**Approval Date: August 10, 2006**

# FREEDOM OF INFORMATION SUMMARY

# ORIGINAL NEW ANIMAL DRUG APPLICATION

# NADA 141-258

## ZILMAX (Zilpaterol Hydrochloride)

## Type A Medicated Article for Cattle Fed in Confinement for Slaughter

**For increased rate of weight gain, improved feed efficiency and increased carcass leanness in cattle fed in confinement for slaughter during the last 20 to 40 days on feed.**

**Sponsored By:**

**Intervet Inc.**
**P.O. Box 318**
**29160 Intervet Lane**
**Millsboro, DE 19966**

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**FREEDOM OF INFORMATION SUMMARY**

**ZILMAX**
**Type A Medicated Article for Cattle Fed in Confinement for Slaughter**

## *1. GENERAL INFORMATION:*

| | |
|---|---|
| a. File Number: | NADA 141-258 |
| b. Sponsor: | Intervet Inc.<br>P.O. Box 318<br>29160 Intervet Lane<br>Millsboro, DE 19966<br><br>Drug Labeler Code: 057926 |
| c. Established Name: | Zilpaterol hydrochloride |
| d. Proprietary Name: | ZILMAX |
| e. Dosage Form: | Type A Medicated Article |
| f. How Supplied: | 10 kg bag |
| g. How Dispensed: | OTC |
| h. Amount of Active Ingredients: | 4.8% (w/w) zilpaterol hydrochloride – 48 g per kg (21.77 g per pound) |
| i. Route of Administration: | Oral, in feed |
| j. Species/Class: | Cattle fed in confinement for slaughter |
| k. Recommended Dosage: | Feed zilpaterol hydrochloride (ZILMAX) at a dietary concentration of 6.8 g per ton (7.5 ppm) of zilpaterol hydrochloride/ton of feed to provide 60-90 mg zilpaterol hydrochloride/head/day on a 90% dry matter basis for the last 20 to 40 days on feed. This is equivalent to 7.6 g per ton (8.3 ppm) on a 100% dry matter basis.<br><br>The label includes the dosage expressed on a 90% dry matter basis in order to provide labeling comparable with other ruminant feed additives presently approved. |

l. Pharmacological Category: Beta adrenergic agonist

m. Indications: For increased rate of weight gain, improved feed efficiency and increased carcass leanness in cattle fed in confinement for slaughter during the last 20 to 40 days on feed.

## *2. EFFECTIVENESS:*

### *a. Dosage Characterization:*

Ten foreign studies were conducted either in the Republic of South Africa or Mexico using 1660 native cattle and typical management practices to measure the effects of zilpaterol hydrochloride (zilpaterol HCl) on rate of weight gain and feed efficiency. Rate of weight gain and feed efficiency data were used to define the optimum dose rate and feeding duration of zilpaterol hydrochloride. Using the optimum dose rate, the average of the initial and final body weights, and the average daily feed consumption, the optimal concentration of zilpaterol hydrochloride to be incorporated in the feed was calculated.

Table 1 presents the dose rate and feeding duration, for each study, when average daily gain and feed efficiency were maximally improved as a result of feeding zilpaterol hydrochloride. Cattle were fed zilpaterol hydrochloride on a milligram per kilogram body weight per day basis. Five of the studies involved titrating the dose and three of studies involved titrating the dose duration. The dose rate when performance was optimal or reached a plateau for the ten studies averaged 0.166 mg zilpaterol hydrochloride/kg body weight (BW)/day. The corresponding average inclusion rate for the ten studies equaled 6.18 g zilpaterol hydrochloride/ton of feed (as fed basis, 82% average dry matter). When corrected to 90% dry matter feed, the inclusion rate equaled 6.78 g zilpaterol hydrochloride/ton of feed. This inclusion rate was calculated to provide a means to deliver zilpaterol hydrochloride to confinement cattle in accordance with commercial feeding practices.

A dose duration range is presented in Table 1 as well.

Based on the data from the ten dose determination studies reported below, an inclusion rate of 6.8 g zilpaterol hydrochloride/ton of feed (90% dry matter basis) and a dosing duration range of 20 to 40 days was tested in the three pivotal dose confirmation efficacy trials.

Table 1. Summary Table of Optimal Dose Rates, Feed and Zilpaterol Consumption Rates, Inclusion Rates and Feeding Duration

| Study | Optimal Dose Rate (mg zilpaterol HCl/kg BW/day) | Average Daily Feed Consumption (kg/d, as-fed basis) | Average of Initial and Final Body Weights (kg) | Average Daily Zilpaterol HCl Consumption (mg/h/d) [1] | Optimal Inclusion Rate (g/ton of feed, as-fed basis) [2] | Dosing Duration (days) |
|---|---|---|---|---|---|---|
| 1 | 0.20 | 8.81 | 435.65 | 87.13 | 8.97 | 56 |
| 2 | 0.15 | 11.05 | 386.52 | 57.98 | 4.75 | 46 |
| 3 | 0.15 | 7.30 | 421.25 | 63.19 | 7.85 | 57 |
| 4 | 0.17 | 10.26 | 363.60 | 61.81 | 5.46 | 54 |
| 5 | 0.17 | 10.18 | 401.17 [3] | 68.20 | 6.08 | 24 |
| 6 | 0.16 | 9.73 | 362.00 | 57.92 | 5.39 | 53 |
| 7 | 0.15 | 9.93 | 424.79 [3] | 63.26 | 5.82 | 30 |
| 8 | 0.15 | 8.82 | 388.40 [3] | 58.26 | 5.98 | 30 |
| 9 | 0.20 | 13.29 | 402.77 | 80.55 | 5.49 | 27 |
| 10 | 0.16 | 9.69 | 403.61 [3] | 64.58 | 6.04 | 28 |
| Mean | 0.166 | 9.90 | 400.0 | 66.33 | 6.18 [4] | Range = 24 - 57 |

[1] Average Daily Zilpaterol HCl Consumption (mg zilpaterol hydrochloride/head/day) = Optimal Dose Rate × Average Body Weight.
[2] Optimal Inclusion Rate (g zilpaterol HCl/ton; as-fed basis) = [(Average Daily Zilpaterol HCl Consumption ÷ Average Daily Feed Consumption) × 907 kg/ton] ÷ 1000
[3] Final body weights only used in calculations.
[4] 6.8 g zilpaterol HCl/ton when corrected to 90% dry matter feed.

***b. Substantial Evidence:***

Data from three replicated, well-controlled steer and heifer trials involving 480 heifers and 480 steers were pooled and analyzed. Trials were conducted in various geographic areas of the United States. All cattle were fed a finishing diet during the pretreatment phase for at least 85 days prior to being treated with zilpaterol hydrochloride. No ionophore or antibiotic was fed during the treatment or withdrawal phase. Zilpaterol hydrochloride was fed either 20 or 40 days prior to slaughter. The cattle were withdrawn from zilpaterol hydrochloride for 5 days prior to slaughter. The cattle were fed a variety of diets that ranged in NEg from 62.3 to 70.9 Mcal/CWT of dry matter. Zilpaterol hydrochloride was administered in the feed at dietary concentrations of 0 or 6.8 g/ton (7.5 ppm) of feed, on a 90% dry matter basis. A mixed model analysis was conducted on data collected from the three trials. The fixed effects for the mixed model analysis were concentration of zilpaterol hydrochloride, duration of treatment, gender, zilpaterol by gender interaction, zilpaterol by duration interaction, and the zilpaterol by gender by duration interaction. Random effects for the mixed model analysis were trial site, weight block, and block within trial site.

Cattle were handled throughout the trials and at the termination of each trial, under conditions, which represent as closely as possible, commercial feedlot practices. Cattle were moved from the feedlot pens to the scale, individually weighed, and moved to the cattle loading area.

Cattle were exposed to typical weather conditions during the winter, spring, summer, and fall months. Cattle were not held off feed prior to slaughter.

Cattle were transported from the feedlot facilities in semi-trucks by commercial livestock haulers. Cattle were exposed to typical weather conditions while being hauled to the slaughter plants. Hauling times ranged from approximately 1 to 18 hours depending on trial site and slaughter facility locations. Cattle were delivered to the commercial beef packing plants, and held co-mingled prior to slaughter. Transportation distances ranged from at least 45 to approximately 900 miles.

A review of the data from the three clinical trials showed no treatment-related adverse effects or indications of concerns for animal safety. No animal safety concerns were identified or observed during the conduct of the feedlot phase, livestock hauling or slaughter of the cattle from these trials. The incidences of digestive death, bovine respiratory disease, and other typical feedlot illnesses were not impacted by treatment. During the loading, transport, unloading, and holding of cattle at the beef packing plants prior to slaughter, no suspect cattle, "downer cattle" or dead on arrivals (DOA) were observed.

A review of the data collected at the slaughter plants showed no adverse treatment related effects or indications of concerns for animal safety. There was no indication of treatment-related elevated stress conditions that may impact the incidence of dark cutting beef, a condition related to and potentially indicative of chronic stress experienced by cattle prior to slaughter.

Carcass leanness was evaluated using carcass percent protein as the composition variable. Carcass percent protein is the measure of the protein content in the carcass soft tissue which is ultimately merchandised as edible beef. Carcasses with increased levels of protein have a higher percentage of lean meat or red meat yield, and thus, more merchandisable red meat. The effects of zilpaterol hydrochloride on carcass percent protein analysis were evaluated on a "wet weight" basis (i.e. calculations were not corrected for dry matter content). Additionally, the effects of zilpaterol hydrochloride on carcass percent protein were evaluated on an "equal weight" basis by collecting treated and control carcasses of similar hot carcass weights and quality grades.

Sides of the "equal weight" carcasses were dissected and weights obtained for soft tissues, and bone (plus heavy connective tissue or any other inedible tissue such as glands, aorta, etc.). Bone and other inedible tissues were discarded after being weighed. The soft tissue from each carcass was ground, blended, frozen in liquid nitrogen, and powdered. Samples were analyzed for moisture, crude protein, ether extractable lipids, and ash. The average composition of the dissected carcass sides (two per pen) represented the pen's carcass composition for statistical analysis. Change in carcass leanness was measured in differences in percent carcass protein which is defined as the tissue percent protein times the tissue weight, divided by the carcass weight, times 100.

b.1. Pooled Data from Three Pivotal Efficacy Trials

Zilpaterol hydrochloride, when fed at 6.8 g/ton (90% DM; 7.5 ppm), increases average daily gain ($P \leq 0.001$), improves feed efficiency ($P \leq 0.001$), and increases carcass leanness ($P \leq 0.01$). Feeding zilpaterol hydrochloride at 6.8 g/ton (90% DM) for 20 to 40 days increases dressing percent, hot carcass weight, ribeye area, and improves yield grade ($P < 0.01$).

Table 2: Pooled Trial Analysis – Performance of Cattle Fed Diets Containing Zilpaterol Hydrochloride – Main Treatment Effects.

| | Zilpaterol Hydrochloride, g per ton (ppm) 90% DM [a] | | |
|---|---|---|---|
| **Claim Variables** | 0 (0) | 6.8 (7.5) | P value [c] |
| Rate of Weight Gain, lb/d | 2.61 | 3.33 | --- [d] |
| Feed Efficiency (feed to gain ratio) | 7.59 | 5.71 | --- [d] |
| Carcass Percent Protein, % | 13.38 | 14.10 | < 0.0001 |
| | | | |
| **Label Panel Variables** | | | |
| Dressing Percentage, % | 60.3 | 61.8 | 0.004 |
| Hot Carcass Weight, lb | 744.5 | 776.0 | --- [e] |
| Ribeye Area, sq. in | 13.23 | 14.35 | < 0.0001 |
| Yield Grade | 2.81 | 2.52 | 0.005 |
| 12th Rib Fat Thickness, in | 0.49 | 0.47 | 0.178 |
| Marbling Score [b] | 4.62 | 4.31 | --- [e] |

[a] Values are least squares means.
[b] Marbling score of 4.00 = Small$^{00}$, 5.00 = Modest$^{00}$ as per USDA marbling scores.
[c] Overall treatment effect
[d] Significant gender by treatment interaction (Table 4)
[e] Significant duration by treatment interaction (Table 3)

Table 3: Pooled Trial Analysis – Performance of Cattle Fed Diets Containing Zilpaterol Hydrochloride for 20 or 40 days, Duration by Treatment Interaction.

| | Duration | | | | |
|---|---|---|---|---|---|
| | 20 Days | | 40 Days | | |
| | Zilpaterol Hydrochloride, g per ton (ppm) 90% DM [a] | | | | |
| **Claim Variables** | 0 (0) | 6.8 (7.5) | 0 (0) | 6.8 (7.5) | P value |
| Rate of Weight Gain, lb/d | 2.55 | 3.33 | 2.66 | 3.32 | --- [b] |
| Feed Efficiency (feed to gain ratio) | 7.63 | 5.64 | 7.55 | 5.77 | --- |
| Carcass Percent Protein, % | 13.30 | 14.03 | 13.39 | 14.14 | --- |
| | | | | | |
| **Label Panel Variables** | | | | | |
| Dressing Percentage, % | 60.2 | 61.5 | 60.4 | 62.1 | --- |
| Hot Carcass Weight, lb | 742.4 | 769.0 | 746.6 | 783.0 | 0.037 |
| Ribeye Area, sq. in | 13.10 | 14.16 | 13.35 | 14.55 | --- |
| Yield Grade | 2.86 | 2.54 | 2.75 | 2.50 | --- |
| 12th Rib Fat Thickness, in | 0.50 | 0.46 | 0.48 | 0.47 | --- |
| Marbling Score [c] | 4.59 | 4.44 | 4.65 | 4.18 | 0.011 |

[a] Values are least squares means.
[b] Refer to Table 2 for pooled analysis main effects
[c] Marbling score of 4.00 = Small$^{00}$, 5.00 = Modest$^{00}$

Table 4: Pooled Trial Analysis – Performance of Steers and Heifers Fed Diets Containing Zilpaterol Hydrochloride, Gender by Treatment Interaction.

| | Gender | | | | |
|---|---|---|---|---|---|
| | Steers | | Heifers | | |
| | Zilpaterol Hydrochloride, g per ton (ppm) 90% DM [a] | | | | |
| **Claim Variables** | 0 (0) | 6.8 (7.5) | 0 (0) | 6.8 (7.5) | P value |
| Rate of Weight Gain, lb/d [b] | 2.62 | 3.56 | 2.60 | 3.09 | 0.003 |
| Feed Efficiency (feed to gain ratio) [c] | 7.94 | 5.61 | 7.40 | 5.88 | 0.012 |
| Carcass Percent Protein, % | 13.58 | 14.17 | 13.11 | 14.00 | --- [d] |
| | | | | | |
| **Label Panel Variables** | | | | | |
| Dressing Percentage, % | 60.2 | 61.8 | 60.4 | 61.8 | --- |
| Hot Carcass Weight, lb | 780.7 | 816.8 | 708.2 | 735.1 | --- |
| Ribeye Area, sq. in | 13.14 | 14.41 | 13.31 | 14.30 | --- |
| Yield Grade | 2.93 | 2.59 | 2.69 | 2.44 | --- |
| 12th Rib Fat Thickness, in | 0.48 | 0.45 | 0.51 | 0.49 | --- |
| Marbling Score [e] | 4.60 | 4.17 | 4.63 | 4.45 | --- |

[a] Values are least squares means.
[b] Treated differed from controls $P \leq 0.0001$ for both heifers and steers.
[c] Treated differed from controls $P \leq 0.0001$ and $P \leq 0.0002$ for steers and heifers, respectively.
[d] Refer to Table 2 for pooled analysis main effects
[e] Marbling score of 4.00 = Small$^{00}$, 5.00 = Modest$^{00}$

b.2. Individual Pivotal Efficacy Trials

Study V-0238-0019. A Dose Confirmation Clinical Effectiveness Trial for Performance Parameters in Confinement Beef Cattle Orally Fed Zilpaterol Hydrochloride ($\beta_2$-agonist). One hundred sixty steers and one hundred sixty heifers considered to be Angus, Angus cross, Red Angus, Red Angus cross, Hereford cross, Hereford, and Charolais cross small to large frame cattle were used in a feeding trial to study the effect of feeding zilpaterol hydrochloride for 20 or 40 days on feedlot performance and carcass effects. The trial was conducted by Dr. Ed Johnson, Johnson Research, Parma ID. Cattle were fed a high concentrate diet based on wheat and corn earlage. One animal was removed from the trial prior to treatment due to severe founder. One animal died during the withdrawal period due to feedlot dry bloat. No adverse reactions due to zilpaterol hydrochloride were observed.

Cattle from this trial site were exposed to spring and summer conditions. Cattle were individually weighed, and moved between approximately 275 and 400 yards from the feedlot research pens to the cattle loading area. Cattle were loaded on semi-trucks and hauled approximately 250 miles from the feedlot to the slaughter plant by a commercial livestock hauler.

Cattle were held co-mingled overnight prior to slaughter. There were no animal safety concerns or treatment-related adverse conditions observed during the study, at the end of the trial or during the slaughter of the cattle.

Table 5: Performance of Cattle Fed Diets Containing Zilpaterol Hydrochloride, Study Number V-0238-0019.

| | Zilpaterol Hydrochloride, g per ton (ppm) 90% DM | | |
|---|---|---|---|
| **Variables** | **0 (0)** | **6.8 (7.5)** | P value |
| No. of Replicates | 16 | 16 | |
| Total No. of Animals Starting on Trial | 160 | 160 | |
| Total No. of Animals Completing Trial | 159 | 159 | |
| Initial Weight [a,b], lb | 1166.3 | 1163.5 | 0.440 |
| Final Weight [a], lb | 1257.8 | 1281.5 | < 0.001 [d] |
| Rate of Weight Gain [a], lb/d | 2.59 | 3.35 | <0.001 |
| Pounds of Feed/ lb of Gain [a] | 7.82 | 5.83 | <0.001 |
| Carcass Percent Protein [a,c], % | 12.71 | 13.52 | < 0.001 |
| Dressing Percentage [a], % | 61.7 | 63.3 | <0.001 |

[a] Values are least squares means.
[b] Initial body weight was collected at initiation of treatment, study day 21 for 40 day treated cattle, and study day 41 for 20 day treated cattle.
[c] A maximum of 2 carcasses per replicate were evaluated for carcass percent protein.
[d] Duration by gender by treatment interaction was significant (P = 0.03) for final weight.

Study V-0238-0020. A Dose Confirmation Clinical Effectiveness Trial for Performance Parameters in Confinement Beef Cattle Orally Fed Zilpaterol Hydrochloride ($\beta_2$-agonist). One hundred sixty steers and one hundred sixty heifers considered to be Angus, Hereford, Charolais, and Limousin small to large frame cattle were used in a feeding trial to study the effect of feeding zilpaterol hydrochloride for 20 or 40 days on feedlot performance and carcass effects. The trial was conducted by Dr. David Bechtol, Agri Research Center, Inc., Canyon,

TX. Cattle were fed a high concentrate diet based on flaked corn. No animal died or was removed during the trial. No adverse reactions due to zilpaterol hydrochloride were observed.

Cattle from this trial site were exposed to early spring and summer conditions. Cattle were individually weighed, and moved between approximately 160 and 300 yards from the feedlot research pens to the cattle loading area. Cattle were loaded on semi-trucks and hauled approximately 45 miles from the feedlot to the slaughter plant by a commercial livestock hauler.

Cattle were held co-mingled prior to slaughter. There were no animal safety concerns or treatment-related adverse conditions observed during the study, at the end of the trial or during the slaughter of the cattle.

Table 6: Performance of Cattle Fed Diets Containing Zilpaterol Hydrochloride, Study Number V-0238-0020.

| | Zilpaterol Hydrochloride, g per ton (ppm) 90% DM | | |
|---|---|---|---|
| **Variables** | **0 (0)** | **6.8 (7.5)** | P value |
| No of Replicates | 16 | 16 | |
| Total No. of Animals Starting on Trial | 160 | 160 | |
| Total No. of Animals Completing Trial | 160 | 160 | |
| Initial Weight [a,b], lb | 1071.4 | 1067.4 | 0.367 |
| Final Weight [a], lb | 1147.5 | 1165.7 | 0.023 |
| Rate of Weight Gain [a], lb/d | 2.17 | 2.78 | <0.001 |
| Pounds of Feed/ lb of Gain [a] | 8.13 | 6.06 | <0.001 |
| Carcass Percent Protein [a,c], % | 13.37 | 14.14 | 0.006 |
| Dressing Percentage [a], % | 60.9 | 62.5 | <0.001 |

[a] Values are least squares means.
[b] Initial body weight was collected at initiation of treatment, study day 21 for 40 day treated cattle, and study day 41 for 20 day treated cattle.
[c] A maximum of 2 carcasses per replicate were evaluated for carcass percent protein.

Study V-0238-0021. A Dose Confirmation Clinical Effectiveness Trial for Performance Parameters in Confinement Beef Cattle Orally Fed Zilpaterol Hydrochloride ($\beta_2$-agonist). One hundred sixty steers and one hundred sixty heifers considered to be Angus, Angus cross, and Hereford cross medium to large frame cattle were used in a feeding trial to study the effect of feeding zilpaterol hydrochloride for 20 or 40 days on feedlot performance and carcass effects. The trial was conducted by Dr. Terry TerHune, HMS Veterinary Development, Inc., Tulare, CA. Cattle were fed a high concentrate diet based on barley, corn and corn silage. One animal died during the pretreatment period due to bovine respiratory disease. No adverse reactions due to zilpaterol hydrochloride were observed.

Cattle from this trial site were exposed to summer through winter conditions. Cattle were individually weighed, and moved between approximately 100 and 200 yards from the feedlot research pens to the cattle loading area. Cattle were loaded on semi-trucks and hauled approximately 900 miles from the feedlot to the slaughter plant by a commercial livestock hauler.

Cattle were held co-mingled overnight prior to slaughter. There were no animal safety concerns or treatment-related adverse conditions observed during the study, at the end of the trial or during the slaughter of the cattle.

Table 7: Performance of Cattle Fed Diets Containing Zilpaterol Hydrochloride, Study Number V-0238-0021.

| | Zilpaterol Hydrochloride, g per ton (ppm) 90% DM | | |
|---|---|---|---|
| **Variables** | **0 (0)** | **6.8 (7.5)** | P value |
| No of Replicates | 16 | 16 | |
| Total No. of Animals Starting on Trial | 160 | 160 | |
| Total No. of Animals Completing Trial | 160 | 159 | |
| Initial Weight [a,b], lb | 1194.4 | 1190.5 | 0.355 [d] |
| Final Weight [a], lb | 1302.1 | 1323.5 | 0.003 |
| Rate of Weight Gain [a], lb/d | 3.06 | 3.85 | <0.001 |
| Pounds of Feed/lb of Gain [a] | 7.82 | 5.83 | <0.001 |
| Carcass Percent Protein [a,c], % | 13.99 | 14.62 | < 0.001 [e] |
| Dressing Percentage [a], % | 58.2 | 59.6 | <0.001 |

[a] Values are least squares means.
[b] Initial body weight was collected at initiation of treatment, study day 21 for 40 day treated cattle, and study day 41 for 20 day treated cattle.
[c] A maximum of 2 carcasses per replicate were evaluated for carcass percent protein.
[d] Duration by gender by treatment interaction was significant (P = 0.05) for initial weight.
[e] Gender by treatment interaction was significant (P = 0.05) for carcass percent protein.

b.3. Summary Comments for Ancillary Sensory and Shear Force Data Collected from Efficacy Trials

Study V-0238-0025. A Dose Confirmation Clinical Effectiveness Trial for Carcass Leanness, Muscle Tenderness, and Sensory Parameters of Confinement Beef Cattle Orally Fed Zilpaterol Hydrochloride ($\beta_2$-agonist). The objective of this study was two fold. In the first phase the site collected carcass sides and conducted the chemical analysis of the soft tissue providing the carcass percent protein values for the carcass leanness claim. The second objective of this study was to determine the effect of feeding zilpaterol hydrochloride on trained sensory panel evaluations and Warner-Bratzler shear force properties of boneless cooked beef strip loin steaks. Dr. Mark Miller, Ph.D., was the investigator for this study, which was conducted at Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX.

Because no single method has been perfected to predict the ultimate consumer satisfaction (either taste panels or Warner-Bratzler shear force), a combination of objective and subjective measurements were employed to evaluate the effects and differences that may be meaningful to consumers. Instrumental objective evaluations (Warner-Bratzler shear force) and cooking loss were conducted on cooked beef strip loin steak samples (*longissimus lumborum*) obtained from representative groups of carcasses from the clinical efficacy trials. Boneless strip loin samples were collected from two carcasses (cattle) per pen, which were slaughtered at the end of the live phase of the zilpaterol hydrochloride feeding. For each pen the two boneless strip loins collected were from the same carcasses (left carcass side) as the carcass sides collected for dissection and chemical analyses (right carcass side). However, sensory evaluations were conducted on cooked beef strip loin steak samples collected from only one randomly selected

trial site (Idaho, V-0238-0019). For this site, Idaho, the sensory evaluations were conducted on samples collected from the same boneless strip loins as the samples in which Warner-Bratzler shear force and cooking loss were determined.

The boneless strip loin steaks evaluated in this study were collected from trials for which the experimental design was a randomized complete block. A block consisted of eight pens, thus eight treatments (two treatment concentrations of zilpaterol hydrochloride, 0 or 6.8 g/ton 90% DM; two duration of treatments, 20 or 40 days; two genders, steer or heifer). For carcass and boneless strip loin selection, four carcasses (two control and two treated) were selected from a replicate consisting of a control and treated pen from the same treatment duration, the same gender, and the same weight block. For each replicate, the four selected carcasses were "equal weight" carcasses of similar weight and USDA quality grade. Thus, for each pen two carcasses were selected for evaluation.

Samples were identified by animal number, and animal number corresponded to trial number and site location. The objective description variables of cooking loss and Warner-Bratzler shear force were evaluated for each sample. Conduct of the sensory panels and preparation of the samples was consistent with the procedures described in the *Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat, (*American Meat Science Association, 1995).[1] Sensory evaluation was conducted by panelists trained to clearly distinguish differing amounts of tenderness, juiciness, flavor and off-flavors. This training enabled differentiation of the variables in increments more discrete and refined than the typical consumer could detect. Panelists were trained to use an eight point descriptive scale (five point descriptive scale for off-flavor). In the opinion of the sensory investigator, the subjective sensory panel variables (eight point scale) must shift by one full point in order for the average consumer to detect a difference. However, while relationships exist between consumer and trained sensory measurements, it is difficult to predict from objective data just how consumers will rate meat at home (Lorenzen et al., 2003).[2] Thus, Warner-Bratzler shear force measurements tend to be slightly more predictive of consumer rating than trained panel scores. Additionally, consumers tend to desire both leanness and palatability in beef, however, these qualities are not necessarily coincidental and tend to be antagonistic (Jeremiah et al., 1992).[3]

Results from this study demonstrated that effects on subjective sensory variables and objective Warner-Bratzler shear force were within the normal variation observed today in the beef industry. No meaningful changes in cooking loss were caused by zilpaterol hydrochloride. For the Texas site only, there was a treatment by gender interaction ($P = 0.003$) for cooking loss in which cooking loss percent was decreased by zilpaterol hydrochloride in heifers and increased in steers.

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[1] AMSA. 1995. Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness of Fresh Meat. National Live Stock and Meat Board, Chicago, IL.

[2] Lorenzen, C. L., R. K. Miller, J. F. Taylor, T. R. Neely, J. D. Tatum, J. W. Wise, M. J. Buyck, J. O. Reagan, and J. W. Savell. 2003. Beef customer satisfaction: Trained sensory panel ratings and Warner-Bratzler shear force values. J. Anim. Sci. 81:143-149.

[3] Jeremiah, L. E., A. K. W. Tong, S. D. M. Jones, and C. McDonell. 1992. Consumer acceptance of beef with different levels of marbling. J. Consumer Studies and Home Econ. 16:375-387.

As shown in Table 8, overall tenderness, juiciness, flavor intensity and beef flavor were all statistically different compared to controls. However, no sensory variables were shifted by more than 0.65 units compared to controls, a change that should not be detectable by the consumer. These differences are considered well within the normal variation observed in the beef industry. Additionally, off-flavor scores were not affected by the treatment. Therefore, it was concluded that no differences in beef palatability would be detected by the average consumer. The cause of the slight decrease in tenderness scores may be partially due to an increase in muscle fiber area.

For the objective variable of Warner-Bratzler shear force, most literature published and cited by meat scientists consider meat to be acceptably tender if the shear force value is below approximately 4.5 to 4.6 kg (Boleman et al., 1997;[4] Platter et al., 2003;[5] Shackelford et al., 1991,[6] 1999;[7] Wheeler et al., 1999).[8] According to most published literature references, a shear force of over 4.5 to 4.6 kg is necessary before a consumer considers meat to be tough. However, Wheeler et al. (1997)[9] reported that comparisons of Warner-Bratzler shear force data among different institutions are not currently valid. Therefore, only threshold levels from Texas Tech University should be used for the current study. Miller et al. (1995)[10] reported the

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[4] Boleman, S. J., S. L. Boleman, R. K. Miller, J. F. Taylor, H. R. Cross, T. L. Wheeler, M. Koohmaraie, S. D. Shackelford, M. F. Miller, R. L. West, D. D. Johnson, and J. W. Savell. 1997. Consumer evaluation of beef of known categories of tenderness. J. Anim. Sci. 75:1521-1524.

[5] Platter, W. J., J. D. Tatum, K. E. Belk, P. L. Chapman, J. A. Scanga, and G. C. Smith. 2003. Relationships of consumer sensory ratings, marbling score, and shear force value to consumer acceptance of beef strip loin steaks. J. Anim. Sci. 81:2741-2750.

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Warner-Bratzler consumer acceptability threshold to be at 4.3 kg while Huffman et al. (1996)[11] reported a Warner-Bratzler shear force of 4.1 kg ensured 98% of consumers found the beef acceptable. In their most recent and comprehensive consumer study Miller et al. (2001)[12] reported that the transition in consumer perception from tender to tough beef occurred between 4.3 and 4.9 kg. Thus, a threshold of 4.1 to 4.3 kg can be used for data collected by Texas Tech University, and the sensory Investigator. The Warner-Bratzler shear force analysis for the current study showed the mean values for zilpaterol hydrochloride were within the normal ranges observed in the beef industry. While the mean Warner-Bratzler shear force was increased ($P < 0.001$) by the treatment, data from this study (see Table 8) shows that the control and treated values were both below either of the threshold levels of shear force. Therefore, it was concluded that no difference in palatability would be detected by the consumer. The cause of the slight increase in Warner-Bratzler shear force may be partially due to an increase in the muscle fiber area.

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Table 8: Pooled Trial Analysis –Summary of Sensory Panel Evaluation and Warner-Bratzler Shear Force of Strip Loin Steaks from Cattle Fed Diets Containing Zilpaterol Hydrochloride.

| | Zilpaterol Hydrochloride, g per ton (ppm) 90% DM[a] | | |
|---|---|---|---|
| **Variables** | 0 (0) | 6.8 (7.5) | P value |
| Overall Tenderness [b] | 6.48 | 5.83 | 0.001 |
| Overall Juiciness [c] | 6.27 | 5.81 | 0.001 |
| Flavor Intensity [d] | 6.56 | 6.30 | 0.002 |
| Beef Flavor [e] | 6.65 | 6.45 | 0.020 |
| Off-Flavor [f] | 1.01 | 1.01 | 0.889 |
| Warner Bratzler shear force, kg | 3.29 | 4.01 | < 0.001 |

[a] Values are least squares means.
[b] Tenderness Scale (1 – 8) : 5 = Slightly Tender; 6 = Moderately Tender; 7 = Very Tender
[c] Juiciness Scale (1 – 8): 5 = Slightly Juicy; 6 = Moderately Juicy; 7 = Very Juicy
[d] Flavor Intensity Scale (1 – 8): 6 = Moderately Intense; 7 = Very Intense
[e] Beef Flavor Scale (1 – 8): 6 = Moderately Characteristic Beef Flavor; 7 = Very Characteristic Beef Flavor
[f] Off-Flavor Scale (1 – 5): 1 = None; 2 = Slight Off-Flavor

## *3. TARGET ANIMAL SAFETY:*

### *A. Pivotal Study:*

A Target Animal Safety Study in Confinement Beef Cattle Orally Fed Zilpaterol Hydrochloride ($\beta_2$-agonist) at 1.5X and 10X Use-Level. Intervet Study number V-0238-0018.
A specifically designed target animal safety study in beef cattle was conducted to address the safety of feeding 68 g/ton (75 ppm; 90% DM) or 10 X the proposed dose, of zilpaterol hydrochloride in the diet during a 40-day feeding period. Forty-eight steers and heifers (8/sex/dose) were fed a complete diet containing 0, 10.2 (1.5 X the proposed dose, to exceed current recommendations for target animal safety studies), or 68 g/ton (0, 11.3, or 75 ppm on a 90% DM basis) of zilpaterol hydrochloride and observed for adverse effects. Dr. C.E. Heard, Ph.D. of Southwest Bio-Labs, Inc., Las Cruces, NM, was the Study Investigator.

The study was designed to determine the toxicological and safety effects (if any) of zilpaterol hydrochloride administered in the diet to cattle. Potential target organs and additional tissues were identified through clinical observations, gross necropsy, and histological examination. Hematological variables, body weight, clinical observations, and feed consumption were other variables of interest.

Data from steers and heifers were pooled and analyzed. The pre-treatment values were used as covariates. For all variables except organ weights, the mixed models included the fixed effects of covariate, treatment, gender, treatment by gender, time, treatment by time, and the random effects of blocks nested within gender, and treatment by blocks within gender. For organ weights, the mixed models included the fixed effects of covariate, treatment, gender, treatment by gender, and the random effects of block within gender, and treatment by block within gender. Sex effects and interactions were declared significant if $\alpha \leq 0.05$. All other effects and interactions, including treatment effects, were declared significant if $\alpha \leq 0.10$.

Feed consumption was decreased by 75 ppm zilpaterol hydrochloride (10 X treatment) during the first week of test article feeding, which was considered to be due to acclimation to the medicated total mixed ration. Test article-related effects at 11.3 ppm (1.5 X dose) and 75 ppm (10 X dose) included an increase in average daily gain over the 40 day treatment period, an increase in platelet count at termination, and a decrease in mean corpuscular volume and prothrombin time at termination. Additionally, the 1.5 X and 10 X doses resulted in an increase of blood creatine kinase, creatinine, and potassium concentrations throughout the feeding trial. The 1.5 X dose resulted in higher albumin concentrations at termination while the 10 X dose resulted in a decrease in hemoglobin concentration, hematocrit, and relative lymphocyte numbers. Daily health observations were considered to be normal except for a test article-related observation of incidence of hyperventilation. The histopathologic evaluations did not reveal any test article-related effects.

A number of the effects of feeding zilpaterol hydrochloride are consistent with what is to be expected when feeding a beta-adrenergic agonist which increases muscle protein accretion. Effects at higher than the intended use dose do not diminish the effectiveness of the drug at the intended use level, however producers and herd health personnel who use this drug should be aware of these effects in the instance of an accidental overexposure. This study has shown that zilpaterol hydrochloride can be safely administered to cattle at the recommended dose of 7.5 ppm in the feed for 40 consecutive days.

***B. Heart Rate Monitoring Study:***

Following randomization in the study above, animals not assigned to the main target animal safety evaluation portion of the study were selected for the heart rate monitoring portion of the study. Twelve animals were randomized into two blocks with two animals per treatment group in each block. Four animals for each treatment group were fitted with heart monitors over three time periods of the 40-day feeding period (beginning, middle, and end) and their heart rate monitored for beats per minute and episodes of tachycardia. Only daily observations were collected from these animals to minimize stress. Results of mean heart rate demonstrated an increase in average post-feeding heart rate, as compared to pre-feeding heart rate in zilpaterol hydrochloride-fed animals.

***C. Corroborative Studies:***

Additional information related to the evaluation of the safety and effectiveness of zilpaterol hydrochloride when fed to cattle under typical feedlot conditions, shipped, and slaughtered under commercial conditions is included in the effectiveness section, as part of the individual study reports. No treatment-related adverse reactions or indications of animal safety concerns were observed in any of the large scale feedlot trials conducted under conditions that represented commercial conditions as closely as possible.

## *4. HUMAN FOOD SAFETY:*

## *A. Toxicology:*

### 1. Summary of Toxicology Studies

#### a. Four-Week Toxicity Study by the Oral Route in Cynomolgus Monkeys. Study No. 9075 TSP.

INVESTIGATOR: F. Sauvez, DVM
Centre International de Toxicologie (C.I.T.)
Miserey-27005 Evreux – France

An oral gavage toxicity study of RU 42173 (zilpaterol hydrochloride) was conducted in cynomolgus monkeys (*Macaca fascicularis*). Groups of two animals per sex were dosed with vehicle (control) 5, 10, 50 or 5000 µg/kg BW/day of RU 42173 for four weeks. Clinical evaluations and food consumption estimates were made daily; body weights were recorded weekly. Ophthalmological evaluations were made prior to the first dose and on day 25. Electrocardiography and blood pressure were recorded before the first dosing and on days 2, 14, and 25. Hematology, blood chemistry, and urinalyses were performed before the first dosing and on day 24. At the end of the treatment period all animals were submitted to a full macroscopic examination, selected organs were weighed, and a microscopic evaluation of designated organs was performed. No deaths occurred and there were no treatment-related clinical signs of toxicity observed. There were no treatment-related findings with regard to food consumption, body weights, and electrocardiographic measurements, hematology, blood chemistry or urinalysis values. No treatment-related abnormalities were found with regard to organ weights, or during macroscopic or microscopic evaluations. Reduction in blood pressure in the 50 and 5000 µg/kg BW/day was accompanied by an increase in heart rate with subsequent decreased QT interval. Consequently, the NOEL for this study was determined to be 10 µg/kg BW/day.

#### b. 13-week Toxicity Study by Oral Route (Gavage) in Rats. Study No. 10167 TCR.

INVESTIGATOR: F. Sauvez, DVM
Centre International de Toxicologie (C.I.T.)
Miserey-27005 Evreux – France

A 13-week oral gavage study was conducted in Sprague-Dawley rats. Rats (20 animals per sex/group) were treated with 0, 0.05, 0.5, and 1 mg zilpaterol hydrochloride/kg BW/day. All animals survived the treatment period. Male and female animals receiving 0.5 and 1.0 mg/kg BW/day had increased body weight gains. There were no treatment-related ophthalmological, hematology, clinical chemistry or urinalysis findings. Lower mean heart rates were observed during week 8 in females given 0.05 or 0.5 mg/kg/day and during week 3 in females given 1 mg/kg/day. This was associated with higher PQ intervals. Higher PQ intervals were noted in males treated with 0.5 or 1.0 mg/kg/day. The differences in mean heart rates were statistically significant on weeks 3, 4, and 8 in females given 1 mg/kg/day, on week 8 in females given 0.5 mg/kg/day and on week 13 in females given 0.05 mg/kg/day.

Lower systolic blood pressure was observed during weeks 3 and 4 in males given 0.5 or 1 mg/kg/day, respectively. Consequently, a LOEL of 0.05 mg/kg/day was determined, based on changes observed in feed consumption, body weight, systemic blood pressure, heart rate intervals, and cardiomyopathy.

**c. 13-Week Toxicity Study by Oral Route in Yucatan Microswine, Study No 10560 TCN.**

INVESTIGATOR: F. Sauvez, DVM.
Centre International de Toxicologie (C.I.T.)
Miserey-27005 Evreux – France

A 13-week oral gavage study was conducted in Yucatan Microswine, with four animals/sex/group. The doses were 0, 1, 50, 1,000, and 10,000 μg zilpaterol hydrochloride/kg BW/day. There were no treatment-related differences in clinical signs, mortality, and food consumption. Body weight increases in the males were observed at 1,000 and 10,000 μg/kg BW/day. Findings at the end of the study were equivalent to those observed before the start of treatment. No treatment-related effects were detected in the ophthalmology exam at the end of the treatment. No abnormalities were observed in the EKG. A higher mean heart rate was observed in the high dose group females (+ 40%); however, a lower heart rate than controls was often noted in males in the high dose group. A higher mean systolic blood pressure was noted in males given 1 μg/kg BW during week 12, however an increase in pressure was not found in other treated groups and a lower blood pressure was observed in males given the high dose. These changes were reported to be spontaneous and not treatment related. Minimal increases in erythrocytes, hemoglobin, and packed cell volume in the high dose group females were detected. Slight increases in plasma glucose and plasma potassium in the high dose females were considered not treatment related. Although there were lung lesions associated with a respiratory disease; these lesions were found in all groups and are commonly found in Yucatan Microswine. Myometrial hypertrophy was observed in females treated with 1,000 and 10,000 μg/kg BW/day. The NOEL in this study was 50 μg/kg BW/day based on biological differences in body weight gains in the males and the myometrial hypertrophy in the females.

**d. 52-Week Toxicity Study by Oral Route (Dietary Admixture) in Rats with 4-Week Recovery Period. Study No. 13529TCR.**

INVESTIGATOR: Dr. J. Richard.
Centre International de Toxicologie (C.I.T.)
Miserey-27005 Evreux – France

A 52-week chronic feeding study was conducted in Sprague-Dawley rats using twenty to forty animals/sex/group. Zilpaterol hydrochloride was fed in the diet at levels to provide 0, 25, 50, 125, and 250 μg/kg BW/day. Satellite groups of ten/sex were fed a diet containing 0, 25, and 250 μg/kg and used for the determination of levels of zilpaterol hydrochloride in plasma and for observations during a 4-week recovery period. The animals were checked daily for mortality and clinical signs. Body weights and food consumption were recorded weekly. Ophthalmological examinations, hematological, and blood biochemistry measurements were conducted during weeks

14, 27, 40, and 52. Electrocardiographic and blood pressure examinations were performed during weeks 2, 5, 13, 26, 39, 51, and 56. At the end of the study, animals were euthanized. Designated organs and tissues were examined macroscopically and samples of the tissues were examined microscopically.

There were no deaths or clinical signs related to treatment. Blood samples collected during weeks 14, 27, and 52 showed that zilpaterol was absorbed; the highest levels in plasma were found in samples from the highest dose group. There were no treatment-related effects observed in ophthalmological, hematology, blood chemistry, macroscopic necropsy or histological examinations. There were slight decreases in heart rate observed in animals treated with 125 and 250 μg/kg and a slight increase in systolic blood pressure observed in animals treated with 250 μg/kg. However, these observations were not considered to be adverse effects and were considered to be findings normally found in the test animals at the age of the observations. Consequently, there were no compound-related adverse findings and the NOEL was 250 μg zilpaterol hydrochloride/kg BW/day.

**e. Two-Generation Study by Oral Route (Dietary Admixture) in Rats. Study No. 13007 RSR.**

INVESTIGATOR: J. Richard, D. Pharm.
Centre International de Toxicologie (C.I.T.)
Miserey-27005 Evreux – France

Reproductive toxicity was determined in a two-generation reproductive performance study in Sprague-Dawley rats. Zilpaterol hydrochloride was administered in the diet at 0, 0.9, 3.6, and 14.4 ppm, continuously for two generations (30 rats/sex). Male and female rats ($F_0$) were treated 71 days and 15 days, respectively, before mating. The females received treatment during mating, gestation, and lactation. The males were treated until weaning of the $F_1$ offspring. $F_0$ animals were observed daily for clinical signs, and signs of morbidity and mortality. Food consumption and body weights were measured weekly. Females were allowed to deliver normally and to raise their progeny until weaning. The $F_1$ offspring were examined daily for physical and reflex development, clinical signs, survival, and body weights. The offspring were standardized to 4 males and 4 females on day 4 post-partum. Necropsy was performed on the parents and the reproductive organs, heart, liver, and kidneys were examined histologically. After weaning, 25 males and 25 females were selected to constitute the $F_1$ generation parents and were administered the same treatments until the end of the study. After attaining sexual maturity, they were paired in a group until mating took place. Females were allowed to deliver normally and to rear their progeny until weaning. Mating and fertility indexes were calculated. The $F_2$ offspring were examined daily for physical and reflex development, clinical signs, survival, and body weights and were standardized to 4 males and 4 females on day 4 post-partum. Necropsy was performed on the $F_1$ parents and $F_2$ offspring. The reproductive organs, heart, liver, and kidneys from the $F_1$ parents were examined histologically.

No clinical signs, deaths or abortions occurred in the $F_0$ or $F_1$ generation. Food consumption of males and females was similar in control and treated groups. Body weight gains of males and females were higher in all treated groups than in the control

group. The reproductive performance, percentage of mating, fertility, and gestation of the $F_0$ and $F_1$ males and females were not affected by treatment. In the $F_1$ and $F_2$ litters, the number of pups born alive, the survival rate to day 4 and day 20, sex ratio, physical and reflex development, and the mean body weight gain of pups were similar in control and treated groups. The macroscopic and microscopic examinations of the parents euthanized at the end of the study did not reveal any changes. In addition, no abnormalities were observed during macroscopic examination of pups.

No effects were noted on the reproductive performance, growth, and development of the offspring at any dose level. The NOEL was 14.4 ppm, a concentration corresponding to a dose level of 0.94 mg zilpaterol hydrochloride/kg BW/day for males and 1.77 mg zilpaterol hydrochloride/kg BW/day for females.

**f. Assessment of Possible Embryotoxic and Teratogenic Effects by Oral Route in Rats. Study No. 7501 RSR.**

INVESTIGATOR: Dr. M. H. Savary
Centre International de Toxicologie (C.I.T.)
Miserey-27005 Evreux – France

An embryotoxic and fetal development study was conducted in pregnant Sprague-Dawley rats. Zilpaterol hydrochloride was administered by daily oral gavage at 0, 0.2, 2, 10, and 50 mg/kg/day BW during days 6 to 15 of pregnancy. There were 28 pregnant females per group. Females were observed for clinical signs, mortality, and abortions. Body weights and food consumption were recorded. On day 20 of pregnancy, females were euthanized and the fetuses delivered by Caesarean section. Parameters recorded were the number of corpora lutea, implantation sites, resorptions, and dead and live fetuses. Live and dead fetuses were examined externally and internally (soft tissue and skeletal tissues).

There were no treatment-related deaths and no abortions occurred. Hypersalivation was observed in animals receiving 2, 10, and 50 mg/kg/day, with the incidence higher in the 50 mg/kg/day treated group. Food consumption was decreased from days 6-9 and increased from days 12-15 in a dose dependent manner. The changes in food consumption for days 6-9 and days 12-15, respectively, were in the 2 mg/kg/day group (0%, +9%), the 10 mg/kg/day group (-17%, +9%), and the 50 mg/kg/day group (-21%, +9%). Water consumption was higher in the high dose group (16-19%). Body weight gains were slightly higher in the 2 and 10 mg/kg/day groups and moderately higher in the 50 mg/kg/day group. These observations were considered maternotoxic effects. There were no treatment-related macroscopic changes. The mean number of corpora lutea, implantation sites, live fetuses, mean fetal body weights, and post-implantation loss were similar in the control and treated groups.

There were no treatment related fetal external formations, soft tissue anomalies or soft tissue malformations noted. The incidence of fetal skeletal variations and anomalies were similar in the control, 0.2, 2, and 10 mg/kg/day groups. In the 50 mg/kg/day group, the incidence of delayed ossification of some bones (5th and 6th sternebrae, skull) and wavy ribs was higher when compared to control fetuses. This effect was considered related to the maternotoxicity noted at this dose level.

The NOEL for effects on pregnant female rats (maternotoxicity) was 0.2 mg zilpaterol hydrochloride/kg BW/day and the NOEL for effects on the fetuses (developmental toxicity) was 10 mg zilpaterol hydrochloride/kg BW/day.

**g. Genetic Toxicology Studies**

Studies have been conducted to assess the genotoxic potential of zilpaterol hydrochloride and of the primary metabolite deisopropyl zilpaterol hydrochloride in order to help establish its safety in human food. The studies, described below, included both *in vitro* and *in vivo* tests for the potential to induce gene mutations, DNA damage or chromosomal damage.

**1) Bacterial Reverse Mutation Test: RU 42173. Study No 14753 MMJ.**

INVESTIGATOR: H. Haddouk
Centre International de Toxicologie (C.I.T.)
Evreux, France

Zilpaterol hydrochloride was evaluated for mutagenic potential in an *in vitro* assay with five strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, TA 1537, and TA 102) and one strain of *Escherichia coli* (WP2uvrA). The test was conducted twice, in accordance with OECD Guidelines. The first trial was a standard plate incorporation assay conducted both in the presence and absence of rat liver S-9 metabolic activation at 312.5, 625, 1250, 2500, and 5000 μg/plate. Based on the absence of a response in the first trial, the second trial was a preincubation assay conducted only in the presence of S-9. No toxicity was seen in these assays and there were no increases in the number of revertant colonies under any condition of testing. It was concluded that zilpaterol hydrochloride was not mutagenic in this test system.

**2) RU 42173 Chinese Hamster Ovary [CHO]/HPRT Locus Assay. Study No RSL 858/920758.**

INVESTIGATOR: K. Adams
Huntingdon Research Center
Cambridgeshire, UK

Zilpaterol hydrochloride was evaluated for the capacity to induce gene mutations in CHO cells, *in vitro*, at the HPRT gene locus. Two separate trials of the assay were conducted. Based on a range-finding study, test doses were 500, 1000, 2000, 3000, 4000, and 5000 μg/ml both in the presence and absence of rat liver S-9. There was evidence of toxicity, in the absence of S-9, with survival ranging from 52-171% and 42-85% in trials 1 and 2 respectively. Less toxicity was observed in the presence of S-9 with survival ranging from 105-132% and 81-102 in trials 1 and 2 respectively. There were no statistically significant treatment-related increases in the mean mutation frequency either in the absence or presence of S-9 metabolic activation. It was concluded that zilpaterol hydrochloride was not mutagenic in this test system.

3) **Mammalian Cell Mutagenesis Testing of RU 42173 Using the L5178Y tk+/- Mouse Lymphoma Cell Assay with Colony Sizing with and without Metabolic Activation. Study V-0238-0003P.**

INVESTIGATOR: A.D. Mitchell
Genesys Research, Inc.
Durham, NC - USA

Zilpaterol hydrochloride was evaluated for the capacity to induce gene or chromosomal mutations in L5178Y mouse lymphoma cells, *in vitro*, at the tk+/- locus. Two separate trials of the assay were conducted in the presence of rat liver S-9. The first trial with S-9 was conducted over a wide range of doses, to facilitate assessment of toxicity, up to 5000 μg/ml. Because the toxicity results did not allow assessment of mutational potential in a range of 10-20% survival, a second trial in the presence of S-9 was conducted. In both trials doses above about 2500 μg/ml were completely lethal. There were no significant treatment-associated increases in mutational frequency in either trial conducted in the presence of S-9. A single trial was conducted in the absence of S-9, wherein significant lethality was observed at 1500 μg/ml and above. There were no significant treatment-associated increases in mutational frequency observed in the trial conducted in the absence of S-9. Due to the absence of an increase in mutant frequency, colony sizing was not conducted. It was concluded that zilpaterol hydrochloride was not mutagenic or clastogenic in this test system.

4) **Genotoxicity Test on RU 42173 in the Assay for Unscheduled DNA Synthesis [UDS] in Rat Liver Primary Cell Cultures with a Confirmatory Trial. Study No 14450 0 447R.**

INVESTIGATOR: M. McKeon
Hazleton Washington
Vienna, VA - USA

The capacity for zilpaterol hydrochloride to induce DNA damage was assessed in primary (Fischer) rat liver cell cultures *in vitro*. Two separate trials were conducted over a wide dose range (0.5 – 5000 μg/ml) to facilitate accounting for cytotoxicity. There was significant cytotoxicity in both trials at 250 μg/ml and greater. Dose levels were scorable at 5, 10, 50, 100, and 250 μg/ml in trial 1, and 25, 50, 75, 100, 175, and 250 μg/ml in trial 2. There was 55.9% and 54.0% survival at the highest dose in trials 1 and 2, respectively. There was no significant treatment-related increase in net nuclear grains, indicative of increased DNA synthesis, in either trial. It was concluded that zilpaterol hydrochloride did not increase DNA synthesis in this test system. Therefore, is not likely to induce DNA damage in mammalian cells *in vitro*.

5) **Dose Range Finding Study on RU 42173 for an *In Vivo* Murine Micronucleus Assay. Study No 14450 0 459PO;** and

6) **Mutagenicity Test on RU 42173 in an *In Vivo* Micronucleus Assay. Study No**

**14450 0-455PO.**

INVESTIGATOR: H. Murli
Hazleton Washington
Vienna, VA - USA

The capacity for zilpaterol hydrochloride to induce chromosomal damage was investigated in a mouse micronucleus assay in ICR strain mice. A dose range finding study was conducted in groups of three animals per sex administered a single dose of 40, 230, 420, 610 or 800 mg/kg BW by oral gavage. There were no signs of toxicity up to 230 mg/kg BW. There was a single death at 420 mg/kg, within 15 min of dosing, 2 deaths (1/sex) at 610 mg/kg BW by 24 hr post-dosing and 4 deaths (2/sex) at 800 mg/kg BW by 1 hr post-dosing. The LD50 was calculated to be 515 mg/kg BW.

For the definitive assay, groups of 5 animals per sex were administered a single dose at 0, 100, 200 or 400 mg/kg BW by oral gavage. Groups of animals were euthanized at 24, 48 or 72 hr post-dosing and bone marrow cells were harvested for preparation of slides. Significant reductions in the ratios of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) combined with the observation of a single death in the 400 mg/kg BW group provided clear evidence of exposure to the test material. The percent of micronucleated cells per 1000 PCEs was not significantly increased under any treatment condition or time. It was concluded that zilpaterol hydrochloride does not induce chromosomal damage *in vivo*, in mice, under the conditions of testing.

**7) *In Vivo* Cytogenetics Testing of RU 42173 Using the Mouse Bone Marrow Micronucleus Test Preceded by Dose Range Finding. Study V-0238-0001P.**

INVESTIGATOR: A.D. Mitchell
Genesys Research, Inc.
Research Triangle Park, NC

In a second, independent study, the capacity for zilpaterol hydrochloride to induce chromosomal damage was investigated in a mouse micronucleus assay in Swiss Webster strain mice. A dose range-finding study was conducted in groups of five animals per sex administered a single dose of zilpaterol hydrochloride up to 1000 mg/kg BW by oral gavage. Lethality was observed at 562 and 1000 mg/kg BW.

In the definitive test, groups of 15 animals per sex were administered a single dose at 0, 31.25, 62.5, 125, 250 or 500 mg/kg BW of zilpaterol hydrochloride by oral gavage. Groups of animals were euthanized at 27, 36 or 45 hr post-dosing and bone marrow tissue slides were prepared. Although there was lethality observed at 500 mg/kg BW, sufficient animals were available for evaluation; therefore, the 125, 250, and 500 mg/kg BW groups were evaluated for the presence of micronuclei. There was a significant reduction in the ratio of PCEs to NCEs providing evidence of toxicity and, therefore, exposure to the test material. There were no significant increases in the percent micronucleated cells per 2000 PCEs at any dose or time

interval. It was concluded that zilpaterol hydrochloride does not induce chromosomal damage *in vivo*, in mice, under the conditions of testing.

8) **Reverse Mutation Assay on Bacterial *Salmonella typhimurium* and *Escherichia coli*: RU 62435. Study 10814 MMJ.**

INVESTIGATOR: B. Molinier
Centre International de Toxicologie (C.I.T.)
Evreux, France

Deisopropyl zilpaterol hydrochloride was evaluated for mutagenic potential in an *in vitro* assay with five strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, TA 1537, and TA 102) and one strain of *Escherichia coli* (WP2uvrA). The test was conducted twice, in accord with OECD Guidelines. The first trial (direct plate) was conducted in the presence (once) and absence (twice) of rat liver (Aroclor-induced) S-9 metabolic activation. Test doses, based on a pre-study toxicity test, were 312.5, 625, 1250, 2500, and 5000 μg/plate for the first trial. Based on the absence of a response in the first trial, the second trial was a preincubation assay conducted only in the presence of S-9 at doses of 37.5, 75, 150, 300, and 600 μg/plate. Slight to moderate toxicity was seen at the highest dose level in the first trial; moderate toxicity was seen at 600 μg/plate in the second trial. There were no increases in the number of revertant colonies under any condition of testing. It was concluded that deisopropyl zilpaterol hydrochloride was not mutagenic in this test system.

9) **Mammalian Cell Mutagenesis Testing of RU 62435 Using the L5178Y tk+/- Mouse Lymphoma Assay with Colony Sizing with and without Metabolic Activation. Study V-0238-0004P.**

INVESTIGATOR: A.D. Mitchell
Genesys Research, Inc.
Research Triangle Park, NC

Deisopropyl zilpaterol hydrochloride was evaluated for the capacity to induce gene or chromosomal mutations in L5178Y mouse lymphoma cells *in vitro*, at the tk+/- locus. Two separate trials of the assay were conducted in the absence of rat liver S-9. The first trial without S-9 was conducted over a wide range of doses, to facilitate assessment of toxicity, up to 5000 μg/ml. Because the toxicity results did not allow assessment of mutational potential in a range of 10-20% survival, a second trial in the absence of S-9 was conducted. In both trials doses at or above about 2800 μg/ml were completely lethal. There were no significant treatment-associated increases in mutational frequency in either trial conducted in the absence of S-9. A single trial was conducted in the presence of S-9, wherein significant lethality was observed at 800 μg/ml, and above. There were no significant treatment-associated increases in mutational frequency observed in the trial conducted in the presence of S-9. Due to the absence of an increase in mutant frequency, colony sizing was not conducted. It was concluded that RU 62435 was not mutagenic or clastogenic in this test system.

10) **Study to Evaluate the Potential of RU 62435 to Induce Unscheduled DNA**

**Synthesis in Isolated Rat Hepatocytes *in vitro*. Study RUF2/RHU.**

INVESTIGATOR: P.J. Ward
Hazleton Microtest
North Yorkshire, England

The capacity for deisopropyl zilpaterol hydrochloride to induce DNA damage was assessed in primary (Wistar) rat liver cell cultures *in vitro*. Two separate trials were conducted over a wide dose range (first trial 0.064 – 5000 µg/ml; second trial 55.99 - 2000 µg/ml) to facilitate accounting for cytotoxicity. There was significant cytotoxicity in the first trial at 1000 µg/ml and greater and in the second trial at 720 µg/ml and greater. Dose levels were scorable at 1.6, 8, 40, 200, and 1000 µg/ml in trial one, and 93.3, 155.5, 259.2, 432, and 720 µg/ml in trial two. There was less than 60% survival at the highest dose in trials one and two, respectively. There was no significant treatment-related increase in net nuclear grains, indicative of increased DNA synthesis, in either trial, with a mean of 4% and 2% of the cell in repair, respectively. It was concluded that deisopropyl zilpaterol hydrochloride did not increase DNA synthesis in this test system; therefore, it is not likely to induce DNA damage in mammalian cells *in vitro*.

11) **Micronucleus Test by Oral Route in Mice. Test Substance RU 62435. Study 11039.**

INVESTIGATOR: B. Molinier
Centre International de Toxicologie (C.I.T.)
Evreux, France

The capacity for deisopropyl zilpaterol hydrochloride to induce chromosomal damage was investigated in a mouse micronucleus assay. For the assay, groups of five animals per sex were administered a single dose at 0 or 1500 mg/kg BW by oral gavage. Groups of animals were euthanized at 24 or 48 hr post-dosing and bone marrow cells were harvested for preparation of slides. Exposure to the test material was evidenced by significant reductions in the ratios of PCEs to NCEs at 24 hr. The percent of micronucleated cells per 1000 PCEs was not significantly increased at 24 hr; however, there was a significant increase at 48 hr when compared to concurrent controls. When the increase seen at 48 hr was compared to historical control ranges, however, the increase in micronucleated cells was not statistically significant. It was concluded that deisopropyl zilpaterol hydrochloride may induce chromosomal damage *in vivo*, in mice, under the testing conditions.

12) ***In Vivo* Cytogenetics Testing of RU 62435 Using the Mouse Bone Marrow Micronucleus Test Preceded by Dose Range Finding. Study V-0238-0002P.**

INVESTIGATOR: A.D. Mitchell
Genesys Research, Inc.
Research Triangle Park, NC

The capacity for deisopropyl zilpaterol hydrochloride to induce chromosomal damage was investigated in a mouse micronucleus assay in Swiss Webster strain

mice. A dose range-finding study was conducted in groups of five animals/sex administered a single dose of deisopropyl zilpaterol hydrochloride up to 1000 mg/kg BW by oral gavage. No lethality was observed at 1000 mg/kg BW.

In the definitive test, groups of 15 animals per sex were administered a single dose at 0, 125, 250, 500, 1000 or 2000 mg/kg BW of deisopropyl zilpaterol hydrochloride by oral gavage. Groups of animals were euthanized at 27, 36 or 45 hr post-dosing and bone marrow tissue slides were prepared. There was lethality observed at 1000 and 2000 mg/kg BW, and there was a significant reduction in the ratio of PCEs to NCEs providing evidence of toxicity and demonstrating exposure to the test material. There were no significant increases in the percent micronucleated cells per 2000 PCEs at any dose or time interval. It was concluded that deisopropyl zilpaterol hydrochloride does not induce chromosomal damage *in vivo*, in mice, under the conditions of testing.

**Summary of the Studies to Assess Genotoxic Potential**

Based on an overall evaluation of the eleven studies described above, it is concluded that zilpaterol hydrochloride and its primary metabolite, deisopropyl zilpaterol hydrochloride are not genotoxic. Consequently, these compounds are unlikely to be genotoxic carcinogens.

**h. Carcinogenicity Study by Oral Route (Dietary Mixture) in Rats. Study No. 12871 TCR.**

INVESTIGATORS: Drs. F. Suavez and J. Richard
Centre International de Toxicologie (C.I.T.)
Miserey-27005 Evreux – France

A 104-week feeding study was conducted in Sprague-Dawley rats to evaluate the potential tumorigenic or carcinogenic activity of zilpaterol hydrochloride. Groups of 66 male and 66 female rats were fed 0, 25, 50, 125, and 250 μg/kg BW/day. Plasma concentrations of zilpaterol at the end of the treatment period increased with increasing dosage and were slightly higher in females. These measurements demonstrate systemic exposure of the test compound.

Zilpaterol hydrochloride was well tolerated at all dose levels except for reduced body weight gains and survival in the highest dose group, and increased ovary weights and incidence/severity of ovarian cysts at 125 and 250 μg/kg BW/day. These observations show that a maximum tolerated dose had been achieved. There were no other increases in the incidence of non-neoplastic lesions in any dose group. There was an increase in the incidence of a benign neoplastic lesion, leiomyoma, in the suspensory ligament of the ovary, observed in 2 of 63 females treated at 125 μg/kg BW/day and 5 of 64 females treated at 250 μg/kg BW/day. It was expected that zilpaterol hydrochloride, a potent $\beta_2$-adrenoreceptor agonist, would induce leiomyomas of the suspensory ligament of the ovary. Leiomyomas are a benign tumor in animals and humans. Studies have shown that the occurrence of leiomyomas can be blocked by the co-administration of an adrenergic β-receptor antagonist; thus, this is understood to be a pharmacologically-based lesion and is considered to be a non-carcinogenic event. The NOEL for toxicity

in this study was 50 μg zilpaterol/kg BW/day.

**i. An 18-Month Oral Gavage Tumorigenicity Study of Zilpaterol Hydrochloride in Female CD-1 Mice. Study No. 00-2657.**

INVESTIGATOR: C. Auletta
Huntingdon Life Sciences
East Millstone, NJ - USA

An 18-month oral gavage study was conducted in female CD-1 mice to evaluate the potential tumorigenic activity of zilpaterol hydrochloride. Groups of 65 female mice were dosed with 0, 10, 20, 50 or 250 μg/kg BW/day in corn oil. In addition, blood samples were obtained from satellite group animals (n=10) to determine plasma concentrations of zilpaterol hydrochloride after one month of dosing and to demonstrate systemic exposure at all doses. After 18 months of treatment, all survivors were euthanized and complete macroscopic examinations and histopathological evaluations of selected tissues were conducted on all animals.

Zilpaterol hydrochloride was well tolerated in this study. Oral administration of zilpaterol hydrochloride to female CD-1 mice for 18 months was not associated with evident signs of toxicity or tumorigenicity. Survival was 66 – 75% for all treatment groups and there were no dose-related treatment differences. Evaluation of clinical signs, body weights, and feed consumption revealed biological variation, but no treatment or dose-related toxic effects of zilpaterol hydrochloride. Macroscopic and microscopic pathology examinations indicated no non-neoplastic or neoplastic changes attributed to treatment with zilpaterol hydrochloride.

Treatment with zilpaterol hydrochloride resulted in some variability in hematology parameters which were only evaluated at the termination of the study. Compared to controls there was a significant increase in hemoglobin (20, 50, and 250 μg/kg BW/day) correlated with a significant increase in the number of red blood cells (50 and 250 μg/kg) BW/day and hematocrit (50 and 250 μg/kg BW/day). Significant diminution of platelets, absolute neutrophils, and monocytes compared to controls were attributable to zilpaterol hydrochloride treatment (50 and 250 μg/kg BW/day). Therefore, the NOEL in this study was 20 μg zilpaterol hydrochloride/kg BW/day.

**j. Human Studies**

**1) An Ascending Dose Tolerance Study of RU 42173 Including an Exploration of Pharmacodynamic Dose-Response Characteristics. Study No. SWIN/287/173.**

INVESTIGATORS: J. Sutton, R. Budhram
Roussel Laboratories, Ltd.
Swindon
Wiltshire, UK

Eight healthy male volunteers were given ascending single aqueous oral doses of 0, 0.25, 0.50, 1.00, or 2.00 mg zilpaterol hydrochloride/person (approximately 0, 4.17, 8.33, 16.67, or 33.33 μg/kg BW) in a four way cross-over design.

Electrocardiogram, arterial blood pressure, and cardiac measurements (cardiac output, stroke volume, ejection velocity) were recorded. Blood glucose, airway caliber, and hematology factors also were measured. Heart rate was slightly increased (6 bpm) at a dose of 0.25 mg per person and increased significantly at higher doses. Systolic blood pressure and airway caliber were significantly increased by doses of 1.00 and 2.00 mg per person. Blood glucose was dose dependent and increased at all dose levels. Hematology was not affected by zilpaterol hydrochloride treatment. The occurrence of tremors was evaluated clinically (subjective and inspective) in all treatment groups. Tremor frequency was significantly increased at doses of 0.5, 1, and 2 mg per person.

2) **Clinical and Biological Tolerance Study of RU 42173 Administered by Oral Route at Repeated doses (0.25 mg x 3/d over 7 Days) in Healthy Volunteers. Study No. F/88/173/06.**

INVESTIGATORS: G. Strauch, A. Venot, M. Letrait, J. Vergne, A. Lurie, F. Bompart, D. deLautre, P. Vivet
ECLIMED-IRT
Hospital CHOCHIN
Paris, France

Thirteen healthy male volunteers participated in this study. Four subjects received the placebo treatment while nine subjects were given a treatment of 0.25 mg zilpaterol hydrochloride (approximately 4.17 µg/kg BW) three times per day for seven consecutive days. One treated subject was removed from the study prior to completing the dosing regimen, leaving eight subjects receiving zilpaterol hydrochloride. Dosing solutions were added to water and swallowed by subjects. Blood pressure, heart rate, clinical chemistry factors, and bronchodilation were measured. Significant changes in heart rate and bronchodilating effect were observed when the volunteers received zilpaterol hydrochloride. A trend of increased tremor findings was observed when volunteers received zilpaterol hydrochloride.

3) **A Study of the Bronchodilating Activity of 3 Single Oral Doses RU 42173 (0.05, 0.10, and 0.25 mg) in Adult Asthmatics. Study No. FF/88/173/05.**

COORDINATOR: P. Vivet
AND AUTHOR: Roussel-UCLAf
Romainville, France

Eleven asthmatic patients were given a single dose of 0.00, 0.05, 0.10, or 0.25 mg zilpaterol hydrochloride per person in a four-way cross-over design. Dosing solutions were added to water and swallowed by patients. Bronchodilation, blood pressure, heart rate, tremor occurrence, blood glucose, and blood potassium were measured. Heart rate was slightly but significantly increased at 0.25 but not at 0.05 and 0.1 mg. Blood pressure was not affected by treatment at any dose level. There were no tremor findings when those were evaluated objectively by the sinusoidal drawing method. Tremors were seen in all dose groups when evaluated clinically.

They were observable but never severe. Bronchodilation was slight, transitory, and reversible at 0.1 mg, but pronounced at 0.25 mg.

4) **Bronchodilating Activity of a Single Oral Dose (0.25 mg) of RU 42173 in Adult Asthmatic Patients. Study No. F/87/173/04.**

COORDINATOR: P. Vivet
AND AUTHOR: Roussel-UCLAF
Romainville, France

Twelve asthmatic patients were given a single dose of 0.0 or 0.25 mg of zilpaterol hydrochloride in a two way cross-over design study. Dosing solutions were added to water and swallowed by patients. Bronchodilation, blood pressure, heart rate, and tremors were measured. Zilpaterol hydrochloride significantly increased bronchodilation. Heart rate was slightly, but significantly increased and diastolic blood pressure was slightly decreased. Short-lasting increases in heart rate (5/12) and tremors were seen in 2/12 patients after treatment. A preexisting tremor in a third patient was not increased following treatment.

**Summary of Human Studies**

In all studies, slight, transitory, marginal, and typical $ß_2$-related pharmacodynamic effects were consistently observed at a dose of 0.25 mg/person/day. Zilpaterol hydrochloride was given as an aqueous drinking solution. It can therefore be expected that a higher $C_{max}$ was reached more quickly than if the compound had been given in or together with the food. Patients were fasted, which also usually accelerates absorption. Tremor evaluation was performed with a sensitive and subjective method detecting tremors pre-treatment and under placebo. These aspects taken together increased the sensitivity of the test system.

**2. Determination of No Observed Effect Level (NOEL)**

A NOEL of 1.7 μg/kg BW (0.1 mg per person) is established for zilpaterol hydrochloride based on induced tremors. However, it is concluded that bronchodilation, rather than tremors, is the most sensitive pharmacological endpoint for toxicity in asthmatic humans. A NOEL of 0.83 μg/kg BW (0.05 mg per person) is established for zilpaterol hydrochloride induced bronchodilation based on increased bronchodilation at higher doses in asthmatic humans.

**3. Acceptable Daily Intake (ADI)**

The final NOEL of 0.83 μg/kg BW is based on increased bronchodilation in asthmatic humans. The safety factor to be used for calculation of the ADI is 10. The resulting acceptable daily intake (ADI) for zilpaterol hydrochloride is (NOEL ÷ Safety Factor) or (0.83 μg/kg BW ÷ 10) = 0.083 μg/kg BW.

**4. Safe Concentrations for Total Residues**

The safe concentration (SC) of residue in an edible tissue is calculated by the procedure described in the Guideline No. 3 General Principles for Evaluating the Safety of

Compounds used in Food Producing Animals (FDA/CVM, revised June 21, 2005):

$$\text{Safe Concentration (SC)} = \frac{\text{ADI x Human Weight}}{\text{Consumption Factor}} \quad \text{or} \quad \frac{0.083\ \mu\text{g/kg x 60 kg}}{\text{Consumption factor}}$$

The average weight of a human is approximately 60 kg. The established consumption factors are 300 grams for muscle, 100 grams for liver, 50 grams for kidney, and 50 grams for fat.

Table 9: Safe Concentrations of Zilpaterol HCl in Edible Tissues.

| Edible Beef Tissue | Daily Consumption (grams) | Safe Concentrations (mg/kg or ppm) |
|---|---|---|
| Muscle | 300 | 0.02 |
| Liver | 100 | 0.05 |
| Kidney | 50 | 0.10 |
| Fat | 50 | 0.10 |

The molecular weight of zilpaterol freebase is 0.878 of the molecular weight of zilpaterol hydrochloride. Because the residue method measures zilpaterol rather than zilpaterol hydrochloride residues in edible tissues, the SC for zilpaterol freebase residues are presented in Table 10.

Table 10: Safe Concentrations of Zilpaterol Freebase Residues.

| Edible Beef Tissue | Daily Consumption (grams) | Safe Concentrations (ppb) |
|---|---|---|
| Muscle | 300 | 18 |
| Liver | 100 | 44 |
| Kidney | 50 | 88 |
| Fat | 50 | 88 |

## *B. Residue Chemistry:*

### 1. Summary of Residue Chemistry Studies

#### a. Total Residue Depletion and Metabolism Studies

Conversion of zilpaterol hydrochloride to zilpaterol freebase is required because the analyte of the residue method is zilpaterol freebase. Therefore, zilpaterol residues for the following studies have been converted to a freebase basis.

Study 1. "Total $^{14}$C Residue in Cattle: $^{14}$C-RU 42173 (Zilpaterol hydrochloride) Pivotal Residue Depletion Study In Steers And Heifers," Study No. XL/97/RU42173/01

Principal Investigator: J. Tulliez, INRA-Laboratoire des Xénobiotiques, Toulouse, France.

Animal Species: Hereford steers and heifers

Number of Animals/Sex: 17; 8 male and 7 female (plus 1 male and 1 female control animals)

Weights of Animals: 200 to 230 kg

Health Status: clinically healthy

Route of Administration: orally

Dose Rate: 0.15 mg/kg body weight per day (equivalent to 6.8 g zilpaterol hydrochloride/ ton in the diet)

Duration of Dosing: 12 or 15 days

Liver, kidneys, muscle, and fat samples were collected and analyzed to determine levels of total radioactivity, unchanged zilpaterol, and a major metabolite, deisopropyl-zilpaterol. The length of time for residues to reach a steady state in tissues, the depletion of total $^{14}$C-zilpaterol residues from tissues and the concentrations of zilpaterol and the principal metabolite, deisopropyl-zilpaterol, in edible tissues were measured. Total radioactivity was measured by combustion and liquid scintillation counting. Since residue levels in tissues were similar after 12 and 15 days of dosing, a steady state was achieved with 12 days of dosing. The data from both groups slaughtered at 12 hr withdrawal were combined for further analysis (Table 11). The total residue data showed that residues deplete most slowly from liver. The target tissue for zilpaterol in cattle was found to be liver.

Table 11: Total Residues in Tissues of Cattle Fed 0.15 mg Fed $^{14}$C-zilpaterol/kg body weight/day for 12 Days (Study No. XL/97/RU42173/01).

| Withdrawal Time (hr) | Liver (ppb ± S.D.) | Kidney (ppb ± S.D.) | Muscle (ppb ± S.D.) | Fat (ppb) |
|---|---|---|---|---|
| 12 hr | 256 ± 49.1 | 161 ± 27.0 | 19 ± 2.1 | 9.2 |
| 24 hr | 180 ± 12.1 | 88 ± 4.3 | 11 ± 2.3 | ND |
| 48 hr | 138 ± 19.8 | 32 ± 21.9 | N.D. | N.D. |
| 96 hr | 99 ± 14.9 | 8 ± 3.1 | N.D. | N.D. |

A mass balance for unchanged zilpaterol and its metabolites in tissues was calculated from the recovery of the radioactivity after different extraction steps. Labeled zilpaterol and labeled metabolites were extracted from liver, kidney, and muscle using an ammonia-acetonitrile-methanol mixture and purified by solid phase extraction. Analysis of the total $^{14}$C-zilpaterol-related residues showed that 40.0 to 57.4% of the residues in liver at 12 and 24 hr and 23.9 to 31.2% at 48 and 96 hr were extractable. In kidney 84.5 to 92.5% of the residues in kidney were extractable at 12 and 24 hr and 37.9 to 73.8% at 48 and 96 hr. Essentially all of the residues in muscle were extractable at the 12 and 24 hr withdrawal periods. The extractable residues in the liver were analyzed by an HPLC method with fluorescence detection and found to be composed of unchanged zilpaterol and deisopropyl-zilpaterol, the only metabolite identified in cattle tissues. Zilpaterol and the metabolite residue levels were 71.5 and

11.9 ppb, respectively, at 12 hr of withdrawal, 35.0 and 5.8 ppb at 24 hr and 12.8 and 2.2 ppb at 48 hr. of withdrawal. Zilpaterol represented 30 to 55% and the metabolite was 5.1 to 9.1% of the extractable residues from tissues obtained at the 12 to 48 hr withdrawal periods. The distribution of zilpaterol freebase residues in liver tissues is presented in Table 12.

Table 12: Distribution of Zilpaterol Freebase Residues in Liver Tissues (Study No. XL/97/RU42173/01).

| Withdrawal (hr) | Extractable $^{14}$C residue from Liver | Extractable $^{14}$C-Zilpaterol Residue by HPLC-F | % Zilpaterol in Extractable Residue | $^{14}$C-De-isopropyl-zilpaterol in Extractable Residue | % Deisopropyl in Extractable Residue |
|---|---|---|---|---|---|
| | ppb | ppb | % | ppb | % |
| 12 hr | 130 ± 22.7 | 71.6 ± 19.2 | 55.1 | 11.9 ± 2.7 | 9.1 |
| 24 hr | 72 ± 4.0 | 35.0 ± 1.1 | 48.6 | 5.8 ± 1.2 | 8.0 |
| 48 hr | 43 ± 18.3 | 12.8 ± 10.4 | 29.8 | 2.2 ± 0.3 | 5.1 |
| 96 hr | 24 ± 2.4 | 1.2 ± 0.13 | 5.2 | 1.0 ± 0.19 | 4.1 |

Study 2. "$^{14}$C-Zilpaterol: Bioavailability in the Rat of Liver Non-Extractable Residues from Cattle", Study No. HST 456/993307.

Principal Investigator: R. Girkin, Huntingdon Life Sciences Ltd., Huntingdon, England.

Animal Species: Sprague-Dawley CD rats surgically altered according to the model of Gallo-Torres

Number of Animals/Sex: 32, 14 male and 14 female (plus 2 male and 2 female control rats)

Weights of Animals: 200 to 239 g

Health Status: clinically healthy

Route of Administration: orally

Dose Rate: pelleted extracted cattle liver, nominal dose level of 1 mg/kg $^{14}$C-zilpaterol hydrochloride in aqueous solution

Duration of Dosing: 3 experimental phases, each 24 hours in duration

A study was conducted to determine the amount of the non-extractable residues (bound residues) in cattle liver that was absorbed by Sprague-Dawley CD rats fed the extracted liver. Extracts were prepared from liver samples obtained from cattle euthanized at 12, 24, 48, and 96 hr withdrawal times in Study No. XL/97RU 42173/01, described above. The extracted tissue was fed to rats that were surgically cannulated to allow collection of bile according to the model of Gallo-Torres (Journal of Toxicology and Environmental Health, 2:827-845, 1977). A second group of rats received a control treatment which was an intragastric dose of $^{14}$C-zilpaterol hydrochloride and an oral

dose of control liver. The amount of radioactivity excreted in the urine, bile, and retained in the carcass was considered to be the amount of bound zilpaterol-related residues that could be bioavailable. The results showed that only 3.28, 2.01, 0.75, and 1.21% of the bound residues from tissues obtained from animals slaughtered at 12, 24, 48, and 96 hr of withdrawal, respectively, were absorbed and considered to be biologically available. When rats fed a control liver diet were administered $^{14}C$-zilpaterol hydrochloride, 91.63% of the radioactivity was absorbed. The amount of total residue, non-extractable residue, and percent bioavailability of bound residue are presented in Table 13.

Table 13: Amount of Total Residue, Non-extractable Residue and Percent Bioavailability of Bound Residue (Study No. HST 456/993307).

| Withdrawal (hr) | Non-Extractable Residues (%) | Bioavailability of Non-extractable Residue (%) |
|---|---|---|
| | | |
| 12 hr | 36.8 | 2.88 ± 1.58 |
| 24 hr | 53.6 | 2.02 ± 0.74 |
| 48 hr | 65.2 | 0.75 ± 0.58 |
| 96 hr | 70.7 | 1.20 ± 0.69 |

A statistical analysis of the bioavailability study data determined that 4.8% of the non-extractable residues could be added to the extractable zilpaterol residue in liver to account for the total zilpaterol-related residue of toxicological concern. The 4.8% absorption represents the upper 95% confidence limit for the 99$^{th}$ percentile of the population which was adjusted for the average percent absorption of $^{14}$C-zilpaterol hydrochloride administered to rats fed a control liver diet. The total non-extractable residues, the bioavailable residues, and the total residues of concern are presented in Table 14.

Table 14: Non-extractable and Total Residues of Concern in Liver of Cattle Fed 0.15 mg $^{14}$C-zilpaterol/kg body weight/day for 12 days or 15 days (Study No. HST 456/993307).

| Withdrawal Time (hours) | Total Nonextractable Residues (ppb) | Bioavailable Residues (ppb) (total nonextractable residues x 4.8%) | Total Residues of Concern (ppb) (bioavailable residues plus extractable residues) |
|---|---|---|---|
| 12 | 125.6 | 6.03 | 136.0 |
| 24 | 107.6 | 5.2 | 77.2 |
| 48 | 94.2 | 4.5 | 47.5 |
| 96 | 75.8 | 3.6 | 27.6 |

Study 3. "Metabolism and Residue Study of $^{14}$C-RU 42173 in the Cattle (Steer and Heifer) After Single Oral Administration." Study No. 90.01.XL

Principal Investigator: J. Tulliez, INRA-Laboratoire des Xénobiotiques, Toulouse, France.

Animal Species: Cattle

Number of Animals/Sex: 8, 3 steer and 3 heifer (plus 1 control steer and 1 control heifer)

Weights of Animals: Mean 295 kg

Health Status: healthy

Route of Administration: orally through medicated feed

Dose Rate: 0.2 mg $^{14}$C-zilpaterol hydrochloride/kg body weight

Duration of Dosing: single administration

A study was conducted to determine the distribution, metabolism and route of excretion of $^{14}$C-zilpaterol hydrochloride after oral administration to cattle. Three groups of two animals (one steer and one heifer) were administered a single oral dose of $^{14}$C-zilpaterol hydrochloride at 0.2 mg zilpaterol hydrochloride/kg BW and slaughtered 12 hr, 48 hr, and 8 days later. The highest levels of zilpaterol freebase residues at 12 hr after dosing were in the liver (98 and 102 ppb for males and females, respectively) and kidney (97 and 104 ppb). Muscle had 15 and 13 ppb, visceral fat 5.7 and 2.2 ppb, and perirenal fat 1.3 and 1.3 ppb of the total residue. At 48 hr of withdrawal the residue levels had declined to an average of 35 ppb in liver, 21 ppb in kidney, 3.1 ppb in muscle, and 1.8 ppb in fat. Of the excreted radioactivity, 88% and 84% was in the urine and 8.7 and 8.6% in feces for males and females, respectively.

Analysis of the radioactivity in the urine showed that unchanged zilpaterol represented more than 60% of the radioactivity with the remainder distributed among four metabolites. In liver, kidney, and muscle tissues unchanged zilpaterol was the main residue and one major metabolite was observed. This metabolite was identified by GC-mass spectral analysis as deisopropyl-zilpaterol and represents about 20% of the extractable residue in tissues and about 13% of the radioactive residue in urine. The metabolites identified in the urine and tissues are listed in Table 15.

Table 15: Metabolites Identified in the Urine and Tissues (Study No. 90.01.XL).

| Metabolite Peak Identification[)] | Name | Cattle Urine | Cattle Liver | Cattle Kidney | Cattle Muscle |
|---|---|---|---|---|---|
| | | Percent Distribution (%) | | | |
| A | Acetylated deisopropyl-zilpaterol | 7.0 | ND | ND | ND |
| B | Unidentified | 11.3 | ND | ND | ND |
| D | Unidentified | 3.5 | ND | ND | ND |
| $E_1$ | Deisopropyl-zilpaterol | 13.2 | 20.6 | 17.9 | 21.2 |
| F | Zilpaterol | 65.7 | 62.3 | 69.5 | 71.9 |

Values are the percentage of radioactivity added to the HPLC column.

Study 4. "Pilot Steady State Study of $^{14}$C-RU 42173 Steer and Heifer." Study No. 94.03.XL.

Principal Investigator: J. Tulliez, INRA-Laboratoire des Xénobiotiques, Toulouse, France.

Animal Species: Cattle

Number of Animals/Sex: 10, 4 steer and 4 heifer (plus 1 control steer and 1 control heifer)

Weights of Animals: 200 to 220 kg

Health Status: healthy

Route of Administration: orally through medicated feed

Dose Rate: 0.15 mg $^{14}$C-zilpaterol hydrochloride/kg body weight

Duration of Dosing: 10, 12, 15, and 21 days

The purpose of this study was to determine the period of dosing required for residues in tissues to reach a steady state and to define the composition of the zilpaterol-related residues in tissues. Four groups of two Charolais animals (one steer and one heifer each) were administered oral doses of $^{14}$C-zilpaterol hydrochloride at 0.15 mg zilpaterol hydrochloride/kg BW for 10, 12, 15, and 21 days and slaughtered 20-24 hr after the last dose. Another group of two animals were non-medicated and served as controls. Analysis of total residues in liver, kidney, and muscle indicated that steady state level was reached by 12 days of dosing. Liver was the tissue with the highest level of residue, followed by kidney and muscle. Total residue levels in the liver after 10, 12, 15, and 21 days of dosing, respectively, were 219, 317, 289, and 336 ppb for males and 256, 366, 400, and 302 ppb for females. The total residue levels in kidney and muscle followed the same pattern. The data are presented in Table 16.

Table 16: Total Residue Levels in Kidney and Muscle (Study No. 94.03.XL).

| Days of Treatment | Tissue Radioactive Residue Levels (ppb of $^{14}$C-Zilpaterol Freebase) | | |
|---|---|---|---|
| | Liver (M/F)[1] | Kidney (M / F) | Muscle (M / F) |
| 10 | 219 / 256 | 113 / 135 | 20 / 16 |
| 12 | 317 / 366 | 161 / 211 | 25 / 28 |
| 15 | 289 / 400 | 200 / 220 | 30 / 27 |
| 21 | 336 / 302 | 205 / 146 | 18 / 19 |

[1]Males / Females

For liver, extractable radioactivity was unchanged zilpaterol (70%), deisopropyl-zilpaterol (12.3%) and one unidentified peak (3.3%). The radioactivity in kidney consisted of 74.9% zilpaterol, 6.8% deisopropyl-zilpaterol, 4.9% acetylated deisopropyl-zilpaterol and 5.7 and 9.1% in two unidentified peaks. The structures of

the two major residues in liver were confirmed by an HPLC-MS method to be the unchanged parent, zilpaterol, and deisopropyl-zilpaterol. The identification and distribution of the metabolites are presented in Table 17.

Table 17: Identification and Distribution of the Metabolites in Liver and Kidney (Study No. 94.03.XL).

| Metabolite Peak | Name | Cattle Liver | Cattle Kidney |
|---|---|---|---|
| | | Percentage Distribution (%) | |
| A | Acetylated deisopropyl-zilpaterol | ND | 4.9 |
| B | Unidentified | ND | 9.1 |
| D | Unidentified | 3.3 | 5.7 |
| $E_1$ | Deisopropyl-zilpaterol | 12.3 | 6.8 |
| F | Zilpaterol | 70.0 | 74.9 |

Values are the percentage of radioactivity added to the HPLC column

**b. Comparative Metabolism Studies**

Study 1. "Metabolic Fate of $^{14}$C-RU 42173 (Zilpaterol Hydrochloride) in the Wistar Rat", Study No. 92/01/XL.

Principal Investigator: J. Tulliez, INRA-Laboratoire des Xénobiotiques, Toulouse, France

Animal Species: Wistar rats

Number of Animals/Sex: 6, 3 male and 3 female

Weights of Animals: 200 to 250 g

Health Status: healthy

Route of Administration: oral gavage

Dose Rate: 0.2 mg $^{14}$C-zilpaterol hydrochloride/kg body weight

Duration of Dosing: single dose

The purpose of this study was to determine the metabolic fate of $^{14}$C-zilpaterol hydrochloride in rats following oral administration of the compound. Six adult Wistar rats (3/sex weighing between 200 and 250 g) each received a single oral dose of 0.2 mg zilpaterol hydrochloride/kg BW of $^{14}$C-zilpaterol hydrochloride by gavage. Two rats (one of each sex) were euthanized at 12 hr, 48 hr, and 8 days post-dosing. The rats eliminated 48.89% of the dose in the urine and 42.29% in the feces. The metabolite profile in the urine from rats collected during the first two days after dosing showed the parent compound and five metabolite peaks. The metabolites were identified as deisopropyl-zilpaterol, acetylated deisopropyl-zilpaterol, conjugated hydroxy-zilpaterol, and 2 unidentified peaks. In day 1 and day 2 urine samples, respectively,

unchanged zilpaterol represented 60.3 and 46.7% of the total radioactivity. The other metabolites in day 1 and day 2 samples, respectively, were deisopropyl-zilpaterol, 19.5 and 14.2%, acetylated deisopropyl-zilpaterol, 4.3 and 4.2%, a conjugate of hydroxy-zilpaterol 9.3 and 22.3%, and two unidentified metabolites with < 3.0% of the radioactivity. The distribution of metabolites in urine is presented in Table 18.

Table 18: Distribution of Metabolites in Rat Urine (Study No. 92/01/XL).

| Metabolite Identification | | Distribution in Rat Urine | |
|---|---|---|---|
| | | Day 1 | Day 2 |
| | | % | % |
| F | Zilpaterol | 60.3 | 46.7 |
| $E_1$ | Deisopropyl-zilpaterol | 19.5 | 14.2 |
| $E_2$ | Hydroxy-zilpaterol | (1) | (1) |
| A | Acetylated Deisopropyl-zilpaterol | 4.4 | 4.2 |
| C | Glucuronate conjugate of hydroxy-zilpaterol | 9.3 | 22.3 |
| B | Unidentified | 2.2 | 0.7 |
| D | Unidentified | 0.5 | ND |

(1) Metabolites $E_1$ and $E_2$ were not separated in this study.
Values are the percentage of radioactivity added to the HPLC column

In fecal sample extracts, unchanged zilpaterol represented less than 16% of the radioactivity on day 1 and 4% on day two. In feces, there was one major metabolite and several minor peaks. The major metabolite was identified as the hydroxylation product of zilpaterol. Structural information obtained using GC-MS indicated that the hydroxylation process occurred in the aromatic ring of zilpaterol. The study shows that hydroxylation is a major pathway in the rat for excretion in the feces. Deisopropyl-zilpaterol was present, although in minor amounts, in the feces from the rat.

Study 2. "Metabolic Fate of $^{14}$C-RU 42173 (Zilpaterol Hydrochloride) in the Sprague Dawley Rat", Study No. 96/01/XL.

Principal Investigator: J. Tulliez, INRA-Laboratoire des Xénobiotiques, Toulouse, France.

Animal Species: Sprague Dawley rats

Number of Animals/Sex: 6, 3 male and 3 female

Weights of Animals: 200 to 250 g

Health Status: healthy

Route of Administration: orally

Dose Rate: 0.2 mg $^{14}$C-zilpaterol hydrochloride/kg body weight

Duration of Dosing: single dose

The purpose of this study was to determine the metabolic fate of $^{14}$C-zilpaterol hydrochloride in Sprague Dawley rats and to compare the metabolites observed in Sprague Dawley rats with those produced by Wistar rats. Sprague Dawley rats were used in the pivotal toxicology studies. Three rats per sex weighing 200-250 g were administered a single oral dose of 0.2 mg/kg BW of $^{14}$C-zilpaterol hydrochloride. Urine and feces were collected for 8 days. The metabolite profile analysis of urine identified five metabolites. The predominant metabolite was unchanged zilpaterol. The other metabolites were deisopropyl-zilpaterol, acetylated deisopropyl-zilpaterol, hydroxy-zilpaterol, a glucuronate conjugate of hydroxy-zilpaterol, and one unidentified peak. Treatment of the glucuronate conjugate with β-glucuronidase degraded the conjugate and produced hydroxy-zilpaterol. The metabolites identified in the urine were the same as those identified in the Wistar rat study. The data are presented in Table 19.

Table 19: Distribution of Metabolites in Rat Urine (Study No. 96/01/XL).

| Metabolite | | Distribution in Rat Urine | |
|---|---|---|---|
| | | (%) | (%) |
| | | Day 1 | Day 2 |
| F | Zilpaterol | 61.6 | 47.9 |
| $E_1$ | Deisopropyl-zilpaterol | 11.8 | 9.1 |
| $E_2$ | Hydroxy-zilpaterol | 4.6 | 11.5 |
| A | Acetylated deisopropyl-zilpaterol | 7.1 | 9.0 |
| C | Hydroxy-zilpaterol glucuronate | 3.8 | 5.6 |
| B | Unidentified | 2.2 | 4.7 |
| D | Unidentified | 0.3 | 0.2 |

Values are the percentage of radioactivity added to the HPLC column

Summary of comparative metabolism studies

Metabolism studies conducted in rats and cattle demonstrated that the metabolism and metabolites produced following oral administration of zilpaterol hydrochloride to the two species are comparable. The compound is readily absorbed and the parent compound and metabolites are readily eliminated, primarily in the urine (80% in cattle, 60% in rats) with the remainder in the feces. Unchanged parent compound is the main compound excreted in the urine of both species and is the main residue found in cattle tissues (liver, kidney, and muscle). In both cattle and rats the main non-parent metabolite excreted is deisopropyl-zilpaterol. This metabolite and unchanged zilpaterol are the only metabolites with >10% of the radioactivity found in edible tissues from cattle. In rats the main metabolite in feces is hydroxy-zilpaterol, however hydroxy-zilpaterol was also present at lower levels in rat urine. Therefore, the results of the studies demonstrate that the metabolic profile of zilpaterol in Sprague Dawley and Wistar rats and cattle are qualitatively similar and the rat is an appropriate model for human safety related studies. The comparison of relative distribution of metabolites in cattle tissues and in Sprague Dawley and Wistar rat urine are presented in Table 20.

Table 20: Comparison of Relative Distribution of Metabolites in Cattle Tissues and in Sprague Dawley and Wistar Rat Urine.

| Metabolite No. | Identification | Cattle Liver[1] (%) | Cattle Kidney[1] (%) | Cattle Urine[2] (%) | Rat Urine[3] (%) | Rat Urine[4] (%) |
|---|---|---|---|---|---|---|
| F | Zilpaterol | 70.0 | 74.9 | 65.7 | 61.6 | 60.3 |
| $E_1$ | Deisopropyl-zilpaterol | 12.3 | 6.8 | 13.2 | 11.8 | 19.5 |
| $E_2$ | Hydroxy-zilpaterol | ND | ND | ND | 4.6 | (4) |
| A | Acetylated deisopropyl-zilpaterol | ND | 4.9 | 7.0 | 7.1 | 4.4 |
| B | Unknown | ND | 9.1 | 11.3 | 2.2 | 9.3 |
| C | Glucuronate conjugate of hydroxy-zilpaterol | ND | ND | ND | 3.8 | 2.2 |
| D | Unknown | 3.3 | 5.7 | 3.5 | 0.3 | 0.5 |

[1]Cattle liver and kidney values from Study No. 94/03/XL
[2]Cattle urine values from Study No. 90/01/XL
[3]Sprague Dawley rat values for day 1 urine from Study No. 96/01/XL.
[4]Wistar rat values for day 1 urine from Study No. 92/01/XL, Metabolite $E_1$ and $E_2$ not separated. Values are the percentage of radioactivity added to the HPLC column.

**c. Residue Depletion Study**

Tissue Residue Depletion Study in Cattle Administered Zilpaterol Hydrochloride Orally via Medicated Feed for 12 Days. Study No. 6187-173.

Principal Investigator: D. L. Hughes, Covance Laboratories Inc., Waunakee and Madison, Wisconsin.

Animal Species: Crossbred beef cattle

Number of Animals/Sex: 18, 8 steers and 8 heifers (including 1 control steer and 1 control heifer)

Weights of Animals: 455 to 595 kg

Health Status: healthy

Route of Administration: orally via medicated feed

Dose Rate: 6.8 g zilpaterol hydrochloride/ton total mixed ration

Duration of Dosing: 12 consecutive days

The concentration of the marker residue, zilpaterol, in the liver (target tissue), muscle, and kidney tissues of cattle was determined in a study wherein cattle were treated for 12 consecutive days with ZILMAX Premix medicated feed at the rate of 6.8 g zilpaterol hydrochloride/ton total mixed ration (90% dry matter basis). Eighteen crossbred beef cattle, 9 steers weighing 455 to 595 kg (1,001 to 1,309 lb) and 9 heifers weighing 480 to 573 kg (1,056 to 1,261 lb) at the initiation of treatment were randomly assigned to four groups (2/sex) with one group euthanized at 12, 24, 48, or 96 hours

after receiving the final medicated feed. The other two animals were non-medicated control animals. Samples of liver, muscle, and kidneys were assayed by a validated HPLC with fluorescence detection procedure. The mean zilpaterol freebase (marker residue) residue levels in liver declined from 24.8 ppb at 12 hours post dose, to 10.0 ppb at 24 hr, 4.0 ppb at 48 hr, and < Limit of Detection (LOD) at 96 hours post-dose. The mean residue levels in muscle at the 12, 24, 48, and 96 hr withdrawal times, respectively, were 4.4 ppb, 1.8 ppb, < Limit of Quantitation (LOQ), and <LOQ. The mean residue levels in kidney at the 12, 24, 48, and 96 hr withdrawal times, respectively, were 44.6 ppb, 11.3 ppb, 5.0 ppb, and <LOQ. These data, in tabular form, are presented in Table 21.

Table 21: Mean Residue Levels in Kidney at the 12, 24, 48, and 96 hr Withdrawal Times (Study No. 6187-173).

| Withdrawal Period (hrs) | Liver [(1)] ppb | Muscle [(1)] ppb | Kidney [(1)] ppb |
|---|---|---|---|
| 12 | 24.8 ± 7.95 | 4.4 ± 1.67 | 44.6 ± 29.10 |
| 24 | 10.0 ± 2.45 | 1.8 ± 0.41 | 11.3 ± 1.35 |
| 48 | 4.0 ± 3.54 | N.D. | 5.0 ± 4.52 |
| 96 | N.D.[(2)] | N.D. | N.D. |

[1]Values in this table are the mean ± SD for n = 4 animals.
[2]N.D. levels below the level of quantification for zilpaterol freebase residue were 3 ppb in cattle liver, 1 ppb in muscle, and 1 ppb in kidney.

**2. Target Tissue and Marker Residues**

Data presented in $^{14}$C-zilpaterol residue depletion study (Study No. XL/97RU 42173/01) demonstrated that the highest total residue level is in the liver and that unchanged zilpaterol freebase is the predominant residue in animals euthanized at 12, 24, and 48 hr withdrawal. Therefore, liver is designated as the target tissue and zilpaterol freebase as the marker residue for the monitoring of residues.

**3. Tolerances**

Liver A statistical analysis of the bioavailability study data determined that 4.8% of the non-extractable residues could be added to the extractable zilpaterol residue in liver to account for the total zilpaterol-related residue of toxicological concern. The 4.8% absorption represents the upper 95% confidence limit for the 99$^{th}$ percentile of the population which was adjusted for the average percent absorption of $^{14}$C-zilpaterol hydrochloride administered to rats fed a control liver diet.

The linear regression of the Total Residues of Concern (extractable zipaterol residues plus bioavailable non-extractable residues) and the zilpaterol freebase residues measured by HPLC-F showed that at the safe concentration of 44 ppb, the liver tolerance is 12 ppb for zilpaterol freebase.

**4. Withdrawal Period**

The liver residue depletion data from Study No. 6187-173 (Tissue Residue Depletion Study in Cattle Administered Zilpaterol Hydrochloride Orally via Medicated Feed for 12

Days) were analyzed with a statistical method which determines the statistical tolerance limit for the 99th percentile of the population with 95% confidence as outlined in CVM's Guideline for Establishing a Withdrawal Period.[13] The tolerance limit falls below the liver tolerance of 12 ppb at 3 days after the last dose. The data support the assignment of a 3 day withdrawal period.

### *C. Microbial Food Safety:*

The impact of the use of zilpaterol hydrochloride [6.8 g/ton (90% dry matter basis) in a Type C Medicated feed, fed the last 20 to 40 days prior to slaughter] for increased body weight gain, improved feed efficiency, and improved carcass leanness in cattle fed in confinement for slaughter on microbial food safety was carefully considered by the Agency. The Agency thinks that this use should not significantly impact public health with respect to antimicrobial resistance, and therefore an evaluation of microbial food safety was not necessary at this time.

### *D. Regulatory Method:*

#### 1. Determinative Procedure

The determinative analytical procedure utilizes HPLC with fluorescence detection that is capable of measuring the marker residue, zilpaterol freebase, in cattle liver at concentrations from 2.6 to 87.8 ppb and in cattle muscle from 0.878 to 31.6 ppb. The procedure successfully completed a sponsor monitored multi-laboratory method trial.

#### 2. Confirmatory Procedure

The sample extraction and preparation for the confirmatory procedure is identical to the sample extraction and preparation for the determinative procedure with the monitoring of four ions by LC/MS/MS. For confirmation, these four ions result in three ions ratios that meet the 10% abundance matching criteria.

#### 3. Availability of the Method

The method is on file with the Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855.

## *5. USER SAFETY CONCERNS:*

The ZILMAX Type A Medicated Article and Type B and C Medicated Feed labels contain the following human warning:

> **WARNING:**
> **The active ingredient in Zilmax® is zilpaterol hydrochloride, a beta$_2$-adrenergic agonist. Not for use in humans. An anti-dust process has been applied to the drug product, Zilmax®, in order to greatly reduce inhalation risk. Extended handling tasks with the potential for dust**

[13] Guidance for Industry #3: General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals, FDA/CVM, revised June 21, 2005.

**generation require respiratory protection. Wear appropriate skin protection (e.g., impervious gloves, apron, overalls) if there is a potential for extended skin contact. Wear protective eye wear, if there is a potential for eye contact. If accidental eye contact occurs, immediately rinse with water and consult a physician.**

## *6. AGENCY CONCLUSIONS:*

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that zilpaterol hydrochloride when fed at a dietary concentration of 6.8 g per ton (7.5 ppm) of zilpaterol hydrochloride/ton of feed to provide 60-90 mg zilpaterol hydrochloride/head/day on a 90% dry matter basis for the last 20 to 40 days on feed, is safe and effective for the claims indicated in section 1 of this FOI Summary.

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the new animal drug has been previously approved.

The drug is to be fed in Type C medicated feeds in accordance with section 1 of the FOI Summary and the Blue Bird labeling that is attached to this document.

The Center for Veterinary Medicine has concluded that, for this product, adequate directions for use by the layperson have been provided and the product will have over-the-counter (OTC) status. Label directions provide detailed instruction in plain language. The drug product is not a controlled substance. Thus, the drug product is assigned OTC status, and the labeling is adequate for the intended use.

ZILMAX is under the following US patent numbers:

| U.S. Patent Number | Date of Expiration |
|---|---|
| 4,585,770 | October 12, 2006 |
| 5,731,028 | June 6, 2016 |

## *7. ATTACHMENTS:*

Facsimile Labeling is attached as indicated below:

ZILMAX Type A Medicated Article
ZILMAX Type A Medicated Article (Front Panel)
ZILMAX Type A Medicated Article (Back Panel)
ZILMAX Finishing Cattle Feed Liquid Type B Medicated Feed
ZILMAX Finishing Cattle Feed Type B Medicated Feed
ZILMAX Finishing Cattle Feed Type C Medicated Feed