CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-498

Pharmacology Review(s)

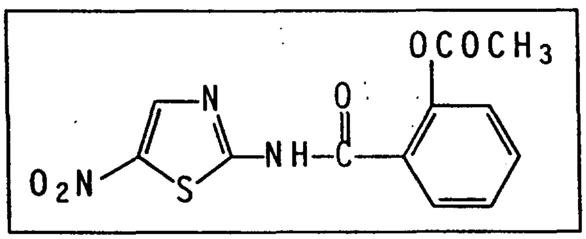
PHARMACOLOGY/TOXICOLOGY REVIEW

NDA number: [redacted] 21-498 (oral suspension)
Review number: 001
Sequence number/date/type of submission: 000/29 May 2002
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Romark Laboratories, LC
6200 Courtney Campbell Causeway
Suite 880
Tampa, Florida 33607

Manufacturer for drug substance : [redacted]

Reviewer name: SKunder
Division name: Special Pathogen and Immunologic Drug Products
HFD #: 590
Review completion date: 6 Nov 2002

Drug:
- Trade name: Cryptaz
- Generic name (list alphabetically): nitazoxanide
- Code name: PH 5776, NTZ
- Chemical name: 2-acetoxy-*N*-(5-nitrothiazol-2-yl) benzamide
- CAS registry number: 55981-09-4
- Mole file number: not provided
- Molecular formula/molecular weight: $C_{12}H_8N_3O_5S$, 307.3
- Structure:



Relevant INDs/NDAs/DMFs: IND [redacted] NDA 20871

Drug class: anti diarrheal

Indication: diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*

Clinical formulation: [redacted] **'ablets, oral suspension (100 mg/5 ml)**

Route of administration: oral

Proposed use: children with diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

Recommendation on Approvability
Nitazoxanide was previously submitted by Unimed Pharmaceuticals Inc in 1998 for cryptosporidosis in AIDS patients (NDA 20-871). The application was not approved due to lack of efficacy. A previous developer of the drug, Romark Laboratories, resubmitted NTZ for cryptosporidial diarrhea in children with a three day course of treatment (200 mg, bid, approximately 11 mg/kg/day in an 11 year old child). The previously submitted preclinical studies were used to support this submission, with an additional 28-day oral dog toxicity study. The previous preclinical studies demonstrated support for the safety of NTZ. The lack of toxicity in animal studies at doses greater than human exposure supports the safety of this drug for the short treatment time. There are no other pharmacology/toxicology issues.

Recommendation for Nonclinical Studies
none

Recommendations on Labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility
Long-term carcinogenicity studies have not been conducted.

Nitazoxanide was not genotoxic in the Chinese hamster ovary (CHO) cell chromosomal aberration assay or the mouse micronucleus assay. NTZ was genotoxic in one tester strain (TA 100) in the Ames bacterial mutagenicity assay

Nitazoxanide did not adversely affect male or female fertility in the rat at 2400 mg/kg/day (approximately 66 times the recommended dose for patients 11 years of age, adjusted for body surface area).

PREGNANCY
Teratogenic Effects
Pregnancy Category B:
Reproduction studies have been performed at doses up to 3200 mg/kg/day in rats (approximately 48 times the clinical dose adjusted for body surface area) and 100 mg/kg/day in rabbits (approximately 3 times the clinical dose adjusted for body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to NTZ. There are, however, no adequate and well-controlled studies in pregnant women.

Summary of Nonclinical Findings

Brief Overview of Nonclinical Findings
Rats receiving 450 mg/kg (HED=75 mg/kg) for 14 weeks demonstrated intense salivation, increased liver and spleen weights, and decreased thymus weights without histopathological differences.

Rats in a 6 month study, demonstrated a NOAEL of 150 mg/kg/day (HED=25 mg/kg). The major toxicity seen was an increase in extramedullary hematopoiesis and pigment deposition in the spleens of males and females receiving 450 mg/kg/day (HED=75 mg/kg). Hematological assays were affected by treatment in the high dose group. Significant increases in leukocytes, lymphocytes, and mean corpuscular volume and decreases in erythrocyte counts and hematocrit were seen in both males and females. High dose females had significant increases in neutrophils and mean corpuscular volume. Clinical chemistry effects were seen in the high dose group. Males had significant decreases in urea nitrogen, sodium, total protein, and calcium and increases in albumin/globulin ratio, total bilirubin, and phosphorus. Females had significantly decreased sodium and glucose and higher total bilirubin.

In the first 28 day dog study (doses= 300, 900, 2700/1800 mg/kg, HED= 150, 450, 1350/900 mg/kg), drug induced effects included weight loss and decreased food consumption, blood in the urine, decreased erythrocyte counts, decreased hematocrit, and decreased organ weights including brain, lung, spleen, heart, lung, kidney, thymus, liver, and testes. Histopathologic findings included lymphoid depletion of the thymus, ulceration of the cecum, and cystic hyperplasia of the gall bladder. A NOAEL was not achieved in this study.

A second 28-day oral dog study at lower doses (7.5-60 mg/kg, HED=3.7- 30 mg/kg) had only gastrointestinal effects including focal hemorrhages and stomach ulceration.

In the 90 day oral dog study (doses=25, 50, 100 mg/kg; HED=12, 25, 50 mg/kg), hematological findings similar to those of the first 28-day dog study occurred. Both weight loss and decreased food consumption were seen. Loose and/or discolored stool was frequently seen. In males, decreased testicular weights and testicular immaturity were seen. The NOAEL for this study was less then 25 mg/kg.

Genetic toxicology studies demonstrated little potential for mutagenicity or clastogenic activity. The battery of studies performed included Ames tests with and without metabolic activation, and also with desNTZ, chromosomal aberration tests with Chinese hamster ovary cell, also with desNTZ and an in vivo mouse micronucleus assay. A positive genotoxic result was seen in the Salmonella-E. coli reverse mutation assay; one strain of S. typhimurium (TA 100) showed slightly higher rates of reversion with both NTZ and desNTZ.

In both rats and rabbits, NTZ did not significantly affect fertility, implantation, pregnancy rates, or cause fetal death. In male rats, histological and functional studies of sperm did not show drug effects. Both species also had maternal effects of decreased food consumption and body weight, possibly due to the bulk amount of drug in the gastrointestinal tract, confounding fetal effects that were seen. A rat study showed no apparent transfer of tizoxanide to fetuses at 30 mg/kg (HED= 5 mg/kg) while fetuses from 2 of 9 dams receiving 300 mg/kg (HED=50 mg/kg) did show tizoxanide.

Reproductive and fertility issues are less of a factor with children while the reproductive toxicology studies do not demonstrate NTZ to be a strong teratogen. Given the target age population and low absorption seen with NTZ, Pregnancy Category B appears to be justified. In all cases, HED values should be compared to the proposed clinical dose of about 11 mg/kg for 3 days.

Pharmacologic Activity

Nitazoxanide exerts antiprotozoal activity by interference with pyruvate:ferredoxin oxidoreductase, an enzyme essential to anaerobic energy metabolism. By this enzyme inhibitory action, sporozoites of *Cryptosporidium parvum* and trophozoites of *Giardia lamblia* are inhibited in growth by NTZ.

Nonclinical Safety Issues Relevant to Clinical Use

NDA ____ ______________________________

Gastrointestinal irritation and bleeding are the major concerns.

Administrative

Reviewer signature: /S/ 11/8/02

Supervisor signature: Concurrence - /S/ 11/8/2002

Non-Concurrence - ______________________________
(see memo attached)

cc: list:

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY: 6

II. SAFETY PHARMACOLOGY: 6

III. PHARMACOKINETICS/TOXICOKINETICS: 7

IV. GENERAL TOXICOLOGY: 9

V. GENETIC TOXICOLOGY: 14

VI. CARCINOGENICITY: 15

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY: 15

VIII. SPECIAL TOXICOLOGY STUDIES: 15

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS: 16

X. APPENDIX/ATTACHMENTS: 17

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: Nitazoxanide is poorly absorbed from the gastrointestinal tract. It remains mostly in the gastrointestinal tract, with mainly fecal elimination by 72 hours. Deacetylation produces the major metabolite, desacetylNTZ. Bioavailability was not determined in animals.

Mechanism of action: antiprotozoal action, may result from interference with pyruvate:ferredoxin oxidoreductase, an enzyme essential to anaerobic energy metabolism

Drug activity related to proposed indication: By the above enzyme inhibitory action, sporozoites of *Cryptosporidium parvum* and trophozoites of *Giardia lamblia* are inhibited in growth

Secondary pharmacodynamics: not applicable

Pharmacology summary: Single dose studies demonstrated in vivo activity including analgesia in the PPQ writhing assay, mortality in rats and mice, antiinflammatory activity, slight effects on increasing HDL and triglycerides, gastrointestinal antisecretory and antiulcer activity and slight histamine activity; in vitro activity included slight antimicrobial activity against S. aureus and P. vulgaris, some antagonism against angiotensin II, leukotriene D4, substance P and acetylcholine.

Pharmacology conclusions: Nitazoxanide appears to exert analgesic, hyperlipidemic, antiinflammatory, antisecretory and antiulcer activity, slight antimicrobial activity against S. aureus and P. vulgaris, and limited antagonism against angiotensin II, leukotriene D4, substance P and acetylcholine.

II. SAFETY PHARMACOLOGY:

In vivo doses were 250 (immunology), 500 or 1000 mg/kg, i.p.; in vitro concentrations ranged from 10μg/ml (antagonism studies) to 20 μg/ml (antimicrobial assays)

Neurological effects: Reflex inhibition, behavioral depression, muscle relaxation, catalepsy, antipentylenetetrazol convulsive activity, anti-electroshock, motor activity, analgesia, tetrabenazine antagonism were evaluated in mice. Analgesia was observed at 500 mg/kg.

Cardiovascular effects: Direct blood pressure, heart rate, and anti-arrhythmia were evaluated in mice. No effects were seen. In vitro study with the guinea pig for atrium contractile force also showed no effect.

Pulmonary effects: In the trachea, bronchodilator antagonism effects (beta II and histamine) were evaluated. Slight antihistamine activity was seen.

Renal effects: Volume diuresis was evaluated in mice. No effects were seen.

Gastrointestinal effects: Antisecretory activity and anti-ulcer activity were evaluated in rats. Gastrointestinal antisecretory and antiulcer activity were seen at 500 mg/kg.

Abuse liability: not determined

Other: Antiinflammatory activity (carrageenan rat paw edema, arachidonic acid ear model, rat gait and antipyretic activity), immunology (immunosuppression, immunoenhancement), antagonism studies (in vitro; ileum: angiotensin II, calcium, leukotriene D4, substance P, acetylcholine, beta (isoproterenol), metabolic assays (cholesterol, glucose tolerance), antimicrobial activity and acute toxicity in mice and rats. Findings included slight effects on increasing HDL and triglycerides, gastrointestinal antisecretory and antiulcer activity (500 mg/kg) and slight histamine activity, and mortality in rats (1/3) and mice (1/3) at 1000mg/kg

Safety pharmacology summary: NTZ was evaluated in a variety of safety pharmacological screens. In vivo activity detected included analgesia (500 mg/kg) in the PPQ writhing assay, mortality in rats (1/3) and mice (1/3) at 1000mg/kg, antiinflammatory activity (500 mg/kg), slight effects on increasing HDL and triglycerides, gastrointestinal antisecretory and antiulcer activity (500 mg/kg) and slight histamine activity; in vitro activity included slight antimicrobial activity against S. aureus and P. vulgaris, some antagonism against angiotensin II, leukotriene D4, substance P and acetylcholine.

Safety pharmacology conclusions:
In vivo activity included analgesia (500 mg/kg) in the PPQ writhing assay, mortality in rats (1/3) and mice (1/3) at 1000mg/kg, antiinflammatory activity (500 mg/kg), slight effects on increasing HDL and triglycerides, gastrointestinal antisecretory and antiulcer activity (500 mg/kg) and slight histamine activity; in vitro activity included slight antimicrobial activity against S. aureus and P. vulgaris, some antagonism against angiotensin II, leukotriene D4, substance P and acetylcholine. The gastrointestinal effects are of interest due to toxicity studies which demonstrate some bowel effects as well.

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters: see table below

pharmacokinetic parameters from 28-day oral toxicity study of daNTZ in dogs and rats

group	AUC day 1 (0-24 hr)	AUC day 28 (0-24 hr)	Cmax day 1	Cmax day 28
900 mg/kg dog	7.73±4.08	14.65±9.95	1.12±0.55	1.58±1.59
2700/1800 mg/kg dog	37.0 ±18.8	20.0±6.6	3.34±1.03	1.37±0.32
800 mg/kg rat			2.38±0.89	
2400 mg/kg rat			3.62±1.37	
4800 mg/kg rat			5.08±2.54	
400 mg/kg rat				2.34±1.45
1200 mg/kg rat				5.78±2.81
2400 mg/kg rat				6.99±1.28
HUMAN - 7.14 mg/kg (500mg dose)	10.10±5.15		2.73±1.73	

mean group values, mg/L ± s.d.

Pharmacokinetic parameters for a single oral dose of C^{14} labeled NTZ (100 mg/kg) in dogs
Cmax=45.5 µg*eqv/ml, males AUC= 245.4 µg*eqv/hr/ml, males $t_{1/2}$ = 22.5 hours, males
=32.9 µg*eqv/ml, females =241.2 µg*eqv/hr/ml, females = 27.9 hours, females

Mean (± SD) plasma pharmacokinetic parameter values following administration of a single dose of Nitazoxanide for Oral Suspension with food to pediatric subjects

		Tizoxanide (desNTZ)			Tizoxanide glucuronide		
Age	Dose*	C_{max} (µg/mL)	T_{max}** (hr)	AUC_{inf} (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{inf} (µg•hr/mL)
12-47 months	100mg	3.11 (2.0)	3.5 (—	11.7 (4.46)	3.64 (1.16)	4.0 (3-4)	19.0 (5.03)
4-11 years	200mg	3.00 (0.99)	2.0 (—	13.5 (3.3)	2.84 (0.97)	4.0 (2-4)	16.9 (5.00)

* Dose: 100 mg/5 mL nitazoxanide, 200 mg/10 mL nitazoxanide
**T_{max} is given as Mean (Range)

The animal studies appear to show greater absorption than pediatric clinical studies.

Absorption: In a single dose C^{14}-labeled NTZ dog study, tissues had negligible recovery of label despite the appearance of drug in plasma. In a study using pregnant rats, C^{14}-labeled NTZ was administered orally on days 6-15 of gestation at doses of 30 and 300 mg/kg (HED= 5 and 50 mg/kg). Fetuses were examined for label and found to be below limits of detection in the 30 mg/kg group, while in the 300 mg/kg group, 2 of the 9 litters examined had detectable label (tizoxanide).

Distribution: radiolabeled NTZ administered orally to rats was found mainly in the digestive tract and kidney for the first 4 hours following dosing, then additionally in the large intestine and thyroid at 8 and 12 hours, followed by thyroid, stomach and kidney at 24 to 48 hours and thyroid, kidney and liver at 72 hours.

Metabolism: A metabolic profile of NTZ in animals has not been conducted. A metabolic profile was determined in human liver microsomal preparations. A dog fecal study did not characterize a consistent pattern of metabolites in 3 dogs. This renders correlations between clinical and nonclinical metabolism difficult; the sponsor used the human metabolite identification to perform animal studies employing the major human metabolite (tizoxanide, or desacetylNTZ) without knowledge of the metabolic pattern in animals.

Excretion: In radiolabeled oral studies in rats, 37.1 % of label was recovered in urine by 24 hours, 28.15 by 72 hours. Fecal recovery was 52% by 72 hours. The carcasses had 0.11% remaining by 72 hours. In dogs, urinary recovery of label was 30.4% in males and 21.1% in females after 72 hours while fecal recovery was 38% in males and 53.3% in females after 72 hours.

Other studies: not conducted

PK/TK summary: Pharmacokinetic studies conducted during 28-day oral toxicity studies in rats and dogs showed similar exposure to NTZ in humans receiving lesser doses. Radiolabel studies show little tissue deposition in dogs or fetal exposure in pregnant rats. Distribution in rats occurred in the gastrointestinal tract, then liver, kidneys and thyroid.. Metabolism was not characterized in experimental animals. Execration of NTZ was demonstrated in dogs and rats as mainly in feces but with substantial urinary excretion as well.

PK/TK conclusions: Absorption appears to be minimal. The pharmacokinetics of NTZ in animals are poorly characterized. The relevance of animal metabolism cannot be correlated accurately with the human metabolic profile as a result.

IV. GENERAL TOXICOLOGY:

See attached NDA, IND reviews for other toxicology studies

Study title: 28-day oral toxicity study in dogs

Key study findings: gastrointestinal irritation (focal hemorrhages and stomach ulceration) at all doses (7.5 -60 mg/kg)

Study no: 0436DR13.001
Volume #, and page #: 11, all
Conducting laboratory and location:

Date of study initiation: 29 Sept 1998
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: lot no. 003
Formulation/vehicle: solution, 0.25% methyl cellulose

Methods (unique aspects): none
Dosing:
Species/strain: dog, beagle (
#/sex/group or time point (main study): vehicle controls, 60 mg/kg, 5; 7.5, 15, 30 mg/kg, 3
Satellite groups used for toxicokinetics or recovery: 2/sex/group, control, 60 mg/kg for 14-day recovery
Age: 5-6 months
Weight: males (7.9-9.3), females (7.3-8.8)
Doses in administered units: 0, 7.5, 15, 30, 60 mg/kg/day (0, 3.75, 15, 30 mg/kg bid)
Route, form, volume, and infusion rate: oral solution, 5 ml/kg bid

Observations and times:
- Clinical signs: twice daily
- Body weights: day 1, 8, 5, 22, 28, 29 (fasted); recovery day 35, 41, 42 (fasted)
- Food consumption: daily
- Ophthalmoscopy: not conducted
- EKG: not conducted
- Hematology: day 29 or day 43
- Clinical chemistry: day 29 or day 43
- Urinalysis: day 29 or day 43
- Gross pathology: day 29 or day 43
- Organs weighed: day 29 or day 43, see table below
- Histopathology: day 29 or day 43, see table below
- Toxicokinetics: not conducted
- Other: not conducted

Results:

Mortality: none

Clinical signs: yellow discolored fur/paws, especially in 60 mg/kg group, due to drug in feces

Body weights: no treatment related effects

Food consumption: no treatment related effects

Ophthalmoscopy: not conducted

Electrocardiography: not conducted

Hematology: no treatment related effects

Clinical chemistry: no treatment related effects

Urinalysis: no treatment related effects

Organ weights: no treatment related effects

Gross pathology: petechiae in colon, ileocolic junction, jejunum, duodenum, and stomach throughout treated groups

Histopathology:

site	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg
focal hemorrhage Colon	1 female, day 29	1 M, day 29		
Cecum		1 female, day 29		
Duodenum			1 female, day 29	
Stomach ulceration				1 male, day 29

Toxicokinetics: not conducted

Summary of individual study findings: The main drug-related finding was gastrointestinal irritation in the form of focal hemorrhages and stomach ulceration in all treatment groups (7.5-60 mg/kg, HED=3.7- 30 mg/kg).

Toxicology summary: Acute toxicity studies were conducted in mice, rats, dogs, and cats. Oral lethal doses by both intraperitoneal and oral routes were determined. In mice, the intraperitoneal LD50 was 105 mg/kg for both males and females. The oral LD50 was 1350 mg/kg in males and 1380 mg/kg for females. The intraperitoneal LD50 in rats was 192 mg/kg in males and 165 mg/kg for females. The oral LD50 was not achieved with the doses used; therefor the LD50 was greater than 10000 mg/kg in both sexes (the lethal oral dose was not achieved in cats). The LD50 was greater than 10 g/kg. The lethal oral dose was also not achieved in dogs. The L\LD50 was greater than 10g/kg. Toxicity was seen as diarrhea and emesis. At necropsy distended gall bladders were seen. Repeat-dose oral toxicity studies were conducted in rats and dogs. Rats were studied for 14 weeks and 6 months. After 14 weeks, rats receiving 450 mg/kg demonstrated intense salivation, increased liver and spleen weights, and decreased thymus weights without histopathological differences. In the 6 month study, a NOAEL of 150 mg/kg/day was observed. The major toxicity seen was an increase in extramedullary hematopoiesis and pigment deposition in the spleens of males and females receiving 450 mg/kg/day. Hematological assays were affected by treatment in the high dose group. Significant increases in leukocytes, lymphocytes, and mean corpuscular volume and decreases in erythrocyte counts and hematocrit were seen in both males and females. Also seen in high dose females were significant increases in neutrophils and mean corpuscular volume. Clinical chemistry effects were seen in the high dose group. Males had significant decreases in urea nitrogen, sodium, total protein, and calcium and increases in albumin/globulin ratio, total bilirubin, and phosphorus. Females had significantly decreased sodium and glucose and higher total bilirubin. In the first 28 day dog study, drug induced effects included weight loss and decreased food consumption, blood in the urine, decreased erythrocyte counts, decreased hematocrit, and decreased organ weights including brain, lung, spleen, heart, lung, kidney, thymus, liver, and testes. Histopathologic findings included lymphoid depletion of the thymus, ulceration of the cecum, and cystic hyperplasia of the gall bladder. A NOAEL was not achieved in this study. A second 28-day oral dog study at lower doses had only gastrointestinal effects including focal hemorrhages and stomach ulceration. In the 90 day oral dog study, hematological findings similar to those of the first 28-day dog study occurred. Bleeding from gastrointestinal hemorrhages may be related to the hematological findings such as decreased hematocrit and erythrocyte counts. Both weight loss and decreased food consumption were seen. Loose and/or discolored stool was frequently seen. In males, decreased testicular weights and testicular immaturity were seen. The NOAEL for this study was less then 25 mg/kg. A salicylate–like effect by NTZ may account for the toxicity seen in dogs. In all of the acute and longer term oral studies, yellow material was recovered form the stomach and intestines. NTZ is a yellow powder; these findings are supportive of the pharmacokinetic studies showing poor absorption. Yellow staining of fur in the urogenital region also was seen in most animals.

Toxicology conclusions: Nonclinical studies provided by the sponsor demonstrate nitazoxanide toxicities including hematotoxicity (rats, 6 months, 450 mg/kg and dogs, 28 days, 300-1800 mg/kg and 90 days, 25-100 mg/kg), gastrointestinal toxicity (dogs, 28 and 90 days) and testicular toxicity (dogs, 90 days, 25-100 mg/kg). The proposed clinical dose is about 11 mg/kg for three days.

Histopathology Inventory for NDA #

Study	28-day oral
Species	Dog
Adrenals	X*
Aorta	X
Bone Marrow smear	
Bone (femur)	X
Brain	X*
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	
Harderian gland	
Heart	X
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	X
Larynx	
Liver	X*
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas	X

Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	
Seminal vesicles	
Skeletal muscle	
Skin	X
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Testes	X*
Thymus	X
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	
Standard List	

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

See attached NDA, IND reviews for other genetic toxicology studies

Genetic toxicology studies demonstrated little potential for mutagenicity or clastogenic activity. The battery of studies performed included Ames tests with and without metabolic activation, and also with desNTZ, chromosomal aberration tests with Chinese hamster ovary cell, also with desNTZ and an in vivo mouse micronucleus assay. A positive genotoxic result was seen in the Salmonella-E. coli reverse mutation assay; one strain of S. typhimurium (TA 100) showed slightly higher rates of reversion with both NTZ and desNTZ.

Labeling recommendations: Nitazoxanide was not genotoxic in the Chinese hamster ovary (CHO) cell chromosomal aberration assay or the mouse micronucleus assay. Nitazoxanide was genotoxic in one tester strain (TA 100) in the Ames bacterial mutagenicity assay

VI. CARCINOGENICITY:

Carcinogenicity studies were not conducted.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

See attached NDA, IND reviews

Reproductive and developmental toxicology summary:
Reproductive toxicity studies were conducted in rats and rabbits. In both species, NTZ did not significantly affect fertility, implantation, pregnancy rates, or cause fetal death. In male rats, histological and functional studies of sperm did not show drug effects. Decreased gravid uterine weights, decreased body weights, and food consumption were seen in females. Fetal abnormalities included protruding tongue, open eyes, and exencephaly in one fetus, macromelia (one fetus), adactylly (one fetus), and protruding tongue and exencephaly (one fetus). Soft tissue malformations included a trend to increased dilation of the brain ventricles and renal pelvic cavitation. Skeletal variations included incomplete skull ossification, fewer than four caudal vertebrae ossified, unossified vertebral centrum, and wavy ribs. These maternal and fetal effects were seen at doses of 600, 1200 and 2400 mg/kg. The maternal effects complicated the interpretation of these data. The range of these doses is equivalent to human doses of 100 to 400 mg/kg, greater than the proposed doses of 11 mg/kg. In the rabbit study, no external abnormalities were seen. Soft tissue abnormalities included internal hydrocephaly (one fetus) and hepatomegaly (one fetus). The NOAEL for the dams was 50 mg/kg and 25 mg/kg for the fetuses, corresponding to 25 and 12 mg/kg, respectively, in humans. In a rat study to determine effects of NTZ on pre- and postnatal development, reduced survival during lactation was seen in pups from mothers receiving 1200 mg/kg. Maternal activity towards the pups was reduced in this group, coinciding with the impaired condition of these dams due to decreased food consumption and body weight. It is unclear whether impaired maternal care and/or in utero and lactation NTZ exposure explain the fetal effects seen in this study. Developmental markers and behavioral assays were not affected. Reproductive performance of the offspring was not affected. The parental NOAEL for this study appeared to be 300 mg/kg/day. It is difficult to determine the F_1 NOAEL due to the maternal effects, otherwise the fetal NOAEL was <300mg/kg/day. The 300 mg/kg day dose is equivalent to a human dose of 50 mg/kg/day.

Reproductive and developmental toxicology conclusions: Reproductive and fertility issues are less of a factor with children while the reproductive toxicology studies do not demonstrate NTZ to be a strong teratogen. Given the target age population and low absorption seen with NTZ, Pregnancy Category B appears to be justified.

Labeling recommendations:

Nitazoxanide did not adversely affect male or female fertility in the rat at 2400 mg/kg/day (approximately 66 times the recommended dose for patients 11 years of age, adjusted for body surface area).

PREGNANCY
Teratogenic Effects
Pregnancy Category B:
Reproduction studies have been performed at doses up to 3200 mg/kg/day in rats (approximately 48 times the clinical dose adjusted for body surface area) and 100 mg/kg/day in rabbits (approximately 3 times the clinical dose adjusted for body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to NTZ. There are, however, no adequate and well-controlled studies in pregnant women.

VIII. SPECIAL TOXICOLOGY STUDIES:

Not conducted

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Nitazoxanide appears safe for the proposed three day, 200 mg bid (11 mg/kg in 11 year olds) indication. Pharmacokinetics in animals remain poorly characterized, particularly absorption, distribution and metabolism. Absorption appears to be less than that seen in humans which provides some reassurance. Reproductive effects are confounded by maternal toxicity and incomplete characterization of pharmacokinetics. Possible gastrointestinal irritation is the main concern at doses in dogs most similar to the proposed clinical dose.

General Toxicology Issues: Nitazoxanide appears to cause gastrointestinal irritation, possibly through metabolism to salicylate-like metabolites at doses (dogs HED=12.5 mg/kg) similar to the proposed clinical dose (11 mg/kg).

Recommendations: Off-label use of nitazoxanide in women of reproductive age may necessitate further characterization in animals of fetal exposure correlating with possible fetal effects.

Labeling with basis for findings:

Carcinogenesis, Mutagenesis, Impairment of Fertility
Long-term carcinogenicity studies have not been conducted.

Nitazoxanide was not genotoxic in the Chinese hamster ovary (CHO) cell chromosomal aberration assay or the mouse micronucleus assay. NTZ was genotoxic in one tester strain (TA 100) in the Ames bacterial mutagenicity assay

Nitazoxanide did not adversely affect male or female fertility in the rat at 2400 mg/kg/day (approximately 66 times the recommended dose for patients 11 years of age, adjusted for body surface area).

PREGNANCY
Teratogenic Effects
Pregnancy Category B:

Reproduction studies have been performed at doses up to 3200 mg/kg/day in rats (approximately 48 times the clinical dose adjusted for body surface area) and 100 mg/kg/day in rabbits (approximately 3 times the clinical dose adjusted for body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to NTZ. There are, however, no adequate and well-controlled studies in pregnant women.

X. APPENDIX/ATTACHMENTS:

Addendum to review: NDA 20-871 review, IND [redacted] reviews

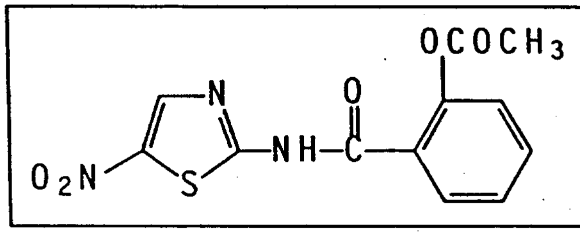
Other relevant materials (Studies not reviewed, appended consults, etc.): none

Any compliance issues: none

PHARMACOLOGIST'S REVIEW

NDA # 20-871

DATE SUBMITTED: December 30. 1997
DATE RECEIVED: January 5, 1998
DATE ASSIGNED: January 7, 1998
DATE REVIEW COMPLETED: June 11, 1998
HFD-590
SPONSOR: Unimed Pharmaceuticals, Inc.
DRUG: Nitazoxanide



RELATED DOCUMENTS: IND ——
INDICATION: Cryptosporidiosis in AIDS patients
DEFINITIONS
NTZ=nitazoxanide
GI= gastrointestinal
desNTZ = desacetylnitazoxanide

INTRODUCTION
Nitazoxanide is a nitrothiazole antiparasitic which has demonstrated activity against protozoa, helminths, and bacteria. Recently NTZ was shown to have in vitro and in vivo activity against Cryptosporidium parvum, a parasitic opportunistic infection affecting AIDS patients. The drug is currently marketed outside the U.S. and has been approved for use in Mexico for infections of the intestinal tract. The drug is manufactured in Columbia. The structure of NTZ demonstrates its relationship to salicylate, an irritant, a factor which may account for the poor tolerance of NTZ by dogs. The metabolism of NTZ is poorly characterized. One metabolite, a deactylated form (desNTZ) has been recovered in dog studies. Most NTZ appears to be eliminated in feces. The mechanism of action for NTZ is not clear. Since NTZ is not well absorbed, a direct effect on either C. parvum or the intestinal wall might be postulated. Direct antimicrobial activity against C. parvum is unlikely as demonstrated by the lack of activity by NTZ or desNTZ in in vitro assays. Speculatively, C. parvum may enter the surface of cells lining the intestinal wall, so that an irritant action might slough these off. The regulatory history of NTZ included several decisions regarding study requirements. A decision was made at an end of phase II meeting with the sponsor to negate the need for the otherwise required six month dog study in view of the toxicity experienced in the 90 day study. Later, at a pre-NDA meeting, the sponsor agreed to perform carcinogenicity studies as Phase IV a commitment. At an advisory committee meeting, the recommendation was made not to approve NTZ due to the lack of efficacy in humans. Subsequently, the Division of Special Pathogens and Immunlogic Drug Products agreed that NTZ was not approvable for lack of demonstrated efficacy.

NONCLINICAL PHARMACOKINETICS

Pharmacokinetics Studies

1. A mass balance and tissue distribution study of ^{14}C-nitazoxanide following a single oral dose to male rats
Study no. [redacted] 6613-100; study dated 5-4-95
2. Determination of desacetylnitazoxanide in dog and rat plasma samples collected during Romark laboratories studies
Study # 195.505; study dated 5-31-95
3. Plasma pharmacokinetics, elimination and tissue distribution of radioactivity following single oral dose administration of 14C-nitazoxanide in dogs
Study # 0831XU13.001; study dated 10-8-97
4. Determination of nitazoxanide and tizoxanide in dog plasma samples collected during [redacted] study no. [redacted] 2712-103
analytical report no. 196.547; study dated 3-7-97

Pharmacokinetics Studies Review

1. A mass balance and tissue distribution study of ^{14}C-nitazoxanide following a single oral dose to male rats
Study no. [redacted] 6613-100
Submitted under IND [redacted] review dated 9-8-95

2. Determination of desacetylnitazoxanide in dog and rat plasma samples collected during Romark laboratories studies
Study # 195.505
Submitted under IND [redacted] review dated 9-8-95

3. Plasma pharmacokinetics, elimination and tissue distribution of radioactivity following single oral dose administration of 14C-nitazoxanide in dogs
Study no. 0831XU13.001
Submitted under IND [redacted] review dated 6-1-98

4. Determination of nitazoxanide and tizoxanide in dog plasma samples collected during [redacted] study no. [redacted] 2712-103
Analytical report no. 196.547
Submitted under IND [redacted] review dated 6-1-98

Summary of Nonclinical Pharmacokinetics Studies

The sponsor attempted to characterize the pharmacokinetic properties of NTZ in radiolabeled studies in dogs and rats. The following table summarizes these studies.

Pharmacokinetic parameters from 28- and 90- day oral toxicity study of desNTZ in dogs and 28-day oral toxicity study in rats

dose, species	AUC (mg.h/L)		Cmax (mg/L)	
	Day 1	**Day 90**	**Day 1**	**Day 90**
25 mg/kg dog	1.22±0.61	1.72±1.26	0.59 ±0.23	0.58±0.31
50 mg/kg dog	4.78±1.06	6.25±3.53	1.27±0.58	1.13±0.52
100 mg/kg dog	16.8±14.3	13.5±9.26	2.10±0.72	1.86±1.22
	day 1 (0-24 hr)	**Day 28 (0-24 hr)**	**day 1**	**Day 28**
900 mg/kg dog	7.73±4.08	14.65±9.95	1.12±0.55	1.58±1.59
2700/1800 mg/kg dog	37.0 ±18.8	20.0±6.6	3.34±1.03	1.37±0.32
800 mg/kg rat			2.38±0.89	
2400 mg/kg rat			3.62±1.37	
4800 mg/kg rat			5.08±2.54	
400 mg/kg rat				2.34±1.45
1200 mg/kg rat				5.78±2.81
2400 mg/kg rat				6.99±1.28
HUMAN - 7.14 mg/kg (500mg dose)	10.10±5.15		2.73±1.73	

mean group values ± s.d.

Metabolism of NTZ has been poorly characterized in animals. Radiolabeled metabolites of NTZ were recovered but, except for the largest fraction described below, uncharacterized. One metabolite desacetylnitazoxanide (desNTZ) has been identified in rats and dogs. Radiolabeled desNTZ has been used to trace

NTZ in the pharmacokinetic studies below. NTZ disappears rapidly in both dogs and rats. Radiolabel concentrations in the plasma peak at about one hour following oral administration. Most radiolabel was found in the gastrointestinal tract. In rats, nearly all radiolabel was eliminated by 72 hours. Most drug was

eliminated by feces and urine in the first 24 hours. In dogs, a single radiolabeled dose was eliminated in feces (0-60%) and urine (0-26%) by 48 hours. Tissue recovery was negligible after 48 hours. As shown in the above table, large oral doses such as 900 and 2700/1800 mg/kg in the dog, do not affect AUC or Cmax when compared to doses such as 25, 50, or 100 mg/kg. Chronic dosing, such as that of the 90 day oral study, did not increase AUC or Cmax either. Plasma protein binding in rats and dogs has not been characterized; human plasma protein binding was reported by the sponsor to be about 95%, thus species differences may produce different pharmacokinetic characteristics between humans and animals. Pharmacokinetic studies employing dosing routes other than oral (i.e. intravenous) would be needed to determine bioavailability of NTZ. Characterization of the remaining metabolites would also permit determination of any changes in the pattern of biotransformation resulting from dose.

NONCLINICAL PHARMACOLOGY

Pharmacology Studies

1. Assay of nitazoxanide for general pharmacological activity
Study # ROM-009; study dated 10-9-92
2. Antiulcerogenic assay in aspirin-treated/pyloric ligated rats
Study # 0251XR13.001; study dated 10-3-96
3. Anti-secretory test in pyloric-ligated Shay rats
Study # 025XR13.001; study dated 10-3-96
4. Study of two compounds on PGE2 secretion
Study # 26034; study dated 11-4-97

Pharmacology Studies Reviews

1. Assay of nitazoxanide for general pharmacological activity
Study # ROM-009;
Submitted under IND — review dated 9-8-95

2. Antiulcerogenic assay in aspirin-treated/pyloric ligated rats
Study # 0251XR13.001
Submitted under IND — ; review dated 6-9-98

3. Anti-secretory test in pyloric-ligated Shay rats
Study no. 025XR13.001
Submitted under IND — review dated 6-9-98

4. Study of two compounds on PGE2 secretion; performed by [redacted] study no. 2603; NTZ batch no. 003; desNTZ batch no. L0795110; 4 November 1997; not GLP

NTZ and desNTZ were evaluated for inhibition of PGE2-secretory activity. The assay was performed using differentiated HL60 cells, with indomethacin as a reference compound. The stimulus for inflammation and PGE2 secretion was A 23187 (5 μM). The test compounds were assayed in duplicate at concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M with an incubation of 30 minutes. PGE2 was determined using commercial kits ([redacted] , and radioactivity determined with a liquid scintillation counter. The percent inhibition of PGE2 by NTZ ranged from 0 [redacted] to 96% [redacted] and by desNTZ from75% [redacted] to 89% [redacted] The IC50 was calculated for NTZ to be 0.10 μM; the IC50 for indomethacin was 0.0056 μM. NTZ would not appear to be an effective inhibitor of PGE2 secretion.

Comment: The experimental procedures provided for this study are minimal, limiting its value to evaluation of this pharmacologic activity of NTZ.

Summary of Nonclinical Pharmacology Studies

The majority of the nonclinical pharmacology studies were reviewed previously and the reviews are included in the Appendix. Slight antimicrobial activity was seen against S. aureus and P. vulgaris. Most notably, NTZ was demonstrated to have analgesic and antiinflammatory activity in rat and mouse in vivo models at a dose of 500 mg/kg, as well as gastrointestinal antisecretory activity and antiulcer activity. Antagonism was demonstrated in vitro against angiotensin II, leukotriene D4, substance P and acetylcholine. The gastrointestinal effects of NTZ are relevant to the potential mechanism of action. These effects were not confirmed in two later studies which examined NTZ antiulcer and antisecretory activity in in vivo rat model . Oral NTZ doses of 100, 300, and 1000 mg/kg were used without any resulting activity.

NONCLINICAL TOXICOLOGY
Toxicity Studies

1. 28-day toxicity study in rats
Study # [redacted] 2712-100 ; study dated 4-27-95
2. Acute intraperitoneal study in mice
Study # ROM-010; study dated 12-78
3. Acute oral toxicity study in mice
study dated 12-78
4. Acute intraperitoneal study in rats
Study # ROM-015; study dated 2-15-77

5. Acute oral toxicity study in rats
Study # ROM-011; study dated 12/78
5. Acute oral toxicity in cats
Study # ROM-013; study dated 5/2/77
6. Acute oral toxicity study in dogs
Study # ROM -012; study dated 5/2/77
7. Acute dermal toxicity study in rabbits
Study # ROM-016; study dated 1/79
8. Primary eye irritation/corrosiveness study in rabbits
Study # ROM-017; study dated 1/79
9. 8-day pilot oral toxicity study of NTZ in rats
Study # — 2647-111; study dated 11/23/92
10. 14-week oral toxicity study in the rat
Study # ROM-018; study dated 5/78
11. Rising dose tolerance study via oral administration of NTZ in dogs
Study # — 2647-112; study dated 11-23-92
12. 28-day oral toxicity study of NTZ in dogs
Study # 2647-114; study dated 12-20-93
14. 90-day toxicity study in dogs with nitazoxanide
Report # — 2712-103; study dated 12-16-96
15. 6-month oral toxicity study in rats
Study no. 0460RU13.001; study dated 7-9-97

Toxicity Studies Review

1. 28-day toxicity study in rats
Submitted under IND — review dated 9-8-95

2. Acute intraperitoneal study in mice
Submitted under IND — review dated 9-8-95

3. Acute oral toxicity study in mice
Submitted under IND — review dated 9-8-95

4. Acute intraperitoneal study in rats
Submitted under IND — review dated 9-8-95

5. Acute oral toxicity study in rats.
Submitted under IND — review dated 9-8-95

6. Acute oral toxicity in cats
Submitted under IND — review dated 9-8-95

7. Acute oral toxicity study in dogs
Submitted under IND — ; review dated 9-8-95

8. Acute dermal toxicity study in rabbits
Submitted under IND — review dated 9-8-95

9. Primary eye irritation/corrosiveness study in rabbits
Submitted under IND — review dated 9-8-95

10. 8-day pilot oral toxicity study of NTZ in rats
Submitted under IND — review dated 9-8-95

11. 14-week oral toxicity study in the rat
Submitted under IND — ; review dated 9-8-95

12. Rising dose tolerance study via oral administration of NTZ in dogs
Submitted under IND — ; review dated 9-8-95

13. 28-day oral toxicity study of NTZ in dogs
Submitted under IND — review dated 9-8-95

14. 90-day toxicity study in dogs with nitazoxanide
Submitted under IND — ; review dated 6-9-98

15. 6-month oral toxicity study in rats
Submitted under IND — review dated 6-1-98

REPRODUCTIVE TOXICOLOGY

Reproductive Toxicology Studies

1. Dose range-finding study for segment II study in rats with NTZ
Study # — 2647-117; study dated 4-1-93
2. Dose range-finding study for segment II study in rabbits with NTZ
Study # — 2647-118; study dated 12-15-93
3. Rabbit developmental toxicity study with nitazoxanide
Study # — 2712-102; study dated 11-19-97
4. Study for the effects of fertility and development in rats with Nitazoxanide
Study # — 2712-104; study dated 12-16-96
5. Study for effects of pre- and postnatal development, including maternal function, in the rat
Study # 6807-100; study dated 10-31-97

Reproductive Toxicology Studies Review

1. Dose range-finding study for segment II study in rats with NTZ
(Study # [redacted] 2647-117)
Submitted under IND [redacted] review dated 9-8-95

2. Dose range-finding study for segment II study in rabbits with NTZ
(Study # [redacted] 2647-118)
Submitted under IND [redacted] review dated 9-8-95

3. Rabbit developmental toxicity study with nitazoxanide
(Study #. [redacted] 2712-102)
Submitted under IND [redacted] review dated 6-9-98

4. Study for the effects of fertility and development in rats with nitazoxanide
(Study # [redacted] 2712-104)
Submitted under IND [redacted] review dated 6-9-98

5. Study for effects of pre- and postnatal development, including maternal function, in the rat; performed by [redacted]; study no. 6807-100; batch no. 003; 31 October 1997; GLP

NTZ was evaluated for oral toxicity in rats from implantation through weaning, in pregnant and lactating females, and the development of the offspring. Male and female Sprague-Dawley rats, ([redacted]), 9 weeks old, were acclimated for one week and then mated. Breeding was confirmed as judged by observation of vaginal sperm or copulatory plug. Mated females were placed in dose groups (25/group). NTZ doses consisted of 0 (vehicle=0.5% methylcellulose), 300, 600, and 1200 mg/kg/day. Dosing was performed daily by oral gavage from gestation day 6 through lactation day 20. Rats were observed twice daily for morbidity and mortality. Body weights were recorded on days 0, 6, 8, 10, 14, 17, and 20 of gestation and on days 0, 4, 7, 10, 14, 17 and 21 of lactation. Food consumption was measured on gestation days 0-6, 6-8, 8-10, 10-14, 14-17, and 14-20. Females were allowed to deliver and raise their litters to day 21 postpartum. At birth, pups were weighed, sexed, and examined for external abnormalities. On days 0, 4 (precull), 7, 14, and 21 of lactation, litter size and pup weights were recorded. On day 4, litters of greater than 8 pups were culled to eight by randomly removing excess pups to leave four males and four females in each litter. The culled pups were sacrificed and examined for cervical, thoracic, or abdominal visceral abnormalities. Litters were examined daily for abnormal behavior or morbidity. At weaning, 23 males and 23 females were selected from each dose group for mating, with at least one pup/sex from each litter (if possible) without sibling matings. Remaining pups were sacrificed. Mating was arranged at seven weeks with up to 21 days of

cohabitation. Mating was confirmed by observation of vaginal sperm or copulatory plug. Mated females were observed twice daily. Body weights were recorded on days 0, 7, 14, and 20, and on lactation day 0. Natural deliveries followed. On day 0 of lactation, litter size and individual sex and weight were recorded. Pups were examined grossly on lactation days 0 and 1, sacrificed on day 1, and examined for cervical, thoracic, and abdominal visceral abnormalities. The F_o females were sacrificed following weaning. A gross examination of cervical, thoracic, and abdominal viscera was performed. Uteri, ovaries, and abnormal tissues were collected. The uterus was examined for implantation sites. The F_1 generation was examined for vaginal opening or cleavage of the balanopreputial gland on day 30 and 35 post partum until all pups were positive. Body weight was recorded with this event. Open field activity was recorded on day 22 postpartum and week 5 postweaning. The pupillary reflex was assessed together with the 3 week open field test and with week 3 postweaning water maze and memory tests. F_1 females and males were sacrificed following weaning. A gross examination of cervical, thoracic, and abdominal viscera was performed. Uteri, ovaries, and abnormal tissues were collected from the females. Abnormal viscera and reproductive organs were collected. During the F_0 gestation, one group 4 female was found dead on day 20, with hypoactivity, coldness, and red/black vaginal discharge prior to death.
Red/black vaginal discharge was also seen in one group 3 female. Yellow staining of the fur of the urogenital region was seen throughout in the treated groups. Dark feces was seen in 19 rats in group 3 and 23 in group 4. Four dams were sacrificed due to the poor condition of their litters, one each in group 1 and 3, and two in group 4. The group 1 pups had no milk visible in their stomachs and died on day 5. The group 3 pups had no remarkable observations prior to death on day 4. One litter of group 4 pups was weak, thin, and without milk visible in their stomachs prior to death on day 9. A second group 4 litter also was weak, thin, and without milk visible in their stomachs prior to death on day 4. The dam also was not attending the litter. Group 4 body weight during gestation was significantly lower than controls on days 6-8, 17-20, 0-20, and 6-20, and higher than controls on days 8-10. During lactation, body weights of the treated dams were significantly higher than controls on day 17. Otherwise, group body weights were similar. Food consumption was similar during gestation for all groups except for days 6-8, when treated groups were significantly lower than controls. Pregnancy rates were similar for all groups. Single litters had stillborn pups in each treated group; the livebirth index was group 1= 100 (291/291), group 2=99(226/229), group 3= 99(269/271) and group 4=99.99(263/264). Four litter deaths occurred during lactation, one each in groups 1 and 3 and two in group 4. The weaning index was: group 1, 94; group 2, 99; group 3, 97; and group 4, 86. More pups died, disappeared/eaten, or were sacrificed in extremis in group 4 than the other groups. Covariate adjusted weights for the group 4 pups were significantly lower than controls throughout lactation. Pathology findings in the F_0

dams included one group 2 dam with mottled kidneys, one group 2 dam with uterine hydrometra (neither pregnant); group 3, two pregnant females with dark areas on the lungs, one dam with distended intestines (sacrificed following total litter death); group 4, one dam found dead with dark kidneys, pale and enlarged adrenals, autolyzed fetuses in the uterus, one dam with an enlarged spleen, one nonpregnant and two pregnant females with distended intestines. Gross pathological findings of the pups included empty stomach (one group 1, one group 2, and three group 4). Bright red or dark lungs were seen throughout, with greatest incidence in group 4. Developmental indicators were unaffected by treatment. One group 4 F_1 female was found dead on day 28 postweaning, with hypoactivity, ataxia, wheezing, ruffled fur, urine stains, and few feces prior to death. Bright yellow stained fur was seen sporadically during the first 3 weeks postweaning. Body weights for group 4 pups of both sexes were significantly lower than controls on postweaning days 0, 7, 14, 21, 28, 35, and 42 (males) and 0, 7, 14, and 21 (females). Open field testing, learning, memory, and reversal learning were unaffected by drug treatment. During gestation, no overt clinical signs were seen in the F_1 females. One group 4 litter died. No other overt clinical signs were seen. F_1 female body weight was unaffected during gestation or lactation. Reproductive performance was unaffected. No gross observations were seen at birth in the litters. One F_1 male (group 2) had a liver mass. Dilated renal pelves were seen in males in all groups (group 1=1, group 2= 2, group 3= 2, group 4=1). Hydometra was seen in three group 1 females, one group 2 female, and three group 3 females. An ovarian cyst was seen in one group 2 female. The group 4 female found dead had a distended urinary bladder with calculi and mottled kidneys with dilated pelves. Food consumption and body weights were particularly sensitive to drug treatment in this study. Toxicities were seen throughout F_0, F_1, and F_2 generations, particularly in the high dose groups. It is unclear whether NTZ is transmitted via lactation or in utero exposure, or whether drug effects were transmitted from generation to generation. The parental NOAEL for this study appeared to be 300 mg/kg/day. It is difficult to determine the F_1 NOAEL due to the maternal effects, otherwise the fetal NOAEL was <300mg/kg/day. The 300 mg/kg day dose is equivalent to a human dose of 50 mg/kg/day. The proposed clinical doses for NTZ are 33-50 mg/kg/day.

GENETIC TOXICOLOGY
Genetic Toxicology Studies

1. Mutagenicity test on NTZ in vivo mammalian micronucleus assay; Study # 15356-0 -455CO; study dated 1-3-94

2. Study of potential mutagenic effects of nitazoxanide using the Ames test with and without metabolic activation
Study # ROM-003; study dated 3-5-92
3. Mutagenicity test with nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay
Study # — 17348-0-409; study dated 5 July 1996
4. Mutagenicity test with desacetyl-nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay
Study # . 17567-0-409; study dated 5 July 1996
5. Mutagenicity test on nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary
Study # — 17348-0-437; study dated 28 June 1996
6. Mutagenicity test on desacetyl-nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary
Study # — 17567-0-437; study dated 27 June 1996

Genetic Toxicology Studies Review

1. Mutagenicity test on NTZ in vivo mammalian micronucleus assay
Study no. 15356-0-455CO
Submitted under IND — review dated 9-8-95

2. Study of potential mutagenic effects of nitazoxanide using the Ames test with and without metabolic activation
Submitted under IND — review dated 4-24-96

3. Mutagenicity test with nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay; performed by —
— : study no. — 17348-0-409; batch no. 003; GLP; 5 July 1996

NTZ was evaluated for the ability to induce reverse mutations at the histidine locus in strains of Salmonella typhimurium and at the tryptophan locus of an Escherichia coli tester strain, both in the presence and absence of metabolic activation by mammalian microsomal enzymes. S. typhimurium tester strains TA98, TA100, TA1535, TA1537, and E. coli tester strain WP2uvrA were used in this study. Positive controls used in S. typhimurium studies were 2-aminoanthracene, 2-nitrofluorene, sodium azide, and ICR-191. Positive controls used in E. coli studies were 2-aminoanthracene and 4-nitroquinoline-N-oxide. The metabolic activation studies used rat liver S9 homogenate prepared from Sprague-Dawley rats treated with Araclor 1254. Vehicle (DMSO) controls were used with all tester strains in the presence and absence of S9. Overnight cultures of each tester strain were used for the studies. All doses of NTZ, vehicle controls, and positive controls were plated in triplicate. A dose finding study was first performed using S. typhimurium strain TA100 and E. coli strain WP2uvrA to select doses of NTZ without cytotoxicity. Based on this study, doses of 250, 100,50, 25, 10, and 5µg per plate were used in S. typhimurium studies with S9 and 500, 100, 50, 25, 10, and 5 µg per plate in studies without S9. In the E. coli studies, doses of 600, 300, 100, 50, 25, and 10µg per plate were used in both the presence and absence of S9. A volume of 50 µl per plate was used for NTZ, positive controls, and vehicle control plates and 500 µl S9 for metabolic activation. Bacterial plates were administered the appropriate test solutions with or without S9 for 48h ±8h, then scored for revertants. All positive controls were successful. NTZ caused a positive response with the S. typhimurium strain TA100 in the presence (3.4 fold increase over vehicle control at 100µg/plate) and absence (4 fold increase over vehicle control at 100 µg/plate) of S9. This is a modest effect relative to the positive control (2-aminoanthracene, with S9, 11-fold increase; sodium azide, without S9, 9-fold increase) increases. No other positive responses were seen in this study.

4. Mutagenicity test with desacetyl-nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay; performed by [redacted] study no. [redacted] 17567-0-409; batch no. 003; 5 July 1996; GLP

APPEARS THIS WAY ON ORIGINAL

desNTZ was evaluated for the ability to induce reverse mutations at the histidine locus in strains of Salmonella typhimurium and at the tryptophan locus of an Escherichia coli tester strain, both in the presence and absence of metabolic activation by mammalian microsomal enzymes. S. typhimurium tester strains TA98, TA100, TA1535, TA1537, and E. coli tester strain WP2uvrA were used in this study. Positive controls used in S. typhimurium studies were 2-aminoanthracene, 2-nitrofluorene, sodium azide, and ICR-191. Positive controls used in E. coli studies were 2-aminoanthracene and 4-nitroquinoline-N-oxide. The metabolic activation studies used rat liver S9 homogenate prepared from Sprague-Dawley rats treated with Araclor 1254. Vehicle (DMSO) controls were used with all tester strains in the presence and absence of S9. Overnight cultures of each tester strain were used for the studies. All doses of NTZ, vehicle controls, and positive controls were plated in triplicate. A dose finding study was first performed using S. typhimurium strain TA100 and E. coli strain WP2uvrA to select doses of NTZ without cytotoxicity. Based on this study, doses of 300, 100,50, 25, 10, and 5 µg per plate were used in S. typhimurium studies with S9 and 100, 50, 25, 10, 5, and 1 µg per plate in studies without S9. In the E. coli studies, doses of 5000, 1000, 500, 250, 100, and 50 µg per plate were used in both the presence and absence of S9. A volume of 50 µl per plate was used for NTZ, positive controls, and vehicle control plates and 500 µl S9 for metabolic activation. Bacterial plates were administered the appropriate test solutions with or without S9 for 48h ±8h, then scored for revertants. All positive controls were successful. NTZ caused a positive response with the S. typhimurium strain TA100 in the presence (3.1-fold increase over vehicle control at 50 μg/plate) and absence (2-fold increase over vehicle control at 25 μg/plate) of S9. This is a modest effect relative to the positive control (2-aminoanthracene, with S9, 12-fold increase; sodium azide, without S9, 7-fold increase) increases. No other positive responses were seen in this study.

5. Mutagenicity test on nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary; performed by [redacted] study no. [redacted] 17348-0-437; batch no. 003; 28 June 1996; GLP

In order to determine the clastogenic potential of NTZ, the in vitro Chinese hamster ovary assay was performed with and without metabolic activation. Chinese hamster ovary cells (CHO-WBL) from a permanent cell line were obtained from the laboratory [redacted] Positive controls used in this assay were mitomycin C (for assays without metabolic activation), and cyclophosphamide (for assays with metabolic activation). The metabolic activation studies used rat liver S9 homogenate [redacted] lot #0583) prepared from Sprague-Dawley rats treated with Araclor 1254. Vehicle (DMSO) controls were used with all tester strains in the presence and absence of S9. A rangefinding assay was first performed to determine an assay concentration range without cytotoxicity and optimal harvest time of treated cells. Based on these preliminary assays, the concentrations of NTZ used in metabolic activated assays were 121, 80.8, 60.6, 40.4, 20.2, 10.1 and 5.05 μg/ml; 1210, 1010, 808, 606, 404, 202, and 101 μg/ml were used without metabolic activation. An optimal incubation time of 20.1 hours was determined. The cells were cultured for 24 hours prior to treatment. Replicate cultures were used for all doses. Cells were incubated for 17.8 hours, then washed and reincubated with colcemid and for two hours. Cells were harvested and prepared on slides with staining for counting. Cytotoxicity occurred in cultures above 80.8 μg/ml without S9 and above 606 μg/ml. One hundred consecutive metaphases from four dose levels with metaphases with and without S9, and from the positive, negative, and vehicle controls were evaluated for the number of cycles through which cells had progressed while in the presence of colcemid. NTZ was negative for induction of chromosomal aberrations in CHO cells except at 60.6 μg/ml with metabolic activation, which was an equivocal positive (% cells with aberrations, mean =9 cells) while the cyclophosphamide positive control had a mean % cells with aberration=52; solvent mean=1).

6. Mutagenicity test on desacetyl-nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary; performed by [redacted]; study no. [redacted] 17567-0-437; batch no.L07-95110; 27 June 1996 June 1996; GLP

In order to determine the clastogenic potential of desNTZ, the in vitro Chinese hamster ovary assay was performed with and without metabolic activation. Chinese hamster ovary cells (CHO-WBL) from a permanent cell line were obtained from the laboratory

Positive controls used in this assay were mitomycin C, for assays without metabolic activation, and cyclophosphamide, for assays with metabolic activation. The metabolic activation studies used rat liver S9 homogenate lot #0583) prepared from Sprague-Dawley rats treated with Araclor 1254. Vehicle (DMSO) controls were used with all tester strains in the presence and absence of S9. A rangefinding assay was first performed to determine an assay concentration range without cytotoxicity and optimal harvest time of treated cells. Based on these preliminary assays, the concentrations of desNTZ used in metabolic activated assays were 999, 799, 599, 400, 200, and 100 μg/ml; 300, 225, 150, 113, 75, 50, and 25 μg/ml were used without metabolic activation. An optimal incubation time of 20.1 hours was determined. The cells were cultured for 24 hours prior to treatment. Replicate cultures were used for all doses. Cells were incubated for 17.8 hours, then washed and reincubated with colcemid and for two hours. Cells were harvested and prepared on slides with staining for counting. Cytotoxicity occurred in cultures above 150 μg/ml without S9. One hundred consecutive metaphases from four dose levels with metaphases with and without S9, and from the positive, negative, and vehicle controls were evaluated for the number of cell cycles through which cells had progressed while in the presence of colcemid. DesNTZ did not produce any positive results in this chromosomal aberration assay.

Summary of the Toxicity, Reproductive, and Genetic Toxicity Studies

Most nonclinical toxicology studies were reviewed previously. Acute toxicity studies were conducted in mice, rats, dogs, and cats. Oral lethal doses by both intraperitoneal and oral routes were determined. In mice, the intraperitoneal LD50 was 105 mg/kg for both males and females. The oral LD50 was 1350 mg/kg in males and 1380 mg/kg for females. The intraperitoneal LD50 in rats was 192 mg/kg in males and 165 mg/kg for females. The oral LD50 was not achieved with the doses used; therefor the LD50 was greater than 10000 mg/kg in both sexes (the lethal oral dose was not achieved in cats). The LD50 was greater than 10 g/kg. The lethal oral dose was also not achieved in dogs. The L\LD50 was greater than 10g/kg. Toxicity was seen as diarrhea and emesis. At necropsy distended gall bladders were seen. Repeat-dose oral toxicity studies were conducted in rats and dogs. Rats were studied for 14 weeks and 6 months. After 14 weeks, rats receiving 450 mg/kg demonstrated intense salivation, increased liver and spleen weights, and decreased thymus weights without histopathological differences. In the 6 month study, a NOAEL of 150 mg/kg/day was observed. The major toxicity seen was an increase in extramedullary hematopoiesis and pigment deposition in the spleens of males and females receiving 450 mg/kg/day. Hematological assays were affected by treatment in the high dose group. Significant increases in leukocytes, lymphocytes, and mean corpuscular volume and decreases in erythrocyte counts and hematocrit were seen in both males and females. Also seen in high dose females were significant increases in neutrophils and mean corpuscular volume. Clinical chemistry effects were seen in the high dose group. Males had significant decreases in urea nitrogen, sodium, total protein, and calcium and increases in albumin/globulin ratio, total bilirubin, and phosphorus. Females had significantly decreased sodium and glucose and higher total bilirubin. In the 28 day dog study, drug induced effects included weight loss and decreased food consumption, blood in the urine, decreased erythrocyte counts, decreased hematocrit, and decreased organ weights including brain, lung, spleen, heart, lung, kidney, thymus, liver, and testes. Histopathologic findings included lymphoid depletion of the thymus, ulceration of the cecum, and cystic hyperplasia of the gall bladder. A NOAEL was not achieved in this study. In the 90 day oral dog study, similar hematological findings occurred. Both weight loss and decreased food consumption were seen. Loose and/or discolored stool was frequently seen. In males, decreased testicular weights and testicular immaturity were seen. The NOAEL for this study was less then 25 mg/kg. A salicylate–like effect by NTZ may account for the toxicity seen in dogs. In all of the acute and longer term oral studies, yellow material was recovered form the stomach and intestines. NTZ is a yellow powder; these findings are supportive of the pharmacokinetic studies showing poor absorption. Yellow staining of fur in the urogenital region also was seen in most animals.

Reproductive toxicity studies were conducted in rats and rabbits. In both species, NTZ did not significantly affect fertility, implantation, pregnancy rates, or cause fetal death. In male rats, histological and functional studies of sperm did not show drug effects. Decreased gravid uterine weights, decreased body weights, and food consumption were seen in females. Fetal abnormalities included protruding tongue, open eyes, and exencephaly in one fetus, macromelia (one fetus), adactylly (one fetus), and protruding tongue and exencephaly (one fetus). Soft tissue malformations included a trend to increased dilation of the brain ventricles and renal pelvic cavitation. Skeletal variations included incomplete skull ossification, fewer than four caudal vertebrae ossified, unossified vertebral centrum, and wavy ribs. These maternal and fetal effects were seen at doses of 600, 1200 and 2400 mg/kg. The maternal effects complicated the interpretation of these data. The range of these doses is equivalent to human doses of 100 to 400 mg/kg, greater than the proposed doses of 33-50 mg/kg. In the rabbit study, no external abnormalities were seen. Soft tissue abnormalities included internal hydrocephaly (one fetus) and