6.61 ± 4.73 | 2.96 ± 0.34 | 1.21 ± 0.14 |
| 48 | <LLOQ | 1.55 ± 0.35 | 1.63 ± 0.38 | <LLOQ |
| 72 | <LLOQ* | <LLOQ* | <LLOQ* | <LLOQ* |

*indicates n=3, all others n=4 LLOQ (lowest point on the calibration curve) is 0.963 ppb

Extractability in tissue samples was low in liver and kidney samples suggesting appreciable protein binding. Protease digestion experiments confirmed that a high proportion of the bound radioactivity could be liberated. Due to low residue concentrations and low apparent extractability, no metabolite profiles could be generated from the tissue samples.

b. Comparative Metabolism Study

(1) Study Number: 196171

(2) Study Dates: April 2013 to June 2014

(3) Study Location: Edinburgh, Scotland, United Kingdom

(4) Objective: The aim of this GLP study was to compare the rate of metabolism and metabolic profile of [$^{14}$C]-lubabegron in cryopreserved hepatocytes and hepatic microsomes prepared from male cattle (Bos indicus and Bos taurus), male Landrace pigs, male Sprague Dawley rats, male Beagle dogs, and mixed gender humans.

(5) Experimental Design: [$^{14}$C]-lubabegron was incubated with cryopreserved hepatocytes or hepatic microsomes prepared from each species. Cells were dosed at 5 or 20 µM [$^{14}$C]-lubabegron and collected at timepoints ranging from 0-240 minutes. After processing, samples were analyzed by HPLC for [$^{14}$C]-lubabegron and potential metabolites. Metabolite analysis was performed by LC-MS/MS.

(6) Results: Based on peaks and retention times, parent lubabegron was confirmed in all samples analyzed. Metabolite M1 (2-[4-amino-2-methyl propyl phenoxyl]pyridine-3-carbonitrile), a cleaved form of the parent compound, was a major metabolite identified in all species. Other metabolites identified in all samples, but in varying concentration between species, were metabolites formed via oxidative metabolism. Metabolism in laboratory animals (rat or dog) produces the same major metabolite of parent lubabegron as those seen in cattle, the target

animal species, thus supporting auto-exposure in laboratory animals to the residues to which humans will be exposed when consuming edible products derived from treated cattle. No species-specific metabolites were observed and the differences in metabolites between species and test systems were quantitative, not qualitative. Therefore, comparative metabolism has been demonstrated.

c. Study to Establish Withdrawal Period

(1) Tissue Residue Depletion Study

(a) Study Number: 016-01556

(b) Study Dates: December 2016 to April 2017

(c) Study Location: Las Cruces, NM (in-life phase)

(d) Objective: The objective of this GLP study was to determine the concentration of lubabegron in edible tissues (muscle, fat, liver, and kidney) of cattle following oral administration as a Type C medicated feed at a dose of 0.37 mg/kg/day for 10 days. Tissue lubabegron concentrations were measured at treatment withdrawal times ranging from 10-72 hours.

(e) Study Animals: Twenty-Six (13 male and 13 female) cross-bred cattle weighing from 225-309 kg were used for the study. Two animals (one male and one female) were used as controls, and the remaining 24 cattle were placed into 4 treated groups of 6 animals (3 male and 3 female) each.

(f) Dosing: Animals in the treated groups were offered Type C medicated feed with 13.0 g/ton of lubabegron fed at 2.6% pen BW for 10 days until withdrawal. Based on the actual feed consumption *per* pen, the average consumed dose was 0.29 to 0.35 mg/kg BW/day.

(g) Experimental Design: The concentration of the marker residue, lubabegron, in the liver (target tissue), muscle, kidney, and fat tissues of cattle was determined in a study wherein cattle were treated for 10 consecutive days with lubabegron Type C medicated feed at the rate of 13.0 g/ton. Control cattle were slaughtered at the beginning of the treatment period. The treated animals were slaughtered at 10-12, 22-24, 46-48, or 70-72 hours after receiving the final medicated feed. Samples of liver, muscle, kidney, and fat were analyzed by a validated LC-MS/MS method.

(h) Results: The liver, muscle, kidney, and fat samples from all animals on the study were analyzed using an LC-MS/MS determinative procedure. The method's limit of quantification (LOQ) was 1.0 ppb. Values that were below the value of the lowest calibration standard (equivalent to 1.2 ppb in tissue) were reported as below the calibration range (BCR). The results from the analysis are presented in Table IV.F.3 below. The mean lubabegron (marker residue) residue levels in liver declined from 2.23 ppb at 12 hours post-dose,

to 2.17 ppb at 24 hours, and BCR at 48 and 72 hours. The mean residue concentrations in muscle at the 12, 24, 48, and 72-hour withdrawal times, respectively, were 1.41 ppb, 1.32 ppb, BCR, and BCR. The mean residue concentrations in kidney at the 12, 24, 48, and 72-hour withdrawal times, respectively, were 2.30 ppb, 2.51 ppb, 1.16 ppb, and BCR. The mean residue concentrations in fat at the 12, 24, 48, and 72-hour withdrawal times, respectively, were BCR, 1.27 ppb, BCR, and BCR.

**Table IV.F.3. Mean concentration of lubabegron (ppb) in cattle tissues in Study 016-01556**

| Withdrawal Period | Fat | Kidney | Muscle | Liver |
|---|---|---|---|---|
| 10-12 hours | BCR | 2.30 | 1.41 | 2.23 |
| 22-24 hours | 1.27 | 2.51 | 1.32 | 2.17 |
| 46-48 hours | BCR | 1.16 | BCR | BCR |
| 70-72 hours | BCR | BCR | BCR | BCR |

2. Target Tissue and Marker Residue

Data presented in the total radioactive residue study (Study Number 196171) show that liver is the edible tissue of cattle in which residues of lubabegron are highest and persist longest. Parent lubabegron was the only metabolite measurable in the study. Thus, the target tissue is liver and the marker residue is lubabegron.

3. Tolerance

Total residues of lubabegron were below the safe concentration of 1.8 ppm at the 12-hour (*i.e.,* 0-day) withdrawal time point in the total residue and metabolism study (Study Number 196171), and a marker to total residue ratio of 0.57% was observed. Using the marker to total ratio and the safe concentration we calculate a tolerance of 10.26 ppb. Rounding to two significant figures, 10 ppb is assigned as the tolerance for lubabegron in cattle liver.

4. Withdrawal Period

The liver residue depletion data from Study Number 016-01556 were analyzed with a statistical method which determines the statistical tolerance limit for the 99$^{th}$ percentile of the population with 95% confidence. The tolerance limit for the single timepoint at 12 hours withdrawal is below the tolerance of 10 ppb. The data support the assignment of a 0-day withdrawal period for doses up to 5 g/ton (4.54 g/ton on a 90% DM basis).

### G. Analytical Method for Residues

1. Description of Analytical Method

a. Determinative Procedure

After adding internal standard to homogenized cattle liver, the sample is extracted twice with methanol:acetonitrile, centrifuged, and brought to volume in methanol:acetonitrile. An aliquot is centrifuged, filtered, and analyzed by LC-MS/MS. Quantitation is based on the m/z 500 → m/z 250 and m/z 518 → m/z 250 transitions for lubabegron and LSN543100, respectively.

b. Confirmatory Procedure

Sample extraction and LC-MS/MS analysis for the confirmatory procedure are identical to those for the determinative procedure. Lubabegron-specific ion transitions (m/z 500 → m/z 250, m/z 500 → m/z 209, and m/z 500 → m/z 187) are monitored to obtain ion ratios, signal to noise ratios, and retention times that meet the required acceptability criteria.

2. Availability of the Method

The method is available from the Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855. To obtain a copy of the analytical method, please submit a Freedom of Information Summary request to: https://www.accessdata.fda.gov/scripts/foi/FOIRequest/requestinfo.cfm.

## V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to Experior™:

**User Safety Warning**: The active ingredient in Experior, lubabegron, is a beta-adrenergic agonist/antagonist. Individuals with cardiovascular disease should exercise special caution to avoid exposure. Not for human use. Keep out of reach of children. When mixing and handling Experior, use protective clothing, impervious gloves, protective eye wear, and a NIOSH-approved dust mask. Operators should wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse thoroughly with water; if wearing contact lenses, rinse eyes first, then remove contact lenses and continue to rinse for 5-20 minutes. If irritation persists, seek medical attention. The safety data sheet contains more detailed occupational safety information. To report adverse drug events, access medical information, or obtain additional product information, call Elanco US Inc. at 1-800-428-4441. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or http://www.fda.gov/AnimalVeterinary/SafetyHealth.

## VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that Experior™, when used according to the label, is safe and effective

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. Additionally, data demonstrate that residues in food products derived from species treated with Experior™ will not represent a public health concern when the product is used according to the label.

**A. Marketing Status**

This product can be marketed over-the-counter (OTC) because the approved labeling contains adequate directions for use by laypersons and the conditions of use prescribed on the label are reasonably certain to be followed in practice.

**B. Exclusivity**

Experior™, as approved in our approval letter, qualifies for FIVE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(i) of the FD&C Act because this is the first time we are approving this active ingredient in a new animal drug application submitted under section 512(b)(1) of the FD&C Act.

**C. Patent Information:**

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.