CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22382Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-382**
SERIAL NUMBER:	**000 (1st submission)**
DATE RECEIVED BY CENTER:	**12/05/08**
PRODUCT:	**Ketorolac Tromethamine Nasal Spray**
INTENDED CLINICAL POPULATION:	**Management of moderate to severe pain (short-term)**
SPONSOR:	**Roxro Pharma, Inc.**
DOCUMENTS REVIEWED:	**Module 4, Vol. 1-6**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Newton H. Woo, Ph.D.**
PHARM/TOX SUPERVISOR:	**Adam Wasserman, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Jessica Benjamin**

Date of review submission to Document Archiving, Reporting & Regulatory Tracking System (DAARTS): August 11, 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical pharmacology and toxicology perspective, NDA 22-382 may be approved.

B. Recommendation for nonclinical studies

No additional nonclinical studies are required at this time based upon the materials reviewed.

(b) (4)

[redacted]

(b) (4)

II. Regulatory Background

Ketorolac tromethamine is a nonsteroidal, anti-inflammatory drug (NSAID) with potent analgesic and moderate anti-inflammatory activity. Originally marketed as Toradol®, ketorolac tromethamine was first approved for intramuscular IM and intravenous IV injection (NDA 19,698; November 1989) and then as oral tablets (NDA 19-645; December 1991). For IM and IV formulations, the indication is for the short-term (<5 days) management of moderately severe acute pain that requires analgesia at the opioid level with the approved dose of 30 mg every 6 hr, with a maximum daily dose not exceeding 120 mg. Current labeling states that ketorolac tromethamine is not indicated for use in pediatric patients. Both NDA

applications were originally submitted by Syntex Corporation but are currently owned by Hoffman-La Roche. Since then NDA 19-698 was withdrawn in 2005. In a Federal register notice that was published in December 19, 2008, it was stated that NDA 19-645 was not withdrawn for reasons of safety or effectiveness. Although Toradol® is no longer marketed, generic ketorolac tromethamine injection products are still available in the United States as are oral tablets and ophthalmic drops.

As an addition to other parenteral routes of administration, Roxro has recently developed the first intranasal formulation of ketorolac tromethamine that produces a pharmacokinetic profile that is within approved IM repeat administration of ketorolac (between 15 mg and 30 mg). For this change in route of administration, a disposable, single-day, multi-dose spray (15% w/w ketorolac tromethamine solution) has been designed to dispense a total of 8 sprays, 1 spray (100 μL, 15.75 mg) to each nostril per dose, for a total of 4 doses (126 mg) and then to be discarded. Roxro first initiated the development of Sprix® under IND 62,829 and have now submitted a 505(b)(1) application seeking approval for the nasal route of administration of ketorolac tromethamine for the indication of short-term (up to 5 days) management of moderate to severe (b) (4) pain in adults that requires analgesia at the opioid level. Roxro has submitted a request for the deferral of pediatric studies to be initiated at a later date. To support this NDA submission (NDA 22-382), Roxro has provided a written right of reference from Hoffmann-La Roche Inc. for NDA 19-645 and NDA 19-698 and has also conducted nonclinical studies to support the change the route of administration and qualify the safety of an identified degradant.

III. Summary of Nonclinical Findings

A. Brief overview of nonclinical findings

To support the intranasal route of administration of ketorolac tromethamine, local tolerance and repeat-dose toxicology studies of up to 28 days in rats and rabbits were conducted. Drug class target organ toxicities, notably gastrointestinal toxicities and renal changes, were observed as previously identified for this and other NSAIDS, but there were no indications of adverse local toxicity in the nasal cavity or respiratory tract or additional safety concerns that arose from the nasal route of administration. Based on these studies, the maximum nasal exposure associated with the animal NOAELs were at least as great as those anticipated in humans, yielding acceptable exposure margins despite the reduced dosing frequency in the animal studies. Systemic exposure levels measure in the rat 28-day study were at least 3-times the maximum recommended human daily dose.

An oxidative degradant identified as (±)-5-benzoyl-1-keto-2,3-dihydro-1H-pyrrolizine (1-keto) exceeded the qualification thresholds as outlined in the "Guidance for Industry: Q3B(R2) Impurities in New Drug Products." As a result, additional studies were conducted and deemed acceptable by this reviewer for the safety qualification of the 1-keto specifications set by stability test results. In two

genotoxicity assays, the degradant tested negative in the Ames (bacterial reverse mutation) assay but positive in the *in vitro* chromosomal aberration assay in CHO cells under conditions of metabolic activation. It is notable that ketorolac itself demonstrated clastogenicity in the chromosomal aberration assay as described in the approved label. In consultation with the Informatics and Computational Safety Analysis Staff (ICSAS) at FDA, computational toxicology analysis with the 1-keto degradant revealed positive predictions for mutagenicity and chromosomal aberrations but negative predictions for carcinogenicity in both the rat and mouse models. Given the (b) (4) indication duration of 5-days, the similarity of findings compared to ketorolac, the negative prediction in carcinogenicity potential by computational toxicology analysis and a negative result in the Ames assay, additional genotoxicity or mechanistic studies were not subsequently required. In addition, a 14-day repeat-dose toxicology study in rats comparing ketorolac tromethamine spiked with 1-keto degradant showed no degradant- related toxicities when compared to ketorolac alone. Taken together, the presence of 1-keto at the proposed maximum specification of NMT (b) (4) HPLC area (b) (4) (b) (4) is not expected to present a significant risk to the intended clinical population.

Analysis of extractables and leachables of the nasal drug product revealed no detectable quantities (detection limit of (b) (4)) of any chemical impurities from the vial or pump device.

B. Pharmacologic activity

Although no new pharmacology studies were conducted for this NDA, it is well established that ketorolac tromethamine, a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory, and anti-pyretic activity, exerts its actions through the inhibition of prostaglandin synthesis by acting as a non-selective competitive blocker of the enzyme cyclooxygenase. Because prostaglandins is involved in multiple homeostatic processes, ketorolac tromethamine like other NSAIDS may induce adverse events involving the gastrointestinal (peptic ulcers, gastrointestinal bleeding, perforation of the stomach or intestines), renal (renal papillary necrosis, renal injury) and blood-clotting systems. Ketorolac tromethamine provides relief from moderate to severe pain and has similar analgesic efficacy to low doses of morphine and meperidine.

C. Nonclinical safety issues relevant to clinical use

There are no outstanding nonclinical safety issues related to the clinical use of the ketorolac tromethamine intranasal solution in the intended clinical population. Toxicology studies demonstrated that administration of ketorolac tromethamine by the intranasal route does not induce local toxicity in the nasal passages and respiratory organs suggesting short-term clinical use would not induce significantly adverse events to the nasal passageway. Furthermore the studies conducted to qualify the safety of the 1-keto degradant demonstrated that this degradant does not pose a significant additional risk to the intended population.

Although both rats and rabbits showed a lack of local toxicity in the nasal passageway and respiratory organs, it is important to note there are differences in the dosing regimen (i.e. more frequent dosing in humans as compared to the dosing regiment used in the animal studies) as well as differences in the anatomy and physiology of the nasal organ between animal models and humans. It was noted in prior clinical trials that there were abnormal nasal exams in a minority of elderly patients. Therefore, it is recommended that local toxicity to the nasal passageways is monitored in patients.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

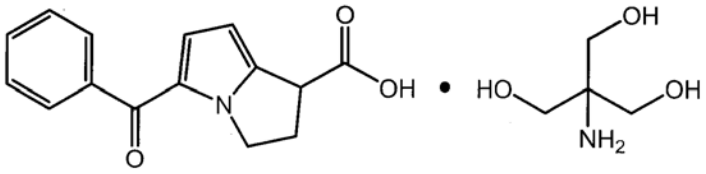
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-382
Review number: 001
Sequence number/date/type of submission: 000/9-JAN-09/Paper
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: ROXRO PHARMA, Inc.
Manufacturer for drug substance: (b) (4)

Reviewer name: Newton H. Woo, Ph.D.
Division name: Division of Anesthesia, Analgesia and Rheumatology Products
HFD #: 170
Review completion date: August 11, 2009

Drug:

Trade name: Sprix®
Generic name: Ketorolac Tromethamine
Code name: LAC-13 (drug substance); ROX-888 (drug product)
Chemical name: 5-(benzoyl)2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxylmethyl)-1,3-proanediol
CAS registry number: 74103-07-4
Molecular formula/molecular weight: 376.40 / $C_{19}H_{24}N_2O_6$
Structure:



Relevant INDs/NDAs/DMFs:

NDA 19-698 (Toradol® IV/IM, Syntex Inc.)
NDA 19-645 (Toradol® Oral, Syntex Inc.)
IND 62,829 (Ketorolac Tromethamine nasal spray; ROXRO PHARMA, Inc.)
DMF (b) (4)
DMF (b) (4)
DMF (b) (4)

Drug class: Non-steroidal anti-inflammatory drug (NSAID)

Intended clinical population: Short-term (up to 5 days) management of moderate to severe pain that requires analgesia at the opioid level in adults.

Clinical formulation and recommended dosage & usage: Clinical formulation of the drug product is summarized below (provided by the sponsor). The drug product is formulated as a multidose, aqueous solution of ketorolac tromethamine with several excipients and is bottled in a Type I clear glass bottle with an attached 100 µL metered pump/snap-on spray closure for intranasal administration.

The metered dose pump is a disposable, single-day, multi-dose spray (15% w/w ketorolac tromethamine solution) designed to dispense a total of 8 sprays, 1 spray (100 µL, 15.75 mg) to each nostril per dose, for a total of 4 doses (126 mg) and then to be discarded. One bottle per day is to be used for a maximum of 5 days. It should be noted that an overfill (b) (4) (b) (4) exists to accommodate five priming sprays.

Table 2.3.P.1-1. Drug Product Unit Composition

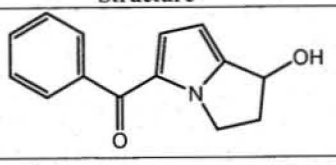
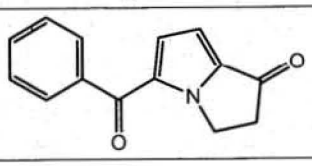
Ingredient	Amount mg/spray[a]	(b) (4)	(b) (4)	(b) ( )	mg/bottle[c] 8 sprays	(b) (4)	Function
Drug Substance:							(b) (4)
Ketorolac Tromethamine USP	15.75	(b)			126.0		
Excipients:		(b)					
Edetate Disodium USP	(b)	(4)			(b)		
Monobasic Potassium Phosphate NF	(b)		(b)	((b)	(b)		
Sodium Hydroxide NF	pH 7.( )	(b)	(b) (4)	(b) (b)	pH ( )		
(b) (4) Water for Injection USP	q.s. ad	(q.)	(b) (4)	( ) (b	q.s. ad		
Total	105	(b	(b)	(b))	840.0		

[a] Based on a nominal spray of 100 uL (=105 mg) per actuation.

(b) (4)

Excipients
There are no novel excipients and all identified excipients are within limits stated in the inactive ingredient guide for FDA approved drug products through the intranasal route.

Stability
Ketorolac tromethamine is degraded predominantly through an oxidative mechanism that produces two major degradation products (see below Table provided by the sponsor) referred to as 1-keto and 1-hydroxy compound.

Name(s)	Structure
(±)-5-benzoyl-1-hydroxy-2,3-dihydro-1H-pyrrolizine (1-hydroxy compound)	
(±)-5-benzoyl-1-keto-2,3-dihydro-1H-pyrrolizine (1-keto compound)	

Stability testing results have revealed the levels of the degradants increase over time, particularly the 1-keto degradant and to a lesser extent the 1-hydroxy compound. From stability tests, it was determined that the levels of the 1-keto degradant exceeded the qualification thresholds stated in the "Guidance for Industry: Q3B(R2) Impurities in New Drug Products" (For a more detailed review, please see CMC review by Dr. Joseph Leginus). Consequently several additional studies were conducted to qualify the 1-keto degradant at the proposed maximum specification of NMT (b) (4) HPLC area (b) (4) which included genotoxicity and repeat-dose toxicity studies. Results of these qualification studies are discussed in-depth in Section 2.6.6 Toxicology.

Extractables and Leachables

No detectable leachables were observed in the stability samples (with a limit of quantitation of (b) (4) which include coverage of up to 18 months of upright and horizontal/inverted storage at 5 C and 6 months of upright and horizontal/inverted storage at 25°C/60% relative humidity. Based on the maximal total daily intake of the drug product (8 x 100 µL), the quantitation limit of (b) (4) corresponds to (b) (4) which is less than the recommended Qualification Threshold of 5 µg per day for an individual leachable in a nasal drug product[1].

Route of administration: Drug product (15% w/w solution of ketorolac tromethamine) is to be delivered through intranasal administration via nasal spray, with 15.75 mg ketorolac tromethamine per actuation (100 µL) per nostril to provide a total dose of 31.5 mg (total of 2 actuations, one to the left and right nostril) per dose, up to 4 doses per day. One bottle per day is to be used for a maximum of 5 days.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Study/Study Title	Volume	Page
TOXICOLOGY		
General toxicology		
KET001 – Ketorolac Tromethamine MTD and 5 Day Intranasal Tolerance Study in Rats	1	1
KET002 – Ketorolac Tromethamine Containing 0.9% 1-keto Degradant: 14 Day Intranasal Toxicity Study in Rats with a 14 Day Recovery Period	2,3	1
KET003 - Ketorolac Tromethamine: 28 Day Intranasal Toxicity Study in Rats with a 28 Day Recovery Period	4,5	1
Genetic toxicology		
KET005 – 1-Keto Degradant: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 100, TA 1537 and TA98 and *Escherichia coli* WP2*uvr*A	6	1
KET006 – 1-Keto Degradant: Chromosome aberrations Assay with Chinese Hamster Ovary Cell Cultures *In Vitro*	6	1

[1] According to the Product Quality Research Institute (PQRI) Leachables and Extractables Working group (Ball et al, 2007).

Local Tolerance		
KET004 – 14-Day Nasal Tolerance Study of Ketorolac Tromethamine Intranasal Formulations in Male and Female Rabbits	6	1
KET007 – Ketorolac Tromethamine: Nasal Absorption and Local Tolerance Study in Rabbits	6	1

Studies <u>not</u> reviewed within this submission: None

2.6.2 PHARMACOLOGY

No new pharmacology studies were conducted for this NDA since there is extensive previous clinical and nonclinical experience with oral ketorolac and similar oral drugs in the NSAID class.

2.6.2.1 Brief summary

Prior pharmacology studies have indicated and subsequently confirmed that ketorolac is a potent analgesic and anti-inflammatory compound acting as a nonspecific COX inhibitor.

2.6.2.2 Primary pharmacodynamics

<u>Mechanism of action</u>:
The mechanism of action of ketorolac, like that of other NSAIDS, is likely through the inhibition of prostaglandin biosynthesis by acting as an inhibitor of the cyclooxygenase pathway of arachidonate metabolism. Normally after injury or trauma, arachidonic acid is formed by phospholipase activity, which is then converted to PGH_2 via PGG_2. Ketorolac blocks cyclooxygenase enzyme that is responsible for converting arachidonic acid to PGG_2, thus inhibiting formation of prostaglandins, which are thought to upregulate the sensitivity of peripheral nociceptors to cause pain.

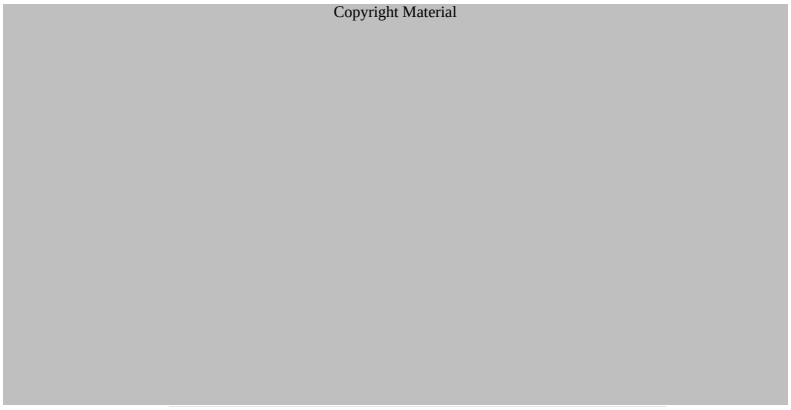


Figure 1: Inhibition of prostaglandin synthesis by Ketorolac.
Adapted from Huntjen et al, 2005

Compared to other NSAIDS, ketorolac tromethamine is relatively a potent inhibitor of cyclooxygenase (COX) isoenzymes (See Figure 1), which inhibits both COX-1 and COX-1. Unlike other NSAIDS such as acetaminophen, naproxen and ibuprofen that have low or moderate potency (Figure 1), ketorolac tromethamine provides analgesia comparable to that of

opioids, such as morphine sulfate and meperidine hydrochloride, but without opioid like side effects (Gillis and Brogden, 1997).

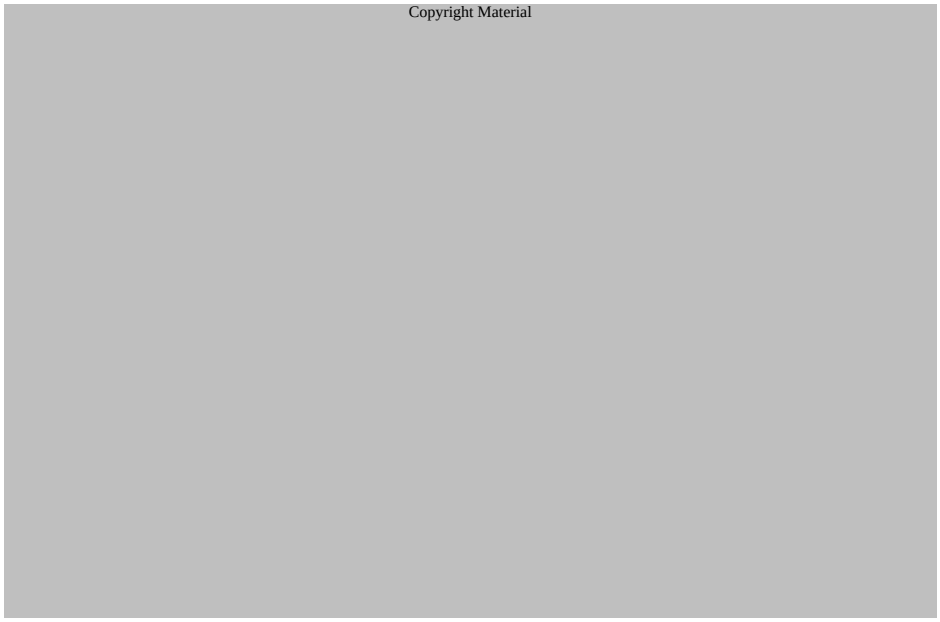


Figure 1: Relative potency and selectivity of various NSAIDS. Concentrations required to inhibit the activity of cylooxygenase 1 and cyclooxygenase 2. Taken from (Radhofer-Welte and Rabasseda, 2000).

Of the two major forms of COX isoenzymes, COX-1, expressed constitutively in cells notably in platelets, enthothelial cells, the GI tract, renal microvasculature, glomerulus, and collecting ducts, is important for homeostatic processes that include regulation of gastric acid secretion, platelet aggregation, and blood flow to the kidney and stomach. Inhibition of COX-1 is believed to be the major mediator of NSAID induced GI toxicity. COX-2 is mostly an inducible form that is believed to play an important role during pain and inflammatory stimuli transduction.

Drug activity related to proposed indication:
Analgesic effects of ketorolac are primarily thought to be the result of cyclooxygenase inhibition, which is known to convert arachidonic acid to endoperoxides, the precursors of prostaglandins, prostacyclin and thromboxanes. By reducing prostaglandin and thromboxanes synthesis, COX inhibition by ketorolac blocks the nociceptive response to endogenous mediators of inflammation arising from tissue injury or insult.

2.6.2.3 Secondary pharmacodynamics
Preclinical studies also indicate that ketorolac influences the actions of Substance P (Ma and Eisenach, 2003) and FAAH (Fowler et al., 1999), endogenous substances related to pain mechanisms. Ketorolac may also exert its analgesic activity via its effects on the central nervous system. Previous studies in various *in vitro* and animal models demonstrate central actions of ketorolac, and propose these central actions may arise from distinct mechanisms that include modulation of opioid peptides (Michel et al., 1996), inhibition of serotonin release (Kaube et al., 1993) or blockade of N-methyl-D-aspartate receptors (Sotgiu et al, 1998).

2.6.2.4 Safety pharmacology

ROXRO has not conducted any nonclinical safety studies for ketorolac since it has been previously approved by the FDA and extensive clinical experience has accumulated. Potential adverse reactions, which are similar to those of other potent NSAIDs, involve the gastrointestinal (peptic ulcers, gastrointestinal bleeding, perforation of the stomach or intestines) and renal (renal papillary necrosis, renal injury) systems. Ketorolac also inhibits platelet aggregation and as a result may prolong bleeding time.

2.6.2.5 Pharmacodynamic drug interactions

No nonclinical drug interaction studies were submitted with this NDA. The following information is reproduced from the latest FDA approved TORADOL® label dated 11 November 2007.

> Ketorolac is highly bound to human plasma protein (mean 99.2%). There is no evidence in animal or human studies that TORADOL induces or inhibits hepatic enzymes capable of metabolizing itself or other drugs. Warfarin, Digoxin, Salicylate, and Heparin The in vitro binding of *warfarin* to plasma proteins is only slightly reduced by ketorolac tromethamine (99.5% control vs 99.3%) when ketorolac plasma concentrations reach 5 to 10 µg/mL. Ketorolac does not alter *digoxin* protein binding. In vitro studies indicate that, at therapeutic concentrations of *salicylate* (300 µg/mL), the binding of ketorolac was reduced from approximately 99.2% to 97.5%, representing a potential twofold increase in unbound ketorolac plasma levels. Therapeutic concentrations of *digoxin, warfarin, ibuprofen, naproxen, piroxicam, acetaminophen, phenytoin* and *tolbutamide* did not alter ketorolac tromethamine protein binding.
>
> In a study involving 12 adult volunteers, TORADOLORAL was coadministered with a single dose of 25 mg *warfarin*, causing no significant changes in pharmacokinetics or pharmacodynamics of warfarin. In another study, ketorolac tromethamine dosed IV or IM was given with two doses of 5000 U of *heparin* to 11 healthy volunteers, resulting in a mean template bleeding time of 6.4 minutes (3.2 to 11.4 min) compared to a mean of 6.0 minutes (3.4 to 7.5 min) for heparin alone and 5.1 minutes (3.5 to 8.5 min) for placebo.
> Although these results do not indicate a significant interaction between TORADOL and warfarin or heparin, the administration of TORADOL to patients taking anticoagulants should be done extremely cautiously, and patients should be closely monitored (see **WARNINGS** and **PRECAUTIONS: Hematologic Effect**).
>
> The effects of warfarin and NSAIDs, in general, on GI bleeding are synergistic, such that the users of both drugs together have a risk of serious GI bleeding higher than the users of either drug alone.
>
> Aspirin
> When TORADOL is administered with aspirin, its protein binding is reduced, although the clearance of free TORADOL is not altered. The clinical significance of this interaction is not known; however, as with other NSAIDs, concomitant administration of ketorolac tromethamine and aspirin is not generally recommended because of the potential of increased adverse effects.
>
> Diuretics
> Clinical studies, as well as postmarketing observations, have shown that TORADOL can reduce the natriuretic effect of furosemide and thiazides in some patients. This response has been attributed to inhibition of renal prostaglandin synthesis. During

concomitant therapy with NSAIDs, the patient should be observed closely for signs of renal failure (see **WARNINGS: Renal Effects**), as well as to assure diuretic efficacy.

Probenecid
Concomitant administration of TORADOLORAL and *probenecid* resulted in decreased clearance and volume of distribution of ketorolac and significant increases in ketorolac plasma levels (total AUC increased approximately threefold from 5.4 to 17.8 µg/h/mL) and terminal half-life increased approximately twofold from 6.6 to 15.1 hours. Therefore, concomitant use of TORADOL and probenecid is contraindicated.

Lithium
NSAIDs have produced an elevation of plasma lithium levels and a reduction in renal lithium clearance. The mean minimum lithium concentration increased 15% and the renal clearance was decreased by approximately 20%. These effects have been attributed to inhibition of renal prostaglandin synthesis by the NSAID. Thus, when NSAIDs and lithium are administered concurrently, subjects should be observed carefully for signs of lithium toxicity.

Methotrexate
NSAIDs have been reported to competitively inhibit methotrexate accumulation in rabbit kidney slices. This may indicate that they could enhance the toxicity of methotrexate. Caution should be used when NSAIDs are administered concomitantly with methotrexate.

ACE Inhibitors/Angiotension II Receptor Antagonists
Concomitant use of *ACE inhibitors and/or angiotension II receptor antagonists* may increase the risk of renal impairment, particularly in volume-depleted patients. Reports suggest that NSAIDs may diminish the antihypertensive effect of ACE inhibitors and/or angiotension II receptor antagonists. This interaction should be given consideration in patients taking NSAIDs concomitantly with ACE inhibitors and/or angiotension II receptor antagonists.

Antiepileptic Drugs
Sporadic cases of seizures have been reported during concomitant use of TORADOL and *antiepileptic drugs* (phenytoin, carbamazepine).

Psychoactive Drugs
Hallucinations have been reported when TORADOL was used in patients taking *psychoactive drugs* (fluoxetine, thiothixene, alprazolam).

Pentoxifylline
When ketorolac tromethamine is administered concurrently with pentoxifylline, there is an increased tendency to bleeding.

Nondepolarizing Muscle Relaxants
In postmarketing experience there have been reports of a possible nteraction between ketorolac tromethamine[IV/ M] and *nondepolarizing muscle relaxants* that resulted in apnea. The concurrent use of ketorolac tromethamine with muscle relaxants has not been formally studied.

Selective Serotonin Reuptake Inhibitors (SSRIs)
There is an increased risk of gastrointestinal bleeding when selective serotonin reuptake inhibitors (SSRIs) are combined with NSAIDs. Caution should be used when NSAIDs are administered concomitantly with SSRIs.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

A pharmacology tabulated summary was not submitted with this NDA.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No new pharmacokinetic studies were conducted for this NDA with the exception of a toxicokinetic evaluation in rats as part of the 28-day toxicology study. In this rat study, intranasal administration of ketorolac tromethamine (3 mg/kg) resulted in a Cmax value[2] of approximately 10,000 ng/mL, which is much greater than previous reported human systemic exposure levels[3]. Generally, Cmax and AUC values were higher in females as compared to male rats with similar Tmax values ranging from 0.5 to 1hr. For a detailed account, please refer to Study KET003 in Section 2.6.6.3.

Reviewer's Comment: This exposure difference in gender was likely an artifact of the dosing regime since the volume administered to the animals was fixed. As a result, lighter females were dosed higher than males and most likely accounts for the higher systemic exposure levels and greater incidences of toxicities reported in females.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

A pharmacokinetics tabulated summary was not submitted with this NDA.

2.6.6 TOXICOLOGY

Ketorolac tromethamine has been approved for intramuscular IM and intravenous IV injection (NDA 19,698; November 1989) and oral tablets (NDA19-645; December 1991). For the new route of administration (intranasal) in the current NDA application, additional nonclinical studies were required to determine local toxicity via the intranasal route. In addition, the intranasal formulation contained an oxidative degradant whose specification exceeded the qualification threshold, and therefore additional studies were also required to qualify the safety of the degradant.

2.6.6.1 Overall toxicology summary

General toxicology:

In support of the change to nasal route of administration, systemic and local toxicity of ketorolac tromethamine was assessed in rabbits and in rats.

In the 14-day repeat dose local assessment study, New Zealand White rabbits were administered ketorolac tromethamine via nasal aspiration (spray pump) at concentrations of 7.5%, 15%, 22.5%, which are equivalent to nasal doses of 0.75, 1.5, and 2.25 mg/cm^2 based on nasal surface area (see Table 1 in Section 2.6.6.10). All animals survived until their scheduled necropsy. There were no treatment related effects observed on any parameters evaluated, including clinical signs, body weight, food consumption, or gross pathology. Microscopic

[2] Achieved after the third intranasal administration (every 4 hours) of ketorolac tromethamine (3 mg/kg) to rats on Day 1

[3] For instance, after a single nasal administration of ketorolac tromethamine (0.5 mg/kg) to humans, a Cmax of 1800 ng/mL was previously reported.

evaluation of the nasal septum and turbinates revealed minimal to mild hemorrhage, nasal epithelial erosion and nasal luminal exudate but were observed in all groups with similar incidences and severity, indicating these findings were not likely test-article related. In a preliminary study in New Zealand white rabbits that aimed to evaluate different formulations of the test article by intranasal administration (at a dose of 12 mg/kg/day for 8 consecutive days by means of a catheter in the right nostril), it was found that ketorolac induced moderate to severe erosions in the mucosa of the nasal septum and slightly increased the incidences of erosions in the mucosa of the turbinates. These findings were confined to the anterior regions of the nasal passageway that were directly exposed to the test-article.

In the 28-day repeat dose toxicity study conducted in rats, animals were dosed with vehicle (EDTA/0.2M potassium dihydrogen phosphate in water) or test article formulation, 10 μL to alternating nostril three times a day using a calibrated pipette. Three formulation strengths of 7.5%, 15.0% and 22.5% were assessed, resulting in maximal nasal exposures[4] of 0.29, 0.58, and 0.87 mg/cm^2/day. Since the volume administered to the animals was fixed, lighter females were dosed higher than males and accounts for the greater systemic exposure levels reported in females. Although no unscheduled deaths in males were observed, 7 of 16 females treated at the HD were either found dead or euthanized moribund by Day 6. Three additional females at the HD exhibited clinical signs on Day 7 similar to those observed in euthanized moribund animals and consequently remaining females were sacrificed. One MD female was euthanized moribund on Day 27 due to adverse clinical signs. Treatment-related clinical signs included hunching mostly in HD and by week 3 in MD females. In addition, swollen abdomen, shallow and labored breathing and piloerection was also observed in the high dose female group and in the euthanized MD female. Females in the LD and MD groups gained less body weight than control females. Several hematology parameters, including reduction in APTT as well as secondary hematology changes related to gastrointestinal toxicity (increases in leukocytes, platelets, and reticulocyte numbers and a reduction in mean cell hemoglobin concentration) were observed. In a dose-dependent manner, urine volume was increased while specific gravity and urinary protein levels were concomitantly reduced in both sexes. Unscheduled necropsy of HD animals as well as scheduled necropsy of MD females exhibited multiple findings in the gastrointestinal tract and mesenteric lymph nodes, which included abnormal cavities with fluid, adhesions, and abnormal contents; intestines with abnormal contents, raised foci, abnormal shape, thickening, reddening, and adhesions. Microscopic findings were not evaluated in HD females but the following histopathological findings were observed in MD females: ulceration/erosion of the stomach, jejunum and ileum; peritonitis of the gastrointestinal organs and mesenteric lymph node, and increased hematopoiesis in the spleen that correlated with increased spleen weights. Minor increases in the incidences and severity of chronic progressive nephropathy and mineralization was observed in LD and MD females. Due to the observed GI toxicities, the conservative NOAEL is the LD, which was associated with $AUC_{0\text{-}4}$ of 23391 ng*hr/mL in females. This dose is 3 times the systemic exposure levels associated with the maximum recommended human daily dose of 126 mg of ketorolac tromemthamine (Table 3 of Section 2.6.6.10). There were no local toxicity in the male rats administered the HD. Unscheduled deaths occurred in the female HD group but it can be assumed that the local NOAEL is also the HD in females since there were not differences in local toxicity findings

[4] Reported maximal nasal exposures refer to one nostril; contralateral nostril is half the nasal respective exposures

between males and females. Maximum nasal exposure in the HD group was 0.87 mg/cm^2/day, which is 1.3 times the maximum recommended human daily dose (0.7 mg/cm^2/day).

For qualification of the 1-keto degradant, a 14 day repeat-dose toxicity study and two *in vitro* genotoxicity studies were conducted.

In the 14-day repeat dose toxicology study, rats were dosed with either Vehicle (Group 1), 15 % ketorolac alone (Group 2) or at one of three doses of 15 % ketorolac spiked with 0.9 % (w/w) 1-keto degradant[5] (Groups 3 – 5) administered to alternating nostril using a calibrated pipette. Doses were escalated by increasing the number of 10 μL nasal administrations, up to three times per day, which corresponded to systemic doses of 18 KT alone, 6.0 KT + 0.054 1-keto, 12 KT + 0.108 1-keto and 18 KT + 0.162 1-keto mg/kg/day. Like the previous study, the volume administered to the animals was fixed, resulting in higher systemic exposure levels in females. Female mortalites were observed in KT alone (3 animals) and HD (1 animal) groups. Clinical signs that were considered test-article related included pale extremities, thin appearance, piloerection, subdued behavior, swollen abdomen, hunched posture and were comparable in KT alone and HD groups. Mean body weight was generally reduced in all dosing female groups. Similar to the 28-day toxicology study, hematology parameters were altered in female groups, which were characterized by reductions in red blood cells, hemoglobin and hematocrit and increases in reticulocytes and highly variable white blood cell counts. These changes in blood cell parameters are most likely secondary changes to gastrointestinal toxicity. Urinalysis revealed differences in both sexes that were likely test-article related, including decreased specific gravity and urinary protein levels with increases in urine volume. These effects were no longer observed after the recovery period. In females, kidney and spleen weights were increased in a dose-dependent manner. Unscheduled necropsy findings of animals that died or were sacrificed prematurely were primarily related to the gastrointestinal tract and included distension, abnormal contents, adhesions, thickening and rupture of the small intestine, adhesion of the abdominal organs, enlargement of the spleen and mesenteric lymph node. Histopathology findings included ulceration/erosions in the gastrointestinal tract, papillary necrosis of the renal medulla, and increased extramedullary hematopoiesis of the spleen and liver. No findings were observed in the nasal cavity or other regions of the respiratory tract. Consequently the maximum nasal exposure of the 1-keto degradant in rats, which was 0.0052 mg/cm^2/day, is approximately equivalent to the estimated local human nasal exposure of 0.0046 mg/cm^2/day (Table 4 in Section 2.6.6.10). Systemically, exposure to the 1-keto degradant in HD rats is almost twice the maxiumum estimated human daily exposure based on body surface area (Table 5 in Section 2.6.6.10).

Genetic toxicology:
The genotoxic potential of ketorolac was not further evaluated as it has been shown to be positive in the Chromosome Aberration Assay (Toradol® label). However because the 1-keto degradant levels in the drug product exceeded ICH guidelines, the sponsor conducted a Bacterial Reverse Mutation Assay (Ames Test) and an *in vitro* Chromosome Aberration Assay using CHO cells. The 1-keto degradant was negative in the Ames Test but tested positive in the *in vitro* Chromosome Aberration assay in the presence of metabolic activation. It was noted

[5] 0.9% 1-keto degradant refers to the percentage weight of ketorolac tromethamine and not the total solution weight

that at the concentrations that produced chromosomal aberrations, 1-keto degradant was also cytotoxic, but overall the weight of evidence indicates that 1-keto degradant is not mutagenic but likely clastogenic. It is important to note that the parent, ketorolac tromethamine also tested positive in the *in vitro* chromosome aberration assay in CHO cells. After consultation with Dr. David Jacobson-Kram, he recommended that the 1-keto degradant be evaluated by computational toxicology analysis, results of which can be found in the Appendix.

Two computational toxicology prediction software models, namely MC4PC and MDL-QSAR, were utilized to assess the 1-keto degradant. To summarize briefly, the degradant was predicted positive by MC4PC in the rodent mutation *in vivo* models but negative at all other endpoints including, *Salmonella* and *E. coli* mutagenicity, micronucleus in vivo, UDS, and chromosome aberrations *in vitro*. Using the MDL-QSAR, the degradant was predicted to be positive for *Salmonella* mutagenicty and chromosome aberrations *in vitro* but negative in chromosome aberrations *in vivo*, micronucleus *in vivo* and UDS. However, the 1-keto degradant was predicted by both MC4PC and MDL-QSAR to be negative for carcinogenicity in both rat and mouse models.

Special toxicology: None

2.6.6.2 Single-dose toxicity
Single-dose toxicology studies were not conducted for this NDA.

2.6.6.3 Repeat-dose toxicity

Study title: Ketorolac Tromethamine MTD and 5 Day Intranasal Tolerance Study in Rats

Key study findings: Two-phase pilot study in SD rats dosed intranasally using an automatic pipette; Phase 1 determined the maximum tolerated dose (MTD) of intranasal administration (0.58, 0.87, 1.3, 2.6 mg/cm^2/day) of ketorolac while Phase 2 examined locally induced toxicity in the upper and lower respiratory tract and gastrointestinal tract following repeat intranasal dosing (0.58, 0.87, 1.3 mg/cm^2/day) for 5 consecutive days.

Phase 1
- observed GI related necropsy findings
- body weight reduction with higher dose groups (3 and 4)

Phase 2
- 3 unscheduled deaths (2 MD, 1 HD)
- GI related necropsy findings and body weight reduction at MD and HD
- tolerated nasal dose: 0.58 mg/cm^2/day (LD) for 5 days

Study no.: KET 001
Volume #, and page #: Module 4 Vol. 1 pg 1-77
Conducting laboratory and location: (b) (4)

Date of study initiation: March 2004
GLP compliance: No
QA report: yes ( ) no (x)

Drug, lot #, and % purity:

- Ketorolac solution 10% w/w, lot# ROX2/02/I, 9.97%
- Ketorolac solution 22.5% w/w, lot# ROX2/022/I, 22.2%

Methods

Phase 1

Doses: The doses used in Phase are summarized in the following Table.
Species/strain: Sprague Dawley (Crl:CD® (SD) IGS BR) male rats
Number/sex/group or time point (main study): 5/group (Repeat Dose)
Route, formulation, volume, and infusion rate: Intranasal 3x per day (at 3 hr intervals) at various volumes (see Table below)
- dosing for animals in Groups 1 and 3 consisted of 10 µL applications per nostril per dose session; group 2 received two 10 µL to one nostril and 10 µL application to the other nostril; Group 4 received two 10 µL applications per nostril per dose session with sufficient time between applications to the same nostril to allow drainage and absorption
Satellite groups used for toxicokinetics or recovery: None
Age: 9-11 weeks
Weight: 232 – 321 g
Sampling times: Blood samples were not taken.
Unique study design or methodology (if any): Utilized male rats only; animals were not randomized by weight

Phase 1 – Maximum Tolerated Dose

Dose Group/ Treatment*	Volume/ Session (µL)	Formulation Concentration	Systemic Dose (mg/kg/day)	Nasal Dose[a] (mg/cm^2/day)	Nasal[b] Rat:Man Ratio
1 – Low Dose	20	10%	24	0.58	1.2
2 – Intermediate Dose 1	30	10%	36	0.87	1.7
3 – Intermediate Dose 2	20	22.5%	54	1.30	2.6
4 – High Dose	40	22.5%	108	2.60	5.2

[a] The surface areas of the rat and human nasal cavities are 10.4 and 181 cm^2 respectively.
[b] The rat to man nasal dose ratio is based upon the anticipated nasal dose in man of 0.5 mg/cm^2/day.

Phase 2

Species/strain: Sprague Dawley (Crl:CD® (SD) IGS BR) male rats
Number/sex/group or time point (main study): 5/group (Repeat Dose)
Route, formulation, volume, and infusion rate: Animals were dosed intranasally for 5 consecutive days 3x per day (at 3 hr intervals) at various volumes (see Table below)
- dosing for animals in Groups 1 and 3 consisted two 10 µL to one nostril and 10 µL application per dose session; Groups 2 and 4 received two 10 µL applications per nostril per dose session with sufficient time between applications to the same nostril to allow drainage and absorption
Satellite groups used for toxicokinetics or recovery: None
Age: 9-11 weeks
Weight: 275 – 382 g
Sampling times: Blood samples were not taken.

Unique study design or methodology (if any): Utilized male rats only; animals were not randomized by weight

Phase 2 – 5 day Repeat Dose

Dose Group/ Treatment	Volume/ Session (µL)	Formulation Concentration	Systemic Dose (mg/kg/day)	Nasal Dose[a] (mg/cm^2/day)	Nasal[b] Rat:Man Ratio
1 – Vehicle Control	30[c]	0%	0	0.00	0
2 – Low Dose	20	10%	24	0.58	1.2
3 – Intermediate	30	10%	36	0.87	1.7
4 – High Dose	20	22.5%	54	1.30	2.6

[a] The surface areas of the rat and human nasal cavities are 10.4 and 181 cm^2 respectively.
[b] The rat to man nasal dose ratio is based upon the anticipated nasal dose in man of 0.5 mg/cm^2/day.
[c] Animals in Group 1 were given 30 µL of placebo, the highest volume dosed amongst groups.

Mortality: Observed twice daily, once in the morning and once in the evening.
Clinical signs: All animals were observed once daily during the pretrial period. During the treatment period, all animals were examined once before dosing, continuously during dosing and approximately 1 hr after dosing.
Body weights: Body weights were recorded once weekly prior to dosing and once daily during the treatment period.
Food consumption: Quantity of food consumed was recorded daily during treatment and once weekly during pretreatment.
Ophthalmoscopy / EKG / Hematology / Clinical Chemistry / Urinalysis: Not examined.
Gross pathology: Necropsy consisted of a complete external and internal examination that includes body orifices (ears, nostrils, mouth, anus, vulva) and cranial, thoracic and abdominal organs and tissues. The respiratory tract was closely examined for signs of irritation. Gross lesions were recorded in descriptive terms included location, size, shape, color, consistency and number. Tissues were not preserved for Phase I animals.
Organ weights (specify organs weighed if not in histopath table):
Kidney, Liver and Lung weights were collected.
Histopathology: Adequate Battery: yes ( ), no (x)
Peer review: yes ( ), no (x)
* did not perform histopathology

Results

Mortality:
There were no unscheduled deaths in Phase 1 but three unscheduled deaths occurred in Phase 2. One animal (702, MD) was euthanized moribund on Day 5, while two animals (705 MD, 804 HD) were found dead on Day 6.
Clinical signs:
No adverse clinical signs were observed in Phase 1.

In Phase 2, animals in vehicle control and LD groups did not exhibit any adverse clinical signs. In the MD group, 3 animals displayed body hunching with animal 702 showing piloerection, labored respiration, and a dark nasal discharge that was euthanized moribund on Day 5. In the HD group, one animal exhibited body hunching and was discovered dead on Day 6.

Body weights:
In Phase 1, a dose-dependent decrease in body weights was observed in rats dosed with ketorolac.

Dose Group/ Treatment		Pretrial (Day)	Treatment Period (Days)			Body Weight Gain or Loss (g) Day 1 to Day 2	Body Weight Gain or Loss (g) Day 1 to Day 3
		-1	1	2	3		
1 Low Dose	Number	6	6	6	3	6	3
	Mean	251	259	262	286	3.8	16.3
	SD	17	18	20	14	3.8	4.7
2 Intermediate Dose 1	Number	6	6	6	3	6	3
	Mean	247	254	256	270	1.3	13.0
	SD	13	14	13	17	6.5	6.2
3 Intermediate Dose 2	Number	6	6	6	3	6	3
	Mean	281	286	279	288	-6.3	-1.3
	SD	16	15	17	16	9.0	8.0
4 High Dose	Number	6	6	6	3	6	3
	Mean	282	290	276	273	-13.8	-24.7
	SD	23	24	24	13	2.7	23.4

Similarly in Phase II, a dose-dependent decrease in body weights was generally observed beginning with rats in the mid and high dose groups (see Table below).

Dose Group/ Treatment	Animal	Pretrial (Day)	Treatment Period (Days)						Body Weight Gain or Loss (g) Day 1 to Day 6
		-1	1	2	3	4	5	6	
1 Vehicle Control	Number	5	5	5	5	5	5	5	5
	Mean	313	317	319	324	328	333	341	24.4
	SD	30	30	32	32	33	35	35	9.9
2 Low Dose	Number	5	5	5	5	5	5	5	5
	Mean	333	339	341	343	348	351	353	14.0
	SD	14	14	16	13	14	16	16	2.5
3 Intermediate Dose	Number	5	5	5	5	5	5	3	3
	Mean	335	342	339	332	332	336	376	17.0
	SD	30	30	36	47	53	58	24	2.6
4 High Dose	Number	5	5	5	5	5	5	4	4
	Mean	338	342	341	334	328	328	340	-5.5
	SD	14	16	22	30	32	38	30	15.2

Reviewer's Comment: As observed in the Pretrial weights, animals were not randomized by weight. The discrepancy for the weight gain is the result of the two unscheduled deaths in the MD group. Body weight gain was calculated for each animal and subsequently averaged. Consequently, the value reported is not simply the averaged weight on Day 6 subtracted by Day 1 weights.

Food consumption:
Similar to body weights, a general dose-dependent decrease in food consumption was also observed with the highest reduction occurring at the high dose group (see Tables below).

Phase 1: Food Consumption (g/animal/day) in rats administered with Ketorolac Tromethamine

Dose Group/ Treatment		Pretrial Period (Days)					Treatment Period (Days)		
		-5	-4	-3	-2	-1	1	2	3
1 Low Dose	N	6	6	6	6	6	6	6	3
	Mean	26.8	26.0	24.3	25.7	27.3	26.8	23.0	28.3
	SD	3.2	2.3	3.4	2.9	2.2	2.6	4.0	0.3
2 Intermediate Dose 1	N	6	6	6	6	6	6	6	3
	Mean	26.0	25.5	26.5	24.7	29.5	24.8	19.5	28.3
	SD	1.5	1.2	1.6	0.7	2.4	0.9	4.7	2.9
3 Intermediate Dose 2	N	6	6	6	6	6	6	6	3
	Mean	25.0	28.2	27.5	28.2	27.8	26.2	16.0	23.3
	SD	1.5	2.8	0.4	1.7	1.4	1.7	5.2	4.0
4 High Dose	N	6	6	6	6	6	6	6	3
	Mean	26.2	29.5	26.2	27.8	27.8	27.8	7.2	10.3
	SD	1.6	2.2	1.7	1.3	0.3	1.4	0.9	5.5

Phase 2: Food Consumption (g/animal/day) in rats administered with Ketorolac Tromethamine

Dose Group/ Treatment	Animal	Pretrial Period (Days)					Treatment Period (Days)					
		-5	-4	-3	-2	-1	1	2	3	4	5	6
1 Vehicle Control	Number	5	5	5	5	5	5	5	5	5	5	5
	Mean	22.4	26.6	26.0	26.4	27.6	27.8	23.0	25.4	25.6	26.2	25.8
	SD	1.9	0.4	0.5	0.5	0.4	2.1	0.5	1.3	1.5	1.6	1.2
2 Low Dose	Number	5	5	5	5	5	5	5	5	5	5	5
	Mean	28.6	29.6	27.6	29.2	28.6	26.6	25.4	24.6	26.8	25.2	25.0
	SD	1.7	2.6	1.3	2.1	0.4	1.3	1.9	1.7	1.6	1.2	0.9
3 Intermediate Dose	Number	5	5	5	5	5	5	5	5	5	5	4
	Mean	25.6	28.4	27.0	27.8	28.2	28.4	18.8	19.2	20.0	19.4	23.8
	SD	1.7	2.8	0.0	2.0	1.6	1.0	0.7	2.0	0.9	1.7	3.8
4 High Dose	Number	5	5	5	5	5	5	5	5	5	5	5
	Mean	27.6	28.4	28.2	28.0	28.0	27.0	20.2	18.4	16.4	17.4	15.2
	SD	0.8	0.5	0.6	1.8	2.3	0.9	4.3	4.0	1.7	3.1	4.3

Ophthalmoscopy / EKG / Hematology / Clinical Chemistry / Urinalysis
Not examined.

Gross pathology:
Summaries of the gross pathology findings are presented in the Tables below.

In Phase 1, there were no findings in Group 1 (Low Dose) and the only finding in Group 2 was dark foci on the lung of one animal. In the mid-dose group, one animal had reddened mucosa in the duodenum, ileum, and jejunum, while another had thickened duodenal mucosa. In both animals, there was prominent lobulation of the liver. A majority of the animals in the high-dose group had signs of intestinal irritation with incidences of stomach lesions and liver lobulation as well as isolated incidence of a pale pancreas and pale liver lobes.

Phase 1

Histopathology Incidence				
	Males			
	LD	**MD**	**MD**	**HD**
Caecum				
Dark				3/6
Contents Dark				1/6
Duodenum				
Thickened			1/6	
Reddened			1/6	1/6
Ileum				
Reddened			1/6	
Jejenum				
Dilated				2/6
Thickened				2/6
Reddened			1/6	4/6
Kidney				
Pale focus				1/6
Liver				
Pale			1/6	1/6
Enlarged			1/6	
Prominent lobulation			1/6	3/6
Lung				
Dark Focus		1/6		
Pancreas				
Pale				1/6

Rectum				
Contents dark				1/6
Stomach				
Pale focus				2/6
Reddened				1/6
Thoracic Cavity				
Contents abnormal			1/6	

In phase 2, there were several incidences of isolated findings including enlarged submandibular and hepatic lymph node, dark lung, and several incidences of enlarged bronchial lymph nodes but are not likely attributable to the test-article due to the lack of dose-dependence. In the HD group, one incidence of a mass in the jejunum was observed. In the unscheduled deaths, one MD animal was associated with a dark liver and one had abnormal cavity contents and another animal had a mass associated with the mesenteric lymph nodes, while in the HD group, one animal was reported to have a mass associated with the jejunum.

Phase 2

Histopathology Incidence						
	Main Study				**Premature Deaths**	
	VC	**LD**	**MD**	**HD**	**MD**	**HD**
Number of Animals	*5*	*5*	*3*	*4*	*2*	*1*
Abdominal Cavity						
Mass						1
Contents Abnormal					1	
Contains fluid						1
Jejenum						
Mass(es)				1		
Kidney						
Dark focus	1					
Liver						
Dark					1	
Lung						
Dark Focus			1			
Dark			1			
Lymph Node (Bronchial)						
Enlarged	2	1				1
Lymph Node (Hepatic)						
Enlarged						1
Lymph Node (Mandibular)						
Enlarged			1			
Lymph Node (Mesenteric)						
Mass					1	

Organ weights
Group mean absolute organ weights are presented below. Treatment with Ketorolac Tromethamine had no effects on relative kidney, liver, or lung organ weights when compared to vehicle control animals.

Group	Body Weight	Kidney*	Liver*	Lung*
1 – Vehicle control	332.6	0.0075	0.049	0.0040
2 – Low Dose	345.4	0.0076 (+1)	0.045	0.0041
3 – Mid Dose	351.3	0.0080 (+7)	0.049	0.0046 (+15)
4 – High Dose	320.4	0.0081 (+8)	0.047	0.0047 (+18)
*ratio organ weight/body weight				

Histopathology: Adequate Battery: yes ( ), no (x)*
Peer review: yes ( ), no (x)
*did not perform histopathological analysis

Toxicokinetics:
Not examined.

Other:
Not applicable.

Study title: Ketorolac Tromethamine Containing 0.9% 1-keto Degradant: 14 Day Intranasal Toxicity Study in Rats with a 14 Day Recovery Period

Key study findings: A 14 Day intranasal study in rats was conducted to support qualification of the 1-keto degradant, examining general, as well as local toxicity and to evaluate the reversibility of any effects after a 14-day recovery period.

- 1-keto degradant at nasal dose of 0.35 mg/cm^2/day with 0.58 mg/cm^2/day ketorolac has a very similar toxicity profile to ketorolac alone, indicating no additive or additional toxicity of the degradant
- no local toxicity in the nasal cavity or respiratory tract with ketorolac alone or in combination with the 1 –keto degradant
- local NOAEL – HD (15% KT + 0.9% 1-keto)

Study no.: KET 002
Volume #, and page #: Module 4 Vol. 2/3 pg 1-393
Conducting laboratory and location: (b) (4)

Date of study initiation: 02 February 2007
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Ketorolac tromethamine, lot# 10680610004, 99.7%

Methods

Doses: Doses used in the study were as follows:

Dose Group/Treatment	Concentration of KT and 1-keto	Total dose Volume per Day (μL)	Dose of KT and Degradant[a] (mg/kg/day)	Nostril Dose (mg/cm^2/day)	Contralateral Nostril Dose (mg/cm^2/day)
1 – Vehicle Control	0% KT 0% 1-keto	3 x 10 μL	0 (KT) 0 (1-keto)	0 (KT) 0 (1-keto)	0 (KT) 0 (1-keto)
2 – KT	15% KT	3 x 10 μL	18 (KT)	0.58 (KT)	0.29 (KT)
3 – KT + Low Dose 1-keto	15% KT 0.9% 1-keto	1 x 10 μL	6 (KT) 0.054 (1-keto)	0.29 (KT) 0.0026 (1-keto)	0 (KT) 0 (1-keto)
4 – KT + Mid Dose 1-keto	15% KT 0.9% 1-keto	2 x 10 μL	12 (KT) 0.108 (1-keto)	0.29 (KT) 0.0026 (1-keto)	0.29 (KT) 0.0026 (1-keto)
5 – KT + High Dose 1-keto	15% KT 0.9% 1-keto	3 x 10 μL	18 (KT) 0.162 (1-keto)	0.58 (KT) 0.0052 (1-keto)	0.29 (KT) 0.0026 (1-keto)
[a] dose illustrated based on a 250 g rat					

Species/strain: Sprague Dawley (Crl:CD® (SD) IGS BR) rats
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Intranasal up to three times (see Table above) per day (at 3 hr intervals) at 10 μL application per nostril. Alternate nostrils were used for each dose session or when a fraction of a dose was administered.
Satellite groups used for toxicokinetics or recovery: 6/sex/group for both recovery and toxicokinetics. For group 5, toxicokinetic animals were used for the 14 day post dose recovery period
Age: Not reported
Weight: 220 - 290 g (males) and 162 - 230 g (Females)
Sampling times: Blood samples were obtained prior to necropsy. For Group 5 animals, blood samples were obtained from all toxicokinetic animals in the HD group on Day 1: predose, 30 min, 1 and 4 hr after the first dose session. 3 animals/sex were used for predose and 1hr timepoints and another 3 animals/sex for 30 min and 4 hr timepoints.
Unique study design or methodology (if any): Groups 3-5 were administered ketorolac tromethamine spiked with the 1-keto degradant. Animals were dosed with intranasal instillation using a calibrated automatic pipette. Nostrils were alternated for dosing for each dose session. The pipette tip was changed between dose groups.

Mortality: Observed twice daily, once in the morning and once in the evening. Animals showing any signs of severe debilitation or determined moribund was euthanized. Any animal that underwent unscheduled euthanasia or was found dead was given a detailed macroscopic examination.
Clinical signs: All animals were observed once daily during the pretrial period as well as the recovery period. During the treatment period, all animals were examined once before dosing, continuously during dosing and approximately 1 hr after dosing.
Body weights: Body weights were recorded once weekly prior to and during the treatment period. However, due to signs of toxicity Group 2 animals were weighed daily for most of the treatment period.
Food consumption: Quantity of food consumed was recorded once weekly starting at the pretreatment phase.

Ophthalmoscopy: Ophthalmoscopic examinations conducted by the veterinarian tested both eyes of all main study animals, and the recovery animals from Groups 1, 2, and 5 were examined pretrial, during week 2 of treatment and the end of the 14 day recovery period. Anterior, lenticular, and fundic areas were evaluated.
EKG: Not examined.
Hematology: hemoglobin concentration; red blood cell count; hematocrit; mean cell volume; mean cell hemoglobulin; mean cell hemoglbulin concentration; reticulocyte counte; platelet count; total white blood cell count; differential white blood cell count; nuetrophils; lymphocytes; monocytes; eosinophils; basophils; large unclassified cells
Clinical chemistry: glucose, urea, creatinine, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, total bilirubin, alanine aminotransferase, aspartate aminotransferase, calcium, phosphate, sodium, potassium, chloride, alkaline phosphatase, triglycerides; lactate dehydrogenase; phosphate; creatinine phosphokinase
Urinalysis: Urine samples were collected over a 4 hr period in the early morning before dosing. The following parameters were evaluated: color; volume; specific gravity; pH; protein; glucose; ketones; urobilinogen; bilirubin; blood pigments; microscopy of the spun deposit
Gross pathology: Necropsy consisted of a complete external and internal examination that include body orifices (ears, nostrils, mouth, anus, vulva) and cranial, thoracic and abdominal organs and tissues. The respiratory tract was closely examined for signs of irritation. Gross lesions were recorded in descriptive terms included location, size, shape, color, consistency and number.
Organ weights (specify organs weighed if not in histopath table):
Kidney, Liver and Lung weights were collected.
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Mortality:
No unscheduled deaths in males were reported. In contrast amongst females, one Group 2 animal (#228) was found dead, while two animals were euthanized for toxicity reasons on Days 5 (#230) and 8 (#229). In addition, one female (#521) in group 5 was euthanized on Day 9.

Unscheduled Deaths	**Group 1 Vehicle control**	**Group 2 KT**	**Group 3 LD KT + 1-Keto**	**Group 4 MD KT + 1-Keto**	**Group 5 HD KT + 1-Keto**
Found Dead	0	1F	0	0	0
Euthanized	0	2F	0	0	1F
Total Deaths	0	3F	0	0	1F

Several common clinical signs were recorded amongst the female unscheduled deaths including pale extremities, thin appearance, piloerection, subdued behavior, markedly swollen abdomen, red staining around nose, hunched posture, swollen abdomen. In addition, two females (229, 230) had dark nasal discharge and skin discoloration whereas animal 228 exhibited staggered movements.

Clinical signs:
Group 2 females showed reversible clinical signs including hunching, swollen abdomen, inactivity, thin appearance, pale discolored skin on extremities and marked piloerection with 2 animals showing dark nasal discharge. Two Group 4 females showed hunched body posture but did not exhibit hunching following the recovery period. One male in group 5 was observed with hunched posture while one female in Group 5 presented with pale extremities, thin appearance, piloerection, swollen abdomen, and was markedly subdued.

Body weights:
No significant different body weight changes were observed in males in any groups. However in females, a reduction in weight was observed in Groups 4 and 5 with a slightly more pronounced reduction in Group 2 (see Table below). These body weight changes were not seen following the recovery period.

Dose Group/ Treatment	Males			Females		
	Pretrial BW (g) (Day -1)	BW Gain (g) (Day 1 to 14)	Recovery BW Gain (g) (Day 14 to 29)	Pretrial BW (g) (Day -1)	BW Gain (g) (Day 1 to 14)	Recovery BW Gain (g) (Day 14 to 29)
1 – VC	253	91	85	191	46	31
2 – HD KT	263	93	77	185	33** (-28%)	31
3 – LD KT + 1-keto	255	87	--	193	39 (-15%)	--
4 – MD KT + 1-keto	257	89	--	191	36* (-22)	--
5 – HD KT + 1-keto	256	96	86	197	37* (-20%)	28
* denotes significance: * $p<0.05$; ** $p<0.01$						

Food consumption:
Generally, no changes in food consumption across groups were observed with the exception of Group 2 that had a small reduction only during Week 1 of treatment as compared to vehicle control animals.

Ophthalmoscopy:
Individual animal ophthalmoscopy revealed several minor findings including hyaloids remnant, persistent papillary membranes as well as small and central opacity but these findings were also present in pretrial and in control animals. Therefore the reviewer agrees with the sponsor's assessment that there were no treatment-related ophthalmoscopy findings.

EKG: Not examined.

Hematology:
Group mean hematology results are summarized in the Table below. Generally, males did not exhibit any changes to any hematology parameter tested. However in females, several

components of the red blood cell system were reduced, including hemoglobin, red blood cell counts, hematocrit. This was observed with a corresponding increase in reticulocytes in groups 2, 4, and 5. After recovery, these changes in the red blood cell system and in reticulocytes were not observed. In addition, female white blood cell counts were increased in groups 2, 4 and 5 with a corresponding increase in neutrophil counts that reversed after the recovery period.
Reviewer's Comment: Decreases in the observed red blood cells parameters are likely secondary effects to the GI toxicities.

Hematology																
	Males								Females							
	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5
Hb g/dL	15.3	15.2	**14.7**	15.1	15.0	14.8	15.0	15.0	14.5	**12.4 (-14)**	14.2	**12.4**	**13.1 (-10)**	15.3	14.9	15.3
RBC $x10^{12}$/L	7.36	7.22	**7.13**	7.39	7.17	7.58	7.9	7.67	7.06	**5.93 (-16)**	6.96	**6.12**	**6.32 (-10)**	7.81	7.55	7.73
Hct L/L	.427	.426	**.412**	.426	.424	.429	.435	.435	.407	**.360 (-12)**	.403	**.358**	**.374 (-8)**	.426	.425	.429
Ret $x10^9$/L	256	285	255	264	276	252	239	256	196	**480 (2.4x)**	239	**396**	**374 (1.9x)**	169	149	163
WBC $x10^9$/L	12.0	12.8	12.8	11.1	12.2	15.4	15.2	10.5	11.1	**17.5 (1.6)**	9.81	16.7	14.0 (1.3)	12.8	**9.54**	**9.00**
Neut $x10^9$/L	1.43	1.86	2.68	1.46	2.24	1.96	2.15	**1.12 (-43)**	1.08	**4.83 (4.5x)**	0.96	**3.45**	2.65 (2.5x)	1.20	.77	**.74**
APTT sec	24	25	26	25	25	22	20	24	20	**17 (-15)**	19	**17**	**18 (-10)**	19	19	18

Note groups 3, 4 and 5 are dose with 1-keto (0.9% w)
shaded columns, recovery groups; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose

Clinical chemistry:
Group mean clinical chemistry results are summarized in the Table below. A slight increase in glucose levels were observed in Groups 4 and 5 males and Groups 3 and 5 females. After the recovery period, glucose levels in Group 5 males were still elevated compared to control animals. It was noted by the sponsor that glucose levels particularly in females were higher than historical controls but the reason for this elevation is not known.

Clinical Chemistry																
	Males								Females							
	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5
Urea mmol/L	4.9	5.4	5.2	5.2	5.7	5.1	5.5	5.7	4.7	**6.3 (1.3x)**	4.9	4.7	**6.6 (1.4x)**	6.1	5.2	**5.4 (-11)**
Glu mmol/L	7.55	7.54	7.76	**8.37 (1.1x)**	**8.41 (1.1x)**	8.70	9.12	**11.0 (1.3x)**	10.9	11.4	**12.8 (1.2x)**	12.0	**12.6 (1.1x)**	9.55	11.2	10.8
LDHIL iu/L	245	184	**161 (-34)**	**136 (-44)**	**139 (-43)**	151	152	213	207	189	**157 (-24)**	**145 (-30)**	**123 (-41)**	190	154	136
Phos mmol/L	2.42	2.42	2.26	2.43	2.47	2.35	2.38	**2.61 (1.1x)**	2.13	**2.36 (1.1x)**	**2.35 (1.1x)**	**2.51 (1.2x)**	**2.35 (1.1x)**	2.17	2.03	2.05

shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose

In both males and females, lactate dehydrogenase levels were significantly reduced in Groups 3, 4, and 5. After the recovery period, levels increased in males but remained slightly reduced in females.

Phosphate levels were slightly increased in all test-article dosed females when compared to control animals but these levels were restored to levels comparable to the control group after the recovery period.

Urea levels in females were significantly increased in Groups 2 and 5 and correlate with the increase in urine volume and decrease in specific gravity as well as kidney histopathology (see below) but reversed after the recovery period.

Urinalysis:
Group mean urinalysis results for specific gravity and urine volume are summarized below. In both males and females, the specific gravity was decreased slightly. Similarly, urine volume was increased in a dose dependent manner, evident in Groups 4 and 5. Significant changes in these parameters were not observed after the recovery period.

Urinalysis - Males								
	VC 1	**KT 2**	**LD 3**	**MD 4**	**HD 5**	**VC 1**	**KT 2**	**HD 5**
SG	1.05	**1.03**	1.05	**1.04**	**1.04**	1.04	1.04	1.04
UV mL	1.5	**3.2 (2.1x)**	1.8 (1.2x)	**2.5 (1.7x)**	**3.2 (2.1x)**	3.0	2.6	3.0
UP mg/dL	106	51 (-52)	89 (-16)	69 (-35)	59 (-44)	61.7	58.3	61.7
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose; SG, specific gravity; UV, urine volume; UP, urinary protein								

Urinalysis - Females								
	VC 1	**KT 2**	**LD 3**	**MD 4**	**HD 5**	**VC 1**	**KT 2**	**HD 5**
SG	1.04	**1.02**	**1.03**	**1.02**	**1.02**	1.03	1.03	**1.04**
UV mL	2.1	3.2 (1 5x)	3.1 (1.5x)	**3.5 (1.7x)**	**4.6 (2.2x)**	3.2	2.1	2.2
UP mg/dL	31.5	16.5 (-46)	13 (-59)	15 (-52)	16.7 (-47)	23.3	16.7	20.0
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose; SG, specific gravity; UV, urine volume; UP, urinary protein								

Gross Pathology
Treatment with Ketorolac tromethamine was associated with various gross pathology findings (summarized in the Table below) relating to the gastrointestinal tract, spleen, and mesenteric lymph node. In group 2, findings included adhesions of the small intestine, accumulation of blood in the abdominal cavity, enlargement of the spleen and enlargement of the mesenteric lymph node in females and rasied focus of the spleen in males. In addition, abnormal contents were observed in the small intestine of females and enlarged mandibular lymph node in males

in Groups 2 and 3 whereas thickening of the small intestine was observed in females in Groups 2 and 4. In groups 2, 3, and 4, males exhibited dark focus in the lungs. Many of these findings occurred in isolated incidences and did not show dose-dependent relationships, indicating such findings were incidental to the test-article administration.

Gross Pathology Findings																
	Males								Females							
	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5
# of Animals	**10**	**10**	**10**	**10**	**10**	**6**	**6**	**6**	**10**	**10**	**10**	**10**	**9**	**6**	**3**	**6**
Abdominal Cavity																
Contains blood										1						
Duodenum																
thickened												1				
Ileum																
Contents abnormal											1					
Intestines																
Contents abnormal										1						
thickened										1		1				
Jejenum																
Pale				2	1					1			1			
Thickened										1						
adhesions										1						
Liver																
Pale										1		1				
Lung																
Dark focus	1	4	2	1			2			1		1	1			
Spongy			1													
Dark					1			1						3	2	4
reddened				1		1		3								
Lymph Node																
Madibular–enlarged		2	1			1			1	1		1				1
Mesenteric-enlarged										3						
Spleen																
Raised focus		1														
enlarged										2						
Thymus																
speckled				1	1				1		1	1				

shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose

Organ Weights

Group mean organ weights changes are presented in the Table below. In all dosed groups administered with ketorolac, kidney and more severely spleen weights were significantly increased. In groups 4 and 5, heart weights in females were slightly lower than those in the vehicle control group. Similarly, uterus weights were significantly reduced in Groups 2, 4 and 5. These organ weight differences were not observed in the recovery animals with the exception of kidney weights, which were still elevated after the recovery period. In the case of the heart and uterus, organ weight changes after the recovery period were opposite to what was observed after the treatment period i.e. heart and uterus weights in the recovery groups were elevated and decreased, respectively.

Relative Organ Weights (% Body weights)								
	Females					Recovery		
Organ	VC	KT	LD	MD	HD	VC	KT	HD
Heart	0.419	0.403 (-4)	0.397 (-4)	**0.389 (-7)**	**0.381 (- 9)**	0.361	0.398 (+ 10)	0.406 (+ 12)
Kidneys	0.796	**0.886 (+ 11)**	0.853 (+7)	**0.875 (+10)**	**0.899 (+ 13)**	0.724	0.816 (+13)	0.776 (+7)
Spleen	0.202	**0.325 (+61)**	0.225 (+13)	**0.300 (+49)**	**0.261 (+29)**	0.203	0.208 (+2)	0.202
Uterus	0.289	**0.210 (-27)**	0.230 (-20)	**0.223 (-23)**	**0.192 (-34)**	0.189	0.251 (+33)	0.300 (+59)
Uterus Abs	0.65	**0.45 (-31)**	0.52 (-20)	**0.49 (-25)**	**0.46 (-29)**	0.51	0.59 (+16)	0.73 (+43)
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose								

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

A summary of the microscopic findings are presented in the Table below. Target organs of toxicity were the gastrointestinal tract, kidney and the mesenteric lymph node. Marked to severe penetrating/perforating ulcers were observed in the duodenum as well as the jejunum whereas non-perforating ulceration was recorded in a Group 2 female and serosal and mural inflammation was present in the ileum of a Group 4 female. Incidence of mild erosion/ulceration was present in the glandular mucosa of a Group 5 male as well as Group 2 and 4 female whereas several incidences of minimal to moderate inflammation of the submucosal region of the glandular stomach in Group 2 and 5 males and was observed in one female in Group 2 and 4. Secondary to the GI toxicities were the increased incidences of extramedullary hematopoiesis of the spleen and liver, which occurred in Groups 2, 4, and 5 for the spleen and Group 2 females for the liver. In addition, inflammatory cell foci were observed in the liver with the highest incidences occurring in the groups administered the HD of ketorolac (Group 2) and more noticeable in group dosed with the HD of ketorolac + 1-Keto (Group 4). Multiple incidences of chronic progressive neuropathy were observed in all treatment groups but several incidences were also observed in the vehicle control groups in both males and females. Isolated incidence of minimal to mild papillary necrosis of the renal medulla was present in a Group 5 male and Group 2 and 4 female. With the exception of agonal congestion/ hemorrhage of the lung and chronic progressive neuropathy in the kidneys, recovery group animals did not exhibit microscopic findings associated with ketorolac, indicating reversibility.

Histopathology Findings																
	Males								Females							
	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5	VC 1	KT 2	LD 3	MD 4	HD 5	VC 1	KT 2	HD 5
# of Animals	**10**	**10**	**10**	**10**	**10**	**6**	**6**	**6**	**10**	**10**	**10**	**10**	**9**	**6**	**3**	**6**
Lung																
Agonal congestion/ hemorrhage	1	3	2	1	1	1	2	4	0	0	0	0	2	3	2	4
Thymus																
lymphocytolysis	0	2	-	-	2	0			0	3	-	-	1	0		
Kidney																
Chronic progressive nephropathy	2	5	6	3	6	4	1	2	6	6	4	6	9	1	1	4
Papillary necrosis	0	0	0	0	1	0			0	1	0	1	0	0		
Stomach																
Degranulation, parietal cell	0	0	0	0	2	0	0	1	0	0	0	0	1	0	0	0
Liver																
Inflammatory cell foci	3	1	-	-	7	0			5	4	-	0	8	0		
Ulcer penetrating																
Duodenum																
Marked	0	0	0	0	0	0			0	0	0	1	0	0		
Severe	0	0	0	0	0				0	1	0	0	0			
Jejunum																
Mod	0	0	0	0	0	0			0	1	0	0	0	0		
Marked	0	0	0	0	0				0	2	0	1	0			
Severe	0	0	0	0	0				0	1	0	0	0			
Ulcer, Mural																
Ileum																
Mod	0	0	0	0	0	0			0	1	0	0	0	0		
Increased Hematopoiesis, extramedullary																
Spleen																
Min	0	0	0	0	0	0			0	0	0	1	2	0		
Mild	0	0	0	0	0				0	2	0	1	0			
Mod	0	0	0	0	0	0			0	1	0	1	0			
Liver																
Min	0	4	-	-	0	0			0	1	-	1	0	0		
Mild	0	0	-	-	0				0	2	-	1	0			
shaded columns, recovery group; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose																

Reviewer's Comment: The design of the study precludes an identification of a systemic and local NOAEL for the 1-keto degradant since the LD and MD had varied levels of ketorolac and 1-keto. However direct comparison between Group 2 (ketorolac alone) and Group 5 (ketorolac + 1-keto) demonstrates that the 1-keto degradant does not appear to elicit novel adverse toxicities and does not appear to increase the drug class toxicities of ketorolac.

Study title: Ketorolac Tromethamine 28 Day Intranasal Toxicity Study in Rats with a 28 Day Recovery Period

Key study findings: A 28 Day intranasal study in rats was conducted to support the change in route of administration, examine general, as well as local toxicity and evaluate the reversibility of any effects after a 28-day recovery period; concentrations of 7.5%, 15% and 22.5% that corresponds to maximum nasal doses[6] of 0.29, 0.58, 0.87 mg/cm^2/day were evaluated.

- no local toxicity in the nasal cavity or respiratory tract was observed with intranasal administration of ketorolac tromethamine
- NSAID class toxicities namely, GI (erosion, ulceration, peritonitis, and kidney (nephropathy, mineralization, increase urinary volume, decrease protein concentration) toxicity
- no additive or additional toxicities was induced by 1 –keto degradant
- systemic NOAEL: males – MD (15%) females – LD (7.5%)
- local NOAEL: HD (22.5%)

Study no.: KET 003
Volume #, and page #: Module 4 Vol. 4/5 pg 1-378
Conducting laboratory and location: (b) (4)

Date of study initiation: 13 February 2006
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

- Ketorolac tromethamine, lot# 10880410007, 99.3%

Methods

Doses: Doses used in the study were as follows:

Dose Group/Treatment[a]	Concentration of Formulation (%)	Dose/Day (mg)	Dose[b] (mg/kg)	Nasal Dose[c] (mg/cm^2/day)
1- Vehicle Control	0	0	0	0
2- Low Dose	7.5	2.25	9	0.29
3- Mid Dose	15.0	4.5	18	0.58
4- High Dose	22.5	6.75	27	0.87

[a] animals were dosed 3 x 10 µL
[b] dose illustrated based on a 250 g rat
[c] dose to one nostril; contralateral nostril is ½ the respective dose

Species/strain: Sprague Dawley (Crl:CD® (SD) IGS BR) rats
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Intranasal three times per day (at 3 hr intervals) at 10 µL application per nostril. Alternate nostrils were used for each dose session.

[6] Reported maximum doses to one nostril; contralateral nostril is half the respective dose

Vehicle control: disodium edentate/0.2M potassium dihydrogen phosphate in water
Satellite groups used for toxicokinetics or recovery: 6/sex/group for both recovery and toxicokinetics.
Age: Not indicated
Weight: 211 - 332 g (males) and 170 - 232 g (Females)
Sampling times: Blood hematology, coagulation, and clinical chemistry were conducted during Week 4 (Day 23) for Main Study animals and towards the end of the recovery period (Day 50 males, Day 49 females) for Recovery animals. Urine was collected for analysis during Week 4 (Day 28 for Main study males and Day 27 for Main Study females) and towards the end of the recovery period (Day 51 males, Day 50 females) for Recovery animals.
Unique study design or methodology (if any): Animals were dosed with intranasal instillation using a calibrated automatic pipette. Nostrils were alternated for dosing for each dose session. The pipette tip was changed between dose groups.

Mortality: Observed twice daily, once in the morning and once in the evening. Animals showing any signs of severe debilitation or determined moribund was euthanized. Any animal that underwent unscheduled euthanasia or was found dead was given a detailed macroscopic examination.
Clinical signs: All animals were observed once daily during the pretrial period as well as the recovery period. During the treatment period, all animals were examined once before dosing, continuously during dosing and approximately 1-2 hrs after dosing.
Body weights: Body weights were recorded once weekly prior to and during the treatment period. However, due to signs of toxicity Groups 3 and 4 animals were weighed daily for most of the treatment period.
Food consumption: Quantity of food consumed was recorded once weekly starting at the pretreatment phase.
Ophthalmoscopy: Ophthalmoscopic examinations conducted by the veterinarian tested both eyes of all main study animals, once during Week 4 of dosing and for Recovery animals towards the end of the 4 week recovery period. Anterior, lenticular, and fundic areas were evaluated.
EKG: Not examined.
Hematology: hemoglobin concentration; red blood cell count; hematocrit; mean cell volume; mean cell hemoglobulin; mean cell hemoglobulin concentration; reticulocyte count; platelet count; total white blood cell count; differential white blood cell count; nuetrophils; lymphocytes; monocytes; eosinophils; basophils; large unclassified cells
Clinical chemistry: glucose, urea, creatinine, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, total bilirubin, alanine aminotransferase, aspartate aminotransferase, calcium, phosphate, sodium, potassium, chloride, alkaline phosphatase, triglycerides; lactate dehydrogenase; phosphate; creatinine phosphokinase
Urinalysis: Urine samples were collected over a 4 hr period in the early morning before dosing. The following parameters were evaluated: appearance (color + turbidity); volume; specific gravity; pH; protein; glucose; ketones; urobilinogen; bilirubin; blood pigments; microscopy of the spun deposit
Gross pathology: Necropsy consisted of a complete external and internal examination that includes body orifices (ears, nostrils, mouth, anus, vulva) and cranial, thoracic and abdominal

organs and tissues. The respiratory tract was closely examined for signs of irritation. Gross lesions were recorded in descriptive terms included location, size, shape, color, consistency and number.

Organ weights (specify organs weighed if not in histopath table):
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x)*, no ( )
*peer review of probable test-item related findings were performed by a second pathologist internally

Results

Toxicokinetics
Toxicokinetic profile of ketorolac administered nasally in male and female rats are summarized below. Due to the fixed volume of the test article via nasal administration, females were dosed much higher than males due to the difference in body weights. Exposure levels of ketorolac were largely observed to be dose-proportional with levels greater in female rats (approx. 2-3 fold) than in males. In both cases, exposure levels were greater at Day 28 as compared to Day 1, with females departing from dose linearity at Day 28.

Dose Group	Day	Gender	Body Weight (g)	Cmax (ng/mL)	Tmax (hr)	AUC0-4 (ng·hr/mL)
2	Day 1	Male	276.2	7388	1.0	19931
		Female	202.2	11034	0.5	38880
	Day 28	Male	403.3	8290	0.5	18632
		Female	257.2	10369	0.5	23391
3	Day 1	Male	279.0	15007	0.5	46277
		Female	191.8	28125	1.0	86418
	Day 28	Male	420,3	17389	0.5	44122
		Female	242.0	43300	0.5	77441
4	Day 1	Male	272.3	24083	1.0	55932
		Female	191.8	43555	0.5	131084
	Day 28	Male	395.5	29657	0.5	63669
		Female	NC	NC	NC	NC

NC - Not calculated (females in the high dose group had died or were sacrificed before Day 28 because of clinical signs).

Group	Day 1 (C_{max}/Dose)		Day 28 (C_{max}/Dose)	
	Males	Females	Males	Females
2	906.5	991.4	1485.7	1185.0
3	930.4	1198.8	1623.6	2328.0
4	971.5	1237.7	1737.4	NC

NC - Not calculated (females in the high dose group had died or were sacrificed before Day 28 because of clinical signs).

Mortality:
No unscheduled deaths in males were reported. Amongst females, 7 of the 16 females treated at the high dose were either found dead or euthanized moribund by Day 6. Three additional females at the HD exhibited clinical signs on Day 7 similar to those observed in previously euthanized moribund animals and as a result all remaining females at the high dose were subsequently sacrificed on Day 8. One MD female (#318) was euthanized moribund on Day 27

due to adverse clinical signs including markedly hunched, subdued with thin appearance, swollen abdomen and marked piloerection.

Clinical Signs:
Males did not exhibit clinical signs after intranasal administration of ketorolac tromethamine with the exception of one male in the recovery group from the HD group, which showed hunched posture from Day 26 to Day 30.

Multiple clinical signs were recorded prior to euthanasia of Group 4 (HD) females which included, markedly hunched posture, pale eyes, thin appearance, marked piloerection, markedly shallow or labored breathing, markedly subdued, markedly swollen abdomen and marked weight loss and dark nasal discharge. Similar signs were also observed in the premature decedent female from Group 3. By week 3, all Group 3 females had markedly hunched body posture. In addition there were several isolated incidences of clinical signs in surviving Group 3 females that included thin body, piloerection and swollen abdomen. After the recovery period, only hunched body posture was observed in Group 3 females.

Body weights:
Both males and females in all groups gained weight during the course of the study. With the exception of an increase in body weights for Group 3 males, there was a general trend of decreased body weights for animals dosed with ketorolac tromethamine. This dose-dependent decrease in body weight was slight in males but more pronounced in females, achieving statistical significance. However, weight gain was similar between Group 3 and control females after the recovery period.

Dose Group/ Treatment	Males			Females		
	Pretrial BW (g) (Day -7)	BW Gain (g) (Day 1 to 29)	Recovery BW Gain (g) (Day 14 to 29)	Pretrial BW (g) (Day -7)	BW Gain (g) (Day 1 to 29)	Recovery BW Gain (g) (Day 14 to 29)
1 – VC	214	132	75	181	67	36
2 – LD	214	124 (-6)		182	56** (-16)	
3 – MD	215	144 (+9)	92	180	54*** (-19)	37
4 – HD	214	118 (-11)	87	182	--	

Food consumption:
Food consumption by females in Groups 2 (Week 3), 3 (Weeks 1 and 3), and 4 (Week 1) were significantly decreased. During the recovery period, there were no differences in food consumption between control and Group 3 or other Group females.

Ophthalmoscopy:
Individual animal ophthalmoscopy revealed several minor findings including fundus pale streaks, hyaloids remnant, persistent papillary membranes as well as small and central opacity. Many of these findings were also present in pretrial and in control animals and recovery

animals did not exhibit any of these ophthalmoscopy findings. Therefore the reviewer agrees with the sponsor's assessment that there were no treatment-related ophthalmoscopy findings.

EKG:
Not examined.

Hematology:
Group mean hematology results are summarized in the Table below. In all dosed females, a dose-dependent reduction in activated partial thromboplastin time was observed. In Group 3 females, several hematology parameters tested were affected that included increases in leukocytes, reticulocyte numbers and reductions in mean cell hemoglobulin concentration and coagulation time. After the recovery period, all of the parameter changes reversed and were comparable to the control group.

Hematology												
	Males							**Females**				
	VC 1	**LD 2**	**MD 3**	**HD 4**	**VC 1**	**MD 3**	**HD 4**	**VC 1**	**LD 2**	**MD 3**	**VC 1**	**MD 3**
APTT sec	22	**21**	**20**	**19**	24	23	20	33	23 (-30)	**19 (-42)**	18	10 (-44)
MCHC g/dL	32.9	33.1	32.6	32.8	35.8	36.0	**36.6**	34.2	33.7	**32.4 (-5)**	36.5	36.6
Ret $x10^9$/L	239	249	269	242	254	254	207	220	208	**438 (2.0x)**	182	**97 (-47)**
WBC $x10^9$/L	8.6	9.6	10.7	8.9	14.2	13.6	12.2	7.02	7.76	**15.6 (2.2x)**	8.4	8.9
Neut $x10^9$/L	1.53	1.61	2.05	1.68	2.32	2.55	2.16	1.23	1.55	**5.74 (4.7x)**	1.2	1.4
Lymph $x10^9$/L	6.5	7.4	7.9	6.6	11.0	10.3	9.3	5.4	5.7	**8.7 (1.6x)**	6.7	6.3
Mono $x10^9$/L	0.21	0.23	0.30	0.21	0.44	0.34	0.33	0.13	0.20	**0.48 (3.7x)**	0.22	0.20
Eos $x10^9$/L	0.14	0.14	0.14	0.13	0.14	0.22	0.15	0.12	0.14	**0.27 (2.3x)**	0.13	0.16
Baso $x10^9$/L	0.19	0.17	0.19	0.17	0.10	0.12	0.10	0.12	0.14	**0.24 (2.0x)**	0.07	0.09
LUC $x10^9$/L	0.06	0.07	0.07	0.06	0.15	0.14	0.11	0.05	0.05	**0.11 (2.2x)**	0.10	0.08

Clinical chemistry:
Group mean clinical chemistry results are summarized in the Table below. A slight increase in glucose levels were observed in Groups 4 and 5 males and Groups 3 and 5 females. After the recovery period, glucose levels in Group 5 males were still elevated compared to control animals. It was noted by the sponsor that glucose levels particularly in females were higher than historical controls but the reason for this elevation is unknown.

Clinical Chemistry												
	Males							Females				
	VC 1	LD 2	MD 3	HD 4	VC 1	MD 3	HD 4	VC 1	LD 2	MD 3	VC 1	MD 3
Urea mmol/L	5.3	**6.0 (1.1x)**	**6.6 (1.2x)**	**6.4 (1.2x)**	6.0	5.8	5.5	6.6	7.3	7.3	7.7	**6.3 (-18)**
ALT iu/L	90	87	85	**70 (-22)**	76	70	74	80	70	67	75	64
ALP iu/L	271	249	238	**208 (-23)**	148	181	156	162	132	129	110	104
AST iu/L	87	95	91	86	90	78	79	108	**80 (-26)**	**85 (-21)**	85	83
LDHIL iu/L	328	282	**243 (-26)**	**212 (-45)**	301	169 (-44)	**123 (-59)**	328	202	235	138	113
TP g/L	69	70	68	67	72	71	70	73	73	**63 (-14)**	75	75
Alb g/L	44	45	**42 (-5)**	**41 (-7)**	44	43	43	49	47	**39 (–20)**	49	48
A/G-Ratio	1.7	1.8	1.7	1.6	1.6	1.5	1.6	2.1	1.9	**1.6 (-24)**	1.9	1.9
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose												

Urinalysis:
Group mean urinalysis results for specific gravity and urine volume are summarized below.

In both males and females, the specific gravity was slightly decreased whereas urine volume was increased in animals administered ketorolac tromethamine. These parameter changes were not observed after the recovery period.

Urinalysis												
	Males							Females				
	VC 1	LD 2	MD 3	HD 4	VC 1	MD 3	HD 4	VC 1	LD 2	MD 3	VC 1	MD 3
SG	1.05	1.03	**1.03**	**1.03**	1.04	1.04	1.04	1.04	**1.03**	**1.02**	1.04	1.03
UV	3.1	3.8 (1.2x)	**5.6 (1.3x)**	4.3 (1.4x)	2.6	3.2	2.6	2.6	3.5 (1.3x)	**6.3 (2.4x)**	2.0	2.1
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose; SG, specific gravity; UV, urine volume; UP, urinary protein												

Gross Pathology
Necropsy findings were observed only in MD females with the majority of findings occurring in the gastrointestinal system. Findings included adhesions, fluids or abnormal contents in the abdominal cavity, intestines with abnormal appearance, raised foci, and adhesions, enlarged mesenteric and madibular lymph nodes, pale mucosa of the stomach. However, these gross findings were not observed in recovery group females, indicating reversibility.

Necropsy Gross Findings: Incidences													
	Males							Females					
	VC 1	LD 2	MD 3	HD 4	VC 1	MD 3	HD 4	VC 1	LD 2	MD 3	HD* 4	VC 1	MD 3
# of Animals	**10**	**10**	**10**	**10**	**6**	**6**	**6**	**10**	**10**	**10**	**16**	**6**	**6**
Abdominal Cavity													
Contents abnormal							**1**			1	2		
Contains fluid											3		
Adhesion(s)											1		
Caecum													
Contents abnormal										1	1		
Duodenum										1			
Raised focus													
Eye													
Pale, both											3		
Ileum													
Abnormal shape											1		
Raised focus										3	1		
Thickened											1		
Reddened											3		
Adhesion(s)											2		
Intestines													
Contents abnormal										1			
Pronounced gut associated lymphoid tissue											1		
Raised focus											1		
Adhesion(s)											2		
Jejenum													
Dark focus											1		
Abnormal shape											1		
Rasied focus										3			
Pale focus											1		
Thickened											2		
Adhesion(s)										1	1		
Liver													
Pale										1	2		
Lung													
Dark focus		1											
Pale, all lobes											2		
Spongy, all lobes											1		
Stomach													
Pale										1			
Dark focus											1		
Contents abnormal											1		
distended											1		
Enlarged Lymph Node													
Bronchial		1									1		
Cervical											2		
Mandibular	1	1				1	1	1	2	1	2		1
Mesenteric										1	1		
Skin and subcutis													
staining											7		

*all HD females were euthanized for found dead by Day 8
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose

Organ Weights

Group mean organ weights changes are presented in the Table below. In group 4 males, pituitary gland weights were reduced by 18% whereas testes weights were increased in the HD group, but these changes in organ weights were not observed in animals following the recovery period. In Group 3 MD females (HD female group was all euthanized or found dead), several organ weight changes were reported including, increases in kidney and lung mean weights and in particular the spleen, which was significantly increased by approximately 50%. In addition, absolute brain weights were increased in MD females but were reduced in HD males. All of the reported organ weight changes were still present after the recovery period, indicating a lack of reversal.

Relative Organ Weights (Males)							
	Main Study				Recovery		
Organ	**VC**	**LD**	**MD**	**HD**	**VC**	**MD**	**HD**
Pituitary Gland	0.0028	0.0028	0.0026	**0.0023 (-18%)**	0.0021	0.0024	0.0020
Testes	0.781	0.841	0.765	**0.849 (+9%)**	0.773	0.704	0.763
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose							

Relative Organ Weights (Females)					
	Main Study			Recovery	
Organ	**VC**	**LD**	**MD**	**VC**	**MD**
Brain	0.701	**0.748 (+7)**	**0.762 (+9)**	0.575	**0.694 (+21)**
Brain (Abs)	1.93	1.92 (-1)	1.96 (+2)	2.01	1.96 (-2)
Kidneys	0.741	0.781 (+5)	**0.861 (+16)**	0.605	**0.733 (+21)**
Lung	0.465	0.485 (+4)	**0.518 (+11)**	0.386	**0.500 (+30)**
Spleen	0.210	0.238 (+13)	**0.316 (+50)**	0.158	0.187 (+18)
shaded columns, recovery group; **bold**, reflects statistical significance; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose					

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

A summary of the microscopic findings are presented in the Table below. Target organs were the gastrointestinal tract, kidney, and spleen. Several minor findings were identified in the nasal cavity, which included minimal hyperplasia of the transitional epithelium, particularly in HD males and minimal inflammatory cell infiltration in MD and HD males but incidences were also observed in the recovery and vehicle control groups. Other findings included, severe thymic atrophy in a MD female and perivascular cell foci in the lungs in HD males and MD females. The majority of histopathological findings were related to the gastrointestinal system, which included moderate ulceration in the glandular stomach of a MD female and minimal to

mild severity in a MD and HD male, with incidences of inflammatory cell infiltration in the lamina propia occurring in all dosed males and females. Marked ulceration in the ileum was observed in a MD female. In addition, several instances of minimal to mild peritonitis occurred in the duodenum of a HD male, as well as the cecum, ileum and jejunum in MD females. Likely secondary to the GI toxicities were the incidences of minimal to mild hematopoiesis in the spleen, which were observed in HD males and in all dosed female groups. Kidney findings included minimal to mild chronic progressive nephropathy in the HD males and MD females and focal mineralization in the LD and MD female groups as well as in the vehicle control group. Other histology findings were observed in treatment groups but were not considered treatment-related as incidences also occurred in the vehicle control group included squamous metaplasia in the larynx, lymphoid hyperplasia in the mesenteric lymph node and plasmocytosis in the mandibular and mesenteric lymph node, interstitial inflammatory cell infiltration of the prostate, focal basophilic tubules of the kidneys, and focal inflammation of the liver.

Reviewer's Comment: In the Pharmacology/Toxicology review of NDA 19-645 dated June 30, 1987, studies in mice administered ketorolac via the oral route reported a similar toxicity profile, which included the GI erosions, ulceration, peritonitis, and adhesions; increased kidney weights and nephritis; enlarged and increased spleen weights. The current study findings indicate that nasal administration does not affect the toxicity profile of ketorolac and does not result in adverse nasal toxicities.

Histopathology Findings: Incidences											
	Males						**Females**				
	VC 1	**LD 2**	**MD 3**	**HD 4**	**VC 1**	**HD 4**	**VC 1**	**LD 2**	**MD 3**	**VC 1**	**MD 3**
# of Animals	**10**	**10**	**10**	**10**	**6**	**6**	**10**	**10**	**10**	**6**	**6**
Nasal Cavity											
Transitional cell hyperplasia, level 1 minimal	0	0	1	4	0	3	0	-	0	3	0
Inflammatory cell infiltration, focal, minimal	0	0	3	3	0		0	-	0	0	
Lung											
Inflammatory cell foci, perivascular minimal	0	-	-	3	0		0	-	1	0	
Spleen											
Hematopoiesis											
min	2	-	-	2	0		2	4	6	0	
mild	0	-	-	0			0	0	2		
Thymus											
Atrophy (severe)	0	-	-	0	0		0	-	1	0	
Kidney											
Chronic progressive nephropathy											
min	2	-	-	0	0	0	1	1	3	1	3
mild	0	-	-	3	0	0	1	0	2	0	0
Mineralization, focal											
min	0	-	-	0			5	7	5	0	2
mild	0	-	-	0			0	1	3	2	1
mod	0	-	-	0			0	0	0	0	1

Stomach											
Ulcer glandular stomach, focal											
min-mild	0	0	1	1			0	0	0		
mod	0	0	0	0			0	0	1		
Inflammatory cell infiltration. Lamina propia											
min	1	2	3	2	1	0	0	1	2	0	0
mild	0	1	0	1	0	0	0	0	0	0	0
Duodenum											
Peritonitis minimal	0	0	0	0			0	0	1		
Jejenum											
Peritonitis											
min	0	0	0	0	R		0	0	3	R	
mild	0	0	0	0			0	0	1		
Ileum											
Ulcer with inflammation											
marked	0	0	0	0	0		0	0	1	0	
Peritonitis											
mild	0	0	0	0	0		0	0	3	0	
Cecum and Colon											
Peritonitis											
mild	0	0	0	0			0	0	1		
shaded columns, recovery group; VC, vehicle control; KT, ketorolac tromethamine; LD, low dose; MD, mid dose; HD, High dose											

Histopathology inventory

Study	KET002	KET003
Species	Rat	Rat
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X*	X*
Bronchial Lymph Node	**X**	
Cecum	X	X
Cervix	X	X
Cervical lymph Node	**X**	
Colon	X	X
Duodenum	X	X
Epididymis	X*	X*
Esophagus	X	X
Eye + Optic Nerves	X	X
Fallopian tube		
Gall bladder		
Gross lesions	X	X
Harderian gland	X	
Heart	X*	X*
Ileum	X	X
Injection site		

Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X+	X
Larynx	**X**	**X**
Liver	X*	X*
Lungs - **left lobe** - **right anterior** - **right middle** - **right posterior** - **accessory**	**X***	**X***
Lymph nodes, cervical	X	X
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity	**X**	**X**
Ovaries	X*	X*
Pancreas	X	X*
Parathyroid	X	X
Peripheral nerve		
Pharynx	**X**	**X**
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland	X*	X*
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle		
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X*
Tongue	X	X
Trachea - **anterior** - **posterior**	**X**	**X**
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained
+, nasolacrimal ducts examined
Bolded are respiratory tract organs

2.6.6.4 Genetic toxicology
The sponsor performed two *in vitro* assays, the Ames test and in the Chromosomal Aberrations Assays, to evaluate the potential mutagenicity and clastogenicity of 1-keto degradant.

Study title: 1-Keto Degradant: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 100, TA 1537, and TA 98 and *Escherichia coli* WP2*uvr*A

Key findings:
- 15% Ketorolac vehicle induced excessive cytotoxicity, therefore DMSO was subsequently used as the vehicle.
- cytotoxicity and precipitate was observed using tester strains TA100 at ≥ 1667 µg test article dissolved in DMSO per plate in the presence or absence of S9 mix
- 1-Keto Degradant was tested in the Ames assay at concentrations of 5, 17, 50, 167, 500 and 1667 µg/plate.
- increases in number of revertants were not detected in any strain at all test article concentrations tested in either the presence or absence of S9 mixture.
- **Under the conditions of this study, A-794738 is considered negative in the bacterial reverse mutation assay.**

Study no.: 780864
Volume #, and page #: Vol. 6, pgs 1-37
Conducting laboratory and location: (b) (4)

Date of study initiation: 30 April 200
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Compound A, Lot No. MCLS1388-13-1, Purity 99.6% (HPLC)

Methods
For reproducibility of all findings, 2 assays were conducted in both the absence and presence of a metabolic activator (S9 mixture). The first test was performed using the Direct Plate Incorporation Method and was repeated if the first test was positive to confirm the response. Otherwise if the first test was negative, the second test was conducted using the Pre-incubation Method.

Strains/species/cell line:
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvr*A

Doses used in definitive study:
Test article concentrations of 5, 17, 50, 167, 500 and 1667 µg/plate for all 5 bacterial strains were tested in both the presence and absence of metabolic activation

Basis of dose selection:
Doses used in the mutagenicity assay were selected on the basis of results of a range finding study conducted on tester strain TA100 with six concentrations of test article ranging from 17 – 5000 μg/plate, either in presence or absence of metabolic activation system (S9). Evidence of cytotoxicity observed as thinning of the bacterial lawn and precipitation were observed at the two highest test concentrations of 1667 and 5000 μg/plate.

Negative controls:
DMSO

Positive controls:
See table below.

Strain	S9 Activation	Positive Control	Concentration (μg/plate)
TA1535, 1537	Present	2-Aminoanthracene	2.0
TA98, 100		2-Aminoanthracene	0.5
WP2*uvr*A		2-Aminoanthracene	20
TA1535, 100	None	Sodium azide	1.0
TA98		2-Nitrofluorene	1.0
TA1537		9-Aminoacridine	80
WP2 *uvr*A		N-Ethyl-N-nitro-N-nitrosoguanidine	2.0

Incubation and sampling times:
Direct Plate Method: 2mL of molten agar was dispensed into a sterile test tube. S9 mix or 0.05M phosphate buffer, pH 7.4 (0.5mL) followed by 0.1 mL of bacteria and finally the test item solution (0.1mL) was added. The tube contents were mized then poured onto minimal medium plates.

Pre-incubation Method: S9 mix or 0.05 M phosphate buffer, pH 7.4 (0.5mL) followed by 0.1 mL of bacteria and finally the test item solution (0.1mL) was added into a sterile test tube. The tubes were placed into a shaking incubator at 37^{o}C for 20 min. Afterwards 2 mL of molten agar was added to each tube, mixed and then poured onto minimal medium plates.

Plates were inverted and incubated at 37^{o}C for 2 to 3 days and then examined. The number of mutant colonies was determined using a Sorcerer Colony Counter and captured electronically.

Results

Study validity:
The study is considered valid. Appropriate test strains and positive controls were used with expected incidences of revertants. Dose selection for the test article was adequately based upon a preliminary study, which demonstrated cytotoxicity and precipitation at doses of 1667 and 5000 μg/plate.

Study outcome:
In a preliminary assay, 15% Ketolorac was used as a vehicle for the 1-Keto Degradant to assess toxicity. However, there was no survival on any plates incubated with the vehicle and test

article, indicating excessive cytotoxicity. As a result DMSO was used as the vehicle for all subsequent tests. In this case, a preliminary assay revealed slight cytotoxicity at the two highest concentrations of test article, 1667 and 5000 µg/plate. It was noted that precipitation at the highest does of 5000 µg/plate was very heavy and was considered excessive and therefore the highest dose was set at 1667 µg/plate.

Summarized in Tables below are the results of the first (Direct Plate) and second (Pre-incubation) mutagenicity assay.

Table 1: First Bacterial Reverse Mutation Assay without S9 Mix (Direct Plate)

			No. Revertants (Mean)				
	Treatment	**Conc. µg/plate**	**TA 1535**	**TA1537**	**TA 98**	**TA 100**	**WP2*uvr*A**
-S9	DMSO	--	15.3	10.0	21.0	85.0	5.7
	1-Keto Degradant	5	12.0	13.0	27.7	80.7	9.0
		17	12.0	14.0	18.7	77.3	11.3
		50	9.7	10.0	13.3	71.3	9.3
		167	12.0	8.3	13.0	69.7	8.7
		500	5.3	9.7	15.7	72.7	12.3
		1667	11.7	2.7	6.7	41.0	13.3
	Positive control	Varied	421.3 (NaN_3)	8570.0 (9-AA)	880.7 (2NF)	1051.7 (NaN_3)	155.0 (ENNG)
NaN3: Sodium Azide (1 µg/plate); **9-AA**: 9-aminoacridine (80 µg/plate) **2NF**: 2-Nitrofluorene (1 µg/plate); **ENNG**: N-ethyl-N-Nitro-N-nitrosoguanidine (2 µg/plate)							

Table 2: First Bacterial Reverse Mutation Assay with S9 Mix (Direct Plate)

			No. Revertants (Mean)				
	Treatment	**Conc. µg/plate**	**TA 1535**	**TA1537**	**TA 98**	**TA 100**	**WP2*uvr*A**
+S9	DMSO	--	11.7	14.0	27.7	80.7	12.7
	1-Keto Degradant	5	10.3	14.7	21.7	76.3	12.0
		17	10.3	12.7	27.0	77.3	10.3
		50	11.0	13.7	22.0	55.3	13.0
		167	10.7	16.3	32.7	64.0	13.7
		500	11.7	11.7	22.7	63.0	10.3
		1667	10.3	7.0	17.7	56.7	11.0
	Positive control-2AAN	Varied	426.7	202.7	299.3	584.0	270.0
2AAN: 2-Aminoanthracene (2µg/plate)							

In both methods, no substantial increases in the number of revertants per plate were observed at all doses of test article either in the presence or absence of S9 mix. At the highest concentration, 1-Keto Degradant precipitated and induced toxicity as seen by the reduction in the number of mutant colonies in the presence or absence of S9 mix. In conclusion, the test article, 1-Keto Degradant, is not likely to be mutagenic in the tested condition of the assay.

Table 3: Second Bacterial Reverse Mutation Assay without S9 Mix (Pre-incubation)

			No. Revertants (Mean)				
	Treatment	**Conc. µg/plate**	**TA 1535**	**TA1537**	**TA 98**	**TA 100**	**WP2*uvr*A**
-S9	DMSO	--	9.7	11.7	18.7	79.7	6.3
	1-Keto Degradant	5	13.0	14.7	22.3	73.3	11.7
		17	8.7	16.3	22.7	78.0	12.7
		50	12.3	18.0	18.3	74.0	13.3
		167	12.0	12.7	18.0	72.3	9.0
		500	13.3	8.3	20.7	74.3	9.0
		1667	4.3	3.3	3.3	36.7	3.3
	Positive control	Varied	521.7 (NaN_3)	7039.7 (9-AA)	994.7 (2NF)	1068.3 (NaN_3)	178.7 (ENNG)
NaN3: Sodium Azide (1 µg/plate); **9-AA**: 9-aminoacridine (80 µg/plate) **2NF**: 2-Nitrofluorene (1 µg/plate); **ENNG**: N-ethyl-N-Nitro-N-nitrosoguanidine (2 µg/plate)							

Table 4: Second Bacterial Reverse Mutation Assay with S9 Mix (Pre-incubation)

			No. Revertants (Mean)				
	Treatment	**Conc. µg/plate**	**TA 1535**	**TA1537**	**TA 98**	**TA 100**	**WP2*uvr*A**
+S9	DMSO	--	8.0	11.3	23.7	87.0	11.3
	1-Keto Degradant	5	14.0	13.0	31.3	81.3	16.0
		17	12.7	15.0	32.0	80.0	17.7
		50	12.0	12.0	33.7	83.0	13.3
		167	15.3	14.3	30.7	68.7	18.3
		500	9.7	9.7	25.7	65.0	8.3
		1667	14.7	9.0	16.0	53.0	13.7
	Positive control - 2AAN	Varied	474.0	295.7	579.3	594.0	450.0
2AAN: 2-Aminoanthracene (2µg/plate)							

Study title: 1-Keto Degradant: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cell Cultures In Vitro

Key findings:

- cytoxicity occurred at doses ≥ 125 µg/mL in the presence of S9 and ≥ 500 µg/mL in the absence of S9.
- definitive study: cells were treated with 63, 125, and 250 µg/mL of test article for 6 hr with metabolic activation or 125, 250, 500, and 1000 µg/ml of test article for 6 hr without metabolic activation.
- **1-keto degradant induced chromosome aberrations in cultured CHO cells only in the presence of metabolic activation at concentrations of 125 and 250 µg/mL. It is noted at these concentrations, the test-article was also observed to be cytotoxic.**
- **Under the conditions of this study, the 1-Keto degradant is clastogenic.**

Study no.: 780885
Volume #, and page #: Vol. 6, pgs 1-23
Conducting laboratory and location: (b) (4)

Date of study initiation: 10 May 2007
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Compound A, Lot No. MCLS1388-13-1, Purity 99.6% (HPLC)

Methods:
The ability for 1-Keto Degradant to induce chromosome aberrations in cultured CHO cells with and without exogenous metabolic activation was determined via a standard assay. Treatments with test article or vehicle control were performed on duplicate cell cultures. After treatment, cells were washed twice with serum free medium and full growth medium added subsequently for the recovery period. After the recovery period colcemid was added to all cultures at a final concentration of 0.1 μg/mL. Three slides per culture were made by dropping a cell suspension on to a slide subsequently stained with 5% Giemsa and finally mounted with DPX under a coverslip.

Strains/species/cell line:
The cell line used was Chinese hamster ovary (CHO 10 B_4) cells.

Doses used in definitive study:
6 hr exposure/19 hr recovery (-S9): 0, 125, 250, 500 and 1000 μg/mL
6 hr exposure/19 hr recovery (+S9): 0, 63, 125, and 250 μg/mL

Basis of dose selection:
Doses used in the chromosomal aberration assay were selected based on the results of a concentration range-finding study using nine concentrations of the test article ranging from 4 to 1000 μg/mL with and without metabolic activation (S9 mix). In the presence of S9, slightly rounded cells and sparse metaphase cells were observed in cultures treated with 125 μg/mL. Cytotoxicity was noted in one set of cultures treated with test article concentration of 250 μg/mL (i.e. cell viability was < 50% of vehicle control cultures). In cultures treated with the two highest concentrations, there were no metaphase cells.

In the absence of S9 mix, cytotoxicity was noted in cultures treated with test article concentrations ≥ 500 μg/mL (i.e. cell viability was < 50% of vehicle control cultures).

Negative controls:
DMSO

Positive controls:
The positive control agents used in the assay were methyl methanesulphonate (10-40 μg/mL) without metabolic activation and cyclophosphamide (20-50 μg/mL) with metabolic activation.

Incubation and sampling times:
CHO cells were incubated with test article, vehicle or positive control for 6 hr in the presence or absence of S9 mix, followed by culture for 16 hr in fresh medium (-S9 or +S9). Colcemide was given 2 hr prior to cell harvesting to stop cells in metaphase. Living cultures were

examined for evidence of changes to cell morphology, at the end of the treatment period and prior to harvesting of cultures.

S9 Mix	Cultures Established	Treatment Period	Recovery Period	Colcemid	Harvest
Presence of S9 mix	*ca* 20 h before exposure	0-6 h	6-22 h	22-24 h	24 h
Absence of S9 mix					

Results

Study validity:
The study appears to be valid. Appropriate positive controls were used and produced expected results. The number of cells and metaphases evaluated were appropriate and in accordance with standard practices. Dose selection was based upon toxicity, solubility and precipitation and deemed acceptable for both non-activated and activated system. Cytotoxicity was assessed with and without metabolic activation through a cell count index and morphology.

Study outcome:
The results of the chromosomal aberration test in the absence and presence of metabolic activation with 1-keto degradant (6 hr treatment) are represented in the summary tables below. The test article did not induce chromosomal aberrations at all concentrations tested in the absence of metabolic activation. However, in the presence of metabolic activation, 1-keto degradant induced greater incidences of chromosomal aberrations that were dose-dependent at the two highest concentrations of 125 and 250 μg/mL as compared to vehicle control. Although cytotoxicity was not assayed concurrently in the chromosomal aberrations assay, a previous dose range finding study demonstrated that 1-keto degradant at the concentrations of 125 and 250 μg/mL induced cytotoxicity as evidenced by the reduction in cell count index and rounded morphology of the cells. It can be concluded that the 1-keto degradant was clastogenic as evidenced by the increased incidences of structural aberrations with Chinese Hamster Ovary cells *in vitro*.

Reviewer's comment: It is important to note that the parent, ketorolac tromethamine also tested positive in the in vitro chromosome aberration assay in CHO cells at higher concentrations. To determine whether 1-keto degradant and its parent compound, ketorolac, share a specific fragment responsible for its clastogenic activity, computational toxicology analysis of the 1-keto degradant and ketorolac was performed. Two software models were used for this evaluation, namely MC4PC and MDL-QSAR. Of the two software programs, only MC4PC has the ability to identify a specific fragment of a test compound that is predicted to be associated with toxicology activity, however, using this test the 1-keto degradant was predicted negative for in vitro aberrations and therefore this relationship could not be determined. In contrast the MDL-QSAR software predicted the 1-keto to be positive but this prediction relies on an electrotopological descriptor-based approach and therefore does not provide evidence that a shared fragment is responsible for clastogenicity (For full detailed computational toxicology report, refer to Appendix 1). In addition, evidence to support distinct mechanisms between the degradant and the parent molecule is that the 1-keto degradant tested positive only in the presence of S9 metabolic activation.

Chromosomal Aberrations in CHO cells with Ketorolac (**6 Hr exposure**) without metabolic activation

	Treatment	**Concentration (µg/mL)**	***Cell count index**	**% cells of aberrations**	
				+g	**-g**
-S9	Vehicle control	--	1.0	1.5	0
	1-Keto Degradant	125	0.92	1.0	0
	1-Keto Degradant	250	0.50	3.0	2.0
	1-Keto Degradant	500	0.49	0.5	0.5
	1-Keto Degradant	1000	0.44	0.5	0
	MMS	30	-	6.0	5.0
	MMS	40	-	26	23

***Performed in a separate preliminary dose range finding study (see below)**

Chromosomal Aberrations in CHO cells with Ketorolac (**6 Hr exposure**) with metabolic activation

	Treatment	**Concentration (µg/mL)**	***Cell count index**	**% cells of aberrations**	
				+g	**-g**
+S9	Vehicle control	--	1.0	2.0	0.5
	1-Keto Degradant	63	1.06	2.0	0.5
	1-Keto Degradant	125	0.66 (slightly rounded cells)	**6.5**	**5.5**
	1-Keto Degradant	250	0.5 (slightly rounded cells, with some rounded cells	**11.5**	**9.0**
	Cyclophosphamide	40	-	15	12
	Cyclophosphamide	50	-	9.0	8.0

***Performed in a separate preliminary dose range finding study**

Toxicity Evaluation: With S9 Mix, 6 h treatment, 24 h harvest

Treatment Group	Conc. (µg/mL)	De-coded No.	Cell Count Data		Observations		Toxic Judge
			No. of Cells ($x10^6$)	Index	Culture	Slide	
Dimethyl-sulphoxide	1%	1	0.95	1.00	Nil toxicity	Nil toxicity	-
		2	0.95	1.00			-
1-keto degradant	4	3	0.98	1.03			-
		4	0.88	0.93			-
	8	5	1.03	1.08			-
		6	0.95	1.00			-
	16	7	0.88	0.93			-
		8	1.00	1.05			-
	31	9	1.10	1.16			-
		10	0.95	1.00			-
	63	11	1.05	1.11			-
		12	0.95	1.00			-
	125	13	0.60	0.63	Slightly rounded cells	Sparse metaphase cells	t
		14	0.65	0.68			t
	250	15	0.50	0.53	Slightly rounded cells, with some rounded cells	Very sparse metaphase cells, large amount of interphase cells	t
		16	0.45	0.47			tt
	500	17	0.33	0.35	Rounded cells	No metaphase cells, large amount of interphase cells	ttt
		18	0.20	0.21			ttt
	1000	19	0.28	0.29			ttt
		20	0.23	0.24			ttt

Toxicity Evaluation: Without S9 Mix, 6 h treatment, 24 h harvest

Treatment Group	Conc. (µg/mL)	De-coded No.	Cell Count Data		Observations		Toxic Judge
			No. of Cells ($x10^6$)	Index	Culture	Slide	
Dimethyl-sulphoxide	1%	25	1.55	0.89	Nil toxicity	Nil toxicity	-
		26	1.95	1.11			-
1-keto degradant	4	27	1.65	0.94			-
		28	1.85	1.06			-
	8	29	1.50	0.86			-
		30	1.63	0.93			-
	16	31	1.63	0.93			-
		32	1.65	0.94			-
	31	33	1.63	0.93			-
		34	1.80	1.03			-
	63	35	1.48	0.85			-
		36	1.40	0.80			-
	125	37	1.58	0.90			-
		38	1.63	0.93			-
	250	39	0.88	0.50			-
		40	0.85	0.49			tt
	500	41	0.85	0.49			tt
		42	0.85	0.49			tt
	1000	43	0.70	0.40			tt
		44	0.83	0.47			tt

2.6.6.5 Carcinogenicity

Carcinogenicity studies were not conducted and are not necessary for acute use products. The following information is found in the approved label for the Ketorolac tromethamine product Toradol®

> An 18-month study in mice with oral doses of ketorolac tromethamine at 2 mg/kg/day (0.9 times the human systemic exposure at the recommended IM or IV dose of 30 mg qid, based on area-under-the-plasma-concentration curve [AUC]), and a 24-month study in rats at 5 mg/kg/day (0.5 times the human AUC) showed no evidence of tumorigenicity.
>
> Ketorolac tromethamine was not mutagenic in the Ames test, unscheduled DNA synthesis and repair, and in forward mutation assays. Ketorolac tromethamine did not cause chromosome breakage in the in vivo mouse micronucleus assay. At 1590 µg/mL and at higher concentrations, ketorolac tromethamine increased the incidence of chromosomal aberrations in Chinese hamster ovarian cells.

2.6.6.6 Reproductive and developmental toxicology

Reproductive and developmental toxicology studies were not conducted for this NDA. The following information is found in the approved label for the Ketorolac tromethamine product Toradol®

> Impairment of fertility did not occur in male or female rats at oral doses of 9 mg/kg (0.9 times the human AUC) and 16 mg/kg (1.6 times human AUC) of ketorolac tromethamine, respectively.

Teratogenic Effects: Pregnancy Category C
Reproduction studies have been performed during organogenesis using daily oral doses of ketorolac tromethamine at 3.6 mg/kg (0.37 times the human AUC) in rabbits and at 10 mg/kg (1.0 times the human AUC) in rats. Results of these studies did not reveal evidence of teratogenicity to the fetus. However, animal reproduction studies are not always predictive of human response.

Nonteratogenic Effects
Because of the known effects of nonsteroidal anti-inflammatory drugs on the fetal cardiovascular system (closure of ductus arteriosus), use during pregnancy (particularly late pregnancy) should be avoided. Oral doses of ketorolac tromethamine at 1.5 mg/kg (0.14 times the human AUC), administered after gestation Day 17, caused dystocia and higher pup mortality in rats. There are no adequate and well-controlled studies of TORADOL in pregnant women. TORADOL should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Labor and Delivery
The use of TORADOL is contraindicated in labor and delivery because, through its prostaglandin synthesis inhibitory effect, it may adversely affect fetal circulation and inhibits uterine contractions, thus increasing the risk of uterine hemorrhage (see CONTRADICTIONS).

Effects on Fertility
The use of ketorolac tromethamine, as with any drug known to inhibit cyclooxygenase/prostaglandin synthesis, may impair fertility and is not recommended in women attempting to conceive. In women who have difficulty conceiving or are undergoing investigation of infertility, withdrawal of ketorolac tromethamine should be considered.

Nursing Mothers
After a single administration of 10 mg TORADOL^{ORAL} to humans, the maximum milk concentration was 7.3 ng/mL, and the maximum milk-to-plasma ratio was 0.037. After 2 day of dosing (qid), the maximum milk concentration was 7.9 ng/mL, and the maximum milk-to-plasma ratio was 0.025. Because of the possible adverse effects of prostaglandin-inhibiting drugs on neonates, use in nursing mothers is contradicted.

2.6.6.7 Local tolerance

Study title: Ketorolac Tromethamine – Nasal Absorption and Local Tolerance Study in Rabbits

Key study findings: A preliminary 7 Day intranasal local tolerance study in rabbits was conducted to evaluate three different formulations A, B, and C versus IV administration; nasal dose evaluated was approximately 1.4 mg/cm^2/day (15% formulation concentration)

- Test article related changes at the level of nasal turbinates included acute inflammation, hemorrhage, erosion and moderate ulcer of the septal mucosa

Reviewer's Comment: This study was conducted early in the drug development nonclinical program to test different formulations of Ketorolac tromethamine and to evaluate local tolerance in rabbits. Information regarding Formulations A, B, and C were NOT provided and therefore the findings to support local tolerance are questionable.

Study no.: KET 007
Volume #, and page #: Module 4 Vol. 6 pg 1-92
Conducting laboratory and location: (b) (4)

Date of study initiation: 20 July 1992
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: Ketorolac tromethamine, lot # E1-MU-002, 99.3%
Formulation/vehicle: For IV administration, Ketorolac tromethamine was solubilized in phosphate buffer solution (pH 8) at a concentration of 4 mg/mL. For intranasal administration, the test article in three different formulations (Formulation A, B, and C) at 15% of the active drug substance was administered twice daily. The administered volume was 0.04 mL/kg and the administered dose was 6 mg/kg.

Methods

Doses: 6 mg/kg/administration (12 mg/kg/day). The test article was administered twice a day for 7 consecutive days with an additional administration on day 8. Group 4 received intranasal administration of saline solution twice daily for 7 days and received a single iv administration of the test article (6 mg/kg).
Reviewer's Comment: Given the average body weight of the rabbits at the time of dosing was approximately 3.6 kg and the total nasal surface area of a rabbit is 60 cm^2, the calculated nasal dose administered was 0.72 mg/cm^2 bid or 1.4 mg/cm^2/day.

Study design: In Groups 1-3, three male New Zealand white rabbits/group were administered different formulations of the test article (12 mg/kg/day) by intranasal administration twice a day for 7 consecutive days by means of a catheter in the right nostril, while the vehicle control was applied in the left nostril. Rabbits in Group 4 received saline administration twice daily for 7 days and subsequently received a single iv dose of ketorolac tromethamine (6mg/kg) by bolus injection into the ear vein. Blood samples (at 0, 0.25, 0.5, 1, 2, 3, 5 hrs after last administration on Day 8) were collected into heparinised tubes and immediately centrifuged at 2500 g for 10 min. Blood cells were discarded and plasma samples were retained. All animals were euthanized and histological examination of the nasal turbinates, pharynx and larynx were conducted.

Results:

Toxicokinetics
Exposure levels of ketorolac after repeated intranasal administration and after a single iv administration are summarized in the Table below. After the single iv administration of ketorolac tromethamine (6mg/kg; Group 4), mean AUC_{0-5} was calculated to be 22,651 ng*hr/mL. In comparison, intranasal administration of the different formulations A, B, and C yielded AUC_{0-5} values of 8897, 9718, and 9974 ng*hr/mL, respectively. Given these exposure levels, bioavailability of the three formulations administrated intranasally were approximately 0.40:1. It should be noted that in Group 2, one rabbit (#57) had considerably lower exposure (4018 vs 15407 and 13286 ng*hr/mL).

Group	Cmax (ng/mL)	AUC_{0-5} (ng*hr/mL)	AUC (ng*hr/mL)	Bioavailability
IV		22651	22964	1
A	4784	8897	9895	0.39
B	5943	9718	10784	0.43
C	4263	9974	10701	0.44

Test article related changes were observed at the level of nasal turbinates and at the site of administration (Summary of the findings are provided in the Table below). Moderate to severe erosions in the mucosa of the nasal septum, and a slight increase in the incidence of mucosal erosions in the turbinates were observed in the right nostril (ketorolac). These changes were confined mostly to the anterior nasal turbinates where the test-article was directly administered. In contrast the distal regions of the nasal passageway, including the posterior turbinates, pharynx and larynx, there were no incidences of erosion and generally fewer findings of edema, hemorrhage and red blood cells.

Histopathology Findings: Incidences and Severity

	Group 1 Formula A		Group 2 Formula B		Group 3 Formula C		Group 4 Saline (IV Keto)	
	L	R	L	R	L	R	L	R
Anterior Nasal turbinates								
Submucosa								
inflammation-subacute	3 (1.3)	3 (1.3)	3 (1.7)	3 (1.7)	1 (1.7)	3 (1.7)	3 (1.0)	3 (1.3)
Inflammation-acute	1 (1.0)	1 (1.0)	1 (1.0)	**1 (2.0)**	1 (1.0)	**2 (1.0)**	0	1 (1.0)
hemorrhage	1 (1.0)	1 (1.0)	1 (1.0)	**2 (1.0)**	1 (1.0)	**2 (1.0)**	0	1 (1.0)
Mucosa								
Erosion	1 (1.0)	**2 (1.5)**	1 (2.0)	**2 (1.5)**	1 (1.0)	**2 (1.5)**	0	1 (2.0)
Septum								
Erosion	0	**3 (2.0)**	0	**2 (2.5)**	0	1 (1.0)	1 (1.0)	0
hemorrhage	0	1 (1.0)	0	**2 (2.0)**	2 (1.5)	2 (1.5)	1 (1.0)	0
Lumen								
Red blood cells	0	0	1 (2.0)	0	0	**2 (1.5)**	0	0
Posterior Nasal Turbinates								
Submucosa								
Inflammation-acute	2 (1.5)	1 (2.0)	1 (1.0)	1 (2.0)	0	3 (1.3)	2 (1.5)	2 (1.0)
edema	0	1 (1.0)	0	0	0	1 (1.0)	1 (2.0)	0
hemorrhage	0	0	1 (1.0)	0	0	1 (1.0)	2 (1.0)	0
Lumen								
Red blood cells	0	**2 (2.0)**	1 (2.0)	2 (1.5)	1 (2.0)	**2 (1.5)**	0	0

L: Vehicle; R: Test article
*Average Severity indicated in ( ) with scoring as follows:
1 (slight) 2 (moderate) 3 (severe)

Study title: 14-Day Nasal Tolerance Study of Ketorolac Tromethamine Intranasal Formulations in Male and Female Rabbits

Key study findings: A 14 Day intranasal study in rabbits was conducted to support the change in route of administration and to determine local tolerance of ketorolac compared to vehicle; doses evaluated were 0.75, 1.5, 2.25 mg/cm^2/day (3 times per day at 4 hr intervals with 100 µL to the left nostril) produced by solutions of 75, 15 and 22.5%, respectively

- no test article related changes at the level of nasal turbinates or nasal septum
- local NOAEL: HD (2.25 mg/cm^2/day; 22.5%)

Study no.: KET 004
Volume #, and page #: Module 4 Vol. 6 pg 1-38
Conducting laboratory and location: (b) (4)

Date of study initiation: 09 Nov 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Ketorolac tromethamine, lot # QFO345, 99.8%
Formulation/vehicle: Test article solutions of 7.5 %, 15%, and 22.5% (w/w) solution were prepared in a solution of 0.02% (w/w) Disodium EDTA. Vehicle control consisted of the same composition as the test article solution with the addition of PBS in place of ketorolac. Intranasal administration was performed using a 100 µL Monospray/ Biodose MK111, PMP VP7/100 spray pump manufactured by Valois, which was provided by the Sponsor.
Reviewer's Comment: The ketorolac solution used in this study is similar to the clinical formulation and that the drug administration was via a nasal spray similar to the clinical setting. In other animal studies, animals were dosed with ketorolac by intranasal instillation using a calibrated automatic pipette.

Methods

Study design and Doses: Five treatment groups with 6 (3M and 3F) Main Study animals and 4 (2M and 2F) Recovery Group animals assigned to each group. Nasal administration was performed 3 times / day to the left nostril, once every 4 hrs for 14 consecutive days and then sacrificed on Day 15. Recovery animals were dosed in the same manner for 14 consecutive days, allowed to recover and then sacrificed on Day 30. Blood samples (at pre-dose, 30 min, 1hr, 2hr, and 4hr from 1 animal per sex after first dose on Day 1 and on Day 8) were collected into heparinised tubes. Plasma samples were collected and retained for possible future toxicokinetic evaluations. All animals were euthanized and histological examination of only the nasal septum and turbinates were conducted, but other standard tissues were preserved.

Treatment Groups	Concentration (% Solution)	Dose Volume (µl/ nostril)*	Number of Animals	
			Main Study**	Recovery Group***
0.9% Saline Control	0	100	3M + 3F	2M + 2F
Vehicle Control	0	100	3M + 3F	2M + 2F
Low Dose	7.5%	100	3M + 3F	2M + 2F
Mid Dose	15%	100	3M + 3F	2M + 2F
High Dose	22.5%	100	3M + 3F	2M + 2F

*100 µL was administered to the left nostril using a metered pump. The right nostril was untreated.

Results:
All animals survived to scheduled necropsy date. There were no clinical signs attributable to test-article. No significant effects on body weight or food consumption were observed in any of the dose groups in either male or female rabbits. There were no adverse gross findings with the exception of sporadic findings of small pituitary, lip lesion, black foci of an ovary. Microscopic findings (summarized in Table below) included minimal to mild hemorrhage, nasal epithelial erosion and nasal luminal exudate but were observed in all groups of rabbit nasal turbinate evaluated. Incidences and severity were similar across groups and across main study compared with recovery animals. Given the similarity across groups and across nostrils, the histopathological findings are not likely treatment related.

Reviewer's comment: An unexpected finding was the incidences of histopathological findings in the untreated nostril (R). The sponsor did not elaborate or propose an explanation. In addition, the sponsor did not specify where incidences of erosion were occurring and which nasal epithelial subtypes were affected.

Histopathology Findings in Males: Incidences and Severity*										
	Saline		Vehicle		7.5%		15%		22.5%	
	L	R	L	R	L	R	L	R	L	R
Day 15										
Fresh Hemorrhage	3 (1.7)	3 (1.7)	3 (1.3)	2 (2)	2 (1.5)	2 (2)	2 (1.5)	1 (1)	3 (1.3)	2 (1)
Luminal Exudate	0	1 (1)	0	0	0	1 (1)	0	2 (1.5)	2 (1)	0
Erosion	3 (1.0)	3 (1)	3 (1.3)	1 (1)	3 (1.7)	2 (1)	3 (1)	2 (1)	2 (1)	2 (1)
Day 30										
Fresh Hemorrhage	1 (2)	2 (2)	2 (1.5)	2 (1.5)	1 (1)	2 (1)	1 (1)	2 (1)	2 (1)	2 (1)
Luminal Exudate	0	1 (1)	0	0	2 (1)	1 (1)	0	1 (1)	0	1\(1)
Erosion	0	0	2 (1)	2 (1)	1 (1)	2 (1)	2 (1)	2 (1)	2 (1.5)	2 (1)
L: Treated; R: Untreated *Average Severity indicated in ( ) with scoring as follows: (1) minimal (2) mild (3) moderate										

Histopathology Findings in Females: Incidences and Severity*										
	Saline		Vehicle		7.5%		15%		22.5%	
	L	R	L	R	L	R	L	R	L	R
Day 15										
Fresh Hemorrhage	2 (1)	2 (1)	2 (1)	3 (1.3)	3 (1)	2 (1.5)	2 (1.5)	1 (2)	2 (1)	2 (2)
Luminal Exudate	1 (1)	0	0	1 (1)	0	0	1 (1)	0	0	1 (1)
Erosion	1 (1)	3 (1)	2 (1)	3 (1)	2 (1.5)	3 (1)	3 (1)	2 (1)	1 (1)	3 (1)
Day 30										
Fresh Hemorrhage	2 (1)	2 (1)	1 (1)	1 (1)	1 (1)	2 (1)	1 (2)	0	1 (2)	1 (2)
Luminal Exudate	0	0	1 (1)	1 (1)	1 (1)	0	1 (1)	0	0	0
Erosion	0	2 (1.5)	2 (1)	1 (1)	2 (1)	1 (1)	2 (1.5)	2 (1)	2 (1)	2 (1)
L: Treated; R: Untreated *Average Severity indicated in ( ) with scoring as follows: (1) minimal (2) mild (3) moderate										

2.6.6.8 Special toxicology studies
None

2.6.6.9 Discussion and Conclusions
In support of NDA 22-382 Ketorolac Tromethamine Nasal Spray (Sprix®), the sponsor conducted a battery of studies that included several local tolerance studies in rabbits and a repeat-dose toxicology study in rats. In addition, qualification studies to establish the safety of an oxidative degradant (identified as 1-keto), which included two *in vitro* genetic toxicology studies and a repeat-dose toxicology study in rats, were completed. Reproductive and developmental toxicology and carcinogenicity studies were not conducted nor required for this NDA application.

Toxicology:
In the 14-day local tolerance study in rabbits, ketorolac (nasal formulation that closely resembles the clinical formulation) at a volume of 100 μL was administered via nasal spray to one nostril three times per day. After 14-days of dosing, no drug related effects on clinical signs, body weight, food consumption, or gross observations were reported. Microscopic evaluation of the nasal septum and turbinates revealed minimal to mild hemorrhage, nasal epithelial erosion and nasal luminal exudate in all groups evaluated with similar incidences and severity, suggesting these findings are not likely drug-related. However, an unexpected observation that was not addressed by the sponsor was the multiple findings reported in the untreated nostril. Unlike rats and mice, rabbits do not have a septal window that connects the two nasal cavities (Gizurarson, 1990). One of several plausible explanations is that systemic levels of ketorolac in the nasal region may have caused the observed erosions or excess ketorolac fluid was transferred to the other side by nose rubbing/picking or through inspiration of condensed fluid into the contralateral nostril. Nevertheless, incidences and severity of the microscopic findings were not dose-dependent or different than what was observed in the saline or vehicle groups. Consequently, the designated NOAEL in this study is the HD, which corresponds to 2.25 mg/cm^2/day (assuming nasal surface area of 30 cm^2 per nostril for rabbits). This nasal dose is approximately 3.2 times the human therapeutic nasal dose of approximately 0.7 mg/cm^2/day (Refer to Table 2 in Section 2.6.6.10). Systemic exposure of ketorolac was not determined in this study, however, a preliminary study (KET 007) reported AUC values of approximately 10,000 ng*hr/mL when dosed twice a day for 7 days with a 15% ketorolac formulation. This systemic exposure level is approximately 1.3 times the systemic exposure level associated with the maximum recommended human daily dose of Sprix. It should be noted that dosing frequency used in this and all animal studies were much less compared to the proposed clinical dosing frequency of two sprays of 100 μL (one spray to each nostril) per dose four times a day. Moreover the volume administered to a nostril of rabbits in this study is approximately twice the recommended volume[7] of 58 μL (Gizurarson, 1990). Significance of these design issues are minimized since there was adequate nasal exposure per spray resulting in daily nasal exposures that are greater than what is predicted in humans.

In the 28-day repeat dose toxicology study in rats, ketorolac was instilled in alternating nostrils three times per day. It should be noted that several anatomical features of the rat affect intranasal dosing. The rat nasal cavity is not divided into individual cavities due to the septal

[7] Based on the maximal recommended volume of 150 μL to be administered into each nostril in humans (Gizurarson, 1990).

window and as a result precludes experiments designed to use one nostril as a control such as those experiments conducted in rabbits. As a result, it is likely the sponsor administered doses to both nostrils using separate animals as control. Moreover rats have a nasal structure known as the nasopalatine that connects the nasal cavity to the mouth, which is located anteriorly in the nose (Gizurarson, 1990) and may account for the high incidences of GI toxicities. Dosing frequency again is noted to be less then that used in the clinical setting, however, sufficient nasal exposure to the test-article are achieved. There were no drug related local toxicities observed in the nasal organ or respiratory tract. Consequently the local NOAEL in the males was the HD group in this study, which corresponded to a maximum nasal exposure of 0.87 mg/cm^2/day. This maximal nasal exposure is 1.3 times the maximum recommended human daily dose (0.7 mg/cm^2/day). Due to the unscheduled deaths in all the HD females, which were GI related, a local NOAEL was not designated in this sex. However, comparison of microscopic findings in the nasal and respiratory tract between males and females revealed no significant differences, arguing the male local NOAEL also applies to females.

Systemically, NSAID class related toxicities were observed, which included the gastrointestinal tract and kidney. In the gastrointestinal tract, minimal to moderate ulceration in the glandular stomach was observed in many treated animals, with one marked incidence of ulceration observed in the ileum. Multiple incidences of inflammatory cell infiltration in the lamina propia as well as minimal to mild peritonitis in the duodenum, cecum, ileum and jejunum were observed. Likely secondary to the GI toxicities, spleen weights were elevated accompanied with minimal to mild extramedullary hematopoiesis in HD males and in all dosed female groups. Kidney findings included minimal to mild chronic progressive nephropathy in the HD males and MD females and focal mineralization in the LD and MD female group, and these incidences persisted following the recovery period. Kidney weights were elevated in females and urinalysis revealed significant differences in urinary volume in both males and females. In light of these results, it is concluded that administration of ketorolac via nasal spray does not induce local adverse toxicities as compared to vehicle control nor cause greater levels of toxicity than those observed with parenteral administration of ketorolac. Due to the observed GI toxicities, the conservative NOAEL is the female LD, which was associated with AUC_{0-4} of 23391 ng*hr/mL. This dose is 3-times the level associated with the maximum recommended human daily dose of 126 mg of ketorolac tromemthamine (Table 3 of Section 2.6.6.10).

Toxicity of the 1-keto degradant was assessed in a 14-day repeat dose toxicity study in rats. Similar treatment related target organs were observed as in the 28-day study in rats, including the gastrointestinal tract and extramedullary hematopoiesis organs- spleen and liver. Comparison of the ketorolac spiked with 1-keto degradant group versus the ketorolac alone group, revealed that the 1-keto degradant does not increase toxicities of ketorolac or induce novel forms of toxicities. Based on the HD group in this study, the local exposure for the 1-keto degradant is equivalent to the total nasal dose of Sprix® in humans while the systemic exposure margin is approximately 2 times as determined by body surface area (BSA) conversion calculations.

Genotoxicity:
The sponsor conducted several studies to asses the genotoxic potential of the 1-keto degradant observed in the drug product. The 1-keto degradant was negative in the Ames Test but tested positive in the *in vitro* Chromosome Aberration assay in CHO cells in the presence of metabolic activation. It was noted that at the concentrations that produced chromosomal

aberrations, 1-keto degradant was also cytotoxic. Overall the weight of evidence indicates that 1-keto degradant is not likely mutagenic but clastogenic. It is important to note that the parent, ketorolac tromethamine also tested positive in the *in vitro* chromosome aberration assay in CHO cells at higher concentrations. After consultation with Dr. David Jacobson-Kram, a computational toxicology analysis of the 1-keto degradant was conducted (Refer to Appendix to see detailed report). The 1-keto degradant was predicted positive in the chromosome aberrations *in vitro*, but was predicted to be negative for carcinogenicity in both rat and mouse models. In light of these results, the acute indication sought in this NDA, and with Dr. David Jacobson-Kram's assent, the specification of the 1-keto degradant is allowed to exceed the 1.5 µg TDI limit normally imposed on genotoxic compounds and no further genotoxicity testing of the degradant is required.

2.6.6.10 Tables and Figures

Table 1: Interspecies Comparison of Nasal Surface Areas

	Rat*	**Rabbit#**	**Human***
Body weight assumption	250 g	3 kg	70 kg
Nasal surface area per nostril (cm^2)	5.2	30	90
Total nasal surface area (cm^2)	10.4	60	180
* Nasal Surface Areas are from Pinkerton et al., 1997; # Nasal Surface Area of rabbits is from Gizurarson et al., 2006			

Table 2: Exposure Margins for Ketorolac Administered via the Nasal Route

Nonclinical Evaluation	**KET004: 14-Day Nasal Tolerance Study of Ketorolac Tromethamine Intranasal Formulations in Male and Female Rabbits Male and Female NOAEL – 22.5%**	
	Per Spray (mg/cm^2)	**Total Nasal Dose (mg/cm^2/day)**
Rabbit	0.75	2.25
Human	0.18	0.7
Local Tolerance Safety Margin	**4.2x**	**3.2x**

Table 3: Safety and Exposure Margins for Ketorolac Administered via the Nasal Route

Nonclinical Evaluation	**KET003: 28-Day Intranasal Toxicity Study in Rats with a 28 Day Recovery Period Systemic NOAEL – Male MD (15.0%) Female LD (7.5%) Local NOAEL – Male HD (22.5%)**					
	Maximum Nasal Exposure		**Systemic (Cmax)**		**Systemic (AUC*)**	
	M (mg/cm^2)	F (mg/cm^2)	M (ng/mL)	F (ng/mL)	M (ng*h/mL)	F (ng*hr/mL)
Rat (Systemic Toxicity)	0.43	0.22	16198	10701.5	44122	23391
Rat (Local Toxicity)	0.87	ND				
Human#	0.7		1805.8		7477.3	
Safety Margin *systemic* *local*	**0.62x 1.3x**	**0.32x ND**	**9.0x**	**5.9x**	**5.9x**	**3.1x**
*AUC values for rat were AUC_{0-4} whereas human AUC are expressed as $AUC_{0-\infty}$, which will produce underestimated safety margins; # Human AUC and Cmax values derive from Study ROX-2001-02 (See Appendix 2); ND – not determined due to deaths of HD females but unlikely different from males.						

Table 4: Exposure Margins for 1-Keto Degradant Administered via the Nasal Route

Nonclinical Evaluation	KET002 14-Day Intranasal Toxicity Study in Rats with a 28 Day Recovery Period (Ketorolac + 1-keto degradant) Local NOAEL: HD (15% KT + 0.9% 1-keto)	
	Per Spray (μg/cm^2)	Maximum Nasal Exposure (μg/cm^2/day)
Rat	2.6	5.2
Human	1.1	4.6
Local Tolerance Safety Margin	**2.4x**	**1.1x**

Table 5: Systemic Exposure Margin for 1-Keto Degradant

Nonclinical Evaluation	KET002 14-Day Intranasal Toxicity Study in Rats with a 28 Day Recovery Period (Ketorolac + 1-keto degradant) NOAEL: HD (15% KT + 0.9% 1-keto)	
	Total daily intake (mg)	HED (mg/m^2)
Rat	0.0405	0.972
Human	0.819	0.505
Exposure Margin		**1.9x**

2.6.7 TOXICOLOGY TABULATED SUMMARY

Local Tolerance –Ketorolac Tromethamine in Rat and Rabbits

Species/ Strain	Method of Administration	Duration of Dosing	Dose	Sample size	Noteworthy findings	Study No.
Rabbit / New Zealand White	Intranasal (nasal spray to the left nostril)	14 days, three times per day (once every 4 hrs)	Group1 (Saline) Group2 (VC) Group3 (7.5%) Group 4 (15%) Group5 (22.5%) Fixed Volume of 10 μL	3M, 3F (main study) 2M, 2F (recovery)	- No adverse effects on BW or food consumption - no clinical signs or mortality - no significant gross pathology or treatment related microscopic findings in the nasal septum or turbinates	KET004

Repeat Dose Toxicology – Ketorolac Tromethamine in Rats

Species/ Strain	Method of Administration	Duration of Dosing	Dose	Sample size	Noteworthy findings	Study No.
Rat / Sprague Dawley	Intranasal (calibrated automatic pipette)	28 days with 28 day recovery period (3 times/day, alternating nostril)	Group 1 (0%) Group 2 (7.5%) Group 3 (15%) Group 4 (22.5%) Fixed volume of 10 μL	10 M, 10 F (main study) 6M, 6F (recovery)	- Treatment related clinical signs included hunching, swollen abdomen and piloerection (MD, HD) - Mortality was observed in MD and HD females Day 5: 1 HD sacrificed moribund Day 6: 2 HD found dead and 4 HD sacrificed moribund Day 8: remaining HD females sacrificed Day 27: 1 MD female sacrificed moribund - GI toxicity in females and males in the MD and HD groups - clinical signs included hunched posture - general trend for reduced body	KET 003

					weight in dosed animals, notably in females - gross pathology correlated with microscopic findings in the GI tract that were reversible - significant dose-dependent increase in spleen weights and spleen hematopoeisis	

Repeat Dose Toxicology – 1-keto degradant in Rats

Species/ Strain	Method of Administration	Duration of Dosing	Dose	Sample size	Noteworthy findings	Study No.
Rat / Sprague Dawley	Intranasal (calibrated automatic pipette)	14 days with 14 day recovery period (alternating nostril)	Vehicle Control Group 1 (3 x 10 µL) KT alone Group 2 (3 x 10 µL) KT + 1-keto Group 3 (1 x 10 µL) Group 4 (2 x 10 µL) Group 5 (3 x 10 µL) Fixed concentration of 15% KT and 0.9% 1-keto	10 M, 10 F (main study) 6M, 6F for Groups 1, 2, 5 (recovery)	- KT dosing achieved by increasing volume of 15% KT with 0.9% 1-keto - Mortality observed in Group 2 and 5 females that appeared related to GI toxicity - 1-keto does not induce local nasal toxicity - 1-keto degradant produced a similar toxicity profile comparable to ketorolac alone, indicating no additive or additional toxicities	KET 002

Genetic Toxicology – 1- keto degradant

Species/ Strain	Test	Experimental Design	[ ] Tested	Sample size	Noteworthy findings	Study No.
Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100; *Escherichia coli* strain WP2uvrA	AMES	Direct plate incorporation and pre-incubation testing with and without S9 activation mix Vehicle control: DMSO Positive control: +S9: 2AAN -S9: NaN3: TA 1535, 100 ENNG: Wp2uvrA 2-NF: TA 98 9-AA: TA 1537	5, 17, 50, 167, 500 and 1667 µg/plate	Triplicate plates	- precipitation and slight toxicity to some of bacterial strains observed as a reduction in the density of background lawn at highest concentration - no mutagenic activity observed in any of the strains in either presence or absence of S9 mix	KET 005
Chinese Hamster Ovary Cells	*In vitro* ChromAbs	6 hr treatment with 16 hr recovery (harvest at 24 h) Vehicle Control: DMSO Positive Control: +S9: CP -S9: MMS	+S9: 63, 125, 250 µg/mL -S9: 125, 250, 500 and 1000 µg/mL	Duplicate cell cultures 3 slides per culture 100 metaphases per culture	- Toxicity noted at 125 to 1000 µg/mL in presence of S9 and at 250 to 1000 µg/mL in the absence of S9 - with S9 mix, **1-keto degradant was clastogenic at 125 and 150 µg/mL** (note cytotoxicity was noted at these concentrations) - without S9 mix, 1-keto degradant was not clastogenic	KET 006

OVERALL CONCLUSIONS AND RECOMMENDATIONS

In this NDA submission, the nonclinical studies submitted satisfy the requirements for both the change in route of administration and the qualification of the 1-keto degradant that exceeds ICH qualification threshold. To support the change in route of administration to intranasal, a 28-day study in rats, along with a 14-day local tolerance study in rabbits were conducted. It was found that these studies demonstrated that the change to nasal route of administration did not produce adverse local toxicity or alter the systemic toxicity profile of ketorolac when administered parenterally and provided adequate safety/exposure margins both locally and systemically for human use. Qualification of the 1-keto degradant was supported by a 14-day study in rats along with two *in vitro* genotoxicity assays. It was demonstrated there were no new systemic or adverse local toxicity attributed to the presence of the 1-keto degradant.

Also see Executive Summary.

Unresolved toxicology issues (if any):
There are no unresolved toxicology issues based upon review of the studies submitted in support of this NDA.

Recommendations:
There are no further pharmacology/toxicology concerns for this NDA. Moreover there are no nonclinical concerns related to the nasal route of administration or attributed to the presence of the 1-keto degradant that required qualification. Therefore, from the nonclinical Pharmacology/Toxicology perspective, based upon the information reviewed by this reviewer, this NDA may be approved.

Suggested labeling:
For the suggested changes, refer to the Executive Summary.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

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Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22382	ORIG 1		KETOROLAC TROMETHAMINE NASAL SPRAY

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

NEWTON H WOO
08/12/2009

ADAM M WASSERMAN
08/12/2009
I concur with Dr. Woo's review and labeling recommendations.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: **22-382** Applicant: **Roxro Pharma** Stamp Date: **Dec 5 2008**

Drug Name: **Sprix (proposed); Ketorolac Tromethamine Nasal Spray** NDA Type: **505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		505(b)(2) with reference to Toradol's NDA 19-645 and 19-698 (Toradol is no longer marketed, although numerous generics are available). intranasal solution contains a higher concentration of ketorolac ~15% w/w, compared to 1.5% w/w for the previously approved Toradol IM/IV solution
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		adjustment of safety margins? Toradol oral (after IV or IM) continuation therapy: up to 40 mg/day; intranasal up to 126 mg/day (replaces IV, 15 or 30 mg, or IM, 15, 30, or 60 mg therapy) q 6 hr?
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		1-keto and 1-hydroxy analogues of ketorolac are controlled at limits of NMT 0.1 % each and studies were conducted to qualify the (b) (4) impurity 1) genetic toxicology (mutagenicity and clastogenicity) studies 2) 14-day repeated dose intranasal administration studies in the rat Refer to Feb 23, 2007 communication
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Reviewing Pharmacologist L. Steven Leshin, DVM, PhD Date

Team Leader/Supervisor Adam Wasserman, PhD Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Lawrence Leshin
1/5/2009 03:52:17 PM
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Adam Wasserman
2/11/2009 02:31:22 PM
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