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APPLICATION NUMBER:

207078Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 207078

Submission date: 5/26/15

Drug: sodium zirconium cyclosilicate

Applicant: ZS Pharma, Inc.

Indication: hyperkalemia

Reviewing Division: Division of Cardiovascular and Renal Products

Discussion:

The primary reviewer and supervisor found the nonclinical information adequate to support the approval of sodium zirconium cyclosilicate for the indication listed above.

The carcinogenicity of sodium zirconium cyclosilicate was not assessed because it was not genotoxic, was not absorbed from the gastrointestinal tract and did not cause local adverse effects in the gastrointestinal tract in a 39-week dog study.

Developmental and reproductive toxicity data were provided from studies in rats and rabbits. However, these data are of little relevance given the lack of systemic absorption of sodium zirconium cyclosilicate.

An acceptable established pharmacologic class for sodium zirconium cyclosilicate would be potassium binder.

Conclusions: I agree that this NDA can be approved from a pharm/tox perspective for the indication listed above. I have provided comments on labeling separately.

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/s/

PAUL C BROWN
05/24/2016

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 207078
Supporting document/s: EDR
Applicant's letter date: 05/26/2015
CDER stamp date: 05/26/2015
Product: Zirconium cyclosilicate
Indication: Treatment of hyperkalemia
Applicant: ZS Pharma, Inc., Fort Worth, TX
Review Division: Cardiovascular and Renal Products
Reviewer: Philip J. Gatti, Ph.D.
Supervisor/Team Leader: Thomas Papoian, Ph.D.
Division Director: Norman Stockbridge, M.D., Ph.D.
Project Manager: Brian Proctor

Template Version: September 1, 2010 (Modified by DCRP: Feb. 6, 2013)

Disclaimer

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1 Executive Summary

1.1 Introduction (and Clinical Rationale)

This application is a 505(b)(1) NDA for Sodium Zirconium Cyclosilicate. The proposed indication is hyperkalemia. Sodium Zirconium Cyclosilicate is a new molecular entity that acts as a selective cation exchanger in the GI tract. Other approved drugs in this class include sodium polystyrene sulfonate. The drug has not been approved outside the U.S.

1.2 Brief Discussion of Nonclinical Findings

Sodium zirconium cyclosilicate was shown to be effective in reducing plasma potassium levels in healthy animals.

Toxicology studies in two species (rat and dog) were conducted to support use of sodium zirconium cyclosilicate for up to 6 months in humans. Initial studies utilized "micro porous zirconium silicate" that resulted in renal tubular degeneration/necrosis and pyelitis in a 14-day repeat dose toxicity study in dogs and rats. Since the drug is not absorbed from the GI tract, these effects are an indirect action of the drug. Formulations of ZS utilized in early toxicology studies contained sodium zirconium cyclosilicate (b) (4)

Toxicology studies performed with these newer formulations did not reveal the renal effects in repeat-dose toxicology studies up to 39 weeks in dogs and 26 weeks in rats. In the long-term repeat-dose toxicology studies in dogs, effects on a variety of parameters including clinical chemistry (e.g., plasma electrolyte abnormalities) were reversed when the animals were supplemented with potassium. Thus, the toxicological effects observed were secondary to hypokalemia produced by the drug.

Sodium zirconium cyclosilicate was not teratogenic in rats and rabbits. No effect on fertility was observed in rats. A pre/postnatal study in rats did not show any detrimental effects. Since the drug is not absorbed from the GI tract, there is no plasma exposure in the animals. However, the studies do show that any secondary effects of the drug on reducing potassium absorption do not impair reproductive function or induce teratogenic effects.

Since the drug is not absorbed from the GI tract, is not genotoxic and does not result in local gastrointestinal effects (i.e., necrosis/regeneration, irritation, inflammation or hyperplasia), carcinogenicity studies of sodium zirconium cyclosilicate were not requested by the division or performed by the sponsor. In the 39-week dog repeat-dose toxicity study, there were no indications of GI inflammation which would indicate possible pre-cancerous effects.

1.3 Recommendations

1.3.1 Approvability

Sodium zirconium cyclosilicate can be approved from the pharm/tox perspective for the indication of hyperkalemia in adults for 6 months or less. Use of the drug for periods longer than 6 months is not limiting from a Pharm/Tox perspective.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

8.1 Pregnancy

Risk Summary

(b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

The following tests for mutagenic potential of sodium zirconium cyclosilicate were negative: (1) the Ames (*S. typhimurium* and *E. coli*) test; (2) chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells; and (3) *in vivo* rat micronucleus assay. Given that zirconium cyclosilicate is not genotoxic, not absorbed from the gastrointestinal tract, and did not cause local gastrointestinal alterations in a chronic toxicity study in dogs, carcinogenicity studies in animals to evaluate tumorigenic potential of sodium zirconium cyclosilicate (b) (4)

Fertility in male and female rats has been assessed at doses up to a Human Equivalent Dose (HED) of 58g per day (the maximum feasible dose) with no adverse effects.

(b) (4)

Labeling edits were submitted through Sharepoint.

2 Drug Information

2.1 Drug

CAS Registry Number: None

Generic Name: Zirconium cyclosilicate

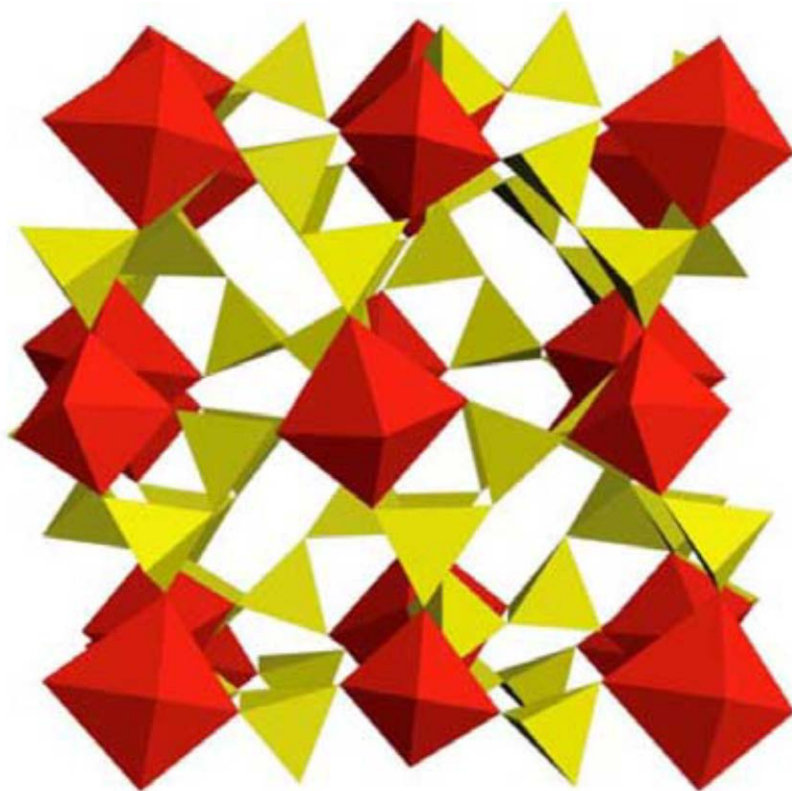
Code Name: ZS (previously ZS-9, UZSi-9)

Chemical Name: Zirconium cyclosilicate

Molecular Formula/Molecular Weight: $\text{Na}_{2.19}\text{ZrSi}_{3.01}\text{O}_{9.11} \cdot 2.71 \text{H}_2\text{O}$ / 420.71 daltons

Structure or Biochemical Description:

The material is a fractionated and protonated form of microporous zirconium silicate.



View down c axis: Red = ZrO_3^{2-} (oct), Yellow = SiO_2 (tet), Cations not shown

Pharmacologic Class: Potassium Binder

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 108951

2.3 Drug Formulation

ZS will be administered orally as a slurry/suspension of the drug substance powder in USP sterile water three times per day (b) (4)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

A single multicenter, prospective, randomized, placebo-controlled, double-blind dose escalating Phase 2 study (ZS-002) was conducted in subjects with chronic kidney disease and mild to moderate hyperkalemia at nine clinical sites within the United States to investigate the safety, tolerability and pharmacodynamics of ZS. Significant dose-dependent reductions in serum potassium were observed in these subjects after administration of the 3 gm TID dose ($p=0.048$) and the 10 gm TID dose ($p<0.0001$). The Phase 3 clinical study (ZS-003) is a double-blind, placebo-controlled study in 750 subjects with entry serum potassium levels between 5.0-6.5 mmol/L. The subjects are randomized to one of four doses of ZS (1.25, 2.5, 5, or 10 gm of ZS TID or placebo on the first two study days (Acute Phase), followed by a maintenance phase of 12 days of treatment with 1.25, 2.5, 5 or 10 gm ZS qd in the subjects that were normokalemic at the end of the acute phase.

2.7 Regulatory Background

At a pre-IND meeting in July 2010, the Division indicated that the 14-day toxicology studies would support human studies up to 14 days in duration, but not longer clinical studies. In December 2010, the sponsor submitted IND 108951. The proposed protocol was intended to demonstrate the effects of ZS-9 on serum potassium levels in a Phase 2, multi-center, placebo-controlled, parallel design dose ranging study in patients with renal insufficiency and hyperkalemia.

On January 18, 2011, the proposed clinical trial was placed on full clinical hold, because the adverse kidney/bladder findings in the rat and dog toxicity studies placed the proposed patient population at additional risk. A letter to the sponsor indicated that determination of accurate NOAELs in the 14-day rat and dog toxicology studies was needed to lift the clinical hold.

In SD 7, the sponsor submitted a draft pathology report that contained the urinary bladder results for all four main groups in the rat 14-day study.

In SD 8, the sponsor submitted a study report for the additional 14-day study (1959-001) in dogs that was requested by the Division. The sponsor concluded that the fractionated and protonated ZS-9 (ZS-9SP) had a NOAEL of 600 mg/kg/dose. Except for substitution of ZS-9SP for ZS-9, the sponsor made no change to the previously proposed protocol for a parallel group study in patients with CKD stage 3-5 (not on dialysis) and moderate acute hyperkalemia. Because of the lack of adequate safety margins for the higher proposed human doses (9 and 18 gm /dose), the Division recommended that the protocol be changed to a dose escalation study. Furthermore, unless an appropriate biomarker of renal injury could be identified to allow the study to be conducted study in healthy volunteers, the study should be conducted in renal failure patients.

In SD 11, the sponsor submitted the results of NAG and NGAL assays from the dog study as well as a revised clinical protocol that proposed dose escalation of ZS-9 administration to healthy volunteers. Because the sponsor did not observe correlation of the levels of NAG or NGAL with the incidence and severity of renal lesions in the subsequent 14-day dog toxicity study (1959-001), the Division recommended conducting the study in patients with renal insufficiency and hyperkalemia. Finally, SD 12 contained a revised clinical protocol (ZS-002) for a dose escalation study in patients

with renal insufficiency and hyperkalemia. The clinical hold was removed on 11/09/2011.

Subsequently, the sponsor submitted a protocol for the on-going study ZS-003. On September 12, 2012, the sponsor met with the Division to discuss their future development plans. On May 15, 2013, the sponsor submitted a protocol for a Phase 3 maintenance study of 29-31 days duration in subjects with hyperkalemia. To support the proposed protocol, the sponsor submitted study reports for 28-day toxicology studies in rats and dogs in that submission.

On 3/3/2014, the sponsor submitted a completed 26-week repeat-dose toxicity study in rats and an interim report on a 39-week repeat-dose toxicity study in beagle dogs.

The final report for the 39-week dog study was submitted on 9/24/2014.

The pre-NDA meeting with the sponsor was held on 1/26/2015. The agency agreed that the completed non-clinical studies were sufficient to support the safety assessment required for filing a NDA for human use of ZS for up to 6 months.

A filing meeting for the NDA was held on 7/7/2015. There were no non-clinical issues.

3 Studies Not Submitted

None

4 Pharmacology

4.1 Primary Pharmacology

The following is the reviewer's modification of the sponsor's pharmacology summary.

The sponsor claims that the in vitro the capacity of ZS-9 to exchange potassium cations is (b) (4) mEq/gram. However, the certificates of analysis show the potassium loading capacities of the lots used in nonclinical studies were (b) (4) meq/gm.

The sponsor states that potassium loading is not affected by the presence of calcium and magnesium cations. Also, the potassium exchange capacity increases proportionally with concentration over the 0.5 to 50 mg/mL range, except in simulated gastric fluid at the lowest dose. The sponsor maintains that pH does not affect potassium exchange capacity at concentrations ≥ 50 mg/mL. However, the potassium exchange capacity decreased by ~91% and 67% in simulated gastric fluid, ~21 and 8% in simulated intestinal fluid at concentrations of 0.5 and 5 mg ZS/mL, respectively, when compared to the exchange capacity in water.

In a non-GLP pharmacology study to determine the efficacy of ZS-9 as an oral sorbent, Sprague Dawley rats, housed in metabolic cages, were alternately fed standard rat chow (4/5 days) and escalating doses of 2, 4 or 6 g ZS/kg/day in rat chow with a 5-day washout of regular rat chow between each dose escalation. ZS-9 treatment in both sexes resulted in significant, dose dependent decreases in urinary potassium and urea nitrogen concentrations, but increases in urinary sodium concentrations concomitant with increases in fecal potassium and urea nitrogen concentrations and decreases in fecal sodium concentrations. ZS-9 treatment did not affect urinary calcium and magnesium concentrations or serum sodium, potassium, calcium, magnesium and blood urea nitrogen levels.

4.2 Secondary Pharmacology

No study examining secondary pharmacology was submitted.

4.3 Safety Pharmacology

Because ZS-9 is insoluble in vehicles compatible with the standard in vitro cardiovascular toxicity assessments, no in vitro cardiovascular study such as the hERG assay was conducted.

No separate in vivo safety pharmacology studies were conducted. The in vivo effect of ZS-9 on respiration rate, heart rate and ECG parameters was measured as part of the GLP 14-day repeat dose toxicity study in dogs (Study ZS 2010-02, see Section 6.2). In this study, ZS-9 treatment had no effect on respiration rate, heart rate, or PR, QRS or QT intervals.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The only ADME study submitted was a non-GLP mass balance study in rats (Study ZSP-1). The amount of zirconium in the urine and feces was determined after a single oral administration of 2000 mg ZS-9/kg to five male Sprague-Dawley rats. To ensure complete consumption of the dose, the requisite amount of ZS-9 was mixed with an amount of pulverized rat diet equivalent to 50% of an individual rat's average daily food intake. Following ZS-9 consumption, rat weight, food and water consumption and urine and feces weights were measured daily for 3 days. The recovery of ZS-9 as zirconium in feces and urine collected over three days indicated that 97-101% of the administered ZS-9 dose was present in the feces and 0.1-0.3% was present in the urine (Table 1).

Table 1: Reviewer's Summary of Zirconium Recovery – Study ZSP-1

	Rat	1	2	3	4	5	Mean
	ZS9 dose, mg	570	547	563	583	553	563
	Zr dose, mg	124	119	122	126	120	122
		Zirconium recovery, % of dose					
Feces	Day 1	72	65	28	31	47	49
	Day 2	25	34	68	69	53	50
	Day 3	0	0	1	0	1	1
	Total feces	97	99	97	100	101	98.8
Urine	Day 1	0.1	0.1	0.1	0.1	0.1	0.1
	Day 2	0	0	0.1	0.1	0	0.04
	Day 3	0	0	0.1	0.2	0.0	0.06
	Total urine	0.1	0.1	0.3	0.3	0.0	0.2
	Total	97.1	99.1	97.3	100.3	101.1	99.0

The sponsor concluded that ZS-9 is not absorbed by the gastrointestinal tract, because the amount of ZS-9 recovered from feces was similar to the recovery of Zr from control samples. The sponsor attributed the amount of zirconium found in urine to food particles

mixed with ZS-9 dose contaminating urine collected in the metabolic cage and not due to gastrointestinal absorption of ZS-9. This reviewer agrees.

5.2 Toxicokinetics

Drug is not absorbed from the GI tract. No TK studies have been submitted by the sponsor.

6 General Toxicology

6.1 Single-Dose Toxicity (Reviewed by Pat Harlow)

6.1.1

Study title: A Maximum Tolerated Dose and 7-Day Repeat Dose Toxicity Study of UZSi-9 Following Oral Administration in Sprague-Dawley Rats

Study no.: 2009-001

Study report location: Vol 2.2, p 121

Conducting laboratory and location: (b) (4)

Date of study initiation: 02/09/09

GLP compliance: Not indicated

QA statement: Not indicated

Drug, lot #, and % purity: UZSi-9, Lot 09614-74-1, purity assumed as 100% (actual purity 87.9%)

Reviewer's Modification of the Sponsor's Summary:

ZS-9 was administered by gavage as a suspension in water at a dose of 2000 mg/kg to 3 Sprague-Dawley rats/sex at a dose volume of 10 ml/kg. Animals were observed daily for 5 days following dose administration for signs of mortality, morbidity, body weight, body weight gain and food consumption and then euthanized without necropsy. No adverse effects were noted and the NOAEL for acute administration of ZS-9 was 2000 mg/kg.

6.1.2

Study title: A Maximum Tolerated Dose and 7-Day Repeat Dose Toxicity Study of UZSi-9 Following Oral Administration in the Beagle Dog

Study no.: 2009-02

Study report location: Vol. 4, p. 1

Conducting laboratory and location: (b) (4)

Date of study initiation: 02/09/09

GLP compliance: Not indicated

QA statement: Not indicated

Drug, lot #, and % purity: UZSi-9 (ZS-9), Lot 09614-74-1, purity assumed as 100% (actual purity 87.9%)

Reviewer's Modification of the Sponsor's Summary:

In the acute phase of this study, ZS-9 was administered once as a suspension in distilled water at a dose volume of 5 ml/kg at a dosage of 2000 mg/kg/day to 1 dog/sex. The dogs were evaluated over a 4-day period for cage side observations, detailed clinical observations, and body weights before being returned to the stock colony. Treatment with ZS-9 did not result in mortality, moribundity or treatment related clinical findings following acute administration. Treatment with ZS-9 had no effect on body weight, body weight gain, or qualitative food consumption. The maximum tolerated single oral dose for ZS-9 in dogs is 2000 mg/kg.

6.2 Repeat-Dose Toxicity**6.2.1 Study title****A 28 Day Oral (Gavage) Toxicity Study with ZS in Rats with a 14 Day Recovery Period (with micronucleus test) (Reviewed by Pat Harlow)**

Conducting laboratory and location: (b) (4)

Study number(s): 524020

Date of study initiation: Dec 13, 2012

Drug lot/batch number: ZS, Batch RD011-53, potassium loading capacity = (b) (4) mEq/g

GLP compliance: Yes

QA statement: Yes

Key Study Findings

In a 28-day repeat dose study, male and female Sprague Dawley rats were treated with ZS alone at 300, 1000, or 2000 mg/kg TID or 2000 mg/kg SID. In the urine of both males and females, sodium and the sodium:creatinine ratio increased while potassium, the potassium:creatinine ratio, chloride, and chloride:creatinine ratio decreased compared with the values in the control groups. Although creatinine excretion did not change significantly in either sex for any treatment group, the ratio of urea clearance to creatinine clearance decreased in males and females in the 2000 mg/kg TID group and the females in the 1000 mg/kg TID and 2000 mg/kg SID groups compared to control values. ZS treatment did not induce excess formation of micronuclei in rat bone marrow cells. Although the sponsor concluded that no microscopic finding was considered related to ZS treatment, the reviewer noted that the incidence of tubular casts was increased in both males and females in the 2000 mg/kg TID group. The sponsor concluded that the NOAEL for ZS was 2000 mg/kg TID.

Purpose

The objective of this study was to evaluate the potential subchronic toxicity of ZS to rats and to evaluate the reversibility, progression, or delayed appearance of any observed changes following a 14-day recovery period.

Methods

Doses: 0, 300, 1000, or 2000 mg/kg/dose (0, 900, 3000, 6000 mg/kg/day) as indicated in Table below.

Frequency of dosing: One or three times a day for 28 consecutive days as indicated in Tables 2 and 3.

Administration route: Oral

Dose volume: 10 mL/kg

Formulation/Vehicle: 0.5% methyl cellulose w/v in water

Species/Strain: Rat/Sprague-Dawley Rat Crl:CD(SD)

Number/Sex/Group: 10 animals/sex/main group

Age: 7 to 8 weeks old,

Weight: Males: 235 - 323 g; Females: 193 and 255 g

Satellite groups: 5 animals/sex in groups 1 and 4 for recovery

Unique study design: Group 5 was administered 2000 mg/kg ZS once daily.

Micronuclei analysis was conducted using bone marrow from the left femur.

Deviation from study protocol: Eight protocol deviations were listed. None had an impact on the outcome of the study.

Table 2: Sponsor's Summary of Study Design

Group No.	Animal Numbers				Treatment	Dosage (mg/kg/dose)	Dosage (mg/kg/day)
	Main Study		Recovery				
	M	F	M	F			
1	1-10	51-60	101-105	111-115	Vehicle TID ^a	0	0
2	11-20	61-70	-	-	ZS TID	300	900
3	21-30	71-80	-	-	ZS TID	1000	3000
4	31-40	81-90	106-110	116-120	ZS TID	2000	6000
5	41-50	91-100	-	-	ZS SID	2000	2000

^a TID = t0, t0 + 6 h, and t0 + 12 h

Parameter	Observation details and times
Mortality	Twice daily
Cage side observations	Five times daily post dosing
Detailed clinical observations	Detailed clinical examinations were performed once weekly during the dosing and the recovery periods (Days -7, 0, 7, 14, 21, 28, 35, 42).
Body weight	Body weights for all animals were measured once pre-test, daily during treatment and weekly during recovery.
Food consumption	Food consumption was measured weekly by cage. The food certificate indicated that the food contained 0.68-0.74% potassium.
Ophthalmoscopy	Ophthalmoscopic examinations were conducted on all animals pretest and during week 4 of treatment.
Hematology	Blood was collected for hematology evaluations via the orbital

Hematology, ctd.	sinus under anesthesia from all animals on the day of necropsy. The animals were not fasted. Hematology parameters included red blood cell count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin concentration, mean cell hemoglobin, reticulocytes, reticulocyte count (absolute), red blood cell distribution width, platelet count, mean platelet volume, white blood cell count, neutrophil, lymphocytes, monocytes, eosinophils, basophil, and large unclassified cells.
Coagulation	Blood was collected for coagulation evaluations from all animals on the day of necropsy. The animals were not fasted. Coagulation parameters included activated partial thromboplastin time, prothrombin time, and fibrinogen
Clinical chemistry	Blood was collected for clinical chemistry from all animals on the day of necropsy. The animals were not fasted. Measured parameters included alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, total bilirubin, urea, creatinine, calcium, phosphate, bicarbonate, total protein, albumin, globulin, albumin/globulin ratio, glucose, cholesterol, sodium, potassium, chloride, magnesium, triglycerides.
Aldosterone/insulin	Blood was collected via the jugular vein during week 4 for possible aldosterone and/or insulin measurements from all animals at necropsy. The samples were not analyzed.
Urinalysis and urine chemistry	Urine was collected for 4 hours from all animals in week 4 and from recovery animals during Week 6. The animals had no access to food or water during the collection period. Parameters at week 4 included colour, turbidity, specific gravity, microscopic evaluation of spun deposit, volume, pH, protein, glucose, bilirubin, ketones, leukocytes, blood pigments, urobilinogen, nitrite, calcium, phosphate, sodium, potassium, chloride, creatinine, magnesium, and urea. Parameters at recovery included calcium, phosphate, sodium, potassium, chloride, creatinine, magnesium, urea, specific gravity, volume, and pH.
Gross pathology	At the end of treatment and recovery, the animals were euthanized using a phenobarbital solution, exsanguinated, and subjected to necropsy. The tissues and organs collected from all animals are summarized in Table 20. The tissues were fixed in neutral buffered formalin, except for the eye (including the optic nerve) and testes, which were fixed using a modified Davidson's fixative. Formalin was infused into the lung.
Organ weights	The following organs from all animals were weighed: brain, adrenal gland, epididymides, heart, kidney, liver, lung, ovary, pituitary, prostate, spleen, testes, thymus, thyroid, and uterus
Histopathology	Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on all protocol-designated tissues indicated in Table 2 from animals in the control (group 1) and 2000

Histopathology, ctd.	mg/kg TID group (group 4). The potential target organs (liver, kidney, heart, stomach, intestinal tract, ureters and urinary bladder) were also examined in the 300 mg/kg TID, 1000 mg/kg TID, and 2000 mg/kg SID groups (2, 3, 5) and the recovery groups.
Micronuclei analysis	At necropsy, the whole left femur from 5 animals/sex in each group was used to obtain bone marrow, which was then subjected to hypotonic treatment and processed to prepare two slides per animal. After Giemsa staining, the slides were evaluated in a blinded manner. Slides previously prepared from animals dosed with cyclophosphamide were randomized into the assessment. At least 2000 polychromatic erythrocytes (PCE) per animal were scored for micronuclei and the frequency of micronucleated cells (MN-PCE) determined. At least 1000 erythrocytes (NCE) per animal were counted and the PCE/NCE ratio determined.
Toxicokinetics	Blood was collected via the jugular vein during week 4 from all animals in Week 4. The samples were stored frozen for possible future analysis of zirconium content. The results were not provided.
Formulation analysis	Formulations were prepared daily. Samples for concentration and homogeneity analyses were collected on Days 2, 10, 15, 22, and 28. Analysis indicated that the mean values of dose formulations from Day 2 ((b) (4) %) and Day 28 ((b) (4) %) were within $\pm$ (b) (4) % of the nominal concentrations. The concentrations in the samples collected from the top, middle and bottom for each formulation were within (b) (4) %.

Table 3: Sponsor's Table of Tissues Collected and Preserved

Animal identification Artery, aorta Bone marrow, femur Bone marrow, sternum Bone, femur Bone, sternum Brain Cervix Epididymis Eye ^a Gland, adrenal Gland, harderian ^a Gland, lacrimal ^a Gland, mammary gland Gland, parathyroid Gland, pituitary Gland, prostate Gland, salivary Gland, seminal vesicle Gland, thyroid Gross lesions/masses Gut-associated lymphoid tissue Heart Kidney Large intestine, caecum Large intestine, colon Large intestine, rectum	Liver Lung Lymph node, mandibular Lymph node, mesenteric Nasal cavity Oesophagus Small intestine, duodenum Small intestine, ileum Small intestine, jejunum Muscle, skeletal Nerve, sciatic Nerve, optic ^a Ovary Pancreas Skin Spinal cord Spleen Stomach Testis ^b Thymus Tongue Trachea Ureter Urinary bladder Uterus including oviduct Vagina
^a Preserved in Davidson's fixative.	^b Preserved in Modified Davidson's fixative.

Results

Mortality: No unscheduled mortality occurred during the study.

Cage side and detailed clinical observations: The sponsor concluded that no clinical findings indicative of systemic toxicity were observed. Light-colored feces were observed in some animals in the ZS-treatment groups (Table 4). In males, the incidence was higher in the 2000 mg/kg TID group than in the 1000 mg/kg TID group. However, in females the incidence was higher in the 1000 mg/kg TID group than in the 2000 mg/kg TID group. The change in feces color was attributed to the excretion of ZS.

Table 4: Reviewer's Summary of Incidence of Light-colored Feces

		Males	Females
Group [#/group/ sex]	Test article/ Doses per day/ mg/kg/dose	Watery feces	Watery feces
1 (15)	Vehicle/TID/0	0	0
2 (10)	ZS/TID/300	0	0
3 (10)	ZS/TID/1000	4	6
4 (15)	ZS/TID/2000	13	3
5 (10)	ZS/SID/2000	0	0
TID = three doses per day; SID = single dose per day			

Body weight

The sponsor concluded that ZS had no effect on body weight in either sex. The mean body weight for males in the 200 mg/kg SID group was significantly higher at the end of the treatment period compared to the mean for the control group (Table 5).

Although the mean body weight for this group on Day 0 was also higher than the mean

for the control group, the mean body weight gain for this group was 9% higher than that for the control group, but was not statistically significant. In contrast, the females in the 2000 mg/kg SID group had an 8% decrease in body weight gain that was not statistically significant.

Table 5: Reviewer's summary of Body Weights and Body Weight Gains

Sex	Group [#/group]	Test article/ Doses per day/ mg/kg/dose	Mean body weight, g (SD)		Mean BW gain, kg	
			Day 0	Day 28	Mean	Min, max
Male	1 (15)	Vehicle/TID/0	282.7 (21.8)	419.5 (26.2)	136.8(16.8)	114.2, 167.6
	2 (10)	ZS/TID/300	271.9 (21.8)	401.7 (35.8)	129.9 (16.8)	104.9, 158.9
	3 (10)	ZS/TID/1000	276.0 (18.1)	411.2 (42.3)	135.2 (29.7)	88.9, 173.2
	4 (15)	ZS/TID/2000	277.7 (10.6)	406.2 (30.3)	128.6 (25.1)	94.3, 168.5
	5 (10)	ZS/SID/2000	297.5 (17.2)	447.0* (34.8)	149.4 (22.7)	117.1, 182.7
Female	1 (15)	Vehicle/TID/0	225.6 (17.4)	286.5 (26.9)	60.9 (13.7)	43.8, 90.5
	2 (10)	ZS/TID/300	219.3 (8.3)	278.1 (15.3)	58.8 (8.8)	45.9, 72.4
	3 (10)	ZS/TID/1000	211.1 (14.7)	271.0 (23.1)	60.0 (11.2)	46.3, 68.5
	4 (15)	ZS/TID/2000	216.9 (19.5)	276.4 (27.6)	59.5 (11.7)	39.7, 92.1
	5 (10)	ZS/SID/2000	210.9 (14.9)	265.0 (18.9)	56.2 (6.8)	47.2, 64.1

TID = three doses per day; SID = single dose per day

Food consumption:

The sponsor concluded that ZS treatment did not affect food consumption. No consistent difference was observed in food consumption by cage among the treatment groups.

Ophthalmoscopy: The detected ophthalmic abnormalities were similar in all animals at the pretest and terminal examinations.

Hematology and coagulation:

The sponsor concluded that no ZS-related effects were observed on hematology and coagulation parameters. However, the sponsor noted the decreases in red blood cell counts, hemoglobin concentrations, and hematocrits in females in the 2000 mg/kg TID and SID groups compared to the values in the control group (Table 6). This effect was not observed in males. The reviewer noted that the females in the 2000 mg/kg TID group also had an increase in mean platelet volume (MPV) that also was not observed in males.

Table 6: Compilation from Sponsor's Tables – Hematology

Group	Males				Females			
	Hb g/dL	RBC x10 ¹² /L	Hct L/L	MPV fL	Hb g/dL	RBC x10 ¹² /L	Hct L/L	MPV fL
1	Mean 14.4 SD 0.4 n 11	Mean 7.69 SD 0.31 n 11	Mean 0.429 SD 0.009 n 11	Mean 7.3 SD 0.6 n 11	Mean 14.2 SD 0.5 n 10	Mean 7.67 SD 0.29 n 10	Mean 0.436 SD 0.012 n 10	Mean 7.9 SD 0.4 n 10
2	Mean 14.6 SD 0.6 n 7	Mean 7.82 SD 0.39 n 7	Mean 0.431 SD 0.019 n 7	Mean 7.7 SD 0.7 n 7	Mean 13.8 SD 0.3 n 8	Mean 7.45 SD 0.13 n 8	Mean 0.421 ^a SD 0.010 n 8	Mean 8.1 SD 0.4 n 8
3	Mean 14.3 SD 0.6 n 8	Mean 7.64 SD 0.56 n 8	Mean 0.431 SD 0.022 n 8	Mean 7.7 SD 0.7 n 8	Mean 14.2 SD 0.6 n 7	Mean 7.66 SD 0.36 n 7	Mean 0.434 SD 0.018 n 7	Mean 8.1 SD 0.3 n 7
4	Mean 14.6 SD 0.5 n 14	Mean 7.82 SD 0.32 n 14	Mean 0.435 SD 0.019 n 14	Mean 7.2 SD 0.4 n 14	Mean 13.9 SD 0.6 n 14	Mean 7.36 ^a SD 0.38 n 14	Mean 0.427 SD 0.018 n 14	Mean 8.4 ^b SD 0.5 n 14
5	Mean 14.8 SD 0.3 n 10	Mean 7.85 SD 0.23 n 10	Mean 0.437 SD 0.011 n 10	Mean 6.9 SD 0.4 n 10	Mean 13.5 SD 0.4 n 8	Mean 7.16 ^c SD 0.22 n 8	Mean 0.413 ^b SD 0.011 n 8	Mean 7.9 SD 0.2 n 8
Significantly different from Group 1 (p<0.05) (p<0.01) (p<0.001)								
Group				1	2	3	4	5
Test Item				Control	ZS	ZS	ZS	ZS once per day
Dose (mg/kg/day)				0	900	3000	6000	2000

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Clinical chemistry

The sponsor concluded that clinical chemistry parameters were unaffected by ZS treatment. However, a few statistically significant differences in group means were noted compared to the means for control groups. These included decreases in sodium in the 300 mg/kg TID females, decreases in urea, triglycerides, and sodium in the 1000 mg/kg TID females, decreases in triglycerides and increases in chloride in the 2000 mg/kg TID and SID females (Table 7). These differences lacked a clear dose response and the sponsor stated that all individual values were within the normal range.

Table 7: Compilation from Sponsor's Tables- Selected Clinical Chemistry Parameters

Group	Males						Females					
	Na mmol/L	K mmol/L	Cl mmol/L	Phos mmol/L	Urea mmol/L	Trig mmol/L	Na mmol/L	K mmol/L	Cl mmol/L	Phos mmol/L	Urea mmol/L	Trig mmol/L
1	Mean 143 SD 1 n 15	Mean 4.7 SD 0.5 n 15	Mean 103 SD 2 n 15	Mean 1.97 SD 0.18 n 15	Mean 5.2 SD 0.6 n 15	Mean 1.74 SD 0.43 n 15	Mean 142 SD 2 n 15	Mean 4.2 SD 0.5 n 14	Mean 104 SD 2 n 15	Mean 1.77 SD 0.20 n 15	Mean 6.4 SD 1.0 n 15	Mean 1.09 SD 0.58 n 15
2	Mean 144 SD 1 n 10	Mean 4.7 SD 0.3 n 10	Mean 104 SD 1 n 10	Mean 1.96 SD 0.19 n 10	Mean 4.9 SD 0.4 n 10	Mean 1.57 SD 0.40 n 10	Mean 141 ^a SD 2 n 10	Mean 4.2 SD 0.3 n 9	Mean 104 SD 1 n 10	Mean 1.76 SD 0.21 n 10	Mean 6.4 SD 0.7 n 10	Mean 1.53 SD 0.33 n 10
3	Mean 142 SD 2 n 10	Mean 5.0 SD 0.7 n 10	Mean 103 SD 1 n 10	Mean 2.00 SD 0.20 n 10	Mean 5.3 SD 0.5 n 10	Mean 1.61 SD 0.29 n 10	Mean 141 ^a SD 1 n 10	Mean 4.3 SD 0.3 n 10	Mean 105 SD 1 n 10	Mean 1.75 SD 0.16 n 10	Mean 5.4 ^b SD 0.9 n 10	Mean 1.12 ^b SD 0.29 n 10
4	Mean 143 SD 2 n 15	Mean 4.7 SD 0.4 n 15	Mean 104 SD 2 n 15	Mean 1.92 SD 0.18 n 15	Mean 4.9 SD 0.6 n 15	Mean 1.59 SD 0.55 n 15	Mean 142 SD 1 n 15	Mean 4.4 SD 0.4 n 15	Mean 105 ^a SD 1 n 15	Mean 1.81 SD 0.24 n 15	Mean 5.9 SD 0.8 n 15	Mean 1.18 ^b SD 0.39 n 15
5	Mean 143 SD 1 n 10	Mean 4.8 SD 0.7 n 10	Mean 103 SD 2 n 10	Mean 1.91 SD 0.15 n 10	Mean 4.8 SD 0.6 n 10	Mean 1.63 SD 0.40 n 10	Mean 142 SD 2 n 10	Mean 4.4 SD 0.5 n 10	Mean 106 ^b SD 2 n 10	Mean 1.79 SD 0.34 n 10	Mean 5.9 SD 0.7 n 10	Mean 1.27 ^a SD 0.65 n 10
Significantly different from Group 1 (p<0.05) (p<0.01) (p<0.001)												
Group						1	2	3	4	5		
Test Item						Control	ZS	ZS	ZS	ZS once per day		
Dose (mg/kg/day)						0	900	3000	6000	2000		

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Urinalysis and Urine Chemistry

Statistically significant decreases in the group mean urinary pH value occurred in males receiving 2000 mg/kg TID and SID compared with the mean for the control group (Table 8). Non-statistically significant decreases in the group mean urinary pH value occurred in females receiving 2000 mg/kg TID and SID and males receiving 1000 mg/kg TID compared with the mean for the control group. However, the sponsor maintained that all individual values were within the normal range and the pH differences were not considered to be treatment related.

However, as expected, ZS treatment affected the urinary excretion of electrolytes. In the urine of both males and females, sodium and the sodium:creatinine ratio increased while potassium, the potassium:creatinine ratio, chloride, and chloride:creatinine ratio decreased compared with the values in the control groups. These changes, except for the decrease chloride:creatinine ratio in males receiving 2000 mg/kg SID, were dose related. Since these changes were consistent with the pharmacodynamic effect of ZS, they not considered toxicologically adverse, particularly since no significant changes occurred in the plasma concentrations of these electrolytes.

The urea:creatinine ratio was decreased in females in the 1000 mg/kg TID and 2000 mg/kg SID groups compared with values for the control group. Because the decrease in the 2000 mg/kg TID female group was not statistically significant, the decrease in urea:creatinine ratio was not considered related to treatment with ZS. However, the reviewer noted that males in the 2000 mg/kg TID group also had a decrease in urea:creatinine ratio, although the difference was not statistically significant.

Table 8: Compilation from Sponsor's Tables of Urinalysis Data

Male	Group / sex		U/Urea mmol/L	UpH	UMg mmol/L	UCrea μmol/L	UCI mmol/L	UNa mmol/L	UK mmol/L	UCa mmol/L	MgC-R	NaC-R	KC-R	ClC-R	CaC-R	UrC-R
	1M	Mean	433.9	8.8	22.70	6985	72.98	44.5	188.49	4.84	3.40	7.86	29.16	12.26	0.8	65
		SD	194.8	0.4	11.22	3806	31.73	18.8	72.69	1.97	0.64	4.81	6.36	6.04	0.5	9
		n	14	14	14	14	14	14	14	14	14	14	14	14	14	14
	2M	Mean	347.8	8.8	15.75	5206	63.40	82.6 ^a	94.39 ^b	3.40	3.24	17.45 ^b	19.44 ^b	13.02	0.7	67
		SD	143.9	0.4	6.37	2048	35.69	36.3	36.48	1.84	0.87	8.67	3.45	7.78	0.3	11
		n	10	10	9	10	10	10	9	10	9	10	9	10	10	10
	3M	Mean	245.1	8.3	10.77	3627	28.99 ^c	72.4	36.92 ^c	3.35	2.96	19.65 ^c	10.52 ^c	7.92	1.0	63
		SD	171.3	0.6	5.63	1630	16.66	40.8	15.34	2.62	0.55	4.66	3.19	2.39	0.5	14
		n	8	9	8	8	8	8	8	8	8	8	8	8	8	8
	4M	Mean	358.3	8.3 ^d	17.15	6218	41.91 ^b	113.1 ^c	32.93 ^c	3.76	2.85	20.60 ^c	5.47 ^c	7.55 ^b	0.7	59
		SD	134.6	0.6	9.10	2493	19.15	39.0	14.50	2.56	1.02	8.94	1.74	3.85	0.5	7
		n	12	13	12	12	12	12	12	12	12	12	12	12	12	12
	5M	Mean	347.5	7.7 ^e	18.84	6169	40.06 ^b	87.7 ^a	53.60 ^c	3.94	3.05	14.44 ^a	10.10 ^c	6.66 ^a	0.7	61
		SD	182.7	0.6	13.30	3708	25.42	53.0	29.99	3.99	0.55	3.70	3.90	2.39	0.6	15
	n	8	9	8	8	8	8	8	8	8	8	8	8	8	8	
Females	Group / sex		U/Urea mmol/L	UpH	UMg mmol/L	UCrea μmol/L	UCI mmol/L	UNa mmol/L	UK mmol/L	UCa mmol/L	MgC-R	NaC-R	KC-R	ClC-R	CaC-R	UrC-R
	1F	Mean	336.8	7.7	11.61	4161	35.91	34.5	92.47	3.50	2.91	9.80	23.58	9.95	1.1	83
		SD	149.8	0.8	5.76	1971	14.58	11.5	42.07	2.80	0.76	4.90	6.62	4.46	1.1	14
		n	14	15	14	14	14	14	14	14	14	14	14	14	14	14
	2F	Mean	279.7	7.9	10.99	3592	31.03	45.9	67.72	4.54	3.10	13.35	19.71 ^a	8.55	1.3	79
		SD	104.3	0.7	4.61	1419	14.60	16.4	20.14	2.67	0.57	3.85	4.32	2.94	0.7	13
		n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	3F	Mean	332.7	7.8	12.72	4666	31.41	62.3 ^b	57.33 ^b	3.45	2.77	16.32 ^a	13.07 ^c	8.30	0.9	71 ^b
		SD	171.1	0.9	6.90	2365	13.02	19.2	27.33	2.03	0.58	9.89	3.79	5.08	0.8	7
		n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	4F	Mean	260.2	7.5	9.83	3493	18.08 ^b	61.3 ^b	26.29 ^c	2.88	2.77	18.55 ^b	8.33 ^c	5.62	0.8	75
		SD	137.5	0.5	5.68	1912	10.57	32.1	12.47	2.30	0.46	7.00	3.03	2.36	0.4	12
		n	13	14	13	13	13	13	13	13	13	13	13	12	13	13
	5F	Mean	229.7	7.0	10.51	3497	30.00	53.5 ^d	37.21 ^c	3.07	3.00	15.40	10.45 ^c	8.38	0.9	65 ^c
		SD	50.0	0.5	2.35	564	20.84	29.2	16.26	1.52	0.37	8.11	3.21	5.34	0.5	5
	n	9	10	9	9	9	9	9	9	9	9	9	9	9	9	
Significance level (p-value) (p < 0.05)				Group	:	1	2	3	4	5						
				Test item	:	Control	ZS	ZS	ZS	ZS						
				Dosage (mg/kg/day)	:	0	900	3000	6000	2000						

Micronuclei analysis

The percentage of micronucleated polychromatic erythrocytes (NMN-PCE) in the vehicle control animals was 0.04%, a value within the historical control range (0.01-0.13% per 5-7 rats) for vehicle/untreated rats (Table 9). The mean MN-PCE frequency in stock slides from rats previously dosed with cyclophosphamide was 2.17%. After ZS

treatment for 28 days the highest incidence of micronuclei was 0.1% in two females in the 1000 mg/kg TID group. These values are within the negative historical control range. Therefore, ZS treatment did not induce excess formation of micronuclei in rat bone marrow cells.

Table 9: Sponsor's Tables- Micronuclei Analysis

Treatment	Sex	No. of Rats Scored	Erythrocytes				
			Normochromatic Cells (NCE)	Polychromatic Cells (PCE)			PCE/NCE
			No. of MN-NCE	PCE Analysed	No. of MN-PCE	% MN-PCE	Mean $\pm$ S.D.
Dose Group 1 (0.5% methyl cellulose w/v in water)	M	5	2	10007	0	0.00	0.81 $\pm$ 0.05
	F	5	0	10007	4	0.04	0.83 $\pm$ 0.10
	M/F	10	2	20014	4	0.02	0.82 $\pm$ 0.08
Dose Group 2 ZS (300 mg/kg/dose; 900 mg/kg/day)	M	5	5	10002	3	0.03	0.82 $\pm$ 0.08
	F	5	0	10005	3	0.03	0.89 $\pm$ 0.11
	M/F	10	5	20007	6	0.03	0.85 $\pm$ 0.10
Dose Group 3 ZS (1000 mg/kg/dose; 3000 mg/kg/day)	M	5	2	10003	0	0.00	0.79 $\pm$ 0.14
	F	5	2	10002	6	0.06	0.78 $\pm$ 0.14
	M/F	10	4	20005	6	0.03	0.79 $\pm$ 0.13
Dose Group 4 ZS (2000 mg/kg/dose; 6000 mg/kg/day)	M	5	2	10005	2	0.02	0.79 $\pm$ 0.05
	F	5	3	10007	2	0.02	0.89 $\pm$ 0.08
	M/F	10	5	20012	4	0.02	0.84 $\pm$ 0.08
Dose Group 5 ZS (2000 mg/kg/dose; 2000 mg/kg/day)	M	5	1	10006	2	0.02	0.87 $\pm$ 0.05
	F	5	2	10002	5	0.05	0.82 $\pm$ 0.13
	M/F	10	3	20008	7	0.03	0.84 $\pm$ 0.09
Micronucleus Positive Control ^a Cyclophosphamide (50 mg/kg/day; 2 days)	M	3	12	6004	130	2.17%	0.53 $\pm$ 0.09

^a Slides from positive control stock.

PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes

MN-PCE = Micronucleated polychromatic erythrocytes
MN-NCE = Micronucleated normochromatic erythrocytes

Negative historical control Data				Positive historical control data	
Frequency of Micronucleated Polychromatic Erythrocytes (MN-PCE) in Vehicle and Untreated Male CD and Female CD Rats				Frequency of Micronucleated Polychromatic Erythrocytes (MN-PCE) in Positive Control Male and Female Treated Rats	
Mean		0.04%		Mean	2.20%
Standard Deviation		0.05%		Standard Deviation	1.03%
Ranges	Individual Rat	Group of 5-7 Rats	Group of 10-12 Rats	Min - Max Frequencies	0.05-6.20%
Min frequency	0.00%	0.01%	0.02%	Number of Polychromatic Erythrocytes Assessed:	300256
Max frequency	0.20%	0.13%	0.11%	Number of Rats Assessed:	231
Number of Polychromatic Erythrocytes Assessed:		10 (8/2)			
Number of Rats Assessed:		539			

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Gross pathology

The gross findings observed were considered unrelated to administration of ZS.

Organ Weights

The sponsor concluded that organ weights were not affected by treatment with ZS.

However, a 12% statistically significant increase was noted for absolute liver weight in the 2000 mg/kg SID males compared with that in the control group (Table 10). Since a statistically significant difference was not observed for relative liver weight, the difference in liver weight was considered to be unrelated to treatment with ZS.

The reviewer noted 12.5% and 5% increases in relative heart weights in males and females, respectively in the 2000 mg/kg TID group. These increases were not

statistically significant; however some dose dependency was evident. No histopathology correlate was observed.

Table 10: Compilation from Sponsor's Tables- Organ Weights

			Absolute weight, gm						Relative to body weight					
Males	Group/ sex		Body Weight (g)	Adrenals	Brain	Heart	Kidneys	Liver	Adrenals	Brain	Heart	Kidneys	Liver	
	1M	Mean	414	0.0534	2.11	1.39	2.74	16.70	0.01295	0.510	0.334	0.660	4.023	
		SD	30	0.0077	0.12	0.13	0.34	2.03	0.00205	0.017	0.019	0.063	0.307	
		n	10	10	10	10	10	10	10	10	10	10	10	
	2M	Mean	394	0.0526	2.13	1.35	2.61	16.03	0.01338	0.544	0.344	0.664	4.068	
		SD	35	0.0098	0.12	0.15	0.33	1.90	0.00231	0.035	0.015	0.057	0.273	
		n	10	10	10	10	10	10	10	10	10	10	10	
	3M	Mean	402	0.0566	2.17	1.46	2.66	16.72	0.01409	0.543	0.362	0.665	4.155	
		SD	42	0.0074	0.09	0.25	0.28	2.24	0.00114	0.050	0.040	0.068	0.240	
		n	10	10	10	10	10	10	10	10	10	10	10	
	4M	Mean	405	0.0511	2.18	1.52	2.65	16.44	0.01269	0.541	0.376	0.653	4.056	
		SD	30	0.0045	0.10	0.20	0.25	1.78	0.00159	0.042	0.054	0.042	0.302	
		n	10	10	10	10	10	10	10	10	10	10	10	
	5M	Mean	436	0.0549	2.10	1.52	2.84	18.78 ^d	0.01270	0.484	0.347	0.651	4.295	
		SD	39	0.0078	0.09	0.21	0.23	2.34	0.00217	0.050	0.038	0.030	0.225	
	n	10	10	10	10	10	10	10	10	10	10	10		

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Females	Group / sex		Body Weight (g)	Adrenals	Brain	Heart	Kidneys	Liver	Adrenals	Brain	Heart	Kidneys	Liver
	1F	Mean	281	0.0697	2.02	1.00	1.84	10.72	0.02498	0.724	0.356	0.658	3.810
		SD	27	0.0112	0.07	0.12	0.16	1.41	0.00420	0.073	0.018	0.053	0.197
		n	10	10	10	10	10	10	10	10	10	10	10
	2F	Mean	271	0.0622	2.04	0.97	1.86	10.72	0.02291	0.752	0.356	0.686	3.948
		SD	13	0.0082	0.10	0.08	0.16	0.82	0.00279	0.041	0.021	0.046	0.167
		n	10	10	10	10	10	10	10	10	10	10	10
	3F	Mean	264	0.0637	2.03	0.96	1.83	10.05	0.02402	0.770	0.365	0.692	3.789
		SD	22	0.0137	0.09	0.10	0.18	1.53	0.00439	0.039	0.023	0.045	0.281
		n	10	10	10	10	10	10	10	10	10	10	10
	4F	Mean	268	0.0641	2.05	1.00	1.89	10.22	0.02406	0.770	0.374	0.707	3.804
		SD	29	0.0101	0.11	0.12	0.25	1.71	0.00378	0.060	0.023	0.057	0.322
		n	10	10	10	10	10	10	10	10	10	10	10
	5F	Mean	261	0.0613	1.99	0.99	1.72	10.28	0.02356	0.763	0.379	0.657	3.935
		SD	19	0.0067	0.11	0.14	0.21	1.09	0.00295	0.059	0.043	0.057	0.291
	n	10	10	10	10	10	10	10	10	10	10	10	

Histopathology

The sponsor concluded that no microscopic finding was considered related to ZS treatment. The reviewer noted that the incidence of tubular casts was increased in both males and females in the 2000 mg/kg TID group (Table 11). No treatment-related findings were observed in the ureter or the bladder.

Table 11: Compilation from Sponsor's Tables- Microscopic Findings

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg /day	Grp 2 900 mg/kg /day	Grp 3 3000 mg/kg /day	Grp 4 6000 mg/kg /day	Grp 5 2000 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 900 mg/kg /day	Grp 3 3000 mg/kg /day	Grp 4 6000 mg/kg /day	Grp 5 2000 mg/kg /day
KIDNEY		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
No abnormality detected		3	6	10	1	5	7	9	9	5	6
Basophilic tubules		7	3	0	7	2	2	1	1	4	2
Tubular casts		1	1	0	4	2	0	0	1	3	1
Inflammatory cell foci		3	1	0	3	1	0	0	1	2	2
Dilatation, tubular, focal		0	0	0	0	2	0	0	0	0	0
Mononuclear cell infiltration, pelvic and papillary		0	0	0	0	0	1	0	0	0	0
Transitional cell hyperplasia, pelvic and papillary		0	0	0	0	0	1	0	0	0	0
Pelvic dilation		0	0	0	0	0	0	0	0	0	1
Mineral deposits, medullary		0	0	0	0	0	0	0	0	1	1

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg /day	Grp 2 900 mg/kg /day	Grp 3 3000 mg/kg /day	Grp 4 6000 mg/kg /day	Grp 5 2000 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 900 mg/kg /day	Grp 3 3000 mg/kg /day	Grp 4 6000 mg/kg /day	Grp 5 2000 mg/kg /day
URETER		(10)	(10)	(9)	(10)	(10)	(9)	(10)	(10)	(10)	(10)
No abnormality detected		10	10	9	10	10	9	10	10	10	10
URINARY BLADDER		(10)	(10)	(10)	(10)	(10)	(9)	(10)	(10)	(10)	(10)
No abnormality detected		10	10	10	10	10	8	10	10	10	10
Transitional cell hyperplasia		0	0	0	0	0	1	0	0	0	0
Mononuclear cell infiltration, mucosal		0	0	0	0	0	1	0	0	0	0

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg /day	Grp 2 900 mg/kg /day	Grp 3 3000 mg/kg /day	Grp 4 6000 mg/kg /day	Grp 5 2000 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 900 mg/kg /day	Grp 3 3000 mg/kg /day	Grp 4 6000 mg/kg /day	Grp 5 2000 mg/kg /day
ADRENAL GLAND		(10)			(10)		(10)			(10)	
No abnormality detected		10			10		10			10	

Summary and Evaluation

In the 28-day repeat dose study, male and female Sprague Dawley rats were treated with ZS alone at 300, 1000, or 2000 mg/kg TID or 1000 mg/kg SID. In the urine of both males and females at all dose of ZS, sodium and the sodium:creatinine ratio increased while potassium, the potassium:creatinine ratio, chloride, and chloride:creatinine ratio decreased compared with the values in the control groups. Although creatinine excretion did not change significantly in either sex for any treatment group, the ratio of urea clearance to creatinine clearance decreased in males and females in the 2000 mg/kg TID group and the females in the 1000 mg/kg TID and 2000 mg/kg SID groups compared to control values. Although the sponsor concluded that no microscopic finding was considered related to ZS treatment, the reviewer noted that the incidence of tubular casts was increased in both males and females in the 2000 mg/kg TID group. The sponsor concluded that the NOAEL for ZS was 2000 mg/kg TID. This NOAEL is 972 mg/kg/day the HED.

6.2.2. A 26 Week Toxicity Study of ZS by Oral Administration in Rats with a 4 Week Recovery Period (Reviewed by Pat Harlow)

Conducting laboratory and location:

(b) (4)

Study number(s): 524942

Date of study initiation: 06 Aug 2013

Drug lot/batch number: ZS, Batch RD011-59, potassium loading capacity = (b) (4) mEq/g

GLP compliance: Yes

QA statement: Yes

Key Study Findings

Male and female Sprague Dawley rats were treated with ZS alone at 333, 1000, or 2000 mg/kg TID in a 26-week repeat dose study. This report describes interim results in rats given ZS for 13 weeks. The only clinical chemistry differences considered related to ZS- treatment were the decreases in serum potassium in the high dose males and females and the decreases in urea in the mid and high dose females. Urine pH increased in all ZS-treated groups. Urine concentrations of sodium and chloride increased with and without normalization with creatinine concentration, whereas urine concentrations of potassium and magnesium decreased. Although the pathologist concluded that no microscopic finding was considered related to ZS treatment, chronic progressive nephropathy was present in 2 of 10 high dose males, but not in the control group. The report concluded that the NOAEL for ZS was 2000 mg/kg TID or 6000 mg/kg/day.

Purpose

The ultimate objective of this study was to evaluate the potential toxicity in rats of ZS administered for at least 26 weeks. This report describes interim results in rats given ZS for 13 weeks.

Methods

Doses: 0, 333, 1000, or 2000 mg/kg/dose (0, 1000, 3000, 6000 mg/kg/day) as indicated in Table 18

Frequency of dosing: Three times a day for 13 weeks as indicated in Table 19.

Administration route: Oral

Dose volume: 10 mL/kg

Formulation/Vehicle: 0.5% methyl cellulose w/v in water

Species/Strain: Rat/Sprague-Dawley Rat Crl:CD(SD)

Number/Sex/Group: 10 rats/sex/group for main groups on Day 91 and 183

Age: 6 to 7 weeks old,

Weight: Males: 202 - 209 g; Females: 174 - 177 g

NDA207078

Philip Gatti, Ph.D.

Satellite groups: 10 animals/sex/group for recovery

Unique study design: None

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Deviation from study protocol: The report listed 13 protocol deviations. No deviation had an impact on the outcome of the study. The most serious deviation was a formulation shortage at the last dose on Day 13. Consequently, high dose males 4023-4025M did not receive the third dose for Day 13 and one other male (4020M), all assigned to Day 183 euthanasia, received only a portion of the third dose on Day 13.

Table 12: Sponsor's Summary of Study Design

Group No.	Number of animals						Treatment (mg/kg/day)	Dosage (mg/kg/dose)
	Day 91 Euthanasia		Day 183 Euthanasia		Recovery - Day 210			
	Males	Females	Males	Females	Males	Females		
1	10	10	20	20	10	10	0	0
2	10	10	20	20	10	10	1000	333
3	10	10	20	20	10	10	3000	1000
4	10	10	20	20	10	10	6000	2000

Parameter	Observation details and times
Mortality	Twice daily
Cage side observations	Five times daily post dosing
Detailed clinical	Detailed clinical examinations were performed once weekly.
Body weight	Body weights for all animals were measured once pre-test, daily up to the start of Week 5, then twice weekly during the dosing and recovery period.
Food consumption	Food consumption was measured weekly by cage. The food certificate indicated that the food contained 0.70-0.72% potassium.
Ophthalmoscopy	Ophthalmoscopic examinations were conducted on all animals pretest and during week 13 of treatment.
Hematology	Blood was collected for hematology evaluations via the orbital sinus under anesthesia or from a lateral tail vein from all animals during weeks 4 and 13. The animals were not fasted. Hematology parameters included red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, reticulocytes, reticulocyte count (absolute), red blood cell distribution width, platelet count, white blood cell count, neutrophil count (absolute), lymphocyte count (absolute), monocyte count (absolute), eosinophil count (absolute), basophil count (absolute), large unstained cells, mean platelet volume, and other cells (as appropriate). A blood smear was also prepared from each hematology sample, but was not examined

Coagulation	Blood was collected for hematology evaluations via the orbital sinus under anesthesia or from a lateral tail vein from all animals during weeks 4 and 13. The animals were not fasted. Coagulation parameters included activated partial thromboplastin time, prothrombin time, and fibrinogen
Clinical chemistry	Blood was collected for clinical chemistry from all animals on the day of necropsy. The animals were not fasted. Measured parameters included urea, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine kinase, lactate dehydrogenase, sodium, potassium, chloride, magnesium, triglycerides, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, creatinine, total bilirubin, calcium, phosphate, bicarbonate
Urinalysis and urine chemistry	Urine was collected overnight during weeks 12-13. Animals were placed into individual cages and were deprived of food during the collection. Parameters included color, appearance/clarity, specific gravity, volume, pH, protein, bilirubin, ketones, blood, leukocytes, microscopic evaluation of spun deposit, urobilinogen, nitrite, calcium*, phosphate*, sodium*, urea*, potassium*, chloride*, creatinine and magnesium* (* Quantitative and as creatinine ratio)
Gross pathology	At the end of treatment and recovery, the animals were euthanized using carbon dioxide, exsanguinated, and subjected to necropsy. The tissues and organs collected from all animals are summarized in Table 14. The tissues were fixed in 10% neutral buffered formalin, except for the eye (including the optic nerve) and Harderian gland, which were fixed using a Davidson's fixative and testes, which were fixed using a modified Davidson's fixative. Bone marrow smears were also prepared, but not evaluated
Organ weights	The following organs from all animals were weighed: brain, adrenal gland, epididymides, heart, kidney, liver, lung, ovary, pituitary, prostate, spleen, testes, thymus, thyroid, and uterus
Histopathology	Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on all protocol-designated tissues indicated in Table 14 from animals in the control (group 1) and 2000 mg/kg/dose group (group 4).
Toxicokinetics	Additional blood and urine samples were collected during pretreatment, week 4 and week 13 from animals assigned to recovery. The samples were stored frozen for possible future analysis of zirconium content. The results were not provided.
Formulation analysis	Formulations were prepared daily. Samples for concentration and homogeneity analyses were collected in weeks 1, 2, 3, 4, 13, 14, 15, 16. The results were not provided in the interim report.

Table 13: Sponsor's Table of Tissues Collected and Preserved

Artery, aorta	Gross lesions/masses	Pancreas
Bone marrow, femur	Gut-associated lymphoid	Skin
Bone marrow, sternum	tissue	Small intestine, duodenum
Bone, femur	Heart	Small intestine, ileum
Bone, sternum	Kidney	Small intestine, jejunum
Brain	Large intestine, caecum	Spinal cord
Cervix	Large intestine, colon	Spleen
Epididymis	Large intestine, rectum	Stomach
Eye ^a	Larynx	Testis ^b
Gland, adrenal	Liver	Thymus
Gland, harderian ^a	Lung	Tongue
Gland, lacrimal	Lymph node, mandibular	Trachea
Gland, mammary	Lymph node, mesenteric	Ureter
Gland, parathyroid	Muscle, skeletal	Urinary bladder
Gland, pituitary	Nerve, optic ^a	Uterus
Gland, prostate	Nerve, sciatic	Vagina
Gland, salivary (mandibular)	Oesophagus	
Gland, seminal vesicle	Ovary	
Gland, thyroid	Oviduct	
^a Preserved in Davidson's fixative.		^b Preserved in Modified Davidson's fixative.

Results

Formulation analysis: The report stated that the dose formulations through week 13 were accurately prepared; however, the data was not provided in this interim report.

Mortality: Four unscheduled deaths occurred during the study (Table 14). All were attributed to injury during dosing. The two animals removed on Day 3 were replaced.

Table 14: Sponsor's Table of Unscheduled Mortality

Group/Treatment (mg/kg/day)	Animal/Sex	Status	Day of Removal	Reason for Removal
1/0	1024/M	Unscheduled	3	Clinical signs
2/1000	2537/F	Unscheduled	3	Clinical signs
3/3000	3504/F	Found dead	38	-
4/6000	4030/M	Found dead	42	-

Cage side and detailed clinical observations: The sponsor concluded that no clinical findings indicative of systemic toxicity were observed. Light-colored feces were observed in males and females receiving 6000 mg/kg/day of ZS between Days 32 and 50. The change in feces color was attributed to the excretion of ZS.

One female (2513F) receiving 1000 mg/kg/day of ZS had hunched appearance and irregular breathing rate from Day 17 to 19 along with a 6% body weight loss from Days 16 to 17. However, this animal fully recovered on Day 20. These findings were considered unrelated to ZS-treatment.

Body weight

The sponsor concluded that ZS had no effect on body weight in either sex. The 7.5% decreases in body weight in body weight gain in the 1000 and 2000 mg/kg/dose groups. were not statistically significant (Table 15). Only two females (F3521:103gm; F 4516: 91 gm) had body weight gains below the concurrent control range of 108 to 246 gm.

Table 15: Compilation of Sponsor's Data Body Weights and Body Weight Gains

Group / Sex		Body weight (gm) on Day				Gain (gm)
		1	14	28	60	
1M	Mean	208.5	302.9	382.4	91	Change 1 - 91
	SD	21.0	29.2	35.8		
	N	40	40	40		
2M	Mean	202.0	300.2	378.2	567.2	358.4
	SD	22.0	30.8	38.7	73.1	64.8
	N	40	40	40	10	10
3M	Mean	203.4	303.4	387.9	555.5	355.9
	SD	22.6	31.7	42.0	58.6	43.6
	N	40	40	40	10	10
4M	Mean	202.4	300.4	384.3	544.5	347.5
	SD	22.1	30.5	37.3	81.3	65.6
	N	40	40	40	10	10
Group / Sex		1	14	28	565.7	356.8
					58.6	45.1
					10	10
1F	Mean	177.0	219.2	257.1	327.7	Change 1 - 91
	SD	13.8	17.8	20.7	36.5	
	N	40	40	40	10	
2F	Mean	176.4	220.0	257.8	314.9	151.4
	SD	12.4	17.4	20.3	26.8	29.8
	N	40	40	40	10	10
3F	Mean	175.4	218.2	254.9	317.9	143.5
	SD	12.8	19.3	22.9	31.9	21.7
	N	40	40	40	9	10
4F	Mean	174.2	217.3	253.5	316.7	140.7
	SD	15.2	19.2	23.9	35.7	18.6
	N	40	40	40	10	9
						140.1
						24.6
						10

Food consumption:

The sponsor concluded that ZS treatment did not affect food consumption. A few occasional differences in the weekly mean food consumption for animals receiving ZS

achieved statistical significance compared with controls. However, the differences were sporadic and had no dose-relationship.

Ophthalmoscopy: The detected ophthalmic abnormalities were similar in all animals at the pretest and terminal examinations.

Hematology and coagulation:

The report concluded that hematology and coagulation parameters were unaffected by ZS administration at 4 and 13 weeks.

At 4 weeks, decreases in mean cell hemoglobin concentration in the high dose males and females were statistically significant compared to the control groups (Table 16). The only other red blood cell parameters with changes were increased hematocrits in high dose males (not statistically significant) and females and increased reticulocyte counts in mid and high dose males and females (not statistically significant). Mean platelet volumes increased in the low and high dose males; whereas mean platelet volumes decreased in the mid and high dose females and females. In addition, the mean activated partial thromboplastin time was increased in the high dose males, but decreased in the high dose females compared with controls. These differences were considered unrelated to ZS-treatment, since the report stated that all values were within the historical control range and clear dose-relationships were lacking.

At 13 weeks, no hematology or coagulation parameter showed statistically significant differences for the ZS-treated groups from the control groups

Table 16: Compilation from Sponsor's Tables - Selected Hematology Parameters

		MALES						FEMALES				
Group			MCHC g/dL	MPV fL	RETIC 10 ⁹ /L	APTT sec	HCT L/L	MCHC g/dL	MPV fL	RETIC 10 ⁹ /L	APTT sec	HCT L/L
Week 4	1	Mean	34.5	6.3	298.8	16.9	0.412	35.3	6.5	204.8	16.5	0.399
		SD	0.6	0.2	58.6	1.1	0.011	0.4	0.3	35.9	1.0	0.014
		N	9	9	9	8	9	10	10	10	9	10
	2	Mean	34.4	6.8b	318.2	17.6	0.413	34.9	6.5	223.3	17.1	0.402
		SD	0.5	0.3	25.4	0.6	0.010	0.8	0.3	31.6	1.8	0.017
		N	10	10	10	9	10	10	10	10	8	10
	3	Mean	34.1	6.5	329.2	16.5	0.418	34.8	6.1a	226.7	16.5	0.411
		SD	0.5	0.2	54.0	0.8	0.015	0.3	0.7	30.9	1.0	0.013
		N	10	10	10	10	10	10	10	10	8	10
	4	Mean	33.9a	6.7b	320.9	18.3b	0.424	34.6b	6.2	233.1	16.2	0.417d
		SD	0.5	0.2	34.4	0.5	0.015	0.2	0.3	56.2	1.0	0.014
		N	10	10	10	8	10	10	10	10	10	10
Group			MCHC g/dL	MPV fL	RETIC 10 ⁹ /L	APTT sec	HCT L/L	MCHC g/dL	MPV fL	RETIC 10 ⁹ /L	APTT sec	HCT L/L
Week 13	1	Mean	34.7	6.3	213.5	18.2	0.433	35.1	6.3	222.9	17.6	0.415
		SD	0.3	0.5	29.1	1.1	0.014	0.4	0.4	39.3	0.8	0.017
		N	10	10	10	10	10	10	10	10	10	10
	2	Mean	34.7	6.6	239.2	19.0	0.433	34.9	6.3	227.7	17.4	0.418
		SD	0.4	0.6	31.4	2.1	0.016	0.4	0.2	37.4	0.9	0.007
		N	10	10	10	8	10	10	10	10	10	10
	3	Mean	34.6	6.4	243.4	19.2	0.438	35.0	6.5	219.3	18.0	0.422
		SD	0.4	0.1	42.1	1.4	0.010	0.3	0.3	19.3	1.0	0.009
		N	9	9	9	10	9	8	8	8	7	8
	4	Mean	34.4	6.5	244.0	19.0	0.429	34.9	6.6	226.8	17.9	0.413
		SD	0.3	0.2	26.5	1.2	0.013	0.4	0.3	38.8	0.9	0.008
		N	9	9	9	9	9	10	10	10	8	10

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)	Group 1 - Control Group 3 - ZS 3000 mg/kg/day	Group 2 - ZS 1000 mg/kg/day Group 4 - ZS 6000 mg/kg/day
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Clinical chemistry

The report concluded that clinical chemistry parameters were unaffected by ZS administration at 4 and 13 weeks.

At 4 weeks, two statistically significant differences were noted in mean clinical chemistry parameters between ZS-treated groups and controls (Table 17). The higher mean creatine kinase activity in the high dose males was statistically significant whereas the higher mean in the high dose females was not. The decreased phosphate concentration in mid dose males was also statistically significant. However, a dose relationship was lacking for both sexes for both parameters. Although not statistically significant, mean urea and magnesium values were decreased in the high dose males and females. Contrary to expectations, mean serum potassium values increased in both the high dose males and females. The report stated that all values were within the historical control range. These differences at week 4 were considered unrelated to ZS-treatment.

However, ZS treatment for 13 weeks resulted in statistically significant decreases in serum potassium in the high dose males and the mid and high dose females. In addition decreases in urea were statistically significant in the mid and high dose females, but were unaffected in the male groups. Likewise, serum phosphate values were not significantly affected by ZS treatment in either males or females. In contrast, serum magnesium values were significantly lower in high dose males, but not females. Other statistically significant differences included lower alkaline phosphatase and creatine kinase values in the high dose males and females, lower lactate dehydrogenase values in the high dose males, and lower albumin:globulin ratios in mid and high dose males. The report stated that clinical chemistry values were within the historical range. The only clinical chemistry differences considered related to ZS-treatment were the decreases in serum potassium in the high dose males and females and the decreases in urea in the mid and high dose females

Table 17: Compilation from Sponsor's Tables - Selected Clinical Chemistry

		Males					Females				
Group		CK U/L	UREA mmol/L	PHOS mmol/L	K mmol/L	Mg	CK U/L	UREA mmol/L	PHOS mmol/L	K mmol/L	Mg
Week 4	1	Mean	194	5.6	2.08	4.4	0.805	171	7.8	1.87	4.0
		SD	71	1.3	0.24	0.2	0.070	53	1.8	0.09	0.2
		N	9	10	10	9		10	10	10	10
	2	Mean	380	5.5	2.11	4.5	0.866	243	7.5	1.82	4.1
		SD	460	0.8	0.21	0.3	0.192	135	1.0	0.16	0.3
		N	10	10	10	10		10	10	10	10
	3	Mean	191	5.3	1.84 _a	4.7	0.777	162	7.5	1.88	4.1
		SD	34	1.0	0.12	0.2	0.061	28	1.2	0.42	0.4
		N	10	10	10	10		10	10	10	10
	4	Mean	329 _a	5.0	1.92	4.7	0.772	310	6.4	1.76	4.3
		SD	122	0.5	0.22	0.2	0.075	304	0.8	0.21	0.2
		N	10	10	10	10		9	9	9	9

Group			CK U/L	UREA mmol/L	PHOS mmol/L	K mmol/L	MG mmol/L	CK U/L	UREA mmol/L	PHOS mmol/L	K mmol/L	MG mmol/L
Week 13	1	Mean	187	5.3	1.90	5.0	0.82	123	6.8	1.55	5.0	0.86
		SD	72	0.7	0.25	0.5	0.05	33	0.8	0.26	0.6	0.04
		N	40	40	40	37	40	40	40	40	40	40
	2	Mean	175	5.3	1.92	5.0	0.83	106	6.4	1.54	5.0	0.86
		SD	62	0.6	0.24	0.4	0.06	21	0.9	0.20	0.5	0.04
		N	39	40	40	38	40	40	40	40	40	40
	3	Mean	165	5.3	1.88	5.1	0.81	132	6.2d	1.57	4.7a	0.86
		SD	49	0.7	0.27	0.4	0.05	53	0.9	0.25	0.4	0.04
		N	39	40	40	38	40	38	38	38	39	38
	4	Mean	131c	5.2	1.84	4.6c	0.78f	109c	6.0e	1.54	4.6b	0.87
		SD	24	0.5	0.29	0.3	0.04	51	1.0	0.28	0.4	0.04
		N	39	39	39	39	39	40	40	40	40	40
Significantly different from control group 1 value : a=p<0.05,b=p<0.01,c=p<0.001 (Dunn)							Group 1 - Control Group 3 - ZS 3000 mg/kg/day			Group 2 - ZS 1000 mg/kg/day Group 4 - ZS 6000 mg/kg/day		

Urinalysis and Urine Chemistry

The report did not note the increases in urine pH. These increases at 13 weeks were statistically significant in both males and females; however, only the females displayed a dose-relationship (Table 18). The increase in urine pH in this study is opposite the decreases in urine pH in the 28-day study in rats, but is similar to the increase in urine pH in the 13 week dog study.

Urine concentrations of creatinine, urea, and calcium did not significantly change, although the urea concentrations were slightly lower at all doses. Mean urinary phosphate concentrations were statistically significantly lower in low dose males, but statistically significantly higher in high dose males compared with controls.

Urine concentrations of sodium and chloride increased with and without normalization with creatinine concentration, whereas urine concentrations of potassium and magnesium decreased. These statistically significant dose-related changes were consistent with the pharmacological action of ZS. The decreased urine potassium concentrations were associated with decreased serum potassium in the higher dose groups. However, no changes in serum concentrations of sodium and chloride were observed.

The report did not consider the changes in electrolyte excretion to be adverse, since no associated adverse clinical signs were observed.

Table 18: Compilation from Sponsor's Tables of Urine Chemistry Data

Males	Group / Sex		NA mmol/L	NA/CREAT	K mmol/L	K/CREAT	CL mmol/L	CL/CREAT	PHOS mmol/L	CA mmol/L	MG mmol/L	UREA mmol/L	CREAT mmol/L	pH
	1M	Mean	65	10.58	103.5	15.96	52	8.18	13.80	0.81	4.36	350.6	6.49	8.05 0.59
		SD	22	3.25	41.9	2.96	23	2.95	7.41	0.38	2.32	121.7	2.42	
		N	40	40	40	40	40	40	40	40	40	40	40	
	2M	Mean	123 ^c	20.45 ^c	64.4 ^a	11.07 ^b	63	10.51 ^a	9.73 ^d	0.91	4.96	335.5	6.16	8.51 [^] 0.49
SD		40	4.48	21.4	3.33	25	3.75	5.94	0.38	3.66	112.4	2.28		
N		40	40	40	40	40	40	40	40	40	40	40		
3M	Mean	178 ^c	30.49 ^c	40.6 ^c	6.80 ^c	68 ^d	11.97 ^c	14.76	0.94	3.59	320.1	6.23	8.33 [*] 0.64	
	SD	41	6.56	20.7	2.21	24	4.69	7.78	0.54	2.31	132.8	2.70		
	N	40	40	40	40	40	40	40	40	40	40	40		
4M	Mean	249 ^c	39.27 ^c	16.2 ^c	2.50 ^c	80 ^f	12.96 ^c	19.78 ^f	0.94	2.87 ^b	328.5	6.51	8.58 [^] 0.52	
	SD	94	6.56	12.4	1.29	36	4.52	7.48	0.45	1.39	134.1	2.64		
	N	39	39	39	39	39	39	39	39	39	39	39		
Females	Group / Sex		NA mmol/L	NA/CREAT	K mmol/L	K/CREAT	CL mmol/L	CL/CREAT	PHOS mmol/L	CA mmol/L	MG mmol/L	UREA mmol/L	CREAT mmol/L	
	1F	Mean	65	13.53	92.0	18.65	48	10.02	19.02	3.48	7.73	402.0	5.14	7.32 0.35
		SD	24	5.07	36.0	4.62	26	5.54	9.32	2.01	3.66	140.3	2.03	
		N	40	40	40	40	40	40	40	40	40	40	40	
	2F	Mean	87	22.28 ^b	59.2 ^b	14.87	48	12.72	13.86	3.44	6.56	337.8	4.20	7.54 [*] 0.50
SD		34	5.99	27.7	4.29	25	5.96	9.88	2.04	2.91	173.7	2.20		
N		40	40	40	40	40	40	40	40	40	40	40		
3F	Mean	137 ^c	35.07 ^c	37.2 ^c	10.04 ^c	54	14.28 ^e	16.91	2.79	5.59 ^a	342.6	4.05	7.79 [^] 0.63	
	SD	57	8.46	18.7	4.96	28	6.20	13.16	1.23	3.04	152.0	1.90		
	N	39	39	39	39	39	39	39	39	39	39	39		
4F	Mean	182 ^c	40.71 ^c	20.2 ^c	3.88 ^c	59	14.12 ^d	19.96	2.76	5.46 ^b	355.7	4.80	8.04 [^] 0.54	
	SD	53	10.74	21.9	2.46	28	7.65	12.57	1.74	3.24	138.5	1.98		
	N	40	40	40	40	40	40	40	40	40	40	40		
Significantly different from control group 1 value: a=p<0.05, b=p<0.01, c=p<0.001 (Dunn) d=p<0.05, e=p<0.01, f=p<0.001 (Dunnnett)							Group 1 - Control Group 3 - ZS 3000 mg/kg/day				Group 2 - ZS 1000 mg/kg/day Group 4 - ZS 6000 mg/kg/day			
Reviewer's analysis – T-test, * p<0.05, **p<0.01, ^ p<0.001														

Gross pathology

The gross findings observed were considered unrelated to administration of ZS.

Organ Weights

The report concluded that organ weights were not affected by treatment with ZS. No statistically significant dose related changes were observed.

Histopathology

The pathologist concluded that no microscopic finding was considered related to ZS treatment. Although chronic progressive nephropathy was present in the high dose males, but not the control group, the incidence was considered low, particularly since the study used ad lib feeding (Table 19). The incidence of chronic progressive nephropathy, a common background lesion in rats, is influenced by caloric intake. The pathologist considered the incidence of chronic progressive nephropathy in the high dose males to be within the background

incidence in rats and unlikely to be related to the administration of ZS. No treatment-related findings were observed in the adrenal gland, ureter or the bladder.

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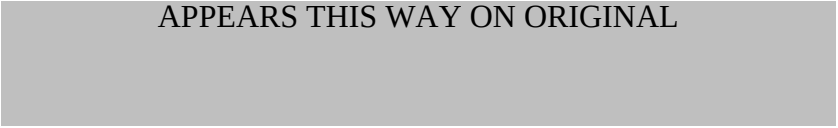


Table 19: Compilation from Sponsor's Tables - Microscopic Findings

Removal Reason: TERMINAL EUTHANASIA	Male				Female			
	0 mg/kg/ day	1000 mg/kg/ day	3000 mg/kg/ day	6000 mg/kg/ day	0 mg/kg/ day	1000 mg/kg/ day	3000 mg/kg/ day	6000 mg/kg/ day
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Number of Animals:	10	10	10	10	10	10	9	10
GLAND, ADRENAL								
Examined	10	0	0	10	10	0	0	10
No Visible Lesions	10	-	-	10	10	-	-	10
KIDNEY								
Examined	10	0	0	10	10	0	0	10
No Visible Lesions	9	-	-	7	10	-	-	8
Fibrosis; cortical	0	-	-	1	0	-	-	0
.... minimal	0	-	-	1	0	-	-	0
Infiltration, mononuclear cell	1	-	-	0	0	-	-	1
.... minimal	1	-	-	0	0	-	-	1
Nephropathy; chronic progressive	0	-	-	2	0	-	-	0
.... minimal	0	-	-	2	0	-	-	0
Cyst; cortical	0	-	-	0	0	-	-	1
.... mild	0	-	-	0	0	-	-	1
URETER								
Examined	10	0	0	10	10	0	0	10
No Visible Lesions	10	-	-	10	10	-	-	10
URINARY BLADDER								
Examined	10	0	0	10	10	0	0	10
No Visible Lesions	10	-	-	10	10	-	-	10

6.2.3. Study title**A 28-Day Repeat Dose Oral Toxicity Study of ZS in Beagle Dogs With A 21-Day Recovery Period (Reviewed by Pat Harlow)**

Conducting laboratory and location: (b) (4)

Study number(s): (b) (4) 1959-007

Date of study initiation: 10/24/2012

Drug lot/batch number: ZS, Lot RD011-53, purity not indicated, potassium loading capacity = (b) (4) mEq/gm

GLP compliance: Yes

QA statement: Yes

Key Study Findings

In a 28-day repeat dose study, male and female Beagle dogs were treated with ZS

alone at 100, 300, or 1000 mg/kg TID or 1000 mg/kg SID and ZS supplemented with potassium chloride at 1000 mg/kg TID. Food consumption was slightly lower in the 1000 mg/kg TID groups with or without KCl supplementation. A statistically significant increase in QTc in females in the 1000 mg/kg TID group was considered possibly treatment related, but was attributed to hypokalemia and not to a direct effect of ZS on ventricular repolarization. Statistically significant increases in actual bicarbonate and total actual carbon dioxide were observed in both sexes in the groups receiving 1000 mg/kg TID and 1000 mg/kg + KCl TID compared to the respective controls. Males and females in the 1000 mg/kg TID group exhibited statistically significant decreases in serum potassium and phosphorus, but increases in bicarbonate compared to the control groups. The effects on serum potassium and phosphorus were ameliorated by potassium supplementation. The mean aldosterone values decreased 77-79% in males and females in the 1000 mg/kg TID group. Treatment with ZS alone reduced fractional excretion of potassium; however, treatment with ZS + KCl increased fractional excretion of potassium and chloride. Although creatinine clearance did not change significantly in either sex for any treatment group, the ratio of urea clearance to creatinine clearance decreased in males and females in the 1000 mg/kg TID group compared to pretest values and control values. Fractional excretion of sodium increased in males and females in the 1000 mg/kg TID group relative to the control groups. In the adrenal glands, the incidence and severity of lipid vacuolation in the zona glomerulosa increased in all ZS-treated groups, except females in the 100 mg/kg TID group. Tubulointerstitial inflammation was present in the kidneys of all males and females in the 1000 mg/kg TID group, but not in the 1000 mg/kg + KCl TID or the 1000 mg/kg SID groups. The sponsor concluded that the NOAEL for ZS alone was 300 mg/kg TID or 1000 mg/kg SID, while the NOAEL for ZS supplemented with potassium was 1000 mg/kg TID.

Purpose

The objective of this study was to evaluate the potential subchronic toxicity of ZS to dogs and to evaluate the reversibility, progression, or delayed appearance of any changes in a 21-day recovery period.

Methods

Doses: 0, 100, 300 or 1000 mg/kg/dose (0, 300, 900, 3000 mg/kg/day) as indicated in Table below.

Frequency of dosing: One or three times a day for 28 consecutive days as indicated in Table below

Administration route: Oral, mixed with food

Dose volume: Not applicable

Formulation/Vehicle: Mixed with 100 gm wet food for each dose

(Note: the wet food contains potassium chloride and potassium sorbate as well as dicalcium phosphate and phosphoric acid as ingredients)

Species/Strain: Dog (*Canis familiaris*)/beagle

Number/Sex/Group: 3 animals/sex/main group

Age: 6-7 months old

Weight: Males: 6.1-7.95 kg; Female: 6.55-8.25 kg on Day -3

Satellite groups: 3 animals/sex in groups 1 and 5 for recovery

Unique study design: Group 4 was administered both 1000 mg/kg ZS as well as supplemental potassium chloride (239 mg/kg) TID

Group 6 was administered 1000 mg/kg ZS once daily.

Deviation from study protocol:

A total of 19 deviations were listed. The study director stated that the deviations did not affect the quality or integrity of the study. The reviewer believes the following deviation impeded full interpretation of the study. At the pretest urine collection, all animals had 8 hour urine collections on Day -4; however, blood collections for clinical chemistry parameters were performed on Day -5. As a result, values for some urine chemistry parameters (creatinine clearance or urine fractional excretions) could not be calculated for the pretest timepoint.

Table 20: Sponsor's Table of Group Assignments- Study 1959-007

Group Assignments					
Group Number	Dose Level (mg/kg)	Regimen	Daily Dose (mg/kg/day)	Number of Animals	
				Male	Female
1	0 ^a	TID	0	6 ^b	6 ^b
2	100	TID	300	3	3
3	300	TID	900	3	3
4	1000 + KCl ^c	TID	3000	3	3
5	1000	TID	3000	6 ^b	6 ^b
6	1000	SID	1000	3	3

^a Administered vehicle only.

^b Three animals per sex were evaluated during the 21-day recovery period.

^c Vehicle was supplemented with 239 mg/kg potassium chloride.

SID – Once daily (in the morning on each day of dosing)

Parameter	Observation details and times
Mortality	Twice daily
Cage side observations	Twice daily
Detailed clinical observations	Detailed clinical examinations were performed once weekly (Days 1, 8, 15, and 22) during the dosing phase and once during the recovery period (Day 43). All dogs received a complete pretest physical examination
Body weight	Body weights for all animals were measured on Days -12, -3, -1, 7, 14, 21, 28, and at necropsy. Body weights for recovery animals were also measured on Days 35, 42, and 49.
Food consumption	Wet food consumption was measured and recorded three times daily at each dosing period. Dry food consumption was measured daily. Wet food was not offered to recovery animals
Ophthalmoscopy	Ophthalmoscopic examinations were conducted on all animals pretest and prior to the scheduled terminal necropsy
ECG	Electrocardiographic examinations were made pretest (Day -6) and on one occasion during Weeks 2 and 4. Using an appropriate lead the RR, PR, and QT intervals, and QRS duration were measured and heart rate was determined. Corrected QT (QTc) interval was calculated using Fridericia's method.
Blood Gas Analysis	Blood was collected for blood gas evaluations (pH, HCO_3^- , and total CO_2) via jugular vein from all animals pretest (Day -5) and during Weeks 2 and 4.
Hematology	Blood was collected for hematology evaluations via the jugular vein from all animals pretest (Day -5) and during Weeks 2 and 4. The animals were fasted overnight, but had access to drinking water. Hematology parameters included total leukocyte count, absolute reticulocyte count, differential leukocyte count, platelet count, erythrocyte count, mean platelet volume, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular concentration (calculated), and blood cell morphology
Clinical chemistry	Blood was collected for clinical chemistry evaluations via the jugular vein from all animals pretest (Day -5), during Weeks 2 and 4, and prior to the scheduled recovery necropsy (Week 7). The animals were fasted overnight, but had access to drinking water. Measured parameters included alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, gamma glutamyl transferase, lactate dehydrogenase, total bilirubin, urea nitrogen, creatinine, total protein, albumin (A), globulin (G), A/G ratio, glucose, total cholesterol, triglycerides, calcium (Ca), chloride (Cl), phosphorus (P), potassium (K), sodium (Na), bicarbonate (HCO_3^-), magnesium (Mg), and anion gap = $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$
Aldosterone	Blood was collected for aldosterone measurements via the jugular vein from all animals pretest (Day -5), during Week 4, and prior to the scheduled recovery necropsy (Week 7). The animals were

	fasted overnight, but had access to drinking water.
Urinalysis and urine chemistry	Urine was collected for 8 hours from all animals pretest, in Weeks 2 and 4, and from recovery animals during Week 7. The animals had no access to food or water during the collection period, except when dosing was required. Additionally, at the terminal and recovery necropsies, urine was collected from the bladder of each animal via cystocentesis for future Zr analysis.
Gross pathology	At the end of treatment and recovery, the animals were euthanized using a phenobarbital solution, exsanguinated, and subjected to necropsy. The tissues and organs collected from all animals are summarized in Table 2. The tissues were fixed in neutral buffered formalin, except for the eye (including the optic nerve) and testes, which were fixed using a modified Davidson's fixative. Formalin was infused into the lung.
Organ weights	The following organs from all animals were weighed: brain, adrenal gland, epididymides, heart, kidney, liver, ovary, pituitary, prostate, spleen, testes, thymus, thyroid/parathyroid
Histopathology	Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on protocol-designated tissues indicated in Table 2 from animals in the control (group 1) and 1000 mg/kg groups (4, 5, 6). As target organs, the adrenal glands and kidneys were also examined in the 100 or 300 mg/kg groups (2, 3).
Toxicokinetics	Blood was collected via the jugular vein from all animals pretest (Day -5) and from animals at 0 and 1000 mg/kg TID in Week 4 (Day 26) and in Week 7. The animals were fasted overnight, but had access to drinking water. The samples were stored frozen for possible future analysis of zirconium content.

Table 21: Sponsor's List of Tissues Collected

- Adrenal (2)* - Aorta - Bone with marrow [femur] - Bone with marrow [rib] - Bone with marrow [sternum] - Bone marrow smear [2 collected]^a - Brain [cerebrum, midbrain, cerebellum, medulla/pons]* - Epididymis (2)* - Eye including optic nerve (2) - Gallbladder - GALT [Gut Associated Lymphoid Tissue] - Gastrointestinal tract: - esophagus - stomach [cardia, fundus, and pylorus] - duodenum^b - jejunum^b - ileum^b - cecum^b - colon^b - rectum^b - Gonads: - ovary (2)* with oviduct (2) - testis (2)* - Gross lesions - Heart* - Joint, tibiofemoral - Kidney (2)* - Larynx	- Liver [3 sections collected; 2 examined]* - Lung with bronchi [collected whole; 2 sections examined] - Lymph nodes: mandibular [2 collected; 1 examined] and mesenteric - Mammary gland [process females only] - Pancreas - Pituitary* - Prostate* - Salivary gland, mandibular [2 collected; 1 examined] - Salivary gland, parotid [2 collected; 1 examined] - Salivary gland, sublingual [2 collected; 1 examined] - Sciatic nerve - Skeletal muscle, biceps femoris - Skin - Spinal cord [cervical, thoracic, and lumbar] - Spleen* - Thymus* - Thyroid/parathyroid (2)* - Tongue - Trachea - Ureter (2) - Urinary bladder - Uterus [both horns]/Cervix - Vagina
(2) Paired organ *Weighed organ	^a Bone marrow smears were collected at necropsy and held. ^b Proximal and distal portions of each segment of the small intestine (duodenum, ileum and jejunum) and proximal and distal portions of each segment of the large intestine (cecum, colon and rectum) were collected from each animal at necropsy and microscopically examined.

Results

Mortality: No unscheduled mortality occurred during the study.

Cage side and detailed clinical observations: The sponsor concluded that no ZS-related clinical findings were observed. The reviewer noted that a higher percentage of animals in the TID 1000 mg/kg group had soft and/or watery feces than in the other groups (Table 22).

Table 22: Reviewer's Summary - Selected Clinical Signs

Group [#/group]	Test article/ Doses per day/ mg/kg/dose	Males			Females			# (%) animals with signs
		Soft feces	Watery feces	Soft or watery	Soft feces	Watery feces	Soft or watery	
1 (12)	Vehicle/TID/0	25/5	3/2	28/5	3/2	0/0	3/2	7 (0.58)
2 (6)	ZS/TID/100	8/3	7/3	15/3	2/1	0/0	2/1	4 (0.67)
3 (6)	ZS/TID/300	19/3	1/1	20/3	2/1	0/0	2/1	4 (0.67)
4 (6)	ZS + KCl/TID/1000	10/2	3/2	23/2	3/2	0/0	3/2	4 (0.67)
5 (12)	ZS/TID/1000	45/5	11/3	56/5	12/6	2/1	14/6	12 (100)
6 (6)	ZS/SID/1000	15/3	10/2	25/3	15/2	27/2	42/2	5 (0.83)

TID = three doses per day; SID = single dose per day

Body weight

The sponsor concluded that ZS had no effect on body weight in either sex (Figure 1). Although the mean body weight gain was lower for males in the 1000 mg/kg TID group than in the control group, the mean body weight gain was higher for females in the

1000 mg/kg TID group than in the control group (Table 23). If the control female with the loss of 0.75 kg is excluded, the mean body weight gain for females in the 1000 mg/kg TID group would be similar to the mean for the control group (0.25 kg).

Figure 1: Sponsor's Body Weight Graphs

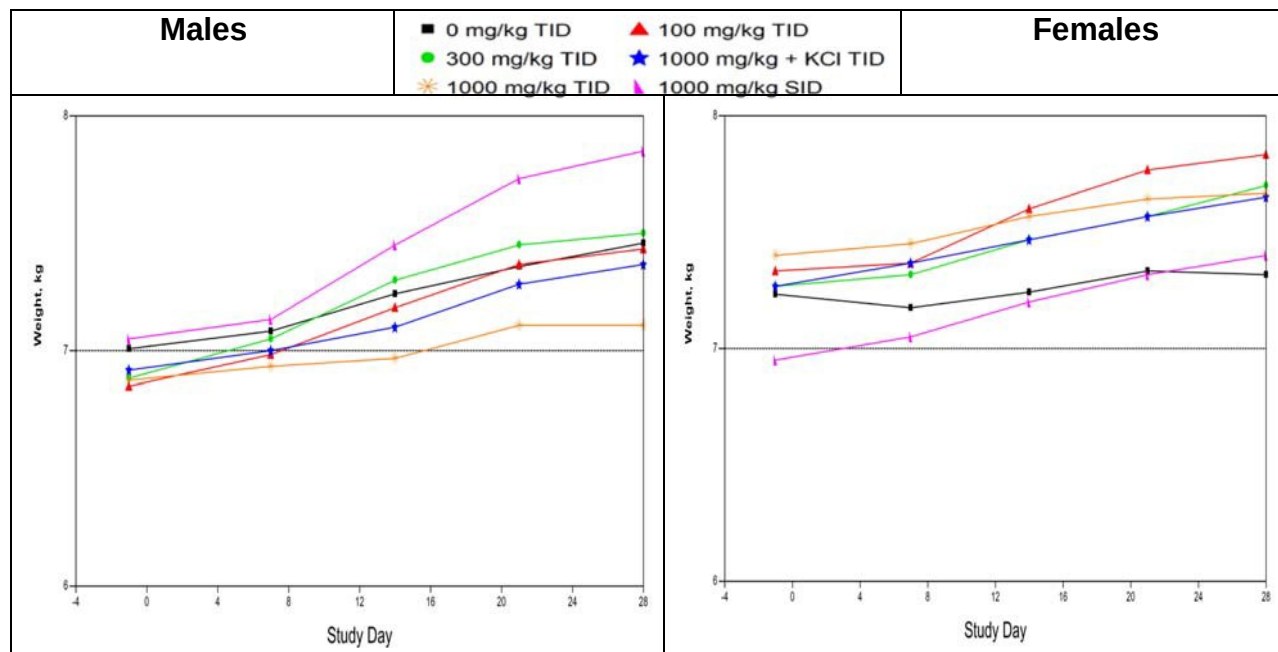


Table 23: Reviewer's Summary of Body Weights and Body Weight Gain

Sex	Group [#/group]	Test article/ Doses per day/ mg/kg/dose	Mean body weight, kg (SD)		Mean BW gain, kg	
			Day -1	Day 28	Mean	Min, max
Male	1 [6]	Vehicle/TID/0	7.01 (0.58)	7.46 (0.36)	0.45	-0.15, 0.95
	2 [3]	ZS/TID/100	6.85 (0.46)	7.43 (0.80)	0.58	0.3, 1.0
	3 [3]	ZS/TID/300	6.88 (0.78)	7.50 (1.14)	0.62	0.1, 1.05
	4 [3]	ZS + KCl/TID/1000	6.92 (0.59)	7.37 (0.32)	0.45	0.2, 0.75
	5 [6]	ZS/TID/1000	6.88 (0.45)	7.10 (0.53)	0.22	0, 0.5
	6 [3]	ZS/SID/1000	7.05 (0.51)	7.85 (0.25)	0.8	0.6, 1.1
Female	1 [6]	Vehicle/TID/0	7.23 (0.56)	7.32 (0.93)	0.09	-0.75, 0.65
	2 [3]	ZS/TID/100	7.33 (0.59)	7.83 (0.70)	0.5	0.45, 0.55
	3 [3]	ZS/TID/300	7.26 (0.72)	7.70 (1.13)	0.44	-0.05, 0.75
	4 [3]	ZS + KCl/TID/1000	7.26 (0.40)	7.65 (0.33)	0.39	0.25, 0.5
	5 [6]	ZS/TID/1000	7.40 (0.56)	7.66 (0.63)	0.26	-0.05, 1.15
	6 [3]	ZS/SID/1000	6.95 (0.49)	7.44 (0.77)	0.49	0.15, 0.7

TID = three doses per day; SID = single dose per day

Food consumption:

The sponsor concluded that ZS treatment did not affect food consumption. All animals, except for two animals in the 1000 mg/kg TID group, consumed all of the wet food containing the ZS treatment. The two animals in the 1000 mg/kg TID group were male 739, who consumed only 200 g of ZS-treated wet food on four days (15, 16, 20, and 21), and female 734, who consumed only 265 g of ZS-treated wet food on Day 12.

The sponsor maintained that dry food consumption was steady throughout the dosing period. The overall mean dry food consumption was slightly lower in the 1000 mg/kg TID male and female groups than in the control groups (Table 24). The number of days with mean dry food consumption less than 100 gm was greater in the 1000 mg/kg TID groups than in the control groups.

Table 24: Reviewer's Summary of Dry Food Consumption

Sex	Group [#/group]	Test article/ Doses per day/ mg/kg/dose	Mean dry food consumption/day, g				Minimum individual daily dry food consumption
			Mean	Minimum	Maximum	Days < 100 g	
Male	1 [6]	Vehicle/TID/0	116.0	102.8	122.5	0	60
	2 [3]	ZS/TID/100	115.1	94.0	122.3	2	40
	3 [3]	ZS/TID/300	115.4	102.0	123.7	0	58
	4 [3]	ZS + KCl/TID/1000	108.5	80.5	121.3	6	46
	5 [6]	ZS/TID/1000	109.8	77.5	119.0	5	0
	6 [3]	ZS/SID/1000	118.5	75.3	127.7	2	47
Female	1 [6]	Vehicle/TID/0	110.2	91.5	117.2	2	28
	2 [3]	ZS/TID/100	112.7	78.0	128.3	3	40
	3 [3]	ZS/TID/300	118.6	103.3	125.7	0	57
	4 [3]	ZS + KCl/TID/1000	99.7	52.3	116.3	11	39
	5 [6]	ZS/TID/1000	100.9	70.7	112.0	13	1
	6 [3]	ZS/SID/1000	104.2	78.3	120.3	9	37

TID = three doses per day; SID = single dose per day

Ophthalmoscopy: No ophthalmic abnormalities were detected in any animal at the pretest or terminal examinations.

ECG parameters:

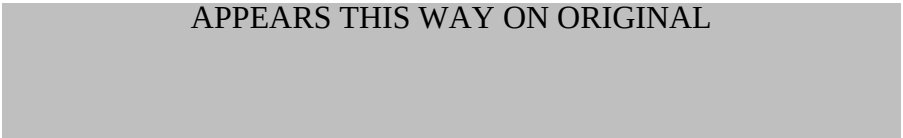
The parameters with statistically significant findings are summarized in Table 25.

The mean RR interval was longer in females in the 1000 mg/kg SID group at pretest and in males in the 100 mg/kg TID group at Week 2 than the means in the respective control groups. Neither change was considered test article-related.

Females in the 1000 mg/kg TID group at Weeks 2 and 4 had statistically significant increases (7.9%, 7.6%) in mean PR interval compared to females in the control group. However, at pretest the 1000 mg/kg TID group also had a 9.4% increase in mean PR

interval compared to the control group. Therefore, the increases in PR interval were not considered test article related.

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In the 1000 mg/kg TID group, the males at Weeks 2 and 4 and females at Week 2 had statistically significant increases in mean QT interval compared to the means for the control groups. When corrected for heart rate using Fridericia's method, the mean QTc interval was statistically significantly longer in females in the 1000 mg/kg TID group at Week 2 and in males in the 1000 mg/kg SID group at pretest compared to the means for the respective control groups. The change in QT interval in the males in the 1000 mg/kg TID group did not translate to a statistically significant change in QTc, although the changes in QTc from the pre-dose mean in the males at both Week 2 and 4 were similar to the changes in QTc from the pre-dose mean in the females in the 1000 mg/kg TID group. Since the statistically significant change in males in the 1000 mg/kg SID group was only observed at pretest, it was not considered test article related. The statistically significant increase in QTc in females in the 1000 mg/kg TID group was considered possibly related to treatment.

The report cited the results of a furosemide-induced model of hypokalemia in which an overall 1 mmol/L decrease in serum potassium was associated with an average QTc increase of 22 msec (Hanton et al. 2007). In the current study, serum potassium was reduced 12.0-12.2% in males and 21-25% in females from pre-dose following the 1000 mg/kg TID dose at Week 2 and 4 (Table 26). These decreases in serum potassium were statistically different from the respective control values in females at Weeks 2 and 4 and in males at Week 2. In the 1000 mg/kg TID group, individual decreases in serum potassium from pretest values ranged from 0.2 to 1.8 mEq/L in females and 0.2 to 1.1 mEq/L in males. In the 1000 mg/kg TID males, potassium decreases of 1.1, 1.0, 0.9 and 0.9 mEq/L from pre-test were associated with increases of 10, 8, 29, and 12 msec, respectively, in QTc from pre-test; however, an increase in QTc of 14 msec in one male was associated with a potassium decrease of 0.3 mEq/L. In the 1000 mg/kg TID females, potassium decreases of 1.8, 1.8, 1.7 and 1.6 mEq/L from pre-test were associated with increases of 19, 13, 20 and 11 msec, respectively, from pre-test; however, an increase in QTc of 25 msec in one female was associated with a potassium decrease of 1.4 mEq/L. In contrast, in control animals, potassium decreases of 0.1 and 0.2 mEq/L from pre-test were associated with increases of 5 and 4 msec in QTc from pre-test in males and females, respectively. The report attributed the increase in QTc in the 1000 mg/kg TID group to hypokalemia and not to a direct effect of ZS on ventricular repolarization.

The report concludes there was no change in serum potassium or QTc in the group receiving 1000 mg/kg TID plus potassium. Overall, the small mean decreases in potassium in males and females in the 1000 mg/kg TID plus potassium group were associated with mean decreases in QTc. However, one male and one female in the 1000 mg/kg TID plus potassium group each had potassium decreases of 0.4 mEq/L from pre-test that were associated with increases of 6 and 2 msec, respectively, in QTc.

The report does not discuss the changes in the 1000 mg/kg SID group in which a potassium decrease of 0.4 mEq/L from pre-test in one male was associated with increase of 19 msec in QTc from pre-test and no change in potassium from pre-test in another male was associated with increase of 20 msec in QTc pre-test.

A clear correlation of the magnitude in potassium decrease with the magnitude in QTc increase was lacking in the current study. In contrast, a correlation of the magnitude in

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potassium decrease with the magnitude in QTc increase was observed by Hanton et al. (2007), who collected blood for potassium and plasma chemistry measurements immediately after each ECG recording. The current study and its protocol indicate that ECG examinations and blood collection for clinical chemistry parameters were conducted pre-test and during weeks 2 and 4. The actual sampling days were not specified. Given the statements that care was taken to avoid causing undue excitement of the animals before the recording of electrocardiograms (ECGs), it is likely that the ECGs and blood collections were performed on different days leading to the lack of a clear correlation.

Table 25: Reviewer's Summary of Selected ECG Parameters

Sex	Article/Doses per day/ mg/kg/dose		RR interval, msec			PR interval, msec			QT interval, msec			QTcf, msec		
			Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4
Male	Vehicle/TID/0	Mean	596.0	574.1	565.3	90.9	87.0	85.6	198.2	190.8	190.4	237.7	231.7	232.0
		SD	76.0	103.0	85.9	18.1	14.7	7.3	7.0	10.6	12.9	4.8	9.4	8.2
	ZS/TID/100	Mean	766.7	746.9¹	665.1	91.0	88.9	90.0	210.0	197.2	192.2	232.3	218.3	221.8
		SD	91.3	30.4	92.0	7.9	8.1	5.7	3.0	4.7	3.1	7.4	6.1	7.7
	ZS/TID/300	Mean	559.9	616.9	577.6	92.1	90.8	93.4	186.5	185.6	187.0	228.4	219.5	225.9
		SD	135.3	40.5	32.0	9.6	9.3	5.8	11.1	7.6	4.6	8.2	8.2	1.5
Female	ZS + KCI/TID/1000	Mean	673.6	660.3	572.5	88.9	85.1	83.2	199.4	189.2	184.2	233.1	220.2	224.7
		SD	177.5	83.0	89.7	3.3	6.1	2.1	13.2	4.4	4.5	13.9	8.8	6.1
	ZS/TID/1000	Mean	696.1	680.8	713.0	89.6	89.9	90.6	200.8	208.2¹	209.1¹	228.9	239.4	236.6
		SD	146.7	95.3	97.3	10.0	10.0	10.0	10.2	9.5	11.0	4.2	9.2	5.7
	ZS/SID/1000	Mean	750.8	549.8	547.8	79.0	83.7	78.9	194.2	186.1	183.1	218.5¹	229.2	226.5
		SD	276.4	87.7	141.7	7.4	8.0	5.5	13.7	11.1	18.0	11.3	13.0	4.6
Female	Vehicle/TID/0	Mean	606.1	604.4	621.3	87.3	86.3	87.0	190.8	189.4	192.2	228.9	226.5	230.4
		SD	113.6	117.4	161.9	2.6	4.5	5.7	6.0	6.5	13.8	7.6	12.0	6.2
	ZS/TID/100	Mean	666.7	719.8	563.0	92.2	92.6	92.3	195.1	197.4	188.8	227.0	223.2	229.4
		SD	123.6	145.8	20.4	18.1	11.6	14.0	1.5	11.0	0.8	14.6	6.0	3.7
	ZS/TID/300	Mean	693.5	699.5	609.4	92.4	90.9	95.1	199.3	198.2	189.0	228.3	225.6	224.9
		SD	132.6	145.5	59.5	2.2	6.9	2.4	11.3	12.9	8.4	1.8	2.8	6.4
Female	ZS + KCI/TID/1000	Mean	664.0	762.5	682.7	97.6	98.6	100.0	208.2	204.7	199.5	241.5	228.0	231.0
		SD	36.9	163.4	137.1	3.9	4.7	6.4	17.5	13.9	13.3	16.8	6.0	2.6
	ZS/TID/1000	Mean	580.2	627.7	579.4	95.5	103.0²	102.7²	195.0	208.4¹	203.3	237.1	245.5¹	245.7
		SD	146.1	110.7	74.4	8.8	10.4	6.7	14.8	15.2	17.7	6.6	11.6	16.1
	ZS/SID/1000	Mean	874.8¹	693.2	794.8	85.6	93.6	93.3	208.4	204.4	201.8	220.0	233.8	220.3
		SD	94.1	107.9	130.4	7.2	1.0	0.5	8.7	5.9	2.5	17.0	13.0	12.4

TID = three doses per day; SID = single dose per day, f = Correction by Fridericia's method

Table 26: Reviewer's Summary - Changes in QTc and Serum Potassium

Sex	Article/Doses per day/ mg/kg/dose		QTcf, msec			Potassium (K), mEq/L			Mean of individual changes from pre-test			
			Pre-test			Pre-test			QTcf, msec		K, mEq/L	
			Pre-test	Wk 2	Wk 4	Pre-test	Wk 2	Wk 4	Wk 2	Wk 4	Wk 2	Wk 4
Male	Vehicle/TID/0	Mean SD	237.7 4.8	231.7 9.4	232.0 8.2	4.98 0.34	4.98 0.39	4.78 0.40	-0.006	-0.006	0.00	-0.20
	ZS/TID/100	Mean SD	232.3 7.4	218.3 6.1	221.8 7.7	5.03 0.15	5.13 0.12	4.87 0.12	-0.014	-0.011	0.10	-0.17
	ZS/TID/300	Mean SD	228.4 8.2	219.5 8.2	225.9 1.5	4.97 0.30	5.03 0.25	4.97 0.32	-0.009	-0.002	0.07	0.00
	ZS + KCl/TID/1000	Mean SD	233.1 13.9	220.2 8.8	224.7 6.1	4.90 0.20	5.17 0.25	4.90 0.20	-0.013	-0.009	0.27	-0.03
	ZS/TID/1000	Mean SD % dif. vs. pre	228.9 4.2	239.4 9.2 4.6	236.6 5.7 3.4	4.98 0.16	4.38 ¹ 0.36 -12.0	4.37 0.31 -12.2	0.010	0.007	-0.60	-0.62
	ZS/SID/1000	Mean SD % dif. vs. pre	218.5 ¹ 11.3	229.2 13.0 4.8	226.5 4.6 3.7	5.13 0.32	5.23 0.15 1.9	5.03 0.06 -1.9	0.009	0.008	0.10	-0.10
Female	Vehicle/TID/0	Mean SD	228.9 7.6	226.5 12.0	230.4 6.2	4.82 0.15	4.98 0.21	4.78 0.31	-0.002	-0.001	0.17	-0.03
	ZS/TID/100	Mean SD	227.0 14.6	223.2 6.0	229.4 3.7	4.63 0.06	4.97 0.12	4.67 0.06	-0.004	0.002	0.33	0.03
	ZS/TID/300	Mean SD	228.3 1.8	225.6 2.8	224.9 6.4	4.97 0.21	5.07 0.32	4.93 0.32	-0.003	-0.003	0.10	-0.03
	ZS + KCl/TID/1000	Mean SD	241.5 16.8	228.0 6.0	231.0 2.6	4.73 0.12	5.03 0.15	4.63 0.06	-0.013	-0.010	0.30	-0.10
	ZS/TID/1000	Mean SD % dif. vs. pre	237.1 6.6	245.5 ¹ 11.6 3.5	245.7 16.1 3.6	4.90 0.42	3.87 ² 0.54 -21.0	3.68 ² 0.55 -25.0	0.009	0.008	-1.03	-1.22
	ZS/SID/1000	Mean SD % dif. vs. pre	220.0 17.0	233.8 13.0 6.2	220.3 12.4 0.1	4.70 0.30	4.80 0.17 2.1	4.47 0.12 -4.9	0.014	0.000	0.10	-0.23

TID = three doses per day; SID = single dose per day, f = Correction by Fridericia's method ¹ p < 0.05 versus control ² p < 0.01 versus control

Blood Gas Analysis

Statistically significant increases in actual bicarbonate and total actual carbon dioxide were observed at week 2 and/or week 4 in both sexes in the groups receiving 1000 mg/kg TID and 1000 mg/kg + KCl TID compared to the respective controls (Table 27). These effects were partly due to decreases in the values for the control groups compared to pre-dose values. No historical values were provided. Although most mean and individual values were within the pre-test ranges, the values for one male (738) and two females (732, 733) were above the pre-test at week 2 and/or week 4. The increased values for actual bicarbonate and total actual carbon dioxide correlated with decreased values for serum potassium in these animals.

Table 27: Reviewer's Summary of Selected Blood Gas Parameters

Sex	Group: Article/Doses per day/ mg/kg/dose		Actual HCO ₃ , mmol/L			Total actual CO ₂ , mmol/L		
			Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4
Male	1: Vehicle/TID/0	Mean	23.27	21.53	22.90	24.55	22.80	24.13
		SD	1.47	0.80	1.20	1.56	0.75	1.23
		Range	21.0-25.1	20.6-22.8	21.6-24.9	22.1-26.5	21.8-23.9	22.8-26.2
	2: ZS/TID/100	Mean	22.30	22.60	22.23	23.63	23.83	23.40
		SD	2.66	1.02	1.32	2.8	1.03	1.38
	3: ZS/TID/300	Mean	22.70	22.33	21.97	23.97	23.60	23.20
		SD	1.55	0.91	1.24	1.60	0.96	1.30
	4: ZS + KCl /TID/1000	Mean	24.23	24.33¹	23.27	25.60	25.67¹	24.47
		SD	0.76	1.32	1.03	0.72	1.43	1.10
	5: ZS/TID/1000	Mean	23.38	24.87²	24.75	24.70	26.13²	26.03
	SD	1.52	1.66	1.58	1.55	1.73	1.66	
	M738	24.9	27.5	26.9	26.4	28.9	28.2	
6: ZS/SID/1000	Mean	24.17	23.40	23.23	25.50	24.70	24.53	
	SD	2.74	2.11	1.97	2.95	2.07	1.97	
Pre-test range in groups 2-6			19.8-26.6			20.9-28.1		
Female	1: Vehicle/TID/0	Mean	23.17	21.72	21.23	24.42	22.95	22.42
		SD	0.34	1.71	0.86	0.37	1.78	0.98
		Range	22.7-23.5	19.4-24.0	20.3-22.4	24.0-24.9	20.5-25.3	21.4-23.7
	2: ZS/TID/100	Mean	25.03	24.57	23.5	26.4	25.83	24.73
		SD	1.11	2.27	0.44	1.15	2.42	0.40
	3: ZS/TID/300	Mean	24.5	22.6	22.37	25.87	23.83	23.6
		SD	1.39	2.21	1.32	1.531	2.29	1.375
	4: ZS + KCl /TID/1000	Mean	23.4	23.57	24.00¹	24.67	24.83	25.23¹
		SD	1.82	0.74	0.87	1.88	0.78	1.00
	5: ZS/TID/1000	Mean	23.67	26.07¹	26.02²	24.95	27.35¹	27.33²
	SD	1.54	2.92	1.58	1.66	2.99	1.73	
	F732	23.7	31.8	26.5	24.9	33.2	28.1	
	F733	26.3	24.5	26.8	27.8	25.6	28.0	
	F736	23.3	26.0	26.2	24.6	27.4	27.6	
6: ZS/SID/1000	Mean	24.37	23.83	23.07	25.7	25.17	24.33	
	SD	3.26	1.94	1.82	3.38	1.97	1.82	
Pre-test range in groups 2-6			20.6-26.4			21.8-27.8		
TID = three doses per day; SID = single dose per day. ¹ p < 0.05 versus control ² p < 0.01 versus control								

TID = three doses per day; SID = single dose per day, ¹ p < 0.05 versus control ² p < 0.01 versus control**Hematology**

The sponsor concluded that no ZS-related effects were observed among the hematology parameters and any statistical differences were not considered ZS-related because of a lack of a dose response. Although most mean and individual values were within the historical control range, the reviewer noted values for a few animals outside the historical individual range. The erythrocyte values for female 715 in the 100 mg/kg TID group at weeks 2 and 4 were above the historical individual range (Table 28), although her values for hemoglobin and hematocrit were within the historical individual range.

The reviewer also noted statistically significant increases in mean hematocrit compared to the control group for males in the 1000 mg/kg TID and 1000 mg/kg SID groups at both weeks 2 and 4 and for females in the 100 mg/kg TID group at week 4. No individual

hematocrit value in these groups was above the maximum in the historical individual range. Mean hematocrit values were also increased for females in the 300 mg/kg TID, 1000 mg/kg TID and 1000 mg/kg SID groups at week 4, but did not attain statistical significance. However, the individual hematocrit value for female 734 was above the maximum in the historical individual range. No change in hematology parameters appeared to correlate with changes in serum potassium.

Table 28: Reviewer's Summary of Selected Hematology Parameters

Sex	Article/Doses per day/ mg/kg/dose		Erythrocytes, 103/uL			Hemoglobin, gm/dL			Hematocrit, %		
			Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4
Male	Vehicle/TID/0	Mean SD	6.80 0.24	7.08 0.20	6.77 0.32	14.43 0.46	15.43 0.14	14.75 0.53	44.67 1.58	47.37 0.36	47.12 1.38
	ZS/TID/100	Mean SD	6.78 0.06	7.3 0.03	7.32 0.43	14.53 0.15	16.03 0.31	16.17 1.16	44.7 0.44	48.6 1.31	51.53a 3.10
	ZS/TID/300	Mean SD	6.71 0.48	7.38 0.085	7.58¹ 0.22	13.73 0.91	15.47 0.55	16.1 0.53	42.53 2.71	47.47 1.06	50.33 1.80
	ZS + KCl/TID/1000	Mean SD	6.85 0.42	7.24 0.35	6.96 0.50	15.07 1.19	16.33 0.98	15.87 1.10	45.83 2.41	49.47 2.22	49.6 3.28
	ZS/TID/1000	Mean SD	7.05 0.23	7.32 0.17	7.26 0.27	14.97 0.54	16 0.28	15.82 0.58	46.38 1.16	49.37¹ 0.86	50.87¹ 1.74
		M738	7.24	7.24	7.53	15.6	16.1	16.6	47.6	49.4	53.4
	ZS/SID/1000	Mean SD	7.08 0.16	7.35 0.21	7.23 0.56	15.47 0.12	16.33 0.60	16.40¹ 1.15	47.77 1.01	50.37¹ 1.88	51.80¹ 2.85
	Historical range	Mean Ind. ^a	5.53-7.64 5.22-8.18			12.8-17.7 11.9-19.3			36.9-51.3 34.0-55.8		
Female	Vehicle/TID/0	Mean SD	7.26 0.31	7.28 0.46	7.12 0.36	15.6 0.39	15.9 0.68	15.57 0.62	47.48 0.94	48.22 1.64	48.85 1.52
	ZS/TID/100	Mean SD	7.65 0.33	8.09¹ 0.46	8.18² 0.36	15.23 0.45	16.73 0.65	17.00 0.36	46.70 1.04	50.90 1.56	53.40¹ 1.51
	F 715		8.03	8.61	8.52	15.2	17.4	17.74	47.3	51.8	54.8
	ZS/TID/300	Mean SD	7.16 0.47	7.32 0.03	7.42 0.28	15.43 1.07	16.3 0.30	16.73 0.57	47.30 2.26	49.27 0.55	51.37 2.05
	ZS + KCl/TID/1000	Mean SD	7.52 0.90	7.4 0.45	7.19 0.26	16.37 2.08	16.27 0.55	16.07 0.71	49.83 6.07	49.47 1.46	50.43 1.71
	ZS/TID/1000	Mean SD	7.29 0.40	7.37 0.22	7.18 0.39	16.13 1.09	16.58 0.67	16.25 1.10	49.27 2.58	50.47 1.78	51.12 3.45
		F732	7.39	7.40	6.80	15.5	15.8	14.4	48.0	48.4	45.7
		F733	6.97	7.18	7.52	15.2	16.0	17.0	47.1	48.8	53.8
		F734	7.00	7.79	7.80	15.3	17.6	17.6	47.2	53.3	55.4
		F736	8.04	7.26	7.05	18.1	16.5	16.3	54.0	50.3	51.2
	ZS/SID/1000	Mean SD	7.28 0.18	7.38 0.23	7.40 0.16	16.20 0.95	16.77 0.90	17.03 0.25	49.30 2.62	50.67 2.95	53.10 0.95
	Historical range	Mean Ind. ^a	5.55-7.63 5.25-8.19			12.8-17.8 11.7-19.6			36.9-51.5 32.3-54.9		

TID = three doses per day; SID = single dose per day, ^a Ind. = individual animal value, ¹ p < 0.05 versus control ² p < 0.01 versus control

Clinical chemistry

Animals in the 1000 mg/kg TID group exhibited statistically significant changes in serum potassium, bicarbonate and phosphorus compared to the control groups (Table 29).

At weeks 2 and 4, serum potassium decreased an average of 12% and 12%, respectively, in males and 20% and 24%, respectively, in females in the 1000 mg/kg TID group compared to pretest values. For individual animals in the 1000 mg/kg TID

group the ranges of potassium decreases compared to pre-test values were 4% to 23% in males and 4.3% to 37% in females, whereas the maximum decreases in the control groups were 8.3% in males and 8.7% in females. Although the means and individual potassium values were within the historical ranges for the males, the means at weeks 2 and 4 and individual potassium values for females 732, 733 and 736 were below the historical control ranges. The maximum individual potassium decreases in the 1000 mg/kg + KCl TID group were 4.1% in males and 4.2% in females indicating that potassium supplementation ameliorated the effect of ZS. In addition, the maximum individual potassium decreases in the 1000 mg/kg SID group were 7.3% in males and 8% in females indicating that a single dose of ZS was insufficient to counteract the potassium in the diet. After the recovery period, serum potassium values were similar in the control and in the 1000 mg/kg TID groups, although values for both groups were decreased relative to the pretest values (Table 29). This effect was attributed to cessation during recovery of the canned wet diet that contained potassium in the ingredients and was administered during the pretest period and the treatment period, but not the recovery period, as the vehicle for ZS.

Like serum potassium, serum phosphorus in the 1000 mg/kg TID group at weeks 2 and 4 decreased an average of 6.5% and 17.9%, respectively, in males and 17.8% and 22.8%, respectively, in females compared to pretest values. For individual animals in the 1000 mg/kg TID group the ranges of phosphorus decreases compared to pre-test values were 5.4% to 27.6% in males and 10.2% to 44% in females, whereas the maximum decreases in the control groups were 11.9% in males and 12.9% in females. The means and individual phosphorus values were within the historical ranges for both sexes. The maximum individual phosphorus decreases in the 1000 mg/kg + KCl TID group were 6% in males and 19.6% in females suggesting that potassium supplementation ameliorated the effect of ZS in males, but not females. In addition, the maximum individual phosphorus decreases in the 1000 mg/kg SID group were 7.5% in males and 8.6% in females. After the recovery period, serum phosphorus values were similar in the control and in the 1000 mg/kg TID groups although values for both groups were decreased relative to the pretest values. This effect was also attributed to cessation during recovery of the canned wet diet that was administered during the pretest period and the treatment period, but not the recovery period, as the vehicle for ZS.

The pathologist suggested that since the effects on serum potassium and phosphorus were ameliorated by potassium supplementation, the effect on serum phosphorus may be linked to hypokalemia. The literature indicates that the association of potassium deficiency and/or hypokalemia with hypophosphatemia and/or an increase in urinary phosphate excretion has been continually reported since the report by Mahler and Stanbury (1956) of a patient with hypokalemia concomitant with hypophosphatemia. For example, studies have been conducted in humans (Sebastian et al. 1990), rats (Wu et al. 1995), and dogs (Morrison et al. 1960). More recently, mechanistic studies of Zajicek et al. (2001) demonstrated that potassium deficiency in the rat caused inhibition of renal sodium/phosphate ion cotransport activity by post-translational mechanisms that are mediated in part through alterations in glucosylceramide content and membrane lipid dynamics.

In contrast to serum potassium and phosphorus, serum bicarbonate in the 1000 mg/kg TID group at weeks 2 and 4 increased an average of 9.2% and 24.2%, respectively, in males and 18.8% and 30%, respectively, in females compared to pretest values. However, compared to the concurrent control group serum bicarbonate in the 1000 mg/kg TID group at weeks 2 and 4 increased an average of 15.3% and 13.7%, respectively, in males and 15.7% and 10.6%, respectively. For individual animals in the 1000 mg/kg TID group the maximum bicarbonate increases at weeks 2 and 4 compared to pre-test values were 28.6% and 47.6%, respectively, in males and 34.8% and 50%, respectively, in females, whereas the maximum increases in the control groups were 9.5% and 28.6%, respectively, in males and 19% and 28.6%, respectively, in females. The mean and some individual values in the 1000 mg/kg TID group were above the maximum of the historical ranges for both sexes at week 2. However, at week 4, mean values for all groups, including the control groups, were above the maximum of the historical ranges for both sexes. The greatest increases in serum bicarbonate were in those animals in the 1000 mg/kg TID group with the greatest decreases in serum potassium, namely male 738 at weeks 2 and 4, female 732 at week 2, and female 733 at week 4. Two exceptions were male 718 and female 714 in the 100 mg/kg TID group that had higher increases in serum bicarbonate, but no decrease in serum potassium.

Table 29: Reviewer's Summary of Serum Potassium, Bicarbonate, and Phosphorus

			Potassium, mEq/L			Bicarbonate, mEq/L			Phosphorus, mg/dL		
Group			Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4
Male	1	Mean	4.98	4.98	4.78	21.30	21.50	24.80	6.48	6.72	6.23
		SD	0.34	0.39	0.40	1.51	0.84	1.47	0.41	0.49	0.27
	2	Mean	5.03	4.98	4.78	20.30	23.30	27.70	6.63	7.03	6.13
		SD	0.15	0.39	0.40	1.53	2.08	1.15	0.31	0.29	1.10
	3	Mean	4.97	5.03	4.97	20.70	22.70	24.00	6.67	6.90	6.53
		SD	0.31	0.25	0.32	2.08	0.58	1.00	0.81	0.53	0.64
	4	Mean	4.90	5.17	4.90	21.70	23.30	26.30	6.10	6.10	5.90
Female		SD	0.20	0.25	0.20	1.15	0.58	1.15	0.56	0.46	0.53
	5	Mean	4.98	4.38¹	4.37	22.70	24.8²	28.2¹	6.42	6.00	5.27¹
		SD	0.16	0.36	0.31	1.86	2.23	2.32	0.82	0.63	0.64
		M738	4.8	3.7	3.8	21	27	31	5.8	5.1	4.2
	6	Mean	5.13	5.23	5.03	22.30	23.30	25.70	6.83	7.07	6.50
		SD	0.32	0.15	0.06	1.15	0.58	3.51	0.15	0.31	0.27
	Historical ^a		4.1-5.2 (3.6-5.4)			20-24 (19-24)			4.0-7.2 (3.1-7.8)		
Female	1	Mean	4.82	4.98	4.78	21.00	22.30	25.50	5.98	6.27	5.93
		SD	0.15	0.21	0.31	0.63	2.16	2.07	0.50	0.57	0.66
	2	Mean	4.63	4.97	4.67	19.70	23.00	26.70	5.70	6.17	5.70
		SD	0.06	0.12	0.06	4.51	0.00	1.53	0.36	0.31	0.27
	3	Mean	4.97	5.07	4.93	22.00	22.30	26.00	6.37	6.50	6.20
		SD	0.21	0.32	0.32	1.73	1.53	1.73	1.00	0.30	0.56
	4	Mean	4.73	5.03	4.63	23.00	23.00	28.30	6.07	6.23	5.40
		SD	0.12	0.15	0.06	1.00	1.00	1.53	0.47	0.74	0.10
	5	Mean	4.90	3.87²	3.68²	21.70	25.8¹	28.20	6.18	5.08²	4.77
		SD	0.42	0.55	0.55	1.21	3.06	2.93	0.23	0.38	1.09
		F731	5.7	4.2	3.9	23	26	30	6.2	5.3	4.5
		F732	4.9	3.1	3.5	23	31	28	6.4	4.6	6.5
		F733	4.6	3.6	2.9	22	27	33	5.9	5.3	3.3
		F734	4.6	4.3	4.4	20	24	25	6.3	4.9	5.2
		F735	4.7	4.5	4.1	21	22	26	6.4	5.6	5.0
		F736	4.9	3.5	3.3	21	25	27	5.9	4.8	4.1

6	Mean SD	4.70 0.30	4.80 0.17	4.47 0.12	23.30 1.53	24.30 0.58	29.00 2.00	5.97 0.47	6.10 0.27	5.70 0.53
Historical ^a		4.0-5.1 (3.8-5.6)			18-25 (18-25)			3.9-6.9 (3.4-8.0)		
Article/Doses per day/mg/kg/dose: 1-Vehicle/T D/0; 2-ZS/TID/100; 3-ZS/TID/300; 4-ZS + KCl /TID/1000; 5-ZS/T D/1000; 6-ZS/SID/1000, TID = three doses per day; SID = single dose per day. ^a Historical control ranges: Mean (individual); ¹ p < 0.05 versus control ² p < 0.01 versus control										

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No effect of ZS treatment was observed on serum urea nitrogen in either sex (Table 30). All mean and individual values were within the historical control ranges. In contrast, serum creatinine in the 1000 mg/kg TID group compared to the concurrent control group at weeks 2 and 4 increased an average of 9.6% and 9.6%, respectively, in males and 13% and 17%, respectively, in females. The increase was statistically significant in females at week 4. The serum creatinine values in the 1000 mg/kg TID group at weeks 2 and 4 increased an average of 23% and 23%, respectively, in males and 11.5% and 12.8%, respectively, in females compared to pretest values. However, at pretest the mean creatinine value for the 1000 mg/kg TID females was 20% higher than the mean for the control females. The means and individual values in all male groups were within the historical ranges for all timepoints. However, the mean values for females in the 1000 mg/kg TID group at weeks 2 and 4 were above the maximum of the historical range. Values for two females 731 and 736 were above the maximum of the historical individual range. The increases in serum creatinine in these females did not appear to correlate with decreases in serum potassium or with specific microscopic changes in the kidney.

Table 30: Reviewer's Summary of Serum Urea Nitrogen and Creatinine

Group			Urea Nitrogen, mg/dL			Creatinine, mg/dL		
			Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4
Male	1-Vehicle/TID/0	Mean	15.0	15.7	16.2	0.63	0.73	0.73
		SD	2.8	2.5	3.0	0.05	0.05	0.05
	2-ZS/TID/100	Mean	14.3	17.7	15.7	0.60	0.73	0.70
		SD	3.2	1.5	2.3	0.10	0.06	0.10
	3-ZS/TID/300	Mean	15.3	17.3	18.3	0.63	0.73	0.77
		SD	4.2	2.5	1.2	0.06	0.06	0.06
	4- ZS + KCl /TID/1000	Mean	17.0	18.3	19.0	0.67	0.73	0.73
		SD	0.0	2.3	3.0	0.06	0.06	0.12
	5-ZS/TID/1000	Mean	14.2	14.7	14.8	0.65	0.80	0.80
		SD	3.1	1.9	2.5	0.06	0.06	0.09
		M738	18	15	14	0.7	0.9	0.9
	6-ZS/SID/1000	Mean	16.0	17.7	18.3	0.67	0.83	0.80
SD		1.0	1.2	2.3	0.06	0.06	0.00	
	Historical ^a		10-22 (9-28)			0.4-0.8 (0.4-1.0)		

			Urea Nitrogen, mg/dL			Creatinine, mg/dL		
Group			Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4
Female	1-Vehicle/TID/0	Mean	16.7	19.5	18.7	0.65	0.77	0.75
		SD	2.1	3.6	3.8	0.06	0.05	0.06
	2-ZS/TID/100	Mean	13.3	18.0	17.3	0.63	0.77	0.70
		SD	2.9	1.0	1.5	0.06	0.06	0.00
	3-ZS/TID/300	Mean	16.7	18.7	20.0	0.60	0.77	0.73
		SD	5.0	5.5	5.0	0.10	0.06	0.06
	4- ZS + KCl /TID/1000	Mean	15.0	15.7	16.3	0.67	0.80	0.73
		SD	2.7	1.2	5.1	0.06	0.10	0.06
	5-ZS/TID/1000	Mean	19.2	14.8	16.5	0.78¹	0.87	0.88¹
		SD	2.3	2.3	2.7	0.08	0.12	0.12
		F731	23	15	17	0.9	1.0	1.1
		F732	18	11	15	0.8	0.7	0.8
		F733	21	14	12	0.7	0.8	0.8
		F734	17	15	19	0.8	0.8	0.9
F735		18	16	17	0.7	0.9	0.8	
F736		18	18	19	0.8	1.0	0.9	
6-ZS/SID/1000	Mean	15.3	17.3	18.0	0.63	0.77	0.73	
	SD	2.3	2.1	2.0	0.06	0.06	0.06	
Historical ^a			10-22 (8-30)			0.4-0.8 (0.4-0.9)		
TID = three doses per day; SID = single dose per day, ^a Historical control ranges: Mean (individual); ¹ p < 0.05 versus control ² p < 0.01 versus control								

Females in the 1000 mg/kg TID group had progressive increases in mean values for AST (3.1-fold) and ALT (2.7-fold) at week 2 and 4 relative to their pre-dose values (Table 31). The mean values for both AST and ALT at week 4 were above the maximum of the group historical controls. The increases were attributable to the females 732, 733, and 736. Although no consistent, specific microscopic liver finding in these animals correlated with the increased enzyme values, these three females were hypokalemic with serum potassium values ≤ 3.6 mEq/L at weeks 2 and 4. The pathologist speculated that the elevations in ALT and AST were secondary to hypokalemia, and indirectly test article-related. Table 31 indicates that the highest enzyme values for each animal correlates best with the lowest potassium values (≤ 3.3 mEq/L). Any literature directly relating hypokalemia and elevation in hepatic enzymes was not obvious in the reviewer's literature searches. Since most of the total body potassium (Greenlee et al. 2009) is distributed to the intracellular fluid primarily in muscle, the liver, and erythrocytes, prolonged hypokalemia may adversely affect these tissues.

Table 31: Reviewer's Summary – Female AST, ALT and Potassium Values

Group	Potassium, mEq/L (% decrease)			AST, U/L			ALT, U/L		
	Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4	Pretest	Wk 2	Wk 4
1	4.82	4.98	4.78	21.5	20.8	25.0	22.5	22.3	22.2
5	4.90	3.87²	3.68²	26.7	48.8	83.5	23.8	39.7	64.2
F731	5.7	4.2 (26.3)	3.9 (31.6)	43	29	33	30	29	35
F732	4.9	3.1 (36.7)	3.5 (28.6)	21	150	57	18	111	85
F733	4.6	3.6 (21.7)	2.9 (37.0)	26	31	169	27	31	105
F734	4.6	4.3 (6.5)	4.4 (4.3)	24	31	39	12	25	24
F735	4.7	4.5 (4.2)	4.1 (12.8)	20	22	26	18	19	20
F736	4.9	3.5 (28.6)	3.3 (32.6)	26	30	177	27	23	116
Historical	4.0-5.1 (3.8-5.6)			16-39 (16-57)			11-42 (9-77)		

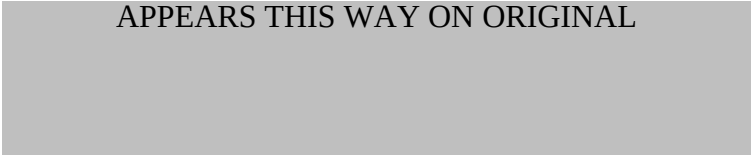
At pretest, male 716 in the 100 mg/kg TID group already had values for alkaline phosphatase (268 U/L) and ALT (133 U/L) that were above the maximum historical individual values (172 U/L and 75 U/L, respectively). At week 2 and 4, the values for male 716 increased further not only for alkaline phosphatase and ALT, but also for AST. By week 4, male 716 had values for alkaline phosphatase, AST and ALT that were 4-, 3-, and 6-fold higher than the maximum historical individual values. At necropsy, this male displayed hepatic lobular atrophy with bile duct hyperplasia consistent with these increases. Since the increases in alkaline phosphatase and ALT were already present at the pretest collection, the liver findings and increases in AST, ALT and alkaline phosphatase were not considered related to ZS treatment, but to a pre-existing condition.

Aldosterone

The mean aldosterone values for the 1000 mg/kg TID group decreased 67% in males and 56% in females compared to the respective values in the control group (Table 32). However, compared to pretest values, aldosterone values in the 1000 mg/kg TID group at week 4 decreased an average of 77% in males and 79%, whereas aldosterone values in the control group decreased an average of 42% in males and 22%. For individual animals in the 1000 mg/kg TID group the ranges of aldosterone decreases compared to pre-test values were 41% to 86% in males and 66% to 91% in females, whereas the maximum decreases in the control groups were 61% in males and 38% in females. The decrease in aldosterone was greater for the 1000 mg/kg TID group and this correlated with the greater decrease in serum potassium at week 4. However, neither absolute values nor decreases from pre-test for aldosterone correlated with serum potassium values for individual animals. The maximum individual aldosterone decreases in the 1000 mg/kg + KCl TID group were 47% in males and 63% in females indicating that potassium supplementation more effectively ameliorated the effect of ZS in males than in females. In addition, the maximum individual potassium decreases in the 1000 mg/kg SID group were 60% in males and 65% in females indicating that single dose administration of ZS induced effects on potassium homeostasis in individual animals. At end of recovery period, three males and two females in the 1000 mg/kg TID recovery group had increases in aldosterone compared to values at week 4;

however, the values were still less than 60% of the pretest values. Furthermore, at the end of recovery, the third female had an aldosterone value at least 90% less than her week 4 value indicating little or no recovery in this animal.

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The sponsor attributed the decreases in serum aldosterone to reductions in aldosterone biosynthesis secondary to ZS-related decreases in serum potassium. These reductions in aldosterone resulted in alterations in renal potassium and sodium excretion and correlated with microscopic changes in the zona glomerulosa of the adrenal gland (intracytoplasmic lipid vacuolation and cystic degeneration).

Table 32: Reviewer's Summary of Potassium and Aldosterone Results

		Potassium, mEq/L				Aldosterone, pg/mL		
Group		Pretest		Wk 4	Rec.	Pretest	Wk 4 (% D)	Rec.
Male	1-Vehicle/TID/0	Mean	4.98	4.78	4.10	113.5	66.3 (42%)	57.0
		SD	0.34	0.40	0.44	75.0	21.2	26.5
	2-ZS/TID/100	Mean	5.03	4.87		106.3	195.7 ² (-)	
		SD	0.15	0.12		29.4	81.6	
	3-ZS/TID/300	Mean	4.97	4.97		80.3	82.3 (-)	
		SD	0.31	0.32		46.8	36.5	
		Range	4.7-5.3	4.6-5.2		48-134	42-113	
	4-ZS + KCl /TID/1000	Mean	4.90	4.90		98.7	71.0 (28%)	
		SD	0.20	0.20		15.7	26.0	
	5-ZS/TID/1000	Mean	4.98	4.37	4.50	97.8	22.2 (77%)	60.0
Female		SD	0.16	0.31	0.0	52.7	8.1	10.4
		M737	5.0	4.5		43	12 (72%)	
		M738	4.8	3.8		80	26 (68%)	
		M739	5.1	4.7		56	33 (41%)	
		M740	5.2	4.3	4.5	176	27 (85%)	66
		M741	5.0	4.4	4.5	84	14 (83%)	48
		M742	4.8	4.5	4.5	148	21 (86%)	66
	6-ZS/SID/1000	Mean	5.13	5.03		104.0	51.0 (51%)	
		SD	0.32	0.06		31.8	12.3	
	Historical ^a	4.1-5.2 (3.6-5.4)				Not provided		
	1-Vehicle/TID/0	Mean	4.82	4.78	4.40	101.0	78.8 (22%)	83.0
		SD	0.15	0.31	0.17	37.6	21.9	36.4
	2-ZS/TID/100	Mean	4.63	4.67		95.3	72.7 (24%)	
		SD	0.06	0.06		47.2	12.9	
	3-ZS/TID/300	Mean	4.97	4.93		150.3	103.0 (31%)	
		SD	0.21	0.32		181.8	96.1	
	4-ZS + KCl /TID/1000	Mean	4.73	4.63		112.0	59.0 (47%)	
		SD	0.12	0.06		69.7	34.4	
	5-ZS/TID/1000	Mean	4.90	3.68²	4.40	162.0	34.5 (79%)	40.0
		SD	0.42	0.55	0.10	64.0	20.1	33.4
		F731	5.7	3.9		250	41 (84%)	
		F732	4.9	3.5		193	18 (91%)	
		F733	4.6	2.9		206	70 (66%)	
		F734	4.6	4.4	4.5	98	19 (81%)	46
		F735	4.7	4.1	4.4	133	21 (84%)	70
		F736	4.9	3.3	4.3	92	38 (59%)	<4
	6-ZS/SID/1000	Mean	4.70	4.47		87.3	120.7 (-)	
		SD	0.30	0.12		54.2	127.7	
	Historical ^a	4.0-5.1 (3.8-5.6)				Not provided		

TID = three doses per day; SID = single dose per day, ¹ p < 0.05 versus control ² p < 0.01 versus control

Urinalysis and urine chemistry

Statistically significant increases in urine volume of 2.2-3.2 fold in males and 3.3-5.7 fold in the 1000 mg/kg TID group occurred at weeks 2 and 4 compared with pretest values with concurrent decreases in urine specific gravity that were considered secondary to the decreases in aldosterone. Smaller increases in urine volume occurred in the 1000 mg/kg + KCl TID group. Urine pH increased up to 2 pH units compared to pretest values in the 1000 mg/kg TID group with smaller increases in the 1000 mg/kg + KCl TID group (Table 33). These ZS-related changes were normalized in the 1000 mg/kg TID group at the end of the recovery period. No clear treatment-related effect was observed among urinalysis parameters in either sex.

Because of a protocol deviation, the values for some urine chemistry parameters (creatinine clearance or electrolyte fractional excretions) could not be calculated for the pre-test timepoint. All comparisons, therefore, are made to the concurrent control value.

Treatment with ZS alone reduced fractional excretion of potassium compared to the control group; however, treatment with ZS + KCl increased fractional excretion of potassium (Table 14). At week 2, fractional excretion of potassium was reduced 23% at 300 mg/kg TID, 28% at 1000 mg/kg SID, and 95% at 1000 mg/kg TID in males and 30% at 100 and 300 mg/kg TID, 43% at 1000 mg/kg SID, and 96% at 1000 mg/kg TID in females. This effect of ZS is consistent with the pharmacological action of ZS. At 1000 mg/kg TID, the highly significant reductions in potassium excretion were considered by the sponsor to be compensatory responses to the decreases in serum potassium concentration. However, values for fractional excretion of potassium in individual animals did not always correlate with their values for serum potassium. The reductions in fractional excretion of potassium at 300 mg/kg TID and 1000 mg/kg SID were not considered adverse by the sponsor, because they were not accompanied by significant changes in serum potassium or aldosterone concentrations.

In contrast, at 1000 mg/kg + KCl TID, fractional excretion of potassium increased 81-87% in males and 53-54% in females. Fractional excretion of chloride also increased 2.6-3.2-fold in males and 2-2.1-fold in females in these groups. The increases in fractional excretion of potassium and chloride were consistent with the KCl supplementation in these groups. Although the ZS administered at 1000 mg/kg TID dose reduced potassium absorption, the potassium chloride supplementation was more than adequate to counteract this effect and prevent reduction in urine potassium excretion and serum potassium concentration, but also resulted in increased potassium excretion.

Fractional excretion of sodium increased 3.5-3.8-fold in males and 2.7-2.8 fold in females in the 1000 mg/kg TID group relative to the control groups. Since these increases did not correspond to significant changes in serum sodium concentrations, the sponsor attributed them to the changes in serum aldosterone concentration.

The ratio of urine calcium excretion to creatinine excretion increased in the 1000 mg/kg TID groups (0.15) compared to pretest values (0.05) and concurrent controls (0.025-0.075) and historical controls (≤ 0.1); however, no effect was observed on serum calcium concentrations.

Table 33: Reviewer's Summary of Selected Urine Chemistry Parameters versus Serum Potassium

		pH			F-Na		F-K		F-Cl		Serum K, mEq/L	
		Pretest	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
Male	1 Mean	5.92	5.67	6.17	0.34	0.39	17.24	16.14	1.00	0.91	4.98	4.78
	SD	0.38	0.26	0.75	0.14	0.07	2.71	2.58	0.16	0.21	0.39	0.40
	2 Mean	6.00	5.83	6.67	0.59	0.46	17.70	12.16	1.31	0.73	4.98	4.78
	SD	0.00	0.58	0.58	0.17	0.12	3.64	4.46	0.14	0.37	0.39	0.40
	3 Mean	5.83	5.83	6.17	0.52	0.71²	13.25	16.03	0.97	1.17	5.03	4.97
	SD	0.29	0.29	0.76	0.22	0.21	2.61	3.72	0.46	0.29	0.25	0.32
	4 Mean	6.17	6.33¹	6.67	1.40²	1.69²	31.23¹	30.18¹	2.58¹	2.88¹	5.17	4.90
	SD	0.58	0.29	0.29	0.29	0.30	6.41	2.80	0.56	0.30	0.25	0.20
	5 Mean	6.08	7.00²	7.25¹	1.29²	1.38²	0.84²	0.82²	0.97	1.04	4.38¹	4.37
	SD	0.38	0.32	0.27	0.20	0.17	0.25	0.24	0.10	0.22	0.36	0.31
	737	6.0	7.0	7.0	1.22	1.38	1.04	1.15	0.81	0.98	4.7	4.5
	738	5.5	7.5	7.5	1.62	1.32	1.25	0.71	1.01	0.82	3.7	3.8
	739	6.5	6.5	7.5	1.42	1.57	0.68	0.8	1.08	1.28	4.5	4.7
Female	740	6.0	7.0	7.5	1.26	1.60	0.61	0.6	1.04	1.33	4.3	4.3
	741	6.0	7.0	7.0	1.12	1.15	0.79	<0.6	0.94	0.83	4.5	4.4
	742	6.5	7.0	7.0	1.11	1.28	0.67	<0.6	0.96	0.97	4.6	4.5
	6 Mean	5.83	5.83	6.00	0.81²	0.87²	12.31	11.61¹	0.92	1.06	5.23	5.03
	SD	0.29	0.29	0.50	0.02	0.02	4.26	0.62	0.26	0.18	0.15	0.06
	1 Mean	6.00	5.75	6.17	0.45	0.52	21.08	19.01	1.19	1.11	4.98	4.78
	SD	0.55	0.42	0.52	0.23	0.25	1.93	3.82	0.19	0.35	0.21	0.31
	2 Mean	6.50	6.00	5.83	0.71	0.68	14.75¹	14.89	1.11	1.16	4.97	4.67
	SD	0.00	0.00	0.29	0.19	0.12	5.23	1.93	0.67	0.27	0.12	0.06
	3 Mean	6.17	5.83	5.83	0.67	0.83	14.75²	15.18	1.13	1.24	5.07	4.93
	SD	0.58	0.29	0.29	0.08	0.22	2.27	1.48	0.30	0.38	0.32	0.32
	4 Mean	6.17	7.00²	7.33¹	1.51²	1.43²	32.56	29.01	2.44	2.18	5.03	4.63
	SD	0.76	0.87	0.76	0.47	0.34	4.13	6.11	0.90	0.49	0.15	0.06
	5 Mean	5.83	7.25²	7.42²	1.268²	1.39²	0.82²	0.95[#]	0.82	0.96	3.87²	3.68²
	SD	0.41	0.42	0.38	0.26	0.31	0.58	NA	0.30	0.31	0.55	0.55
	731	5.5	7.0	7.5	1.42	1.96	0.71	<0.6	0.95	1.43	4.2	3.9
	732	6.5	7.5	7.5	0.89	1.35	<0.6	<0.6	0.44	1.04	3.1	3.5
	733	6.0	7.5	8.0	1.09	0.99	0.55	<0.6	0.65	0.47	3.6	2.9
	734	6.0	7.5	7.0	1.18	1.39	0.37	<0.6	0.57	0.98	4.3	4.4
	735	5.5	6.5	7.0	1.53	1.33	1.66	0.95	1.18	1.02	4.5	4.1
	736	5.5	7.5	7.5	1.50	1.34	<0.6	<0.6	1.10	0.82	3.5	3.3
	6 Mean	6.33	5.67	6.17	0.48	0.54	11.94²	12.52²	1.01	0.88	4.80	4.47
	SD	0.58	0.29	0.29	0.16	0.22	6.99	1.85	0.29	0.29	0.17	0.12

Article/Doses per day/ mg/kg/dose by group: 1-Vehicle/TID/0; 2-ZS/TID/100; 3-ZS/TID/300; 4-ZS + KCl /TID/1000; 5-ZS/TID/1000; 6-ZS/SID/1000, TID = three doses per day; SID = single dose per day, ¹ p < 0.05 versus control ² p < 0.01 versus control

The sponsor did not specifically address the creatinine and urea clearance results. At weeks 2 and 4, creatinine clearance did not change significantly in either sex for any treatment group (Table 34). However, the ratio of urea clearance to creatinine clearance decreased an average of 17% and 12%, respectively, in males and 27% and 16%, respectively, in females in the 1000 mg/kg TID group compared to pretest values. Compared to concurrent control groups, the decreases were an average of 21% and

18%, respectively, in males and 35% and 28%, respectively, in females in the 1000 mg/kg TID group. For individual animals in the 1000 mg/kg TID group, the maximum decreases in ratio of urea clearance to creatinine clearance compared to pre-test values were 30% in males (M742) and 29% in females (F732), whereas the maximum decreases in the control groups were 13% in males and 1.5% in females. Furthermore, the females in the 1000 mg/kg + KCl TID had a 31% decrease in week 2 relative to the control group.

Table 34: Reviewer's Summary of Creatinine and Urea Clearance

		Serum K, mEq/L			Cr Clearance, mL/min		Urea N/Cr			
		Pretest	Week 2	Week 4	Week 2	Week 4	Pretest	Week 2	Week 4	
Male	1	Mean	4.98	4.98	4.78	14.21	24.98	14.02	13.82	14.23
		SD	0.34	0.39	0.40	4.54	16.14	2.41	1.28	2.23
		Range				7.9-21.9	13.8-57.3	10.9-16.8	11.3-14.6	10-16.4
	2	Mean	5.03	4.98	4.78	18.24	15.58	14.10	15.03	13.40
		SD	0.15	0.39	0.40	0.50	10.30	0.27	2.20	3.31
	3	Mean	4.97	5.03	4.97	18.33	17.90	13.90	14.30	15.73
		SD	0.31	0.25	0.32	8.72	5.32	0.79	1.21	1.64
	4	Mean	4.90	5.17	4.90	19.86	21.72	13.77	14.57	15.40
		SD	0.20	0.25	0.20	5.98	16.50	2.44	2.54	1.45
	5	Mean	4.98	4.38 ¹	4.37	14.10	15.93	13.20	10.95 ¹	11.63
		SD	0.16	0.36	0.31	6.37	5.93	1.18	1.09	1.06
		M737	5.0	4.7	4.5	15.2	5.4	13.6	12.6	12.4
		M738	4.8	3.7	3.8	19.2	13.7	14.2	11.8	10.6
		M739	5.1	4.5	4.7	2.0	18.5	11.6	9.9	11.3
		M740	5.2	4.3	4.3	18.3	17.9	14	10.9	12.3
		M741	5.0	4.5	4.4	12.6	17.3	11.8	10.7	12.9
		M742	4.8	4.6	4.5	17.3	22.9	14	9.8	10.3
	6	Mean	5.13	5.23	5.03	12.72	15.04	13.63	14.03	15.23
		SD	0.32	0.15	0.06	5.49	3.28	1.60	1.46	2.06
Female	1	Mean	4.82	4.98	4.78	16.07	16.69	14.43	16.63	17.00
		SD	0.15	0.21	0.31	3.40	8.47	2.83	2.64	3.94
		Range				10.0-20.0	6.1-29.5	12.5-19.7	13.4-19.4	12.1-23.4
	2	Mean	4.63	4.97	4.67	15.94	24.10	14.77	15.47	17.03
		SD	0.06	0.12	0.06	8.09	4.58	1.71	0.59	2.34
	3	Mean	4.97	5.07	4.93	14.45	19.37	15.70	16.33	17.70
		SD	0.21	0.32	0.32	1.72	8.08	3.78	1.47	2.21
	4	Mean	4.73	5.03	4.63	20.66	22.87	12.20	11.43 ²	13.47
		SD	0.12	0.15	0.06	2.18	5.25	0.46	0.15	4.90
		725	4.8	5	4.7	23.2	26.0	11.8	11.3	10.2
		726	4.8	5.2	4.6	19.7	16.8	12.7	11.6	11.1
		727	4.6	4.9	4.6	19.2	25.8	12.1	11.4	19.1
	5	Mean	4.90	3.87 ²	3.68 ²	16.71	18.88	14.63	10.75 ²	12.25
		SD	0.42	0.55	0.55	3.19	4.01	1.26	1.13	2.07
		F731	5.7	4.2	3.9	16.8	17.0	11.8	10.4	12
		F732	4.9	3.1	3.5	14.6	12.4	12.7	9	10.2
		F733	4.6	3.6	2.9	18.4	21.5	12.1	10.3	9.6
		F734	4.6	4.3	4.4	14.4	22.0	13.7	11.7	14.2
		F735	4.7	4.5	4.1	13.9	17.4	14.9	12.2	12.8
		F736	4.9	3.5	3.3	22.2	22.9	14.5	10.9	14.7

6	Mean	4.70	4.80	4.47	13.38	15.35	14.03	14.77	15.33
	SD	0.30	0.17	0.12	8.51	9.85	1.05	1.80	1.08

Doses per day/ mg/kg/dose by group: 1-Vehicle/TID/0; 2-ZS/TID/100; 3-ZS/TID/300; 4-ZS + KCl /TID/1000; 5-ZS/TID/1000;
Article/ID/1000, TID = three doses per day; SID = single dose per day, ¹ p < 0.05 versus control ² p < 0.01 versus control

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Fecal occult blood

No difference in the incidence of fecal occult blood was considered to be related to administration of ZS.

Gross pathology

No macroscopic observation was considered to be related to administration of ZS.

Organ weights

The sponsor concluded that no difference in mean organ weights was considered to be related to administration of ZS. The only statistically significant difference in mean organ weights was an increase in ovary absolute (0.758 gm) and relative weight (0.0100) compared to body weight for the 1000 mg/kg + KCl TID female group compared to the values for the control group (0.521 gm, 0.0071, respectively). However, the pathologist attributed changes in ovarian weights to phases of the estrous cycle in females. In the males, the pathologist attributed the variable organ weights for reproductive organs (epididymides, prostate) to stage of maturity, because they were associated with lower testes weights and microscopic characteristics of immaturity.

The pathologist did note that mean adrenal weights (absolute, relative to body weight) were lower for both sexes in all ZS-treated groups (Table 35). However, these differences were not considered ZS-related, because the differences were not dose-related and were not affected by KCl supplementation. In females, the differences were attributed to control female 702, who had high adrenal weights (1.985 gm, 0.0244%) compared to other females of the control group, although no histopathology abnormality was observed in her adrenal gland. Furthermore, histopathologic findings in the adrenal gland of individual dogs did not consistently correlate with individual changes in adrenal weight.

The reviewer noted increases in spleen relative weights in male, but not female, treated groups compared to the control groups. Examination of spleen weights for individual dogs indicated that one control male 707 had low spleen weights (32.32 gm, 0.4225%) in the absence of any histopathology findings compared to other males of the control group.

In addition, the mean relative kidney weight in the 1000 mg/kg TID female group was 17% higher than that in the control female group. The increase was attributable to 21% and 26% increases for females 732 and 733, respectively, both of whom had mild tubulointerstitial inflammation in their kidneys compared to minimal tubulointerstitial inflammation in female 731 in the same group and no tubulointerstitial inflammation in the kidneys of control females. Female 733 also had transitional cell hyperplasia and increased numbers of neutrophils compared to other animals of the same group.

Table 35: Reviewer's Summary of Selected Organ Weights

Group		BW, kg	Adrenal, g	Adrenal, % BW	Kidney, g	Kidney, % BW	Spleen, g	Spleen, % BM
Male	1 Mean	7.57	1.264	0.017	42.47	0.561	50.323	0.666
	SD	0.08	0.011	0.0001	6.306	0.078	18.438	0.247
	2 Mean	7.5	1.05	0.014	38.785	0.515	59.467	0.782
	SD	0.88	0.062	0.002	8.568	0.085	17.487	0.148
	3 Mean	7.45	1.062	0.014	37.755	0.515	69.377	0.918
	SD	1.13	0.365	0.004	4.154	0.100	20.672	0.154
	4 Mean	7.27	1.019	0.014	35.901	0.494	69.177	0.951
	SD	0.32	0.06	0.001	3.38	0.037	6.336	0.050
	5 Mean	7.1	1.008	0.014	41.106	0.580	65.86	0.937
	SD	0.35	0.098	0.002	1.305	0.029	16.635	0.285
	737	7.3	0.915	0.0125	42.56	0.583	61.61	0.844
	738	7.3	1.110	0.0152	40.06	0.549	51.76	0.709
	739	5.7	0.998	0.0149	40.69	0.607	84.21	1.257
	6 Mean	7.77	1.048	0.014	37.888	0.487	66.564	0.858
	SD	0.25	0.13	0.002	3.125	0.027	6.73	0.098
Female	1 Mean	7.3	1.405	0.019	37.036	0.511	58.107	0.786
	SD	1.34	0.506	0.005	5.522	0.041	16.212	0.093
	2 Mean	7.78	1.035	0.013	39.697	0.509	61.84	0.795
	SD	0.63	0.202	0.002	4.66	0.024	8.138	0.089
	3 Mean	7.63	1.013	0.014	36.696	0.482	55.948	0.702
	SD	1.12	0.069	0.002	7.321	0.065	30.953	0.321
	4 Mean	7.58	1.094	0.014	34.26	0.452	61.779	0.815
	SD	0.31	0.245	0.003	1.722	0.029	5.505	0.066
	5 Mean	7.32	1.27	0.017	43.677	0.599	62.903	0.854
	SD	0.38	0.301	0.004	2.55	0.063	16.575	0.178
	731	7.75	1.336	0.017	40.98	0.528	82.04	1.058
	732	7.10	0.942	0.013	43.98	0.620	53.58	0.745
	733	7.10	1.533	0.022	46.06	0.648	53.09	0.747
	6 Mean	7.32	1.117	0.015	37.853	0.516	65.102	0.905
	SD	0.79	0.148	0.001	4.908	0.014	8.779	0.224

Article/Doses per day/ mg/kg/dose by group: 1-Vehicle/TID/0; 2-ZS/TID/100; 3-ZS/TID/300; 4-ZS + KCl /TID/1000; 5-ZS/TID/1000; 6-ZS/SID/1000, TID = three doses per day; SID = single dose per day,

Histopathology

The pathologist concluded that the adrenal glands and kidneys contained ZS-related microscopic findings.

In the adrenal glands, the incidence and severity of lipid vacuolation in the zona glomerulosa increased in all ZS-treated groups, except females in the 100 mg/kg TID group (Table 36). In addition, cystic degeneration in the zona glomerulosa was present in one male and all three females in the 1000 mg/kg TID group. These findings in the zona glomerulosa were attributed to the decreased aldosterone synthesis resulting in accumulation of 11-deoxycorticosterone and other precursors (Mitani et al. 2005). In addition, two control animals (one male, one female) in the recovery group had

increased lipid vacuolation in the adrenal zona glomerulosa indicating this finding has a background incidence.

Tubulointerstitial inflammation was present in the kidneys of all males and females in the 1000 mg/kg TID group. The pathologist described the finding as multifocal areas in the medulla and cortex with tubular and interstitial degenerative and inflammatory changes, including vacuolation/degeneration of tubules with or without concomitant expansion of the interstitium by inflammatory cells (primarily lymphocytes, macrophages, and/or fibroblasts). The pathologist also stated that some animals had dilation of tubules with attenuation of tubular epithelium; however, this finding was not documented in the record of individual animal data. In contrast, the ZS-related kidney changes in the 1000 mg/kg TID group were absent in the 1000 mg/kg + KCl TID group. Based on this observation and the literature (Conn and Johnson 1956), the pathologist concluded that the microscopic kidney findings were consistent with kidney changes in animals subjected to prolonged hypokalemia.

Female 733 in the 1000 mg/kg/dose TID group also had hemorrhage in the renal medulla, hyperplasia of transitional epithelium lining the renal pelvis, and increased neutrophils in addition to the other inflammatory cells in the interstitium. These findings are consistent with the increased relative kidney weight in this animal.

Three females in the 1000 mg/kg TID group had increased alanine aminotransferase (ALT) and aspartate transaminase (AST) values at weeks 2 and/or 4. Although female 732 had a small area of minimal inflammation/necrosis and female 733 had sinusoidal leukocytosis, the pathologist concluded that no significant microscopic findings in the livers of these animals correlated with the increased serum liver enzyme values. Since all three dogs with elevated liver enzyme values were hypokalemic, these elevations were considered to be secondary to the effects of hypokalemia.

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Table 36: Reviewer's Compilation from Sponsor's Pathology Tables – Main Study

		0 mg/kg T1D	100 mg/kg T1D	300 mg/kg T1D	1000 mg/kg + KCl	1000 mg/kg T1D	1000 mg/kg S1D
Males	Tissue	T1D					
	Observation						
	Severity						
	Number of Animals Examined	3	3	3	3	3	3
	adrenal glands	(3)	(3)	(3)	(3)	(3)	(3)
	degeneration, cystic, zona glomerulosa	0	0	0	0	1	0
	vacuolation, increased lipid, zona glomerulosa	0	1	2	3	3	2
	- minimal	0	1	2	3	2	2
	- mild	0	0	0	0	1	0
	within normal limits	3	2	1	0	0	1
	kidneys	(3)	(3)	(3)	(3)	(3)	(3)
	anomaly, developmental	0	1	0	1	0	0
	basophilic tubules, cortex	1	2	0	2	0	3
	cast, hyaline, tubular	1	2	1	2	0	0
	cyst	0	1	0	0	0	1
	infiltration, mononuclear cells, interstitial	2	3	1	2	0	0
	inflammation, tubulointerstitial	0	0	0	0	3	0
	mineralization, tubular	3	3	3	2	3	2
	liver	(3)	(1)	(0)	(3)	(3)	(3)
	atrophy, diffuse	0	1	0	0	0	0
	hyperplasia, bile duct	0	1	0	0	0	0
	infiltration, mononuclear cell	3	0	0	2	2	3
	inflammation, subacute/chronic	0	0	0	1	0	0
	within normal limits	0	0	0	1	1	0
	spleen	(3)	(0)	(0)	(3)	(3)	(3)
	within normal limits	3	0	0	3	3	3

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	Tissue		0 mg/kg TID	100 mg/kg TID	300 mg/kg TID	1000 mg/kg + KCl TID	1000 mg/kg TID	1000 mg/kg SID
	Observation	Severity						
Females	Number of Animals Examined		3	3	3	3	3	3
	adrenal glands		(3)	(3)	(3)	(3)	(3)	(3)
	degeneration, cystic, zona glomerulosa		0	0	0	0	3	0
		- minimal	0	0	0	0	1	0
		- mild	0	0	0	0	2	0
	vacuolation, increased lipid, zona glomerulosa		0	0	2	2	1	1
		- minimal	0	0	2	2	0	1
		- mild	0	0	0	0	1	0
	within normal limits		3	3	1	1	0	2
	kidneys		(3)	(3)	(3)	(3)	(3)	(3)
	anomaly, developmental	- no grade	0	0	0	1	1	0
	basophilic tubules, cortex	- minimal	0	0	1	2	0	1
	cast, hyaline, tubular	- minimal	0	2	1	0	0	0
	hemorrhage	- mild	0	0	0	0	1	0
	hyperplasia, transitional cell	- minimal	0	0	0	0	1	0
	infiltration, mononuclear cells, interstitial	- minimal	0	3	0	0	0	0
	inflammation, tubulointerstitial	- mild	0	0	0	0	3	0
	lipidosis, glomerular	- minimal	0	0	0	0	0	1
	mineralization	- minimal	0	0	1	0	0	0
	mineralization, tubular	- minimal	3	3	3	3	3	3
	liver		(3)	(0)	(0)	(3)	(3)	(3)
	infiltration, mononuclear cell	- minimal	2	0	0	3	3	3
	inflammation/necrosis	- minimal	0	0	0	0	1	0
	leukocytosis, sinusoidal	- minimal	0	0	0	0	1	0
	within normal limits		1	0	0	0	0	0
	spleen		(3)	(0)	(0)	(3)	(3)	(3)
	pigment, increased	- minimal	0	0	0	1	0	0
	within normal limits		3	0	0	2	3	3

Table 18: Compilation from Sponsor's Pathology Tables - Recovery

Males			Recovery			Females		
Tissue		Severity	0 mg/kg TID	1000 mg/kg TID	Tissue		0 mg/kg TID	1000 mg/kg TID
Observation					Observation			
Number of Animals Examined			3	3	Number of Animals Examined		3	3
adrenal glands			(3)	(3)	adrenal glands		(3)	(3)
vacuolation, increased lipid, zona glomerulosa			1	0	vacuolation, increased lipid, zona glomerulosa		1	0
within normal limits			2	3	within normal limits		2	3
kidneys			(3)	(3)	kidneys		(3)	(3)
basophilic tubules, cortex			1	1	basophilic tubules, cortex		1	3
cast, hyaline, tubular			1	0	infiltration, mononuclear cells, interstitial		0	1
infiltration, mononuclear cells, interstitial			2	1	lipidosis, glomerular		0	1
inflammation, perivascular/vascular			0	1	mineralization, tubular		2	3
mineralization, tubular			3	3	within normal limits		1	0
liver			(3)	(3)	liver		(3)	(3)
infiltration, mononuclear cell			3	3	infiltration, mononuclear cell		2	2
					inflammation/necrosis		0	1
					vacuolation, diffuse		1	2
					within normal limits		1	0

Toxicokinetics

The toxicokinetic data were not included in the report.

6.2.4. Study title**A 39-week Toxicity Dietary Study with ZS (Sodium Zirconium Cyclosilicate) in Dogs with a 28-Day Recovery Period**

Study number: 1959-008
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date study initiation: 07/12/2013
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot # and % purity: Zirconium Silicate; RD011-61; undisclosed

Key Study Findings

In the interim 13-week results that had been previously reported, the major finding was tuberointerstitial inflammation in males receiving the dose of 2000 mg/kg/day (without KCl supplementation). Thus, the NOAEL was 1000 mg/kg/day at this 13-week time point.

The terminal results at 39 weeks also noted this effect in the kidneys at 2000 mg/kg/day (both sexes). The incidence was 5/8 without KCl supplementation (this effect improved during recovery period) and 2/8 with KCl supplementation. In addition, the severity of the inflammation was reduced in KCl supplemented animals. The NOAEL was still 1000 mg/kg/day, however, in both sexes at this 39-week time point. The addition of KCl demonstrated that these renal effects were due predominantly to the potassium-depleting effect of the drug. Thus, in patients who will be given the drug to treat hyperkalemia, there should be less of a probability for these renal effects to occur, provided potassium is not lowered below normal levels.

The sponsor states in the letter to the Division (Sept. 24, 2014), that "The dog urine has been analyzed from this study and no quantifiable levels of ZS were noted. Also, quantifiable ZS levels were not found in human urine analyzed from ZS-004". These findings further indicate that the toxicity observed in the dog study was produced by the drug's desired and expected effect to decrease plasma potassium.

Methods

Doses: 0, 300, 1000, 2000 mg/kg/day; 2000 mg/kg/day + 253 mg/kg/day
 KCl

Route of administration: Dietary

Species/strain: Male and female Beagle dogs

Table 37: Number/sex/group:

Group number	Dose level (mg/kg/day)	Male	Female
1	0 (vehicle only)	10	10

2	300	7	7
3	1000	7	7
4	2000	10	10
5	2000 + KCl (vehicle supplemented with 253 mg/kg/day potassium chloride)	7	7

Three animals in groups 1 and 4 remained on the study for the 28-day recovery period after the last dose.

Three animals in all 5 groups were submitted to necropsy on Day 90 for interim evaluations.

Four animals in all 5 groups were submitted to necropsy on Day 274 for terminal evaluations.

Age: 5-6 months
 Weight: 6.19-8.60 kg
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: None

Observations and Results

Mortality

All animals survived the duration of the study.

Clinical Signs

In the animals receiving 2000 mg/kg/day, decreased activity, inappetence, impaired limb function, ataxia and rigid body were intermittently observed between weeks 31 and 37. These effects were attributed to ZS-induced hypokalemia. These effects resolved after a 1-2 day drug holiday and did not occur in the group receiving supplemental potassium chloride along with this dose.

Body Weights

Female dogs treated with 2000 mg/kg/day without KCl supplementation had slightly lower body weights compared to control animals.

Food Consumption

No significant findings were noted

Ophthalmoscopy

No significant findings were noted

ECG

No significant findings were noted

Hematology

No significant findings were noted

Clinical Chemistry and Urinalysis

At 2000 mg/kg/day (without KCl), there were minimal reversible decreases in serum potassium and increases in HCO₃ and tCO₂ levels. There were mild reversible decreases in aldosterone levels in this group. There were mild reversible increases in urinary pH in animals receiving $\geq$ 1000 mg/kg/day both with and without KCl supplementation. There were minimal increases in urine volume in animals treated with

2000 mg/kg/day with and without KCl supplementation. Marked reversible decreases in renal potassium excretion were observed at all doses without KCl supplementation. Finally, mild reversible increases in sodium excretion was observed in males receiving ≥ 1000 mg/kg/day and females receiving ≥ 300 mg/kg/day with and without KCl supplementation.

Gross Pathology

In one male given 2000 mg/kg/day without KCl supplementation, irregular surface in both kidneys was observed and this correlated with bilateral moderate tubulointerstitial inflammation and mild bilateral infarcts.

Organ Weights

Mean absolute kidney weights were higher in males receiving 2000 mg/kg/day and this effect was lower in animals receiving KCl supplementation. Pituitary gland weights were higher in controls (compared to all doses in females and doses ≥ 1000 mg/kg/day in males). These effects occurred in animals both with and without potassium supplementation.

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

Kidneys: Tubulointerstitial inflammation was observed in animals given 2000 mg/kg/day in dogs with and without KCl supplementation. This was observed in multiple areas in both renal cortex and medulla and included vacuolation, degeneration and regeneration of renal tubules. One male and one female exhibited renal medullary interstitial fibrosis at the highest dose. Without KCl, 5/8 dogs exhibited these effects (grade of 2.0) while only 2/8 that received KCl exhibited this effect (grade 1.5).

Adrenal gland: Bilateral atrophy of the zona glomerulosa was observed in 1 male at 1000 mg/kg/day and 1 male and 1 female at 2000 mg/kg/day. Intracytoplasmic lipid vacuolation was observed in cells in the z. glomerulosa in 2 males from all dose groups and 2/4 females at 2000 mg/kg/day. These effects were not observed in the groups that received KCl supplementation.

Recovery: In the animals receiving 2000 mg/kg/day without KCl supplementation, following the 4 week recovery period, there were no adrenal findings and the renal tubulointerstitial inflammation and increased medullary interstitial matrix were less severe.

7 Genetic Toxicology (reviewed by Pat Harlow)

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: ZS-9: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Echerichia coli WP2uvrA

Study no.: 789296

Study report location: Volume 2.6, p 1

Conducting laboratory and location: (b) (4)

Date of study initiation: August 12, 2010

GLP compliance: Indicated

QA statement: Indicated

Drug, lot #, and % purity: ZS-9, Batch No. 5332-04310-A, purity 87.1% after correction for water content

Reviewer's Summary of Key Study Findings

In valid assays, ZS-9 (microporous zirconium silicate) did not induce excess bacterial reversion in either the absence or the presence of metabolic activation in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and in *Escherichia coli* WP2uvrA either when a suspension of ZS-9 in water was applied directly to the strains using the plate incorporation method or when a DMSO extract of ZS-9 was applied to the strains using the pre-incubation method.

Table 38: Sponsor's Tabulated Summary of Study 789296

Test Article: ZS-9		Page 1 of 3
Report Title:	Testing for Mutagenic Activity with <i>Salmonella typhimurium</i> TA 1535, TA 1537, TA 98 and TA 100 and <i>Escherichia coli</i> WP2uvrA	Study No: 789296
Test for Induction of:	No. of Independent Assays: 2	Testing Facility (b) (4)
Reverse Mutation in Bacterial Cells		
Strains:	No. of Replicate Cultures: 3	
<i>Salmonella typhimurium</i> TA 1535, TA 1537, TA 98, TA 100 and <i>Escherichia coli</i> WP2uvrA WP2uvrA		
Metabolizing System: Aroclor 1254-induced rat liver S9 mix (9 parts cofactor with 1 part S9 (v/v))	No. of Cells Analyzed/Culture: n/a	GLP Compliance: yes
Vehicles:	Test Article:	Positive Controls:
	Test 1: Aqueous suspension	Sodium azide: sterile water
	Test 2 DMSO extract	9-Aminoacridine/2-Nitrofluorene: DMSO
	(200 mg ZS-9/ml DMSO incubated at 37 °C for 3 days)	N-Ethyl-N-nitro-N-nitrosoguanidine: DMSO
		2-Aminoanthracene: DMSO.
Treatment:	Test 1: Direct plate incorporation method ± S9	Date of Treatment: Test 1: 17 August 2010
	Test 2: Preincubation method ± S9	Test 2: 10 September 2010
Cytotoxic Effects:	Test 1 = Not toxic	
	Test 2 = Toxic in the absence of S9 mix at 37.5% to 75% of 100 µL of the undiluted extract per plate	
Genotoxic Effects: None		
Comments: Test 1 – Precipitation noted at all concentrations. Test 2 – No precipitation.		

Table 3 continued		Genotoxicity: In Vitro	Study No. 789296		Page 2 of 3		
Metabolic Activation	Test Article	Dose Level (µg/plate)	Test #1				
			Revertant Colony Counts (Mean ± SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
Without Activation	Water	100	13 ± 6	9 ± 6	20 ± 7	82 ± 10	8 ± 3
	ZS-9	17	14 ± 5 P	8 ± 2 P	24 ± 6 P	86 ± 4 P	9 ± 2 P
		50	15 ± 2 P	7 ± 7 P	22 ± 10 P	73 ± 7 P	11 ± 4 P
		167	10 ± 0 P	12 ± 1 P	20 ± 5 P	90 ± 6 P	10 ± 2 P
		500	9 ± 4 P	6 ± 2 P	22 ± 4 P	77 ± 15 P	7 ± 4 P
		1667	9 ± 2 P	5 ± 0 P	13 ± 2 P	68 ± 7 P	8 ± 1 P
		5000	12 ± 4 P	5 ± 2 P	11 ± 2 P	70 ± 12 P	4 ± 2 P
	Sodium azide	1	437 ± 54	-	-	1050 ± 46	-
	9-Aminoacridine	80	n/a	5666 ± 664	-	-	-
	2-Nitrofluorene	1	n/a	-	807 ± 43	-	-
N-Ethyl-N-nitro-N-nitrosoguanidine	2	n/a	-	-	-	244 ± 30	
With Activation	Water	100	15 ± 2	9 ± 4	33 ± 5	93 ± 1	5 ± 2
		17	12 ± 2 P	7 ± 4 P	28 ± 7 P	96 ± 6 P	7 ± 3 P
		50	10 ± 4 P	11 ± 1 P	36 ± 11 P	102 ± 18 P	5 ± 1 P
		167	12 ± 3 P	10 ± 5 P	37 ± 7 P	104 ± 21 P	3 ± 2 P
		500	11 ± 2 P	8 ± 5 P	30 ± 8 P	110 ± 6 P	6 ± 1 P
		1667	11 ± 2 P	7 ± 3 P	28 ± 8 P	103 ± 12 P	8 ± 4 P
		5000	10 ± 2 P	6 ± 2 P	21 ± 6 P	91 ± 5 P	5 ± 2 P
	2-Aminoanthracene	0.5	-	-	551 ± 53	782 ± 44	-
	2-Aminoanthracene	2	639 ± 36	269 ± 14	-	-	-
	2-Aminoanthracene	20	-	-	-	-	631 ± 38

P – precipitate; n/a – not applicable

P – precipitate; n/a – not applicable

Table 3 continued	Genotoxicity: In Vitro	Study No.	789296					Page 3 of 3
Metabolic Activation	Test Article	Dose Level	Test #2 Revertant Colony Counts (Mean ± SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2uvrA	
Without Activation	DMSO	100 µl	15 ± 3	16 ± 2	23 ± 2	87 ± 8	7 ± 6	
	ZS-9 DMSO	2.34%	16 ± 4	13 ± 5	28 ± 5	78 ± 15	5 ± 4	
	Extract	4.69%	19 ± 2	10 ± 7	22 ± 5	93 ± 5	7 ± 4	
		9.38%	21 ± 4	8 ± 2	19 ± 6	92 ± 2	6 ± 2	
		18.75%	13 ± 2	8 ± 2	22 ± 9	70 ± 8	6 ± 1	
		37.5%	19 ± 3	4 ± 2 (ST)	22 ± 5	21 ± 3 (TL)	9 ± 4 (ST)	
		75%	19 ± 4 (ST)	VTNC	13 ± 8 (ST)	VTNC	5 ± 2 (VT)	
	Sodium azide	1 µg	518 ± 16	-	-	1134 ± 20	-	
	9-Aminoacridine	80 µg	-	4374 ± 607	-	-	-	
	2-Nitrofluorene	1 µg	-	-	867 ± 81	-	-	
	N-Ethyl-N-nitro-N-nitrosoguanidine	2 µg	-	-	-	-	443 ± 48	
	With Activation	DMSO	100					
TBC3711		3.13%	9 ± 3	23 ± 1	37 ± 9	97 ± 23	5 ± 1	
		6.25%	16 ± 1	16 ± 5	41 ± 2	95 ± 2	5 ± 3	
		12.5%	16 ± 4	18 ± 3	46 ± 14	99 ± 5	9 ± 2	
		25%	16 ± 1	18 ± 2	33 ± 14	97 ± 2	7 ± 5	
		50%	16 ± 3	18 ± 2	31 ± 6	109 ± 10	6 ± 2	
		100%	14 ± 2	18 ± 2	38 ± 4	84 ± 6	6 ± 2	
2-Aminoanthracene		0.5 µg	16 ± 1	20 ± 7	50 ± 9	86 ± 13	6 ± 4	
2-Aminoanthracene		2 µg	-	-	489 ± 25	772 ± 68	-	
2-Aminoanthracene		20 µg	479 ± 46	284 ± 41	-	-	-	
ST – Slightly Thin Lawn		TL – Thin Lawn	VT – Very Thin Lawn	NC – Not counted due to resistant colonies				

ST – Slightly Thin Lawn

TL – Thin Lawn

VT – Very Thin Lawn

NC – Not counted due to resistant colonies

7.2 *In Vitro* Assays in Mammalian Cells

Study title: ZS-9: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cell Cultures In Vitro

Study no.: 789301

Study report location: Volume 2.6, p 63

Conducting laboratory and location: (b) (4)

Date of study initiation: August 12, 2010

GLP compliance: Indicated

QA statement: Indicated

Drug, lot #, and % purity: ZS-9, Batch No. 5332-04310-A, purity 87.1% after correction for water content

Reviewer's Summary of Key Study Findings

Two valid GLP chromosomal aberration assays with Chinese hamster ovary cell cultures evaluated the clastogenic potential of ZS-9. In Test 1, ZS-9 was administered as a suspension in culture medium at concentrations of up to 5000 µg/mL. In Test 2, a dimethylsulphoxide (DMSO) extract of ZS-9, prepared by incubating 200 mg ZS-9 per mL of DMSO for 3 days at 37°C with shaking, was applied up to a maximum of 1% (v/v) of the culture medium. Both tests were conducted in the presence and absence of metabolic activation and used concurrent cyclophosphamide or methyl methanesulphonate as positive controls.

In Test 1, precipitation or undissolved ZS-9 was observed in cultures treated with 156-5000 µg/mL in the presence of S9 mix and 78-5000 µg/mL in the absence of S9 mix. No toxicity was observed in the cultures treated in the presence of S9 mix but toxicity was observed at 156 µg/mL and above in the absence of S9 mix. In Test 2, precipitation was observed in cultures treated with ZS-9 eluate at 0.5 and 1% (v/v) in both the presence and absence of S9 mix. No toxicity was noted in any of the cultures treated with the ZS-9 eluate.

Neither ZS-9 nor the extract from ZS-9 induced excess structural chromosomal aberrations or polyploidy in either the presence or absence of metabolic activation indicating that ZS-9 was not clastogenic when tested in vitro with Chinese hamster ovary cells.

Table 39: Sponsor's Tabulated Summary of Study

Genotoxicity: In Vitro		Test Article: ZS-9		Page 1 of 4
Report Title:		ZS-9: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cell Cultures In Vitro		
Test for Induction of: Chromosomal Aberrations		No. of Independent Assays: 2		
Strains: Chinese Hamster Ovary cells (CHO)		No. of Replicate Cultures: 2 (vehicle/ZS-9) 1 (positive controls)		Study No. 789301
Metabolizing System: Aroclor 1254-induced rat liver S9 (10%)		No. Cells Analyzed/Culture: 100 Tests 1 & 2 300 Test 2 (Polyploidy)		
Vehicles:	Test Article: Test 1: Ham's F-10 (suspension) Test 2: DMSO extract	Positive Controls: Cyclophosphamide (+ S9 mix): Ham's F-10 Methyl methanesulphonate (– S9 mix): Ham's F-10		GLP Compliance: yes
Treatment: Continuous for 6 (+ S9) or 22 (– S9) hours. Cultures harvested 24 hours (Test 1 and 2) or 48 hours (Test 2) post treatment.		Date of Treatment: Test 1: 12 August 2010 Test 2: 24 August 2010		
Cytotoxic Effects: Test 1: None (+S9 mix), Dose related decreases in cell counts at ZS-9 concentrations ≥ 156 µg/mL (– S9 mix) Test 2: None (± S9 mix)				
Genotoxic Effects: None				

Assay 1 ¹ (24 hour harvest)						
Metabolic Activation	Test Article	Concentration (µg/mL)	Cytotoxicity (% of Control)	Aberrant Cell Frequency (%)	Abs/Cell	Total Polyploid Cells (% of Cells)
Without Activation	Ham's F10 Medium	0	100	0.0	0.00	0.0
	ZS-9	39	100	0.0	0.01	0.0
		78	97	0.0	0.00	0.5
		156	51	0.0	0.01	0.0
	Methyl methanesulphonate	20	—	5.0	0.10	0.0
		30	—	17.0	0.28	0.0
With Activation	Ham's F10 Medium	0	100	0.0	0.01	0.5
	ZS-9	78	83	0.0	0.01	1.0
		1250	84	0.0	0.00	0.0
		2500	91	0.0	0.00	0.0
	Cyclophosphamide	5000	63	0.5	0.01	0.5
		30	—	6.0	0.11	0.0
		40	—	7.0	0.10	0.0

¹— Mean values from 2 cultures except for positive controls.
 — Not determined

Assay 2 ¹ (24 hour harvest)						
Metabolic Activation	Test Article	Concentration (%)	Cytotoxicity (% of Control)	Aberrant Cell Frequency (%)	Abs/Cell	Total Polyploid Cells (% of Cells)
Without Activation	DMSO	1	100	0.0	0.00	0.0
	ZS-9 (DMSO extract)	0.25	80	0.0	0.01	0.0
		0.5	89	0.0	0.01	0.0
		1	88	0.0	0.00	0.0
	Methyl methanesulphonate	20 g/ml	—	2.0	0.06	0.0
		30 g/ml	—	14.0	0.24	0.0
With Activation	DMSO	0	100	0.5	0.01	2.0
	ZS-9 (DMSO Extract)	0.25	97	0.0	0.00	3.0
		0.5	118	0.0	0.01	4.0
		1.0	119	0.5	0.01	2.5
	Cyclophosphamide	30 g/ml	—	4.0	0.08	0.0
		40 g/ml	—	9.0	0.12	0.0

¹— Mean values from 2 cultures except for positive controls.
 — Not determined

Assay 2 ¹ (48 hour harvest)						
Metabolic Activation	Test Article	Concentration (%)	Cytotoxicity (% of Control)	Aberrant Cell Frequency (%)	Abs/Cell	Total Polyploid Cells (% of Cells)
Without Activation	DMSO	1	100	0.0	0.00	0.0
	ZS-9 (DMSO Extract)	0.25	86	0.0	0.00	0.0
		0.5	74	0.0	0.00	0.0
	Methyl methanesulphonate	1.0	75	0.0	0.00	0.0
		20 g/ml	—	9.0	0.18	0.0
		30 g/ml	—	40.0	1.02	0.0
Assay 2 ¹ (24 hour harvest) Polyploidy Data						
With Activation	DMSO	1	—	—	—	1.15
	ZS-9 (DMSO Extract)	0.25	—	—	—	2.12
		0.5	—	—	—	1.64
		1.0	—	—	—	2.44
Assay 2 ¹ (48 hour harvest) Polyploidy Data						
Without Activation	DMSO	1	—	—	—	0.00
	ZS-9 (DMSO Extract)	0.25	—	—	—	0.17
		0.5		—	—	0.66
		1.0		—	—	1.16

¹— Mean values from 2 cultures except for positive controls.
 — Not determined.

7.3 In vivo Micronucleus Assay

Conducted as part of 28- day repeat dose rat toxicology study.

8 Carcinogenicity

Not performed since ZS is not genotoxic, not absorbed from the gastrointestinal tract, intended treatment duration is less than 6 months, and the drug did not cause local GI effects (e.g., inflammation, irritation, necrosis/regeneration, hyperplasia) in dog 39-week repeat-dose toxicology study.

9 Reproductive and Developmental Toxicology

Male fertility studies were performed in the rat 26 week repeat-dose toxicology study

9.2.1 Study title: The Effect of Oral (gavage) Sodium Zirconium Cyclosilicate on Female Fertility and Embryo-fetal Development in Rats

Conducting laboratory and location: (b) (4)

Study number(s): 496748

Date of study initiation: 02/17/2014

Drug lot/batch number: PD 13007

GLP compliance: Yes

QA statement: Yes

Key Study Findings

Oral administration of sodium zirconium cyclosilicate (0, 1000, 3000 and 6000 mg/kg/day) 14 days prior to mating through gestation day 16 did not produce in-life or post-mortem evidence of toxicity in female rats (estrus cycling, mating performance, fertility or pregnancy). This regimen also did not affect fetal growth or development. Thus, the NOAEL was considered to be 6000 mg/kg/day, the highest dose tested.

Purpose

The purpose of the study was to assess the effect of sodium zirconium cyclosilicate (ZS) on female fertility and pregnancy in female Sprague-Dawley rats. Another aim of the study was to assess the effects of ZS on embryo-fetal development in rats.

Methods

Doses: 0, 1000, 3000, 6000 mg/kg/day total dose

Frequency: 3X/day

Dose volume: 10 ml/kg

Route of administration: oral gavage

Formulation/vehicle: 0.5% methylcellulose in water (w/v)

Species/strain: Sprague-Dawley rat

Number/group: 20/group

Satellite groups: No

Study design:

Table 40: Effect of ZS on Female Fertility and teratogenicity in Rats: Experimental Design

Group No.	Treatment (mg/kg/day)	Dosage (mg/kg/dose)	Animal Numbers
			Females
1	0	0	1-20
2	1000	333	21-40
3	3000	1000	41-60
4	6000	2000	61-80

Deviation from study protocol: None

Results

Mortality: None

Clinical signs: None

Body weight and feed consumption: None

Toxicokinetics: Not performed (drug not absorbed from GI tract)

Dosing solution analysis:

Table 41: Effect of ZS on Female Fertility and Teratogenicity in Rats: Dose Formulation Analysis Results

Nominal Concentration (mg/mL)	Mean Measured Concentration (mg/mL)	Mean Accuracy (% Recoveries)	Mean Precision (% CV)
<u>Week 1</u>			

33	32	-2.5	1.8
100	106	5.6	2.6
200	139	-30.6	2.3
<u>Week 3</u>			
33	32	-3.1	0.7
100	102	2.4	2.1
200	196	-1.8	0.9

Necropsy: No gross pathology effects were observed.

Fertility Data:

Estrus cycle length, mating performance, fertility, pregnancy performance: No effect

Pregnancy Performance

Mean number of corpora lutea graviditatis, number of live implants, number of dead implants: No effect

Offspring

Fetal growth, Fetal Development: No effect

9.2.2 Study title: Developmental Toxicity Study of Sodium Zirconium Cyclosilicate by Oral Gavage Administration in the Rabbit

Conducting laboratory and location:

(b) (4)

Study number(s): 496863

Date of study initiation: 08/04/2014

Drug lot/batch number: ZS 140002

GLP compliance: Yes

QA statement: Yes

Key Study Findings

Oral administration of sodium zirconium cyclosilicate (0, 1500, 3000 and 6000 mg/kg/day) from gestation day 6 through 18 did not produce in-life or post-mortem evidence of toxicity in female rabbits (as well as pregnancy performance: mean number of corpora lutea graviditatis, number and type of uterine contents). This regimen also did not affect fetal growth or development. Thus, the NOAEL was considered to be 6000 mg/kg/day, the highest dose tested.

Purpose

The purpose of the study was to assess the effect of sodium zirconium cyclosilicate (ZS) on maternal toxicity in female New Zealand White rabbits. Another aim of the study was to assess the effects of ZS on embryo-fetal development in rabbits.

Methods

Doses: 0, 1500, 3000, 6000 mg/kg/day total dose

Frequency: 3X/day

Dose volume: 10 ml/kg

Route of administration: oral gavage

Formulation/vehicle: 0.5% methylcellulose in water (w/v)

Species/strain: New Zealand White

Number/group: 20/group

Satellite groups: No

Study design:

Table 42: Teratogenicity Study in Rabbits: Experimental Design

Group No.	Treatment (mg/kg/tid)	Treatment (mg/kg/day)	Animal Numbers
1	0	0	1-20
2	500	1500	21-40
3	1000	3000	41-60
4	2000	6000	61-80

Deviation from study protocol: None

Results

Mortality: One dam died during dosing due to a gavage error.

Clinical signs: None

Body weight and feed consumption: None

Toxicokinetics: Not performed (drug not absorbed from GI tract)

Dosing solution analysis:

Table 43: Teratogenicity Study in Rabbits: Dose Formulation Analysis Results

Nominal Concentration (mg/mL)	Mean Measured Concentration (mg/mL)	Mean Accuracy (% Recoveries)	Nominal Concentration (mg/mL)	Mean Measured Concentration (mg/mL)	Mean Accuracy (% Recoveries)
<u>Week 1 (delivery 1)</u>			<u>Week 1 (delivery 2)</u>		
50	49.2	98.3	50	48.5	96.9
100	95.7	95.7	100	95.6	95.6
200	184.0	92.0	200	172.3	86.3

<u>Last week of dosing (delivery 1)</u>			<u>Last week of dosing (delivery 2)</u>		
50	48.2	96.4	50	48.7	97.5
100	98.4	98.6	100	96.7	96.6
200	176.0	88.1	200	172.3	86.3

Necropsy: No gross pathology effects were observed.

Pregnancy Performance

Mean number of corpora lutea graviditatis, number and type of uterine contents:

No effect

Offspring

Fetal growth and sex distribution, Fetal Development: No effect

9.3. Study title: A Pre and Post Natal Study Study of Orally (gavage) Administered Sodium Zirconium Cyclosilicate in Rats

Conducting laboratory and location:

(b) (4)

Study number(s): 496753

Date of study initiation: 04/07/2014

Drug lot/batch number: ZS 140002

GLP compliance: Yes

QA statement: Yes

Key Study Findings

Oral administration of sodium zirconium cyclosilicate (0, 1000, 3000 and 6000 mg/kg/day) from gestation day 6 until weaning did not produce in-life or post-mortem evidence of toxicity in female rats (F0) as well as the F1 generation assessing body weight, vaginal opening preputial separation, performance or functional tests and reproductive parameters. No effects were observed in the F2 generation looking at survival and weights to LD 14. Thus, the NOAEL was considered to be 6000 mg/kg/day, the highest dose tested.

Purpose

The purpose of the study was to assess in mated female Sprague-Dawley rats (F0) given oral doses of sodium zirconium cyclosilicate (ZS) from the time of implantation until weaning on the pregnant/parturient/lactating F0 female. Another aim of the study was to assess the effects of ZS on the development of the conceptus and offspring (F1) to sexual maturity.

Methods

Doses: 0, 1000, 3000, 6000 mg/kg/day total dose

Frequency: 3X/day

Dose volume: 10 ml/kg

Route of administration: oral gavage

Formulation/vehicle: 0.5% methylcellulose in water (w/v)

Species/strain: Sprague-Dawley rat

Number/group: 24/group

Satellite groups: No

Study design:

Table 44: A Pre-and Post Natal Study of ZS in Rats: Experimental Design

Group No.	ZS Treatment (mg/kg/day)	ZS Dosage (mg/kg/dose)	Animal Numbers
			Females
1	0	0	1-24
2	1000	333	25-48
3	3000	1000	49-72
4	6000	2000	73-96

Two spares were provided, however they were not required for replacement and were considered not to be part of the study

Group No.	F1 Male Numbers	F1 Female Numbers
1	101-119	201-219
2	125-146	225-246
3	149-171	249-271
4	173-192	273-292

Deviation from study protocol: None

Results

Mortality: Four dams killed prematurely: One control animal that was not using its hindlimbs; two high dose animals that exhibited clinical signs such as hunched posture, piloerection and rolling gait; and one high dose animal experiencing parturition difficulties

Clinical signs: Pale feces in high dose animals

Body weight and feed consumption: None

Toxicokinetics: Not performed (drug not absorbed from GI tract)

Dosing solution analysis:

Table 45: A Pre- and Post Natal Study of ZS in Rats: Dose Formulation Analysis Results

Nominal Concentration (mg/mL)	Mean Measured Concentration (mg/mL)	Mean Accuracy (% Recoveries)
<u>Week 1</u>		
33.3	33.1	99.5
100	87.6	87.7
200	169.7	84.8
<u>Week 5/6</u>		
33.3	35.4	106.3
100	89.6	89.7
200	166.7	83.3

Necropsy: No gross pathology effects were observed.

Pregnancy Performance: Duration of F0 gestation, number of implants, number of pups born, weights of litters or pups born, fetal abnormalities: No effect

F1 generation: pup survival over LD 0-21, pre-weaning physical or functional developmental landmarks (pinna detachment, upper incisor eruption, open eyes, mean litter percent of pups reaching criterion for negative geotaxis, auditory and visual function), post weaning clinical observations, post weaning body weights, age and weight at sexual maturity, post weaning neuronal behavior, mating performance, fertility and gestation length, mean number of implant sites, litter weights and necropsy : No effect

F2 generation: viability index, pup weight, abnormalities and necropsy of F2 animals: No effect.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Brief Background / Introduction

Zirconium Cyclosilicate (ZS) is a new molecular entity developed for the treatment of hyperkalemia in patients with chronic kidney disease. Chemically, it is a selective cation exchanger that acts in the gastrointestinal tract to trap potassium ions as well as ammonium ions in exchange for sodium ions. The indication will be for hyperkalemia

(b) (4)

Pharmacology

In vitro the exchange capacity of ZS-9 for potassium cations is (b) (4) mEq/gram. A 5-day pharmacology study in rats showed that the daily oral administration of ZS-9 at doses of 2, 4, or 6 gm/kg/day to rats decreased the urinary and increased the fecal concentration of potassium and urea nitrogen and increased urinary and decreased the fecal sodium concentration in a dose dependent manner. No effect was observed on urinary or fecal calcium and magnesium excretion or on serum calcium, magnesium, sodium, potassium or urea nitrogen concentrations.

A non-GLP mass balance study in rats showed the absence of systemic absorption following a single dose of 2000 mg ZS-9/kg when administered in food. Measurement of the amount of zirconium in feces and urine indicated that 97-101% of the administered ZS-9 dose was present in the feces and 0.1-0.3% was present in the urine collected over three days.

No safety pharmacology studies were performed with ZS.

Toxicology

In rat and dog maximum tolerated dose and 7-day repeat-dose toxicity study, the NOAEL was the highest dose administered (2000 mg/kg).

In the 28-day repeat-dose toxicity study in rats (with a 14-day recovery period), the incidence of renal tubular casts was increased in both sexes at the highest dose tested (2000 mg/kg, TID). This effect was not observed in the 26-week toxicology study in rats. This was most likely due to a change in the formulation (b) (4)

(b) (4) In this 28-day rat study, ZS did not induce excess formation of micronuclei in rat bone marrow cells.

In the 26-week repeat-dose rat study (with a 4-week recovery period), most of the effects observed were related to the pharmacological effect of lowering plasma potassium in healthy animals. Along with decreases in plasma potassium, there were decreases in plasma urea, increases in urinary sodium and chloride, and decreases in urinary potassium and magnesium.

In the dog 28-day repeat-dose toxicology study (with a 21-day recovery period), doses were administered up to 100 mg/kg TID. One group was supplemented with potassium chloride while receiving the highest dose. An increase in QTc observed in the highest dose group was obtunded by potassium chloride supplementation. Thus, this effect was due to hypokalemia. In addition, tuberointerstitial inflammation was present in all animals in the 1000 mg/kg TID group. This effect was eliminated in the group that received the same dose with potassium supplementation. Thus, many of the adverse effects observed in this study were reversed by potassium supplementation. Therefore, the NOAEL was 300 mg/kg TID for ZS alone but 1000 mg/kg TID with potassium supplementation.

In the 39-week dog repeat-dose toxicology study (with a 28-day recovery period), renal tuberointerstitial inflammation was also observed in 5/8 animals administered the highest dose (2000 mg/kg, TID). However, in animals supplemented with potassium chloride (253 mg/kg/day), the incidence fell to 2/8 at the highest dose tested. Again, as

in the previous study in canines, the major effects observed were subsequent to plasma potassium depletion and were reversed by co-administration of potassium chloride. Genotoxicity studies such as Ames test and chromosomal aberration assays with CHO cells were negative.

Reproductive toxicity tests were also negative. These studies included a male fertility study (rat), female fertility study (rat), teratogenicity studies in rat and rabbit and a pre- and post-natal study in rats.

Carcinogenicity studies were not performed.

Conclusions

Zirconium cyclosilicate is acceptable for approval from a pharmacology/toxicology perspective. It is effective in reducing plasma potassium. The drug is not absorbed from the GI tract. Most of its adverse effects in animals relate to its pharmacological effect since these adverse effects can be obtunded by potassium chloride supplementation in the major dog repeat-dose toxicological studies. There appear to be no mutagenic and carcinogenic concerns with the compound. I recommend approval.

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/s/

PHILIP J GATTI
01/08/2016

THOMAS PAPOIAN
01/08/2016
Concur.