CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	209363
Supporting document:	1
Applicant's letter date:	January 17, 2017
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Product:	SOLOSEC® (secnidazole)
Indication:	Treatment of bacterial vaginosis
Applicant:	Symbiomix Therapeutics, LLC
Review Division:	Division of Anti Infective Products
Reviewer:	Owen G. McMaster, Ph.D.
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TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

SOLOSEC® (secnidazole) is a nitroimidazole antimicrobial that is approved in several countries outside of the US for bacterial vaginosis, giardiasis, and amoebiasis. Symbiomix Therapeutics, LLC seeks to market SOLOSEC in the US as a single-dose, oral treatment of bacterial vaginosis in adult females. The 5-nitroimidazoles enter the bacterial cell as inactive prodrugs and are subsequently reduced to a radical anion form that exerts the primary antimicrobial activity.

1.2 Brief Discussion of Nonclinical Findings

There were no nonclinical data that preclude the approval of SOLOSEC. The nonclinical toxicology program supports marketing of SOLOSEC as a single-dose oral treatment of bacterial vaginosis. The nonclinical studies conducted were appropriate and adequate per ICH guidelines and included *in vitro* and *in vivo* studies using rats, dogs and rabbits for up to 28 days in addition to evaluations of safety pharmacology, genotoxicity, fertility, embryofetal toxicity and pre- and postnatal development. Study conduct was acceptable and pivotal studies were GLP-compliant. Secnidazole resulted in toxic effects in the brain, kidney, liver, thymus, adrenals, spleen, testes and DNA, but these findings were only seen after high and/or repeated doses.

In safety pharmacology studies secnidazole resulted in reduced motor activity, a slight, transient decrease in blood pressure with reflex tachycardia and lower P-R interval in dogs.

Reduced food intake, with concomitant reductions in body weight gain were observed in several animal studies. These findings are consistent with the nausea, dysgeusia and metallic taste that were among the most common adverse events reported in the US clinical trials and in the label of the French secnidazole product SECNOL®.

Minimal hyaline droplet accumulation in the proximal tubules was seen at the lowest dose in the 7 day rat study. This finding may represent an accumulation of secondary lysozymes within the cytoplasm containing proteins (such as α_{2u} globulin) reversibly bound to secnidazole or a metabolite. These findings are not considered to be predictive of human toxicity.

In a 7 day dog study, doses about 5 times the clinical exposure showed morbidity within 4 days and symptoms including ataxia, decreased activity, partially closed eyes, vomiting, tremors, seizures, recumbence and brain lesions including vacuolar degeneration and gliosis of the lateral reticular nuclei, medial vestibular nuclei, dentate nuclei, and the vestibulo-cerebellar tract. Neurotoxic effects, including seizures and peripheral neuropathy, have been reported in patients after 5 to 7 days of dosing with another 5-nitroimidazole drug, metronidazole (6 to 10 g, every other day), equivalent to about 2 to 5 times the clinical dose for trichomoniasis.

Excessive reductions in maternal body weight gain in several reproductive toxicology studies indicated that effects seen could be secondary to reduced body weight gain. Reduced bodyweight gain and stress are known to be associated with fetal skeletal effects such as supernumerary ribs and delayed ossification in pups. At nontoxic maternal doses, there were no adverse effects on the fetuses with SOLOSEC. This lack of findings is consistent with the reproductive toxicology data for metronidazole. High doses of secnidazole have been associated with changes in the male reproductive system (including sperm morphology, epididymis and testes), but these changes are irrelevant to the current indication for bacterial vaginosis in adult women.

Although there are no nonclinical data to indicate that SOLOSEC would be harmful to the unborn fetus, there are no clinical trial data in pregnant women. Since animal studies are not always predictive of human toxicities, the (b) (4)

Secnidazole is genotoxic but only a single dose is to be administered at a particular time. Although the genotoxicity of nitroimidazoles has raised concerns about the risk of prenatal exposure, metronidazole is used in children for treatment of anaerobic infections and amoebiasis and an oral suspension of secnidazole has been studied in Venezuelan children, 2 to 11 years old, infected with *Giardia intestinalis*.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology or toxicology data that preclude the approval of SOLOSEC.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical pharmacology or toxicology studies of SOLOSEC are being recommended at this time.

1.3.3 Labeling

The labeling review team has recommended the following wording for SOLOSEC® prescribing information:

(b) (4)

12 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

2 Drug Information

2.1 Drug

CAS Registry Number: 3366-95-8

Generic Name: Secnidazole

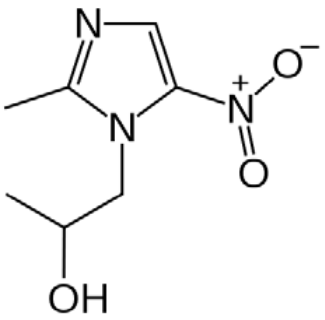
Code Name: SYM-1219

Chemical Name: 1-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol

Molecular Formula: $C_7H_{11}N_3O_3$

Molecular Weight: 185.18

Structure or Biochemical Description



Pharmacologic Class: nitroimidazole antimicrobial

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 117,811
DMF (b) (4)

2.3 Drug Formulation

SOLOSEC is supplied as a packet with 4.8 g of off-white to slightly yellowish granules, which contain 2 g of secnidazole and the following inactive ingredients: Eudragit NE30D (ethyl acrylate methyl methacrylate copolymer, (b) (4) mg), polyethylene glycol 4000 ((b) (4) mg), povidone ((b) (4) mg) , sugar spheres ((b) (4) mg), and talc ((b) (4) mg).

2.4 Comments on Novel Excipients. There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

The only identified impurity in the drug product is a (b) (4) . It is a process impurity and is controlled in the API. It is not controlled with a

specification in the drug product. This compound has been measured in clinical batches at (b) (4) %. The specified limits for any single unknown impurity (NMT(b) (4)%) and for total unknown impurities (NMT (b) (4)%) are consistent with those in ICH Q3B (R2) for a daily dose of 2g.

2.6 Proposed Clinical Population and Dosing Regimen

SOLOSEC oral granules are proposed as a single-dose oral treatment of bacterial vaginosis

2.7 Regulatory Background

Secnidazole has never been approved for use in the US. It is approved for use in several other countries and this granule formulation (b) (4).

The following is a summary of the regulatory history for NDA 209363 relevant to this review:
• 12/18/2013: IND 117811 submitted for secnidazole for the treatment of BV
• 11/18/2014: Qualified Infectious Disease Product (QIDP) designation granted under IND 117811
• 08/12/2015: Fast Track Designation granted
• 06/09/2016: Applicant informed that a Type C face to face meeting would not be granted since issues were primarily related to CMC, and follow-up would be through written responses. The need for REMS was not discussed at any of the pre-NDA meetings
• 01/17/2017: NDA 209363 submission received

3 Studies Submitted

3.1 Studies Reviewed

Study # 030622:	Effect of SYM-1219 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
Study # 030623:	SYM-1219: Single Oral Dose Cardiovascular Safety Pharmacology Study in Female Beagle Dogs
Study # 030624:	SYM-1219: Single Oral Dose CNS Safety Pharmacology Study in Rats
Study # 030625:	SYM-1219: Single Oral Dose Respiratory Safety Pharmacology Study in Rats
Study # 030613:	SYM-1219: Oral Rising-Dose and 7-Day Oral Repeat-Dose Toxicity and Toxicokinetics study in rats
Study # 030614:	SYM-1219: Oral Rising-Dose and 7-Day Oral Repeat-Dose Toxicity and Toxicokinetics study in dogs
Study # 030615:	SYM-1219: 28-Day Oral Toxicity and Toxicokinetic Study in Rats with a 14-Day Recovery period
Study # 030616:	SYM-1219: 28-Day Oral Repeat-Dose Toxicity and Toxicokinetic Study in Dogs with a 14-Day Recovery Period
Study # 030627:	Bacterial Reverse Mutation Assay

Study # 030629:	*In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay)
Study # 030630:	*In Vivo* Micronucleus Assay in Rats
Study # 993006:	An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation of SYM-1219 in Rats
Study # 993002:	An Oral (Gavage) Study of the Effects of SYM-1219 on Embryo/Fetal Development in Rats
Study # 993005:	An Oral (Gavage) Study of the Effects of SYM-1219 on Embryo/Fetal Development in Rabbits
Study # 993008:	An Oral (Gavage) Study of the Effects of SYM-1219 on Pre and Postnatal Development Including Maternal Function in Rats

3.2 Studies Not Reviewed

Dose range finding studies and non-pivotal studies were not reviewed.

3.3 Previous Reviews Referenced: N/A

4 Pharmacology

4.1 Primary Pharmacology

Secnidazole, like other 5-nitroimidazoles enters the microbial cell as an inactive prodrug where the nitro group is reduced to toxic radical anions and nitrite ions, both of which are believed to be responsible for antibacterial activity. The primary mechanism of action is believed to be the interaction of the toxic radical ion with microbial DNA, which interferes with its synthesis, leading to cell death. Please see the clinical microbiologist's review for additional details.

4.2 Secondary Pharmacology

No secondary pharmacology studies were performed.

4.3 Safety Pharmacology

Study title:	Effect of SYM-1219 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
Study report location:	eCTD 4.2.1.3
Conducting laboratory :	(b) (4)
Date of study initiation:	February 5, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	F120044
Purity (%):	99.7
Vehicle	HEPES-buffered physiological saline (HB-PS) solution composed of: NaCl, 137 mM ; KCl, 4.0 mM; CaCl2, 1.8 mM; MgCl2, 1 mM; HEPES, 10 mM; Glucose, 10 mM; pH adjusted to 7.4 with NaOH and supplemented with 0.3% DMSO.
Positive control	Terfenadine 60 nM in vehicle.
Reference substance	E-4031, 500 nM

Key Study Findings: Secnidazole did not inhibit IKr at concentration up to 300 µM.

Methods

In this study, whole-cell voltage clamp technique was used to determine the effects of secnidazole (three concentrations ranging from 10 to 300 x 10^{-6} M) on the IKr-like membrane potassium current in a human embryonic kidney cell line (HEK293) transfected with the human *ether-à-go-go*-related gene (hERG).

Results

Data show that secnidazole concentrations up to 300 µM had no effect on the membrane K+ current (IKr) in HERG-transfected HEK293 cells compared to solvent. Under the same conditions, terfenadine resulted in the inhibition of the IKr at nanomolar concentrations.

Figure 1: Peak current amplitude during application of vehicle control, 300 µM secnidazole (SYM-1219) and the reference substance E-4031.

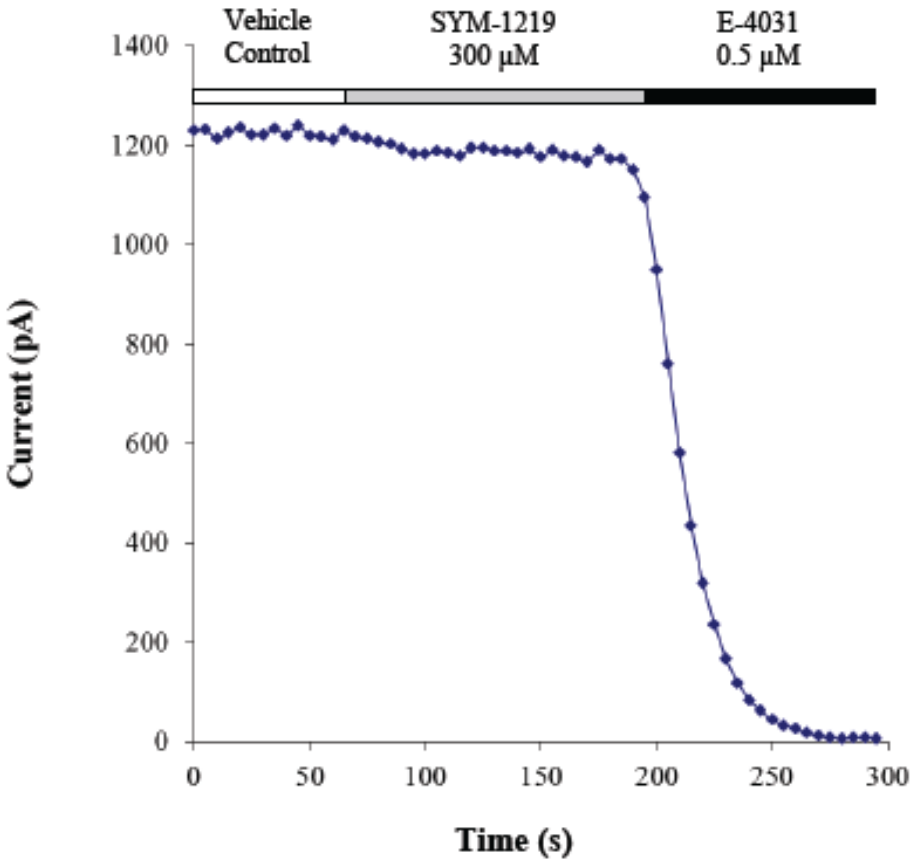


Table 1: Effect of increasing concentrations of secnidazole on HERG-mediated K+ current in HEK293 cells compared to terfenadine

Test compound	Concentration (μM)	Mean percent inhibition (%)	SD (%)
Vehicle control		1.6	± 1.3
Secnidazole	10 μM	2.9	±1.7
Secnidazole	300 μM	3.0	±2.4
Terfenadine	60 nM	78.0	±2.7

Conclusion
Secnidazole did not inhibit IKr at doses that were above 300 μM while terfenadine inhibited IKr by 78 % at 60 nM. All formulations were homogeneous and concentrations were within ± 15.0% of nominal concentrations.

Study title:	SYM-1219: Single Oral Dose Cardiovascular Safety Pharmacology Study in Female Beagle Dogs
Study no.:	030623
Document location	eCTD 4.2.1.3
Conducting laboratory :	(b) (4)
Date of study initiation:	29-May-2013
GLP compliance:	GLP
QA report :	Yes
Drug, lot #,	F120044.
% purity:	99.7 %
Key study findings:	A single dose of secnidazole orally administered to dogs resulted in a slight, transient decrease in blood pressure with a reflex tachycardia and lower P-R interval at 200 mg/kg.

Methods

Doses:	0, 20, 60 and 200 mg/kg
Species/strain:	*Canis familiaris,* beagle dogs
Age	1-3 years
Weight	6.90 to 8.35 kg
Route	Oral gavage
Formulation	Test article was suspended in 2% CMC (carboxymethylcellulose, 400 c.p.)
Volume	10 mL/kg
Number/sex/group	Four female dogs

Secnidazole or vehicle was administered once per day, by oral gavage, with a 3-day washout between doses, until all doses were administered to each dog. See Table 2, below

Table 2: Study design for Single Oral Dose Cardiovascular Safety Pharmacology Study in Female Beagle Dogs

Animal ID	Dose event			
	1	2	3	4
	Secnidazole dose			
1501	0 (vehicle)	20 mg/kg	200 mg/kg	60 mg/kg
1502	20 mg/kg	60 mg/kg	0 (vehicle)	200 mg/kg
1503	60 mg/kg	200 mg/kg	20 mg/kg	0 (vehicle)
1504	200 mg/kg	0 (vehicle)	60 mg/kg	20 mg/kg

Results

Clinical observations

No animals died or were deemed moribund during the study. The dogs for this study were previously surgically implanted with Data Sciences International (DSI) transmitters which were used to record ECG and other cardiovascular parameters. .

At 200 mg/kg, dogs experienced reductions in systolic blood pressure (as low as -15% compared to concurrent controls) between 30 minutes and 5 hours postdose. Although these pressures at the high dose were consistently lower than the concurrent controls at those time points, the range of blood pressures at the high dose (about 128 to 158 mmHg) were only slightly outside the range of blood pressures of the concurrent controls (136 to 162 mmHg).

Mean Blood Pressure was also significantly lower (as much as -12 %, compared to concurrent controls) for the dogs receiving 200 mg/kg from 30 minutes to 1.75 hours, and at the 2.5- and 3.25-hour time points. The range of blood pressures at the high dose (about 94 to 119 mmHg) was similar to the range of blood pressures of the concurrent controls (98 to 122 mmHg).

Heart rate was significantly increased (up to +44%), from 45 minutes to 1.25 hours and 2 hours postdose for dogs receiving 200 mg/kg SECNIDAZOLE . The range of heart rates in the high dose group (74-133 bpm) was marginally higher than the range observed in the controls (70 to 113). The increased heart rate was ascribed to a baroreflex response to the drop in systolic and mean blood pressure.

Significantly lower P-R interval means were observed from 45 minutes to 1.25 hours, 2 to 3.25 hours, and 3.75 to 4 hours postdose for the high dose group. The range of PR intervals in the high dose (85 to 99 msec) was marginally outside of the range of the controls in this study (92 to 101 msec). The shorter PR interval was considered to be related to the increased heart rate.

In conclusion, a single dose of secnidazole orally administered to dogs resulted in a slight, transient decrease in blood pressure with a reflex tachycardia and lower P-R interval at 200 mg/kg.

Study title:	SYM-1219: Single Oral Dose CNS Safety Pharmacology Study in Rats
Study no.:	030624
Document location	eCTD 4.2.1.3
Conducting laboratory :	(b) (4)
Date of study initiation:	21-May-2013
GLP compliance:	GLP
QA report :	Yes
Drug, lot #,	F120044.
% purity:	99.7 %
Key Study findings:	Motor activity was significantly (-60%) lower than controls 1 to 2 hours after dosing with 1000 mg/kg secnidazole in rats. These transient changes were not seen 24 hours postdose. These findings were consistent with the recumbency/ataxia findings in other studies of high dose secnidazole.

Methods

Doses:	0, 100, 300 and 1000 mg/kg
Species/strain:	*Rattus norvegicus,* Crl:CD(SD) rats
Age	8 weeks
Weight	Females: 149-224 Males: 214-269g
Route	Oral gavage
Formulation	Test article was suspended in 2% CMC (carboxymethylcellulose, 400 c.p.)
Volume	10 mL/kg
Number/sex/group	10

Observation Items:

Functional Observational Battery (FOB)

The Functional Observational Battery (FOB) was comprised of 4 sets of observations. The battery included home cage observations, handling observations, open-field observations and handling/specific testing of the animal.

Home cage observations:
Posture, involuntary motor movements, biting, palpebral closure, vocalizations

Reflex determinations:
Approach response, touch response, auditory response, tail pinch response, eye blink response righting reflex, hind limb extensor strength response, and pupillary size.

Observations made while handling animals:

Ease of removing animal from cage, ease of handling animal, lacrimation, color of tears, salivation, piloerection, fur-appearance, palpebral closure, exophthalmos, respiration

Open-field evaluations:
Mobility, posture, involuntary motor movements, gait abnormalities, reactivity to environment (arousal), stereotypical behavior - any repetitive action, bizarre behavior, number of rears, defecation, urination, vocalizations.

Motor Activity Test
Each rat was placed in an Opto-Varimex Animal Chamber designed to measure motor activity, which were recorded every minute during a 15-minute session.

Results:

FOB parameters
Rats receiving 100, 300, or 1000 mg/kg secnidazole had no test article-related changes in FOB parameters at 1 to 2 hours or 23.5 to 24.5 hours postdose.

Motor activity
Motor activity in male rats receiving 1000 mg/kg secnidazole had Bursts of Stereotypic Movement (BMS), Vertical Breaks (V1C), and Vertical Counts (V1B) that were significantly lower than control at 1 to 2 hours postdose (Table 3). These findings represent a general decrease in level of activity and were considered test article related. Female rats dosed with 1000 mg/kg secnidazole had Horizontal Counts (HC), Vertical Breaks (V1C), and Vertical Counts (V1B) that were significantly lower than control at 1 to 2 hours postdose. These findings represent a general decrease in level of activity and were considered drug-related. None of these changes were detectable at the 23.5 to 24.5 time point.

Conclusion

Secnidazole did not induce any changes in FOB parameters in males or females at any dose at any time point but induced a significant, transient reduction in motor activity at 1000 mg/kg 1 to 2 hours after dosing. This (up to 60 %) reduction was not observed at 23.5 to 24.5 hours. No drug-related effects were noted on motor activity in male or female rats at doses below 1000 mg/kg.

Study title:	SYM-1219: Single Oral Dose Respiratory Safety Pharmacology Study in Rats
Study no.:	030625
Document location	eCTD 4.2.1.3
Conducting laboratory :	(b) (4)
Date of study initiation:	21-May-2013
GLP compliance:	GLP
QA report :	Yes
Drug, lot #,	F120044.
% purity:	99.7 %
Key study findings:	NOAEL for respiratory effects was a single 1000 mg/kg dose of secnidazole in rats.

Methods

Doses:	0, 100, 300 and 1000 mg/kg
Species/strain:	*Rattus norvegicus*. Crl:CD(SD) rats
Age	7-8 weeks
Weight	6234-287 g
Route	Oral gavage
Formulation	Test article was suspended in 2% CMC (carboxymethylcellulose, 400 c.p.)
Volume	10 mL/kg
Number/sex/group	Four male rats

A single dose of secnidazole or vehicle was administered by oral gavage to 4 groups of Crl: CD (SD) rats (4 males /group). Respiratory data were collected using whole body plethysmographs and a data collection software system. Respiratory parameters collected were respiratory rate, tidal volume, and minute volume. Respiratory data were collected at predose (1 hour duration), from 0 to 6 hours postdose, and for one hour including the 24-hour time point.

Results

No animals died or were deemed moribund during the study. There were no changes in respiratory rate, tidal volume, or minute volume for male rats receiving 100, 300, or 1000 mg/kg secnidazole by oral administration.

Figure 2: Respiratory Rate (breaths/min) response in male CD rats following 0, 100, 300, or 1000 mg/kg p.o. secnidazole

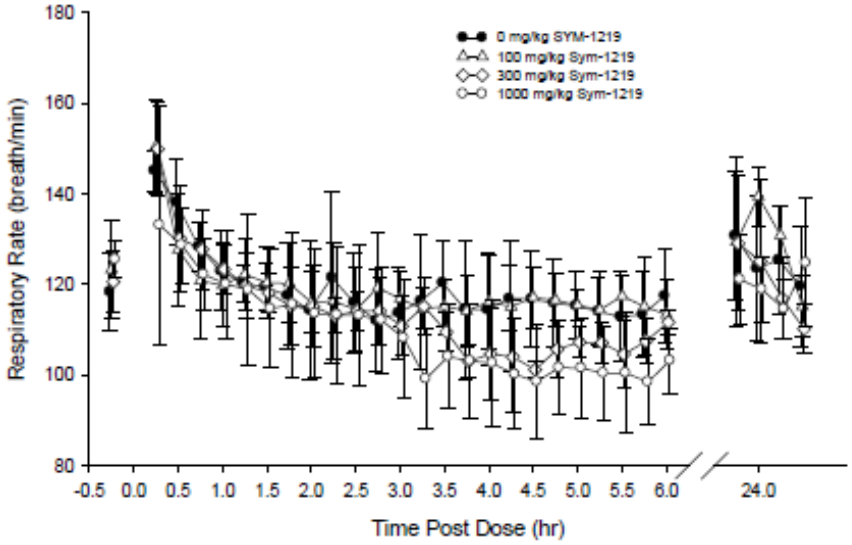


Figure 3: Tidal Volume (mL/breath response in male CD rats following 0, 100, 300, or 1000 mg/kg p.o. secnidazole

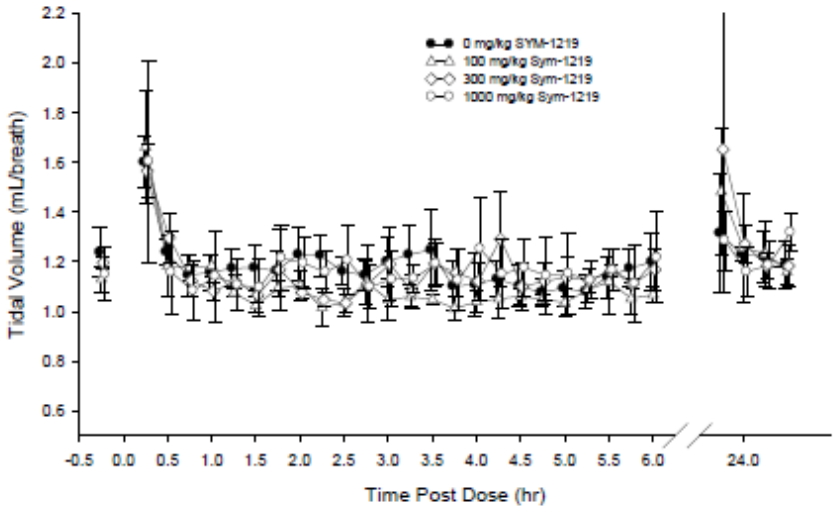
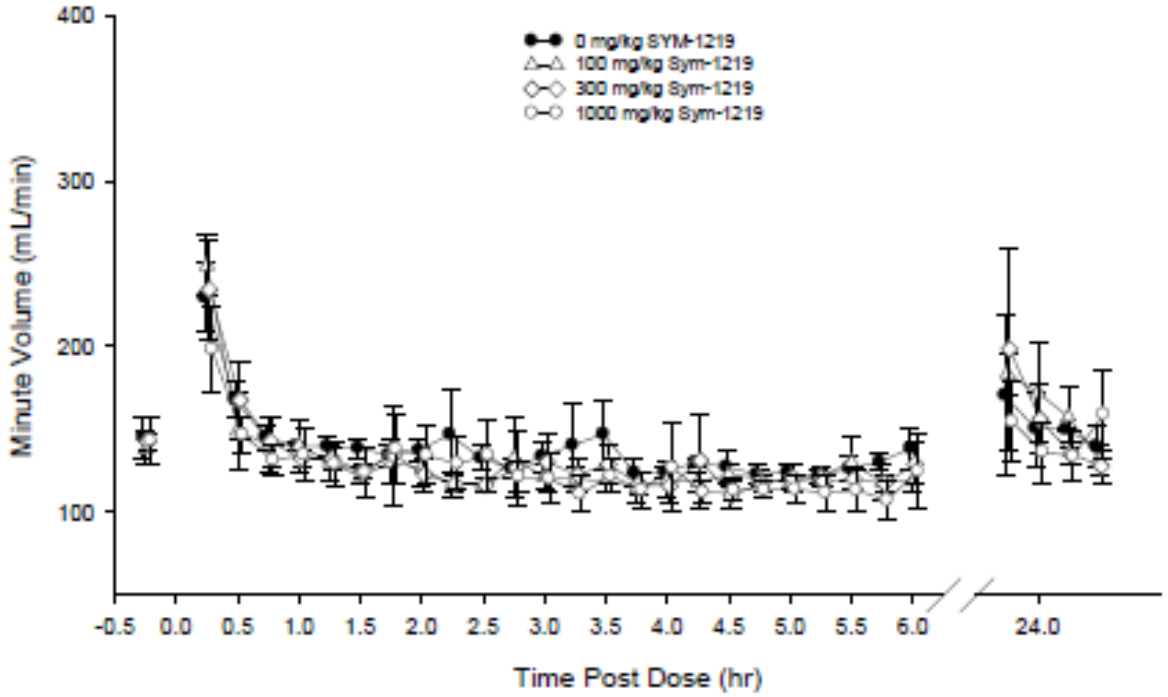


Figure 4: Minute Volume (mL/min) response in male CD rats following 0, 100, 300, or 1000 mg/kg p.o. SYM-1219



Discussion:

In conclusion, a single dose of secnidazole orally administered to rats had No-Observable Effect on respiratory parameters at 1000 mg/kg.

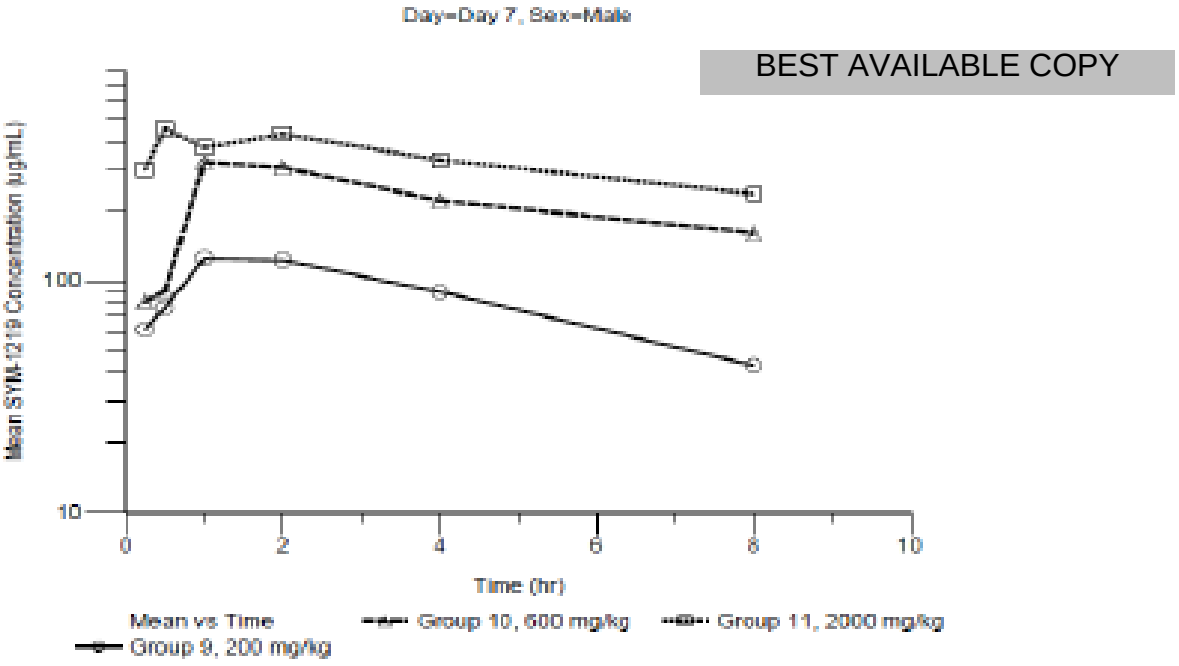
5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

No dedicated nonclinical pharmacokinetics studies were conducted. Secnidazole absorption and excretion data are described in the toxicokinetics data accompanying the toxicology studies. Typically T_{max} was reached within 0.5 to 2 hours after a single oral gavage administration. The plasma concentrations (C_{max}) increase was generally less than proportional to dose, but AUC increase was generally greater than proportional to dose on Day 1 in rats and dogs. A single, oral 2 g dose of SOLOSEC in healthy adult female subjects resulted in a C_{max} of 45 µg/ml, $AUC_{0\text{-}inf}$ of 1332 µg•hr/mL and T_{max} of 4 hours.

Figure 5: Mean plasma secnidazole concentrations versus time (Day 7) in male rats



A single oral dose of 2 g of SOLOSEC in healthy adult female subjects, following an overnight fast and admixed with (4 oz.) of applesauce, resulted in a mean (%CV) secnidazole peak plasma concentration (C_{max}) of 45.4 (16.8%) µg/ml and mean (%CV) systemic exposure ($AUC_{0\text{-}inf}$) of 1331.6 (17.3%) µg•hr/mL. Median (range) time to peak concentration (T_{max}) was 4.0 (3.0-4.0) hours. Following administration of the 2 g dose, mean secnidazole plasma concentrations decreased to 22.1 µg/mL at 24 hours, 9.2 µg/mL at 48 hours, 3.8 µg/mL at 72 hours, and 1.4 µg/mL at 96 hours.

Effect of Food

Administration of 2 g of SOLOSEC admixed with applesauce followed by a high fat meal resulted in no significant change in secnidazole exposure (C_{max} or AUC) as compared to administration when admixed applesauce and taken under fasted conditions.

Distribution

Nonclinical tissue distribution, protein binding, and placental transfer studies for secnidazole have not been conducted. In human subjects, the apparent volume of distribution of secnidazole is approximately 42 L. The plasma protein binding of secnidazole is <5%.

Metabolism

Metabolism information from animals is not available. *In vitro,* secnidazole is metabolized via oxidation by human hepatic CYP450 enzyme system with ≤ 1% conversion to metabolites. See the Clinical Pharmacology review for additional information.

Excretion:

Studies evaluating the excretion of secnidazole have not been performed. In patients, approximately 15% of a 2 g oral dose of SOLOSEC is excreted as unchanged secnidazole in the urine.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title:	SYM-1219: Oral Rising-Dose and 7-Day Oral Repeat-Dose Toxicity and Toxicokinetic Study in Rats
Study no.:	030613
Document location	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	06-Mar-2013
GLP compliance:	Non GLP
QA report :	Yes
Drug, lot #,	F120044.
% purity:	99.7 %
Key study findings:	The maximum nonlethal single dose was 2000 mg/kg. Minimal hyaline droplet accumulation in the proximal tubules was observed in females at doses of 600 mg/kg and higher and at all doses in males. These kidney findings are not considered to be predictive of human toxicity.

Methods

Doses:	Phase 1: 1000, 2000 mg/kg (single dose) Phase 2: 0, 200, 600, 2000 mg/kg/day (7 daily doses)
Species/strain:	*Rattus norvegicus*/Crl:CD(SD)
Number/sex/group :	Phase 1: 2/sex/dose group Phase 2: 5/dose group (main study), 6/sex/dose group (TK animals)
Age	8 weeks old
Weight	167 to 282g
Route	Oral gavage
Formulation	Test article was suspended in 2% CMC (carboxymethylcellulose, 400 c.p.)
Volume	10 mL/kg
Deviation from study protocol:	There were no protocol deviations that impacted the results or interpretation of the study

Table 3: Group assignments for 'Oral rising-dose and 7-day oral repeat-dose toxicity study in rats'.

Group	Dose (mg/kg)	# of animals M/F	Dose conc. (mg/mL)	Dose volume (mL/kg)
1	1000	2/2	100	10
2	2000	2/2	200	10
3	NT	-	-	-
4	NT	-	-	-
5	0	5/5	0	10
6	200	5/5	20	10
7	600	5/5	60	10
8	2000	5/5	200	10
9 (TK)	200	6/6	20	10
10 (TK)	600	6/6	60	10
11 (TK)	2000	6/6	200	10

Animals from groups 1 and 2 were observed for 72 hours postdose. The animals were terminated at the end of the observation period and discarded without necropsy.

Results**:**

Mortality and Clinical signs

No animals died or were deemed moribund. There were no test article-related observations for animals given single doses of 1000 mg/kg or 2000 mg/kg or 7 doses at 200- or 600-mg/kg. Both male and females administered secnidazole at 2000 mg/kg showed darker than normal feces on Days 4 through 7.

For Phase 1, cage side observations were recorded predose and at 1, 2, 4, 24, 48, and 72 hours after dosing. For Phase 2, cage side observations were recorded predose and at 1, 2, and 4 hours after dosing and once daily on nondosing days (during the acclimation period and the day of necropsy).

Body weights:

There were no test article-related changes in body weights (Table 4). Body weights were measured at least once during the acclimation period. During Phase 1, the rats were weighed on Day 1 and Day 4. During Phase 2, the rats were weighed on Days 1 and 7. A fasted body weight was collected on the day of necropsy for organ-to-body weight evaluation.

Table 4: Mean body weights in males in 'Oral rising-dose and 7-day oral repeat-dose toxicity study in rats'.

Sex: Male		Day(s) Relative to Start Date			
		-5	1	7	8
Group 5 - 0 mg/kg	Mean	195.28 [1]	240.92 [1]	278.88 [1]	258.14 [1]
	SD	6.79	13.01	24.56	23.45
	N	5	5	5	5
Group 6 - 200 mg/kg	Mean	198.06	246.66	288.40	266.12
	SD	12.90	20.46	25.29	21.51
	N	5	5	5	5
Group 7 - 600 mg/kg	Mean	201.30	245.20	284.94	268.68
	SD	4.33	5.64	15.48	16.84
	N	5	5	5	5
Group 8 - 2000 mg/kg	Mean	194.78	241.58	283.54	265.74
	SD	8.27	11.85	12.04	14.64
	N	5	5	5	5

Table 5: Mean body weights in females in 'Oral rising-dose and 7-day oral repeat-dose toxicity study in rats'.

Sex: Female		Day(s) Relative to Start Date			
		-5	1	7	8
Group 5 - 0 mg/kg	Mean	171.00 [1]	181.58 [2]	191.70 [2]	179.84 [2]
	SD	10.70	10.53	10.28	9.47
	N	5	5	5	5
Group 6 - 200 mg/kg	Mean	176.34	192.72	201.42	192.48
	SD	5.81	5.23	6.68	5.37
	N	5	5	5	5
Group 7 - 600 mg/kg	Mean	174.56	192.66	201.80	193.62
	SD	7.13	4.48	5.95	6.04
	N	5	5	5	5
Group 8 - 2000 mg/kg	Mean	167.30	184.98	206.94	194.24
	SD	8.76	15.56	20.41	17.99
	N	5	5	5	5

Hematology and coagulation:

After 7 daily doses of 2000 mg/kg secnidazole, higher mean absolute neutrophil count (ANEUT) (mean +81%, both sexes) and higher mean absolute monocyte (ABMONO) count (+90 %, both sexes) were recorded.

The following parameters were evaluated in blood samples collected from all repeat dose animals prior to necropsy, after an overnight fast, from the retro-orbital plexus under CO_2/O_2 anesthesia): Red blood cells (RBC, count and morphology), mean corpuscular volume (MCV), white blood cells (WBC, total and differential absolute values), mean corpuscular hemoglobin (MCH), hemoglobin concentration (HGB), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), platelet count (PLAT),

reticulocyte count (ABSRET), mean platelet volume (MPV), fibrinogen (FIB), prothrombin time (PT), and activated partial thromboplastin time (APTT).
Serum chemistry:

At 2000 mg/kg, secnidazole resulted in higher marginally increased mean alanine aminotransferase activity (ALT) values (+33%) and increased mean phosphorus concentration (+28%, females only). Other statistically significant changes were either slight or of questionable toxicological significance.

The following parameters were evaluated in blood samples collected on Day 8, after an overnight fast, from the retro-orbital plexus under CO_2/O_2 anesthesia): Sodium, calcium, potassium, inorganic phosphorus, chloride, glucose total bilirubin, urea nitrogen, alkaline phosphatase, total protein, lactate dehydrogenase, albumin, aspartate aminotransferase, globulin, alanine aminotransferase, albumin/globulin ratio, gamma-glutamyltransferase, cholesterol, creatine phosphokinase, triglycerides, creatinine.

Gross pathology:

Distention of the cecum was observed all male and female high dose (2000 mg/kg) animals. The histologic correlate appeared to be mild, diffuse atrophy of the mucosal folds of the cecum.

Organ Weights

Mean absolute and relative liver weights were increased by 39 to 48 % in males and females rats dosed at 2000 mg/kg for 7 days. These changes likely relate to known enzyme induction associated with secnidazole. Testes weights were decreased by about 20 % in high dose males. In high dose females adrenal weights (absolute and relative) were decreased by about 20 % and kidney weights (absolute and relative) were increased by 11 to 20%.

Table 6: Tissue collection table for 'Oral rising-dose and 7-day oral repeat-dose toxicity study in rats'.

Study	Collected at necropsy	Organ weighed	Histopathology
Bone marrow smear (femur)	X		
Brain	X	X	X
Cervix	X	X	
Epididymides	X	X	
Esophagus	X		
Eye	X	X	
Gall bladder	X		
Gross lesions	X		X
Heart	X	X	X
Intestines, Cecum	X		X
Intestines, Colon	X		X
Intestines, Duodenum	X		X
Intestines, Jejunum	X		X
Intestines, ileum	X		X
Intestines, rectum	X		X
Kidneys	X	X	X
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, cervical	X		
Lymph nodes mesenteric	X		
Mammary Gland	X		
Nerves, optic	X		
Nerves, sciatic	X		
Ovaries	X	X	
Pancreas	X		
Parathyroid	X	X	
Pituitary	X	X	
Prostate	X		
Salivary gland (mandibular)	X	X	
Skeletal muscle (biceps femoris)	X		
Skin	X		
Spinal cord, cervical	X		
Spinal cord, thoracic	X		
Spinal cord, lumbar	X		
Spleen	X	X	X
Stomach	X		X
Testes	X	X	
Thymus	X	X	
Thyroid	X	X	
Tongue	X		
Trachea	X		
Urinary bladder	X		
Uterus	X	X	
Vagina	X		

Histopathology: Adequate Battery: Yes
Peer review: No

Dose-related hepatic observations consisted of mild, hypertrophy of centrilobular hepatocytes in all 600 mg/kg males and moderate hypertrophy of centrilobular hepatocytes in all males and females at 2000 mg/kg. Mild, diffuse atrophy of the mucosal folds was observed in the cecum at 2000 mg/kg (4/ 5 males and 1/5 females).

In the kidneys, dose-related diffuse hyaline droplet accumulation in the proximal tubules was observed. Findings were minimal (2 /5 at 200 mg/kg in males, 1/5 at 600 mg/kg in males, 1/5 at 600 mg/kg females, and 5/5 at 2000 mg/kg in females. Mild, diffuse hyaline droplet accumulation in the proximal tubules was observed in 2 of 5 600 and 2000 mg/kg males; and moderate, diffuse hyaline droplet accumulation in the proximal tubules was seen in 3 of 5 males at 2000 mg/kg.

Toxicokinetics:

Table 7: Toxicokinetics for 'Oral rising-dose and 7-day oral repeat-dose toxicity study in rats'.

Day	Group	SYM-1219 Dose (mg/kg)	Sex	T_{max} (hr)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	AUC_{last} (hr*µg/mL)	$AUC_{0-\infty}$ (hr*µg/mL)
7	9	200	Male	1.00	3.94	126	677	921
7	9	200	Female	1.00	NR[1]	154	811	NR
7	10	600	Male	1.00	NR	326	1750	NR
7	10	600	Female	2.00	NC[2]	324	1800	NC
7	11	2000	Male	0.50	NR	456	2650	NR
7	11	2000	Female	4.00	NC	549	3580	NC

The Tmax ranged from 0.5 to 4.0 hours. Half-life values were not reported (due to extensive extrapolation) or could not be calculated (due to insufficient data points for the elimination phase) for most group/sex combinations. The half-life was 3.94 hours for Group 9 (200 mg/kg) males. On Day 7, increases in Cmax and AUC_{last} were less than dose proportional for males and females. Cmax and AUC_{last} values were similar between males and females.

Discussion

In this non-GLP study, the maximum nonlethal single dose was 2000 mg/kg. In the 7-day study, minimal hyaline droplet accumulation in the proximal tubules was observed at all doses in males and at doses of 600 mg/kg and higher in females. The exposure at the lowest dose was 744 µg*h/mL, about 0.6 times the clinical exposure, based on AUC comparisons. The hyaline droplet accumulation was not considered to be predictive of human toxicity.

Study title:	SYM-1219: Oral Rising-Dose and 7-Day Repeat-Dose Toxicity and Toxicokinetic Study in Dogs
Study no.:	030614
Document location	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	6 March, 2013
GLP compliance:	Non-GLP
QA report :	Yes
Drug, lot #,	F120044
% purity:	99.7 %
Key study findings:	No overt clinical signs were observed after a single 500 mg/kg dose of secnidazole or 7 daily doses of 200 mg/kg/day (about 1.1 times the clinical exposure, based on AUC comparisons). The C_{max} and AUC_{last} of animals on Day 7 for the 60- and 200-mg/kg doses were lower than exposure on Day 1, indicating potential induced clearance or saturation of absorption of secnidazole with multiple dosing.

Methods

In the first phase of the study, secnidazole was administered by oral gavage at 1000 mg/kg to one dog/sex/dose for determination of the maximum tolerated dose. Based on the findings during a 72 hour observation period of these first set of animals, a second group was dosed at 300 mg/kg. After a 1-week washout period, a 500 mg/kg dose was administered to the first set of dogs, and the 750 mg/kg dose was administered to the second set of dogs. Based on the results of Phase 1, animals received vehicle or 60, 200, 600 mg/kg secnidazole daily for 7 consecutive days. Animals were terminated the day following completion of dosing.

Doses:	Phase 1: Single dose :1000, 300, 500, 750 mg/kg Phase 2: Repeat dose: 0, 60, 200, 600 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	2% CMC (carboxymethylcellulose, 400 c.p.,
Species/Strain:	*Canis familiaris,* beagle
Number/Sex/Group:	1
Age:	6.8 to 24 months of age
Weight:	6 to 10 kg
Deviation from study protocol:	There were no protocol deviations that impacted the results or interpretation of the study

Observations and Results

Mortality

There were no deaths in Phase 1 of the study. In Phase 2, the 600 mg/kg female was euthanized moribund on Day 4 and the 600 mg/kg male was euthanized moribund on Day 6. The sponsor was unable to

determine the cause of mortality/moribundity in any animals. All other animals survived to the scheduled termination date (Day 8).

Clinical signs

Phase 1. At 750 mg/kg dogs showed ataxia, decreased activity and vomiting. At 1000 mg/kg signs included vomiting, partially closed eyelids, ataxia, salivation, and coolness to touch. .

Phase 2. On day 1, ataxia was observed in 600-mg/kg male and female. The females also showed decreased activity, partially closed eyes and vomiting. On day 4, the female showed tremors, seizures, recumbence, ataxia, decreased activity, and partially closed eyelids and the male was recumbent with tremors and seizures.

All animals were observed daily for viability in the morning and afternoon. Cage side observations were performed at 1, 2, and 4 hours postdose on dosing days, to evaluate general signs of clinical health, behavior, illness, and stress. For Phase 2, animals were observed predose on the days of dosing, starting on Day 5.

Body Weights

There were no biologically significant secnidazole-related effects on body weight in Phase 1 or at 60 and 200 mg/kg in Phase 2. Since 600 mg/kg dogs were terminated prior to Day 7 body weight measurements, no assessment of body weight could be made at that dose.

Body weights were measured during acclimation, prior to dose and on Day 9 during Phase 1. For Phase 2, body weights were measured for each animal prior to dose administration on the first and last days of dosing and after fasting, prior to necropsy.

Clinical Pathology

No adverse hematology or serum chemistry findings were observed at dose levels of 0, 60, or 200 mg/kg. Sporadic differences observed were within historical control ranges. Dogs administered 600 mg/kg were euthanized moribund on this study without clinical pathology samples being collected.

Blood samples for evaluation of hematology, coagulation, and serum chemistry parameters were collected from all Phase 2 animals, using the jugular vein, during the acclimation period, and prior to necropsy on Day 8. The animals were fasted overnight prior to blood collection.
Hematology and coagulation blood samples were analyzed for the following parameters: Red blood cells (RBC, count and morphology), mean corpuscular volume (MCV), white blood cells (WBC, total and differential absolute values), mean corpuscular hemoglobin (MCH), hemoglobin concentration (HGB), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), platelet count (PLAT), reticulocyte count (ABSRET), mean platelet volume (MPV), fibrinogen (FIB), prothrombin time (PT), and activated partial thromboplastin time (APTT).

Serum Chemistry

There were no biologically significant changes in clinical chemistry parameters. Any differences were either marginal or not statistically significant or not dose related.

Blood samples were analyzed for the following parameters.): Sodium, calcium, potassium, inorganic phosphorus, chloride, glucose, total bilirubin, urea nitrogen, alkaline phosphatase, total protein, lactate

dehydrogenase, albumin, aspartate aminotransferase, globulin, alanine aminotransferase, albumin/globulin ratio, gamma-glutamyltransferase, cholesterol, creatine phosphokinase, triglycerides and creatinine.
Gross Pathology

Thickened duodenum was recorded in the Group 6 female that was euthanized on Day 4. There were no macroscopic abnormalities in any of the control, 60 mg/kg or 200 mg/kg male and female dogs that survived and were necropsied on Day 8.

Gross necropsy evaluation included examination of the carcass and muscular/skeletal system, external surfaces and orifices, cranial cavity and external surface of the brain, neck with associated organs and tissues, thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Organ Weights

Absolute

At 200 mg/kg there was a 16 % reduction of the absolute heart weight and 18 % reduction in the relative (to body weight) heart weight in males but not females. The toxicological significance of this finding is unclear since this finding was detected in just one animal and the finding was not seen in the female. There were no statistically or biologically significant differences in terminal body weight or absolute organ weights for the 60 mg/kg group.

For all animals euthanized at scheduled necropsies, the designated organs listed on Table 8 were weighed. Paired organs were weighed together. Organ weights were not taken from animals euthanized moribund. Organ/body weight ratios were calculated using the terminal fasted body weight obtained prior to necropsy.

The following organs were weighed: adrenals, brain, epididymides, heart, kidney, liver, lungs, ovaries, parathyroid gland, pituitary, salivary gland, spleen, testes, thymus, thyroid and uterus.

Histopathology Findings

Moderate depletion of the lymphocytes of the spleen (periarteriolar sheaths and marginal Zone) was observed in both male and female Group 6 dogs. Minimal, multifocal necrosis of the epithelium of the renal proximal tubules was noted in the Group 6 male. Although these findings appear to be drug related, they were not severe enough to account for the moribundity seen in these two dogs. All other microscopic findings were considered to be incidental. The brain, heart, kidney, liver, lungs, spleen were subjected to histopathology evaluations.

Adequate Battery: Yes for this non-GLP study.
Peer Review: No

Table 8: Tissue collection table for ‘Oral rising-dose and 7-day oral repeat-dose toxicity study in dogs.

Study	Collected at necropsy	Organ weighed	Histopathology
Esophagus	X		
Eye	X		
Gall bladder	X		
Gross lesions	X		
Heart	X	X	X
Intestines, Cecum	X		
Intestines, Colon	X		
Intestines, Duodenum	X		
Intestines, Jejunum	X		
Intestines, ileum	X		
Intestines, rectum	X		
Kidneys	X	X	X
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, cervical	X		
Lymph nodes mesenteric	X		
Mammary Gland	X		
Nerves, optic	X		
Nerves, sciatic	X		
Ovaries	X	X	
Pancreas	X		
Parathyroid	X	X	
Pituitary	X	X	
Prostate	X		
Salivary gland (mandibular)	X	X	
Skeletal muscle (biceps femoris)	X		
Skin	X		
Spinal cord, cervical	X		
Spinal cord, thoracic	X		
Spinal cord, lumbar	X		
Spleen	X	X	X
Stomach	X		X
Testes	X	X	
Thymus	X	X	
Thyroid	X	X	
Tongue	X		
Trachea	X		
Urinary bladder	X		
Uterus	X	X	
Vagina	X		

Toxicokinetics

The TK data were of limited utility since only one animal per sex per dose was used. The Tmax ranged from 0.5 to 2.0 hours on say 1 and 7. On Day 1, half-life values ranged from about 6 to 8 hours for the 60- and 200-mg/kg doses. For the 600-mg/kg dose, the half-life values were higher, ranging from 10 to 11.5 hours. On Day 7, half-life values for the 60- and 200-mg/kg doses were lower than on Day 1, ranging from 2.11 to 4.79 hours.

On Day 1 C_{max} and AUC_{last} was approximately dose proportional between doses of 60 and 200 mg/kg, and were less than dose proportional between doses of 60 and 600 mg/kg. For a roughly 3-fold increase in dose from 60 to 200 mg/kg, C_{max} increased by around 3-fold for males and 3-fold for females, and AUC_{last} increased by about 5-fold for males and 3-fold for females. Between doses of 60 and 600 mg/kg, increases in C_{max} were slightly less than dose proportional, and increases in AUC_{last} were slightly less than dose proportional for males and slightly greater than dose proportional for females. For a10-fold increase in dose from 60 to 600 mg/kg, C_{max} increased 7-fold for males and 7-fold for females, and AUC_{last} increased 8-fold for males and 11-fold for females. C_{max} and AUC_{last} values were generally similar between males and females.

On Day 7, between doses of 60 and 200 mg/kg, increases in C_{max} and AUC_{last} were approximately dose proportional. C_{max} and AUC_{last} of animals on Day 7 for the 60- and 200-mg/kg doses were lower than exposure on Day 1, indicating potential induced clearance or saturation of absorption of secnidazole with multiple dosing. No toxicokinetic data was collected after Day 1 for the high dose dogs.

Table 9: Toxicokinetics for 'Oral rising-dose and 7-day oral repeat-dose toxicity study in dogs'.

Day	Group	SYM-1219 Dose (mg/kg)	Sex	Animal Number	T_{max} (hr)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	AUC_{last} (hr*µg/mL)	$AUC_{0-\infty}$ (hr*µg/mL)
1	4	60	M	1821947	1.00	8.14	68.6	619	703
1	4	60	F	1752163	0.50	6.04	76.3	596	635
1	5	200	M	1979699	1.00	NR[1]	233	3010	NR
1	5	200	F	2093520	2.00	5.96	212	1960	2090
1	6	600	M	1983726	0.50	10.08	463	5140	6330
1	6	600	F	1755669	1.00	11.51	565	6850	8970
7	4	60	M	1821947	1.00	4.79	65.9	476	490
7	4	60	F	1752163	1.00	3.49	69.8	428	431
7	5	200	M	1979699	1.00	4.44	216	1990	2040
7	5	200	F	2093520	1.00	2.11	199	1040	1050

The AUC values corresponding to the 60, 200 and 600 mg/kg doses were 452, 1515 and 5995 µg*h/mL. Since $AUC_{(0\text{-}24h)}$ value in patients treated with a single 2g dose of secnidazole was about 1331 µg*h/mL, these exposures are equivalent to about 0.3, 1.1 and 4.5 times the clinical exposure, based on AUC comparisons.

Discussion:

The data in this study are of limited utility since only one animal per sex per dose was studied.

In the absence of mortality at the 2000 mg/kg dose, the maximum nonlethal single dose of secnidazole was determined to be 2000 mg/kg in dogs. In the 7-day study, the 600 mg/kg dose was associated with mortality and moribundity in both sexes. Ataxia, decreased activity, partially closed eyes, vomiting, tremors, seizures, recumbence, tremors and seizures were seen at this high dose. The cause of

moribundity was undetermined. The moderate depletion of the lymphocytes of the spleen and minimal, multifocal necrosis of the epithelium of the renal proximal tubules noted in the Group 6 animals were not cited as causes of the moribundity seen in these animals. The slight (16-18%) reduction in the absolute and relative heart weight was seen in the male but not the female dog. Since the finding was detected in one animal and since there was also no effect on heart weight at this dose in the females in the 28 day study (reviewed below), the finding is considered to be of no toxicological significance. No overt clinical signs were observed at 200 mg/kg, which is equivalent to about 1.1 times the clinical exposure after a single dose, based on AUC comparisons.

6.2 Repeat-Dose Toxicity

Study title:	SYM-1219: 28-Day Oral Toxicity and Toxicokinetic Study in Rats with a 14-Day Recovery Period
Study no.:	030615
Document location	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	23-Apr-2013
GLP compliance:	GLP
QA report :	Yes
Drug, lot #,	F120044.
% purity:	99.7 %
Key study findings:	The lowest dose tested was 100 mg/kg, equivalent to 0.4 times the recommended dose based on AUC comparisons. The NOAEL could not be determined for this study since all males showed increases in pituitary weights, diffuse hypertrophy of the acidophils of the pars distalis of the pituitary gland and increased thyroid/ parathyroid weights and all females showed reduced mean body weight gains (by 18 to 35 % in females at all doses relative to the control animals) and decreased absolute (-22 %) and relative (-20 %) thymus weights at 100 mg/kg. After a 14 day non-dosing period high dose recovery period, findings in the brain (varying degrees of vacuolar degeneration of the lateral reticular nuclei, medial vestibular nuclei, dentate nuclei, and the vestibule-cerebellar tract and varying degrees of gliosis of these same structures), adrenal gland, spleen, epididymides and seminiferous tubules persisted in some animals.

Methods

Species/strain:	*Rattus norvegicus*/Crl:CD(SD)
Doses	0, 100, 300, 1000 mg/kg/day
Number/sex/group :	10-15
Age:	8 weeks old
Weights:	167-229 (females) and 233 to 279g (males)
Route:	Oral gavage
Formulation:	2% carboxymethylcellulose
Volume	10 mL/kg
Satellite groups:	Recovery animals were terminated 14 days after the completion of dosing.
Protocol Deviations:	Deviations did not impact the study validity.

Table 10: Group assignments for 28-Day Oral Toxicity and Toxicokinetic Study in Rats with a 14-Day recovery period.

Group	Dose (mg/kg)	# of animals M/F	Dose conc. (mg/mL)	Number of animals for necropsy	
				Terminal (Day 29)	Recovery (Day 43)
1	0	15/15	0	10/10	5/5
2	100	10/10	10	10/10	5/5
3	300	10/10	30	10/10	5/5
4	1000	15/15	100	10/10	5/5
5 (TK)	0	3/3	0		
6 (TK)	100	9/9	10		
7 (TK)	300	9/9	30		
8 (TK)	1000	9/9	100		

Results

Mortality

Mortality and significant moribundity was observed in rats treated with secnidazole at 1000 mg/kg. One female was found dead on Day 28. Four high dose females were euthanized moribund. Two female rats had head tilt and circling consistent with vestibular lesion, so were euthanized on Day 20 for humane reasons. Another female was euthanized on Day 26 hyper responsive to stimulus with wide base stance on all legs. A fourth female was euthanized after head tilt and rolling on Day 26. One high dose male was euthanized moribund on Day 26. The cause of death in these animals was considered to be degeneration of the brain, specifically in the medulla oblongata and the cerebellar peduncles.

Clinical Signs

Secnidazole -related clinical signs were limited to the high-dose animals.

Clinical signs in moribund animals included ataxia; head tilt , abnormal/ uncoordinated /exaggerated gait; straub tail, hyper responsivity /hyper reactivity to stimulus; splayed legs/wide stance; darker than normal and/or decreased feces; orange discolored urine; salivation; anogenital staining; labored breathing; loss of righting ability; circling; rolling; stained/rough facial, snout, or forelimb haircoat; decreased activity; hunched, recumbent posture; partially closed eyelids; chromodacryorrhea; vocalization; and/or small testes.

Among surviving animals, drug-related clinical signs included dark feces (14 males and 10 females between Days 9 and 29), abnormal, uncoordinated, or exaggerated gait (three males and three females between Days 18 and Day 28); ataxia (six males and six females on days 1 to 28); decreased activity (nine males and nine females on Days 2 to 28) eyelids partially closed (one male on Day 24); decreased feces (three males and three females between Days 21 to 29); rough hair coat (five males on Day 28); stained (red or dark red) facial hair coat (seven males and two females between Days 18 to 28); chromodacryorrhea (one male on Day 28); hyper reactive to stimulus (one male on Day 20 and one female on Day 18); straub tail (one male on Day 20) splayed legs (two males on Day 28 and three females between Days 18 and 21); hunched posture (two males between Days 25 and 29) recumbent posture (one male on Day 28); anogenital staining (one male on Day 28 and one female on Day 18); discolored (orange) urine (five males between Days 25 and 27); vocalization (one male on Days 21 and 28); and

small testes (six males on Day 21 and eight males on Day 28). The attending veterinarian concluded that the head tilt and circling symptoms were consistent with vestibular damage or lesion. Many of these clinical signs are consistent with motor changes related to vestibulo-cerebellar lesions noted below.

During the recovery period dark feces persisted to Day 31 for four males and two females and small testes persisted for four males to day 42.

Body Weights

Body weights of male and female high dose animals were between 17 and 11 % less than controls on Day 29. Mean body weight gains (Day -1 to Day 28) were reduced by 41 % at 1000 mg/kg in males and by 18 to 35 % in females at all doses relative to the control animals.

Table 11: Mean body weight gains (grams) in male rats for 28-Day oral toxicity study

Bodyweight Gain - grams (g)

Sex: Male		Day(s) Relative to Start Date					
		-1 → 7	7 → 14	14 → 21 aa[1]	21 → 28 aa[1]	-1 → 28 aa[1]	28 → 42
Group 1 - 0 mg/kg	Mean	56.71	42.27	41.31	30.38	170.66	55.64
	SD	10.62	8.84	11.57	7.25	35.10	14.04
	N	15	15	15	15	15	5
Group 2 - 100 mg/kg	Mean	54.51	39.25	38.85	28.40	161.01	.
	SD	8.86	8.30	10.32	5.52	28.66	.
	N	10	10	10	10	10	.
Group 3 - 300 mg/kg	Mean	52.79	41.06	36.77	26.82	157.44	.
	SD	7.97	8.56	7.22	9.70	25.56	.
	N	10	10	10	10	10	.
Group 4 - 1000 mg/kg	Mean	52.06	38.17	21.36 dd[2]	-8.86 dd[2]	100.86 dd[2]	66.35
	SD	5.42	4.97	19.79	32.59	27.94	5.20
	N	15	15	15	14	14	4

Statistical Test: Anova & Dunnett's Test

Table 12: Mean body weight gains (grams) in female rats in 28-Day oral toxicity study.

Bodyweight Gain - grams (g)

Sex: Female		Day(s) Relative to Start Date					
		-1 → 7 aa[1]	7 → 14	14 → 21 aa[1]	21 → 28	-1 → 28 aa[1]	28 → 42*
Group 1 - 0 mg/kg	Mean	27.33	20.56	16.11	10.65	74.65	25.66
	SD	10.91	11.30	7.80	5.25	25.57	10.60
	N	15	15	15	15	15	5
Group 2 - 100 mg/kg	Mean	11.98 dd[2]	15.07	15.81	6.01	48.87 dd[2]	.
	SD	8.85	9.21	6.06	3.08	16.50	.
	N	10	10	10	10	10	.
Group 3 - 300 mg/kg	Mean	21.02	14.52	17.29	7.85	60.68	.
	SD	7.06	7.39	7.86	5.51	15.90	.
	N	10	10	10	10	10	.
Group 4 - 1000 mg/kg	Mean	29.13	14.79	-2.13 dd[2]	5.63	51.73 d[2]	32.95
	SD	8.21	9.84	12.59	15.41	14.09	21.57
	N	15	15	13	10	10	2

Statistical Test: Anova & Dunnett's Test

During the recovery period, mean body weights in high dose animals remained marginally (-11 %) lower when compared to the controls but body weight gain in these high dose animals was 20 to 29 % higher compared to control animals.

Body weights were measured twice during the acclimation period and weekly during the remainder of the study. At necropsy, body weights were collected from animals fasted overnight, for calculation of organ/body weight ratios.

Feed Consumption
Feed consumption in surviving high dose males and females was decreased by about 25 % between Days 21 and 28 for the surviving Group 4 males and females.

Food consumption was measured for 7 days during the acclimation period, and weekly during the remainder of the study.

Ophthalmoscopy
There were no abnormalities in the ophthalmology data that were considered related to test article administration. Observed corneal changes were considered typical for this strain of rats.

An ophthalmic examination was conducted during the acclimation period, on all terminal and recovery animals on one occasion during Week 4, and on the recovery animals during the second week of the recovery period. Biomicroscopy and indirect ophthalmoscopy were performed by a board-certified veterinary ophthalmologist.

Clinical pathology
Blood samples for evaluation of hematology, coagulation, and serum chemistry parameters were collected from all surviving Group 1-4 animals from the retro-orbital plexus prior to necropsy (Day 29 for terminal animals and Day 43 for recovery animals). Retro-orbital bleeding was generally performed no more than twice, after which, samples were obtained from the abdominal aorta. The animals were fasted overnight prior to blood collection.

Hematology and Coagulation
There were no drug related hematology changes at doses of 100, 300, and 1000 mg/kg/day in male or female rats.

Blood (1mL) was collected in KEDTA-containing tubes and analyzed for the following parameters: Red blood cells, (count and morphology), mean corpuscular volume, white blood cells (total and differential absolute values), mean corpuscular hemoglobin, hemoglobin concentration, mean corpuscular hemoglobin concentration, hematocrit, platelet count, reticulocyte count and mean platelet volume.

Clinical Chemistry
There were no biologically significant drug-related Clinical Chemistry changes at doses of 100, 300, and 1000 mg/kg/day in male or female rats. Changes were generally marginal or within the range of historical controls.

A target volume of 1 mL of blood was collected in tubes without anticoagulant. The sample was allowed to clot and then was centrifuged to obtain serum. The following parameters were evaluated in blood samples collected after an overnight fast, from the retro-orbital plexus under CO_2/O_2 anesthesia): Sodium, calcium, potassium, inorganic phosphorus, chloride, glucose total bilirubin, urea nitrogen, alkaline phosphatase, total protein, lactate dehydrogenase, albumin, aspartate aminotransferase, globulin, alanine aminotransferase, albumin/globulin ratio, gamma-glutamyltransferase, cholesterol, creatine phosphokinase, triglycerides, creatinine.

Urinalysis
There were no biologically significant differences for any urine parameter for any of the groups of male and female rats administered secnidazole for 28 days, when compared to the controls. There were also no differences after a 14 day recovery period.

Urinalysis was performed on all terminal animals during the last week of dose administration and on recovery animals prior to recovery termination. The following urinalysis parameters were recorded and evaluated for each animal while housed individually: bilirubin, nitrite, clarity occult blood, color, pH, glucose, protein, ketones, specific gravity, leukocytes, urobilinogen and microscopic examination of sediment.

Gross Pathology
Gross pathology findings in high dose males included bilateral enlargement of the adrenal glands (correlated with hypertrophy of adrenal cortex) and bilateral small epididymides. High dose females showed small thymus.

Organ Weights
Secnidazole administration at 1000 mg/kg was associated with reduction in thymus weights (-33 to-38 3%, males and females), absolute heart weight (-14%, males only), absolute testes weights (-62%), absolute epididymides weights (-39%) and absolute spleen weights (-[12 - 24 %], males and females) in addition to increases in absolute liver weight (+10-14 %, males and females), and absolute kidney weight (+11-15 %, males and females). Males in all dose groups showed increases in pituitary weights (increased 39-48%) and thyroid/parathyroid weights (16-31%). Thymus weights were reduced by 17 to 33% in females at all doses.

Histopathology

Adequate Battery Yes

Table 13: Tissue collection table for 28-day study in rats.

Study	Collected at necropsy	Organ weighed	Histopathology
Adrenals (2)	X	X	X
Aorta	X		X
Bone (femur)	X		X
Bone (sternum)	X		X
Bone Marrow (femur)	X		X
Bone Marrow (sternum)	X		X
Bone Marrow smear (sternum)	X		
Brain	X	X	X
Cervix	X	X	X
Epididymides	X	X	X
Esophagus	X		X
Eye	X		X
Gall bladder	X		X
Gross lesions	X		X
Heart	X	X	X
Intestines, Cecum	X		X
Intestines, Colon	X		X
Intestines, Duodenum	X		X
Intestines, Jejunum	X		X
Intestines, ileum	X		X
Intestines, rectum	X		X
Kidneys	X	X	X
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, cervical	X		X
Lymph nodes mesenteric	X		X
Mammary Gland	X		X
Nerves, optic + sciatic	X		X
Ovaries	X	X	X
Pancreas	X		X
Parathyroid	X	X	X
Pituitary	X	X	X
Prostate	X		X
Salivary gland (mandibular)	X	X	X
Skeletal muscle	X		X
Skin (inguinal)	X		X
Spinal cord, cervical/ thoracic	X		X
Spinal cord, lumbar	X		X
Spleen	X		X
Stomach	X		X
Testes	X	X	X
Thymus	X	X	X
Thyroid	X	X	X
Tongue	X		X
Trachea	X		X
Urinary bladder	X		X
Uterus	X	X	X
Vagina	X		X

Peer Review: Not indicated

At the end of the dosing period, test article-related histopathology findings were found in the adrenal glands, brain, epididymides (males), kidneys, liver, pituitary gland (males only), spleen, testes, and thymus.

There were no adverse histopathology findings in any low dose (100 mg/kg) female rats. Almost all the males in all dose groups (10/10 low dose, 10/10 mid dose, and 9/10 high dose males) showed mild, diffuse hypertrophy of the acidophils of the pars distalis of the pituitary gland. It is uncertain what the biological significance of this change denotes; there did not appear to be any other lesions in these animals which correlated with this observation.

Mid dose males (7/10 males) also showed mild, diffuse lymphoid depletion in the spleen and all mid dose animals showed mild, diffuse bilateral hypertrophy of the adrenal cortex.

High dose animals (about 4 times the clinical exposure) showed lesions in the brain (9/10 males and 8/8 females, varying degrees of vacuolar degeneration of the lateral reticular nuclei, medial vestibular nuclei, dentate nuclei, and the vestibulo-cerebellar tract and varying degrees of gliosis of these same structures as well as diffuse hypertrophy of the acidophils of the pars distalis of the pituitary gland in 29/30 male rats), kidneys (minimal, diffuse, bilateral anisokaryosis of the epithelial cells of the proximal tubules, 8/10 male and 5/8 females), liver (minimal or mild , diffuse hypertrophy of centrilobular hepatocytes in all animals), thymus (diffuse lymphoid depletion of the thymic cortex ; mostly mild [10/18 animals] but some moderate [3/18 animals]), epididymides (diffuse hypospermia/ degeneration localized to the efferent ducts in all high dose males), seminiferous tubules (marked, diffuse bilateral degeneration of the spermatogenic elements lining the seminiferous tubules), and adrenals (mild or moderate, diffuse, bilateral hypertrophy of the adrenal cortex in all animals).

Recovery animals

After a 14 day non-dosing period (Day 43), high dose recovery animals were evaluated for reversibility. Brain lesions (varying degrees of vacuolar degeneration of the lateral reticular nuclei, medial vestibular nuclei, dentate nuclei, and the vestibule-cerebellar tract and varying degrees of gliosis of these same structures) persisted in in 2 of 4 males and both females. The adrenal gland lesion (moderate, diffuse, bilateral hypertrophy of the adrenal cortex) persisted in 1 of 2 high dose females. Findings in the spleen (mild, diffuse lymphoid depletion) persisted in all males, but were not seen in the 2 females. Findings in the epididymides (marked, bilateral hypospermia/ degeneration localized to the efferent ducts and mild, multifocal bilateral degeneration of the spermatogenic elements lining the seminiferous tubules of the testes persisted in most males and moderate, multifocal bilateral degeneration of the spermatogenic elements lining the seminiferous tubules of the testes persisted in 1 of 4 males.

Table 14: Summary Histopathology Data for 28 day rat study

	Males				Females			
Group #	1	2	3	4	1	2	3	4
Number of animal in study	10	10	10	10	10	10	10	8
Secnidazole dose (mg/kg)	0	100	300	1000	0	100	300	1000
Adrenal gland hypertrophy; cortical; bilateral; diffuse								
mild	0	0	10	1	0	0	10	4
moderate	0	0	0	9	0	0	0	4
Pituitary gland: hypertrophy; acidophils; pars distalis; diffuse, mild	0	10	10	9	0	0	0	0
Epididymides, hypospermia/degeneration; efferent ducts (head/body); bilateral; diffuse, marked	0	0	0	10	-	-	-	-
Liver hypertrophy; hepatocellular; centrilobular; diffuse								
minimal	0	0	0	0	0	0	0	6
mild	0	0	0	10	0	0	0	2
Spleen; depletion; lymphoid; diffuse								
Mild	0	0	7	1	0	0	0	4
Moderate	0	0	0	9	0	0	0	4
Testes; degeneration; spermatogenic; seminiferous tubule; bilateral; diffuse								
marked	0	0	0	10	_	_	_	_
Thymus; depletion; lymphoid; cortex; Diffuse								
Mild	0	0	0	5	0	0	0	5
Moderate	0	0	0	2	0	0	0	1
Kidneys: basophilia; tubular; cortex; Multifocal, minimal	1	2	3	5	0	0	0	0
Kidneys: anisokaryosis; proximal tubule; bilateral; diffuse, minimal	0	0	0	8	0	0	0	5
Brain: degeneration; vacuolar; dentate nucleus; vestibulo-cerebellar tract; bilateral; multifocal;								
minimal	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
marked	0	0	0	3	0	0	0	1
Brain: degeneration; vacuolar; medial vestibular nuclei; unilateral; Multifocal								
minimal	0	0	0	1	0	0	0	2
Brain: degeneration; vacuolar; medial vestibular nuclei; bilateral; Multifocal								
Mild	0	0	0	1	0	0	0	2
Moderate	0	0	0	1	0	0	0	0
Brain: degeneration; vacuolar; lateral reticular nucleus; bilateral; multifocal								
Moderate	0	0	0	2	0	0	0	2
Marked	0	0	0	2	0	0	0	0

Table 14: Summary Histopathology Data for 28 day rat study

	Males				Females			
Group #	1	2	3	4	1	2	3	4
Number of animal in study	10	10	10	10	10	10	10	8
Secnidazole dose (mg/kg)	0	100	300	1000	0	100	300	1000
Brain: gliosis; dentate nucleus; medial vestibular nuclei; bilateral; diffuse								
Mild	0	0	0	0	0	0	0	1
Moderate	0	0	0	3	0	0	0	0
Brain: gliosis; dentate nucleus; Vestibulo-cerebellar tract; bilateral; diffuse								
Moderate	0	0	0	0	0	0	0	2
Marked	0	0	0	0	0	0	0	1
Brain: gliosis; lateral reticular nucleus; bilateral; diffuse								
Mild	0	0	0	0	0	0	0	1
Moderate	0	0	0	4	0	0	0	1
Brain: gliosis; lateral reticular nucleus; unillateral; diffuse								
Mild	0	0	0	1	0	0	0	0
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	0	1
Brain, necrosis; neuronal; dentate nucleus unilateral; diffuse								
Mild	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Brain, necrosis; neuronal; dentate nucleus bilateral; diffuse								
Mild	0	0	0	2	0	0	0	0

Toxicokinetics

T_{max} ranged from 1 to 2 hours for both sexes at all doses on Day 1 and Day 28 except for the high dose males on Day 28, when Tmax was 4 hours. $T_{½}$ values ranged from 3.2 h to 5.4 hours for both sexes at all doses on Day 1 and Day 28 except for the high dose animals on Day 1 when $T_{½\ was}$ 17 to 22 hours.

On Days 1 and 28, increases in Cmax were less than dose proportional and increases in AUC were greater than dose proportional. Cmax and AUC values were generally similar between males and females. Exposure $AUC_{(0\text{-}\infty)}$ of animals on Day 28 was somewhat lower than exposure on Day 1, indicating no accumulation of secnidazole with multiple dosing.

There was no measurable exposure of control animals to secnidazole on Days 1 or 28. Secnidazole concentrations in all predose samples from secnidazole-treated groups (Groups 6-8) on Day 1 were also below the lower limit of quantitation, indicating no exposure of these animals to secnidazole prior to dosing.

Table 15: Toxicokinetic parameters in 28-Day oral toxicity study in rats: Day 1

Dose (mg/kg)	Males			Females		
	$T_{½}$ (h)	C_{max} (µg/mL)	AUC $_{(0-\infty)}$	$T_{½}$ (h)	C_{max} (µg/mL)	AUC $_{(0-\infty)}$
100	4.65	63.4	488	4.42	67.2	457
300	3.67	191	1980	4.64	203	1890
1000	17.4	410	9040	22.0	419	9740

Table 16: Toxicokinetics in 28-Day oral toxicity study in rats: Day 28

Dose (mg/kg)	Males			Females		
	$T_{½}$ (h)	C_{max} (µg/mL)	AUC $_{(0-\infty)}$ (µg•hr/mL)	$T_{½}$ (h)	C_{max} (µg/mL)	AUC $_{(0-\infty)}$ (µg•hr/mL)
100	4.15	74.3	565	3.90	80	510
300	5.37	204	1920	3.49	220	1780
1000	3.20	458	5060	5.46	578	5720

Dosing Solution Analysis

Dose samples were analyzed for secnidazole. At Week 1, the 10 and 30 mg/mL formulations met the accuracy and homogeneity criteria for suspensions. One 100 mg/mL aliquot in the Week 1 sample set assayed at 144% of the nominal that is believed to have been an aliquoting or dilution error; this formulation concentration met the accuracy criteria but failed homogeneity. Excluding the 144% aliquot produces assay results in line with the other two formulation concentrations. In the end, this failure had no impact on the overall study results or study interpretation. At Week 4, all formulations met the accuracy criteria. All system suitability criteria were met. The retention times of the formulations agree with the standard solutions and this confirms the identity of the test article as secnidazole.

Conclusion

Secnidazole is being proposed as a single dose treatment foe bacterial vaginosis. Findings observed in this 28 day study are not considered predictive of human toxicity after a single dose. Secnidazole (gavage) administration to male and female Sprague-Dawley rats at dose levels of 0, 100, 300, or 1000 mg/kg/day for up to 28 consecutive days resulted in morbidity and moribundity at 1000 mg/kg, about 4-fold the clinical exposure, based on AUC comparisons.. The deaths were preceded by clinical signs of motor effects that were consistent with histologic changes in the vestibule-cerebellar tract. Other target organs included the pituitary gland, spleen, kidney, liver, thymus, sternal bone marrow, epididymis, testes, thyroid/parathyroid glands, and/or adrenal glands.

Mean body weight gains (Day -1 to Day 28) were reduced by 41 % at 1000 mg/kg in males and by 18 to 35 % in females at all doses relative to the control animals. No NOAEL could be determined due to

findings at the lowest dose, 100 mg/kg, 0.4 times the clinical exposure based on AUC comparisons. In females, reduced body weight gain was observed at all doses and marginal decreases in absolute (-22 %) and relative (-20 %) thymus weights were observed at the 100 mg/kg dose. No NOAEL could be determined for males since all dose groups showed increases in pituitary weights (increased 39-48%) and thyroid/parathyroid weights (16-31%). Since there were no histologic correlates for these organ weight differences in either the males or females, the significance of these findings is unclear.

Table 17: Organ weights in 28 day rat study.

Group /Sex	Absolute Thymus Weight (g)	% of Control	Relative Thymus Ratio (to body weight) (g/kg)	% of Control	Relative Thymus Ratio (to brain weight) (g/g)	% of Control
1M	0.49490		1.256		0.2498	
2M	0.53660	108.4	1.357	108.0	0.2737	109.6
3M	0.49030	99.1	1.237	98.5	0.2535	101.5
4M	0.30600	61.8	0.923	73.5	0.1556	62.3
1F	0.49420		1.942		0.2640	
2F	0.38610	78.1	1.684	86.7	0.2113	80.0
3F	0.40710	82.4	1.748	90.0	0.2213	83.8
4F	0.32950	66.7	1.503	77.4	0.1886	71.4

The $AUC_{0\text{-}inf}$ values measured in rats exposed at 100, 300 and 1000 mg/kg were 538, 1850 and 5390 µg•hr/mL. A single oral dose of 2 g of SOLOSEC in healthy adult female subjects, resulted in a mean systemic exposure ($AUC_{0\text{-}inf}$) of 1331.6 (17.3%) µg•hr/mL.

Study title: SYM-1219: 28-Day Oral Toxicity and Toxicokinetic Study in Dogs with a 14-Day Recovery Period

Study no.:	030616
Document location	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	19-Apr-2013
GLP compliance:	GLP
QA report :	Yes
Drug, lot #,	F120044.
% purity:	99.7 %
Key study findings:	The NOAEL was estimated to be 60 mg/kg, with a mean AUC_{last} of 313 µg*h/mL, which is about 0.2 times the clinical exposure. The high dose, which was about 1.4 times the clinical exposure, resulted in moribundity and mortality.

Methods

Doses:	0, 20, 60, 200 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	2% CMC (carboxymethylcellulose, 400 c.p.,
Species/Strain:	*Canis familiaris,* beagle
Number/Sex/Group:	4-6
Age:	5.7 to 6.7 months of age
Weight:	4.70 to 7.95 kg
Satellite groups:	Recovery animals were retained untreated from Days 29 to 43
Deviation from study protocol:	There were no protocol deviations that impacted the results or interpretation of the study

Table 18: Group assignments for 28-Day Oral Repeat-Dose Toxicity and Toxicokinetic Study in Dogs

Group	Dose (mg/kg)	# of animals M/F	Dose conc. (mg/mL)	Number of animals for necropsy	
				Terminal (Day 29)	Recovery (Day 43)
1	0	6/6	0	4/4	2/2
2	20	4/4	2	4/4	
3	60	4/4	6	4/4	
4	200	6/6	20	4/4	2/2

Dose volume was 10 mL/kg for all dose groups

Observations and Results
Mortality

Five males and one female from the 200 mg/kg group were terminated in a moribund condition on Days 19, 19, 20, 28, 29, and 28, respectively. Clinical observations in these animals included ataxia, reluctance to rise, weakness, hyper reflexes, nystagmus, limited and/or loss of mobility, trembling and/or tremors, abnormal gait, and splayed legs.

The sponsor was unable to determine the cause of mortality/moribundity in any animals. There were no test article associated postmortem or histopathology changes or adverse findings in clinical pathology parameters which correlated with the moribundity seen in these animals.

Clinical Signs

The surviving 200 mg/kg dose male showed was stiff gait, hopping on both rear limbs when placed on floor, mild nystagmus, and dog was tense and in extension upon handling. There were no other drug-related clinical signs in the surviving animals. Emesis, salivation and changes in fecal characteristics did not show dose relatedness, were isolated occurrences, and/or were of a similar incidence when compared to control animals.

Body Weights

There were no biologically significant secnidazole -associated effects on body weight.

Body weights were measured for each animal twice during the acclimation period and at least once weekly for the remainder of the study. On the day of necropsy, body weights were collected from animals fasted overnight to allow for calculation of organ/body weight ratios.

Feed Consumption

Food consumption was very variable (including 27 % differences between groups, predose) but generally lower in high dose dogs only (by as much as 66% in high dose males on Day 27 compared to control animals).

Food consumption was measured daily following assignment to study and continued through completion of participation. Qualitative wet food consumption was also monitored for those animals deemed in need by the Study Director and the staff veterinarian.

Certified canine diet was provided daily in amounts appropriate for the size and age of the animals. Pedigree ® Brand wet dog food was provided to those animals deemed in need by the Study Director and staff veterinarian. Feed was offered for approximately 1 hour each day during the acclimation and the dosing periods. The animals were fasted 17 to 24 hours prior to dosing and were not offered food until at least 2 hours postdose. Animals were fasted as required for specific study procedures. Tap water was available *ad libitum,* except as specified below for study procedures or for activities requiring removal of animals from their home cage.

Ophthalmoscopy

There were no abnormalities in the ophthalmology data that were considered related to test article administration.

An ophthalmic examination was conducted on all animals during the acclimation period, during the final week of test article administration, and during the final week of the recovery period. Biomicroscopy and indirect ophthalmoscopy was performed by a board-certified veterinary ophthalmologist. Biomicroscopy and indirect ophthalmoscopy were performed with a Keeler All-Pupil Indirect Ophthalmoscope and a Zeiss HSO 10 Slit Lamp / Kowa SL-15 Slit Lamp. Prior to the examination, an ophthalmic mydriatic solution (1% tropicamide) was instilled into each eye to facilitate the examination.

ECG

Secnidazole administration did not result in any biologically significant effects on the electrocardiogram in dogs treated for 28 days. All dogs maintained sinus rhythms throughout the study.

On Study Day 27, the QTc interval in Group 4 females was statistically longer than Group 1 females ($p < 0.01$) with a mean difference from control of 19 msec (8%), but the prolongation in QTc interval observed in Group 4 females was considered to be not toxicologically significant since the change was small and the value was very similar to the observed QTc value on Pretest Study Day -9.

Table 19: Mean ECG parameters in dogs dosed daily with secnidazole. Day 27

	Males				Females			
SYM-1219 (mg/kg)	**0**	**20**	**60**	**200**	**0**	**20**	**60**	**200**
Heart rate (bpm)								
Pretest	68	84	81	74	78	69	75	92
Study Day 27	84	100	93	88	92	90	81	90
QT interval (msec)								
Pretest	228	223	229	216	212	225	221	212
Study Day 27	199	189	211	215	197	185	203	218
QTc (msec)								
Pretest	235	244	249	231	228	233	238	239
Study Day 27	222	221	240	238	225	214	223	244*

** statistically different from Group 1 at $p < 0.01$*
Statistical analysis for QRS in males and HR in females at Pretest and PR and QRS in females on Study Day 27 were nonparametric.

Clinical Pathology

Blood samples for evaluation of hematology, coagulation, and serum chemistry parameters were collected from all animals from the jugular vein during the acclimation period (Day -8), during the final week of test article administration (Day 27), and during the final week of the recovery period (Day 43). The animals were fasted overnight prior to blood collection. Additionally, for animals considered moribund, blood samples for evaluation of hematology, coagulation, and serum chemistry parameters were collected prior to termination.

Hematology and Coagulation

On Day 27, 200 mg/kg dose dogs showed lower platelet counts (-31 % males to -41 % females). Other changes at other time points were slight or sporadic or not statistically significant.

Hematology and coagulation blood samples were analyzed for the following parameters: Red blood cells (RBC, count and morphology), mean corpuscular volume (MCV), white blood cells (WBC, total and differential absolute values), mean corpuscular hemoglobin (MCH), hemoglobin concentration (HGB), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), platelet count (PLAT), reticulocyte count (ABSRET), mean platelet volume (MPV), fibrinogen (FIB), prothrombin time (PT), and activated partial thromboplastin time (APTT).

Serum Chemistry

There were no biologically significant changes in clinical chemistry parameters. Increases in glucose, protein, albumin and globulin were either marginal or not statistically significant or not dose related.

Blood samples were analyzed for the following parameters: Sodium, calcium, potassium, inorganic phosphorus, chloride, glucose, total bilirubin, urea nitrogen, alkaline phosphatase, total protein, lactate dehydrogenase, albumin, aspartate aminotransferase, globulin, alanine aminotransferase, albumin/globulin ratio, gamma-glutamyltransferase, cholesterol, creatine phosphokinase, triglycerides and creatinine.

Urinalysis

There were no biologically significant difference in urinalysis between dosed and control animals at any time during the study.

Urinalysis was performed pretest and on all terminal animals during the last week of dose administration and on recovery animals prior to recovery termination. The following urinalysis parameters were recorded and evaluated for each animal while housed individually: Bilirubin, nitrite, clarity occult blood, color, pH, glucose, protein, ketones, specific gravity, leukocytes, urobilinogen and microscopic examination of sediment.

Gross Pathology
There were drug-related macroscopic observations. The most common finding 3 of the 6 dogs euthanized moribund consisted of red streaking of the colon which was considered to be nonspecific. Cause of death was undetermined in all moribund/found dead animals. There were no test article associated microscopic alterations or adverse findings in clinical pathology parameters which correlated with the moribundity seen in these animals.

Animals were terminated the day following completion of dosing or following a 14-day recovery period. All animals (whether sacrificed moribund or terminally sacrificed) were terminated by deep anesthesia induced with sodium pentobarbital (Fatal-Plus), followed by exsanguination. A necropsy with tissue collection was conducted on all animals. The necropsy included examination of the carcass and muscular/skeletal system, all external surfaces and orifices, cranial cavity and external surface of the brain, neck with associated organs and tissues, thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Organ Weights

High dose (200 mg/kg) females showed increased mean absolute liver weight, (+36 %,) mean absolute lungs weight (+20%), and the mean absolute thymus weight (+253 %) compared to the controls.

Other parameters which were shown to be statistically different in the treated groups of animals from the control group were considered to be due to individual animal variation rather than test article

administration. There were no microscopic findings which correlated with any of the organ weight parameters shown to be different from the controls.

For all animals euthanized at scheduled necropsies, the designated organs listed in Table 20 were weighed. Paired organs were weighed together. Organ weights were not taken from animals euthanized moribund. Organ/body weight ratios were calculated using the terminal fasted body weight obtained prior to necropsy. Organ/brain weight ratios also were calculated.

Histopathology

Adequate Battery: Yes

Peer Review: No

Histopathology Findings

Diffuse depletion of lymphoid elements in the spleen (3 mild and 2 moderate) was reported among the 5 Group 4 males euthanized moribund. This finding was attributed by the sponsor to a stress response (rather than a direct test article effect on lymphocytes) since there were no test article effects in the clinical pathology data or other lymphoid organs such as the thymus. Among the animals that survived to terminal necropsy, there were no histopathology findings which could be attributed to test article administration. There were no findings in the spleen at the lower doses. All other microscopic observations were considered to be incidental.

Table 20: Tissue collection table for 28-day study in dogs.

Study	Collected at necropsy	Organ weighed	Histopathology
Adrenals (2)	X	X	X
Aorta	X		X
Bone (femur)	X		X
Bone (sternum)	X		X
Bone Marrow (femur)	X		X
Bone Marrow (sternum)	X		X
Bone Marrow smear (sternum)	X		
Brain	X	X	X
Cervix	X		X
Epididymides	X	X	X
Esophagus	X		X
Eye	X		X
Gall bladder	X		X
Gross lesions	X		X
Heart	X	X	X
Intestines, Cecum	X		X
Intestines, Colon	X		X
Intestines, Duodenum	X		X
Intestines, Jejunum	X		X
Intestines, ileum	X		X
Intestines, rectum	X		X
Kidneys	X	X	X
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, cervical	X		X
Lymph nodes mesenteric	X		X
Mammary Gland	X		X
Nerves, optic + sciatic	X		X
Ovaries	X	X	X
Pancreas	X		X
Parathyroid	X	X	X
Pituitary	X	X	X
Prostate	X		X
Salivary gland (mandibular)	X	X	X
Skeletal muscle	X		X
Skin (inguinal)	X		X
Spinal cord, cervical/ thoracic	X		X
Spinal cord, lumbar	X		X
Spleen	X		X
Stomach	X		X
Testes	X	X	X
Thymus	X	X	X
Thyroid	X	X	X
Tongue	X		X
Trachea	X		X
Urinary bladder	X		X
Uterus	X	X	X
Vagina	X		X

Special Evaluation

Table 21: Toxicokinetics for 28-day study in dogs.

Day	Group	SYM-1219 Dose (mg/kg)	Sex	T_{max} (hr)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	AUC_{last} (hr*µg/mL)	$AUC_{0-\infty}$ (hr*µg/mL)
1	2	20	M	0.75	3.83	20.1	105	109
1	2	20	F	0.50	2.65	18.9	70.2	80.9
1	3	60	M	0.63	4.35	57.8	410	418
1	3	60	F	0.75	3.60	59.0	337	360
1	4	200	M	0.83	9.18	215	2070	2480
1	4	200	F	0.58	5.33	208	1720	1810
28	2	20	M	0.75	3.33	19.4	110	98.7
28	2	20	F	1.50	2.44	14.7	54.7	75.3
28	3	60	M	0.63	3.21	58.1	322	323
28	3	60	F	0.63	2.91	55.6	303	311
28	4	200	M	2.25	2.57	148	1080	1090
28	4	200	F	0.70	2.69	186	866	999

On Day 1, increases in C_{max} were approximately dose proportional and increases in AUC_{last} were greater than dose proportional. On Day 28, increases in C_{max} were less than dose proportional for males and greater than dose proportional for females, and increases in AUC_{last} were approximately dose proportional for males and greater than dose proportional for females. C_{max} and AUC_{last} values were generally similar between males and females, except for Group 2 on Days 1 and 28, where AUC_{last} values were higher in males than in females. Exposure of animals on Day 28 was generally similar to exposure on Day 1, except for Group 4, where exposure was lower on Day 28 than on Day 1, indicating no accumulation of secnidazole with repeat dose administration.

Discussion:

The 200 mg/kg dose resulted in mortality and moribundity in males and females. Cause of moribundity was undetermined in all animals. There were no test article associated microscopic alterations or adverse findings in clinical pathology parameters which correlated with the moribundity seen in these animals. All brains examined were described as within normal limits except one high dose female which exhibited focal perivascular cuffs of mononuclear cells in the pons. The reversibility data were confounded because of the mortality and reduced numbers of animals at the high dose. The NOAEL was estimated to be 60 mg/kg, with a mean AUC_{last} of 313 µg*h/mL, which is about 0.2 times the clinical exposure.

7 Genetic Toxicology

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

Study title:	Bacterial Reverse Mutation Assay
Report no.:	030627
Study report location:	eCTD 4.2.3.3
Conducting laboratory:	(b) (4)
Date of study initiation:	22 February 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #,:	F120044
Purity:	>99%

Key Study Findings: Secnidazole was positive in the Ames assay.

Methods	
Strains:	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A.
Concentrations in definitive study:	33, 100, 333, 1000, 3333 and 5000 μg per plate
Basis of concentration selection:	In the preliminary toxicity assay, dose levels tested were 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 μg per plate. Increases in revertant counts (ranging from 3.3- to 29.6-fold maximum increases) were observed with all test conditions except tester strain TA1537 in the presence of S9 activation. Neither precipitate nor background lawn toxicity was observed.
Negative control:	DMSO
Positive control:	2-Nitrofluorene, 1 μg/plate Sodium azide, 1 μg/plate 9-amino-acridine, 75 μg/plate methyl methanesulfonate, 1000 μg/plate
Formulation/Vehicle:	Drug was dissolved in DMSO
Incubation time:	Plates were counted after 48 to 72 hours of incubation at 37°C.

Study Validity

Study was valid because the bacterial tester strains were selected based on ICH guidelines and positive controls showed expected response. Dose selection was adequate based on the lack of precipitate or background lawn toxicity. Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was purchased commercially from (b) (4). Each bulk preparation of S9 was assayed for its ability to metabolize benzo (a) pyrene and 2-aminoanthracene to forms mutagenic to *Salmonella typhimurium* TA100. In addition, the sponsor also set the following criteria for validity, which were met: All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21;

WP2 *uvr*A, 10 - 60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.3x109 cells/mL. The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels is required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

A preliminary toxicity assay was used to assess toxicity for the selection of dose levels in the mutagenicity assay. Neither precipitate nor background lawn toxicity was observed at concentrations up to 5000 μg per plate. Based on the findings of the toxicity assay, the sponsor appropriately selected a maximum dose of 5000 μg per plate.

Results

In the definitive mutagenicity assay, positive mutagenic responses (ranging from 5.0- to 29.7-fold maximum increases) were observed with all test conditions in all strains.

Discussion

The sponsor concluded that the results were equivocal in tester strain TA98 in the absence of S9 activation and tester strain TA1537 in the presence and absence of S9 activation. A reproducible dose-responsive increase was observed with tester strain TA98 in the absence of S9 activation but the revertant counts at the peak of the response were only slightly above the historical vehicle control range. Therefore, the sponsor's overall conclusion with this strain and activation condition was equivocal. The increases observed with tester strain TA1537 in the presence and absence of S9 activation, although reproducible, were not considered indicative of mutagenic activity because (1) the increases were not dose-responsive, (2) the vehicle control values were on the low end of the acceptable range and (3) the revertant counts at the peak of the response were still within the historical vehicle control range. Therefore, the conclusion with this strain (TA1537) and activation conditions was negative.

This reviewer considered the test article secnidazole to be positive with tester strains TA100, TA1535, and WP2 uvrA in the presence and absence of S9 activation and tester strain TA98 in the presence of S9 activation in the Bacterial Reverse Mutation Assay. An increase in revertant counts observed with tester strain TA98 in the absence of S9 activation was only slightly above the historical vehicle control range and the conclusion with this strain and activation condition is equivocal. The increases observed with tester strain TA1537 in the presence and absence of S9 activation, although reproducible, were not considered indicative of mutagenic activity because (1) the increases were not dose-responsive, (2) the vehicle control values were on the low end of the acceptable range and (3) the revertant counts at the peak of the response were within the historical vehicle control range. Therefore, the conclusion with this strain (TA1537) and activation conditions was negative.

Table 22: Revertant counts in plate incorporation test in the presence and absence of S9 activation.

Test Article	Conc (µg/plate)	Tester strain TA98	TA100	TA1535	TA1537	WP2uvrA
		Number of revertant colonies				
Without metabolic activation						
DMSO	50	11	92	8	5	14
SECNIDAZOLE	33	14	148	15	4	22
SECNIDAZOLE	100	13	274	20	4	40
SECNIDAZOLE	333	18	524	52	7	65
SECNIDAZOLE	1000	21	710	121	9	161
SECNIDAZOLE	3333	52	306	5	11	255
SECNIDAZOLE	5000	11	23	5	0	289
2-NF[1]	1.0	154				
Na azide[2]	1.0		509	475		
9-AA[3]	75				360	
MMS	1000					316
With metabolic activation						
DMSO	50	20	88	9	4	15
SECNIDAZOLE	33	17	189	16	8	33
SECNIDAZOLE	100	21	357	25	5	50
SECNIDAZOLE	333	33	640	68	4	105
SECNIDAZOLE	1000	45	679	127	9	252
SECNIDAZOLE	3333	100	184	6	13	395
SECNIDAZOLE	5000	60	13	14	2	446
[5]2-AA	1.0	335		68	37	
[5]2-AA	2.0		428			
[5]2-AA	15					330

[1]2-Nitrofluorene, [2] Sodium azide, [3]9-Amino-Acridine, [4]4-Nitroquinoline-N-Oxide
[5]2-amino-anthracine, MMS, methylmethanesulfonate;

Conclusion
The sponsor's criteria for positive included 'a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations'. Secnidazole was therefore positive in the Ames assay.

7.2 In Vitro Assays in Mammalian Cells

Study title:	*In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay)
Study report no.:	030629
(b) (4) Study Number:	(b) (4)
Conducting laboratory :	(b) (4)
Date of study initiation:	08 February 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	F120044
Purity (%)	>99%

Key Study Findings: Secnidazole was negative in the L5178Y/TK+/- Mouse Lymphoma Assay.

Methods

Cell line:	Mouse lymphoma L5178Y cells, clone 3.7.2C, obtained from (b) (4)
Concentrations in definitive study:	4-hour incubation: 50, 75, 100, 150 and 185 µg/mL 24- hour incubation: 50, 75, 100, 150 and 185 µg/mL
Basis of concentration selection:	In a preliminary toxicity assay cells were exposed to secnidazole at a maximum concentration of 185 µg/mL (1 mM). No visible precipitate was present in the treatment medium and the sponsor reports that the concentration selection for subsequent studies was based on reduction of suspension growth relative to the solvent control. Suspension growth relative to the solvent control was 105% and 90% at 185 µg/mL for the non-activated and S9-activated cultures with a 4-hour exposure, respectively. The concentrations used in the definitive mutagenesis assay ranged from 5 to 185 µg/mL for the non-activated and S9-activated cultures with a 4-hour exposure and the non-activated cultures with a 24-hour exposure. This is consistent with ICH S2 (R1) which recommends a maximum top concentration of 1 millimolar when not limited by solubility in solvent or culture medium or by cytotoxicity.
Negative control:	DMSO
Positive control:	Methyl methanesulfonate MMS
Formulation/Vehicle:	DMSO

Incubation & sampling time:

L5178Y/TK+/- cells (6 x 10^6) were combined with F0P medium or S9 activation mixture, and test article or positive control article in solvent or solvent alone. Treatment tubes were incubated with mechanical mixing for 4 or 24 hours at 37 °C.

For the preliminary toxicity assay only, after a 4-hour treatment in the presence and absence of S9 activation, the cells were washed with culture medium and cultured in suspensions for two days post-treatment, with cell concentration adjustment on the first day after exposure to the test article. After a 24-hour treatment in the absence of S9 activation, the cells were washed with culture medium and immediately readjusted to 3 x 10^5 cells/mL. Cells were, then, cultured in suspension for an additional two days post-treatment with cell concentration adjustment on the first day after exposure to the test article.

For the definitive assay only, at the end of the exposure period, the cells were washed with culture medium and collected by centrifugation. The cells were resuspended in 20 mL F10P, and incubated under standard conditions for two days following treatment.

Toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures after 2 or 3 days.

For expression of the TK-/- cells, cells were placed in cloning medium (C.M.) containing 0.24% dissolved Noble agar in F0P plus 20% horse serum. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT (trifluorothymidine, the selective agent) or VC (viable count).

For expression of the mutant phenotype in the 4-hour exposure, the cultures were counted using an electronic cell counter and adjusted to 3 x 10^5 cells/mL, 1 and 2 days after treatment in 20 and 10 mL total volume, respectively. For the 24-hour exposure, cultures were adjusted to 3 x 10^5 cells/mL in 20 mL immediately after test article removal, then 2 and 3 days after treatment in 20 and 10 mL total volume, respectively.

After the incubation period, the VC plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were then counted for each culture with ≥10% total relative growth (including at least one concentration between 10% and 20% total growth, if possible). The diameters of the TFT-resistant colonies for the positive and solvent controls were determined over a range of 0.2 to 1.1 mm.

Results

No cloned cultures exhibited induced mutant frequencies ≥90 mutants per 10^6 clonable cells. There was no concentration-related increase in mutant frequency. Tables 23 and 24 show the mutant frequencies for vehicle control, secnidazole and the positive controls.

Table 23: Summary for L5178Y/TK+/- mouse lymphoma cells treated with secnidazole in the presence of metabolic activation. Mutagenesis Assay (4-hour exposure)

DOSE LEVEL (µg/mL)	PRECIP.	% SUSP. GROWTH	VC COLONIES PLATE COUNTS 1	2	3	MEAN	TFT COLONIES PLATE COUNTS 1	2	3	MEAN	TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% RELATIVE TOTAL GROWTH
SOLVENT A		100	221	194	204	**206**	76	53	58	**62**	60	N/A	100
SOLVENT B			199	211	226	**212**	70	61	59	**63**	60		
50 A		96	174	169	210	**184**	64	68	62	**65**	70	**10**	85
50 B		102	164	152	172	**163**	70	73	85	**76**	93	**33**	79
75 A		91	185	187	236	**203**	72	101	84	**86**	85	**24**	88
75 B		97	209	196	200	**202**	58	80	65	**68**	67	**7**	94
100 A		95	205	210	181	**199**	60	61	91	**71**	71	**11**	90
100 B		96	215	214	229	**219**	78	67	67	**71**	64	**4**	100
150 A		87	217	220	200	**212**	79	65	90	**78**	73	**13**	89
150 B		93	214	203	199	**205**	54	56	85	**65**	63	**3**	91
185 A		93	182	184	185	**184**	64	64	53	**60**	66	**6**	82
185 B		89	218	218	206	**214**	97	80	83	**87**	81	**21**	91
POSITIVE CONTROL: 7,12-dimethylbenz(a)anthracene (DMBA) (µg/mL)													
1.5		27	136	116	151	**134**	281	236	286	**268**	399	**338**	18
1.0		50	161	157	163	**160**	245	246	284	**258**	322	**262**	39
MEAN SOLVENT TOTAL SUSPENSION GROWTH: 18.6													
MEAN SOLVENT CLONING EFFICIENCY: 105%													
MEAN SOLVENT MUTANT FREQUENCY: 60 (PER 10^6 CELLS)													

Solvent = DMSO A and B are duplicate cultures

$$\text{Mutant frequency per } 10^6 \text{ surviving cells} = \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$$

$$\text{Induced mutant frequency per } 10^6 \text{ surviving cells} = \text{mutant frequency} - \text{average mutant frequency of solvent controls}$$

$$\text{Total suspension growth} = \frac{\text{Day 1 cell conc.}}{0.3\times10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

$$\text{\% control cloning growth (not shown)} = \frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$$

$$\text{\% total growth} = \frac{(\text{\% suspension growth})(\text{\% cloning growth})}{100}$$

Table 24: Data summary for L5178Y/TK+/- mouse lymphoma cells treated with secnidazole in the absence of exogenous metabolic activation. Mutagenesis Assay (24-hour exposure)

DOSE LEVEL (µg/mL)	PRECIP.	% SUSP. GROWTH	VC COLONIES PLATE COUNTS 1	2	3	MEAN	TFT COLONIES PLATE COUNTS 1	2	3	MEAN	TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% RELATIVE TOTAL GROWTH
SOLVENT A		100	184	227	214	**208**	29	30	29	**29**	28	N/A	100
SOLVENT B			236	230	231	**232**	26	32	27	**28**	24		
50 A		105	259	248	217	**241**	26	21	24	**24**	20	**-7**	**115**
50 B		110	174	184	171	**176**	24	24	37	**28**	32	**6**	**88**
75 A		110	212	184	197	**198**	26	24	27	**26**	26	**0**	**98**
75 B		106	215	200	205	**207**	47	29	30	**35**	34	**8**	**100**
100 A		102	224	231	228	**228**	42	27	36	**35**	31	**4**	**105**
100 B		108	220	206	184	**203**	29	30	36	**32**	31	**5**	**100**
150 A		103	200	200	226	**209**	50	29	26	**35**	34	**7**	**98**
150 B		109	202	171	198	**190**	32	21	24	**26**	27	**1**	**94**
185 A		102	227	206	189	**207**	37	31	33	**34**	32	**6**	**96**
185 B		111	223	186	163	**191**	21	31	21	**24**	26	**-1**	**96**
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (µg/mL)													
7.5		59	111	93	97	**100**	239	233	215	**229**	456	**430**	**27**
5		80	126	118	117	**120**	220	204	223	**216**	358	**332**	**44**

MEAN SOLVENT TOTAL SUSPENSION GROWTH: 44.5
MEAN SOLVENT CLONING EFFICIENCY: 110%
MEAN SOLVENT MUTANT FREQUENCY: 26 (PER 10^6 CELLS)

Solvent = DMSO A and B are duplicate cultures

$$\text{Mutant frequency per } 10^6 \text{ surviving cells} = \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$$

$$\text{Induced mutant frequency per } 10^6 \text{ surviving cells} = \text{mutant frequency} - \text{average mutant frequency of solvent controls}$$

$$\text{Total suspension growth} = \frac{\text{Day 0 cell conc.}}{0.3\times10^5 \text{ cells/mL}} \times \frac{\text{Day 1 cell conc.}}{\text{Day 0 adjusted cell conc.}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

$$\text{\% control cloning growth (not shown)} = \frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$$

$$\text{\% total growth} = \frac{(\text{\% suspension growth})(\text{\% cloning growth})}{100}$$

Study Validity
The study was deemed valid. The applicant's criteria for a valid test were met and were reasonable.

Criteria for a valid test:

(1) Negative Controls: The average spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 35 to 140 TFT-resistant mutants per 10^6 surviving cells. Low spontaneous mutant

frequencies, i.e., 20 to 34 mutants per 106 surviving cells, are considered acceptable if small colony recovery is demonstrated. The average cloning efficiency of the solvent (or vehicle) controls must be between 65% and 120% and the total suspension growth between 8-32 for the 4-hour exposure and 20-180 for the 24-hour exposure

(2) Positive controls: The mutant frequency for at least one dose of the positive controls must meet the criteria for a positive response and induce an increase in small colony mutants according to the following criteria: Induced Mutant Frequency (IMF) positive control ≥300 x 10-6 mutants with 40% small colonies **or** small colony IMF for positive control ≥150 x 10^{-6}

(3) Test Article-Treated Cultures: Cultures treated with a minimum of four concentrations of test article must be attained and their mutant frequencies reported. Results may be accepted, with justification, when only three concentrations of test article are evaluated and otherwise meet the other criteria for a valid test. The highest test article concentration must produce 80% to 90% toxicity unless limited by solubility or the maximum required concentration.

Criteria for positive

A result was considered positive if a concentration-related increase in induced mutant frequency was observed in the treated cultures and one or more treatment conditions with 10% or greater total growth exhibited induced mutant frequencies of ≥ 90 mutants per 10^6 clonable cells (based on the average mutant frequency of duplicate cultures).

A result was considered negative if the treated cultures exhibited induced mutant frequencies of less than 90 mutants per 10^6 clonable cells (based on the average mutant frequency of duplicate cultures) and there was no concentration-related increase in mutant frequency.

Study Outcome

The secnidazole concentrations chosen for cloning were 50, 75, 100, 150 and 185 µg/mL, with the top dose being equivalent to 1mM. No cloned cultures exhibited induced mutant frequencies ≥90 mutants per 106 clonable cells and there was no concentration-related increase in mutant frequency. In the same assays, the positive control substances methyl methanesulfonate (MMS) and N-nitrosodimethylamine (DMN) induced significant mutant frequency (mean mutant frequencies ≥300 x 10^{-6} mutants). These data show the sensitivity of the test and the metabolizing activity of the S9-mix.

Conclusion

Under the conditions of this study, test article secnidazole was negative in the L5178Y/TK+/- Mouse Lymphoma Assay.

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title:	*In Vivo* Micronucleus Assay in Rats
Study no.:	030630
Conducting laboratory:	(b) (4)
Date of study initiation:	07 February 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	F120044
% purity:	>99%

Key Study Findings: Secnidazole was nongenotoxic in the rat micronucleus assay.

Methods

Doses in definitive study:	500, 1000 and 2000 mg/kg
Frequency of dosing:	Single
Route of administration:	Oral
Dose volume:	0.2 mL/10g
Positive control dose volume	0.1 mL/10g
Formulation/Vehicle:	2% Carboxy methylcellulose
Species/Strain:	*Rattus norvegicus* (Sprague-Dawley (Hsd:SD))
Number/Sex/Group:	25/sex/dose group
Age	6-8 weeks
Body weights	140-350g
Basis of dose selection:	There was no mortality at 2000 mg/kg in the dose range-finding assay, and so a dose of 2000 mg/kg was tested as the high dose in the definitive micronucleus assay. The mid and low doses were 1000 and 500 mg/kg.
Negative control:	2% Carboxy methylcellulose (CMC; 400 cP), medium viscosity
Positive control:	Cyclophosphamide (40 mg/kg)

The study was conducted in two phases: a dose range-finding assay that evaluated the toxicity of the test article and a definitive micronucleus assay that evaluated the genotoxic potential of secnidazole.

In the dose range-finding assay, three rats/sex were exposed to secnidazole at an initial dose of 1000 mg/kg, based on toxicity information provided by the Sponsor. Due to the absence of mortality at 1000 mg/kg, an additional three rats/sex were exposed to the test article at the maximum OECD guideline recommended dose level of 2000 mg/kg. No mortality was observed. Lethargy and piloerection were noted in male and female rats at 1000 and 2000 mg/kg. Prostration was also noted in one male rat at 2000 mg/kg. Since no substantial differences in the clinical signs of toxicity between the sexes were observed in the dose range-finding assay, only male mice were used in the definitive micronucleus assay.

In the definitive micronucleus assay, groups of rats (5/sex/dose) were administered either vehicle or with the secnidazole at 500, 1000 or 2000 mg/kg on two consecutive days. The second dose was administered approximately 24 hours after the first dose. A fifth group of five was administered with the positive control article once on the second day. Animals were euthanized by carbon dioxide inhalation after which

the femurs were exposed, cut just above the knee, and the bone marrow was aspirated into a syringe containing fetal bovine serum.

The bone marrow was transferred to a centrifuge tube containing approximately 3 mL of fetal bovine serum, the cells were pelleted by centrifugation and the supernatant was drawn off leaving a small amount of fetal bovine serum with the pellet. Cells were re-suspended and a small drop of the bone marrow suspension was spread onto a clean glass slide. Two slides were prepared from each animal, air dried and fixed by dipping in methanol. One set of slides was stored at room temperature and stained with acridine orange for microscopic evaluation. The other set of slides was kept as backup. Each slide was identified by the harvest date, study number, and animal number. Slides were coded using a random number table by an individual not involved with the scoring process.

Scoring
Bone marrow was evaluated by fluorescent microscopy. The criteria for the identification of micronuclei are those of Schmid (1975). Scoring was based upon the micronucleated cell, not the micronucleus; thus, occasional cells with more than one micronucleus were counted as one micronucleated PCE (mnPCE), not two (or more) micronuclei.

At least 2000 PCEs/animal were scored for the presence of micronuclei (mnPCEs) whenever possible. In addition, whenever possible, at least 1000 total erythrocytes (PCEs + NCEs) were scored per animal to determine the proportion of PCEs as an index of bone marrow cytotoxicity. PCE proportions <20% of vehicle control value were considered excessively cytotoxic and the animal data were excluded from evaluation.

Criteria for positive response

The result was to be considered positive in the micronucleus test if it induced a statistically significant ($p<0.05$) and dose-related increase in the number of micronucleated PCEs either in the combined data for both sexes or in the data for male or female mice separately.

Study Validity

The sponsor's criteria for determination of a valid Test were that (1) the mnPCE frequency of the vehicle controls must be within the historical vehicle control range, and (2) the positive control must induce a significant increase ($p \leq 0.05$) in mnPCE frequency as compared to the concurrent vehicle control.

The dosing was deemed adequate since it was consistent with the dose selection guidance in ICH Guidance for Industry 'S2 (R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use' which recommends a limit dose of 2000 mg/kg. Five animals per group were available for analysis. Negative and positive control animals produced the expected responses, verifying that laboratory was able to reliably detect micronuclei in the test rats.

Results

Clinical signs.

Lethargy and piloerection were noted at 2000 mg/kg only. All other animals in the remaining treatment groups appeared normal during the study period.

Toxicokinetics

No toxicokinetics evaluations were conducted.

Table 25: Summary of Bone Marrow Micronucleus Analysis following secnidazole in Sprague Dawley rats.

Test article	PCE/Total erythrocytes	Number mnPCEs/PCE scored
2 Carboxy methylcellulose	0.545	7/10,000
500 mg/kg Secnidazole	0.588	9/10,000
1000 mg/kg Secnidazole	0.572	8/10,000
2000 mg/kg Secnidazole	0.570	10/10,000
40 mg/kg Cyclophosphamide	0.564	*139/10,000

PCE: Polychromatic Erythrocytes; mnPCE: Micronucleated Polychromatic Erythrocytes
*Statistically significant increase compared to the vehicle control, $p \leq 0.05$ (Kirshenbaum-Bowman Tables)

Discussion

No reductions in the ratio of PCEs to total erythrocytes in the test article groups compared to the vehicle control group were observed, suggesting the test article did not inhibit erythropoiesis. No statistically significant increase in the incidence of mnPCEs in the test article-treated groups relative to the vehicle control group was observed at 24 hours after final dose administration ($p > 0.05$, Kastenbaum-Bowman tables). CP, the positive control, induced a statistically significant increase in the incidence of micronucleated PCEs ($p \leq 0.05$, Kastenbaum-Bowman tables). Under the conditions of the study conduct as described in this report, oral administrations of secnidazole at doses up to and including a dose of 2000 mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow of male Hsd:SD rats. Therefore, secnidazole was negative in the rat micronucleus assay.

7.4 Other Genetic Toxicity Studies

8 Carcinogenicity

The course of treatment is a single dose and, by ICH guidelines, the applicant is not required to conduct carcinogenicity studies with secnidazole. However, several nitroimidazoles have been associated with carcinogenicity. For example, tumors affecting the liver, lungs, mammary, and lymphatic tissues have been detected in several studies of metronidazole in rats and mice. Carcinogenicity is seen as a class effect for nitroimidazoles.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title	An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation of SYM-1219 in Rats
Report no.:	(b) (4)-993006
Study no:	SYM1219.TOX.945.RPT1022
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	09 July 2013
Date of first dosing:	05 August 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	F130006
% purity:	99.9%

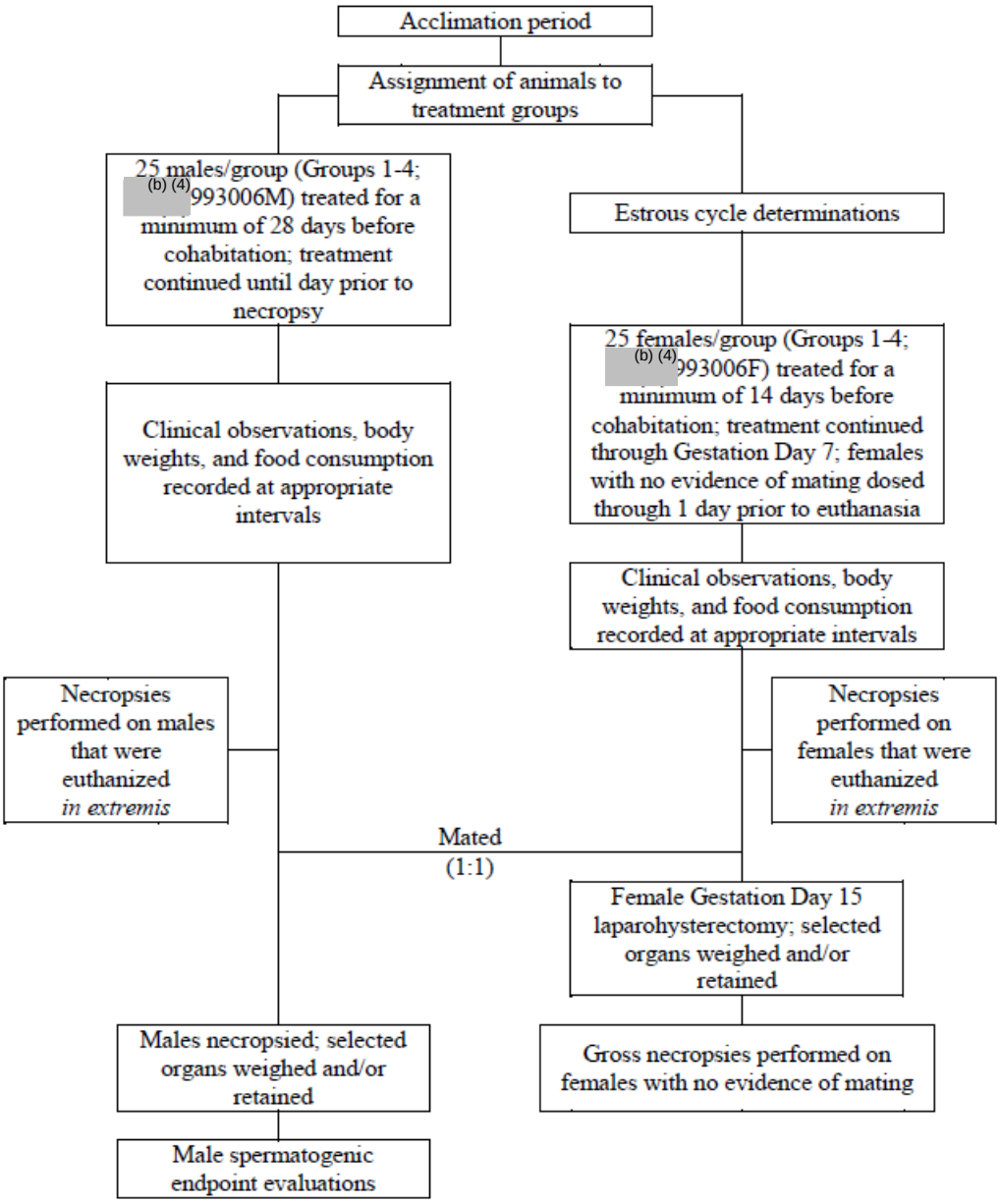
Key study findings

The NOAEL for reproductive toxicity was 100 mg/kg/day for males and females. The 300 mg/kg dose caused lower bodyweight gain in female rats. The 1000 mg/kg dose was associated with mortality and moribundity in males and females. At 300 mg/kg, rats showed reduced sperm motility, reduced epididymis weights (-19%) and increased numbers of abnormal sperm (normally shaped head separated from flagellum) compared to controls.

Methods	
Doses:	0, 100, 300 and 1000 mg/kg/day.
Frequency of dosing:	Males: Daily, a minimum of 28 days prior to cohabitation through 1 day prior to scheduled euthanasia Females: Daily, a minimum of 14 days prior to cohabitation through gestation day 7.
Dose volume:	10 mL/kg/day
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% Carboxymethylcellulose sodium, medium viscosity, 400-800 cps, in deionized water.
Species:	Rat (*Rattus norvegicus*)
Strain:	Sprague-Dawley Crl:CD®
Number/Sex/Group:	25 rats/sex/dose group
Satellite groups:	None
Deviation from study protocol:	There were no study deviations that impacted the quality or integrity of the study.

Males were treated for at least 28 days before cohabitation, throughout the mating period through one day prior to necropsy (63-64 doses). Females were dosed for at least 14 days before cohabitation, one male with one female. The day when evidence of mating was identified was termed Gestation Day 0. For the females, treatment continued through Gestation Day 7 and females were subjected to laparohysterectomy on Gestation Day 15. See Figure 6 for additional details.

Figure 6: Study design for 'An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation of SYM-1219 in Rats'



Observations and Results

Mortality

Three males in the 1000 mg/kg/day group were euthanized *in extremis* on Study Days 28, 32, and 48, respectively. Clinical signs in these animals included head tilt, circling, labored respiration red, clear, and/or yellow material around the nose, mouth, forelimbs, and/or urogenital area.

Four females in the 1000 mg/kg/day group were euthanized *in extremis* approximately 4 hours following dose administration on Study Day 14 or 23. Clinical observations included a cool, limp, and/or pale body, labored respiration, lacrimation, and red/clear material around the mouth. Based on the macroscopic findings noted for these females at necropsy, the moribundity for females in the 1000 mg/kg/day group was attributed by the sponsor to intubation error.

Clinical Signs

Test article-related clinical findings noted for surviving males in the 1000 mg/kg/day group indicated that this dose was above the maximum tolerated dose. Head tilt, body drags, and/or rocking, lurching, or swaying while ambulating were noted for 9 surviving males at 1000 mg/kg/day during Study Days 53-64 at the daily examinations and/or approximately 2 hours following dose administration. In addition, increased incidences of red, yellow, and/or clear material around the nose, mouth, forelimbs, and/or urogenital area were noted for 21 surviving males in the 1000 mg/kg/day group. Test article-related increased incidences of red, yellow, and/or clear material around the nose, mouth, forelimbs, dorsal head, and/or urogenital area were noted for 23 surviving females in the 1000 mg/kg/day group.

Body Weight

There was a statistically significant (13 to 18%) decrease in bodyweight in high dose females on Days 13 and 15 of gestation. During the gestation treatment period (Day 0-7), bodyweight gain was reduced by 47% in high dose females compared to controls. Final body weights were decreased by 19 % at the end of the study and body weight gains were 45 to 100 % reduced in high dose animals in all phases of dosing. Body weight gain was only sporadically reduced at the mid dose and no different from controls at the low dose.

Table 26: Summary of bodyweights (g) in secnidazole-treated pregnant females: Fertility and Early Embryonic Development study in Rats

	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Bodyweight on gestation Day 15 (g)	311	302	293	254
% difference from control		-2.9	-5.8	-18

Table 27: Bodyweight gains in secnidazole-treated pregnant females during gestation. Fertility and Early Embryonic Development study in Rats.

	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Day 0-7	36	36	33	19
% difference from control			-8%	-47%
Day 7-15	41	38	30	0
% difference from control		-7%	-27%	-100%

Table 28: Summary of bodyweights and bodyweight gains in secnidazole-treated males. Fertility and Early Embryonic Development study in Rats.

	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Bodyweight on day 63 (g)	536	528	514	432
% difference from control		-1%	-4%	-19%
Bodyweight gain Day 0-63 (g)	240	234	221	138
% difference from control		-2%	-8%	-43%

Feed Consumption

There were no drug related or biologically significant differences in food consumption between control and drug treated female animals. The observed differences in mean food consumption in males (up to 27 %) were sporadic.

Dosing Solution Analysis

The formulation sets submitted for analysis on average met the accuracy and homogeneity criteria for suspensions. The one sample outside the accuracy criteria deviated by only 1% and did not impact the study.

Necropsy

Females euthanized *in extremis in* 1000 mg/kg/day group.
At necropsy, two females, euthanized *in extremis* on Study Day 14, showed esophageal perforations and intubation error was determined to be the cause of death. Two females (euthanized *in extremis* on Study Day 23) had clear fluid contents in the thoracic cavity. Based on the internal findings, the death of these females was attributed to suspected intubation error.

Three males euthanized *in extremis* in the 1000 mg/kg/day group.
At necropsy, all 3 males showed small and/or soft left and right epididymides and/or testes. One male had dark red areas on the lungs, a pale spleen with rough surfaces, and a diaphragmatic hernia. Based on the congenital defect noted for this male at necropsy, the death of this male was not attributed to test article administration. All other moribundity noted at 1000 mg/kg/day was considered test article-related.

Scheduled Necropsy
At the scheduled male necropsy, 20 of 22 surviving males in the 1000 mg/kg/day group showed small and/or soft left and right epididymides and/or testes. No other test article-related macroscopic findings were noted for males at any dosage level. Other macroscopic findings observed in the test article-treated groups occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not dose-related.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Changes in male fertility parameters were largely restricted to the 1000 mg/kg dose, although there were a few marginal changes in sperm parameters at 300 mg/kg. In the high dose males, the mating index was reduced by 27 %, the fertility index was reduced to 9 % (compared to 92 % in controls), and the number of males that sired a litter was reduced to 12.5 %, compared to 100 % in controls.

Table 29: Male reproductive performance in in Fertility and Early Embryonic Development study in Rats

Parameter	Dosage Level (mg/kg/day) 0	100	300	1000	(b) (4) HC[a] Mean (Range)
Male Mating Index (%)	92.0	100.0	96.0	72.7	97.1 (88.0-100.0)
Male Fertility Index (%)	92.0	100.0	88.0	9.1[††]	93.8 (87.5-100.0)
Male Copulation Index (%)	100.0	100.0	91.7	12.5[††]	96.6 (88.0-100.0)

[a] = (b) (4) historical control data
[††] = Statistically significant at 0.01 compared to the control group using Chi-square test.

Definitions:

Mating index (%) $\frac{\text{No. of animals with evidence of mating}}{\text{No. of animals used for mating}} \times 100$

Male fertility index (%) $\frac{\text{No. of males siring a litter}}{\text{Total number of males used for mating}} \times 100$

Male copulation index $\frac{\text{No. of males siring a litter}}{\text{No. of males with evidence of mating}} \times 100$

Female fertility index (%) $\frac{\text{No. of females with confirmed pregnancy}}{\text{No. of females with evidence of mating}} \times 100$

Female conception index (%) $\frac{\text{No. of females with confirmed pregnancy}}{\text{No. of females with evidence of mating}} \times 100$

At 1000 mg/kg, lower testis (-61%) and epididymis (-58%) weights were consistent with the lower sperm concentrations in the testes (-97%) and cauda epididymis sperm concentrations (-88%). Sperm production rate was also reduced by 97%. The percentage of normal sperm was also reduced by 66 %.

At 300 mg/kg, rats showed reduced sperm motility (65 % vs 85 % in controls), reduced epididymis weights (-19%) and increased numbers of abnormal sperm (normally shaped head separated from flagellum) compared to controls.

Table 30: Sperm Evaluations in Fertility and Early Embryonic Development study in Rats

Parameter	Dosage Level (mg/kg/day) 0	100	300	1000	(b) (4) HC[a] Mean (Range)
Motility (%)	82	84	65+	0	87.3 (78.9-94.0)
Left Cauda Epididymis Weight (grams)	0.3138	0.3175	0.2527**	0.1318**	0.3197 (0.2655-0.3706)
Left Cauda Epididymis Concentration (milllions/gram)	874.4	947.7	802.4	106.3**	896.4
Left Testis Concentration (millions/gram)	127.7	128.1	128.3	4.1**	79.2 (65.9-102.5)
Left Testis Weight (grams)	1.80	1.84	1.78	0.69**	1.76 (1.66-1.88)
Sperm Production Rate (millions/gram/day)	20.9	21.0	21.0	0.7**	13.0 (10.8-16.8)
Normal Sperm (%)	99.7	99.4	94.9++	34.0++	99.3 (97.1-99.9)
Normally Shaped Head Separated from Flagellum (%)	0.2	0.3	2.0	17.0	0.4 (0.0-1.8)
Head Absent with Normal Flagellum (%)	0.0	0.3	3.2	49.0	0.3 (0.0-1.4)

[a] = (b) (4) historical control data
+ = Statistically significant at 0.05 compared to the control group using Dunn's test.
++ = Statistically significant at 0.01 compared to the control group using Dunn's test.
** = Statistically significant at 0.01 compared to the control group using Dunnett's test.

In the females, reproductive capacity was also affected only at the high dose. The female fertility index was reduced by 91% and the female conception index was reduced by 90 %. The mean precoital interval was increased from 2.4 days in controls to 5.8 days in the high dose.

Table 31: Female reproductive performance in Fertility and Early Embryonic Development study in Rats.

Parameter	Dosage Level (mg/kg/day) 0	100	300	1000	(b) (4) HC[a] Mean (Range)
Female Mating Index (%)	92.0	100.0	100.0	91.3	99.4 (95.0-100.0)
Female Fertility Index (%)	92.0	100.0	92.0	8.7††	96.0 (88.0-100.0)
Female Conception Index (%)	100.0	100.0	92.0	9.5††	96.7 (88.0-100.0)
Estrous Cycle Length (days)	4.0	4.0	4.0	4.3**	4.2 (3.7-5.2)
Pre-Coital Interval (days)	2.4	1.8	2.8	5.8**	2.9 (2.0-3.9)

[a] = (b) (4) historical control data
** = Statistically significant at 0.01 compared to the control group using Dunnett's test.
†† = Statistically significant at 0.01 compared to the control group using Chi-square test.

Table 32: Summary of embryonic data for rats in Fertility and Early Embryonic Development study in Rats

Dosage Group	Vehicle	100 mg/kg	300 mg/kg	1000 mg/kg
No of gravid females	23	25	23	2
Viable embryos	326	359	330	4
Dead embryos	0.0	0.0	0.0	0.0
Early resorptions/dam	19	18	19	1
Late resorptions/dam	0.0	0.0	0.0	0.1
Pre-implantation loss	15	9	24	19
Post-implantation loss	19	18	19	1
No. of implantations	345	377	349	5
Number of corpora lutea	360	386	373	24

Discussion

Adverse clinical findings in males at 1000 mg/kg included moribundity and reductions in mean body weights and body weight gains. Three high dose males were sacrificed moribund and frank decreases in bodyweights (-19%) and bodyweight gain (-47%).

In males treated with 300 and 1000 mg/kg secnidazole, poor male reproductive performance, reduced spermatogenic parameters, reduced male reproductive organ weights, and/or adverse macroscopic findings in the epididymides and testes were observed (See Tables 30 and 31, above).

In females, the two highest doses, 300 and 1000 mg/kg resulted in 27% and 100% reductions in bodyweight gain compared to controls. Per ICH Guideline 'Detection of Toxicity to reproduction for medicinal products and toxicity to male fertility S5 (R2)', factors limiting the high dosage include reduction in bodyweight gain. The maternal toxicities recorded at these doses indicate that the 300 and 1000 mg/kg doses were higher than what should have been the high dose for female rats. Poor female reproductive performance (only 2 females were determined to be gravid), lower numbers of corpora lutea and implantation sites and increased pre- and post-implantation loss, reduced ovary weights were observed in females in the 1000 mg/kg/day group.

Conclusion:

The NOAEL for reproductive toxicity was 100 mg/kg/day for males and females. The 300 and 1000 mg/kg doses resulted in excessive maternal toxicity based on reduction in bodyweight gain in female rats. The 1000 mg/kg dose resulted in mortality and moribundity in males. At 300 mg/kg, rats showed reduced sperm motility (65 % vs 85 % in controls), reduced epididymis weights (-19%) and increased numbers of abnormal sperm (normally shaped head separated from flagellum) compared to controls.

9.2 Embryonic Fetal Development

Study title:	An Oral (Gavage) Study of the Effects of SYM-1219 on Embryo/Fetal Development in Rats
Study no:	(b) (4)-993002
Study report location:	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	01 July 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	F130006
% purity:	99.9 %

Key Study Findings: There were no effects on embryofetal development in rats at 0.25 times the clinical exposure based on AUC comparisons.

Methods

Doses:	100, 300, and 1000 mg/kg/day
Frequency of dosing:	Daily from gestational Day 6 to 17 inclusive
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% Carboxymethylcellulose sodium, medium viscosity, 400-800 cps, USP in deionized water.
Species:	*Rattus norvegicus*
Strain:	Sprague Dawley Crl:CD(SD)
Number/Group:	25 rats/dose group
Satellite groups:	8 female rats/dose group for toxicokinetics
Study design:	Four groups of pregnant rats were treated orally by gavage, with secnidazole or vehicle between gestation Days 6 to 17 inclusive. All surviving rats were euthanized by carbon dioxide inhalation on Gestation Day 20 and abdominal contents examined. Reproductive parameters are shown on Table 36, below. Satellite animals were bled on Days 6 and 20 at 0 (pre-dose), 1, 2, 4, 8, and 24 hours after dose administration for toxicokinetics evaluations.
Deviation from study protocol:	There were no deviations occurred which compromised the validity of the study results

Table 33: Group assignments in Embryo/Fetal Development study in Rats

Group Number	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Females	
					Main	TK
1	Vehicle control	0	0	10	25	8
2	SYM-1219	100	10	10	25	8
3	SYM-1219	300	30	10	25	8
4	SYM-1219	1000	100	10	25	8

Observations and Results

Maternal mortality and Clinical signs

All females in the control, 100, 300, and 1000 mg/kg/day groups survived to the scheduled necropsy on Gestation Day 20. Red material around the nose and mouth was noted in the 1000 mg/kg/day group at the daily observations and/or approximately 2 hours following dose administration beginning as early as Gestation Day 7 and continuing through Gestation Day 20.

All rats were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded daily from Gestation Days 0 through 20 (prior to dose administration during the treatment period). Animals were also observed for signs of toxicity approximately 2 hours following dose administration.

Body Weight

Overall mean maternal body weight gains in the 300 and 1000 mg/kg/day group were 12 and 16 % lower compared to the control group over the Gestation Days 6-18. Individual maternal body weights were recorded on Gestation Days 0, 6-18 (daily), and 20 for the embryo/fetal development phase and on Gestation Days 0 and 6-17 (daily) for the toxicokinetic phase.

Feed Consumption

Slight transient reductions in feed consumption were observed at 100, 300 and 1000 mg/kg between days 6-9. Feed consumption in 1000 mg/kg animals between days 6-18 was 10 % lower than controls.

Toxicokinetics

T_{max} (h) values were between 1-2 hours after dosing on Day 6 or Day 17. $AUC_{(0\text{-}24h)}$ values increased approximately in proportion with dose on both evaluation days. C_{max} values increased approximately in less than proportionally with dose on both days. Systemic exposure, in terms of AUClast, was similar on GD 6 and GD 17; accumulation ratios ranged from 1.1 to 1.3, indicating negligible drug accumulation. Actual terminal half-life values were between 3-5 hours. approximately 4 hours at 300 mg/kg/day on GD 6, and 3 and 5 hours at 300 and 1000 mg/kg/day on GD 17. Three values had to be estimated since the data failed to meet acceptance criteria. See Table 34 below.

Table 34: Mean plasma concentrations and pharmacokinetics parameters on gestation Day 6 in Embryo/Fetal Development study in Rats

Dosage Group	Vehicle	Low 100 mg/kg/day	Medium 300 mg/kg/day	High 1000 mg/kg/day
C_{max} (µg/mL)	ND	76	213	386
T_{max} (h)	ND	1	1	1
$T_{½}$ (h)	ND	3.4 *	3.6	13*
$AUC_{(last)}$ µg·h/mL	ND	331	1650	4140

*Estimated: Failed to meet acceptance criteria

Table 35: Mean plasma concentrations and pharmacokinetics parameters on gestation Day 16/17 in Embryo/Fetal Development study in Rats.

Dosage Group	Vehicle	Low 100 mg/kg/day	Medium 300 mg/kg/day	High 1000 mg/kg/day
C_{max} (µg/mL)	ND	78	210	521
T_{max} (h)	ND	1	1	2
$T_{½}$ (h)	ND	3.9*	3.3	4.9
$AUC_{(last)}$ µg·hr/mL	ND	352	1920	5370

*Estimated: Failed to meet acceptance criteria

Stability and Homogeneity

The analyzed dosing formulations were within [redacted] (b) (4) SOP range for suspensions (85% to 115%), were homogeneous, and were stable following 7 days of refrigerated storage. The test article was not detected in the vehicle formulation that was administered to the control group (Group 1).

Necropsy

Cesarean Section Data

Litter data presented on Table 36, show that mean fetal weight (-10%) was reduced at the high dose compared to control animals. Also at the high dose there was a lower mean litter proportion of cervical centrum no. 1 ossified, higher mean litter proportions of sternebra(e) nos. 1, 2, 3, and/or 4 unossified and sternebra(e) nos. 5 and/or 6 unossified. Although the findings of delayed ossification in the 1000 mg/kg/day group were considered test article-related, they were not considered adverse because ossification delays resolve with growth. The incidence of 7th cervical rib was also increased at the 1000 mg/kg dose, compared to concurrent and historical controls.

Table 36: Litter data for rats dosed with secnidazole between gestational Days 6 to 17 in Embryo/Fetal Development study in Rats.

Dosage Group	Vehicle	100 mg/kg	300 mg/kg	1000 mg/kg
Number of live fetuses/dam	13.8	14.8	14.1	14.1
Number of dead fetuses/dam	0.0	0.0	0.0	0.0
No. of early resorptions/dam	0.7	0.5	0.7	0.6
No. of late resorptions/dam	0.0	0.0	0.0	0.1
No of gravid females	24	23	23	24
Pre-implantation loss	0.7	0.6	2.1	0.8
Post-implantation loss	0.7	0.5	0.7	0.8
No. of implantations/dam	14.5	15.3	14.8	14.9
Number of corpora lutea/dam	15.2	15.9	17.0	15.7
Weight of male fetuses (g)	4.0	3.8	3.8	3.6
Weight of female fetuses (g)	3.8	3.7	3.6	3.4
Overall Fetus weight	3.9	3.8	3.7	3.5
Sex ratio (% male fetuses)	51	53	50	48
Fetal anomalies				
Gross external malformations	0	1	0	0
Visceral anomalies	2	0	0	0
Skeletal anomalies	0	0	0	0
7th cervical rib (pups/litter)	1/1	7/5	7/6	16/8
7th cervical rib (%/litter)	0.3	2.0	2.6	4.8
Sternebrae #1, #2, #3 and/or #4 unossified (pups/litters)	0/0	0/0	2/2	8/4
Cervical centrum #1 ossified (pups/litters)	73/18	46/15	34/14	28/11
Sternebrae #5 and/or #6 unossified (pups/litters)	4/2	8/6	6/4	57/17

Malformations and Variations
Sporadic occurrences of abnormalities were not considered to be related to treatment.

Laparohysterectomies and macroscopic examinations were performed blind to treatment group. All females were euthanized on Gestation Day 20 by carbon dioxide inhalation. The thoracic, abdominal, and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined.

Each viable fetus was examined externally, individually sexed, weighed, euthanized by a subcutaneous injection of sodium pentobarbital in the scapular region, and tagged for identification. The detailed external examination of each fetus included, but was not limited to, an examination of the eyes, palate, and external orifices, and each finding was recorded. Crown-rump measurements and degrees of autolysis were recorded for late resorptions, a gross external examination was performed (if possible), and the tissues were discarded. Each viable fetus was subjected to a visceral examination to include the heart and major blood vessels. All carcasses were eviscerated and fixed in 100% ethyl alcohol after which each

fetus was macerated in potassium hydroxide and stained with Alizarin Red S. Fetuses were then examined for skeletal malformations and developmental variations.

Discussion

Overall mean maternal body weight gains in the 300 and 1000 mg/kg/day group were 12 and 16 % lower compared to the control group over the Gestation Days 6-18. These frank reductions in bodyweight gain indicate maternal toxicity and that the high dose should have been lower than 300 mg/kg.

In high dose animals, mean fetal weight was reduced (-10%) compared to control animals. Also at the high dose, the incidence of 7th cervical rib was increased compared to controls. Lower mean litter proportion of cervical centrum no. 1 ossified and higher mean litter proportions of sternebra(e) nos. 1, 2, 3, and/or 4 unossified and sternebra(e) nos. 5 and/or 6 unossified were also recorded. The findings of reduced ossification in the 1000 mg/kg/day group were not considered adverse because ossification delays resolve with growth.

The NOAEL was estimated at 100 mg/kg, based on the reduction in bodyweight gain at 300 mg/kg. This dose, ($AUC_{(last)}$ 352 µg·h/mL) is equivalent to about 0.3 times of the exposure observed at the clinical dose (1331 µg·h/mL).

Conclusion:
There were no effects on embryofetal development in rats at 0.3 times the clinical exposure based on AUC comparisons.

Study title:	An Oral (Gavage) Study of the Effects of SYM-1219 on Embryo/Fetal Development in Rabbits
Study no:	(b) (4)-993005
Sponsor Report no:	SYM1219.TOX.945.RPT1023
Study report location:	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	30 August 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	F130006
% purity:	99.9 %

Key Study Findings: The NOAEL for maternal toxicity was 30 mg/kg/day and the NOAEL for embryo/fetal development was 100 mg/kg/day when secnidazole was administered orally to pregnant New Zealand White rabbits during organogenesis.

Methods

Doses:	10, 30, and 100 mg/kg/day
Frequency of dosing:	Daily from gestational Day 7 to 20 inclusive
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% Carboxymethylcellulose sodium, medium viscosity, 400-800 cps, USP in deionized water.
Species:	***Oryctolagus cuniculus***
Strain:	New Zealand White [Hra:(NZW)SPF]
Number/Group:	22 rabbits/dose group
Satellite groups:	4 female rats/dose group for toxicokinetics
Deviation from study protocol:	There were no deviations occurred which compromised the validity of the study results

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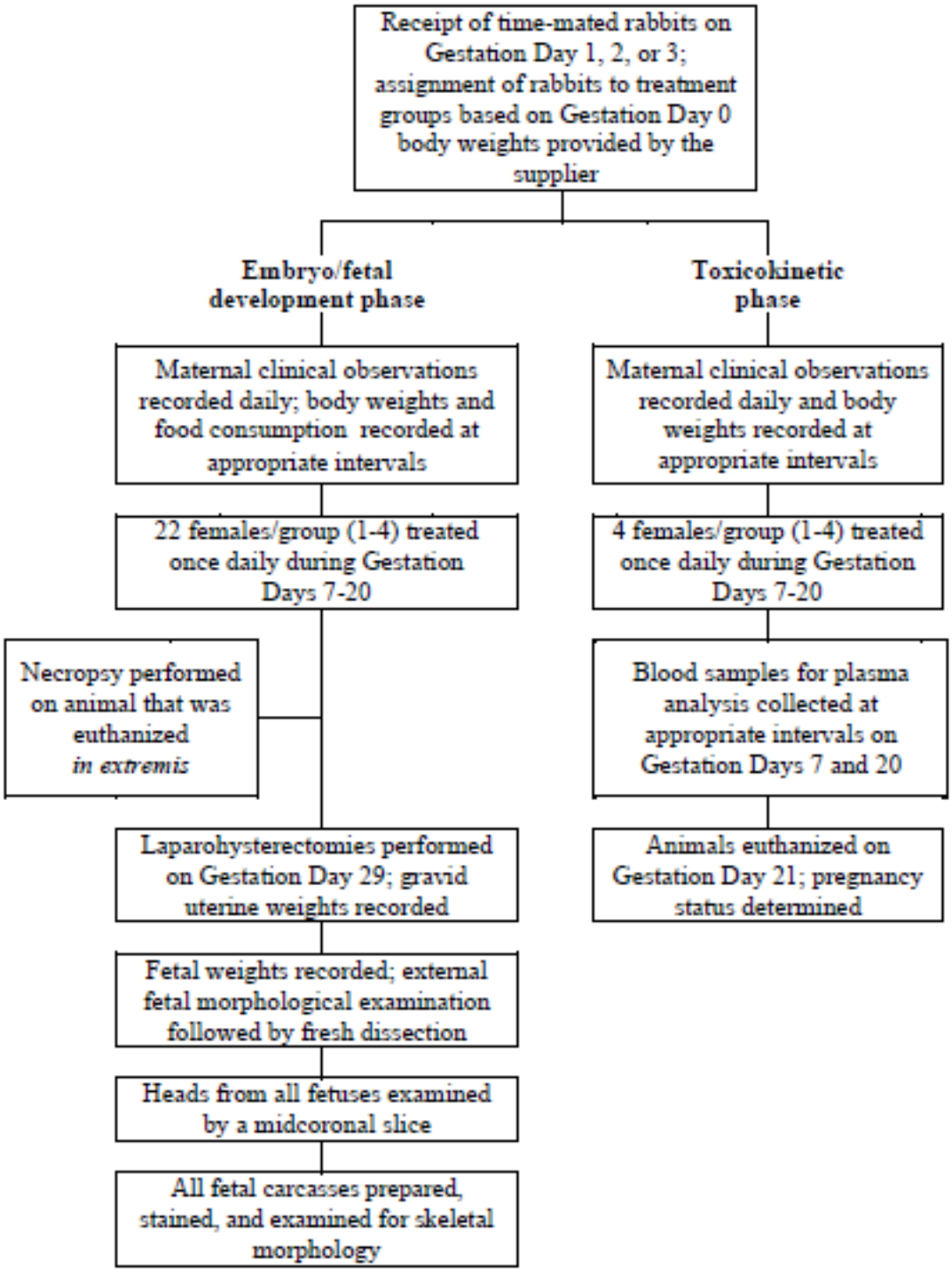
Figure 7: Study outline Embryo/Fetal Development study in Rabbits



Observations and Results

Maternal mortality and Clinical signs

One female in the 100 mg/kg/day group was euthanized *in extremis* on Gestation Day 28. Rales, labored respiration, brown material on the urogenital area, decreased defecation, small feces, and soft stool were recorded in this animal. These findings were attributed to the possible aspiration of test article during dosing procedures and were not observed for surviving females in this group. At necropsy this female was noted with a cystic oviduct and 9 live fetuses with no apparent malformations *in utero*. This death was not considered test article-related.

All rabbits were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded daily from the day of receipt through Gestation Day 29 (prior to dose administration during the treatment period). Animals were also observed for signs of toxicity approximately 2 hours following dose administration.

Clinical signs

Dose-related increased incidences of decreased defecation, small feces, and/or soft stool were noted in the secnidazole groups at the daily examinations primarily during Gestation Days 16-29. Slightly higher incidences of brown material on the urogenital area were also noted for females in the secnidazole groups

Body Weight

Overall mean maternal body weight gain in the 1000 mg/kg/day group was significantly lower (-64 %) compared to the control group between gestation days 7 and 21. Absolute mean body weights in this group were comparable to the control group throughout the treatment period despite the body weight gain differences.

Table 37: Summary of bodyweights changes (g) in secnidazole-treated pregnant rabbits.

	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Bodyweight gain Day 7-21	244	240	256	87
% difference from control		-2%	+5 %	-64%

Individual maternal body weights were recorded on Gestation Days 0, 4, 7-21 (daily), 24, and 29 for the embryo/fetal development phase and on Gestation Days 0, 4, and 7-20 (daily) for the toxicokinetic phase. Group mean body weights were calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for Gestation Days 7-10, 10-13, 13-21, 7-21, and 21-29 for the embryo/fetal development phase.

Individual maternal body weights were recorded on Gestation Days 0, 6-18 (daily), and 20 for the embryo/fetal development phase and on Gestation Days 0 and 6-17 (daily) for the toxicokinetic phase.

Feed Consumption

Feed consumption was 18 % lower in high dose animals compared to controls. There were no other changes at any other doses.

Toxicokinetics

T_{max} (h) values were between 1-2 hours after dosing on Day 7 or Day 20. AUC $_{(0\text{-}24h)\ and}$ C_{max} values increased more than dose-proportionally on both evaluation days. Systemic exposure, in terms of AUClast, was generally similar on GD 7 and GD 20 following dosing at 10 and 30 mg/kg/day, but was higher on GD 20 than on GD 7 at 100 mg/kg/day. Accumulation ratios appeared to increase with increasing dose 1.6, 1.9 and 2.8 at 10, 30 and 100 mg/kg respectively, indicating some drug accumulation at the higher doses. See Table 38 and 39 below.

Table 38: Mean pharmacokinetics parameters in pregnant rabbits on gestation Day 7.

Dosage Group	Vehicle	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
C_{max} (µg/mL)	ND	0.99	5.8	40
T_{max} (h)	ND	1.0	1.0	1.0
$AUC_{(last)}$ µg·h/mL	ND	1.5	7.4	69

Table 39: Mean pharmacokinetics parameters in pregnant rabbits on gestation Day 20.

Dosage Group	Vehicle	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
C_{max} (µg/mL)	ND	1.4	8.7	53
T_{max} (h)	ND	1.0	1.0	1.5
$AUC_{(last)}$ µg·h/mL	ND	1.8	14	194

Stability and Homogeneity

The analyzed dosing formulations were within 21 % of the target ranges for accuracy and within 13% for homogeneity. Although these were outside the established criteria for accuracy and homogeneity, they were not considered to impact the interpretability of the toxicology study. The accuracy criterion for dose formulations that are suspensions is +/- 15% and the homogeneity criterion for suspensions is ≤ 10% relative standard deviation. The test article was not detected in the vehicle formulation that was administered to the control group (Group 1).

For the assessment of batch homogeneity and concentration, quadruplicate (4) samples (0.5 mL each) were withdrawn from the top, middle and bottom strata of each batch of test article formulations prepared during the in-life phase of the study. In addition, a single 10 mL sample was withdrawn from the middle of the vehicle control group used to prepare the batch of test article formulations (each time test article formulations are sampled).

Necropsy

Cesarean Section Data

Table 40: Litter data for rabbits dosed with secnidazole between gestational Days 6 to 20.

Dosage Group	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Number of live fetuses/dam	8.7	8.9	9.0	9.1
Number of dead fetuses/dam	0.0	0.0	0.0	0.0
No. of early resorptions/dam	0.5	0.3	0.2	0.4
No. of late resorptions/dam	0.1	0.0	0.1	0.1
No of gravid females	22	19	21	20
Pre-implantation loss	0.8	0.8	1.0	0.6
Post-implantation loss	0.6	0.3	0.4	0.5
No. of implantations/dam	9.4	9.2	9.3	9.5
Number of corpora lutea/dam	10.2	9.9	10.4	10.1
Weight of male fetuses (g)	42	43	42	40
Weight of female fetuses (g)	41	42	40	39
Overall Fetus weight	42	43	41	40
Sex ratio (% male fetuses)	53	51	49	56
Fetal anomalies				
Total affected fetuses (litters)	8 (6)	5 (4)	3 (3)	6 (3)
Gross external malformations	1	0	0	0
Visceral anomalies	6	2	1	6
Skeletal anomalies	2	3	2	0

Malformations and Variations
Sporadic occurrences of abnormalities were not considered to be related to treatment.
Skeletal developmental variations, consisting of 13th full rib(s), 13th rudimentary rib(s),sternebra(e) nos. 5 and/or 6 unossified, 27 presacral vertebrae, hyoid arch(es) bent, extra site of ossification anterior to sternebra no. 1, 7th cervical rib(s), sternebrae with thread-like attachment, sternebra(e) misaligned (slight or moderate), extra site of ossification ventral to cervical centrum no. 2, sternebra(e) nos. 1, 2, 3, and/or 4 unossified, 25 presacral vertebrae, hyoid body and/or arch(es) unossified, and 7th sternebra were noted similarly in the test article-treated groups and the concurrent control group and the mean litter proportions were not statistically significantly different from the concurrent control group and/or were within the (b) (4) historical control data ranges. Therefore, these skeletal developmental variations were not considered to be test article-related.

Table 41: Skeletal and visceral malformations in rabbits.

	FETUSES			
DOSE GROUP:	1	2	3	4
NUMBER EXAMINED EXTERNALLY	192	169	188	181
CLEFT LIP	1	0	0	0
NUMBER EXAMINED VISCERALLY	192	169	188	181
DIAPHRAGMATIC HERNIA	1	0	0	0
LUNGS- LOBULAR AGENESIS	5	2	1	6
NUMBER EXAMINED SKELETALLY	192	169	188	181
STERNEBRAE FUSED	1	1	2	0
STERNEBRA(E) MALALIGNED (SEVERE)	0	1	0	0
COSTAL CARTILAGE ANOMALY	0	1	0	0
VERTEBRAL CENTRA ANOMALY	1	0	0	0
TOTAL NUMBER WITH MALFORMATIONS				
EXTERNAL :	1	0	0	0
SOFT TISSUE :	6	2	1	6
SKELETAL :	2	3	2	0
COMBINED :	8	5	3	6

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 30 MG/KG/DAY 4- 100 MG/KG/DAY

All surviving rabbits were euthanized on Gestation Day 29 by an intravenous injection of sodium pentobarbital via the marginal ear vein. The thoracic, abdominal, and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined.

Discussion

There were no adverse effects on embryofetal development in rabbits at doses up to 100 mg/kg. At 100 mg/kg/day, body weight gain was 64% reduced in secnidazole-treated rabbits compared to controls. This dose was clearly associated with excessive maternal toxicity and indicated that the high dose selected was too high. High dose animals in this study showed secnidazole exposures ($AUC_{(0\text{-}24h)}$ values) of up to 194 µg*h/mL. Since $AUC_{(0\text{-}24h)}$ value in patients treated with a single 2g dose of secnidazole was about 1331 µg*h/mL, this dose was equivalent to about 15 % of the clinical dose based on AUC comparisons.

Conclusion:
The NOAEL for maternal toxicity was 30 mg/kg/day and a dosage level of 100 mg/kg/day was considered to be the NOAEL for embryo/fetal development when secnidazole was administered orally by gavage to time-mated New Zealand White rabbits during organogenesis.

9.3 Prenatal and Postnatal Development

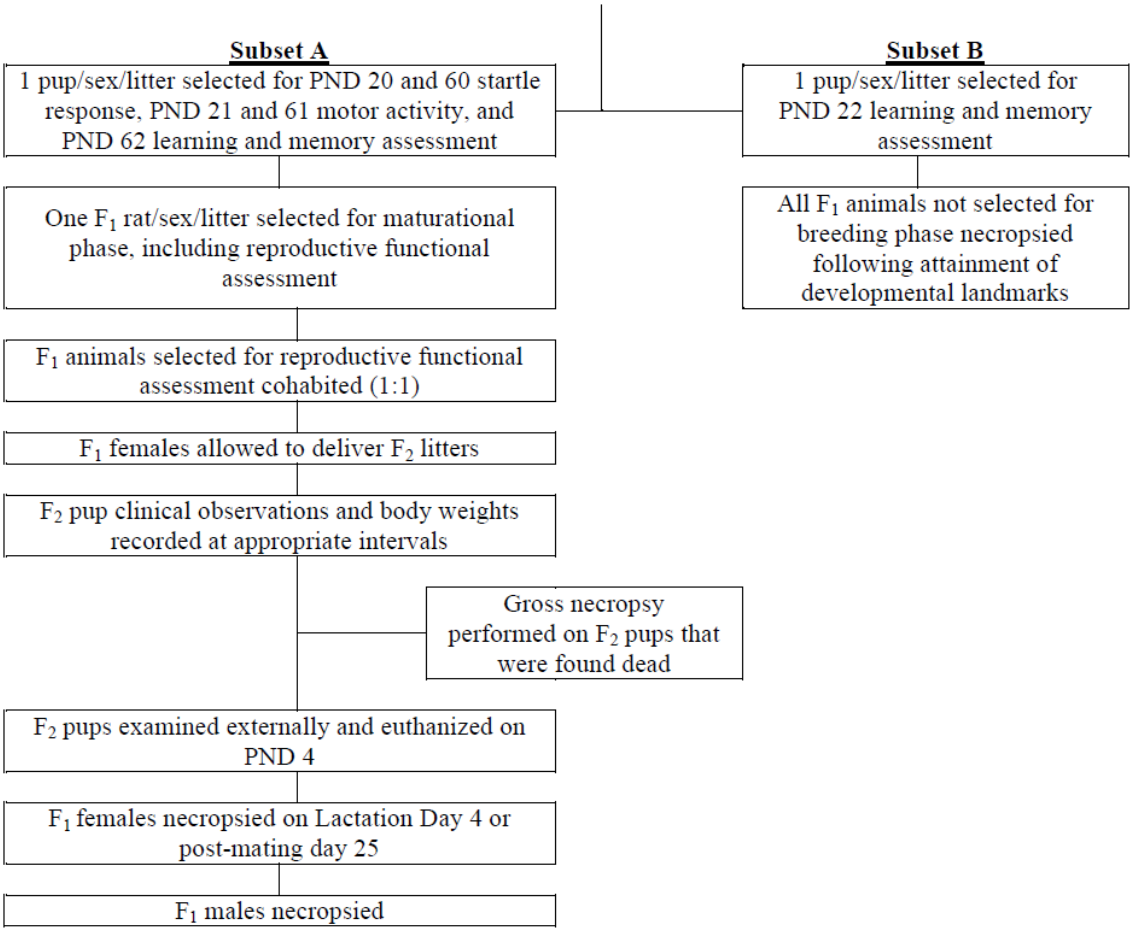
10	Study title:	An Oral (Gavage) Study of the Effects of SYM-1219 on Pre and Postnatal Development, Including Maternal Function in Rats
	(b) (4) Research Study no:	(b) (4) -993008
	Study report location:	eTCD Module 4.2.3.5
	Conducting laboratory:	(b) (4)
	Date of study initiation:	January 16, 2015
	GLP compliance:	Yes
	QA statement:	Yes
	Drug, batch #, % purity:	F130006, 99.9 %
	Key study findings	
		Secnidazole was administered to the F0 females as a single daily dose from Gestation Day 6 through Lactation Day 20, inclusively.
	F0 generation/	No treatment related necropsy observations were observed in F0 generation female rats. Mean bodyweight gain showed statistically significant reductions (-13 %) at 100 and 300 mg/kg/day. Lactation bodyweight gains were increased by+ 46 to +66 % in all secnidazole-dosed groups compared to controls. Gestation food consumption was reduced by -10 and -14 % at the mid and high dose compared to controls. Lactation food consumption was increased by +9 to +13 % in all secnidazole-dosed groups compared to controls.
	F0/F1 preweaning)	Treatment with secnidazole did not adversely affect mean litter parameters including litter size, number of implantations, post implantation survival index, mean number of live born pups/litter, live birth index, postnatal survival to Day 4, postnatal survival to weaning, sex ratio and pup weights.
	F1 generation (post-weaning)	There were no drug related internal findings at the necropsies of F1 pups that were found dead or euthanized *in extremis* or euthanized on day 21. There were no drug related differences in bodyweight (g) PND 21-84, balanopreputial separation, sensory function, motor activity, learning and memory.
	F1 generation (fertility/mating):	There was no difference between drug-treated and control animals in mean number of days to mating, mean number of males/females mated, mean number of fertile males, mean age of vaginal patency, number of females sperm-positive , number of pregnant females, mean number of corpora lutea, mean number of implantations, mean % preimplantation loss, mean duration of gestation.

Maternal NOAEL	The maternal NOAEL was 30 mg/kg, based on the reduction in body weight gain at 100 and 300 mg/kg
Developmental NOAEL	The developmental NOAEL was 300 mg/kg based on the lack of adverse findings in the F1 pups
Methods	
Doses:	0, 30, 100, and 300 mg/kg/day
Frequency of dosing:	Pregnant dams dosed once daily from Gestation Day 6 through Lactation Day 20
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	2% carboxymethylcellulose.
Species/Strain:	*Rattus norvegicus.* Crl:CD(SD)
Number of dams/Group:	25 pregnant dams
Deviation from study protocol:	There were no deviations from the protocol that impacted the interpretation of the study

Study design

F_0 females were bred with males after which groups of females (25/dose) were treated with secnidazole by oral gavage from Gestation Day 6 through Lactation Day 20. Clinical observations, body weights, and food consumption were recorded at appropriate intervals.
F1 generation: To reduce variability among the litters, each litter was randomly culled to 8 pups, 4 per sex when possible, on PND 4. Females were allowed to litter and rear their offspring to weaning and surviving F_0 females were sacrificed and necropsied on Lactation Day 21. F1 pup clinical observations and body weights were recorded at appropriate intervals. 2 male and 2 female pups, when possible, were selected per litter from the F_1 generation; 1 pup/sex/litter assigned to Subset A and other assigned to Subset B. See Figure 8 below: Selected F_1 pups were evaluated for attainment of landmarks of sexual maturity, reproductive function, learning and memory.

Figure 8: F1 Evaluations in Pre and Postnatal Development study



Observations and Results

Analysis of dose formulations

Stability:	Documentation regarding the purity and stability of the test article was provided by the Sponsor. The test article was stored at room temperature, protected from light, with desiccant and was considered stable under these conditions.
Formulation homogeneity:	The analyzed dosing formulations were within (85% to 115%) and were homogeneous. The test article was not detected in the vehicle formulation that was administered to the control group (Group 1).
F0 Survival:	One control F0 female was euthanized *in extremis* on Gestation Day 23 due to pale body during parturition, having delivered no pups by that time. All rats were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded daily (prior to test article administration during the treatment period) for each F0 female from Gestation Day 0 until necropsy. All animals were also observed for signs of toxicity approximately 2 hours following dose administration each day.
Clinical signs:	Secnidazole-treated groups showed hair loss or scabbing on various body surfaces and red material around the nose and/or mouth. These findings occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not dose-related. One female in the 300 mg/kg/day group had a mass on the left lateral trunk that regressed over time; the mass could not be located at the time of necropsy.
Body weight:	Although F0 bodyweights were similar across groups between days 6 and 20, mean bodyweight gain showed statistically significant reductions (-13 %) at 100 and 300 mg/kg/day. Lactation bodyweight gains were increased by 46 to 66 % in all secnidazole-dosed groups compared to controls.
Feed consumption	Gestation food consumption was reduced by 10 and 14 % at the mid and high dose compared to controls. Lactation food consumption was increased by 9 to 13 % in all secnidazole-dosed groups compared to controls.
Necropsy observations	No adverse test article-related necropsy findings were observed at any dosage level.
Uterine content	Two females in the control group and 1 female in the 100 mg/kg/day group failed to deliver and were determined to be nongravid.
Natural delivery and litter observations	Pregnancy occurred in 23, 25, 24 and 25 of the 25 mated female rats in the 0, 30, 100, and 300 mg/kg/day groups respectively. All pregnant dams delivered litters. Natural delivery observations were not affected by test article as high as 300 mg/kg/day. Numbers of dams delivering litters and the duration of gestation were comparable among the 4 groups.

F1 Litter parameters

Treatment with secnidazole did not adversely affect mean litter size, number of implantations, mean number of live born pups/litter, post implantation survival, postnatal survival to Day 4, postnatal survival to weaning, sex ratio and pup weights. (See Table 42) The total number of pups found dead per dose group appeared to be increased in secnidazole-treated animals compared to controls (1, 9, 6, and 5) but there were generally1 to 2 deaths/litter and survival/litter was not significantly different from controls. Survival relative to number born was not different from controls at any intervals examined.

Table 42: F0/F1Litter data for rats dosed with secnidazole between gestational Days 6 to Lactation day 20.

Dosage Group	Vehicle	30 mg/kg	100 mg/kg	300 mg/kg
Females on study	25	25	25	25
Females found dead	0	0	0	0
Females euthanized	1	0	0	0
Females that failed to deliver	1	0	1	0
Number gravid	23	25	24	25
Females gravid (%)	92	100	96	100
Gestational length	21.8	21.8	22.0	22.0
Number of dams with viable pups	22	25	24	25
No. of implantations/dam	15.0	15.4	15.0	15.4
Number of pups born /dam	13.8	14.7	14.0	14.2
Live litter size	13.8	14.5	14.0	14.2
Females with total litter loss	0	0	0	0
Unaccounted sites	1.3	0.7	1.0	1.3
Sex ratio (% male fetuses)	48	50	48	47
Survival (% per litter) to PND 4	99.7	99.5	98.6	99.0
Number of pups found dead	1	9	6	5
Number of litters w/ dead fetuses	1	8	5	5
Milk not present	0	6	1	1
Partially cannibalized	1	0	0	0
Survival (% per litter) to PND 4	99.7	99.5	98.6	99.0
Missing	2	2	9	6
Litters with missing pups	2	2	6	5
Pup weight (g) PND1 (males)	7.5	7.1	7.3	7.4
Pup weight (g) PND1 (females)	7.2	6.8	7.1	7.0
Pup weight (g) PND21 (males)	56	56	60	58
Pup weight (g) PND21 (females)	54	55	57	55

F1 Clinical signs:	There were no drug related clinical signs in F1 pups
F1 necropsy evaluations:	No internal findings that could be attributed to F0 maternal administration to the test article were noted at the necropsies of F1 pups that were euthanized on PND 21, found dead or euthanized *in extremis*.
F1 mortality	One control and one 300 mg/kg/day male were found dead on PND 79 and 67, respectively. These males had no clinical findings prior to death. The deaths were not considered test article-related. All other F1 parental animals in the control, 30, 100, and 300 mg/kg/day groups survived to the scheduled necropsy.
F1 post weaning evaluations:	There were no drug related differences in bodyweight (g) PND 21-84, preputial separation (PND), sensory function, motor activity, learning and memory. The auditory startle response habituation paradigm was conducted as a longitudinal assessment with selected F1 animals on PND 20 and again at PND 60. Motor activity patterns (total activity as well as ambulatory activity counts) were evaluated on PND 22 and 62. Values obtained from the 6 subintervals evaluated (0-10, 11-20, 21-30, 31-40, 41-50 and 51-60 minutes) and the overall 60-minute test session values were compared to the concurrent control values and the (b) (4) historical control data. Swimming ability and mean time to locate the submerged platform were evaluated in the Biel maze assessment on PND 22 or PND 62.
F1 mating/fertility evaluations:	There were no drug related differences in mean number of days to mating, mean number of males/females mated, mean number of fertile males, mean age of vaginal patency, number of females sperm-positive , no. of pregnant females, mean number of corpora lutea, mean number of implantations, mean % preimplantation loss, mean duration of gestation or abnormal parturition.
F2 Litters:	In the F2 litters, There were no drug related differences in survival, clinical signs, mean number of live pups/litter, fetal bodyweight changes or fetal sex ratios or fetal anomalies. The number of pups found dead +and litters with Dead Conceptuses was increased at 100 mg/kg but not at 300 mg/kg

Table 43: F1 Sexual development and Reproductive performance in rats dosed with secnidazole between gestational Days 6 to Lactation Day 20.

Dosage Group	Vehicle	30 mg/kg	100 mg/kg/day	300 mg/kg/day
Balanopreputial separation (days)	43.1	43.5	43.6	43.7
Vaginal patency	31.9	32.3	32.6	33.4
Male Mating Index (%)	90	96	100	100
Female Mating Index (%)	100	100	100	100
Male fertility Index (%)	90	92	96	90
Female fertility Index (%)	100	96	96	96
Male copulation Index (%)	100	96	96	96
Female conception Index (%)	100	96	96	96
Estrous cycle length (days)	4.3	4.0	4.3	4.3
Precoital interval (days)	3.9	2.8	2.7	2.7
Gestational length	21.9	21.6	21.7	21.8

Table 44: F2Litter data for rats dosed with secnidazole between gestational Days 6 to Lactation Day 20.

Dosage Group	Vehicle	30 mg/kg	100 mg/kg	300 mg/kg
Number of dams with viable pups	20	24	23	23
Number of pups born /dam	14.9	14.2	16.1	14.5
Live litter size	14.0	14.2	15.7	14.4
Sex ratio (% male fetuses)	54	51	48	48
Survival (% per litter) to PND 4	97	98	95	92
Number of pups found dead	3	1	14	7
Milk not present			11	4
Number of litters w/ dead fetuses	2	1	9	3

There were no drug-related abnormal findings in the F1 or F2 pups.

Discussion

F0 Dams tolerated the secnidazole at 30 mg/kg/day during gestation, but the 100 and 300 mg/kg/day were toxic and resulted in reduced food consumption and bodyweight gain.

Secnidazole did not result in any effects on physical development in F1 or F2 pup weights at any stage of development. There were also no adverse effects on sensory function, motor activity, learning and memory in F1 rats at any stage of development. Sexual maturation was normal as assessed by vaginal opening for females and preputial separation in males. There were no drug related differences in precoital

interval, mating performance and fertility or litter characteristics between secnidazole-treated and control animals. Despite higher number of pups found dead at the higher secnidazole doses in F1 and F2, the finding was not dose related and there were generally only 1-2 dead pups per litter. Also, in several instances these deaths were associated with an absence of milk in the stomach. It is not clear if the metallic taste known to be associated with secnidazole may have transferred to the milk and discouraged feeding.

Conclusion.

Administration of secnidazole to animals in utero and during early postnatal development resulted in no adverse effects on the offspring. The maternal NOAEL was 30 mg/kg, based on the reduction in body weight gain at 100 and 300 mg/kg. The developmental NOAEL was 300 /kg based on the lack of adverse findings in the F1 or F2 pups.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Symbiomix Therapeutics, LLC seeks to market SOLOSEC (secnidazole), a nitroimidazole antimicrobial, as a single-dose oral treatment of bacterial vaginosis. SOLOSEC is supplied as oral granules containing 2 g of secnidazole, which are intended to be administered with soft food such as applesauce. Secnidazole has been studied for over 3 decades and is approved in several countries outside of the US for bacterial vaginosis, giardiasis, and amoebiasis. There are extensive clinical safety data available for secnidazole. A single, oral 2 g dose of SOLOSEC in healthy adult female subjects resulted in a C_{max} of 45 µg/ml, AUC_{0-inf} of 1332 µg•hr/mL and T_{max} of 4 hours. The safety implications of nonclinical studies were based on the exposure data achieved with the proposed dose and duration.

Bacterial vaginosis (BV) is the most common vaginal infection in women between 15 and 44 years old. BV describes a condition in which *Lactobacillus* bacteria are replaced by other types of bacteria that normally are present in smaller concentrations in the vagina. Some specialists consider it to be a sexually transmitted disease, but CDC reports that 19% of the women with BV have not reported sexual intercourse. BV is a serious infection which may increase the risk for acquisition and transmission of HIV, acquisition of other STDs such as gonorrhea and chlamydia, pelvic inflammatory disease and preterm births. Early treatment of BV by clindamycin was effective in preventing preterm birth and treatment of BV with metronidazole has been shown to reduce the risk of preterm birth in women who are at high risk of preterm birth. SOLOSEC has therefore been designated a Qualified Infectious Disease Product for the treatment of BV, but has not been studied in pregnant women. Like Clindesse® (clindamycin phosphate) vaginal cream, the course of treatment is a single dose. BV is also known to recur and patients may experience BV again several months after successful treatment. Retreatment of patients is therefore anticipated.

The Applicant has not submitted data showing the mechanism of action of secnidazole, but hypothesized that the mechanism of action is similar to that of metronidazole since they have similar structures and are both 5-nitroimidazole antimicrobials. Metronidazole inhibits nucleic acid synthesis by disrupting DNA and causing strand breakage. The 5-nitroimidazoles enter the bacterial cell as inactive prodrugs. Under anaerobic conditions, the nitro group of 5-nitroimidazoles is reduced by ferredoxin to a radical anion form that exerts the primary antimicrobial activity. The anion from reduced metronidazole has been shown to disrupt nucleic acid leading to DNA degradation in a metronidazole-sensitive isolate of *Bacteroides distasonis* and *H. pylori* under anaerobic conditions. The disruption in DNA synthesis leads to death of the bacterial cell. Given this DNA-targeting mechanism of action, it is not surprising that the nitroimidazole class of drugs is associated with various tumors in laboratory animals. For example metronidazole has been associated with lung tumors, liver tumors and malignant lymphomas in mice and mammary and liver tumors in rats.

The nonclinical toxicology program supports marketing of SOLOSEC as a single-dose oral treatment of bacterial vaginosis and there were no adverse findings that preclude its approval. The nonclinical studies conducted were adequate for the indication and included *in vitro* and *in vivo* studies using rats, dogs and rabbits. The applicant evaluated the effects of secnidazole administration for up to 28 days in rats and dogs as recommended by ICH. There were also evaluations of safety pharmacology, genotoxicity, fertility, embryofetal toxicity and pre- and postnatal development. The studies were conducted in GLP compliant facilities by qualified personnel who adequately characterized the toxicity of secnidazole. Study designs, including species selection and study durations were appropriate given the single dose clinical regimen. In several instances the doses tested were too high and exceeded the maximum tolerated dose. Study conduct was acceptable and pivotal studies were GLP-compliant.

In a CNS safety pharmacology study of secnidazole in rats, a single oral 1000 mg/kg dose resulted in reduced motor activity. Vertical breaks, vertical counts, bursts of stereotypic movement, and horizontal

counts were significantly lower than control at 1 to 2 hours postdose. These transient changes were not seen 24 hours postdose. In the cardiovascular safety pharmacology study secnidazole resulted in a slight, transient decrease in blood pressure with reflex tachycardia and lower P-R interval in dogs at 200 mg/kg (about 2 times the clinical exposure). No adverse findings were detected in the respiratory safety pharmacology studies.

Single doses of secnidazole resulted in lethargy and piloerection at 1000 mg/kg in the rat micronucleus study although no adverse symptoms were observed after a single 2000 mg/kg dose in another toxicology study. After 7 daily doses of 600 mg/kg and higher, changes in the kidney (minimal hyaline droplet accumulation) were observed in the proximal tubules in female rats. In male rats, these findings were seen at 200 mg/kg. The 600 mg/kg dose (1775 µg•hr/mL) is equivalent to about 1.3 times the clinical exposure of 1332 µg•hr/mL. Proximal tubule changes were therefore observed at exposures similar to the clinical dose in female rats dosed for 7 times longer than the clinical duration.

The single 500 mg/kg dose was not associated with overt clinical signs in dogs but the 750 mg/kg dose resulted in ataxia, decreased activity and vomiting. After 7 daily doses there were no overt clinical signs at 200 mg/kg in dogs, where the exposure was similar to the clinical exposure, based on AUC comparisons. At higher doses (600 mg/kg, equivalent to about 5 times the clinical exposure) observations included mortality, moribundity, ataxia, decreased activity, partially closed eyes, vomiting, tremors, seizures, recumbence, moderate depletion of the lymphocytes of the spleen and minimal, multifocal necrosis of the epithelium of the renal proximal tubules. The sponsor was unable to determine the cause of moribundity in these dogs. Secnidazole was reasonably well tolerated in dogs at exposures about double the clinical exposure for seven times the proposed clinical duration.

In the 28 day rat study, no NOAEL was determined. All doses showed reduced mean body weight gains (-18 to 35 % at all doses relative to the control animals) and decreased absolute (-22 %) and relative (-20 %) thymus weights at the lowest dose (100 mg/kg). In males all dose groups (100, 300 and 1000 mg/kg) showed increases in pituitary weights (increased 39-48%) and diffuse hypertrophy of the acidophils of the pars distalis of the pituitary gland and increased thyroid/parathyroid weights (16-31%). The maximum nonlethal dose in males was 300 mg/kg due to mortality at 1000 mg/kg. The AUC at the 100 mg/kg dose (538 µg•hr/mL) was equivalent to about 0.4 times the clinical exposure. At about 4 times the clinical exposure (1000 mg/kg/day, for up to 28 consecutive days) secnidazole resulted in mortality and moribundity. The deaths were preceded by clinical signs of motor effects that were consistent with histologic changes in the vestibule-cerebellar tract. Other target organs included the pituitary gland, spleen, kidney, liver, thymus, sternal bone marrow, epididymis, testes, thyroid gland parathyroid glands, and adrenal glands.

In the 28 day dog study, the NOAEL was estimated to be 60 mg/kg, with a mean AUC_{last} of 313 µg*h/mL, which is about 0.2 times the clinical exposure. Repeat dosing of secnidazole was not well tolerated for 28 days at doses above one third the clinical exposure. Long term (28 day) dosing at 200 mg/kg (about 0.7 times the clinical exposure) was very toxic and resulted in mortality and moribundity, characterized by ataxia, reluctance to rise, weakness, hyper reflexes, nystagmus, limited and/or loss of mobility, trembling and/or tremors, abnormal gait, and splayed legs. As with the rats, the effects were consistent with effect on the vestibulo-cerebellar tract.

The rat was slightly more sensitive to secnidazole than the dog. Although no overt clinical signs were observed at 2000 mg/kg in the rat in study # 030613, lethargy and piloerection were noted at 1000 mg/kg (but not at 500 mg/kg, about 2 times the clinical exposure based on body surface area comparisons) in the rat micronucleus assay (study # 030630). After the single dose in dogs, no overt findings were observed at 500 mg/kg (approximately 8 times the clinical dose based on body surface area comparisons). Minimal hyaline droplet accumulation in the proximal tubules was observed at all doses in the 7 day rat study

(lowest dose was 200 mg/kg, about 0.6 times the clinical exposure based on AUC comparisons. In the 7 day dog study, no overt toxicities were observed at 200 mg/kg (about 0.7 times the clinical exposure), but tremors, seizures, recumbence, ataxia, decreased activity, and partially closed eyelids were observed at 600 mg/kg. The 28 day NOAEL's in rats was <100 mg/kg (< 0.4 times the clinical exposure) and in dogs it was 60 mg/kg (about 0.3 times the clinical exposure).

In the fertility and early embryonic development study in rats, there were no adverse effects on reproductive function in females at 100 mg/kg (about one fifth of the clinical exposure, based on AUC comparisons). At 300 mg/kg (with an exposure about 1.4 times the clinical exposure, based on AUC comparisons), male rats showed reduced sperm motility (65 % vs 85 % in controls), reduced epididymis weights (-19%) and increased numbers of abnormal sperm (normally shaped head separated from flagellum) compared to controls. There were no adverse effects on reproductive function in males at 100 mg/kg (about one fifth of the clinical exposure, based on AUC comparisons).

In the embryofetal study in rat, where rats were treated during the period of organogenesis, there were no effects on embryofetal development in rats at one quarter the clinical exposure based on AUC comparisons. The higher doses exceeded the maximum tolerated dose due to reduced bodyweight gain compared to control. In rabbits, secnidazole did not result in adverse effects on embryo-fetal development in rabbits at doses up to 100 mg/kg, equivalent to about 15 % of the clinical dose based on AUC comparisons. In the pre-post-natal study in rats, pregnant rats were dosed once daily from Gestation Day 6 through Lactation Day 20. The F1 offspring were evaluated for development, learning, memory and reproductive capacity. Secnidazole did not result in any adverse effects on physical development in F1 or F2 pup weights at any stage of development. There were also no biologically significant adverse effects on sensory function, motor activity, learning and memory in F1 rats at any stage of development. Sexual maturation was normal as assessed by vaginal opening for females and preputial separation in males.

The genotoxic potential of secnidazole was evaluated in the Bacterial reverse mutation assay and it was found to be positive. This is similar to metronidazole and not unexpected given the mechanism of action of this chemical class of drugs. Secnidazole was concluded to be negative in the L5178Y/TK+/- Mouse Lymphoma Assay and negative in the mouse micronucleus assay.

Adverse events of special interest

Reduced food intake

Several studies were associated with reduced food intake and concomitant reductions in body weight gain. These nonclinical findings are consistent with the clinical findings of nausea, dysgeusia and metallic taste reported in the US clinical trials and the adverse events reported in the label of the French secnidazole product SECNOL. In several reproductive toxicology studies, reductions in food intake were associated with reductions in body weight gain which impacted the overall health of the pregnant animals. These reductions in bodyweight gain impacted the maximum tolerated dose in several studies.

Kidney

Minimal hyaline droplet accumulation in the proximal tubules was seen in all male rats (but no females) at 200 mg/kg (the lowest dose in the 7 day study). This finding was only seen in 1/5 females at 600 mg/kg (equivalent to the clinical exposure). Hyaline droplets accumulation is not considered predictive of human toxicity.

Reproductive toxicology

Excessive body weight reductions in several reproductive toxicology studies indicated that the high dose selected for these studies were too high, since they resulted in excessive maternal toxicity. Per ICH S5(R2) 'Some minimal toxicity is expected to be induced in the high dose dams' and 'factors limiting the high dosage ……from preliminary reproduction studies could include reduction in bodyweight gain'. Excessive body weight loss, with the resulting poor health of the dams, is known to adversely affect fetuses (Khera, K.S., Maternal toxicity: A possible etiological factor in embryo-fetal deaths and fetal malformations of rodent-rabbit species. Teratology.1985. 31:129-153). At nontoxic doses, there were no adverse effects on the fetuses with SOLOSEC. This lack of findings is consistent with the reproductive toxicology data for metronidazole where rat reproduction studies revealed no evidence of impaired fertility or harm to the fetus at doses up to five times the human dose and no fetotoxicity when metronidazole was administered orally to pregnant mice at 20 mg/kg/day, approximately one and a half times the most frequently recommended human dose (750 mg/day) based on mg/kg body weight.

Bacterial vaginosis therapies such as Clindesse are indicated for Bacterial vaginosis in 'non-pregnant, adult women'. There are no nonclinical data to indicate that SOLOSEC would be harmful to the unborn fetus. However, there are no adequate and controlled clinical trials in pregnant women. In the absence of clinical trial data in pregnant women and since animal studies are not always predictive of human toxicities, (b) (4).

Although secnidazole has been associated with changes in the male reproductive system (including sperm morphology, epididymis and testes), these changes are irrelevant to the current indication for bacterial vaginosis in adult women.

No juvenile toxicology studies were conducted. This is acceptable since the results of the nonclinical studies raised no specific development concerns given the proposed indication in adult women. Also, in the currently available rat studies, dosing began when rats were 6-8 weeks old (equivalent to humans about 12 to 16 years old). The need for juvenile studies may be revisited if males or subjects younger than 12 were to be studied in the US (since 19 % of the patients report no sexual activity). There are published reports that an oral suspension of secnidazole (20 mg/kg) has already been studied in 2-11 year old Venezuelan children infected with *Giardia intestinalis* (also known as *Giardia lamblia)*.

CNS toxicity

In the CNS safety pharmacology study, motor activity was reduced at 1000 mg/kg. In the 7-day dog study, doses about 5 times the clinical exposure showed morbidity after as few as 4 days in males and 6 days in females. Symptoms included ataxia, decreased activity, partially closed eyes, vomiting, tremors, seizures and recumbence The sponsor was unable to determine the cause of moribundity in these animals but high doses in the 28 day rat study showed lesions in the brain including varying degrees of vacuolar degeneration of the lateral reticular nuclei, medial vestibular nuclei, dentate nuclei, and the vestibulo-cerebellar tract and varying degrees of gliosis of these same structures as well as diffuse hypertrophy of the acidophils of the pars distalis of the pituitary gland. Although it seems unlikely that these findings could be replicated in patients receiving a single dose, the five-fold safety margin is very low and so this may indicate a risk to patients if SOLOSEC were administered for longer than the proposed duration. This low safety margin is consistent with those seen with other imidazole class drugs. Neurotoxic effects, including seizures and peripheral neuropathy, have been reported after 5 to 7 days of doses of 6 to 10 g of metronidazole every other day (equivalent to about 2 to 5 times the clinical dose for trichomoniasis).

SOLOSEC is likely to be used repeatedly in patients with recurrent BV. Although no additional studies are being recommended at this time, a 12 month, once-monthly treatment with secnidazole in rats at clinical exposures for one year could characterize the potential adverse effects of intermittent retreatment

with SOLOSEC. Such a study may be indicated if the clinical use of the drug should change, such as if the applicant decided to market the drug for prophylaxis in patients with recurrent BV.

Genotoxicity/carcinogenicity

Secnidazole is genotoxic and although ICH guidelines don't recommend that the applicant conduct carcinogenicity studies because of the short duration of dosing, tumors affecting the liver, lungs, mammary, and lymphatic tissues have been detected after lifetime exposure to other structurally similar nitroimidazoles in rats and mice. International Agency for Research on Cancer (IARC) considers the evidence sufficient to consider metronidazole as an animal carcinogen, but not sufficient to consider it a human carcinogen despite several human studies.

The genotoxicity of nitroimidazoles has raised concerns about the risk of prenatal exposure. In one retrospective cohort study of children younger than 5 years, although there was no increase in risk for all cancers associated with in utero exposure to metronidazole, the observed increased risk for neuroblastomas, although not significant, was recommended for further evaluation (Purushottam et al, *Cancer* 1998;83:1461-1468). Despite a theoretical risk, metronidazole is used in children for treatment of anaerobic infections and amoebiasis. An oral suspension of secnidazole (20 mg/kg) has been studied in Venezuelan children infected with Giardia intestinalis (2 to 11 years old).

Conclusion
There are no nonclinical data that preclude the approval of SOLOSEC for use a single dose treatment for bacterial vaginosis in non-pregnant adult women. The prescribing information will be updated with the relevant information for health care providers.

Table 45 Safety margins for adverse events of special interest

Toxicity	Species	NOAEL (mg/kg) Dose	AUC (µg•hr/mL) or BSA equivalent	Safety Margin (Basis)
CNS (Lethargy, ataxia)	Rat (single dose)	500	80 (BSA)	2.4
	Dog (single dose)	500	270 (BSA)	8
Mortality (7 day)	Rat	2000	3115	2.3
	Dog	200	1515	1.1
Reduced food consumption	Rat, (embryofetal)	300	1920	1.4
	Rat (Fertility)	300	1920	1.4
	Rat (28 day)	300	1850	1.4
	Rat (pre-postnatal)	30	4.8 (BSA)	0.1
	Rabbit (embryofetal)	30	14	0.01
Reduced body weight	Rat (7 day)	2000	3115	2.3
	Dog (7 day)	60	452	0.3
	Rat (28 day)	<100	<538	<0.4
	Dog (28 day)	200	973	0.7
	Rat, (embryofetal)	100	352	0.3
	Rat (Fertility)	300	1920	1.4
	Rat (pre-postnatal)	30	4.8 (BSA)	0.1
	Rabbit	30	14	0.01

*AUC in human: 1332 µg•hr/mL at 2g/day (33 mg/kg/day).
BSA: based on body surface area comparisons.

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/s/

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08/29/2017

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