Date of Approval: May 25, 2021

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 140-269

KETOFEN®

ketoprofen

Injectable Solution

Beef heifers, beef steers, beef calves 2 months of age and older, beef bulls, replacement dairy heifers, and dairy bulls

This supplement provides for the addition of a new target animal and indication to the label

Sponsored by:

Zoetis Inc.

Executive Summary

KETOFEN® (ketoprofen) is approved for the control of pyrexia associated with bovine respiratory disease (BRD) in beef heifers, beef steers, beef calves 2 months of age and older, beef bulls, replacement dairy heifers, and dairy bulls. KETOFEN® is not for use in reproducing animals over one year of age or calves less than 2 months old. This supplemental approval adds cattle as an additional species for which KETOFEN® is approved. KETOFEN® is already approved for the alleviation of inflammation and pain associated with musculoskeletal disorders in the horse.

Ketoprofen is in the propionic acid class of non-narcotic, nonsteroidal anti-inflammatory drugs (NSAIDs) and has characteristic antipyretic activity. Like many NSAIDs, ketoprofen works by inhibiting the enzyme cyclooxygenase, which in turn leads to decreased synthesis of prostaglandins. Prostaglandins are present throughout the body and contribute to signs of inflammation, such as fever. Prostaglandins have other important functions, such as protecting the lining of the gastrointestinal tract, helping to maintain blood flow to the kidneys, and supporting platelet function.

Proprietary Name	Established Name	Dosage Form	Application Type and Number	Sponsor
KETOFEN®	ketoprofen	Injectable solution	New Animal Drug Application (NADA) 140-269	Zoetis Inc.

Safety and Effectiveness

The sponsor conducted a multi-site, clinical field study to show that KETOFEN® controls pyrexia associated with BRD. The study included crossbred beef calves sourced from multiple livestock auctions that were at high risk for developing BRD. Cattle were enrolled if they had clinical signs of BRD, including defined levels of depression and respiratory distress as well as a fever of at least 104.5 °F. Cattle in the treated group were administered a single dose of 3 mg/kg body weight (BW) ketoprofen subcutaneously. Cattle in the control group were administered a single dose of sterile saline subcutaneously. An animal was considered a treatment success if its rectal temperature was at least 2 °F lower 6 hours after treatment compared to its rectal temperature at enrollment. Compared to the control group, significantly more cattle in the treated group were treatment successes for the control of pyrexia. No adverse reactions related to KETOFEN® were seen in the study.

The sponsor conducted a safety study in young, healthy male and female beef calves. The calves were dosed subcutaneously at 0X, 1X, 3X, or 5X the recommended dose of KETOFEN® once daily for 9 consecutive days. Overall, KETOFEN® was well-tolerated by cattle in all treatment groups. The drug caused mild, regenerative changes to the kidneys that were considered reversible and not clinically significant. The drug also caused abomasal ulceration, which was generally mild except for in one calf in the 5X treatment group that, at necropsy, was found to have severe peritonitis caused by a chronic abomasal ulcer. These adverse effects on the kidneys and gastrointestinal tract were expected based on the drug's mechanism of action, and the safety profile was considered acceptable. The label instructs veterinarians to stop giving KETOFEN® if fecal blood is observed and to not use the drug in cattle that are dehydrated or with known kidney disease.

The sponsor conducted an injection site safety study in growing beef calves that showed KETOFEN® caused swelling at the site of injection that resolved by 28 days after treatment. These reactions may result in trim loss of edible tissue at slaughter.

The sponsor did not conduct safety studies in pregnant or lactating cattle, in cattle of reproductive age intended for breeding, or in calves less than 2 months of age. Therefore, the label states that KETOFEN® is not for use in reproducing animals over one year of age or for calves less than 2 months of age.

Human Food Safety

FDA conducted a human food safety assessment to ensure that any residues of ketoprofen in the edible tissues of treated animals are at a concentration that present a reasonable certainty of no harm to people when KETOFEN® is used according to the label. The human food safety evaluation is conducted from the perspectives of toxicology, residue chemistry, and microbial food safety.

FDA determined the sponsor did not need to develop or submit for review additional data to address the use of ketoprofen in cattle on microbial food safety (antimicrobial resistance).

After review of pivotal toxicology data covering systemic toxicity, developmental and reproductive toxicity, genotoxicity, carcinogenicity, and pharmacokinetics, FDA established the toxicological acceptable daily intake (ADI) and the acute reference dose (ARfD) for ketoprofen as 5 µg/kg body weight/day and 20 µg/kg body weight, respectively. Because ketoprofen is not an antibacterial drug and has no or negligible antibacterial activity, a microbiological ADI was not needed. The toxicological ADI (5 µg/kg body weight/day) is established as the final ADI for total residue of ketoprofen.

The sponsor conducted 3 metabolism studies and 1 residue depletion study to assess the quantity and nature of the residues in tissues derived from cattle treated with ketoprofen. FDA determined that ketoprofen is the marker residue and kidney is the target tissue. FDA used the information from these studies, in combination with the ADI and safe concentration, to establish a tolerance of 360 µg/kg or 0.36 ppm of ketoprofen in cattle kidney and a tissue withdrawal period of 48 hours. FDA evaluated the validated analytical method and found its use acceptable.

KETOFEN® is not for use in lactating cattle or calves less than 2 months of age; therefore, a milk discard time was not established and a tissue withdrawal period was not established in pre-ruminating calves.

Conclusions

Based on the data submitted by the sponsor for the approval of KETOFEN®, FDA determined that the drug is safe and effective when used according to the label.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 140-269

B. Sponsor

Zoetis Inc.
333 Portage St.
Kalamazoo, MI 49007

Drug Labeler Code: 054771

C. Proprietary Name

KETOFEN®

D. Drug Product Established Name

ketoprofen

E. Pharmacological Category

Non-steroidal anti-inflammatory

F. Dosage Form

Injectable solution

G. Amount of Active Ingredient

100 mg/mL

H. How Supplied

50 mL and 100 mL multiple dose bottles

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

The recommended dosage is 3 mg/kg (1 mL/33.3 kg) or 1.36 mg/lb (1 mL/74 lb) of body weight. Treatment is administered by subcutaneous injection once daily and may be repeated for up to three days if pyrexia persists.

K. Route of Administration

Subcutaneous

L. Species/Class

Cattle: beef heifers, beef steers, beef calves two months of age and older, beef bulls, replacement dairy heifers, and dairy bulls. Not for use in reproducing

animals over one year of age, dairy calves, or veal calves. Not for use in lactating dairy cattle or calves less than two months of age.

M. Indication

KETOFEN® is indicated for the control of pyrexia associated with bovine respiratory disease (BRD) in beef heifers, beef steers, beef calves two months of age and older, beef bulls, replacement dairy heifers, and dairy bulls. Not for use in reproducing animals over one year of age, dairy calves, or veal calves. Not for use in lactating dairy cattle or calves less than two months of age.

N. Effect of Supplement

This supplement provides for the addition of a new target animal and indication to the label.

II. EFFECTIVENESS

A. Dosage Characterization

1. Lipopolysaccharide (LPS) Challenge Model

The dose of ketoprofen for control of pyrexia in calves was first evaluated in a LPS challenge model study.

Sixty-four healthy, Holstein steers (approximately 100 kg body weight) were challenged subcutaneously with 5.0 µg/kg LPS. Immediately after the LPS challenge, all animals were randomized to treatment groups and were treated with their allotted treatment (sterile saline; 1 mg/kg ketoprofen; 3 mg/kg ketoprofen; 6 mg/kg ketoprofen). Rectal temperatures were measured and recorded at time 0 (prior to LPS injection) then at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours post challenge.

Plasma samples were collected from 24 randomly selected calves at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 hours post challenge, and were analyzed for ketoprofen using a validated UPLC-MS/MS assay. Estimates of maximum concentration (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration curve to the last time point of quantifiable drug concentration (AUC_{last}), and half-life ($t_{½}$) were made for each animal using a non-compartmental pharmacokinetic analysis. The C_{max} and AUC_{last} were tested for dose proportionality, and the AUC_{last} was found to be dose proportional over a range of 1-6 mg/kg administered subcutaneously.

Relative to saline treated controls, treatment with 1 mg ketoprofen/kg significantly reduced rectal temperatures at 1 hour through 6 hours post dosing. Treatment with 3 mg ketoprofen/kg significantly reduced rectal temperatures compared with saline treated controls at 1 hour through 8 hours post dosing and 12 hours post dosing. Treatment with 6 mg ketoprofen/kg had significantly reduced temperatures at 1 hour post dosing through 12 hours post dosing compared to the saline treated controls.

Under the conditions of this LPS challenge model study, doses of 1, 3, and 6 mg/kg of ketoprofen were efficacious for control of pyrexia in calves. The 3 mg/kg dose was selected due to the variability associated with the 1 mg/kg

dose and similar efficacy in reducing pyrexia compared with the 6 mg/kg dose.

2. Pilot Field Effectiveness

   A single-site pilot study was conducted to evaluate efficacy of 3 mg/kg of ketoprofen for control of pyrexia associated with naturally occurring bovine respiratory disease (BRD). Sixty crossbred, beef calves (133 to 221 kg in weight) were randomized to treatment (sterile saline; vs. 3 mg/kg ketoprofen).

   Enrollment criteria included a rectal temperature ≥104.5 °F and a Respiratory and Attitude Clinical Score of 1 or 2. At 4, 6, and 8 hours post-treatment, rectal temperatures and clinical scores for all calves were recorded. The percent of animals with at least a 2 degree decrease in rectal temperature from baseline was significantly lower ($P<0.0001$) for 3 mg/kg ketoprofen vs saline [13.3% in saline and 96.7% in ketoprofen treated animals at 4 hours, 13.3% in saline and 100% in ketoprofen treated animals at 6 hours and 6.7% in saline and 100% in ketoprofen treated animals 8 hours post-dosing]. Mean rectal temperatures of ketoprofen treated animals were significantly lower than saline animals at 4, 6, and 8 hours post- dosing ($P<0.0001$).

   This study demonstrated the effectiveness of 3 mg/kg of ketoprofen for control of pyrexia associated with BRD.

   Based on the results of these studies, the dose of 3 mg/kg of ketoprofen was selected for use in the clinical field study conducted to provide substantial evidence of effectiveness.

B. Substantial Evidence

1. Type of Study: Multi-site Bovine Respiratory Disease Clinical Field Study in Feedlot Cattle

   Title: Efficacy of Ketoprofen 100 mg/mL Sterile Injectable Solution for Control of Pyrexia Associated with Naturally Occurring Bovine Respiratory Disease (Study Number A131C-US-15-343)

   Study Dates: December 1, 2015, through December 19, 2015

   Study Locations: Oakland, NE and Parma, ID

   Study Design:

   Objective: The objective of this study was to demonstrate field effectiveness of ketoprofen injectable solution (100 mg/mL) for control of pyrexia associated with naturally occurring bovine respiratory disease (BRD).

   Study Animals: 202 crossbred beef, mixed sex calves, 136.5 to 347.5 kg in weight, at high risk for developing BRD, were sourced from multiple livestock auctions in Kentucky, Tennessee, Washington, and Idaho.

   Experimental Design: Approximately 25 animals were enrolled per treatment per site. A total of 101 animals were enrolled per treatment group across four

sites. Within each site, animals were allocated to pens and treatments according to a generalized randomized block design. Animals were randomly assigned to pens such that there were three to five consecutive blocks of animals (6-10 animals) housed in each pen. Treatment groups evaluated were a saline group (0.028 mL/kg, T01) or a ketoprofen group (3 mg/kg, T02) at each study site. The study was conducted according to Good Clinical Practices (GCP) guidelines.

Drug Administration: The test article was KETOFEN® (ketoprofen) Injectable Solution,100 mg/mL given by a subcutaneous injection at a dose of 3 mg ketoprofen/kg body weight (BW). The negative control was 0.9% sterile saline given by a subcutaneous injection at a dose of 0.028 mL/kg BW.

Measurements and Observations: Enrollment criteria were a rectal temperature ≥104.5 °F, an attitude score of ≥2 (see Table II.1 for attitude scoring chart) and a respiratory score of ≥2 (see Table II.2 for respiratory scoring chart). Animals were dosed by body weight. Six hours post-treatment, the animals were clinically scored and had their rectal temperatures measured.

Table II.1 Attitude Clinical Scoring Chart

Clinical Score	Attitude
0	Normal - bright, alert, responsive.
1	Mildly Depressed: May stand isolated with head down, ears drooping, but responsive to stimulation.
2	Moderately Depressed: May remain recumbent or stand isolated with head down, may show signs of muscle weakness (standing cross-legged, knuckling or swaying when walking), depression obvious when stimulated.
3	Severely Depressed: May be recumbent and reluctant to rise, or if standing, is isolated and reluctant to move; when moving, ataxia, knuckling or swaying evident; head carried low with ears drooping; eyes dull, possible excess salivation/lacrimation, obvious gauntness.
4	Moribund: Unable to stand; approaching death; highly unlikely to respond to any antimicrobial therapy.

Table II.2 Respiratory Clinical Scoring Chart

Clinical Score	Respiration
0	Normal: no abnormal respiratory symptoms. Rate and effort are appropriate for the environment.
1	Mild respiratory distress: Serous nasal discharge or ocular discharge and/or cough.

Clinical Score	Respiration
2	Moderate Respiratory Distress: Mucous or mucopurulent nasal or ocular discharge and/or an increase in respiratory rate or effort.
3	Severe Respiratory Distress: Marked increase in respiratory rate or effort, with one or more of the following: open-mouth breathing; abdominal breathing, and/or extended head.

Statistical Analysis: Treatment with KETOFEN® (ketoprofen) Injectable Solution was deemed successful if there was a statistically significantly greater proportion of animals with a ≥2 °F reduction in rectal temperature in the treated group compared to the control group.

For each animal, it was determined if it has at least a 2 °F reduction in rectal temperature at 6 hours post-treatment compared to baseline (1=yes, 0=no). This variable was analyzed using a generalized linear mixed model (SAS Proc GLIMMIX) with binomial error distribution and logit link function. The model included fixed effect of treatment and random effects of site, treatment by site interaction and pen within site.

Back-transformed least squares means, 95% confidence intervals, and number of animals with data were reported for each treatment group. Comparisons were conducted after requiring a significant (P≤0.05) treatment effect.

Additionally, rectal temperature was analyzed using a general linear mixed model (SAS Proc MIXED). The model included fixed effects of treatment. Random effects included site, treatment by site interaction, and pen within site. The baseline temperature measurement was included as a covariate in the model.

Comparisons were made between treatments after testing for a significant (P≤0.05) treatment effect. Least squares means, 95% confidence intervals, ranges, and the number of animals with data were reported for each treatment.

Results:

Fever Reduction: The percent of animals with a ≥2 degree decrease in rectal temperature at 6 hours post-treatment was significantly different and higher (P=0.0215) in the ketoprofen treated group (74.3%, T02) vs. the saline treated group (5.9%, T01).

Rectal Temperature: Least squares mean rectal temperature at 6 hours post-treatment was significantly different and lower (P=0.0075) in the ketoprofen treated group (102.8 °F, T02) vs. the saline treated group (104.8 °F, T01).

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusions: This study demonstrates that KETOFEN® (ketoprofen) Injectable Solution, 100 mg/mL, administered as a single subcutaneous injection of 3.0 mg/kg BW, was effective for control of pyrexia associated with bovine respiratory disease (BRD) in beef heifers, beef bulls, beef steers, dairy replacement heifers, and dairy bulls. Not for use in reproducing animals over one year of age, dairy calves, or veal calves. Not for use in lactating dairy cattle or calves less than two months of age.

III. TARGET ANIMAL SAFETY

A. Type of Study: Injection Site Irritation Study

Title: Injection Site Tolerance of Ketoprofen Administered Subcutaneously to Beef Cattle (Study No. A335N-US-15-357)

Study Dates: July 2015 to January 2017

Study Location: Parma, ID

Study Design:

Objective: The objective of this study was to demonstrate the injection site tolerance of ketoprofen when injected subcutaneously (SC) to beef cattle at a dose of 3 mg/kg body weight (BW), three times at 24-hour intervals.

Study Animals: Sixteen growing beef cattle (eight heifers and eight steers), from 381 to 447 kg BW

Experimental Design: Sixteen growing beef cattle were injected with either saline or ketoprofen. Eight steers were housed in one pen and eight heifers were in the other pen. Within each sex, animals were randomly assigned to treatment group. Saline was injected at 1 mL/33 kg BW and ketoprofen was injected at 3 mg/kg BW, each for three consecutive days. Each animal received injections at three different sites. On Days 7, 14, 28, and 42 one heifer and one steer were randomly selected from each treatment group for euthanasia and necropsy. Study personnel making subjective evaluations were masked to treatment allocation. The gross pathologist was also masked during necropsy and gross pathological evaluation. The histopathologist was unmasked during the histopathological evaluation. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR Part 58).

Drug Administration: Both saline and ketoprofen were administered as a subcutaneous injection in the neck. Saline was injected at 1 mL/33 kg BW and ketoprofen was injected at 3 mg/kg BW, each for 3 consecutive days. The first dose was given on the left cranial neck, the second dose was administered in the right cranial neck, and the third dose was administered in the left caudal neck.

Measurements and Observations: Veterinarians conducted clinical observations on Days -13, -7, -1, 0, 1, 2, 7, 14, 28, and 42. The general health of each calf was evaluated at least once daily throughout the study. Comprehensive injection site evaluations [redness, heat, sensitivity, hardness, and volume of swelling (calculated)] were conducted on all animals on Days -13, -7, -1, 0 to 5, 7, 14,

21, 28, 35, and 42. On days of treatment administration (Days 0, 1, 2), injection site observations occurred before injections were given. Body weights were recorded on days -13, -7, -1, and 7, 14, 28, and 42. On Days 7, 14, 28, and 42 one heifer and one steer were randomly selected from each treatment group for euthanasia and necropsy. At necropsy, gross pathology and histopathology were evaluated. In addition, lesion volumes were calculated.

Statistical Methods: General health observations and clinical observations were summarized by treatment and time point. The number and percent of animals that were normal were summarized by treatment group and time point.

For injection site observations, frequency distributions were used to summarize the categorical variables: redness, heat, sensitivity, and hardness by injection site, time point, and treatment group. Data for evaluation of skin appearance were summarized by listing descriptors for each injection site (normal, necrosis, drainage, scaling, erosion) for each animal at each time point.

The following formula was used to calculate the swelling volume for each animal at each injection site and time point that observations were collected and for the volume of grossly altered tissue at necropsy, according to the following formula:

$$\text{Volume} = \frac{\pi \text{ x length x width x depth}}{6}$$

Swelling volume was summarized with summary statistics (mean, standard deviation, minimum, and maximum) by injection site, time point, and treatment group.

The volume of grossly altered tissue was summarized using summary statistics (mean, standard deviation, minimum, and maximum) by injection site, time point, and treatment group.

Results:

Administration of ketoprofen had no negative effects on general animal health.

Injection Site Observations:

No redness or sensitivity was observed in any animal during the study.

Heat at the injection site was only diagnosed twice during the study. On Day 1 a heifer in the saline treatment group and a heifer in the ketoprofen treatment group were both diagnosed with heat at the left cranial injection site.

Hardness

Palpation of hardness at all three injection sites post-treatment was a common finding, in both treatment groups, during the study.

Table III.1: Number of animals from each treatment group with hardness at the respective injection sites

Study Day (Total Number of Animals Eligible)	Saline Treated Left Cranial Injection Site	Ketoprofen Treated Left Cranial Injection Site	Saline Treated Right Cranial Injection Site	Ketoprofen Treated Right Cranial Injection Site	Saline Treated Left Caudal Injection Site	Ketoprofen Treated Left Caudal Injection Site
1 (8)	5	5	N/A	N/A	N/A	N/A
2 (8)	2	1	1	3	N/A	N/A
3 (8)	2	3	0	3	2	0
4 (8)	2	5	0	5	0	1
5 (8)	0	5	0	5	0	3
7 (8)	0	4	0	6	0	3
14 (6)	0	4	0	5	0	4
21 (4)	0	3	0	3	1	3
28 (4)	0	3	0	2	1	2
35 (2)	0	2	0	1	0	2
42 (2)	0	1	0	0	0	0

In summary, some saline-treated animals did have injection site hardness at all three injection sites within one-day post-injection. However, the frequency quickly decreased with hardness observed in only one injection site after Day 4. While the initial rates of injection firmness in the ketoprofen-treated animals were similar to saline-treated animals, hardness persisted in the ketoprofen-treated animals, with one site still firm on Day 42. The extended injection site firmness in the ketoprofen-treated animals was correlated with observed injection site swelling as described below.

Swelling Volume

Injection site swelling was observed at all three injection sites. All injection site swellings were approximate volumes, based on measurements obtained from the cattle while restrained in a cattle chute.

There were only two injection site swellings in the saline-treated group, with both occurring in the same animal on Day 3. Both reactions were small with a volume of 0.07 cm^3 and the swelling was resolved by the next day.

Injection site swelling in the ketoprofen-treated animals was common and typically began 1 to 3 days post-treatment. The reactions peaked between Days 5 and 21, with all swelling resolved by Day 28. Swelling volumes ranged from 1.31 cm^3 to 40.48 cm^3.

Table III.2: Number of animals from each treatment group with swelling at the respective injection sites

Study Day (Total Number of Animals Eligible)	Saline Treated Left Cranial Injection Site	Ketoprofen Treated Left Cranial Injection Site	Saline Treated Right Cranial Injection Site	Ketoprofen Treated Right Cranial Injection Site	Saline Treated Left Caudal Injection Site	Ketoprofen Treated Left Caudal Injection Site
1 (8)	0	1	N/A	N/A	N/A	N/A
2 (8)	0	1	0	1	N/A	N/A
3 (8)	1	1	1	1	0	0
4 (8)	0	1	0	1	0	0
5 (8)	0	3	0	2	0	2
7 (8)	0	3	0	3	0	3
14 (6)	0	3	0	5	0	2
21 (4)	0	1	0	2	0	1
28 (4)	0	0	0	0	0	0
35 (2)	0	0	0	0	0	0
42 (2)	0	0	0	0	0	0

Gross Pathology Findings:

Volume of Altered Injection Site Tissue

On the scheduled necropsy Days 7, 14, 28, and 42, none of the saline -treated animals had any altered injection site tissues and hence all volumes were zero.

All ketoprofen-treated animals had a clinically significant amount of altered tissue at all three injection sites on Days 7 and 14 of necropsy. However, by Day 28, only one of the six injection sites (2 animals each with 3 injection sites) had altered tissue and by Day 42, two of six injection sites had altered tissue.

Table III.3: Altered Tissue Volume for Ketoprofen-Treated Cattle at Necropsy

Necropsy Day	Gender	ID	Left Cranial Injection Site (cm^3)	Right Cranial Injection Site (cm^3)	Left Caudal Injection Site (cm^3)	Average of 3 sites (cm^3)
7	Heifer	275	9.90	7.85	34.56	17.4
7	Steer	17	62.83	15.71	12.96	30.5
14	Heifer	257	1.60	18.33	43.20	21.0
14	Steer	7	5.24	36.65	19.63	20.5
28	Heifer	242	0.00	0.00	0.00	0.0
28	Steer	13	0.00	3.21	0.00	1.1
42	Heifer	287	0.63	0.27	0.00	0.3
42	Steer	15	0.00	0.00	0.00	0.0

The color and location of the altered tissue was also described. None of the saline-treated animals had tissue discoloration at necropsy. For the ketoprofen-

treated animals, all 12 injection sites evaluated on Days 7 and 14 had tissue discoloration and involved the subcutaneous tissues. On Day 7, all six injection sites also involved the skin and three injection site lesions involved the extraneous muscle. On Day 14, five of the six injection sites involved the extraneous muscle; however, none of the injection site lesions involved the skin. On Day 28, only one of six injection sites had discoloration. The discoloration only involved the subcutaneous tissue. On Day 42, only two injection sites from one animal had discoloration. The discoloration only involved the subcutaneous tissues.

Histopathological Findings

There were a few microscopic tissue changes in the saline-treated animals, described as low grade severity and of a nature inconsistent with saline injection. The observed changes were attributed to repeated handling through the chute and associated tissue trauma. The lack of gross necropsy changes in the saline-treated animals matched the microscopic findings.

Numerous findings were documented in the ketoprofen-treated animals. On Day 7, four out of six injection sites from the ketoprofen-treated animals had microscopic evidence of fibrosis, hemorrhage, fibrin accumulation, necrosis of adipose tissue, and mixed cell inflammation.

Edema and thrombosis of minimal to moderate severity were also found in three out of 6 injection sites on Day 7. The mixed cell inflammation was mainly observed on Day 7 and was characterized by macrophages and occasional lymphocytes, plasma cells, and/or neutrophils. On Day 7, the right cranial injection site from one steer was documented with skin ulceration due to thrombosis of a medium-sized artery in the dermis.

On Day 14, six out of six ketoprofen-injection sites were characterized by the mild to moderate accumulation of fibrin and mild to marked fibrosis. Also, four out of six injection sites on the same day had hemorrhage, necrosis of adipose tissue, and granulomatous inflammation that varied from minimal to moderate severity. The mild to marked fibrosis was correlated with tissue repair following injury. The mild to moderate fibrin accumulation was correlated with either hemorrhage or exudation. The minimal to moderate granulomatous inflammation was associated with injured adipose tissue.

On Day 28, microscopic changes were fibrosis and minimal to mild granulomatous inflammation. On Day 42, microscopic changes were minimal to mild fibrosis.

Conclusions:

Injection of ketoprofen had no negative effects on general animal health. Administration of ketoprofen resulted in injection site reactions which resolved clinically by Day 28 post-injection. Injection site lesions after ketoprofen injection were evident grossly on necropsy evaluation, with discoloration involving the skin and subcutaneous tissue on Day 7. By Day 42, discoloration was limited to subcutaneous tissue. Microscopically, evidence of fibrosis, hemorrhage, fibrin accumulation, necrosis of adipose tissue, and mixed cell inflammation was

present on Day 7. By Day 42, microscopic changes were minimal to mild fibrosis. Subcutaneous injection of ketoprofen can cause a transient local tissue reaction. These reactions may result in trim loss of edible tissue at slaughter.

B. Margin of Safety Study

Title: Safety of Ketoprofen in Cattle (Study Number A332N-US-15-361)

Study Dates: January 2016 to January 2017

Study Location: Parma, ID

Study Design:

Objective: The objective of this study was to characterize the safety of ketoprofen when administered subcutaneously to cattle up to 5 times the maximum recommended dose at 24-hour intervals for nine consecutive days.

Study Animals: 32 growing beef cattle (16 male, 16 female), 5-7 months of age, 4 animals per sex per treatment group, and averaging 268.3 kg (females) and 269.7 kg (males) body weight

Experimental Design: Animals were randomly allocated to one of four treatment groups of eight animals per group (4 per sex). Blocking was based on pre-treatment body weight and pen location with the individual animal as the experimental unit for treatment. Calves were administered ketoprofen or saline, once daily for nine days. Animals were housed individually. Study personnel making subjective evaluations, including clinical observations, daily general health observations, physical examinations, clinical pathology analysis, and fecal occult blood analysis were masked to treatment. The pathologist was also masked during necropsy and gross pathological evaluation, but was unmasked for histopathological evaluation. Individuals involved in animal dosing were unmasked and therefore did not assist in other study activities. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR Part 58).

Drug Administration: Animals were injected with saline or ketoprofen via subcutaneous injection in the neck according to the table below. Each animal received injections at nine different sites.

Table III.4: Summary of Treatment Groups

Group	Treatment	Dosing Regimen	Number of Animals
T01 (0X)	Saline (0.03 mL/kg BW)	9 subcutaneous injections (24 hours apart in the neck)	8 (4M/4F)
T02 (1X)	3 mg/kg ketoprofen (0.03 mL/kg BW)	9 subcutaneous injections (24 hours apart in the neck)	8 (4M/4F)
T03 (3x)	9 mg/kg ketoprofen (0.09 mL/kg BW)	9 subcutaneous injections (24 hours apart in the neck)	8 (4M/4F)
T04 (5X)	15 mg/kg ketoprofen (0.15 mL/kg BW)	9 subcutaneous injections (24 hours apart in the neck)	8 (4M/4F)

Measurements and Observations: General Health Observations were conducted at least once daily from Day -14 though the last day of the in-life phase. A veterinarian conducted clinical observations and/or physical examinations including injection site observations at least once on Day -14, and once daily from Day -1 through the last day of the in-life phase. Blood was collected for clinical pathology evaluation (hematology, coagulation, and serum chemistry) and urine was collected for urinalysis on Days -14, 0, 3, 7, and 10 or 11. Feces were collected from each animal for occult blood testing on Days -14 (+/- 2 days) and 0 through 10 or 11. Blood was collected for plasma ketoprofen levels from all animals prior to the first dose on Day 0, 45 minutes post-treatment, and 6 hours post-treatment; on Day 1, 24 hours after Day 0 dosing, just prior to dosing; on Day 3, 24 hours after Day 2 dosing, just prior to dosing; on Day 5, 24 hours after Day 4 dosing, just prior to dosing; on Day 7, 24 hours after Day 6 dosing, just prior to dosing; on Day 8, 24 hours after Day 7 dosing, 45 minutes post-treatment, 6 hours post-treatment; and on Day 9, 24 hours after Day 8 dosing. Individual body weights were recorded on Day -14, Day 0, and on the last day of the in-life phase. Individual food and water consumption was recorded daily, beginning 14 days prior to dosing. On Days 10 or 11, at 24 to 48 hours after the last dose, animals were euthanized for gross pathology evaluation and sampling of selected tissues for histopathological evaluation. The selected tissues included esophagus, trachea, pituitary gland, spleen, brain (cerebrum, midbrain, cerebellum and brainstem), adrenal gland (2), bone and marrow (from a rib), lung (2 section: 1 peripheral and 1 with bronchus), thyroid gland (1 lobe) with parathyroid, rumen, reticulum, omasum, abomasum, spinal cord (cervical, thoracic and lumbar), pancreas, bone marrow smear, skeletal muscle (gluteal), eyes with optic nerves (2), heart (left ventricle, right ventricle, interventricular septum, right atrium, and left atrium), kidney (2), duodenum, liver (2 sections: one from the left lobe and one from the right lobe), ileum, gallbladder, jejunum, lymph node (mesenteric), cecum, urinary bladder, colon, testis, epididymis, prostate, seminal vesicle, lymph node (prescapular), uterus, salivary gland (submandibular), thymus, skin (from the area over the semi-tendinosis), and ovary.

Statistical Methods: The experimental unit was the individual animal.

A two-sided 0.10 alpha level was used for all comparisons unless otherwise specified. Data and observations from animals removed from the study after Day 0 were included in the statistical analysis.

Numerical clinical pathology data were analyzed using a general linear mixed model for repeated measures. The model included fixed effects of treatment, time, sex, and all interactions among these effects. Random effects included block within sex, treatment by block within sex interaction (the animal term), and error. Where appropriate, a baseline covariate was included in the model.

Body weights measured on Day 10 or 11 were analyzed by using a general linear mixed model. The model included fixed effects of treatment, sex, and treatment by sex interaction. Random effects included block within sex and error. The baseline body weight measured on Day 0 was included as a covariate in the model.

Selection of correlation structure for repeated measures models described above was based on the smallest Corrected Akaike Information Criterion (AICC) among the following structures: Compound Symmetry (CS), First-Order Autoregressive AR(1), Heterogeneous Compound Symmetry (CSH), Heterogeneous First-Order Autoregressive (ARH(1)), and Unstructured Covariance (UN). If time points are unequally spaced Spatial Power (SP(POW)) were tested rather than AR(1) and ARH(1).

Results:

General Health Observations, Physical Examinations, and Clinical Observations

There were no mortalities and no animals were removed from the study post-treatment. Only one animal developed clinical signs that were considered treatment related. On Day 8, one male in the 5X dose group developed clinical signs of diarrhea, inappetence, and fever (104.6 °F). The clinical signs continued until scheduled necropsy and by Day 10 the animal was depressed and had frank blood in the feces. At necropsy the animal was found to have a severe peritonitis caused by a chronic abomasal ulcer. Due to the peritonitis multiple clinical pathology analytes were abnormal.

Other infrequent study findings of abnormal general health observations, clinical observations, and physical examination parameters were considered non-treatment related.

Body Weights, Feed Consumption, and Water Consumption

There were no statistically significant test article related effects on body weight, feed consumption, or water consumption.

Injection Site Evaluations

Multiple ketoprofen-treated animals had swelling at the injection site. The severity of the injection site reactions tended to correlate with the amount of test article administered, with the high dose group (5X) having the largest number of animals with an injection site reaction (See Table Below).

Three animals had heat at the injection site, one animal in the 1X treatment group on Day 5, one animal in the 3X treatment group on Day 10, and one animal in the 5X treatment group on Day 10.

Table III.5: Animals with injection site swelling by treatment group:

Treatment Group	**Number of animals with injection site swelling at least one time post-treatment**
T01 (0X)	0
T02 (1X)	4
T03 (3X)	5
T04 (5X)	6

Hematology, Coagulation, and Clinical Chemistry

Clinically relevant treatment related abnormalities were found for the following parameters:

Albumin

Albumin was significantly lower in the 5X treatment group when compared to the controls (P=0.0014). However, upon reviewing the individual animal plots for the 5X treatment group, if the animal with peritonitis is excluded, there is no clinically relevant difference between treatment groups.

Total Protein

A review of the individual animal and treatment group plots found only one value outside the reference range of 5.6-7.8 g/dL occurring after Day 0, this being the one animal with peritonitis in the 5X group.

Urinalysis

There were no test article related changes in the urinalysis results.

Fecal Occult Blood Test

Two males in the 3X treatment group were the only positive samples. One animal was positive on Days 4 through 7 and the other was positive only on Day 6. All 5X and 1X dose group animals were negative including the 5X animal diagnosed with peritonitis at necropsy. Both males with positive fecal occult blood results had abnormal abomasal mucosa at necropsy and one was confirmed to have a focal chronic moderate abomasal ulcer by microscopic examination. However, additional animals with abomasal ulcers at necropsy were negative for fecal occult blood and the severity of ulcers did not correlate to a positive fecal occult blood test.

Gross Pathology and Histopathology Examination

Injection Site Reactions

Eight injection sites were grossly abnormal, one animal in the 1X dose group, two animals in the 3X dose group, and five animals in the 5X dose group. On histopathology, the abnormal injection sites contained edema, fibrin accumulation, mixed inflammation, hemorrhage, adipose tissue necrosis, degeneration and regeneration of skeletal muscle, fibrin thrombi in vessels, suppurative inflammation, and fibroplasia. The injection site from one animal in the 3X dose group, did have an area with bacterial colonies.

Abomasal Ulcerations and Erosions

Table III.6: Incidence of Gross and Microscopic Abomasal Lesions

Treatment Group	Gross finding- Abnormal pyloric mucosa	Microscopic Finding- Chronic focal abomasal ulcers	Microscopic Finding- Abomasal erosions
T01 (0X)	0	0	2
T02 (1X)	1	0	4
T03 (3X)	7	6	1
T04 (5X)	5	6[1]	1

[1] One animal had an abomasal ulcer that was histologically different from the other ulcers and not considered treatment related. This ulcer was caused by foreign material.

In the 3X and 5X groups, a common gross observation at necropsy and upon microscopic analysis was change in the pyloric mucosa of the abomasum that correlated with test article associated mucosal ulceration.

The microscopic features of the abomasal ulcers were described as loss of the gastric mucosa with mixed infiltrates of neutrophils, eosinophils, lymphocytes, and fibroblasts into the submucosa. Fibroplasia was common throughout the lesions and was indicative of ongoing repair and scarring. The ulcers were all moderate in severity and chronic.

In addition to test article related abomasal ulceration, a second common finding in the abomasum were areas of mucosal erosions. These erosions were considered not test article related as they occurred in all treatment groups including controls and were of minimal severity.

Renal Alterations

The most frequent gross renal lesion was pale multifocal discoloration of the renal cortex usually measuring 1 mm by 2 mm. These gross lesions usually correlated with areas of renal tubular degeneration and regeneration. The microscopic renal tubular degeneration and regeneration lesions were minimal to mild in severity, except the lesions in one animal in the 5X treatment group were classified as moderate. These lesions extended from the corticomedullary junction through the renal cortex. The epithelial tubular degeneration was characterized with one or more morphologic changes including flattening of the tubular epithelium,

cytoplasmic blebbing, individual necrotic cells, and the presence of sloughed epithelial cells in the tubular lumina.

Secondary renal findings were mononuclear inflammation in the interstitium and mononuclear cell inflammation in the peri-calyx region of the kidney. The most likely cause of chronic nephritis in calves is hematogenous bacterial infections and the most likely cause of mononuclear cell inflammation in the peri-calyx is urinary tract infections. These findings were most likely not test article related due to the incidence in the control group and the suspected causes (hematogenous bacterial infections and urinary tract infections). For the purposes of the table below, animals with either renal interstitial inflammation, peri-calyx inflammation, or both were included in the same column as animals that had non test article related renal lesions.

Table III.7: Incidence of Gross and Microscopic Renal Lesions

Treatment Group	Gross finding- Pale multifocal discoloration of the renal cortex	Microscopic Finding- Renal tubular degeneration and regeneration	Microscopic Finding- Either renal interstitial or peri-calyx inflammation
T01 (0X)	0	1	7
T02 (1X)	5	5	8
T03 (3X)	4	6	8
T04 (5X)	6	7	8

One control female had minimal multifocal renal tubular degeneration and regeneration that was similar to that seen in the test article dose groups. Even though one control animal had tubular dilation, degeneration, and regeneration; the tubular dilation, degeneration, and regeneration in the treated groups was considered test article related due to the increased incidence in the test article dose groups.

These microscopic changes are indicators of transient tubular insult with no evidence of widespread tubular necrosis. The test article related lesions did not increase in severity with increasing test article dose. The distribution and characteristics of the test article related findings are likely the result of test article induced hemodynamic changes leading to multifocal ischemia. The renal tubular lesions are regenerating and reversible because there is no necrosis and the epithelium basement membrane is intact. The minimal to mild severity of the lesions indicate that removal of test article would likely result in resolution of the renal lesions. Histologically there is no loss of nephrons.

Pharmacokinetics

Plasma samples collected on Days 1, 3, 5, 7, and 8, were analyzed for ketoprofen using a validated UPLC-MS/MS assay. Estimates of maximum concentration (C_{max}), time to C_{max} (T_{max}), half-life ($t_{1/2}$) and area under the plasma concentration curve following the ninth dose to the last time point of quantifiable drug concentration (AUC_{last}) were made for each animal using a non-compartmental pharmacokinetic analysis. The C_{max} and AUC_{last} were tested for dose proportionality. Both C_{max} and AUC_{last} were found to be dose proportional over a

range of 3-15 mg/kg SC. Drug accumulation was tested by comparing the AUC and C_{max} of the first and last doses for each treatment group. No substantial accumulation was observed based on AUC or C_{max}. However, the accumulation based on C_{max} was slightly greater in females than in males.

Conclusions:

The study supports an acceptable safety profile for the use of ketoprofen in beef heifers, beef steers, beef calves 2 months of age and older, beef bulls, replacement dairy heifers, and dairy bulls when used at the labeled dose and duration. At 1X the dose (3 mg/kg/day) for nine days, ketoprofen was well tolerated and caused minimal regenerative changes to the renal system. These changes are considered reversible and not clinically significant.

IV. HUMAN FOOD SAFETY

A. Microbial Food Safety

The Agency evaluated the need to address the impact of the use of ketoprofen on antimicrobial resistance among bacteria of public health concern in or on ketoprofen-treated cattle. After reviewing information (literature, data, etc.) both submitted by the sponsor and available in the public domain, the Agency determined:

- Ketoprofen is not regularly considered to have properties that would exert pressure towards the emergence or selection of resistant bacteria of public health concern in food-producing animals,
- Ketoprofen is not used to treat zoonotic gastroenteritis or other bacterial diseases in humans,
- Ketoprofen (or a similar class representative) is not under development to treat a bacterial disease in humans, and
- Ketoprofen is not indicated for a bacterial disease in a food-producing animal species.

Therefore, the Agency determined there was no need to provide additional microbial food safety (antimicrobial resistance) information or data regarding this approved use of ketoprofen in beef heifers, beef steers, beef calves 2 months of age and older, beef bulls, replacement dairy heifers, and dairy bulls.

B. Toxicology

1. Summary of Toxicology Studies

   Ketoprofen (also referred to as PF-0344989, or RP-19583, 19,583 R.P.) is a chiral molecule, existing in the R- and S-enantiomers. The toxicology studies on ketoprofen summarized below, except as noted, were based on racemic ketoprofen. Toxicity studies also were conducted on ketoprofen methyl ester (KME, also referred to as PF-06452795; a prodrug of ketoprofen) to support the toxicological evaluation of ketoprofen.

a. Subchronic Oral Toxicity Study in Rodents

Title: A 28-Day Oral Gavage Toxicity and Toxicokinetic Study of PF-06452795 and PF-0344989 in Wistar Han Rats

Study Number: 344103

Report Number: A291N-US-13-139

Report Date: May 2, 2016

Study Location: Ashland, OH

Study Design: This GLP study was to determine the potential toxicity and toxicokinetic profile of KME (purity 99.3%) when administered *via* oral daily gavage for 28 days to Wistar Han rats. Ketoprofen (purity 99.7%) was used for comparison. For the toxicity study, rats (5/sex/group) were orally administered by gavage at 2, 4, and 8 mg/kg BW/day of either ketoprofen or KME. For the toxicokinetic study, rats (3/sex for the control, and 6 rats/sex for the treated group) were administered by gavage at 2, 4, and 8 mg/kg BW/day of either ketoprofen or KME. The control group received the vehicle (carboxymethylcellulose and Tween® 80). Animals were euthanized after either 28 or 29 day of consecutive dosing.

For the toxicology assessment, animals were observed twice per day for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Body and food weights were recorded weekly. Functional observation battery and locomotor data were collected during week 3 of the study. All clinical pathology parameters were analyzed at necropsy. Complete necropsies were conducted on all animals and selected organs were weighed. Selected tissues were examined microscopically from the control and high dose groups. All animals had their stomach, kidney and gross lesions examined.

For the toxicokinetic evaluation, blood was collected from control animals at 2 hours after dose administration and from 3 animals/sex/group/time point in the test article groups prior to dose administration and at approximately 0.5, 1, 2, 6, and 24 hours after dose administration on days 0 and 27. All animals were euthanized following final blood collection (study day 28). Samples were analyzed for ketoprofen and a metabolite (ZTS-00102401).

Results and Conclusion: All animals survived until termination. Significant histopathology effects were seen at all doses, with more prominent effects at the highest doses. For either test article and at all doses, higher absolute and relative kidney weights was seen in all males and mean relative kidney weights in all females. At 8 mg/kg BW/day for both test articles, decreased food consumption and body weight gains were seen in females. Females at this dose exhibited lower absolute and relative heart weights when treated with ketoprofen relative to KME. There were ulcers in this dose in both groups as well. Similarly, some changes in hematological and clinical chemistry parameters related to inflammation were present in females at this dose. In males at this dose, more severe signs of inflammation from histopathology were present. Inflammation in the stomach and hypertrophy in the kidney occurred in a dose and severity dependent fashion in males across all doses. In females, inflammation in the stomach was observed starting at 4 mg/kg

BW/day in the KME article, and at 2 mg/kg BW/day in ketoprofen treated rats and proceeded in a dose and severity dependent fashion. Body weight was decreased in all dose groups in females in both test articles. This decrease in KME treated rats was dose dependent from 3.5% in the 2 mg/kg BW/day group to 5.3% in the 8 mg/kg BW/day group. In ketoprofen treated females, the decrease was not dose dependent in severity but was present across all doses. Valid toxicokinetic data were not gathered due to measurement errors.

A lowest-observed-effect level (LOEL)/lowest-observed-adverse-effect level (LOAEL) of 2 mg/kg BW/day for both test articles was identified based on the effects seen at the lowest dose tested. These effects included hypertrophy of the kidney beginning a dose dependent trend) and eosinophil inflammation (beginning a dose dependent trend) in the stomach in males, a reduction in body weight with a corresponding reduction in food consumption in females and increases in kidney weights (absolute and/or relative) in both males and females.

b. Subchronic Oral Toxicity Study in Non-Rodents

Title: PF-0344989: 13-Week Oral Gavage Toxicity and Toxicokinetic Study in Rats with a 4-Week Recovery Phase

Study Number: 8325787

Report Number: A291N-US-15-320

Report Date: June 27, 2017

Study Location: Madison, WI

Study Design: This GLP study was conducted according to the Organization for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals No. 408. The purpose was to evaluate the toxicity and determine the toxicokinetics of the test article, PF-0344989 (ketoprofen, purity 99.7%), when administered daily *via* oral gavage to rats for 13 weeks. Sprague Dawley rats (10/sex/group) were administered ketoprofen *via* oral gavage once daily at a dose level of 0, 0.05, 0.1, 0.5, or 1 mg/kg BW/day for 13 weeks. Additional animals (5 rats/sex/group) were dosed the vehicle control article (1.0% [w/v] medium viscosity carboxymethylcellulose and 0.5% [v/v] Tween® 80 in reverse osmosis water) or ketoprofen at 1 mg/kg BW/day for 13 weeks followed with a 4-week recovery period. For the toxicokinetic evaluation, another set of animals (6 rats/sex/ketoprofen treated group, 3 rats/sex/group for the control group) were administered ketoprofen at a dose level of 0, 0.05, 0.1, 0.5, or 1 mg/kg BW/day *via* oral gavage for 13 weeks.

For the toxicity and recovery study, individual animals were weighed weekly. Food consumption was measured periodically. All animals were observed twice a day for behavior, physical appearance, and survival. Ophthalmic examinations were conducted once during the predose, week 13, and during recovery phase. Hematology, coagulation, blood chemistry, urine analysis, and fecal analysis were performed on all animals at termination. At the end of the dosing period and the end of the recovery period, all surviving animals

were anesthetized, exsanguinated and subjected to histopathology evaluations. Organ weights, macroscopic and microscopic evaluations were conducted on all tissues and lesions.

For the toxicokinetic study, blood samples were collected at day 1, week 6 and week 13 at predose, and 1, 2, 6, and 24 hours postdose. The plasma concentrations of ketoprofen and its metabolite (ZTS-00102401) were measured.

Results and Conclusion: No significant treatment related differences were noted in survival rates, body weights, food consumptions, clinical signs, ophthalmic examination, hematology, coagulation, clinical chemistry, urine analysis, fecal evaluation, organ weights, and macroscopic examinations.

Incidences of erosion/ulceration on the glandular stomach and degeneration/necrosis of the renal papilla and renal tubules in the kidney were found at 0.5 mg/kg BW/day and above. These findings were resolved during the recovery period except that the degeneration/necrosis of renal papilla at 1 mg/kg BW/day did not fully resolve post-treatment. Toxicokinetic analysis indicated that increased AUC_{0-last} and C_{max} were in a dose-dependent manner within the dose range of treatment. Female rats were exposed to higher levels of ketoprofen than male rats with the difference increasing over time due to accumulation. T_{max} was generally reached at 1.0–2.0 hours on all sampled days at all doses. The metabolite of ketoprofen, ZTS-00102401, was found with low blood concentration, generally less than $1/20^{th}$ of the ketoprofen concentration.

The no-observed-effect level (NOEL)/ no-observed-adverse-effect level (NOAEL) was 0.1 mg/kg BW/day based on the erosion/ulceration in the glandular stomach and degeneration/necrosis of the renal papilla and renal tubules in the kidney observed at the next higher dose of 0.5 mg/kg BW/day at the end of the dosing period.

c. Chronic Oral Toxicity Study in Rodents

Title: 19,583 R.P. Dietary Administration to Rats for 78 Weeks

Study Number: 5049/72/484

Report Number: A291R-GB-14-203

Report Date: April 13, 1973

Study Location: Huntingdon, England

Study Design: This non-GLP study was to evaluate the chronic toxicity of 19,583 R.P. (ketoprofen) administered daily *via* diet to rats. Male and female SPF:CD rats (35 rats/sex/group for the control, low, and mid dose groups, and 50 rats/sex for the high dose group) were administered ketoprofen (purity unreported) *via* diet at a dose level of 0, 4.5 (low), 7.5 (mid), or 12.5 (high) mg/kg BW/day of ketoprofen for 78 weeks. Individual animals were weighed weekly. Food consumption was determined weekly by each cage of rats. All animals were observed for behavior, physical appearance, and survival. Ophthalmic examinations were conducted once during the predose,

and at weeks 4, 8, 13, 26, 52 and 77. Hematology was performed on blood samples taken from 10 animals/sex/group of the control and high dose groups at the end of weeks 4, 8, 13, 26, 52, and 78. Blood samples also were taken from 10 rats/sex/ group of the low and mid-dose groups at the end of weeks 13 and 53 for hematology, and from 10 males of mid-dose group and 10 females of low and mid-dose groups at the end of week 78 for measuring erythrocytic value only. Blood chemistry was performed on samples taken from the control and high dose groups at the end of weeks 4, 13, 26, 52, and 78. Urinalysis was performed on the control and high dose groups at the end of weeks 4, 8, 13, 26, 52, and 78. Fecal analysis was performed on 5 rats/sex/group of all groups at the end of weeks 2, 4, 8, 13, 26, 52, and 78, and 5 rats/sex of the control and high dose groups at the end of week 20. At the end of week 26, 10 rats/sex of each group were sacrificed for interim evaluation. At the end of week 78 (terminal sacrifice), all surviving animals were anesthetized, exsanguinated and subjected to histopathology evaluations.

Results and Conclusion: Twenty-nine (58%) males and 35 (70%) females of the high dose group died during treatment. Seven (29%) females of the mid dose group died between weeks 27 and 52. Between weeks 26 and 51, rats in the high dose group and a few rats in the mid dose group showed reduced grooming activity (percentage of the affected animals was unknown). No treatment related effects were noted for the eye examinations, body weight, food consumption, and clinical chemistry. Decreases in packed cell counts, hemoglobin, and red blood cell counts and increases in reticulocyte and neutrophil counts were noted in the high dose group. Blood was identified sporadically in the urine of the high dose group (4/5 males and 4/5 females). Two out of 5 rats in the mid and high dose groups, respectively, showed blood in feces sporadically during the 78 weeks. At both interim and final sacrifice, enlarged and irregular kidney cortex were observed in the low (1/20 males), mid (1/20 males), and high (6/11 males) dose groups but not the control group. In the high dose group, adhesions, pallor, and congestions of gastro-intestinal tract in (3 /11 males) were found; enlargement of mesenteric lymph nodes (3/5 females) were present. The weights of the kidney and spleen were increased at the high dose group, while only the kidney weights were increased at low (10%) and mid (20%) dose groups. At the terminal sacrifice, ulceration of the small intestine and severe renal damages was noted in both the mid (4/10 females) and high dose groups (1/11 males and 2/5 females). Severe renal damages (papillary necrosis, degenerated tubules in the renal papilla, and tubular and glomerular loss) were noted in the mid (4/20 males, 2/10 males) and high dose groups (7/11 males, 3/5 females). Degeneration of the renal papilla were identified in the low dose group (2/20 males).

The NOEL/NOAEL could not be established because of enlarged and irregular kidney cortex, kidney weight increases, and degeneration of the renal papilla found at the lowest dose tested (4.5 mg/kg BW/day); this lowest dose was considered the LOEL/LOAEL of this study.

d. Chronic Oral Toxicity Study in Non-Rodents

Title: PF-0344989: 13-Week Oral Gavage Toxicity and Toxicokinetic Study in Cynomolgus Monkeys

Study Number: 8338165

Report Number: A271N-US-16-112

Report Date: August 11, 2017

Study Location: Madison, WI

Study Design: The purpose of this GLP-compliant study was to evaluate the toxicity and determine the toxicokinetics of the test article, PF-0344989 (ketoprofen; purity 99.3%), when administered daily *via* oral gavage to cynomolgus monkeys for at least 13 weeks. Monkeys (4/sex/group) were orally dosed by gavage at 0, 3, 9 and 27 mg/kg BW/day for 13 weeks. Vehicle was 1.0% (w/v) medium viscosity carboxymethylcellulose and 0.5% (v/v) Tween® 80 in reverse osmosis water. Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, qualitative food consumption, ophthalmic observations, clinical chemistry, hematology tests, urinalysis, fecal occult blood evaluation, electrocardiographic (ECG) measurements, blood pressure measurements, organ weights, and clinical and anatomic pathology. Blood samples were collected for toxicokinetics, bioanalytical and chiral analysis.

Results and Conclusion: There were no compound-related effects on mortality, hematologic parameters, body weight, clinical chemistry, ophthalmic, blood pressure, ECG measurements, organ weights, fecal occult blood, or food consumption. Isolated increases in the incidence of vomiting and emesis, fibrinogen concentration, and chloride excretion were reported at 27 mg/kg BW/day, however, these effects were determined not to be of biological or toxicological importance. The NOEL/NOAEL for this study was 27 mg/kg BW/day, the highest dose tested, based on the lack of treatment related findings.

e. Oral Developmental Toxicity Study in Rodents

Title: An Oral (Gavage) Prenatal Developmental Toxicity Study of PF-0344989 in Rats

Study Number: 344130

Report Number: A291N-US-15-358

Report Date: February 2, 2017

Study Location: Ashland, OH

Study Design: This study was designed to evaluate the effects of ketoprofen in mated rats and their developing embryos or fetuses following its administration during gestation, and to identify NOEL/NOAEL. Five groups (Toxicity Study Groups) of mated Sprague Dawley rats (25 per treatment group) were administered ketoprofen (purity 99.7%) in vehicle suspension at 0, 0.3, 1, 4 or 12 mg/kg BW/day by daily gavage from gestation days (GDs) 6

to 19. A dose volume of 10 mL/kg BW was used and adjusted daily to the actual body weight. Control rats were administered the vehicle only (1.0% [w/w] carboxymethylcellulose and 0.5% [w/w] Tween® 80 in reverse osmosis-deionized water). Five toxicokinetic groups (TK Study Groups) of six mated females per treatment group were administered the test article from GDs 6 to 20, on a comparable basis.

Mortality, clinical signs, body weight and food consumption were evaluated daily during the dosing period from GDs 6 to 19; dams also were weighed on GDs 0, 4, and 20. Gross necropsy was performed on females that were found dead, euthanized in extremis, or terminated early. The number and location of implantation sites, corpora luteum, and viable fetuses were recorded. On GD 20, all female rats were humanely sacrificed. The thoracic, abdominal, and pelvic cavities were opened, and the contents were examined. The uterus and ovaries were excised and the number of corpora luteum was counted. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. The placentae also were examined. Uteri with no macroscopic evidence of implantation were opened and placed in solution for detection of early implantation loss. Maternal tissues were preserved for future histopathological examination.

Each viable fetus was removed, examined externally, sexed, weighed and euthanized. Each viable fetus was subjected to a visceral examination. Fetal kidneys were examined and graded for renal papillae development. The head from approximately one-half of the fetuses in each litter was placed in fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The head from the remaining one-half of the fetuses was examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Fetuses were then examined for skeletal malformations and developmental variations.

For toxicokinetics groups, blood was collected from the jugular vein (~0.5 mL each) on GD 19 from all animals at 0 (pre-dose) and approximately 0.5, 1, 2, 6, and 24 hours post-dosing. In addition, blood samples (~1.0 mL each) were collected from each toxicokinetic phase female *via* the vena cava, followed by euthanasia on GD 20, ~2 hours post-dosing. Blood samples were collected from each viable fetus *via* the umbilical veins and samples pooled by litter.

Results and Conclusion: On GD 20, ketoprofen (parent) and its metabolite ZTS-00102401 were detected in maternal and fetal blood plasma samples, confirming systemic exposure to both analytes. The AUC_{last} values indicated that exposure of maternal animals to the metabolite was <1% relative to the parent compound. In the fetus, the metabolite plasma concentrations represented <2% of parent concentrations. Systemic exposure to parent and metabolite, in terms of AUC_{last} and C_{max}, increased in a nearly dose-proportional manner as the dose of ketoprofen increased from 0.3 to 4 mg/kg BW/day. Plasma ketoprofen concentrations in maternal samples were generally 2-fold higher than in fetal samples. Plasma ZTS-00102401 concentrations in dams and fetuses were generally similar.

Maternal Outcomes: Two females died, and one was euthanized in extremis; the remaining 22 rats of the 12 mg/kg BW/day dose group were euthanized

on between GDs 6 to 10. At this dose, all females experienced body weight loss of 5.3% to 15.6% and reduced food consumption of zero to 18 g feed/day from GD 6 to death/euthanasia. The major clinical signs of toxicity were red material around the nose (porphyrin) in 8 females and one animal was observed as pale with cool body. At necropsy, the major gross observations were intestinal adhesions, distended stomach or intestine, brown areas on the intestine, yellow discoloration of Peyer's patches or adipose tissue, and yellow or clear fluid contents in the abdominal cavity. All examined females in the 12 mg/kg BW/day group were determined to be gravid and had 13-19 normally developing implantations in utero.

At 4 mg/kg BW/day, gross macroscopic findings included fused placentae in one female, enlarged lobes of the liver of two females, and enlarged kidneys, kidneys cyst and spleen anomalies in one female. At 12 mg/kg BW/day, the mean body weight was reduced by 4.4 to 16.7% from GDs 7 until the animals were euthanized on GD 10. This weight reduction corresponded to lower food consumption during this period. Mean food consumption, mean body weights, body weight gains, net body weight gains and gravid uterine weights were unaffected at 0.3, 1 or 4 mg/kg BW/day groups.

Developmental outcomes: No evaluation of intrauterine parameters at 12 mg/kg BW/day group was carried out due to early group termination. Intrauterine growth and survival were similar to the control group at dosage levels of 0.3, 1 and 4 mg/kg BW/day. None of the fetal parameters (post-implantation loss, live litter size, means fetal body weights, fetal sex ratios, fetal morphology, external malformations and variations, visceral malformations and variations, and skeletal malformations and variations) evaluated at necropsy on GD 20 at 0.3, 1 and 4 mg/kg BW/day were significantly different from the control group. In addition, the mean numbers of corpora luteum and implantation sites, and the mean litter proportions of pre-implantation loss were generally similar across all dose groups.

The NOEL/NOAEL for maternal toxicity was 1 mg/kg BW/day based on gross macroscopic findings at the next highest dose level. The NOEL/NOAEL for embryo-fetal developmental toxicity was 4 mg/kg BW/day.

f. Oral Developmental Toxicity Study in Non-Rodents

Title: An Oral (Gavage) Prenatal Developmental Toxicity Study of PF-06452795 in Rabbits

Study Number: 344094

Report Number: A2J1N-US-13-021

Report Date: February 26, 2016

Study Location: Ashland, OH

Study Design: This study was designed to evaluate the effects of KME (PF-06452795; purity 99.7%) in time-mated rabbits and their developing embryos or fetuses following its administration during gestation, and to identify the NOEL/NOAEL. Four groups of time-mated New Zealand White rabbits [Hra :(NZW) SPF] (25 per treatment group) were administered

ketoprofen in vehicle suspension at 0, 1, 4 or 8 mg/kg BW/day by daily gavage from GDs 7 to 28. A dose volume of 5 mL/kg BW was used and adjusted daily to the actual body weight. Control rabbits were administered the vehicle only (1.0% [w/w] carboxymethylcellulose and 0.5% [w/w] Tween® 80 in reverse osmosis-deionized water). Four toxicokinetic groups of four non-pregnant females per treatment group were administered the test article from GDs 7 to 28, on a comparable basis.

Mortality, clinical signs, food consumption and body weight were evaluated during the study. On GD 29, all female rabbits were weighed and humanely sacrificed. The thoracic, abdominal and pelvic cavities were opened, and the contents were examined. The uterus and ovaries were excised and the number of corpora luteum counted. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. The placentae also were examined. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss. Maternal tissues were preserved in 10% neutral-buffered formalin for histopathological examination, if needed.

Each viable fetus was removed, examined externally, sexed, weighed and euthanized. Each viable fetus was subjected to a visceral examination using appropriate dissection techniques. Fetal kidneys were examined and graded for renal papillae development. The head from each fetus was examined by a midcoronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Fetuses were then examined for skeletal malformations and developmental variations.

For rabbits in the toxicokinetic groups, blood was collected *via* a marginal ear vein (~1.0 mL each) for toxicokinetics on GD 21 from all animals at 0 (pre dose) and approximately 0.5, 1, 2, 6, and 24 h post-dosing.

Results and Conclusion: Toxicokinetic: On GD 21, PF-0344989 (ketoprofen, active metabolite of KME) was measured in maternal blood plasma samples, confirming systemic exposure to the active metabolite following administration of KME. Mean exposure to the active metabolite, in terms of AUC_{0-t}, was ~3000 to 5000-fold higher than exposure to KME, with no apparent trend related to dose. Moreover, exposure to the KME and its active metabolite, in terms of AUC_{0-t} and C_{max} increased in a nearly dose-proportional manner as the dose of KME increased from 1 to 8 mg/kg/day.

Maternal Outcomes: Dams in all treatment groups showed no significant overt clinical signs. At 8 mg/kg BW/day, there was a significantly lower mean body weight gain from GDs 7–9 and 20–21 as well as from GDs 7–29, with a 35% reduction noted. In addition, a significantly lower mean gravid uterine weight (19%) that was attributed to lower fetal body weights and fewer viable fetuses was noted. At 4 mg/kg BW/day, the mean body weight change from GDs 7–29 was reduced by 23% when compared to concurrent control value. Mean food consumption at 8 mg/kg BW/day group was lower during GDs 9–11 and the 7–0 cumulative interval as well as GDs 7–29, corresponding to the body weight losses noted in this group during these periods.

Developmental outcomes: The corpora luteum/dam and implantation sites/dam were not affected at 1, 4 and 8 mg/kg BW/day. However, the pre-implantation loss per dam increased in a dose-related manner at 1, 4 and 8 mg/kg BW/day by 0.8, 0.9 and 0.8, respectively, when compared to control value of 0.4. This difference was not considered test article-related because the rabbits were dosed after implantation (GD 7). Post-implantation loss per dam at 1, 4 and 8 mg/kg BW/day increased in a dose-dependent manner to 0.25, 0.68 and 0.96, when compared to concurrent control value of 0.2. The number of dams affected with post-implantation loss per treatment group were 2/22 (9%), 4/22 (18%), 10/24 (42%) and 13/22 (59%) for control, 1, 4 and 8 mg/kg BW/day, respectively. The mean number of viable fetuses per liter was not affected at 1 and 4 mg/kg BW/day but was reduced at 8 mg/kg BW/day (8.7/litter vs 9.7/litter in the control group). Fetal morphology, external malformations and variations, visceral malformations and variations, and skeletal malformations and variations, at 0.3, 1 and 4 mg/kg BW/day were not different from the control group.

The NOEL/NOAEL for maternal toxicity was established at 1 mg/kg BW/day based on changes in maternal body weight and gravid uterine weight at the next highest dose level tested. The NOEL/NOAEL for embryo-fetal developmental toxicity was established at 1 mg/kg BW/day based on the post-implantation loss seen at higher doses.

g. Oral Maternal Parturition Study in Rats with Ketoprofen and Dosing Holiday

Title: An Oral (Gavage) Maternal Parturition Toxicity Study of PF-0344989 with a Non-Dosing Period during Parturition in Sprague-Dawley Rats

Study Number: 344122

Report Number: A291N-US-15-309

Report Date: March 23, 2016

Study Location: Ashland, OH

Study Design: The aim of this study was to determine the effect of a dosing holiday by ketoprofen on the process of parturition following administration from GDs 10 to 16 or GDs 11 to 17 and then from lactation day (LD) 2 to 6 in female rats. On GD 10 or 11, 10 pregnant female rats per treatment group were administered the vehicle (vehicle containing 1.0% [w/v] carboxymethylcellulose and 0.5% [v/v] Tween® 80 in water) by gavage or ketoprofen (purity 99.7%) at 0.1, 0.5, 1.0 and 2.0 mg/kg BW/day in 2 mL/kg BW dose volume.

Parameters measured included daily observation for moribundity and mortality, clinical observation for toxicity, body weights, food consumption, plasma monitoring for exposure analysis and parturitional factors. Gross macroscopic examination was performed on animals not surviving until scheduled euthanasia, gross necropsy on surviving females. Implantation sites, corpora lutea, unaccounted for sites, and other pathologic findings were examined. Litters were evaluated for clinical observation body weights, sex

determination, live litter size, postnatal survival between and postnatal survival between birth and all other intervals.

Results and Conclusion: All animals survived to scheduled necropsy. The GDs 10–16 2.0 mg/kg BW/day group had lower mean maternal body weight during both gestation and lactation, decreased food consumption during gestation, lower mean implantation sites and increased number of unaccounted for sites. The GDs 1–17 2.0 mg/kg BW/day group had a lower mean number of pups born.

The dams in the GDs 10–16 1.0 mg/kg BW/day group showed decreased food consumption, and one pup had mandibular micrognathia, microstomia and an open eyelid. The dams in the GDs 11–17 1.0 mg/kg BW/day group had lower mean food consumption, longer mean gestation length, and lower mean number of implantation sites; there were lower mean number of pups born, higher mean birth weight in males (postnatal day (PND) 1) and lower mean body weight gains in females and males (PNDs 4–7).

A maternal NOEL/NOAEL of 0.5 mg/kg BW/day was set based on decreased food consumption and longer mean gestation length at the next highest dose. A developmental NOEL/NOAEL of 0.5 mg/kg BW/day was set based on lower number of implantation sites, lower number of pups born, higher birth weight in male pups and lower body weight gains in female and male pups next highest dose.

h. Oral Maternal Parturition Study in Rats with Ketoprofen and No Dosing Holidays

Title: An Oral (Gavage) Maternal Parturition Toxicity Study of PF-0344989 in Sprague-Dawley Rats

Study Number: 344116

Report Number: A291N-US-15-300

Report Date: March 23, 2016

Study Location: Ashland, OH

Study Design: The aim of this study is to determine the NOEL/NOAEL of ketoprofen on the process of parturition following administration from GD 14 to LD 6 in female rats. On GD 14 until LD 6, including parturition, 25 pregnant female rats were dosed by gavage at 0 (control vehicle containing 1.0% [w/v] carboxymethylcellulose and 0.5% [v/v] Tween® 80 in water) or ketoprofen (purity 99.7%) at 0.05, 0.1, 0.3, 0.5 and 1.0 mg/kg BW/day in 2 mL/kg BW dose volume.

Parameters measured examined included moribundity, mortality, toxicity observations, body weights, food consumption, plasma monitoring for exposure analysis, parturitional factors, gross necropsy on animals not surviving until scheduled euthanasia, gross necropsy on surviving females, implantation sites, corpora lutea, unaccounted for sites, and other pathologic findings. Litters were evaluated for clinical observation, body weights, sex

determination, litter size, postnatal survival between birth and postnatal survival between birth and all other intervals.

Results and Conclusion: The 1.0 mg/kg BW/day group exhibited dystocia, longer mean gestation length, three pups with gross necropsy findings and a decrease in the number of pups born and mean litter size as well as decreased pup survival. Two dams in this group were euthanized in extremis.

The 0.5 mg/kg BW/day group exhibited a slight increase in gestation length, one animal with total litter loss, lower mean postnatal survival and two pups with gross necropsy findings.

A maternal NOEL/NOAEL of 0.3 mg/kg BW/day was established based on the increase in gestation length and total litter loss at the next highest dose. A developmental NOEL/NOAEL of 0.1 mg/kg BW/day was established based on increased pup mortality in a dose dependent fashion (both the total number and on liter basis) and number of dead pups/litters.

i. Oral Maternal Parturition Study in Rodents with Ketoprofen Methyl Ester and No Dosing Holiday

Title: An Oral (Gavage) Maternal Parturition Toxicity Study of PF-06452795 in Sprague-Dawley Rats

Study Number: 344121

Repot Number: A291N-US-15-307

Report Date: October 14, 2016

Study Location: Ashland, OH

Study Design: The aim of this study is to determine the NOEL/NOAEL of ketoprofen on the process of parturition following administration from GD 14 to LD 6 in female rats. On GD 14 until LD 6, including during parturition, 25 pregnant female rats per treatment group were dosed by gavage with 0 (vehicle control: 1.0% [w/v] carboxymethylcellulose and 0.5% [v/v] Tween® 80 in water) or ketoprofen (purity 99.29%) at 0.05, 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0 mg/kg BW/day in 2 mL/kg BW dose volume.

Parameters examined included daily observation for moribundity and mortality, clinical observations for toxicity, body weights, food consumption, plasma monitoring for exposure analysis and parturitional factors. Gross macroscopic examination was performed on animals not surviving until scheduled euthanasia, gross necropsy on surviving females. Implantation sites, corpora lutea, unaccounted for sites, and other pathologic findings were examined. Litters were evaluated for clinical observation body weights, sex determination, live litter size, postnatal survival between and postnatal survival between birth and all other intervals.

Results and Conclusion: Two females in the 1.0 mg/kg BW/day group and nine females in the 2.0 mg/kg BW/day group exhibited premature mortality. There was no significant difference in body weights and food consumption

during gestation. During lactation, there was a significant difference in mean food consumption in the 1.0 and 2.0 mg/kg BW/day groups.

A test article-related increase in mean gestation length was noted in the 2.0 mg/kg BW/day group relative to controls (22.2 to 21.7). No effects at any dose were noted on mean number of pups born or the sex ratio. The 2.0 mg/kg BW/day group had a decreased mean live litter size at PND 0 (10.4 pups per dam); this value was lower than both concurrent and historical controls. Mean pup viability also on PND 0, PNDs 0–1, PNDs 1–4 and birth to PND 4 was lower than the concurrent and historical controls.

Lower mean pup viability was seen in the 1.0 mg/kg BW/day group during PNDs 0–1 and from birth to PND 4. These values were below the concurrent and historical controls.

A maternal NOEL/NOAEL of 0.5 mg/kg BW/day was established based on maternal mortality and a decrease in mean body weight gain at higher dose. A developmental NOEL/NOAEL of 0.5 mg/kg BW/day was established based on lowered mean pup viability from PNDs 0–1 and birth to PND 4 at higher dose.

j. Two-Generation Oral Reproductive Toxicity Study in Rats

Title: An Oral (Gavage) Two-Generation Reproductive Toxicity Study of PF-0344989 in Sprague-Dawley Rats

Study Number: 344123

Report Number: A291N-US-15-312

Report Date: August 16, 2017

Study Location: Ashland, OH

Study Design: Young male and female Sprague-Dawley rats (7 weeks of age), designated as the F0 generation, were dosed with ketoprofen (purity 99.7%) for 70 days by daily gavage administration at the doses of 0 (vehicle control), 0.1, 0.3, 1.0, and 3.0 mg/kg BW/day (25 sex/dose). One male and one female rat in the same dose group were then paired for up to 2 weeks. Following positive evidence of mating, the F0 females were individually housed during pregnancy and, after delivery, with the litter until weaning on PND 21. The F0 males and females continue to receive the daily treatment during mating, and the subsequent period, except that from GD 18 to PND 1, the pregnant dams were not dosed. Following birth, the F1 litters were culled to 8, with the sexes balanced if possible, on PND 4 and were weaned on PND 21. The F0 animals were killed at necropsy at the weaning of the F1 pups. The F0 rats were evaluated with clinical chemistry, macroscopic and microscopic examinations.

Following weaning, 25/sex/dose of the F1 animals were selected as the F1 breeders and continued to receive daily treatment, starting from PND 21 for 70 days when the mating of the F1 rats commenced. F1 animals not selected as breeders were necropsied for macroscopic examinations. Pregnant F1 rats were given the dosing holiday from GD 18 to PND 1 and were allowed to

deliver. The F1 parental rats and F2 litters were treated and evaluated in the same way as for the F0 and F1 pups.

Overall, the F0 males were dosed for 128–134 consecutive days, and F0 females were dosed for 121–134 days in total (not counting the dosing holiday). F1 males were directly dosed for 128–145 consecutive days, and F0 females were directly dosed for 121–143 days in total. A dose volume of 2 mL/kg was used. Individual dosages were based on the most recently recorded body weights.

The animals were evaluated for moribundity, mortality, clinical observations, body weights, food consumption, plasma monitoring for exposure analysis, parturition, gross necropsy of animals at scheduled and unscheduled necropsies. Data also were collected on organ weights of all necropsied animals, reproductive performance, littler data, sperm parameters, clinical chemistry and histopathological evaluations. Blood samples were collected form a group of F1 male and female rats (5/sex/dose) on approximately PND 90 for determination of plasma ketoprofen concentrations.

Results and Conclusion: Ketoprofen at 3.0 mg/kg BW/day caused multiple unscheduled deaths in the F0 females; the cause of death was peritonitis resulting from jejunal and cecal ulceration. At this dose level, there were reduced mating in F0, reduced fertility in F0 and F1 animals, and decreased implantation sites and smaller litter size for the F0 dam/F1 litters. F0 females were affected for body weight gain during late gestation and food consumption during lactation. Clinical chemistry parameters, mostly those associated with anemic and inflammatory responses were altered at the 3.0 mg/kg BW/day dose level.

Ketoprofen, at 1.0 mg/kg BW/day and 3.0 mg/kg BW/day was associated with prolonged gestation in both F0 and F1 pregnant dams, which corresponded to increased pup body weight at birth.

Kidney weight was increased in the F0 male at 1.0 and 3.0 mg/kg BW/day and in the females at 3.0 mg/kg BW/day. Microscopically, papillary necrosis was seen in the F0 males and females at 1.0 and 3.0 mg/kg BW/day, and exacerbation of CPN in the males was seen at 1.0 and 3.0 mg/kg BW/day and in the females at 3.0 mg/kg BW/day. In the F1 rats, kidney organ weight increases were seen in all dose groups, and treatment-related renal papillary necrosis was observed, where incidences in female rats were greater than males. An over 10% occurrence of renal papillary necrosis was observed in F1 females at the lowest dose 0.1 mg/kg BW/day.

A NOEL/NOAEL for parental systemic toxicity could not be established for this study due to the finding of papillary necrosis in the kidney tissue at all doses. The dose of 0.1 mg/kg BW/day was identified as the LOEL/LOAEL for systemic toxicity. The NOEL/NOAEL for parental reproductive toxicity was 0.3 mg/kg BW/day based on the prolonged gestation at 1.0 and 3.0 mg/kg BW/day for the F0 generations in this study. The dose of was identified as the overall LOEL/LOAEL for the study 0.1 mg/kg BW/day.

k. Genetic Toxicity Studies

The findings from the genotoxicity testing are presented in Table IV.B.1a and 1b. and described in detail below. Based on results of the genetic toxicity tests for ketoprofen and its metabolite (ZTS-00102401), it is concluded that ketoprofen residues are not of genetic toxicity concerns. Additionally, the prodrug KME was evaluated for their genetic toxicity potentials and were concluded to be not of genetic toxicity concerns.

Table IV.B.1a: Summary of Ketoprofen Genotoxicity Studies

Study Type	Study Number	Results
Bacterial Reverse Mutation Assay (Ames Test)	A2X1R-FR-14- 166	Negative
In Vitro Mammalian Chromosome Aberration Test (Chinese Hamster Ovary Cells).	A2X1N-FR-14- 164	Negative
In vivo Mammalian Erythrocyte Micronucleus Test (Rat Peripheral Blood)	A291N-US-15- 363	Inconclusive (Positive finding possibly due to excessive toxicity)
In vivo Mammalian Erythrocyte Micronucleus Test (Rat Bone Marrow)	A291N-US-16- 433	Negative

Table IV.B.1b: Summary of ZTS-0012401 (Ketoprofen Metabolite) and KME (Ketoprofen Prodrug) Genotoxicity Studies

Study Type	Study Number	Results
ZTS-00102401: Bacterial Reverse Mutation Assay (Ames Test)	A2X1N-US-16- 370	Negative
ZTS-00102401: *In vitro* Micronucleus Assay (Human Lymphocyte	A2X1N-US-17- 399	Negative
KME: Bacterial Reverse Mutation Assay (Ames Test)	A2X1N-US-13- 111	Negative
KME: *In Vitro* Mammalian Chromosome Aberrations Test (Chinese Hamster Ovary Cells)	A2X1N-US-13- 112	Negative
KME: Mammalian Erythrocyte Micronucleus Test (Rat Bone Marrow)	A291N-US-13- 140	Negative
KME: *In vivo* Comet Assay in Rat Liver Tissue	A291N-US-14- 196	Negative

(1) Bacterial Reverse Mutation Assay (Ames Test)

Title: Ketoprofen (RP-19,583) – Bacterial Mutagenicity Ames Test

Report Number: A2X1R-FR-14-166

Report Date: June 26, 1979

Study Location: Vitry-Sur-Seine, France

Study Design: The GLP-compliant study was conducted according to the OECD Test Guideline No. 471. The objective was to evaluate the potential mutagenic activity of ketoprofen.

Ketoprofen sodium salt in aqueous solution was tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538. The assay was conducted both with and without an Aroclor 1254-induced rat (Sprague-Dawley) liver S9 using three plates per condition. In the initial toxicity/mutagenicity assay, ketoprofen (Batch BOS 618) was tested in the bacterial strains without the inclusion of the S9 fraction; the ketoprofen concentrations tested ranged from 5 to 5000 µg/plate using the plate incorporation method. The second plate incorporation test was conducted in the dose range of 125 to 1000 µg/plate, with and without metabolic activation. The third experiment was a spot test, in which ketoprofen was applied as a spot on the top of the agar layer. The ketoprofen concentration used for the spot test was 1000 µg/10 µL solution per spot. The positive controls were β-propiolactone (50 µg) for TA1535, hycanthone (50 µg) for TA1537 and TA1538, and niridazole (0.05 µg) for TA98 and TA100 under the condition of without S9; for the experiments with S9, ethidium bromide (60 µg) was used for TA98 in the plate incorporated experiment, and 2-nitrofluorene (10 µg) was used for TA1538 and TA98 in the spot test.

Results and Conclusion: At 5000 µg/plate, ketoprofen was cytotoxic in the initial assay without S9 and completely inhibited the growth of TA 1537, TA 1538, and TA 98 strains. At 1000 µg/plate with and without S9, ketoprofen was cytotoxic to strains TA1538 and TA98 in the presence of S9. No positive mutagenic response was observed for ketoprofen in any strain with or without metabolic activation either in the plate incorporation method or in the spot test. It was concluded that ketoprofen did not induce gene mutations in this assay.

(2) *In Vitro Metaphase Chromosome Aberration Test*

Title: Ketoprofen (RP – 19583) – Chromosome Aberration Test in Chinese Hamster Ovary Cells

Report Number: A2X1N-FR-14-164

Report Date: May 7, 1985

Study Location: Vitry-Sur-Seine, France

Study Design: The GLP-compliant study was conducted according to the OECD Test Guideline No. 473. The objective was to evaluate the potential induction of structural chromosome aberrations effect of ketoprofen.

A preliminary cytotoxicity assay of ketoprofen on the CHO-K1 cells was conducted with concentrations of ketoprofen (Batch 955 CA 84 19100; purity unstated, dissolved in the culture media) ranging from 0.01 to 10,000 µg/mL. Cultured cells in duplicate plates (seeded with 400 cells per plate) were treated with ketoprofen for 4 hours and then cultured in media without the test article for 10 days before being scored for growth of colonies. The treatment was conducted with and without metabolic activation (inclusion of S9 fraction of liver homogenate from rats treated with Aroclor). Based on the results of the cytotoxicity assay, ketoprofen was tested at the concentrations of 0, 150, 400, and 650 µg/mL in the chromosome aberration assay in the CHO-K1 cells. The assay was performed both with and without an Aroclor 1254-induced rat (Sprague-Dawley) liver S9 using duplicate culture per condition. The positive controls were cyclophosphamide (CP) and methylmethanesulfonate (MMS) for tests with and without metabolic activation, respectively. The cells treated with culture medium alone constituted the negative control.

Results and Conclusion: In the cytotoxicity test, no colonies were observed at concentrations of 650 µg/mL and higher. At the concentrations of 150 and 400 µg/mL, the survival colonies were 87% and 81% for the cultures without metabolic activation and 81% and 71% for the cultures with metabolic activation, respectively. The percentages of cells with aberrations were 2–6% for the negative control and for the ketoprofen-treated cultures, including samples harvested at the 18 and 42 hours post treatment, with and without metabolic activation; in contrast, the MMS-treated cultures had 62–74% cells with aberrations, and the CP-treated cells had 46–74% aberrations. It was concluded that ketoprofen was negative in the *in vitro* chromosome aberration assay.

(3) *In Vivo* Micronucleus Assay in Peripheral Blood of Rats

Title: *In vivo* Mammalian Micronucleus Assay in Rats with Flow Cytometry Analysis in Peripheral Blood Reticulocytes

Study Number: AE48AV.125021FLPBICH.BTL

Report Number: A291N-US-15-363

Report Date: April 07, 2017

Study Location: Rockville, MD

Design and Method: The GLP-compliant study was conducted according to the OECD Test Guideline No. 474. The objective was to evaluate the potential induction of micronuclei in peripheral blood of ketoprofen.

Both male and female Sprague-Dawley rats (5 animals/sex/group; 8 rats/sex in the high dose group; 5/8 were analyzed for the mutagenic effect) were treated with ketoprofen (Lot No. 108115009; purity 99.3%) by gavage once daily for two days at doses of 10, 20, and 40 mg/kg BW/day. Ketoprofen was prepared in vehicle (1.0% [w/v] carboxymethylcellulose and 0.5% [v/v] Tween 80 aquatic solution). A

single dose of 40 mg/kg BW cyclophosphamide (CP) was administered to a positive control group (5/sex) on study day 2. Positive and negative controls were run concurrently and met the testing criteria. The animals were observed for mortality and signs of morbidity throughout the study. Body weights were recorded prior to the first dose and before euthanasia. Forty-eight hours after the last dose, peripheral blood was collected from the retro-orbital sinus and the animals were euthanized. Blood cells were prepared for flow cytometry analysis and up to 20,000 PBRs per animal were analyzed to determine the frequency of micronucleated PBRs (MN-PBRs).

Results and Conclusion: Ketoprofen treatment at 40 mg/kg BW/day resulted in piloerection, body weight loss of about 10% for both males and females; one female died at this dose. At 20 and 40 mg/kg BW/day, increases in %PBRs were observed; the increases were 3 and 6 folds in males at 20 and 40 mg/kg BW/day, respectively. The %MN-PBR measurements were (male/female) 0.06/0.06, 0.05/0.05, 0.12/0.10, and 0.19/0.19 for the control, 10, 20, and 40 mg/kg BW/day groups, respectively; the corresponding values for the control group were 0.39/0.32. Statistically significant two-fold increases in the incidence of MN-PBR were observed in females in the 20 mg/kg BW/day dose group, and in both males and females at 40 mg/kg BW/day (a three-fold increase). In the positive control group, increase of the MN-PBR was greater than five folds. The dose of 10 mg/kg BW/day was not associated with an increase in either %PBR or MN-PBR.

The increased incidence of %MN-PBR at 20 (males) and 40 (males and females) mg/kg BW/day dose levels were attributed to the possibility of rebound erythropoiesis at the gastrointestinal toxic doses of ketoprofen. The positive finding was interpreted to be secondary to the excessive toxicity noted at these dose levels; the results were considered questionable, and a follow-up study (see the *in vivo* rat bone marrow micronucleus assay, Study Number A291N-US-16-433) with modified design was deemed necessary to provide further information.

(4) *In Vivo* Micronucleus Assay in Bone Marrow of Rats

Title: PF-0344989: *In vivo* Rat Bone Marrow Micronucleus Assay

Report Number: A291N-US-16-433

Report Date: July 24, 2017

Study Location: Greenfield, IN

Study Design: The GLP-compliant study was conducted according to the OECD Test Guideline No. 474. The objective was to evaluate the potential induction of micronuclei in bone marrow of ketoprofen as a follow up investigation of a previous study (A291N-US-15-363).

Ketoprofen (Lot no. 108115009; purity 99.3%) was prepared in vehicle (1.0% [w/v] carboxymethylcellulose and 0.5% [v/v] Tween® 80 aquatic solution). Male Sprague-Dawley rats (12 animals/group) were treated with

ketoprofen by gavage once at doses of 2.5, 5, 10, 20, and 40 mg/kg BW. Cyclophosphamide was used as the positive control. Positive and negative controls were run concurrently and met the testing criteria.

After treatment, bone marrow was collected from right femur at two sampling times (24 and 48 hours) with 6 animals/group/condition from ketoprofen treated animals and 24 hours from the negative and positive control treated animals. Based on the evaluation of hematology, clinical pathology, and polychromatic erythrocytes (PCE) to normochromatic erythrocytes ratio (NCE) ratio, dose groups of 0, 2.5, 5.0, and 10 mg/kg ketoprofen, along with the positive control group, were selected for the determination of % micronucleated PCE (%MN-PCE). At least 4000 PCEs were examined per animal. In addition, blood samples collected from all animals at the 24- and 48-hour sampling times were analyzed for plasma concentrations of ketoprofen and the metabolite ZTS-00102401 using previous validated methods.

Results and Conclusion: Ketoprofen concentrations in the plasma ranged from 620 to 10,100 ng/mL for the 24-hour samples and 132 to 3770 ng/mL for the 48-hour samples, corresponding to the oral dose range of 2.5 to 40 mg/kg BW; the plasma concentrations were approximately dose proportional. The corresponding values for the metabolite of ZTS-00102401 were 31 to 555 ng/mL and 7.5 to 214 ng/mL for the 24 hour and 48-hour samples, respectively.

No mortalities or adverse clinical signs were observed with ketoprofen treatment at any dose levels. There were slight reductions in body weight at necropsy for the 20 and 40 mg/kg dose groups (7% and 8%, respectively). At doses levels ≥15 mg/kg BW, bone marrow toxicity was noted. Because the hematological changes observed are known to confound the interpretation of the micronucleus assay, MN-PCE enumeration was not carried out on samples from the 15, 20, or 40 mg/kg BW groups. The dose groups evaluated for micronucleus formation were 2.5, 5.0, and 10.0 mg/kg BW. The results indicated that there were no significant increases in MN-PCE frequencies following ketoprofen treatment, with either the 24- or 48-hour post-dose samples. It was concluded that ketoprofen was negative in the *in vivo* bone marrow micronucleus assay.

(5) Other Genetic Toxicology Studies

In addition to the genetic toxicology studies on ketoprofen listed above, genetic toxicity potential was evaluated for the ketoprofen metabolite ZTS-00102401 and KME.

Studies on Ketoprofen Metabolite ZTS-00102401:

The studies on the ketoprofen metabolite ZTS-00102401 included an Ames test and an *in vitro* micronucleus in cultured human lymphocytes. The Ames test (Study Report No. A2X1N-US-16-370, November 10, 2016), an FDA GLP-compliant study that is in full accordance with the current genotoxicity test guidance, demonstrated that the test article was

not mutagenic in bacterial reverse mutation assay. In the *in vitro* micronucleus assay (Study Report No. A2X1N-US-17-399, January 25, 2017), treatment with ZTS-00102401 of cultured human lymphocytes did not increase the formation of micronucleus in the absence of metabolic activation. While treatment with ZTS-00102401 in the presence of metabolic activation resulted in statistically significantly increased micronucleus incidences, such increases lacked consistence between the replicate samples, and the measured values were within historical controls; therefore, the putative positive finding was not deemed to be toxicologically significant. It was concluded that ZTS-00102401 was negative in the Ames test and *in vitro* micronucleus assay.

Studies on Ketoprofen Prodrug - Ketoprofen Methyl Ester:

The studies on KME included an Ames test, an *in vitro* chromosome aberration assay, an *in vivo* micronucleus assay in bone marrow of rats, and an *in vivo* Comet assay in the liver tissue. The Ames test of KME (Study Report No. A2X1N-US-13-111, December 11, 2013) was negative. The *in vitro* chromosomal aberration assay (Study Report No. A2X1N-US-13-112, December 11, 2013) showed that KME was positive for inducing structural aberrations in CHO-WBL cells when a 3-hour treatment was carried out in the presence of metabolic activation in serum-free cell culture media, and the result become negative when serum was included in the culture media. Two *in vivo* tests, the bone marrow micronucleus assay in rats (Study Report No. A291N-US-13-140, July 31, 2014) and the liver tissue comet assay in rats (Study Report No. A291N-US-14-196, October 28, 2014) showed no genotoxic effect of KME. It was concluded that KME was negative in the genotoxicity studies.

l. Oral Carcinogenicity Study in Mice

Title: Carcinogenicity Study of Ketoprofen (19,583) in The Mouse

Study Number: 18471

Report Number: A291R-FR-14-199

Report Date: December 19, 1975, updated July 27, 1979

Study Location: Vitry-Sur Seine, France

Study Design: The carcinogenicity study was conducted prior to enactment of GLP regulations. Four consecutive experiments were conducted to assess the carcinogenicity potential of ketoprofen. In Experiment I, C57B1/6/Rho-Ico mice (20/sex/group) the toxicity of ketoprofen was examined were administered by stomach tube ketoprofen (purity unreported) at 64, 128 and 256 mg/kg BW/day. The surviving animals were kept under observation for 106 weeks. Animals were evaluated for clinical observations, body weight, gross pathology and histopathology. In Experiment II (a pilot study), the maximum quantity of the compound acceptable to mice was examined by administering ketoprofen in drinking water at 32 and 64 mg/kg BW/day to 20 mice per sex per group for 43 days and then, they were kept with no drug administration for an additional 21 weeks; this pilot study established the

maximum concentration of ketoprofen in drinking water at 60 mg/L for the mice to consume 32 mg/kg BW/day of the compound, the maximum dosage selected for Experiment III. In Experiment III, ketoprofen was orally administered in drinking water to C57B1/6/Rho-Ico mice (50 per sex per group) at doses of 4, 8, 16 and 32 mg/kg BW/day for 105 weeks. Clinical observations, body weight, gross pathology and histopathology were evaluated. In an independent parallel experiment (Experiment IV), benzo-a-pyrene (positive control) at 250 or 500 mg/kg BW/day was administered orally to 50 mice per sex per group for 58 weeks; the control groups of 50 mice per sex were administered saline solution.

Results and Conclusion: Two preliminary studies, a toxicity study (Experiment I) and determination of the sub-toxic dose and the maximal tolerated dose (Experiment II) were used to determine the doses for the main carcinogenicity study of ketoprofen (Experiment III). In Experiment III, no clinical observations were reported. Mean body weights of male and female mice of all treated groups were slightly lower than the control mice, especially at the end of the study. There were no neoplastic findings attributable to ketoprofen treatment. Most of the non-neoplastic lesions observed were benign inflammatory lesions. In Experiment IV, benzo-α-pyrene induced epidermoid carcinoma of the non-glandular part of the stomach. Based on the results obtained in Experiment III, ketoprofen was not carcinogenic in mice under the conditions of the study. Similar results also were found in Experiment I. A NOEL/NOAEL was not identified.

m. Oral Carcinogenicity Study in Rats

Title: Orudis (Ketoprofen) Two Year Rat Carcinogenicity Study

Study Number: 17443

Report Number: A291N-GB-14-205

Report Date: May 25, 1990

Study Location: Cambridgeshire, England

Study Design: This study was conducted in compliance with GLP and in accordance with OECD Test Guideline No. 451. The carcinogenic potential of ketoprofen (purity 99.8%) was assessed in 100 control and 50 CD-Sprague-Dawley rats per sex per test article group at dietary doses of 0, 1.5, 3.0 and 6.0 mg/kg BW/day for 104 weeks. The test-article administration in females at the highest dose group was stopped at 81 weeks and the animals were killed at 87 weeks due to low survival rates. Pre-treatment aortic blood samples were collected from five males and five females for red blood cell count, and total and differential white blood cell counts. In addition, blood samples were collected from 5 males and 5 females from each satellite group prior to commencement, and on days 7 and 28 for assessment of plasma ketoprofen concentrations.

In-life examinations included detailed daily clinical observations, palpation to record palpable masses, mortality and moribundity, body weights, and food consumption. Blood samples were taken from the orbital sinus of 10 rats per

sex per group in weeks 38, 76 and on the day of termination for hematological and coagulation testing. During week 42, urine stained cage paper from 3 rats per sex from the control and high dose groups were analyzed qualitatively for the presence of heme pigments, a breakdown product of hemoglobin. All surviving rats were necropsied at sacrifice followed by determination of organ weights and histopathology.

Results and Conclusion: The most frequently observed clinical sign was the higher frequency of the passage of red/brown discolored urine (heme or porphyrin) in all males and females from the test article groups. No significant differences were noted in the survival rates of males and females, except for females at the 6 mg/kg BW/day dose, their survival rate was significantly reduced, and causing treatment removal at week 81 and animal were sacrificed at week 87. Body weights, food consumption and blood smear were not significantly affected throughout the study. There was an increased in the levels of circulating neutrophils in males during week 38 at all dose levels and among females at 6 mg/kg BW/day.

The most frequently observed macroscopic pathology of the kidneys was bilateral enlargement, pallor and cortical scarring in both male and female rats. Renal and mesenteric lymph node, forestomach and stomach-corpus mucosa were noted in male and female rats at 3 and 6 mg/kg BW/day. The test-article resulted in the appearance of white forestomach, depression, increased thickening of the corpus mucosa and pale corpus mucosa. In male rats, the incidence of small and flaccid testes increased at all dose levels. The incidence of small prostate increased at 3.0 and 6.0 mg/kg BW/day dose levels. Toxic responses also were noted in the adrenals of males administered 3.0 and 6.0 mg/kg BW/day as well as the heart and pituitary of males at 6.0 mg/kg BW/day dose.

No significant test-article related neoplastic findings were observed in male and female rats. The major significant dose-related non-neoplastic lesions occurred in the kidneys, adrenals, stomach, liver, lymph nodes, thymus and colon of both male and female rats. The effects on the kidneys induced other significant secondary effects on parathyroid, bone marrow/sternum, femur/joint, lungs and heart. In addition, significant male specific effects (increases in the incidence of prostatitis, testes periarteritis and tubular atrophy of the testes as well as atrophy of the epididymides) were considered secondary renal effects. The major factors, excluding progressive glomerulonephrosis, pyelonephritis or renal hemorrhage contributing to death or euthanasia of moribund animals was "renal lesions", in all test article group but not in the control group.

It was concluded that ketoprofen was not carcinogenic in rats. A NOEL/NOAEL could not be established because of a plethora of non-neoplastic macroscopic and microscopic findings in the kidneys, adrenals, stomach and lymph nodes as well as other male specific findings on the testes, prostate and epididymites. The lowest dose of this study, 1.5 mg/kg BW/day, was considered the LOEL/LOAEL of the study.

n. Other Studies

A GLP-compliant pharmacokinetic study (Study Report No. A4J1N-US-15-032, October 20, 2016) of ketoprofen and KME was conducted in rabbits. This study was designed to determine the pharmacokinetics of a single equal molar doses of ketoprofen (2.0 mg/kg) or KME (2.11 mg/kg) administered orally to female rabbits (24 animals/group). Pharmacokinetic parameters for C_{max}, t_{max}, $AUC_{0-\infty}$, $AUC_{0-t(last)}$, and $t_{½}$, were determined for each animal using non-compartmental analysis. Based on the $AUC_{0-\infty}$ values, exposure to the metabolite ZTS-00102401 was 16–18% of ketoprofen following both ketoprofen and KME administration. For ketoprofen, the bioavailability of KME relative to ketoprofen was 92.2% based on $AUC_{0-t(last)}$ and 75.9% based on C_{max}. For ZTS-00102401, the bioavailability of KME relative to ketoprofen was 99.3% based on $AUC_{0-t(last)}$ and 93.6% based on C_{max}. It was concluded that ketoprofen and KME have similar pharmacokinetic characteristics.

A GLP-compliant pharmacokinetic study (Study Report No. A491N-US-16-432, March 2, 2018) of ketoprofen and KME was conducted in rats. Single doses of ketoprofen (0.5 mg/kg BW) and KME (0.53 mg/kg BW) were administered to rats through gavage, and blood samples were collected following dosing at a series of time points up to 96 hours. Plasma concentration profiles of the (R) and (S) enantiomers of ketoprofen and total ketoprofen followed similar trends for both ketoprofen and KME. Following treatment with ketoprofen, the mean C_{max} was 691 ng/mL for (R) ketoprofen and 1110 ng/mL for (S) ketoprofen with both occurring at less than 20 minutes post-dose. The mean terminal $t_{1/2}$ was 1.37 hours for (R) ketoprofen and 12.6 hours for (S) ketoprofen. The $AUC_{0-t(last}$) was 768 ng·h/mL for (R) ketoprofen and 9900 ng·h/mL for (S) ketoprofen. Based on $AUC_{0-\infty}$ values, (S) ketoprofen consistently represented approximately 93% of the total ketoprofen exposure for all rats. The toxicological equivalence factor was approximately 1 based on the relative bioavailability of KME to ketoprofen.

A non-GLP study (Study Report No. A491R-FR-14-213, October 18, 1973) in rats examined enterohepatic circulation in rats. Tritium labeled ketoprofen (specific activity 270 µCi/mmol) was administered orally at 1 mg/kg to either intact rats, rats with a biliary fistula, or rats with a laparotomy (2 rats/group). Blood was collected from the retroorbital sinus over 24 hours following dosing (0.5, 1, 3, 6, 12, and 24 hours) for measuring ketoprofen plasma concentrations. After reaching peak concentrations, rats with biliary fistulas (not having enterohepatic circulation) demonstrated a rapid decrease in ketoprofen plasma concentrations, while the decrease was much slower in control or laparotomized animals. After 12 hours, the plasma elimination rates were similar for both groups of animals. Enterohepatic circulation was concluded to be responsible for the pharmacokinetic properties of ketoprofen in the plasma of rats.

A GLP-compliant pharmacokinetic study (Study Report No. A291N-US-16-438, February 5, 2018) was performed to characterize the toxicology and toxicokinetic profile of racemic (R+S)-ketoprofen in comparison to the metabolite ZTS-00102401 and to assess the contribution of the chiral enantiomers (R-ketoprofen and S-ketoprofen) to (R+S)-ketoprofen toxicity, in addition to evaluate the conversion of R-ketoprofen to S-ketoprofen in the rat. In this study, 3 to 6/sex/group rats were administered daily through gavage (R+S)-ketoprofen (12 mg/kg BW/day), R-ketoprofen

(6 mg/kg BW/day), S-ketoprofen (6 mg/kg BW/day), and ZTS-00102401 (1.2 and 12 mg/kg BW/day) for 28 consecutive days and evaluated for signs of toxicity along with pharmacokinetic parameters. The results indicated that exposure to the (R+S)-, R-, and S-ketoprofen did not lead to difference in clinical and behavioral toxicity. Analysis of the AUC_{0-24} values in the toxicokinetic group demonstrated that for rats treated with R+S-ketoprofen, the predominant enantiomer in the plasma of male and female rats after Day 1 (92% both sexes) and Day 15 (96% both sexes) was S-ketoprofen. For both sexes of R-ketoprofen-treated rats, after Day 1, the predominant enantiomer was S-ketoprofen (83%). After Day 27, S-ketoprofen made up over 93% of the total enantiomer in both sexes. No conversion was seen in the S-ketoprofen treated rats, as AUC_{0-24} for R-ketoprofen was 0.15% and 0.18% in females and males, respectively, in this group after Day 15. In the limited data obtained from rats of both sexes treated with ZTS-001203401, 40–50% of the low-dose and 30–40% of the high-dose metabolite was converted to achiral ketoprofen after Day 1. The results of this study supported that R-ketoprofen is largely converted to S-ketoprofen in rats.

A GLP-compliant pharmacokinetic study (Study Report No. A271N-US-16-129, August 11, 2017) was conducted in monkeys to determine the pharmacokinetics of ketoprofen and the metabolite ZTS-00102401 following a single dose i.v. administration of ketoprofen (1 mg/kg BW) and a single dose oral administration of ketoprofen (3 mg/kg BW) and KME (3.17 mg/kg BW) in a cross-over design. For the i.v. administration of ketoprofen, the systemic clearance of ketoprofen was 169 mL/kg/hour; V_{dss} was 166 mL/kg; the elimination half-life was 5.76 hours. For the oral administration of ketoprofen, ketoprofen was rapidly absorbed with a C_{max} of 24 µg/mL, t_{max} of 19 minutes post-dose, and the elimination half-life of 5.23 hours. For the oral administration of KME, C_{max} for ketoprofen was 15 µg/mL; t_{max} was approximately 30 minutes post-dose; the elimination half-life was 6.33 hours. Oral treatment with ketoprofen or KME exhibited similar elimination profiles and half-life compared to that of intravenous administration of ketoprofen. Based on $AUC_{0-t(last)}$, the bioavailability of ketoprofen and KME were 99.7% and 90.6%, respectively. The toxicological equivalence factor was 0.909 based on the relative bioavailability of KME to ketoprofen. The exposure to ZTS-00102401 was approximately 12% of that to ketoprofen based on $AUC_{0-\infty}$ *via* both intravenous and oral administration.

2. Determination of Toxicological Point-of-Departure for Chronic Exposure

Studies considered for determination of the toxicological NOEL/NOAEL or LOEL/LOAEL for chronic exposure to total residues of ketoprofen are summarized in Table IV.B.2. Among all the toxicology studies conducted on ketoprofen, its metabolites, and the prodrug KME, the most sensitive toxicity finding was the renal papillary necrosis caused by ketoprofen exposure in the two-generation reproduction study in Sprague-Dawley rats (Study No. A291N-US-15-312). The ketoprofen dose of 0.1 mg/kg BW/day was identified as the overall LOEL/LOAEL for the study.

In order to establish the point-of-departure (POD) for this renal papillary necrosis effect, BMD analyses were conducted on this endpoint, using U.S. EPA's Benchmark Dose Software (BMDS), Version 2.7.0.4. With 5% chosen as

the benchmark response (BMR), the $BMDL_5$ was estimated to be 0.04 mg/kg BW/day, which was established as the POD for the finding of renal papillary necrosis in the F1 rats of the two-generation reproductive toxicity study.

Table IV.B.2: Pivotal Toxicology Studies Supporting Human Food Safety Assessment for Ketoprofen

Report No.	Type of Study	Test Article	NOEL/NOAEL or LOEL/LOAEL or
A291N-US-15-358	Developmental; rat; GLP	Ketoprofen	NOEL/NOALE for fetal developmental toxicity at 4 mg/kg BW/day (absence of findings); NOEL/NOAEL for maternal toxicity at 1 mg/kg BW/day (macroscopic maternal findings)
A2J1N-US-13-021	Developmental; rabbit; GLP	Ketoprofen methyl ester	NOEL/NOAEL for maternal toxicity at 1 mg/kg BW/day (body weight and uterine weight); NOEL/NOAEL of embryo-fetal developmental toxicity at 1 mg/kg BW/day (post-implantation loss)
A291N-US-15-300	Reproductive; maternal parturition; rat; GLP	Ketoprofen	Maternal NOEL/NOAEL of 0.3 mg/kg BW/day (gestation length and litter loss); developmental toxicity NOEL/NOAEL of 0.1 mg/kg BW/day (pup mortality)
A291N-US-15-307	Reproductive; maternal parturition; rat; GLP	Ketoprofen methyl ester	Maternal NOEL/NOAEL of 0.5 mg/kg BW/day (maternal mortality, etc.); developmental NOEL/NOAEL of 0.5 mg/kg BW/day (postnatal survival)
A291N-US-15-309	Reproductive; maternal parturition; rat; GLP	Ketoprofen	Maternal NOEL/NOAEL of 0.5 mg/kg BW/day (decreased food consumption and prolonged gestation); developmental NOEL/NOAEL of 0.5 mg/kg BW/day (lower numbers of implantation sites and pup per litter)

Report No.	Type of Study	Test Article	NOEL/NOAEL or LOEL/LOAEL or
A291N-US-15-312	Two-generation reproductive toxicology; rat; GLP	Ketoprofen	LOEL/LOAEL of 0.1 mg/kg BW/day for maternal toxicity (rental papillary necrosis in F1 adults); NOEL/NOAEL for reproductive toxicity at 0.1 mg/kg BW/day (prolonged gestation)
A291N-US-13-139	28-day oral toxicity study; rat; GLP	Ketoprofen and ketoprofen methyl ester	LOEL/LOAEL for both at 2 mg/kg BW/day (hypertrophic kidney and inflammation in stomach)
A271N-US-16-112	13-week oral toxicity study; monkey; GLP	Ketoprofen	NOEL/NOAEL of 27 mg/kg BW/day (absence of effects)
A291N-US-15-320	13-week oral toxicity study; rat; GLP	Ketoprofen	NOEL/NOAEL of 0.1 mg/kg BW/day (stomach ulceration and renal papillary necrosis)
A271R-GB-14-049	1-year oral toxicity study; monkey; 1972; non-GLP	Ketoprofen	NOEL/NOAEL of 9 mg/kg BW/day (gastric effects and reduction in body weight)
A291R-GB-14-203	78-week oral carcinogenicity study; 1973, non-GLP; rat	Ketoprofen	LOEL/LOAEL of 4.5 mg/kg BW/day (renal effects)
A291N-GB-14-205	2-year oral carcinogenicity 1990; rat	Ketoprofen	LOEL/LOAEL of 1.5 mg/kg BW/day (renal and gastric effects)

3. Toxicological Acceptable Daily Intake (ADI)

The toxicological ADI is determined based on the point-of-departure (POD) from the most appropriate study and an overall safety factor.

$$\text{Toxicological ADI} = \frac{\text{POD}}{\text{Safety Factor}}$$

As discussed above, the POD is the $BMDL_5$ of 0.04 mg/kg BW/day based on the finding of renal papillary necrosis in the F1 rats of the two-generation reproductive toxicity study.

Instead of the default overall safety factor of 100 for interspecies and intraspecies extrapolations of toxicological data, data-derived extrapolation factors (DDEFs, see Table IV.B.3), also referred to as chemical specific adjustment factors (CSAFs), were used to determine the overall safety factor.

Table IV.B.3. Data-Derived Extrapolation Factors

Extrapolation	Toxicokinetics	Toxicodynamics
Interspecies (animal to human), EF_A	EF_{AK}	EF_{AD}
Intraspecies (within human), EF_H	EF_{HK}	EF_{HD}

a. Interspecies Toxicokinetic Extrapolation Factor (EF_{AK})

The interspecies extrapolation of toxicokinetics from rats to humans is determined by the ratio of the external dose to the animals (D_A) that leads to the area-under-the-curve (AUC) at or near the POD and the external dose to humans (D_H) at the central tendency that leads to the same AUC as measured in the animals.

$$EF_{AK} = D_A/D_H = Slope_H/Slope_A$$

Data Source: The ketoprofen blood concentration data for animals were from the two-generation reproductive toxicity study in rats (Study No. A291N-US-15-312), for which the ketoprofen toxicity POD for the ADI determination was based on. For the EF_{AK} analysis, the measured blood S-ketoprofen concentration data from the two generations were pooled for each sex (no dose-by-generation interaction), and the sexes were modeled separately. In a simple linear regression model, both male and female data showed satisfactory linearity (>98% in both cases) between the external doses and the corresponding AUCs. The EF_{AK} was inversely related to the slope of the external dose-AUC regression line; the more conservative slope value of 31.96 from the male (rather than 41.68 from the female) was taken as the input data point for estimating the EF_{AK}.

For pharmacokinetics of ketoprofen in humans, the dose matrices are similar under repeated dose conditions as those following a single dose exposure. Therefore, AUC data from experiments with a single dose exposure were chosen for the model. The linear regression model for human data incorporated studies with healthy adults administered a single dose of ketoprofen. Twelve experiments from 10 studies were included to extract the AUC and ketoprofen dose data, covering the dose from 0.21 to 3.3 mg/kg BW racemic ketoprofen (5.25 to 82.5 times higher, respectively, than the proposed POD of 0.04 mg/kg BW racemic ketoprofen in the rat study). The regression model based on data from healthy humans excluding the highest dose resulted in a R^2 value of 98.1% and a slope of 5.97.

Estimation of EF_{AK} Based on Rat and Human TK Data: Based on the outcome of the linear regression analysis that included the data from male rats and humans, the interspecies TK extrapolation subfactor EF_{AK} is calculated as follows:

$$D_A/D_H=Slope_H/Slope_A=5.97/31.96=0.19$$

Considering that certain vulnerable subpopulations may not be represented in the dataset, an EF_{AK} of 0.4, instead of the 0.19, was adopted.

b. Intraspecies Toxicokinetic Extrapolation Factor (EF_{HK})

Pharmacokinetic variations in the human population are accounted for by the intra-species toxicokinetic extrapolation factor EF_{HK}.

Data Source: Twenty-five sets of data from 17 publications concerning the ketoprofen pharmacokinetics in humans were included in this analysis. Subjects tested in those studies included healthy adult volunteers and those with arthritis or other disease conditions. Ten of those experiments included individual AUC values for S-ketoprofen from each subject; others included only summary statistics. Among the 17 studies, the S-enantiomer dose levels ranged from 0.21 to 3.33 mg/kg BW; 14 used a single dose, and three used repeated dosing.

Available data indicated that human pharmacokinetics of ketoprofen differs with that of rats; specifically, there is an enterohepatic recirculation of ketoprofen in rats but not in humans, and there is limited conversion of R- to S-ketoprofen in humans. Upon oral dosing, the half-life of racemic ketoprofen is shorter in humans (2.62 hours) than that in rats (12.6 hours for S-ketoprofen and 1.37 hours for R-ketoprofen).

There was little difference in AUC values in humans following single doses and repeated doses in most of the published literature, with one exception that was seen in end stage renal disease (ESRD) patients. ESRD patients administered repeated doses of ketoprofen showed significantly different pharmacokinetic profile (a 3-fold difference in AUC values after multiple doses compared to that of those patients receiving a single dose).

Estimate the 50^{th} and 95^{th} Percentile Values of the AUC in Human: The pooled AUC means and standard deviations from the ketoprofen studies in humans were used to estimate the EF_{HK} through Monte Carlo simulation; random values of means and deviations were generated to match the means and deviations reported in published studies. Using this simulation and re-sampling technique, a set of data for each study was generated for each dose level based on the sample size, mean and standard deviation values of the studies at each dose level. For each dose level, 50,000 iterations of the simulation were performed, yielding 50,000 set of EF_{HK} estimates. All the EF_{HK} estimates were then combined by weighting the estimates at each dose level based on their respective sample sizes, and the resulting median value of the combined EF_{HK} estimates across all dose levels was considered the overall estimate for the intra-species EF_{HK}.

Analysis of EF_{HK} following Acute Exposure: Three studies of healthy adults at the dose level of 12.5 mg (0.21 mg/kg BW) were included in the analysis; at 50 mg (0.83 mg/kg BW), four experiments with healthy subjects and seven experiments with subjects in various disease conditions were included. The three experiments at the 12.5 mg dose level had sample sizes of 12, 12, and 24; the two subgroups of the subjects at the 50 mg dose level had samples sizes of 41 and 48, respectively.

The simulation routine was invoked to randomly sample observations from the log-normal distribution matching the specific mean and standard deviation of the experiment. At the 12.5 mg dose level, the 48 records (based on the available cohort size) of the simulated outcome were used to calculate the

50th and 95th percentile. This set of 48 records was one of many plausible sets of AUC values consistent with the reported means and standard deviations from these S-enantiomer experiments at this dose level. Simulations were conducted to assess the level of variability of the EF_{HK} derived from these three experiments, with a total of 50,000 iterations carried out, each having 48 randomly drawn records from log-normal distributions with the reported means and standard deviations of these three experiments. The 50th and 95th percentile values were calculated from each sample, and the distribution of these 50,000 percentile values was calculated.

Dividing the 95th percentile (representing the relatively sensitive population) by the 50th percentile (representing the general population) provided an estimate of the EF_{HK} for human variability in toxicokinetics. At the 12.5 mg ketoprofen dose level, the 50,000 EF_{HK} had a median value of 1.76 with an empirical 95% confidence interval of 1.44–2.27. The same process of determining the EF_{HK} was repeated for 25, 50, 150, and 200 mg dose levels. EF_{HK} was shown to be largely stable across different dose levels, with the median values ranged from 1.41 at the 200 mg dose level to 1.78 at the 25 mg dose level. When all the human S-enantiomer studies from all dose levels available in the human database were combined to estimate an overall EF_{HK}, a new database of 300,000 records were created, based on 50,000 EF_{HK} estimates at each dose level and with each of the six dose groups weighted for their sample sizes. Based on this approach, EF_{HK} was estimated to be 1.73, with a 95% confidence interval of 1.42–2.23.

Those estimates were based on single dose studies in healthy human population. It was shown in a study (Crubb *et al*., Br J Clin Pharmacol. 48(4):494-500. 1999) that in patients with end-stage renal diseases (ESRD), the AUC of the S-ketoprofen in repeated dose of 50 mg (3 times a day for 8 days) was estimated to be 2.4 times higher than the AUC associated with healthy subjects given a single dose. Because the 95th percentile simulated AUCs may not have provided adequate protection for the sensitive subpopulations, an EF_{HK} value of 2.4, as derived for the ESRD subjects, was used in lieu of 1.73.

c. DDEF or Overall Safety Factor

The default toxicokinetic extrapolation factors (EFs) were modified based on analyses of EF_{AK} and EF_{HK} using the rat and human toxicokinetic/ pharmacokinetic data on ketoprofen. The default toxicodynamic extrapolation factors for interspecies (EF_{AD}=2.5) and intraspecies (EF_{HD} =3.16) were applied.

For animal to human extrapolation: $EF_A = EF_{AD} \times EF_{AK} = 2.5 \times 0.4 = 1.0$

For human variability: $EF_H = EF_{HD} \times EF_{HK} = 3.16 \times 2.4 = 7.6$

The overall safe factor or DDEF: $DDEF = EF_A \times EF_H = 1.0 \times 7.6 = 7.6$

The toxicological ADI for total residue of ketoprofen is calculated based on the POD of 0.4 mg/kg BW/day from the two-generation reproductive toxicity study and a safety factor of 7.6. A safety factor of 7.6 was applied to account

for a 1-fold factor for animal-to-human variability and a 7.6-fold factor for human-to-human variability.

$$\text{Toxicological ADI} = \frac{\text{POD}}{\text{Safety Factor}} = \frac{0.04 \text{ mg/kg bw/day}}{7.6}$$
$$= 0.005 \text{ mg/kg bw/day} = 5 \text{ µg/kg bw/day}$$

The calculated toxicological ADI for total residue of ketoprofen is 5 µg/kg BW/day.

4. Acute Reference Dose (ARfD)

A study of ketoprofen in human subjects was chosen as the basis upon which to determine the ARfD. The study (Onset and duration of analgesia for low dose ketoprofen in the treatment of postoperative dental pain. Sunshine, et al. J. Clin. Pharmacol. 38:1155–1164. 1998) evaluated the analgesic efficacy of single dose ketoprofen at three dosages in post-operative dental patients. This clinical investigative study utilized a double-blind, placebo-controlled, and parallel-group design. The results showed that the lowest dose of ketoprofen used in the trial, 6.25 mg, was effective in reducing dental pain. This dose of 6.25 mg was therefore considered as the LOEL/LOAEL for this study. This study was noted to have excluded pregnant women, nursing mothers, and patients with allergy to ketoprofen and other NSAIDs. Also excluded were patients with impairments in renal, hepatic, endocrine, pulmonary, cardiac, neurologic, or cerebral functions, as were patients with peptic ulcerations or other gastrointestinal bleeding. In addition, the exclusion also was extended to patients with many categories of medication or life-style history. The subjects in the human study were presumably given racemic ketoprofen, whereas human exposure to ketoprofen through consuming edible tissues from treated cattle would be exposed to higher proportions of S-ketoprofen. A safety factor of 5 was applied to account for both a lack of NOEL/NOAEL, and human-to-human intraspecies variability in the determination of the ARfD.

LOEL/LOAEL = 6.25 mg/person ÷ 60 kg BW = 0.1 mg/kg BW

$$\text{ARfD} = \frac{\text{LOEL/LOAEL}}{\text{Safety Factor}} = \frac{0.1 \text{ mg/kg bw}}{5}$$
$$= 0.02 \text{ mg/kg bw} = 20 \text{ µg/kg bw}$$

C. Establishment of the Final ADI and ARfD

Because ketoprofen is not an antibacterial agent and has no or negligible antibacterial activity, a microbiological ADI was not needed. The toxicological ADI (5 µg/kg BW/day) is established as the final ADI for total residue of ketoprofen.

20 µg/kg BW is assigned as the ARfD for total residue of ketoprofen.

D. Safe Concentrations for Total Residues in Edible Tissues and Injection Sites

The calculation of the tissue safe concentrations is based on the General Principles for Evaluating the Human Food Safety of New Animal Drugs used in Food-Producing Animals (FDA/CVM Guidance for Industry #3, revised June 2018) and reflects the partition (25% of the ADI to the meat, and 75% of the ADI to the milk) requested by the drug sponsor. The safe concentration of total residues of ketoprofen in each edible tissue of cattle:

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

Table IV.B.4 Safe Concentrations for Edible Tissues

Edible Tissue	Partition	Consumption Values (g)	Safe Concentration (µg/g or ppm)
Meat	25%		
Muscle		300	0.25
Liver		100	0.75
Kidney		50	1.5
Fat		50	1.5
Milk	75%	1500	0.15

Based on an ARfD of 20 µg/kg BW, the safe concentration for total residue of ketoprofen in muscle at the injection sites is calculated as:

Safe Concentration (injection sites)
= (ARfD x Human Body Weight) ÷ Food Consumption Value
= (20 µg/kg BW X 60 kg BW) ÷ 300 g
= 4 ppm

E. Residue Chemistry

1. Summary of Residue Chemistry Studies

   a. Total Residue and Metabolism Studies

   Title: Pivotal Total Residue Depletion Study in Beef Cattle Treated Subcutaneously in the Neck with Three Daily Doses of [^{14}C]-Ketoprofen at a Dose Rate of 3 mg/kg Body Weight/Day. (Study Number A432N-GB-15-339).

   Study Dates: May 15, 2015, to March 8, 2017

   Study Locations: Tranent, East Lothian, United Kingdom and Kalamazoo, MI.

   Study Design:

   Objective: The purpose of this study was to determine the total radioactive residues of [^{14}C]ketoprofen in liver, kidney, muscle, fat, injection site, injection site surround, urine and feces from beef cattle treated subcutaneously in the neck with three daily doses of 3 mg/kg Body Weight/Day of [^{14}C]ketoprofen.

Study Animals: 29 animals were considered for the study. Twelve Simmental X, ten Aberdeen Angus X, five Limousine X, one Charolais and one Luing. 27 animals were selected and randomized for the study. The animals were between 7 and 16 months old and weighted between 230 and 312 kg.

Experimental Design: In a GLP-compliant study, the 27 animals selected for the study were randomly separated into nine groups of three animals each (either two males and one female or two females and one male). The groups were slaughtered at 10, 24, 48, 72, 96, 168, 240, 336, and 432 hours after last dose. Liver, kidney, muscle, fat, injection site and injection site surround (from the site of last injection) tissue samples were collected, processed and stored at -20°C. Urine and feces were collected at 0, 1, 2, 3, 4, 5, 6, 7, and 8 days from the 168-hour slaughtered group, placed in metabolism crates, and stored at -20°C.

Drug Administration: [^{14}C]ketoprofen was administered by three consecutive daily doses of subcutaneous injections. The site of administration of each dose was: dose 1, left side bottom; dose 2, right side; dose 3, left side top of the neck. The target dose was 3.0 mg [^{14}C]ketoprofen/kg Body Weight (BW). The mean dose level achieved across all animals over the 3 doses was 3.16 mg [^{14}C]ketoprofen/kg BW and the range of the dosing was between 3.04-3.50 mg [^{14}C]ketoprofen/kg BW.

Measurements and Observations: Liver, kidney, muscle, injection site and injection site surround samples aliquots were analyzed for total radioactivity by combustion followed for by liquid scintillation count (LSC). Fat samples aliquots were sonicated and analyzed by LSC. Aliquots of urine samples were analyzed directly by LSC, while fecal samples were homogenized with water and allowed to dry before combustion and LSC.

Results: The administered dose was primarily excreted in urine (92.7%) and feces (10%). The mean recovery of total radiolabeled residue (TRR) in urine during the three dosing days was about 30% and dropped to less than 1% for the remainder of the collection period. The mean recovery of TRR in feces was 2-3% during the dosing period and between 0.1-1.5% for the rest of the collection period.

From the edible tissues, kidney had the highest observed concentrations at 10, 24 and 48 hrs., followed by liver, fat and muscle (Table IV.E.1.a.1). The highest mean TRR concentrations in tissues were observed in injection site and injection site surround at 10 hrs., decreasing rapidly thereafter (Table IV.E.1.a.2).

Table IV.E.1.a.1: Concentrations of Total Radioactive Residues (mean ± standard deviation; in µg equiv./kg, parts per billion (ppb)) in Edible Tissues, from Cattle Treated with Three Daily doses of 3 mg/kg Body Weight of [^{14}C]Ketoprofen.

Withdrawal Period (hours)	Liver	Kidney	Muscle	Fat
10	762 ± 28	3323 ± 858	67 ± 14	270 ± 79
24	159 ± 44	767 ± 432	10 ± 5	49 ± 22
48	71 ± 8	132 ± 10	3 ± 1	54 ± 26
72	62 ± 8	91 ± 18	3 ± 1	16 ± 6
96	64 ± 9	75 ± 17	2 ± 1	17 ± 14
168	44 ± 10	56 ± 6	2 ± 1	25 ± 9
240	26 ± 6	31 ± 5	2 ± 1	56 ± 47
336	25 ± 1	35 ± 4	1 ± 0	18 ± 13
432	17 ± 2	20 ± 3	2 ± 1	10 ± 9

Table IV.E.1.a.2: Concentrations of Total Radioactive Residues (mean ± standard deviation; in µg equiv./kg, ppb) in Injection Site and Injection Site Surround, from Cattle Treated with Three Daily doses of 3 mg/kg Body Weight of [^{14}C]Ketoprofen.

Withdrawal Period (hours)	Injection Site	Injection Site Surround
10	47658 ± 9531	10412 ± 13201
24	2226 ± 909	339 ± 392
48	177 ± 52	25 ± 17
72	238 ± 171	15 ± 10
96	177 ± 90	14 ± 10
168	168 ± 147	6 ± 6
240	70 ± 43	22 ± 14
336	66 ± 44	3 ± 0
432	56 ± 37	13 ± 13

Aliquots of tissue samples were analyzed for parent ketoprofen by LC-MS/MS. Only samples with total radioactivity residue concentrations of >500 dpm/g were analyzed. The results are presented in Table IV.E.1.a.3.

Table IV.E.1.a.3: Mean Concentrations of Ketoprofen (mean ± standard deviation; in µg/kg, ppb) in Bovine Edible Tissues, Injection Site and Injection Site Surround from Cattle Treated with Three Daily doses of 3 mg/kg Body Weight of [^{14}C]Ketoprofen.

Withdrawal Period (hours)	Liver	Kidney	Muscle	Fat	Injection Site	Injection Site Surround
10	38.4 ± 41.5	2087 ± 336	34.7 ± 10.6	143.8 ± 71.0	46533 ± 18390	13642 ± 17039
24	BLOQ	215 ± 102	BLOQ	9.8 ± 6.7	1522 ± 995	546 ± 525
48	5.8	31.4 ± 9.1	N/A	BLOQ	28.9 ± 11.1	21.1*
72[#]	-	-	-	-	-	-
96	N/A	9.5 ± 4.2	N/A	N/A	11.9*	N/A
168	N/A	8.8*	N/A	N/A	6.4*	N/A

BLOQ: Below Limit of Quantitation, 5 ppb.
N/A: Not Applicable. Samples were not analyzed because total radiolabeled residue were below threshold of 500 dpm/g.
* Reflects result from one sample the was above LOQ.
[#] These samples were left at room temperature and were not considered for the analysis of parent ketoprofen.

Title: Residue Profiling of Tissues and Excreta Collected from the Pivotal Total Radioactive Residue Depletion Study in Beef Cattle Treated Subcutaneously in the Neck with Three Daily Doses of [^{14}C]Ketoprofen at a Dose Rate of 3 mg/kg Body Weight/Day. (Study Number A432N-US-15-356).

Study Dates: June 26, 2015, to April 19, 2016

Study Location: Kalamazoo, MI

Study Design:

Objective: The objective of the study was to profile tissues and excreta collected from the total radioactive residue depletion study (Study Number A432N-GB-15-339) described above.

Experimental Design: In a GLP-compliant study, excreta and tissue samples generated in Study Number A432N-GB-15-339 were processed for metabolite profiling by HPLC when levels of total radioactivity were >500 dpm/g. Standards for ketoprofen and ketoprofen metabolite ZTS-00102401 were used to verify retention times.

Results: Parent [^{14}C]ketoprofen, and metabolites ZTS-00102401 (dihydro ketoprofen), M2 (isomer of hydroxy ketoprofen) and M5 exceeded 5% of the total urine profile, while parent [^{14}C]ketoprofen and ZTS-00102401 were the most abundant residues identified in feces. Analysis of edible

tissues in cattle yielded, in addition to parent [^{14}C]ketoprofen, two metabolites that were considered major metabolites based on their concentrations. Metabolite ZTS-00102401 (dihydro ketoprofen) was the most abundant metabolite present in all edible tissues at the earliest withdrawal period (10 h) and in multiple edible tissues at later withdrawal periods. Metabolite M1 (isomer of hydroxy ketoprofen) presence was detected in liver at 10-, 24-, and 48-h withdrawal periods, and kidney at 10-, 24-, 48-, and 96-h withdrawal periods. In addition, metabolite M2 was observed in liver at 10- and 24-h withdrawal. Photodecomposition products of parent [^{14}C]ketoprofen were identified in the dose formulation, M4 (3-acetyl benzophenone) and M7 (dimer of ketoprofen).

b. Comparative Metabolism Study

Title: Comparative Metabolite Profiling and Identification of [^{14}C]Ketoprofen in Rat and Bovine Liver Microsomes. (Study Number A4X4R-US-20-720).

Study Date: November 10, 2020

Study Location: Indianapolis, IN

Study Design:

Objective: The objective of the study was to investigate the comparative *in vitro* metabolism of [^{14}C]ketoprofen in liver microsomes from rat and bovine by radioprofiling and to characterize the observed metabolites by mass spectrometry.

Test System: Mixed gender Sprague Dawley rat liver microsomes and mixed gender bovine (*Bos taurus*) liver microsomes were used in the study.

Experimental Design: In a non-GLP compliant study, rat and bovine microsome solutions at 1 or 2 mg/mL protein concentration were incubated with 2 or 5 µM ketoprofen solution containing [^{14}C]ketoprofen at 37°C for different timepoints.

Measurements and Observations: Parent ketoprofen and metabolites identification was performed using Accurate mass LC/MS and LC-MS/MS in both positive and negative ion modes.

Results: In addition to parent ketoprofen, metabolites ZTS-00102401 (dihydro ketoprofen), M1 and M2 (isomers of hydroxy ketoprofen), were identified in rat and bovine liver microsomes. These results indicated that laboratory animals used in the toxicological studies were exposed to the same metabolites to which humans will be exposed when consuming edible products derived from treated animals (auto-exposure). Results are summarized in Table IV.E.1.b.1.

Table IV.E.1.b.1: % Radioactivity Among Metabolites Identified in 1 mg/mL Rat and Bovine Liver Microsomes Incubated With 5 µM [^{14}C]Ketoprofen for 0 and 1 hour.

Peak	Rat Liver Microsomes 0 hr	Rat Liver Microsomes 1 hr	Bovine Liver Microsomes 0 hr	Bovine Liver Microsomes 1 hr
Ketoprofen	96.58	95.22	96	82.91
ZTS-00102401	NQ	0.84	0.99	13.82
M1	NQ	0.49	0.05	0.08
M2	NQ	0.32	0.13	0.40

NQ: Not quantifiable (threshold 10 cpm in peak height).

c. Study to Establish Withdrawal Period

Tissue Residue Depletion Study

Title: Pivotal Tissue Residue Decline Study in Beef Cattle Treated Subcutaneously in the Neck with 3 Daily Doses of Ketoprofen at a Dose Rate of 3 mg/kg Body Weight/day. (Study Number A230N-US-17-572).

Study Dates: May 22, 2018, to June 29, 2018

Study Location: Tulare, CA

Study Design:

Objective: The purpose of the study was to measure concentrations of ketoprofen in edible cattle tissues (muscle, liver, kidney, fat, injection site, and injection site surround) at 12, 24, 48, 72, 96, and 120-hour withdrawal after 3 once-daily subcutaneous injections of ketoprofen (3 mg/kg BW).

Study Animals: Twenty-six animals representative of U.S. commercial production between 8 to 14 months of age and weighting between 402 to 465 kg at the time of dosing were used in the study.

Experimental Design: In a GLP-compliant study, animals were assigned randomly to six groups that received the same treatment regimen but had different withdrawal periods. Group 1, formed by 3 females and 3 males, was slaughtered at 12 hours after receiving the last dose. Groups 2 to 6 were formed by 2 females and 2 males, and were slaughtered at 24, 48, 72, 96, and 120 hours after last dose respectively. Liver, kidney, muscle, fat, injection site and injection site surround (from the site of last injection) were collected, processed and stored at -20°C.

Drug Administration: ketoprofen was administered as three consecutive daily doses of subcutaneous injections. The sites of administration were on the left, right, right, and left side of the neck. The target dose was 3.0 mg ketoprofen/kg Body Weight. The mean dose level achieved across all animals over the 3 doses was 3.08 mg ketoprofen/kg Body Weight and the range of the dosing was between 3.00-3.17 mg ketoprofen/kg BW.

Measurements and Observations: Kidney, liver, muscle, fat, injection site and injection site surround samples aliquots were analyzed by a validated LC-MS/MS method. The method's limit of quantitation (LOQ) was 5 µg/kg (5 ppb).

Results: Kidney, liver, muscle, fat, and injection site samples from all animals on the study were analyzed. Values that were below the limit of quantitation of 5 µg/kg were reported as BLOQ. The results are presented on Tables IV.E.1.c.1 and IV.E.1.c.2. The mean ketoprofen (marker residue) residue concentration in kidney declined from 1,245 µg/kg at 12 hours withdrawal time to 123 and 9 µg/kg at 24 and 48 hours withdrawal time respectively. Only one animal at 72 hours withdrawal time contained ketoprofen residues concentration above LOQ, 7.68 µg/kg ketoprofen.

Table IV.E.1.c.1: Mean Concentrations of Ketoprofen (mean ± standard deviation; in µg/kg, ppb) in Edible Cattle Tissues Treated with Three Daily doses of 3 mg/kg Body Weight of Ketoprofen.

Withdrawal Period (hours)	Kidney	Liver	Muscle	Fat
12	1245 ± 741	12 ± 5	26 ± 9	41 ± 19
24	123 ± 74	85 ± 107	BLOQ	8 ± 3
48	9 ± 3	BLOQ	BLOQ	BLOQ
72	7.68*	BLOQ	BLOQ	BLOQ
96	BLOQ	BLOQ	BLOQ	BLOQ
120	BLOQ	BLOQ	BLOQ	BLOQ

BLOQ: Below Limit of Quantitation, 5 ppb.
* Reflects result from one sample the was above LOQ.

Table IV.E.1.c.2: Mean Concentrations of Ketoprofen (mean ± standard deviation; in µg/kg, ppb) in Cattle Injection Site and Injection Site Surround Tissues from Treated with Three Daily doses of 3 mg/kg Body Weight of Ketoprofen.

Withdrawal Period (hours)	Injection Site	Injection Site Surround
12	64551 ± 76525	2624 ± 4800
24	5215 ± 5114	31.2 ± 18.7
48	181 ± 146	BLOQ
72	34 ± 27	BLOQ
96	32 ± 0.8	BLOQ
120	15 ± 5.7	BLOQ

BLOQ: Below Limit of Quantitation, 5 ppb.

Conclusion: Tissue residue data from study A230N-US-17-572 were analyzed using a statistical tolerance limit algorithm that determines the upper tolerance limit for the 99th percentile of the population with 95% confidence. The data support assignment of a 48-hour withdrawal period. A withdrawal period of 48 hours is consistent with depletion of residues at the injection site.

2. Target Tissue and Marker Residue

   Data presented in the total radioactive residue study (Study Number A432N-GB-15-339) show that kidney is the edible tissue in cattle in which residues of ketoprofen are highest and persist longest. Thus, the target tissue is kidney and the marker residue is ketoprofen.

3. Tolerance

   The tolerance assigned to ketoprofen in cattle kidney is 360 µg/kg or 0.36 ppm, representing 0.24% of the kidney safe concentration for total radiolabeled residues. Ketoprofen concentration data in kidney from the residue depletion study (Study Number A230N-US-17-572) showed that all the samples evaluated at 48 hours withdrawal yielded a ketoprofen concentration lower than the bovine kidney tolerance (mean = 9 ± 3 µg/kg).

4. Withdrawal Period

   Bovine Kidney residue depletion data from Study Number A230N-US-17-572 were analyzed using a statistical algorithm that calculated the upper tolerance limit for kidney ketoprofen concentrations for the 99^{th} percentile with 95% confidence. The data support the assignment of 48-hour withdrawal period.

F. Analytical Method for Residues

1. Description of Analytical Method

   a. Determinative Procedure

      Homogenized cattle kidney (1 g) is fortified with the internal standard (ketoprofen-D3) and extracted with 5 mL of a mixture of acetonitrile and 10 mM aqueous ammonium acetate pH 9 (80:20; v/v). An aliquot (2 mL) of the tissue extract is diluted to 10 mL and processed by solid phase extraction (SPE), evaporation of eluate, and reconstitution in 50:50 acetonitrile:0.1% aqueous formic acid (v/v). The extract is analyzed for ketoprofen by negative-ion electrospray ionization LC-MS/MS. The following transitions are monitored for quantitation:

      Ketoprofen: 253 → 209
      Ketoprofen-D3: m/z 256 → m/z 212

   b. Confirmatory Procedure

      Sample extraction for the confirmatory procedure is identical to the one for the determinative procedure. Ketoprofen is detected by LC-MS/MS in the positive-ion mode. The following ketoprofen-specific ion transitions are monitored to obtain ion ratios, signal to noise ratios and retention time reproducibility that meet the required acceptability criteria:

      m/z 255 → m/z 209 (reference ion)
      m/z 255 → m/z 194
      m/z 255 → m/z 177

2. Availability of the Method

   The validated analytical method for analysis of residues of ketoprofen is on file at the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855. To obtain a copy of the analytical method, please submit a Freedom of Information request to: https://www.accessdata.fda.gov/scripts/foi/FOIRequest/requestinfo.cfm

V. USER SAFETY

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

"USER SAFETY WARNINGS: Not for human use. Keep this and all drugs out of the reach of children. The Safety Data Sheet (SDS) provides more detailed occupational safety information. To obtain a Safety Data Sheet contact Zoetis Inc. at 1-888-963-8471."

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that KETOFEN®, when used according to the label, is safe and effective for the control of pyrexia associated with bovine respiratory disease (BRD). Additionally, data demonstrate that residues in food products derived from species treated with KETOFEN® will not represent a public health concern when the product is used according to the label.

A. Marketing Status

This product may be dispensed only by or on the order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required to monitor the safe use of this product.

B. Exclusivity

This supplemental approval for KETOFEN® qualifies for THREE years of marketing exclusivity under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act because the supplemental application included safety and effectiveness studies. This exclusivity begins as of the date of our approval letter and only applies to the indication for the control of pyrexia associated with bovine respiratory disease in beef heifers, beef steers, beef calves two months of age and older, beef bulls, replacement dairy heifers, and dairy bulls. Not for use in reproducing animals over one year of age, dairy calves, or veal calves. Not for use in lactating dairy cattle or calves less than two months of age.

C. Supplemental Applications

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

D. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.