CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204684Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on N204684 Miltefosine (Impavido)

From A. Jacobs, AD

Oct 4, 2013

1. I concur that there are no pharm/tox approval issues

2. I concur that a pregnancy category (b) is appropriate.

3. I note that the leydig cell tumors seen after 1 year in rats are probably secondary to reduced testosterone, and are probably not relevant to humans

4. My previously conveyed comments have been addressed as appropriate

---

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/s/

ABIGAIL C JACOBS
10/04/2013

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	204684
Supporting document/s:	SD#7
Applicant's letter date:	4/19/2013
CDER stamp date:	4/19/2013
Product:	Miltefosine (Impavido)
Indication:	Visceral and Cutaneous Leishmaniasis
Applicant:	Paladin Therapeutics Inc.
Review Division:	Division of Anti-infective Products
Reviewer:	James S. Wild, Ph.D.
Supervisor/Team Leader:	Wendelyn Schmidt, Ph.D.
Division Director:	Sumathi Nambiar, M.D.
Project Manager:	Gregory DiBernardo

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Miltefosine is a structural analog of alkyl (lyso) phospholipids (ALP). ALPs show cytostatic and immunomodulatory effects and originally were investigated for their use in the treatment of cancer. Subsequently, ALPs were tested *in vitro* for their antileishmanial activity, and miltefosine was carried forward into antileishmanial clinical trials. The original indication was visceral leishmaniasis (VL). Subsequent clinical trials were conducted in cutaneous (CL) and mucosal leishmaniasis (ML). Miltefosine (Impavido®) has been widely studied and obtained marketing authorization in 2002 in India as an oral treatment of VL. Subsequently, marketing authorization was granted for VL and/or CL in 12 other countries. Miltefosine has been tested in more than ten phase 2, 3, and 4 clinical trials with more than 350 adult patients dosed with miltefosine. Paladin Labs Inc. (Montreal, Canada) acquired Impavido~ from Aeterna Zentaris (Frankfurt, Germany), the company responsible for the discovery of miltefosine and the clinical trials until that time. The rationale for seeking an NDA in the United States is that there is a need for alternative therapies particularly an oral therapy like miltefosine for the patients with visceral leishmaniasis, and an unmet medical need for the treatment of mucosal and cutaneous leishmaniasis for which there are currently no approved therapies.

From a clinical perspective, visceral leishmaniasis is a life-threatening disease and patients are seriously ill at the beginning of treatment. Cutaneous leishmaniasis, depending on infecting Leishmania species, is either quickly or slowly self-healing, and either restricted to a localized skin ulcer or disseminating to local lymph nodes or more rarely to the oro-nasal mucosa (mucosal disease).

1.2 Brief Discussion of Nonclinical Findings

- Oral miltefosine is 93% bioavailable in dogs and binds well to plasma proteins (95% bound) from rats, dogs, and humans.
- Distribution studies in rats indicate that miltefosine accumulates with repeated-dosing with relatively more accumulation in some tissues including nervous tissue and reproductive organs.
- Miltefosine has a long $t_{1/2}$ in plasma (approximately 80 hours in rats and 160 hours in dogs) with longer $t_{1/2}$ values in some tissues particularly nervous tissue.
- The only detected metabolite is choline and miltefosine is excreted mainly in urine and feces at a slow rate.
- In 8- and 52-week toxicology studies in rats, the main target organs for toxicity were kidney (chronic nephropathy), GI tract (hyperplasia of stomach chief cells, hyperplasia and hypertrophy of intestinal mucosa), male reproductive organs (atrophy of testes, Leydig cell hyperplasia and adenomas, atrophy of prostate, epididymides, and seminal vesicles, many spermatogenic granulomas in epididymides), female reproductive organs (ovarian cysts, hydrometra, mucometra, and pyometra of the uterus, hyperplasia of cervical and vaginal mucosa) and the eye (corneal inflammatory changes, homogenization of the lens

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nucleus, swelling and vacuolization of lens fibers, and retinal degeneration). Toxicities occurred in a dose-dependent manner and in the high-dose group (21.5 mg/kg/day) in the 52-week study, chronic nephropathy, testicular atrophy and retinal degeneration did not fully reverse during Recovery. NOAEL values in both studies were ≤ 4.64 mg/kg/day which corresponds to a HED of 0.742 mg/kg/day based on body surface area (BSA) comparisons which is equal to 0.2 times the maximum recommended human dose (MRHD = 3.33 mg/kg/day).

- Juvenile rats exhibited more sensitivity to miltefosine toxicity compared to adults. In 13-week toxicology studies, retinal degeneration was noted in some juvenile animals receiving 2.15 mg/kg/day miltefosine, but not in adult rats receiving 4.64 mg/kg/day miltefosine. A number of other toxicities occurred in juvenile rats at the miltefosine dose of 4.64 mg/kg/day including toxicities in kidney, retina, small intestine and seminal vesicles whereas toxicities in adult rats receiving 4.64 mg/kg/day were limited to alopecia and nasal and conjunctival secretions/red incrustations.
- Dogs also experienced GI tract toxicity (vomiting, diarrhea, reduced food consumption, and hyperemia of the intestinal mucosa) but a different scope of male and female reproductive-organ toxicity compared to rats. In a 52-week toxicology study, dogs experienced GI tract toxicity and miltefosine-related female reproductive-organ toxicity including increased numbers of atretic follicles in the ovaries, and cycle arrest in the uterus, vagina, and mammary gland with morphology consistent with anestrus or diestrus. Both the GI tract and female reproductive organ toxicity were reversed during Recovery. Some male reproductive organ toxicity was observed including multifocal atrophy and degeneration of seminiferous tubules in high-dose (6.19 mg/kg/day) males in the 52-week study, and also prostate atrophy in mid- (3.16 mg/kg/day) and high-dose (10 mg/kg/day) males in a 13-week toxicity study. These effects were reversed during Recovery; however, in the 52-week study, reduced testicular weights and testicular atrophy were noted in 2/2 Recovery males, but not in the Main Study animals. Generally the scope and severity of male reproductive organ toxicity was reduced in dogs compared to rats.
- A notable toxicity in rats but not in dogs was dose-dependent retinal degeneration. With sufficient duration of dosing, retinal degeneration characterized by complete damage to photoreceptors inclusive of nuclei and the consecutive loss of inner retinal structures was not reversible.
- Miltefosine has been evaluated in Ames and DNA Amplification assays and a V79 mammalian cell gene mutation assay *in vitro*, and *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay and *in vivo* in a micronucleus assay. The results for all the studies were negative except for the V79 mammalian cell gene mutation assay. Because miltefosine was negative in the micronucleus assay it is considered to be of low genotoxic potential in humans.
- Because miltefosine is scheduled to be administered for less than 6-months, formal rodent carcinogenicity studies were not performed. However, in a 52-week oral-toxicity study in rats, tumors were observed at the high-dose of 21.5 mg/kg/day miltefosine (HED of 3.44 mg/kg/day or roughly equal to the MRHD). Tumors included: benign basal cell adenoma of the skin, multiple histiocytic

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sarcoma, squamous cell carcinoma in the uterus and malignant adenoacanthoma in the uterus that was observed in separate females (each in 1/30 females) and testicular Leydig cell adenoma observed in 3/30 males.

- In a male fertility study in rats, miltefosine produced a dose-dependent impairment of the male reproductive system including reduced copulation index, dramatically reduced fertility, reduced sperm number and viability, increased morphologically altered sperm, and atrophied testes, prostate, and seminal vesicles. Testicular histopathology included slight to massive diffuse tubular atrophy with degenerative spermatocytes and spermatogonia. The NOAEL dose was considered to be 3.16 mg/kg (HED of 0.51 mg/kg/day or approximately 0.15 fold the MRHD). After a 10-week recovery period, the effects were reversed in rats receiving 8.25 mg/kg miltefosine, but most effects were not reversed in the rats receiving the high dose of 21.5 mg/kg.
- In a female fertility study, estrus cycle arrest in the metestrus or diestrus phases occurred with the high-dose of 21.5 mg/kg. Reproductive performance was further impacted in a dose-dependent manner at doses of 6.81 and 21.5 mg/kg in the form of increased numbers of embryonic and fetal resorptions and the proportion of dead fetuses. Substantial fetal visceral and skeletal malformations also occurred at these doses.
- In embryo-fetal studies in rats and rabbits, miltefosine doses ≥ 6.0 mg/kg caused pronounced fetal resorption in dams treated during the period of organogenesis. Miltefosine was a potent teratogen in rats when administered at doses of ≥1.2 mg/kg/day. Malformations included: undeveloped cerebrum, lumina of the skull filled with hemorrhagic fluid and in a few fetuses further malformations including cleft palate and generalized edema. In another study, a fertility study in female rats, a miltefosine dose of 6.14 mg/kg/day produced dramatic visceral (misshaped cerebral structures, cerebral ventricles filled with brown masses, misshaped spinal cord, and misshaped and malpositioned eyes, hypophysis, absent inner ear) and skeletal (cleft palate, dumbbell shaped ossification of thoracic vertebral centers, increased sizes for skull bones, and markedly dilated suturae) abnormalities in fetuses.
- In rabbits, one dam receiving a miltefosine dose of 2.4 mg/kg/day aborted and two dams exhibited reddened gastric mucosa. However, miltefosine doses of ≤ 2.4 mg/kg/day did not cause maternal toxicity or influence prenatal parameters (number of fetuses, number of resorptions, fetal and placental weights), increase variation rates, or cause malformations in rabbits.
- Serious toxicities have been shown to occur in nonclinical toxicology studies including retinal degeneration, testicular atrophy and dramatically impaired male fertility in rats and follicular atresia in the ovaries of female dogs at doses similar to or below the clinical dose. However results from the many human studies suggest that the retinal degeneration effects noted in rats are attenuated or absent in humans administered the MRHD. Less data is available regarding the male and female reproductive organ effects in humans and it remains to be seen if these toxicities will present serious challenges to clinical administration of miltefosine at therapeutic doses.

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1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, this product is approvable once the HPTLC methodology for the measurement of the substance impurities, (b) (4) has been validated according to the FDA recommendations communicated in the June 20, 2013 Information Request.

1.3.2 Additional Non Clinical Recommendations

The product label should include information about the major rat and dog toxicities including retinal effects in rats, male and female reproductive and developmental toxicities, and the Leydig cell carcinogenicity data.

1.3.3 Labeling

8.1 Pregnancy

Current Applicant Version

(b) (4)

Proposed Revisions

Pregnancy Category (b) (4)
Pregnancy Category (b) (4)

Miltefosine administration in rat embryo-fetal toxicity studies during early embryonic development (b) (4) indicate an embryotoxic, fetotoxic, and teratogenic risk with miltefosine dosages of ≥ 1.2 mg/kg/day (0.06 times the MRHD based on BSA comparisons). Teratogenic effects included undeveloped cerebrum, lumina of the skull filled with hemorrhagic fluid (b) (4) cleft palate and generalized edema. Embryotoxic and fetotoxic findings were also observed in rabbits after oral administration of miltefosine at doses ≥ 2.4 mg/kg/day (0.2 times the MRHD based on BSA comparisons). No live fetuses were obtained in rats or rabbits at miltefosine doses ≥ 6.0 mg/kg/day (0.3 or 0.6 times the MRHD based on BSA comparisons for rats and rabbits respectively).

(b) (4)

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13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Current Applicant Version

Mutagenicity/Carcinogenicity: Miltefosine tested negative in the AMES-Salmonella test, DNA-amplification test, chromosomal aberration test in vitro, UDS-test in vivo/in vitro, and oral mouse micronucleus test in vivo. The V 79 mammalian cell HPRT gene mutation test showed an increase in mutant frequency without dose dependency. In view of all mutagenicity test results, the single positive finding in the V 79 HPRT test is considered to be not of toxicological relevance with respect to a mutagenic risk to humans.

(b) (4)

(b) (4)

Proposed Revisions

Mutagenicity/Carcinogenicity: Miltefosine tested negative in the AMES-Salmonella test, DNA-amplification test, chromosomal aberration test *in vitro*, UDS-test *in vivo/in vitro*, and oral mouse micronucleus test *in vivo*. The V 79 mammalian cell HPRT gene mutation test showed an increase in mutant frequency without dose dependency. In view of all mutagenicity test results, the single positive finding in the V 79 HPRT test is considered to be not of toxicological relevance with respect to a mutagenic risk to humans.

Carcinogenicity studies were not performed. In a 52-week oral toxicity study in rats, testicular Leydig cell adenoma was observed in 3 of 30 male rats with daily administration of 21.5 mg/kg/day miltefosine (1.0 times the MRHD based on BSA comparison). The carcinogenic potential of miltefosine in humans is unknown.

In a Segment I fertility study in male rats, testicular atrophy and impaired fertility were observed in rats following daily oral doses of ≥ 8.25 mg/kg (0.4 times the MRHD based on BSA comparison). These findings were reversible within a recovery period of 10 weeks except at the highest dose tested, 21.5 mg/kg/day (1.0 times the MRHD based on BSA comparison), where effects were not fully reversible.

In a female fertility study in rats, estrus cycle arrest in the metestrus or diestrus phases occurred with the high-dose of 21.5 mg/kg. Reproductive performance was further impacted in a dose-dependent manner at doses of 6.81 and 21.5 mg/kg in the form of increased numbers of embryonic and fetal resorptions and the proportion of dead fetuses. In a 52-week toxicology study in dogs, increased numbers of atretic follicles in

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the ovaries, and cycle arrest in the uterus, vagina, and mammary gland with morphology consistent with anestrus or diestrus was observed at doses ≥ 1 mg/kg/day (0.2 times the MRHD based on BSA comparisons).

13.2 Animal Toxicology and/or Pharmacology

Current Applicant Version

Toxicological studies with miltefosine have been performed in mice, rats, dogs, and rabbits. Adverse reactions not observed in clinical studies but seen in animals at exposure levels similar to clinical exposure levels and with possible relevance to clinical use were as follows:

Acute and chronic toxicity: The oral administration of miltefosine in rats was associated with lesions especially affecting the eyes (retinal degeneration), (b) (4) after 8 weeks treatment at doses of 10 mg/kg/day. (b) (4)

(b) (4)

Proposed Revisions

General toxicological studies with miltefosine have been performed in mice, rats, and dogs. Adverse reactions not observed in clinical studies but seen in animals at exposure levels similar to clinical exposure levels and with possible relevance to clinical use were as follows:

Acute and chronic toxicity: The oral administration of miltefosine in rats was associated with lesions affecting the eyes (retinal degeneration). These alterations were observed after 8 weeks treatment at doses of 10 mg/kg/day (0.5 times the MRHD based on BSA comparisons). Juvenile rats were more sensitive to the miltefosine-induced effects, especially on eyes and kidneys, than adult rats.

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2 Drug Information

2.1 Drug

CAS Registry Number: 58066-85-6

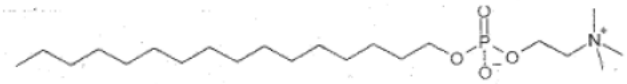
Generic Name: miltefosine

Code Name: D 18506

Chemical Name:
2{[(Hexyldecyloxy)hydroxyphosphenyl]oxy}N,N,Ntrimethylethylammonium inner-complex salt

Molecular Formula/Molecular Weight: $C_{21}H_{46}NO_4P$/ 407.57

Structure or Biochemical Description



Pharmacologic Class: Anti-parasitic compound

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 105430

2.3 Drug Formulation

Table 1: Miltefosine Product Formulation (Applicant's Table)

Component	Composition		Function
	mg/capsule	wt %	
Miltefosine	50.0	(b) (4)	Active ingredient
Colloidal Silicon Dioxide NF	(b) (4		(b) (4)
Microcrystalline Cellulose NF (b) (4)			
Lactose Monohydrate NF			
Talc NF			
Magnesium Stearate NF			
Opaque red hard gelatin capsule size 2*			
(b) (4) white ink**			
Total***	190.0		

* The capsule shells nominally consist of Gelatin NF (b) (4) Titanium Dioxide USP (b) (4) Ferric Oxide NF Red (b) (4) and Purified Water USP (b) (4) with a typical empty capsule shell weight of about (b) (4) Additional information for the capsule shell is incorporated by reference to (b) (4) Type IV DMF (b) (4) with an authorization letter provided in *Section 1.4.2* of this submission.

** The (b) (4) white ink used for imprinting on the hard gelatin capsules consists of (b) (4) (b) (4) Additional information for the imprinting ink is provided in (b) (4) Type IV DMF (b) (4) for which a DMF authorization letter is provided in the (b) (4) DMF.

*** The total weight includes (b) (4)

2.4 Comments on Novel Excipients

The product will not contain any novel excipients. The product excipients are shown in Table 1. Each of the excipients have been used at greater or equal concentrations in approved products as noted in the FDA inactive ingredient search engine (Inactive Ingredient Search for Approved Drug Products).

2.5 Comments on Impurities/Degradants of Concern

The drug substance impurities, degradants and specifications are shown below in Table 2. The drug product impurities, degradants and specifications are shown in Table 3. All of the acceptance criteria fall within the thresholds recommended in the ICH Guidances Q3A, Q3B(R), and Q3C.

However, in a June 20, 2013 information request from the FDA, the Applicant was informed that the HPTLC method used to measure two impurities, (b) (4) in the drug substance was not sufficiently validated for quantitative testing and corrective measures were requested. Until this deficiency is corrected and verification can be obtained that levels of (b) (4) in the drug substance remain within the qualification threshold amounts recommended in the ICH-Q3A (R2) Guidance, the drug product will not be approvable.

Table 2: Impurities, Degradants and Specifications in the Drug Substance. (Applicant's Table)

Test	Acceptance Criteria	Method
Description	White (b) (4) essentially free of visible foreign matter	Visual inspection
Identification (IR)	IR spectrum corresponds to that of standard similarly measured	USP <197>
Identification (HPLC)	HPLC retention time corresponds to that of standard similarly measured	*Section 3.2.S.4.2.1*
(b) (4)	NMT (b) (4)	USP (b) (4) Method (b) (4)
Assay (HPLC)	(b) (4)	*Section 3.2.S.4.2.1*
Impurities: (b) (4) Total (HPLC + HPTLC)	NMT (b) (4) NMT NMT NMT NMT NMT (b) (4) NMT (b) (4)	*Section 3.2.S.4.2.1* (HPLC); *Section 3.2.S.4.2.2* (HPTLC)
(b) (4)	NMT (b) (4)	*Section 3.2.S.4.2.3*
	NMT	*Section 3.2.S.4.2.3*
Heavy Metals	NMT (b) (4)	USP <231>, Method II
Residual Solvents (GC):* (b) (4)	NMT (b) (4) NMT NMT	*Section 3.2.S.4.2.4*

* The above acceptance criteria correspond to the ICH Q3C(R5) limits for Class (b) (4) solvents. Alert limits of NMT (b) (4) NMT (b) (4) and NMT (b) (4) have been established.

Table 3: Impurities, Degradants, and Specifications for the Drug Product.
(Applicant's Table)

Test	Acceptance Criteria	Method
Description	White powder, essentially free of foreign matter, encapsulated in a size 2 red opaque hard capsule with "PLB" imprinted on the capsule body and "MILT 50" imprinted on the cap in white.	Visual inspection
Identification (HPTLC)	R_f corresponds to that of standard similarly measured	*Section 3.2.P.5.2.1*
(b) (4)	NMT (b) (4)	USP (b) (4) Method (b) (4)
Assay (HPTLC)	(b) (4)	*Section 3.2.P.5.2.1*
Degradants (HPTLC)	1-Hexadecanol: NMT (b) (4) Unknowns: NMT Total: NMT	*Sections 3.2.P.5.2.2, 3.2.P.5.2.3*
Weight Variation	Conforms to USP <905>	Gravimetric
Dissolution (USP <711>, HPTLC)	NLT (b) (4) dissolved in (b) (4)	*Section 3.2.P.5.2.2*
Microbial Limits:	Total Aerobic Count: NMT (b) (4) Total Yeasts and Molds: NMT (b) (4)	USP <61>/<62>

2.6 Proposed Clinical Population and Dosing Regimen

Miltefosine will be taken daily for 28 days with food in the form of 50 mg tablets to adolescents and adults ≥ 12 years of age and weighing ≥ 30 kg. The number of 50 mg capsules per day is determined by bodyweight. For a body weight of 30-44 kg the dose is: two 50 mg capsules daily; 1 capsule taken with breakfast and 1 with dinner. For a body weight of ≥ 45 kg the dose is: three 50 mg capsules daily; one taken with breakfast, lunch, and dinner. These doses correspond to a maximum daily dose of 3.33 mg/kg/day.

2.7 Regulatory Background

This product was first submitted for review in IND 105430.

3 Studies Submitted

3.1 Studies Reviewed

Secondary Pharmacology

1. Studies on the effect of miltefosine on *Toxoplasma gondii*-mediated inhibition of apoptosis in a cell-free system.
2. Effects of D 18506 on PAF-induced edema in the rat hind paw (Study Report No.: 63005).
3. Investigations of D 18506 for bronchospasmolytic activity against PAF-induced bronchospasm in guinea pigs (Konzett-Rossier Method) (Study Report No.: 63109).

4. Investigation of D 18506 for bronchospastic activity in anesthetized guinea pigs (Konzett-Rossler Method) (Study Report No.: 63018).

Safety Pharmacology

1. Testing the antipyretic activity of D-18506 (Report #: 63012).
2. Antinociceptive activity in the hot-plate-test of D-18506 in the mouse (Report #:63011).
3. Influence of D-18506 on the spontaneous motility in the mouse (Report #: 63014).
4. Testing of ataxic effects by D-18506 on the rotating rod in the mouse (Report #: 63010).
5. Testing the anticonvulsive activity of D-18506 (Report #: 63013)
6. Electrophysiological investigation of the effects of compounds on the HERG mediated potassium current (Report GEP_ZTS_0406_03).
7. The effects of D-18506 after intraduodenal application on the cardiovascular function in the anaesthetized pig (Report #: 63006).
8. The effects of D 18506 after intraduodenal application on the respiratory system of anesthetized, spontaneous breathing cats (Report # 63007).
9. Investigation of D 18506 for stimulating effect on salivary secretion in anesthetized rats. (Report #: 63017).
10. Development of gastric erosions in fasted rats 24 hours after single intragastric application of D-18506 and indomethacin (Report #: 63001).
11. The development of intestinal ulcers in non-fasted rats 24 hours after single intragastric application of D-18506 (Report #: 63004).
12. Development of gastric erosions after 3-day intragastric pretreatment of D-18605 in fasted rats (Report #: 63002).
13. The effects of D 18506 on the gastrointestinal transit in fasting mice (Report #: 63003).
14. Investigation of D 18506 for spasmogenic activity on isolated guinea pig ileum (Report #: 63015).
15. Investigation of D 18506 for spasmolytic activity against histamine- and barium chloride-induced spasm of the isolated guinea pig ileum (Report #: 63016).

Pharmacokinetics

Absorption

1. D-18506: Study on the absolute bioavailability and pharmacokinetics in male rats following a single oral or intravenous administration of 10 mg/kg (Report #: 7000010038, 9/2000).
2. D-18506: Plasma pharmacokinetics in male Wistar rats after single oral administration in the range of 1 to 10 mg/kg (Report #:7001009041, 8/2001).
3. D-18506: Study on the absolute bioavailability and pharmacokinetics in male Beagle dogs following a single oral administration (Report #: 700065040, 8/2001).

Distribution

1. [^{14}C]Miltefosine: *In vitro* protein binding in plasma of rats, dogs, and human
2. [^{14}C]Miltefosine: A study of distribution in the rat by quantitative whole-body autoradiography after single and multiple oral dosing (Study Report No.: 9321010047; Study No.: 010047).

Reference ID: 3384901

Metabolism

1. [^{14}C]Miltefosine: *In vitro* metabolism studies with reconstituted enzymes (phospholipases A-D, CYP monooxygenases) and hepatocytes (human, rat, dog). (Study Report No.: D18506/9321010056).
2. [^{14}C]Miltefosine: Metabolite profiling in feces and urine in male albino Wistar rats following a single oral or intravenous administration (Study Report No.: D-18506/93210200075; samples from excretion Study No. 010038)
3. [^{14}C]Miltefosine: Metabolite profiling in plasma and selected tissues of male albino Wistar rats following a single oral or intravenous administration. (Study Report No.: D-18506/9321020076; samples from Study No.: 010038).

Excretion

1. [^{14}C]Miltefosine: Excretion balance in male albino Wistar rats following a single oral or intravenous administration. (Study No.: 010038; Study Report No.: 9321010038).

Pharmacokinetic Drug Interactions

1. Influence of D-18506 on hepatic cytochrome P450 isoenzymes of the CYP3A family in rats (Study No.: FB21099).

General Toxicology

Single-dose Toxicology (only summary results for these studies)

1. D 18506 - Testing the acute toxicology after single oral administration in mice (Study No: 871830; Study Report No.: 3000871830).
2. D 18506 - Pilot study on the acute toxicity after single intraperitoneal administration in mice (Study Report No.: 24064).
3. D 18506 - Pilot Study on the acute toxicity after single oral administration in rats (Study Report No.: 240066).
4. D-18506 – Pilot study on the acute toxicity after single intravenous administration in rats (Study Report No.: 240069).
5. D-18506 – Pilot study on the acute toxicity after single intraperitoneal administration in rats (Study Report No.: 240068).

Repeated-Dose Toxicity

1. 8-week oral repeat dose toxicity study in Wistar rats (dose finding study) (Study No.: 864494).
2. 13-week oral repeat dose toxicity study in Beagle dogs with a subsequent 6-week recovery period (Study No.: 859408).
3. Miltefosine (D-18506); 52-week oral toxicity study after repeated administration in rats and subsequent 6-week recovery period (Study No.: 861344).
4. D-18506 (miltefosine): 52-week oral toxicity study after repeated-administation in Beagle dogs and subsequent 6-week recovery period (Study No.: 873450).

Genetic Toxicology

1. Investigations into the mutagenic effect of HPC on *Salmonella Typhimurium* (Study Report No.: 3000514563).
2. *In vitro* assessment of the clastogenic activity of D-18506 (miltefosine) in cultured human peripheral lymphocytes (Study Report No.: 3000876857; Study No.: 6057/90).
3. Mammalian Cell V79 Gene Mutation Test on D18506 (Study No: 876868; Study Report No.: 3000876868).

Reference ID: 3384901

4. D-18506: Mouse micronucleus test (single oral administration) (Study No.: 871841, Study Report No.: 3000871841).
5. *In vivo/in vitro* unscheduled DNA synthesis (UDS) assay on D-18506 (Study No.: 914073; Study Report No.: 3000914073).

Reproductive and Developmental Toxicology

1. Miltefosine (D-18506): Examination of the influence on the fertility and general reproductive performance after oral administration in male Wistar rats (Study No.: 9191528; Study Report No.: 3000919528).
2. Miltefosine (D-18506): Examination of the influence on the fertility and general reproductive performance as well as the early embryonic development after oral administration in female Wistar rats (Study No.: 919934; Study Report No.: 3000919934).
3. Orientating Dose-Range-Finding Study to determine the dose-levels for an examination of the influence of hexadecylphophocholine in the pregnant rat and the foetus by oral administration (Segment II) (Study No.: 874282, Study Report No.: 3000874282).
4. Orientating Dose-Range-Finding Study to determine the dose-levels for an examination of the influence of hexadecylphophocholine on the pregnant rabbit and the foetus by oral administration (Segment II) (Study No.: 874293, Study Report No.: 3000874293)

Special Toxicology Studies

1. Miltefosine (D-18506) 8-week oral toxicity study after repeated administration in juvenile rats and subsequent 6-week recovery period (Study No.: 916266; Study Report No.: 3000916266).
2. Examination on the formal pathogenesis of eye changes in rats. (Study No.: 871828; Study Report No.: 3000871828).
3. Examination on the reversibility of retina changes induced in pigmented rats by repeated oral administration of miltefosine (D-18506). (Study No.: 892855; Study Report No.: 3000892855).

3.2 Studies Not Reviewed

1. [^{14}C]Miltefosine: Determination of radiochemical and chemical purity (Study No.: 010037).
2. Preparative details of [^{14}C]Miltefosine (Study No.: 010147).
3. The establishment and validation of a high performance thin layer chromatography (HPTLC) assay with densitometric detection for measurement of miltefosine in rat plasma samples (Study No.: 9458 0078-94).
4. Validation of an HPLC/MS/MS method in combination with automated solid phase extraction (SPE) for the determination of D-21266 in human, rat and dog plasma, and in human urine, and of D-18506 in human plasma (Study No.: 97050).
5. Preliminary results of the investigations into the acute and subacute toxicity of hexadecylphophocholine (C16PC) (Study No. 511762).
6. Hexadecylphosphocholine (D 18506) – toxicological study after 90-day oral administration with the diet to rats (Study NO.: 513011).
7. Miltefosine (D-18506) – 52-week oral toxicity study after repeated administration in rats to determine the “non toxic effect dose” (Study No.: 892866).

Reference ID: 3384901

Previous Reviews Referenced

IND 105430 Review by Dr. Terry Miller (6/25/2010 Nonclinical Review in DARRTS)

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies were reviewed by the Microbiology reviewer.

4.2 Secondary Pharmacology

The effects of miltefosine on inhibition of apoptosis, induced edema and bronchspams have been examined in several secondary pharmacology studies. The results of these studies are briefly summarized below.

1. **Studies on the effect of miltefosine on *Toxoplasma gondii*-mediated inhibition of apoptosis in a cell-free system.**
   Miltefosine had no effect on the caspase 3-caspase 9 cascade downstream of induction. Miltefosine when pre-incubated with *Toxoplasma gondii* was able to block the *T. gondii*-mediated inhibition of the caspase cascade in a cell-free system.

2. **Effects of D 18506 on PAF-induced edema in the rat hind paw** (Study Report No.: 63005).
   Miltefosine (D 18506) inhibited (>40%) PAF-induced edema in the hind paws of Sprague-Dawley rats 1 hour after a single intraperitoneal administration at a dose of ≥ 8 mg/kg. Higher doses of miltefosine (32, 64 mg/kg) produced greater inhibition in a roughly dose-dependent manner.

3. **Investigations of D 18506 for bronchospasmolytic activity against PAF-induced bronchospasm in guinea pigs (Konzett-Rossier Method)** (Study Report No.: 63109).
   Miltefosine (D 18506) at an intravenous dose of 1 mg/kg was administered 5 minutes prior to PAF (platelet activating factor) to anesthetized and ventilated Pirbright white guinea pigs and PAF-induced bronchospasm was measured. Under the conditions of this study, miltefosine was shown to have no effect on PAF-induced bronchospasm.

4. **Investigation of D 18506 for bronchospastic activity in anesthetized guinea pigs (Konzett-Rossler Method)** (Study Report No.: 63018).
   Miltefosine (D18506) administered intravenously in doses of 0.1 to 3 mg/kg demonstrated weak bronchospastic activity in anesthetized and ventilated Pirbright white guinea pigs. The maximum bronchspastic effect produced by miltefosine was approximately 10-fold lower than the magnitude of bronchospasm produced by 30 mg/kg carbachol. The miltefosine effect was not dose-proportional and bronchospasm increased only marginally with dose.

Reference ID: 3384901

4.3 Safety Pharmacology

A number of safety pharmacology studies have been performed to examine the neurobehavioral, cardiovascular, respiratory, and gastrointestinal effects of miltefosine. Renal safety pharmacology studies were not performed. For each category of the safety pharmacology studies that were performed, the studies are listed, and the results are briefly summarized.

Neurobehavioral

Miltefosine was tested in a number of safety pharmacology studies to evaluate its antipyretic, antinociceptive, and anticonvulsant activity and its effects on motor coordination and spontaneous motility in mice and rats.

1. **Testing the antipyretic activity of D-18506.** (Study Report #: 63012)

Orally administered miltefosine (D-18506) at concentrations ≤ 400 mg/kg did not affect normal temperature in female Sprague–Dawley rats, but did reduce febrile temperatures in a dose-dependent manner in the yeast-induced fever test.

2. **Antinociceptive activity in the hot-plate-test of D-18506 in the mouse.** (Study Report #: 63011)

Single oral administration of 25, 50 and 100 mg/kg miltefosine produced an antinociceptive effect at 100 mg/kg in the mouse hotplate test with approximately 50% of the efficacy of codeine.

3. **Influence of D-18506 on the spontaneous motility in the mouse.** (Study Report #: 63014)

In male mice, spontaneous motility was slightly inhibited (31.5%) by miltefosine (D-18506) at the highest tested oral dose of 200 mg/kg compared to vehicle treated animals.

4. **Testing of ataxic effects by D-18506 on the rotating rod in the mouse.** (Study Report #: 63010)

In male mice, a single oral dose of miltefosine (D-18506) at doses up to 400 mg/kg did not produce ataxia in the rotating rod test.

5. **Testing the anticonvulsive activity of D-18506.** (Study Report #: 63013)

A single oral dose of 80 mg/kg miltefosine (D-18506) did not produce anticonvulsant activity in female Sprague-Dawley rats (6 rats/dose group) compared to the vehicle control treatment.

Cardiovascular

1. **Electrophysiological investigation of the effects of compounds on the hERG mediated potassium current.** (Report GEP_ZTS_0406_03)

In this GLP-compliant study, miltefosine appeared to produce cytotoxic effects at concentrations ≥ 10 μM which interfered with the assay. At the miltefosine concentrations that did not interfere with the assay (0.1, 1 and 3 μM), no significant effect on hERG-mediated potassium current was observed.

Reference ID: 3384901

2. **The effects of D-18506 after intraduodenal application on the cardiovascular function in the anaesthetized pig.** (Report #: 63006)

Single intraduodenal doses of miltefosine (D 18506) in anesthetized male pigs (3-5/group) produced a dose-dependent decrease in blood pressure and cardiac output, but heart rate and left ventricular contractility were not affected. Doses of 10 and 20 mg/kg miltefosine slightly decreased blood pressure and cardiac output, but larger doses of 50 and 200 mg/kg produced marked effects that increased with time to as much as 30% below baseline values.

Respiratory

1. **The effects of D 18506 after intraduodenal application on the respiratory system of anesthetized, spontaneous breathing cats.** (Study Report # 63007)

In three spontaneously breathing, anesthetized female cats, intraduodenal application of 20 mg/kg miltefosine did not produce changes in the inspiratory or expiratory flow, tidal volume or $C0_2$ content of expired air, but did slightly increase respiratory rate and minute volume by about 10% from baseline. Heart rate was not affected, but blood pressure decreased by as much as 20% three hours after dosing.

Gastrointestinal System

1. **Investigation of D 18506 for stimulating effect on salivary secretion in anesthetized rats.** (Study Report #: 63017)

Anesthetized Sprague-Dawley rats were administered 100 mg/kg miltefosine (D 18506) or vehicle (1% methocel) orally and spontaneously produced saliva was collected over two hours. Vehicle-treated rats (n = 7) did not produce saliva, and only 1/12 rats receiving miltefosine produced saliva suggesting miltefosine did not greatly induce spontaneous saliva secretion.

2. **Development of gastric erosions in fasted rats 24 hours after single intragastric application of D-18506 and indomethacin.** (Study Report #: 63001)

Fasted male Sprague-Dawley rats treated with single intragastric doses of 25 to 400 mg/kg miltefosine (D-18506) did not experience intestinal ulcers at any dose. Animals treated with 200 and 400 mg/kg miltefosine demonstrated severely inflated stomachs, diarrhea, and severe erythema of the gastric mucosa as well as death for 1/12 animals treated with 200 mg/kg and 5/12 animals treated with 400 mg/kg miltefosine.

3. **The development of intestinal ulcers in non-fasted rats 24 hours after single intragastric application of D-18506.** (Study Report #: 63004)

Non-fasted male Sprague-Dawley rats treated with single intragastric doses of 25 to 400 mg/kg miltefosine (D-18506) did not experience intestinal ulcers at any dose, but demonstrated severely inflated stomachs and intestines at 200 and 400 mg/kg. The 400 mg/kg dose also produced partial intestinal erythema in 2/6 rats.

4. **Development of gastric erosions after 3-day intragastric pretreatment of D-18605 in fasted rats.** (Study Report #: 63002)

Reference ID: 3384901

Three daily intragastric applications of miltefosine (D18605) at doses ≥ 50 mg/kg/day in fasted Sprague-Dawley rats produced non-dose related gastric mucosal lesions. Animals experienced diarrhea at doses ≥ 100 mg/kg/day. In the animals treated with 200 mg/kg/day, 3/6 animals died and all 6 animals treated with 400 mg/kg/day died.

5. **The effects of D 18506 on the gastrointestinal transit in fasting mice.** (Study Report #: 63003)

Miltefosine (D 18506) administered by oral gavage in doses of 12.5, 25, 50, 100, 200 and 400 mg/kg to fasted NMRI white mice strongly inhibited gastrointestinal transit 80 minutes after administration. All of the doses of miltefosine produced more than the approximately 35% inhibition produced by the positive-control compound, atropine sulfate. The strong degree of inhibition produced by miltefosine was not dose-proportional and increased incrementally from approximately 40% with the lowest dose to approximately 65% with the highest dose.

6. **Investigation of D 18506 for spasmogenic activity on isolated guinea pig ileum.** (Study Report #: 63015)

Miltefosine (D 18506) at concentrations as high as 134 μg/ml did not induce spasmogenic activity in isolated guinea pig ileum as measured by ileac contraction. In contrast, a positive control agent, 1 μM acetylcholine, produced marked contraction in the same test system.

7. **Investigation of D 18506 for spasmolytic activity against histamine- and barium chloride-induced spasm of the isolated guinea pig ileum.** (Study Report #: 63016)

Miltefosine (D 18506) was tested for its ability to inhibit histamine-induced and barium chloride-induced spasm in isolated guinea pig ileum. Miltefosine produced a weak inhibition of histamine-induced spasm with an IC_{50} value of 2.79 μg/ml, about 300 fold less than the 9.76 ng/ml IC_{50} for the positive control agent, atazoline. Against barium chloride-induced spasm, miltefosine produced no spasmolytic activity at concentrations as high as 10 μg/ml. In contrast, a positive control agent, papaverine, demonstrated an IC_{50} of 2.32 μg/ml for inhibition of barium chloride-induced spasm.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Three single-oral dose absorption studies (Reports No.: 7000010038, 7001009041, and 7000065040), two in rats and one in dogs, are reviewed below.

Study Title: D-18506: Study on the absolute bioavailability and pharmacokinetics in male rats following a single oral or intravenous administration of 10 mg/kg. (Study Report #: 7000010038; Study No.: 00010)

Methods

Reference ID: 3384901

Rats (6 for each route) were administered 10 mg/kg miltefosine (D-18506) by the oral and intravenous routes. Blood samples were collected predose, and at 0.083, 0.5, 1.5, 4, 8, 24, 48, 96, 168, 264, 360, and 432 hours post administration. Miltefosine plasma concentrations were assessed using a validated HPLC/MS/MS technique.

Results
The absolute bioavailability in male rats after a single administration was 81.9%. As shown in Table 4 clearance values were low compared to hepatic or renal plasma flow, and the mean plasma $t_{1/2}$ value was long (84.3 hours). T_{max} values following oral dosing were variable ranging from 24 to 48 hours. The estimated volumes of distribution at steady state (Vss) and during the terminal phase (Vz) were about 10-fold greater than the expected volume of the vascular compartment of rats (≈ 60 ml/kg) suggesting substantial distribution of miltefosine to extravascular tissues.

Table 4: Pharmacokinetic Parameters for Miltefosine Following Intravenous and Oral Administration of 10 mg/kg to Male Rats. Values are expressed as means plus 95% lower and upper confidence interval limits in parentheses. (Applicant's Table)

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Intravenous, 10 mg/kg				
$t_{1/2}$ [h]	AUC [ng·h/ml]	CL [ml/min·kg]	V_{ss} [l/kg]	V_z [l/kg]
82.5 (67.6–95.5)	1104180 (889352-1370902)	0.15 (0.12 – 0.19)	0.96 (0.76-1.21)	1.07 (0.87-1.31)

Oral, 10 mg/kg			
C_{max} [ng/ml]	t_{max} [h]	$t_{1/2}$ [h]	AUC [ng·h/ml]
6770 (6279-7299)	24.0 (24.0-48.0)	84.3 (71.9-94.1)	904533 (732417-1117098)

Study Title: D-18506: Plasma pharmacokinetics in male Wistar rats after a single oral administration in the range of 1 to 10 mg/kg. (Study Report #: 7001009041; Study No.: 01009)

Methods
Male Wistar rats (4 per dose) received single-oral administrations of 1, 5, and 10 mg/kg miltefosine (D-18506). Blood samples were collected predose, and at 1, 2, 4, 8, 24, 48, 96, 168, 264, 360, and 432 hours after administration. Plasma concentrations of miltefosine were determined using a validated HPLC-MS/MS method (lower limit of quantification of 5 ng/ml).

Results
C_{max} and AUC values increased in a dose-proportional manner between doses of 1 and 10 mg/kg (Table 5). The $t_{1/2}$ values were long for each dose and similar to the value determined in a previous study (84.3 hours). Also as in the previous study, T_{max} values were variable (8-48 hours).

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Table 5: Pharmacokinetic Parameters for Single-Oral Doses of 1, 5, and 10 mg/kg Miltefosine in Rats. (Applicant's Table)

Dose [mg/kg]	n = 4	C_{max} [ng/ml]	t_{max} [h]	$t_{1/2}$ [h]	AUC [ng·h/ml]	MRT [h]
1	Median	702	8.0	89.4	70628	114.8
	(Min - Max)	(480 – 841)	(8.0 – 48.0)	(77.9 - 96.2)	(65927 - 76467)	(102.6 – 136.0)
5	Median	3515	8.0	83.5	321885	114.9
	(Min - Max)	(3357 - 4165)	(8.0 - 24.0)	(75.9 - 93.6)	(308257 - 384336)	(106.7 – 120.0)
10	Median	6647	24.0	74.2	746956	104.6
	(Min - Max)	(6417 - 6842)	(8.0 - 24.0)	(71.6 - 77.6)	695725 - 843820)	(102.0 – 109.6)

Study Title: D-18506: Study on the absolute bioavailability and pharmacokinetics in male Beagle dogs following a single oral administration (Study Report No.: 7000065040; Study No.: 00065).

Methods

Beagle dogs (n = 4) were administered a single-oral or a single-IV dose of miltefosine (D-18506; 1 mg/kg for each route) in a crossover design with a 77 day washout period, and blood samples were collected predose and at 0.17 (IV only), 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 168, 216, 264, 360, 432, 52, and 672 hours after dosing. Plasma samples were analyzed using a validated HPLC-MS/MS technique, and the oral bioavailability and pharmacokinetic parameters for plasma miltefosine were calculated.

Results

The median absolute oral bioavailability of miltefosine in dogs was 93.6%. As was the case for oral doses of miltefosine in rats, T_{max} values were variable between 8 and 24 hours for oral administration in dogs (Table 6). Also $t_{1/2}$ values were long, averaging 165 hours or about twice as long as for rats in agreement with a low plasma clearance. As for rats, the estimated values for the volume of distribution (Vz = 0.53 L/kg; Vss = 0.48 L/kg) were greater than the estimated blood volume for dogs (≈ 90 ml/kg) suggesting substantial tissue distribution. The C_{max} value for a 1 mg/kg oral dose of miltefosine in dogs (2271 ng/ml) was approximately 3 times greater than the C_{max} (702 ng/ml) for the same oral dose in rats. The estimated values for the AUC value in dogs, assuming calculations were for the same time period as for rats, appeared to be about 5-6 times greater than the rat value for the same dose (1 mg/kg).

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Table 6: Pharmacokinetic Parameters of Miltefosine in Dogs after Oral and Intravenous Administration. (Applicant's Table)

PK parameters of D-18506 in male dogs (n=4) Intravenous (1 mg/kg)							
C_{max} [ng/ml]	t_{max} [h]	$t_{1/2}$ [h]	AUC [ng·h/ml]	MRT_{iv} [h]	CL [ml/min/kg]	V_z [l/kg]	V_{ss} [l/kg]
6068 (4976-7672)	0.17 (0.17-0.17)	165 (142-187)	427274 (380054-524761)	204 (189-238)	0.04 (0.03-0.04)	0.53 (0.50-0.62)	0.48 (0.43-0.52)

PK parameters of D-18506 in male dogs (n=4) Oral (1 mg/kg)				
C_{max} [ng/ml]	t_{max} [h]	$t_{1/2}$ [h]	AUC [ng·h/ml]	MRT_{po} [h]
2271 (1984-2789)	24.0 (8.0-24.0)	159 (156-192)	410430 (360627-484780)	212 (190-232)

Distribution

Study Title: [^{14}C]miltefosine: *In vitro* protein binding in plasma of rats, dogs, and human (Study Report No.: 9321010041; Study No.: 010041).

Methods
[^{14}C]miltefosine at concentrations of 0.1, 1, and 10 μg/ml was incubated with plasma from rats, dogs, or humans for 30 minutes at 37°C followed by ultracentrifugation. The unbound fractions of radioactivity in plasma prior to ultracentrifugation and in the protein-free supernatant after ultracentrifugation were determined with liquid scintillation counting and compared to determine the degree of plasma-protein binding.

Results
[^{14}C]miltefosine was > 95% bound to plasma proteins in plasma from all three species (rats, dogs, and humans) independent of [^{14}C]miltefosine concentration (Table 7).

Table 7: *In Vitro* Plasma Protein Binding of [^{14}C]Miltefosine in Plasma from Rat, Dog, and Human. (Applicant's Table)

	In vitro protein binding [%]		
Conc. [μg/ml]	Rat plasma	Dog plasma	Human plasma
10.0	95.6	96.0	97.5
1.0	95.5	95.9	97.4
0.1	95.7	96.3	97.6

Study Title: [^{14}C]Miltefosine: A study of distribution in the rat by quantitative whole-body autoradiography after single and multiple oral dosing. (Study Report No.: 9321010047; Study No.: 010047)

Methods

Male rats were dosed orally with 5 mg/kg [^{14}C]miltefosine according to multiple single- and repeated-dose regimens as shown in the Applicant's table below (Table 8). The rats used in this study were Sprague-Dawley (albino) and Lister Hooded (pigmented). Upon euthanasia of animals at specific time-points, the carcasses were subjected to whole-body autoradiography and radioactivity concentrations in tissues were quantified from whole-body autoradiograms using a validated image analysis system.

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Table 8: Single- and Repeated-Dose Regimens and Sacrifice Schedules for the Quantitative Whole-Body Autoradiography Study for [^{14}C]Miltefosine in Rats. (Applicant's Table)

Animals	Dosing regimen	Kill time after dosing	Number of animals sampled at each time
Albino rats	single dose	8 hours, 1, 2, 4, 7 and 21 days	3
Pigmented rats	single dose	8 hours, 1, 4, 7, 21, and 35 days	1
Albino rats	7 daily doses	1 day	3
Albino rats	14 daily doses	1 day	3
Albino rats	21 daily doses	1, 2, 4, 7, 14, 21 and 28 days	3

Results

Following a single oral administration of [^{14}C]miltefosine in albino rats, radioactivity was widely distributed into tissues at 8 hours post-dose and peak concentrations in most tissues occurred at either 1 or 2 days after dosing, except in tissues of the central nervous system where concentrations did not peak until 7 days after dosing (Table 9). The tissues exhibiting the highest concentrations of radioactivity one day after dosing were: the outer cortex of the kidney, liver, preputial gland, small intestine mucosa, and optic nerve. High levels of radioactivity, approximately 5-10 fold lower than in the most radioactive tissues were also noted in the cartilage of the articular surfaces of the spine in the region of the intervertebral disks. The tissues with the lowest concentrations of radioactivity one day after dosing were: brain, meninges, spinal cord, white fat, muscle, and lens, but these were among the tissues that continued to accumulate radioactivity for up to seven days after dosing. Radioactivity slowly declined with quantifiable amounts of radioactivity remaining in all the measured tissues 21 days after dosing. Terminal half-life values for total radioactivity for most tissues were calculated to be between 4 and 7 days.

Table 9: Mean Concentration of Radioactivity in the Tissues of Albino Male Rats (n = 3) after a Single Oral Administration of [^{14}C]Miltefosine at a Dose of 5 mg/kg.
(Applicant's Table)

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Tissue type	Tissue / Kill time	µg equivalent of Miltefosine/g of tissue					
		8 hour	1 day	2 day	4 day	7 day	21 day
Vascular/ lymphatic	Blood	2.44	2.42	2.14	1.66	1.18	0.44
	Aorta	2.99	3.27	3.01	2.63	1.82	0.76
	Lymph	3.95	7.44	4.84	3.48	3.33	2.21
	Mandibular lymph nodes	4.07	5.86	4.74	3.98	2.60	0.50
Metabolic/ excretory	Kidney cortex (inner)	18.04	19.48	17.80	8.23	4.22	1.05
	Kidney cortex (outer)	*46.78*	*48.66*	42.43	25.01	15.20	1.42
	Kidney medulla	7.81	11.36	9.74	6.78	4.62	0.62
	Liver	11.17	10.86	10.21	7.80	5.76	0.85
CNS	Brain	0.30	0.64	0.98	1.33	1.71	1.59
	Choroid plexus	3.59	4.01	2.69	3.72	2.11	1.67
	Meninges	1.44	1.91	2.67	2.67	1.75	0.87
	Pineal body	4.25	7.41	8.29	5.69	3.58	0.59
	Spinal cord	0.34	0.77	1.21	1.49	1.82	1.51
Endocrine	Adrenal	4.42	6.50	7.04	5.57	4.11	0.63
	Pituitary	4.49	5.83	6.78	9.75	5.53	1.69
	Thymus	3.57	4.94	4.04	3.14	2.38	0.54
	Thyroid	3.88	6.06	5.90	4.37	3.10	0.77
Secretory	Exorbital lachrymal gland	2.79	4.38	4.01	3.80	2.72	0.70
	Eyelid/meibomium gland	4.59	10.59	9.31	7.45	5.08	1.06
	Intra-orbital lachrymal gland	2.95	4.22	4.02	3.73	3.36	0.70
	Harderian gland	2.92	7.25	7.79	9.78	6.95	0.98
	Mucus gland	2.71	4.49	4.71	3.99	2.96	0.70
	Salivary gland	4.14	5.30	4.56	3.96	2.75	0.62
Fatty	Brown fat	2.38	2.75	2.67	1.84	1.63	0.47
	White fat	0.76	1.12	0.95	0.75	0.67	0.27
Gonads	Bulbo-urethral gland	4.18	5.12	3.90	4.49	2.47	0.88
	Epididymis	3.60	4.19	5.27	4.32	3.83	0.87
	Preputial gland	23.63	27.26	34.58	24.09	23.86	6.85
	Prostate	2.35	3.98	3.31	2.48	1.99	0.52
	Seminal vesicles (contents)	1.17	2.85	3.43	2.78	3.93	1.07
	Seminal vesicles (wall)	9.47	17.65	18.05	12.40	8.52	1.43
	Testis	1.22	2.22	2.61	2.74	2.80	0.90
Muscular	Muscle	1.17	1.45	1.46	1.34	1.19	0.63
	Myocardium	2.58	3.02	2.71	2.24	1.74	0.68
	Tongue	2.37	3.10	2.76	2.38	1.86	0.64
Ocular	Lens	0.05+	0.22	0.50	0.74	0.69	0.88
	Optic nerve	1.90	7.24	13.62	9.00	9.65	5.95
	Retina	1.61	3.02	3.04	2.41	1.71	0.55
Unclassified	Articular surface (spine)	4.86	8.89	11.53	12.23	10.50	3.95
	Bone marrow	3.72	5.71	3.73	3.81	2.71	0.78
	Lung	4.89	5.77	5.00	3.65	2.51	0.53
	Nasal mucosa	1.66	2.26	3.16	2.14	2.44	0.53
	Non-pigmented skin	2.65	5.12	3.94	2.73	2.31	1.61
	Pancreas	3.74	5.22	4.55	4.29	3.11	0.74
	Peridontal membrane	2.94	4.16	3.19	2.62	1.99	1.19
	Tooth pulp	3.39	3.35	3.09	2.80	2.53	0.40
	Spleen	5.76	7.26	6.02	4.73	3.25	0.60
Gastrointestinal	Stomach mucosa (fundus)	7.18	8.66	6.63	4.90	3.81	0.59
	Stomach mucosa (non-fundic)	5.28	2.82	2.96	2.12	1.73	0.46
	Small intestine mucosa	33.43	15.39	9.93	4.97	3.49	0.47
	Caecum mucosa	4.39	5.97	4.20	3.03	2.21	0.41
	Large intestine mucosa	5.38	5.00	4.16	3.45	3.12	0.36
	Rectum mucosa	3.85	6.72	5.58	3.65	2.22	0.63
	Lower limit of quantification =	0.06	0.06	0.06	0.06 or 0.02	0.06	0.06
	Upper limit of quantification =	59.03 or 45.88 µg equiv/g data					

Italicised values - some individual measurements above upper limit of quantification
+ - Some individual measurements below lower limit of quantification

In pigmented rats following a single oral administration of [^{14}C]miltefosine, tissue concentrations of radioactivity were similar to those of albino male rats (Table 10). Melanin-containing tissues (uveal track/retina and pigmented skin) did not accumlate increased radioactivity indicating no melanin binding of radiolabelled miltefosine. At the final sampling time of 35 days, most tissues still contained low but quantifiable levels of radioactivity. The tissues with the highest concentrations of radioactivity on Day 35 were: the optic nerve, articular surface of the spine, skin, lymph, and the spinal cord.

Table 10: Mean Concentration of Radioactivity in the Tissues of a Pigmented Male Rat (n = 1) after a Single Oral Administration of [^{14}C]Miltefosine at a Dose of 5 mg/kg. (Applicant's Table)

		µg equivalents of Miltefosine/g of tissue					
	Animal number and sex	20M	21M	22M	23M	24M	25M
Tissue type	Tissue / Kill time	8 hour	1 day	4 day	7 day	21 day	35 day
Vascular/ lymphatic	Blood	2.38	2.86	1.71	1.26	0.37	0.32
	Aorta	2.47	4.06	2.57	1.57	0.65	0.52
	Lymph	5.70	12.98	3.84	3.71	2.98	1.27
	Mandibular lymph nodes	4.16	6.44	3.62	2.55	0.51	0.31
Excretory/ metabolic	Kidney cortex (inner)	15.28	15.56	7.00	4.53	1.00	BLQ
	Kidney cortex (outer)	45.44	*51.38*	27.57	12.81	1.47	0.39
	Kidney medulla	7.10	11.76	5.21	4.32	0.65	0.25
	Liver	11.01	15.35	8.41	5.01	0.80	0.28
CNS	Brain	0.38	0.82	1.47	1.89	1.47	1.00
	Choroid plexus	1.56	3.06	2.60	4.77	BLQ	BLQ
	Meninges	1.45	3.72	1.88	1.82	BLQ	BLQ
	Pineal body	5.40	12.22	6.45	4.98	0.52	BLQ
	Spinal cord	0.45	0.94	1.87	2.01	1.34	1.23
Endocrine	Adrenal	3.99	6.54	5.12	3.73	0.59	0.28
	Pituitary	5.68	10.06	7.58	4.49	0.86	0.33
	Thymus	2.92	5.97	3.37	2.44	0.48	0.44
	Thyroid	3.60	6.65	4.44	3.18	BLQ	BLQ
Secretory	Exorbital lachrymal gland	2.57	4.82	3.35	2.74	0.66	0.28
	Eyelid/meibomium gland	5.61	7.93	7.33	6.78	0.84	0.50
	Intra-orbital lachrymal gland	2.63	4.94	3.60	2.61	0.73	0.33
	Harderian gland	2.33	5.69	6.31	5.24	0.99	0.42
	Mucus gland	2.77	5.69	4.10	3.02	BLQ	BLQ
	Salivary glands	3.70	6.15	3.81	2.83	0.63	0.24
Fatty	Brown fat	2.31	3.11	2.27	1.61	0.33	0.23
	White fat	0.81	0.98	0.48	0.54	0.27	0.21
Gonads	Bulbo-urethral gland	3.85	3.65	NS	2.54	0.45	0.72
	Epididymis	2.23	4.76	6.28	4.56	1.01	0.35
	Preputial gland	21.66	*49.48*	31.68	27.74	2.05	NS
	Prostate	2.89	3.37	2.63	2.13	0.51	0.70
	Seminal vesicles (contents)	1.04	2.27	3.40	3.47	1.41	0.64
	Seminal vesicle (wall)	10.81	22.37	13.03	7.22	BLQ	BLQ
	Testis	1.20	2.33	3.21	2.90	1.02	0.36
Muscular	Muscle	1.02	1.65	1.29	1.18	0.60	0.41
	Myocardium	2.29	3.62	2.19	1.75	0.61	0.36
	Tongue	2.24	3.71	2.61	2.06	0.68	0.34
Ocular	Lens	NS	0.08	0.36	0.24	0.29	0.71
	Optic nerve	8.45	7.00	11.03	11.94	7.36	6.70
	Retina	2.06	2.98	2.08	1.36	0.47	0.41
Dermal	Non-pigmented skin	2.26	4.01	2.58	2.80	1.34	1.03
	Pigmented skin	3.25	5.14	3.36	3.30	1.68	1.31
Unclassified	Articular surface (spine)	3.59	12.14	15.60	11.95	4.15	4.38
	Bone marrow	2.75	3.72	2.45	2.79	0.49	0.41
	Lung	4.61	7.42	4.34	2.87	0.44	0.31
	Nasal mucosa	1.97	2.69	2.69	2.84	0.67	0.26
	Pancreas	3.38	6.06	4.00	3.30	0.69	0.29
	Peridontal membrane	2.21	4.74	3.12	2.08	1.37	0.90
	Tooth pulp	3.50	4.50	2.77	1.98	0.59	0.15
	Spleen	5.28	9.03	4.98	3.23	0.57	0.26
Gastrointestinal	Stomach mucosa (fundus)	2.87	6.17	5.30	3.24	0.56	0.32
	Stomach mucosa (non-fundic)	3.72	2.78	1.28	1.51	0.32	0.24
	Small intestine mucosa	38.93	9.09	7.72	2.94	0.56	0.22
	Caecum mucosa	2.97	6.94	3.45	2.07	0.43	0.31
	Large intestine mucosa	4.07	6.31	2.57	1.97	0.39	0.26
	Rectum mucosa	3.19	7.37	4.23	2.54	0.50	0.28
	Upper limit of quantification	45.85	45.85	45.85	45.85	45.85	45.85
	Lower limit of quantification	0.06	0.06	0.06	0.06	0.06	0.06

NS – Tissue not sectioned
BLQ – Radioactive concentration below lower limit of quantification
Italicised values above upper limit of quantification

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Tissue Distribution Following Repeated-Dose Administration

In albino rats receiving 7 daily oral administrations of [^{14}C]miltefosine, the general pattern of tissue distribution of radioactivity one day after the last of 7 doses was similar to that following single oral administrations except tissue concentrations were higher (Table 11). Following repeated dosing for 7 days, tissue concentrations were generally in the range of 5- to 9- fold higher than those after a single administration. More accumulation up to 16-fold higher than after a single administration also occurred in

some tissues including brain, lens, harderian gland, nasal mucosa, epididymis, spleen, spinal cord, non-fundic region of the stomach mucosa, optic nerve, seminal vesicles and articular surface of the spine.

In albino rats receiving 14 daily doses of [^{14}C]miltefosine, tissue distribution was qualitatively similar to that seen for a single dose and 7 daily doses, but tissue concentrations of radioactivity were somewhat higher than after 7 daily doses. On the first day following 14 daily doses, tissue concentrations were generally in the range of 7- to 12-fold higher than those after a single administration. Some tissues demonstrated higher ratios than after a single administration including lens (39 fold), brain (38 fold), spinal cord (25 fold), contents of seminal vesicles (20 fold) testes and optic nerve (both approximately 16 fold).

Results were similar following 21 daily doses of [^{14}C]miltefosine, but the highest tissues concentrations of radioactivity were seen with this dosage regimen. On the first day following 21 daily doses, tissue concentrations of radioactivity were generally in the range of 7- to 15-fold higher than after a single dose with some tissues demonstrating higher ratio. Tissues with the highest ratios included: lens (68 fold), brain (50 fold), spinal cord (37 fold), optic nerve (28 fold), epididymis, contents of seminal vesicles, testis (all about 21 fold), and choroid plexus (20 fold). Two days after the end of dosing, tissue concentrations of radioactivity had generally declined, but for some tissues concentrations continued to increase including inner kidney cortex, choroid plexus, meninges, adrenal, white fat, preputial gland, prostate, testis, lens, bone marrow, parts of the stomach and cecum, brain, spinal cord, and contents of the seminal vesicles.

Nearly 18% of the administered radioactivity was still present in tissues at 21 days after the final dose of 21 daily doses indicating long tissue half-lives (Table 12). Terminal half-life values for tissue radioactivity were mostly in the range of 8 to 16 days with only the prostate (6.7 days), Harderian gland (6.5 days), and pituitary (6.3 days) having lower values. Tissues with the longest $t_{1/2}$ values were: spinal cord (47 days), brain (39 days), and nonfundic region of the stomach mucosa, blood, aorta, bulbo-urethral gland, muscle, bone marrow, and skin (all with $t_{1/2}$ values in the range of 16-20 days).

Reference ID: 3384901

Table 11: Mean Concentrations of Radioactivity in the Tissues of Albino Male Rats (n = 3) 1 Day After Single and Repeat Daily Oral Administrations of [^{14}C]Miltefosine at a Nominal Dose Level of 5 mg/kg Body Weight. (Applicant's Table)

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		µg equivalents of Miltefosine/g of tissue			
	Group identification	A	C	D	E
Tissue type	Tissue / Number of doses	1	7	14	21
Vascular/ lymphatic	Blood	2.42	13.84	20.56	25.33
	Aorta	3.27	22.47	31.05	35.60
	Lymph	7.44	14.47	84.60	17.43
	Mandibular lymph nodes	5.86	34.17	44.85	50.10
Metabolic/ excretory	Kidney cortex (inner)	19.48	62.36	108.0	116.5
	Kidney cortex (outer)	*48.66*	*222.2*	*244.7*	*237.8*
	Kidney medulla	11.36	61.58	84.75	84.28
	Liver	10.86	74.54	93.06	114.6
CNS	Brain	0.64	10.13	21.01	32.20
	Choroid plexus	4.01	37.80	45.69	81.94
	Meninges	1.91	14.12	25.09	29.78
	Pineal body	7.41	54.21	69.50	86.00
	Spinal cord	0.77	9.82	19.47	28.16
Endocrine	Adrenal	6.50	44.50	68.08	68.36
	Pituitary	5.83	31.37	77.38	116.4
	Thymus	4.94	27.29	40.15	47.27
	Thyroid	6.06	43.53	55.99	69.60
Secretory	Exorbital lachrymal gland	4.38	30.64	46.28	53.91
	Eyelid/meibomium gland	10.59	94.14	98.68	107.6
	Intra-orbital lachrymal gland	4.22	63.65	94.07	54.63
	Harderian gland	7.25	30.86	43.75	109.3
	Mucus gland	4.49	34.39	47.66	54.62
	Salivary glands	5.30	32.38	49.12	56.16
Fatty	Brown fat	2.75	20.29	34.78	32.89
	White fat	1.12	4.83	7.22	8.87
Gonads	Bulbo-urethral gland	5.12	29.52	49.62	58.95
	Epididymis	4.19	56.58	56.14	87.38
	Preputial gland	27.26	*208.8*	166.9	200.3
	Prostate	3.98	19.84	38.24	38.68
	Seminal vesicles (contents)	2.85	28.87	57.00	59.38
	Seminal vesicle (wall)	17.65	194.0	144.4	164.3
	Testis	2.22	21.48	35.64	46.51
Muscular	Muscle	1.45	10.47	18.56	22.25
	Myocardium	3.02	19.30	29.39	35.36
	Tongue	3.10	22.83	33.10	38.46
Ocular	Lens	0.22	3.48	8.61	15.04
	Optic nerve	7.24	85.51	118.0	204.8
	Retina	3.02	22.75	24.00	34.35
Unclassified	Articular surface (spine)	8.89	94.00	131.5	165.8
	Bone marrow	5.71	33.69	46.67	52.64
	Lung	5.77	31.94	41.44	48.10
	Nasal mucosa	2.26	31.51	30.52	37.06
	Non-pigmented skin	5.12	18.32	35.36	47.18
	Pancreas	5.22	34.01	57.08	62.57
	Peridontal membrane	4.16	24.52	33.29	45.64
	Tooth pulp	3.35	41.53	56.19	43.87
	Spleen	7.26	23.91	33.50	63.94
Gastrointestinal	Stomach mucosa (fundus)	8.66	57.51	62.39	80.53
	Stomach mucosa (non-fundic)	2.82	33.58	30.70	42.00
	Small intestine mucosa	15.39	107.5	110.5	142.8
	Caecum mucosa	5.97	30.39	42.59	40.69
	Large intestine mucosa	5.00	36.08	38.85	48.85
	Rectum mucosa	6.72	36.83	41.05	51.59
	Lower limit of quantification	0.06	0.32	0.32	0.32
	Upper limit of quantification	59.03 or 45.88	225.8	225.8	225.8 or 290.6

Italicised values - some individual measurements above upper limit of quantification

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Table 12: Mean Concentrations of Radioactivity in the Tissues of Albino Male Rats (n =3) at Varying Time Points Following the Last of 21 Daily-Oral Administrations of [^{14}C]Miltefosine, Each at a Dose Level of 5 mg/kg. (Applicant's Table)

		µg equivalents of Miltefosine/g of tissue						
Tissue type	Kill time	1 day	2 day	4 day	7 day	14 day	21 day	28 day
Vascular/ lymphatic	Blood	25.33	24.18	19.79	14.83	11.46	8.87	7.12
	Aorta	35.60	31.76	29.30	22.38	16.03	13.02	9.06
	Lymph	17.43	17.01	22.01	10.63	4.48	5.31	3.97
	Mandibular lymph nodes	50.10	49.65	39.86	28.06	13.87	9.75	6.16
Metabolic/ excretory	Kidney cortex (inner)	116.5	121.5	97.99	65.11	24.10	16.49	9.81
	Kidney cortex (outer)	*237.8*	*236.7*	*228.2*	147.6	39.82	20.31	12.59
	Kidney medulla	84.28	83.84	66.35	40.54	18.51	13.06	7.46
	Liver	114.6	104.2	81.02	56.31	25.01	13.85	8.83
CNS	Brain	32.20	32.81	34.66	34.89	31.22	27.31	24.34
	Choroid plexus	81.94	93.68	66.94	BLQ	BLQ	BLQ	BLQ
	Meninges	29.78	33.90	24.17	31.61	BLQ	BLQ	BLQ
	Pineal body	86.00	75.19	62.20	43.95	15.97	9.54	6.89
	Spinal cord	28.16	29.61	31.31	32.00	27.70	27.16	22.52
Endocrine	Adrenal	68.36	70.69	55.65	37.87	17.95	11.41	7.32
	Pituitary	116.4	95.83	102.0	44.90	46.78	13.89	10.07
	Thymus	47.27	44.70	37.04	28.18	13.41	8.63	5.35
	Thyroid	69.60	63.19	52.62	38.32	19.73	14.60	8.36
Secretory	Exorbital lachrymal gland	53.91	53.62	44.23	33.63	17.78	11.18	7.95
	Eyelid/meibomium gland	107.6	87.71	78.29	58.66	28.65	15.44	15.38
	Intra-orbital lachrymal gland	54.63	52.80	44.37	33.32	18.74	13.09	8.06
	Harderian gland	109.3	101.9	89.38	90.25	34.18	14.50	9.83
	Mucus gland	54.62	52.62	44.81	32.49	17.35	11.17	6.52
	Salivary glands	56.16	50.28	45.19	33.77	18.20	10.81	7.16
Fatty	Brown fat	32.89	32.42	30.50	23.50	12.43	9.50	5.28
	White fat	8.87	19.99	13.32	7.29	5.87	6.39	3.84
Gonads	Bulbo-urethral gland	58.95	44.26	43.21	28.05	16.46	13.18	9.62
	Epididymis	87.38	61.97	58.45	51.76	17.54	13.10	9.40
	Preputial gland	200.3	*230.9*	*204.4*	171.7	74.22	43.49	42.25
	Prostate	38.68	46.28	30.28	42.82	18.16	8.26	4.28
	Seminal vesicles (contents)	59.38	61.03	57.02	69.81	37.66	21.33	12.23
	Seminal vesicle (wall)	164.3	161.1	124.6	27.05	21.40	22.65	12.66
	Testis	46.51	49.34	46.80	40.28	25.78	14.91	9.32
Muscular	Muscle	22.25	22.73	20.18	17.57	15.59	12.50	9.50
	Myocardium	35.36	35.09	30.66	25.46	18.37	13.44	8.85
	Tongue	38.46	36.90	30.63	27.98	17.64	13.94	9.54
Ocular	Lens	15.04	22.43	11.26	9.81	9.91	8.97	13.28
	Optic nerve	204.8	181.6	166.4	187.9	102.2	105.6	117.0
	Retina	34.35	29.88	30.02	29.67	14.12	13.35	7.19
Unclassified	Articular surface (spine)	165.8	166.0	156.7	151.8	93.87	99.77	94.49
	Bone marrow	52.64	55.35	40.74	29.14	14.44	10.54	8.75
	Lung	48.10	45.67	36.99	24.09	14.26	9.93	7.70
	Nasal mucosa	37.06	36.39	33.56	50.85	19.78	9.13	7.52
	Non-pigmented skin	47.18	46.48	43.90	34.48	25.68	24.08	14.49
	Pancreas	62.57	60.40	47.88	39.84	19.93	12.57	8.11
	Peridontal membrane	45.64	42.29	35.79	25.27	23.73	26.66	14.20
	Tooth pulp	43.87	33.54	35.95	25.10	15.66	8.77	5.31
	Spleen	63.94	55.48	47.50	33.40	15.84	10.47	7.70
Gastrointestinal	Stomach mucosa (fundus)	80.53	77.29	52.89	49.70	19.13	12.59	8.20
	Stomach mucosa (non-fundic)	42.00	43.19	31.37	20.79	12.56	10.14	7.57
	Small intestine mucosa	142.8	122.7	92.82	56.48	16.18	9.54	5.93
	Caecum mucosa	40.69	41.75	32.55	32.34	13.57	8.78	5.39
	Large intestine mucosa	48.85	39.94	30.87	27.79	14.81	8.97	6.02
	Rectum mucosa	51.59	39.54	36.02	31.04	16.86	10.67	8.00
	Upper limit of quantification	225.8 or 290.6 µg equiv/g						
	Lower limit of quantification	0.32 µg equiv/g						

BLQ - radioactivity concentration below lower limit of quantification
Italicised values - some individual measurements above upper limit of quantification

Conclusions

Repeated-daily dosing of [^{14}C]miltefosine produced similar patterns of tissue distribution for all dosing durations, but tissue accumulation of radioactivity increased with the

duration of dosing. A plateau for accumulation was not clearly evident with the greatest accumulation occurring after 21 daily doses. Tissue $t_{1/2}$ values also increased to a degree with the duration of dosing ranging from 4 to 10 days after a single dose and 8 to 16 days after 21 daily doses. The tissue distribution of [^{14}C]miltefosine clearly indicates that miltefosine and/or its metabolites cross the blood-brain barrier, accumulate in nervous tissue, and are only slowly removed from nervous tissues. The report for this study makes the point that a metabolite of miltefosine, choline, is part of intermediary phospholipid metabolism which may partially explain the accumulation and slow elimination of radioactivity in this study given the prevalence of phospholipids in cell membranes. Although only one pigmented rat was evaluated, the comparison of distribution in tissues of albino and pigmented rats suggest that miltefosine and/or its metabolites do not bind melanin.

Metabolism

Study Title: [^{14}C]Miltefosine: *In vitro* metabolism studies with reconstituted enzymes (phospholipases A-D, CYP monooxygenases) and hepatocytes (human, rat, dog). (Study Report No.: 9321010056)

Methods
In this non-GLP study conducted in Dec., 2002 by the (b) (4) biotransformation of miltefosine was examined in reconstituted enzyme systems (Phospholipase A-D and CYP-450 isozymes) and in cultures with human, rat and dog hepatocytes by radio-HPLC and HPLC-MS methods. The reconstituted CYP-450 isozymes tested were CYP1A1 CYP1A2, CYP1B1, CYP 2A6, CYP2B6, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A1.

Results
None of the CYP-450 isozymes produced measureable oxidative metabolism of miltefosine. Phospholipase A_2, B, and C enzymes were similarly inactive, but miltefosine was approximately 83% metabolized to choline by phospholipase D. A low level of up to approximately 30% miltefosine metabolism to choline occurred in culture with human, rat and dog hepatocytes. The extent of metabolism was highest with human hepatocytes (2 donors; ≈20-30 % metabolism), followed by rat (2 donors; ≈10-20% metabolism, then dog (4 donors; ≈1-2% metabolism).

Study Title: [^{14}C]Miltefosine: Metabolite profiling in feces and urine in male albino Wistar rats following a single oral or intravenous administration (Study Report No.: 93210200075; samples from excretion Study No. 010038)

Methods
[^{14}C]Miltefosine was administered IV at 1 mg/kg and orally at 5 mg/kg to male albino Wistar rats (4 rats/dosage route). Fecal samples were collected from 0-24 hours, 48-72 hours, 96-120 hours, and 216-264 hours, and urine samples were collected from 4-8

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hours, 12-24 hours, 48-72 hours, 96-120 hours, and 216-264 hours following both administrations and analyzed with radio-HPLC analysis.

Results
After 264 hours, 14.89% and 16.11% of the total radioactivity was excreted in urine and 5.36% and 5.31% in feces for the IV and oral doses respectively. Choline, the only detectable metabolite, was detected in all the fecal and urine samples collected at all the time points. The amount of choline present in each sample was not determined. Miltefosine was absent from all the samples at all time-points except in the fecal samples collected from 216 to 264 hours.

Study Title: [^{14}C]Miltefosine: Metabolite profiling in plasma and selected tissues of male albino Wistar rats following a single oral or intravenous administration. (Study Report No.: 9321020076; samples from Study No.: 010038).

Methods
Following oral administration of 5 mg/kg miltefosine a number of tissues were collected at 8, 24 and 48 hours following administration. Tissues included: plasma, brain, liver, kidneys, stomach, large intestine, small intestine, testes, epididymes, prostate, and seminal vesicles (glanulae vesicularis). Miltefosine concentrations and concentrations of potential metabolites in each tissue were determined using Radio-HPLC and HPLC-MS methods.

Results
Miltefosine was detected in all tissue samples, and a single metabolite, choline was detected in all tissues except plasma, brain, and prostate where no metabolism was detected (Table 13).

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Table 13: Relative Amounts of Miltefosine and Choline in Tissue Samples of Male Wistar Rats Which Were Treated with 5 mg [^{14}C]Miltefosine/kg. (Applicant's Table)

Tissue	Animal No.	Ratio choline/Miltefosine [%]		
		8 h	24 h	48 h
Plasma	01	0/100	0/100	0/100
	02	0/100	0/100	0/100
Brain	01	0/100	0/0	0/100
	02	0/100	0/0	0/100
Prostate	01	0/100	0/100	0/100
	02	0/100	0/100	0/100
Liver	01	3/97	3/97	7/93
	02	8/92	1/99	15/85
Kidneys	01	7/93	12/88	14/86
	02	7/93	13/87	11/89
Stomach	01	15/85	80/20	89/11
	02	60/40	90/10	92/8
Large intestine	01	30/70	59/41	90/10
	02	22/78	94/6	100/0
Small intestine	01	8/92	11/89	17/83
	02	4/96	40/60	38/62
Testes	01	0/100	20/80	15/85
	02	0/100	7/93	23/70
Epididymes	01	0/100	4/96	5/95
	02	0/100	0/100	4/96
Seminal vesicles	01	0/100	72/28	34/66
	02	0/100	100/0	59/41

Excretion

Study Title: [^{14}C]Miltefosine: Excretion balance in male albino Wistar rats following a single oral or intravenous administration. (Study No.: 010038; Study Report No.: 9321010038)

Methods
Male albino Wistar rats were administered [^{14}C]miltefosine, 1 mg/kg IV and 5 mg/kg oral (n = 5 for both routes), and the rate, routes, and extent of excretion were studied. Fecal samples were collected in 24 hour intervals from 0 to 264 hours and urine samples were collected predose and from 0-2, 2-4, 4-8, 8-12, 12-24, 48-72, 96-120, 120-144, 144-168, 168-192, 192-216, and 216-240 and 240-264 hours following both administrations and analyzed with radio-HPLC analysis. Cage wash was performed in 24 hour intervals with water.

Results
After intravenous injection of miltefosine, excretion was slow but occurred predominantly in urine (14.89% in urine after 96 hours) and feces (5.36% after 264 hours). A similar pattern occurred after oral administration with 16.11% excreted by

urine and 5.31% excreted in feces. The majority of recovered radioactivity remained in the carcass (Table 14) and in particular in muscle and skin tissue (Table 15).

Table 14: The Percent of Administered Radioactivity Excreted in Urine and Feces Following Administration of 1 mg/kg IV and 5 mg/kg Oral [^{14}C]Miltefosine.
(Applicant's Table)

Specimen	[^{14}C]Fraction [% of admin. dose] excreted within 264 h	
	intravenous (1 mg/kg)	oral (5 mg/kg)
Urine + cage wash	14.89	16.11
Feces	5.36	5.31
Carcass*	45.92	40.08
Recovery#	65.05	61.78

* Carcass in sum with the [^{14}C]fractions in the removed tissues
# Mean of the individual recoveries, differs from sum of means above

Table 15: The Percent of Administered Radioactivity Distributing to Tissue Following Administration of 1 mg/kg IV and 5 mg/kg Oral [^{14}C]Miltefosine.
(Applicant's Table)

Specimen	[^{14}C]Fraction [% of admin. dose]			
	01-iv	05-iv	average	01-po
Plasma	0.92	0.94	0.93	0.69
Brain*	0.30	0.34	0.32	0.19
Pancreas*	0.16	0.14	0.15	0.15
Liver*	4.49	4.74	4.62	3.96
Lungs*	0.27	0.35	0.31	0.22
Spleen*	0.11	0.13	0.12	0.08
Kidneys*	1.79	2.07	1.93	1.35
Muscle	13.57	13.72	13.65	9.36
Skin	12.80	11.25	12.02	9.06
Adrenals*	0.01	0.01	0.01	0.01
Heart*	0.12	0.13	0.13	0.09
Adipose tissue	3.12	2.36	2.74	1.78
Blood	1.88	1.82	1.85	1.49
Sum	**39.54**	**38.00**	**38.77**	**28.42**
Sum of tissues to be added to carcass-fraction (*)	7.25	7.91	7.58	6.04
Sum of carcass tissues	**32.29**	**30.09**	**31.19**	**22.38**

* tissues that have been removed from the respective carcass before shreddering

Pharmacokinetic Drug Interactions

Study Title: Influence of D-18506 on hepatic cytochrome P450 isoenzymes of the CYP3A family in rats (Study No.: FB21099).

Methods

Wistar rats (4/sex/group) were administered 3.16, 10.0, and 31.6 mg/kg of miltefosine (D-18506) or the vehicle for miltefosine (water) orally on a daily basis for 5 days. Two other groups (2/sex/group) received daily intraperitoneal injections of a positive control agent, 20 mg/kg dexamethasone, or the corn oil vehicle for dexamethasone, daily for five days. One day after the last administration, rats were sacrificed and the liver was removed, homogenized, and liver microsomal membranes were isolated by centrifugation. Total protein, total CYP-450 content, and erythromycin N-demethylase activity as a measure of CYP3A activity were assessed in microsomal suspensions.

Results
Miltefosine at any dose did not substantially change total microsomal CYP-450 content (nmol/mg protein) or CYP3A activity in the livers of either male or female rats compared to its vehicle control group. In contrast, treatment with dexamethasone substantially reduced total CYP-450 liver content and substantially increased CYP3A activity.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies included oral- and intraperitoneal-administration studies in mice, and oral-, intravenous-, and intraperitoneal-administration studies in the rat. These studies are summarized below in Table 16.

Table 16: Single-Dose Toxicology Studies and LD_{50} Values.

Species	Route	Doses (mg/kg)	LD_{50}
Male and female NMRI mice (5/sex/group)	oral	21.5, 215, 464, 1000	680 mg/kg in male mice 603 mg/kg in female mice
Male CD2F1 mice (2/group)	IP	21.5, 46.4, 100, 215, 464, 1000	46.4 mg/kg
Male and Female Sprague Dawley rats (10/sex/group)	oral	0, 100, 159, 252, 400	Estimated to be 246 mg/kg
F344 rats	oral	100, 215, 464	Estimated to be 316 mg/kg
Sprague Dawley rats	IV	21.5, 46.4, 100	Estimated to be 68.1 mg/kg
	IP	68.1, 100, 147	< 68.1 mg/kg

6.2 Repeat-Dose Toxicity

The Applicant conducted an 8-week toxicology study in Wistar rats and a 13-week toxicology study in Beagle dogs. These studies were reviewed by Dr. Terry Miller in a 6/24/2010 Nonclinical Review submitted to DARRTS. Summary findings from Dr. Miller's reviews are shown below. In addition 52-week toxicology studies were performed in rats and dogs and these studies are reviewed below.

Study Title: 8 week oral repeat dose toxicity study in Wistar rats (dose-finding study). (Study No.: 864494)

Reference ID: 3384901

Key Findings
Significant dose-related clinical signs, clinical pathology, and histopathology occurred at miltefosine doses ≥ 10.0 mg/kg. Target organs included the GI tract, WBC, kidneys, eye and gonads. Because low dose (4.64 mg/kg) animals exhibited alopexia and nasal and conjunctival secretions/red incrustations, a NOAEL value was not determined.

Methods
This GLP-compliant study was initiated on 4/25/1998 and conducted by (b) (4) Male and female Wistar rats (5/sex/group) were administered miltefosine for 8-weeks (or as noted) in daily oral gavage doses of 4.64, 10.0, 21.5/31.6 (dose elevation on Day 39 to 31.6 mg/kg) and 46.4 mg/kg (lethal dose, surviving animals were euthanized on Day 26). A full range of assessments including mortality, clinical signs, hematology, blood chemistry, gross pathology, organ weights, and histopathology were conducted.

Results
Mortality: Mortality occurred for 3/5 males and 1/5 females receiving the high-dose of 46.4 mg/kg in Weeks 3 and 4 and all remaining high-dose animals were euthanized on Day 26.

Clinical Signs: The incidence and severity of clinical signs increased with dose. Low-dose animals receiving 4.64 mg/kg miltefosine exhibited secretions and red incrustations around the nostrils and eyes as well as alopecia. The mid-dose groups (10.0 and 21.5/31.6 mg/kg) demonstrated similar findings, but with increased incidence and severity. At 21.5/31.6 mg/kg red salivation and increased urinary production occurred. High-dose (46.4 mg/kg) animals demonstrated increased severity and incidence of clinical signs noted in the mid-dose groups, and also piloerection, skin pallor, retracted flanks, and prolapsed penis.

Body weight and Food Consumption: High-dose animals demonstrated a significant reduction in body weight and food consumption compared to control animals. At Week 2 and Week 4, mean body weights were reduced approximately 15% and 27-37% respectively compared to controls. Food consumption was also reduced 25-65% for all weeks evaluated (once-weekly measurements), but the effect was most pronounced in early weeks with food consumption trending toward control values in Weeks 3 and 4. Females receiving 21.5/31.6 mg/kg demonstrated an approximate 16-20% reduction in food consumption in Weeks 1 and 2 and a 25% reduction in Week 8.

Hematology: High-dose animals exhibited increased WBC counts in Week 4 with 70 and 132% increased WBC compared to controls in males and females respectively. Other changes in high-dose animals at Week 4 included a significantly increased percentage of segmented neutrophils (↑ 450-500%) and a reduction in the percent lymphocytes (↓ 30%). Male and female animals receiving 21.5/31.6 mg/kg miltefosine demonstrated similar patterns at Week 8 with a 58-70% increase in WBC counts, a

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significant increase of 225-300% in the percentage of segmented neutrophils, and a smaller decline of 13 to 30% in the percentage of lymphocytes compared to controls.

Serum Chemistry: In high-dose animals, liver ASAT and ALAT significantly increased 150 to 200% and 360 to 550% respectively in both genders compared to controls. High-dose males and also demonstrated increased TBIL (↑25%) and BUN levels (↑60%) and females showed increased GGT (↑12%). At Week 8, male and female animals receiving 21.5/31.6 demonstrated similarly high levels of ASAT and ALAT with BUN and creatinine increased by >50% above control values. All mid-dose (21.5/31.6 mg/kg) and high-dose animals demonstrated a dose-dependent decrease in serum phosphate (↓25-70%) possibly as a result of decreased food consumption.

Organ weights: Organ weights were generally decreased compared to controls and historical background data in all high-dose animals at 4 weeks and animals receiving 21.5/31.6 mg/kg miltefosine at 8 weeks. In contrast adrenal and liver weights were increased in both genders in the high- (46.4 mg/kg/day) and mid-dose(21.5/31.6 mg/kg) groups.

Gross Pathology: Findings included reddened and thickened musosa in the stomach and small intestine of some mid- and high-dose animals. Other gross pathology findings occurred in the skin, uterus, prostate, seminal vesicles, spleen, stomach, and intestines. These findings correlated with histopathology detailed below.

Histopathology: Histopathology was multifold in high-dose animals at Week 4 and mid-dose (21.5/31.6mg/kg) animals at Week 8 with some findings in all dose groups. Findings included: retinal atrophy (degeneration and necrosis of the outer granular layer), degeneration and maturation abnormalities of hair follicles (all dose groups), moderate to marked tubular degeneration/necrosis and secondary hyperplasia in testes, prostate and seminal vesicle atrophy, kidney hyperplasia with associated inflammatory cell infiltrate, small uteri associated with an estrus cycle stage of di/anestrus in some mid- and high-dose females, mucosal hypertrophy and hyperplasia in the duodenum and jejunum, moderate to marked reduction of lymphatic tissue (high-dose animals that died), mild to moderate cortical hypertrophy and hyperplasia in adrenals, and slight thymic atrophy in mid-dose animals only. The latter two findings may have been stress related. No significant liver histopathology was observed that clearly correlated with the changes in liver enzymes.

Study Title: 13-week oral repeat dose toxicity study in Beagle dogs with a subsequent 6-week recovery period. (Study No.: 859408).

Key Study Findings

Significant miltefosine-related clinical signs, hematology, and histopathology findings occurred at dose ≥ 3.16 mg/kg in dogs receiving day oral doses for 13 weeks.

- Clinical signs included vomiting and excess salivation.

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- Changes in peripheral WBC (segmented neutrophils and lymphocytes) occurred with miltefosine doses of 10 mg/kg, but bone marrow histopathology was not evident.
- Decreased prostate weights and prostate atrophy was noted in 2/5 males in each of the 3.16 and 10.0 mg/kg dose groups.
- Miltefosine high-dose (10.0 mg/kg) produced local irritation of GI mucosa in 2/5 females.
- The NOAEL was considered to be 1.0 mg/kg/day.

Methods

This GLP-compliant study was initiated on 12/07/1987 by (b) (4) Male and Female Beagle dogs received daily oral gavage doses of 0, 1.0, 3.16, and 10 mg/kg miltefosine (Main Study Groups 1-4 respectively, 4/sex/group) for 13-weeks. Recovery animals consisted of 2/sex/group for groups 1 and 4 that were maintained for an additional 6 weeks without dosing. A full range of assessments including mortality, clinical signs, EEG, hematology, blood chemistry, gross pathology, organ weights, and histopathology were performed on Main Study and Recovery animals.

Results

Mortality and Clinical Signs: No mortality occurred in this study. Groups 2 (3.16 mg/kg) and 4 (10 mg/kg) exhibited vomiting, salivation, diarrhea, and retracted flanks (caused by weight loss). Vomiting occurred 1-2 hours after dosing, but salivation persisted throughout the day. Symptoms decreased in Weeks 3-6 such that Group 3 animals showed little or no treatment related symptoms later in the study.

Body Weights and Food Consumption: Body weight and food consumption did not change substantially during the study.

EEG: ECG, body temperature, and heart rate were not affected by miltefosine treatment.

Hematology: In the high-dose group, mean WBC values nearly doubled in Week 13 in conjunction with a large increase in the mean absolute and relative numbers of segmented neutrophils in both genders (↑ 138-157%) and decreased absolute and relative numbers of lymphocytes (↓ 7-30%) relative to controls. By week 20, mean values for the high-dose group trended closer to control values. Prothrombin time values decreased at some times and doses, but platelets were not affected and clear dose-response was not evident.

Serum Chemistry: The serum chemistry changes that occurred generally did not occur in a dose-dependent manner or at multiple time-points and were generally considered not toxicologically significant. Blood was collected for serum chemistry analysis in Weeks 1, 6, 13 (Main Study), and 19 (Recovery). Cholesterol increased up to 50% in mid-dose males in Weeks 6 and 13, in high-dose males in Week 13, and in low-, mid- and high-dose females in Week 6 only. Serum glucose was lower approximately 10% in

high-dose males compared to controls. Increased serum albumin was noted in mid- and high-dose males and females in Week 13. Alpha-1 globulin was significantly reduced in high-dose males and females it Week 13 compared to controls and in the same animals, alpha-2 globulin was significantly increased in Weeks 6 and 13. Serum chemistry changes did not occur in high-dose recovery animals compared to controls.

Organ Weights: Absolute organ weights were generally unchanged with treatment. However, two males each in the mid- and high-dose miltefosine groups as well as one high-dose recovery male demonstrated decreased prostate weight.

Gross Pathology: The prostate of one high-dose male appeared small, two high-dose females demonstrated reddened and thickened mucosa in the small intestine. Also two high-dose males exhibited reddish abdominal adipose tissue.

Histopathology: Possible miltefosine-related changes in the prostate, small intestine, adipose tissue, liver, and lungs. In each of the mid- and high-dose groups, 2/5 Main Study animals exhibited moderate prostate atrophy. In the recovery group, 1/2 control males and 1/2 high-dose males demonstrated minimal prostate atrophy. Two high-dose males in the Main Study demonstrated atrophy of abdominal adipose tissue probably as a result of weight loss. Two high-dose females in the Main Study exhibited increased congestion in the proximal small intestine correlating with gross pathology findings of reddened mucosa. Liver pathology occurred in all groups, but no treatment-related patterns were evident.

Study title: Miltefosine (D-18506); 52-week oral toxicity study after repeated administration in rats and subsequent 6-week recovery period.

Study no.:	861344
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 10, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Miltefosine (D-18506), Batch # PaX 8303, purity of 98.9%.

Key Study Findings

- Miltefosine-related toxicity included clinical signs (alopecia, swollen testicles, red urine, eye opacities, and distended abdomen), increased reticulocytes and WBCs, numerous clinical chemistry changes, ophthalmology changes (enlarged choriocapillaris vessels, thinned retinal vessels, lens opacities), retinal degeneration, chronic nephropathy, hypertrophy and hyperplasia of GI mucosa, ovaian cysts, uterine mucometra, pyrometra, and metritis/perimetritis and mucosal hyperplasia, mucosal hyperplasia in cervical and vaginal tissue and testicular, epididymidal, and seminial vesicle atrophy.

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- The effects occurred in a dose-dependent manner and all of the changes were at least partially reversed in high-dose Recovery animals except the retinal degeneration, chronic nephropathy, male organ atrophy, and the changes to uterus, cervix, and vagina.
- In conjunction with high-dose administration several benign (Leydig cell adenomas of the testes in 3/30 males and basal cell ademoma in 1/30 females) and malignant (histiocytic sarcoma/metastases in 1/30 females; squamous cell carcinoma in the uterus in a second female and malignant adenoacanthoma of the uterus/metastasis in a third female) neoplasms occurred.
- The NOAEL was considered to be < 4.64 mg/kg/day (low dose) because the ophthalmology findings and retinal degeneration occurred with the low dose.

Methods

Doses:	0, 4.64, 10.0, and 21.5 mg/kg body weight
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	2.15 ml/kg body weight.
Formulation/Vehicle:	Ampuwa®, Aqua bidestillata ad injectabilia
Species/Strain:	Rat/ Bor:WISW (SPF (b) (4)
Number/Sex/Group:	20/sex/group
Age:	Males were 7 weeks old and females were 8 weeks old at the start of dosing.
Weight:	Males weighed 147 to 190 g and females weighed 126 to 159 g at the start of dosing.
Satellite groups:	Recovery animals (10/sex/group) were included in the control and high-dose groups.
Unique study design:	See Table 17. Control animals did not receive the vehicle, but instead received 2.15 ml/kg body weight drinking water by gavage. The treatment animals received daily doses for 52 weeks. Main Study animals were sacrificed 52-weeks after the initiation of dosing (dosing on day of sacrifice), and Recovery animals were euthanized 6-weeks later (Week 58).
Deviation from study protocol:	Yes. Multiple deviations from the study protocol occurred and were noted. However, the deviations were not considered to have altered the results or lessened the integrity of the study.

Table 17: Study Design for Study No.: 861344

	Control Group	Low Dose	Mid Dose	High Dose
Number/group (Main Study)	20/sex/group	20/sex/group	20/sex/group	20/sex/group
Number/group (Recovery)	10/sex/group	0	0	10/sex/group
Dose mg/kg body weight	0	4.64	10.0	21.5

Dose Concentration (mg/ml)	0	2.15	4.64	10.0

Observations and Results

Table 18: Observations Schedule

Parameter	**Schedule**
Mortality	Twice daily for duration of study. Animals found dead were necropsied immediately.
Clinical Signs	Daily for duration of study
Body Weight	Once per week beginning with pre-test period
Food Consumption	Once per week beginning with pre-test period
Reflexes	Pain, pinna, and corneal reflexes were tested once per week.
Hearing and dental examinations	Before first dose and in Weeks 26 and 52. Teeth were checked and hearing was checked with a simple noise test.
Ophthalmological Exams	Before first dose and in Week 58 for Groups 1 and 4 and in Weeks 13/14, 23, 39, and 52 for all animals.
Clinical Pathology blood collection	In Weeks 13, 26, and 52 as well as from Recovery animals in Week 58.
Urine collection	In Weeks 12, 25, and 50 from all animals, in Week 57 for recovery animals.
Toxicokinetics	Blood collection in Week 52, 4-5 hours after dosing.
Necropsy	At the end of the treatment period (Week 52) and the Recovery period (Week 58).

Mortality

Several animals were found dead with more in the treatment groups compared to the control with a rough pattern of dose-dependency (Table 19).

Table 19: Group Incidence of Mortality

Group	**Found Dead**		**Sacrificed in Extremis**	
	Male	**Female**	**Male**	**Female**
Group 1(control)				1(R)
Group 2-Low dose	2(MS)	1(MS)		1(MS)
Group 3-mid dose	3(MS)		2(MS)	
Group 4-high dose	3 (MS), 3(R)	2 (MS)	3(MS), 1(R)	2 (MS), 2(R)
MS = Main Study; R = Recovery				

Clinical Signs

Animals in all groups exhibited alopecia, but the incidence and severity increased with increasing miltefosine dose. The alopecia was thought to be caused by the animals pulling their own fur, so the alopecia may represent an anxiety reaction. Mid-dose and high-dose males exhibited reddish or blackish scrotums midway through the study and later swollen testicles and red urine. Individual females demonstrated reddish

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incrustation of their noses and head tilt. Other clinical signs apparently associated with miltefosine administration in the mid-and high-dose groups included: loss of cleaning behavior, piloerection, pale skin, eye opacities, inflamed eyelids, and distended abdomen.

Body Weights

Body weight gain for male animals decreased in a miltefosine dose-dependent fashion compared to controls. The decreased weight gain was significantly different from controls from Week 11 onward for the mid-dose group and from Week 1 onward for the high-dose group. Body weights for high-dose males in the Recovery Study were also reduced.

In contrast, body weights in Main Study female animals did not change for any of the miltefosine groups relative to controls. Body weights for high-dose females in the Recovery Study were reduced about 10% relative to controls.

Feed Consumption

Mid-dose males and females demonstrated a trend toward increased food consumption with significant differences relative to control animals for many weeks after Week 6. Males and females in the high-dose group initially consumed less food than controls, then following the change in diet from hard pellet to powder in Week 5 began to consume more food than controls.

Ophthalmoscopy

Animals were assessed for ophthalmic effects by slit lamp examination and ophthalmoscopy with Fundus Camera RC2 after generation of mydriasis.

Pronounced ophthalmology changes which developed progressively with dosing were noted in all miltefosine-treatment groups. In the low-dose group, eye findings were first noted at Week 23 including enlarged vessels of the choriocapillaris, thinned retinal vessels, and increased fundus reflex. Lens opaciites were also noted in Week 39. In Week 52, miltefosine-related findings consisting mainly of slight to diffuse lens opacity, and increased reflection of the eye fundus were noted in nearly all male and about two thirds of the female rats.

Eye findings were noted in mid- and high-dose animals by Week 13/14 including red coloration and enlarged vessels of the choriocapillaris, thinned retinal vessels, and lens opacities. The incidence was greater in the high-dose group which also demonstrated increased reflection of the fundus. All mid- and high-dose animals were afflicted with one or more eye findings by Week 23 when the incidence and the severity of findings was notably increased. The incidence and severity of the ocular changes increased progressively with more and worse findings at Week 39 and again at Week 52. After the 6-week recovery period (Week 58 examination), severe eye changes were still present in high-dose rats.

ECG: Not performed

Hematology

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Blood was collected from the retro-orbital venous plexus of one eye under anesthesia for all groups. The hematology parameters shown in Table 46 were measured.

Several hematology changes occurred in a miltefosine-related and dose-dependent manner. Changes which occurred to a significant degree in the mid-dose males and females included: decreased red blood cell parameters (RBC, hemoglobin, hematocrit) in Weeks 26 and 52. In addition, mid-dose females demonstrated mildly increased reticulocytes in Week 13 and slightly increased platelets in Weeks 13, 26, and 52.

High-dose animals of both genders exhibited hematology parameter changes that encompassed the changes observed in mid-dose but beginning earlier in the dosing cycle. Reticulocytes were significantly increased in a 2/21 males in Week 13, 4/19 males in Week 26, and in 14/22 females in Week 13. White blood cells, particularly neutrophils but also lymphocytes were increased in Weeks 13, 26, and 52 in high-dose animals of both genders.

In females all hematology changes returned to control levels during the Recovery period (Week 58), but in males RBCs and platelets remained slightly increased and MCV and MCH were slightly decreased in Recovery.

Clinical Chemistry

The clinical chemistry parameters shown in Table 47 were measured.

Dose-dependent clinical chemistry changes were consistent with renal and hepatic effects. Parameter changes in the low-dose group were generally within the physiological range and not considered toxicologically significant. Many parameters were altered in the mid-dose and high-dose groups with a qualitatively similar pattern but to a higher degree or extent in the high-dose group (Table 20). Most of the serum chemistry changes returned to control levels during Recovery with the exception of total protein (females), BUN (males), triglycerides (males), albumin (females), and α1, α2, and β-globulins (males). Renal histopathology in the form of chronic nephropathy correlated with the serum chemistry changes in renal-associated parameters, but liver histopathology correlates were absent.

Table 20: Serum Chemistry Changes in the 52 Week Rat Toxicology Study

	Mid-dose (10 mg/kg)	High-dose (21.5 mg/kg)
CK		Moderate increase in both sexes in Weeks 13, 26, and 52
ASAT	Mild increase in Weeks 13, 26, and 52.	Moderate to severe increase in both sexes in Weeks 13, 26, and 52
ALAT	Mild increase in Weeks 26, and 52.	Moderate to severe increase in both sexes in Weeks 13, 26, and 52
AKP	Increased in females in Week 52	Slight to moderate increase in both sexes in Weeks 13, 26, and 52
Gamma GT	Slightly decreased in Week 13 in females	Slight to moderate increase in males in Weeks 13, 26, and 52

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Inorganic Phosphate	Slightly decreased in males in Week 13	Slight increase in both sexes in Weeks 13 and 26 and in males in Weeks 52 and 58
Total Protein		Slight to moderate decrease in both sexes in Weeks 13, 26, 52 and 58 (females)
BUN	Moderately increased in males and slightly increased in females in Weeks 13, 26, and 52.	Moderate increase in both sexes in Weeks 13, 26, 52 and 58 (males)
Creatinine	Slightly increased in Week 13 (females) and Week 26 (males)	Slight increase in both sexes in Week 13 and in males in Weeks 26, 52, and 58
Chloride		Slight increase in males in Weeks 13, 26, and 52
Calcium	Increased in males in Weeks 13 and 26	Slight increase in males in Weeks 13 and 26.
Triglycerides	Slightly decreased in Week 13, and 52 (both sexes) and Week 26 (females)	Moderate to slight decrease in triglycerides in both sexes in Weeks 13, 26, and 52 as well as in males in Week 58.
Serum Albumin	Slightly decreased in females in Weeks 13, 26, and 52	Slight (males) and moderate (females) in Weeks 13 (females only) 26, 52 and 58 (females only)
α1-globulins, α2-globulins, β-globulins	Slight to moderate decreases and increases at several time-points in males and/or females	Slight to moderate decrease in both sexes in Weeks 13, 26, and 52 as well as males in Week 58
CK = creatinine kinase; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; AKP = alkaline phosphatase; Gamma GT = gamma-glutamyl transferase; BUN = blood urea nitrogen		

Urinalysis

Each urinalysis animal was pretreated with tap water (10 ml/kg BW) before sampling. Each urinalysis included measurement of bilirubin, glucose, hemoglovin/erythrocytes, ketones, leucocytes, nitrite, osmolality, pH, protein, and urobilinogen. Microscopic examinations of urine sediment was performed in animals that showed pathological changes in hemoglobin/erythrocytes, leucocytes, or protein.

Miltefosine-related changes in urinalysis parameters occurred predominately in the high-dose group. Urinalysis changes included: slight to moderately increased leucocytes, protein, and hemoglobin/erythrocytes in high-dose males and females. In addition, in low–dose males, urine protein was increased, and urine protein and hemoglobin/erythrocyte values were increased compared to controls in the mid-dose group. Also erythrocytes were increased in mid- and high-dose males and high-dose females. These changes correlated with a finding of chronic nephropathy.

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Gross Pathology

All animals including those found dead or sacrificed for humane reasons were examined for external and internal gross pathology.

Miltefosine-related gross pathology was noted in a number of organs including the kidney, urinary bladder, skin, GI track, and reproductive organs in both genders. The extensive gross pathology corresponded to histopathology which is summarized in detail below.

Organ Weights

The organ listed in Table 48 were weighed after sacrifice and trimming. Absolute organ weight values and organ weights relative to body weight were determined. Organs from animals found dead or sacrificed for humane reasons were not weighed.

Dose-dependent changes in absolute organ weights were observed for both genders (Table 21). In high-dose males many absolute organ weights were decreased including weights for brain, thymus, heart, liver, testes, and prostate/seminal vesicles. However, high-dose males in the Main study lost a great deal of body weight (32% weight loss compared to controls), and relative organ weights were often increased suggesting that reductions in absolute organ weights were often the result of overall body weight loss. The organs that had decreased relative weights in high-dose males from the Main study were testes (also mid-dose males), prostate/seminal vesicles, and thymus.

Unlike males, high-dose females in the Main Study did not lose significant body weight compared to control females. In these females, many absolute and relative organ weights were increased including for liver, spleen, adrenals, and ovaries, but the relative weights for pituitary and thymus were decreased.

Both male and female high-dose animals from the Recovery study lost body weight (approximately 30% for males and 10% for females) relative to control animals. In Recovery high-dose males the only relative weights that decreased were those for testes and prostate/seminal vesicles. In Recovery high-dose females, the only organ with a decreased relative weight was the thymus.

Table 21: Absolute and Relative Organ Weight Changes in Main Study and Recovery Animals.

Organ	Group 2 (4.64 mg/kg/day)		Group 3 (10.0 mg/kg/day)		Group 4 (21.5 mg/kg/day)		Group 4 (Recovery	
	Male	Female	Male	Female	Male	Female	Male	Female
Body Weight			↓		↓		↓	↓
Brain (Absolute)			↓		↓	↓	↓	
Brain (Relative)					↑		↑	↑
Heart (Absolute)	↓		↓		↓			
Heart (Relative)						↑	↑	↑
Pituitary (Absolute)								
Pituitary (Relative)					↑			
Liver (Absolute)					↓	↑	↓	
Liver (Relative)					↑	↑	↑	↑

Thymus (Absolute)					↓	↓	↓	↓
Thymus (Relative)						↓		↓
Kidney (L) (Absolute)				↑		↑		↑
Kidney (L) (Relative)			↑	↑	↑	↑	↑	↑
Kidney (R) (Absolute)				↑		↑		↑
Kidney (R) (Relative)			↑	↑	↑	↑	↑	↑
Adrenal (L) (Absolute)				↑	↓	↑		
Adrenal (L) (Relative)					↑		↑	↑
Adrenal (R) (Absolute)				↑	↓	↑		
Adrenal (R) (Relative)				↑	↑	↑		
Spleen (Absolute)						↑		
Spleen (Relative)					↑	↑	↑	
Testis (L) (Absolute)					↓		↓	
Testis (L) (Relative)			↓		↓		↓	
Testis (R) (Absolute)					↓		↓	
Testis (R) (Relative)			↓		↓		↓	
Prostate/Seminal Vesicle (Absolute)					↓		↓	
Prostate/Seminal Vesicle (Relative)					↓			
Ovary (L) Absolute						↑		
Ovary (L) Relative						↑		
Ovary (R) Absolute								
Ovary (R) Relative								
↓: Significantly decreased relative to the respective control group. ↑: Significantly increased relative to the respective control group.								

The absolute and relative weight changes for male reproductive organs for the Main Study and Recovery Study animals are shown below in Table 22 and Table 23 respectively.

Table 22: Absolute and Relative Reproductive Organ Weight Changes in Main Study Males in the 52-Week Toxicology Study

	Absolute Weight			**Relative Weight**		
	Left Testis	**Right Testis**	**Prostate/ Seminal Vesicle**	**Left Testis**	**Right Testis**	**Prostate/ Seminal Vesicle**
Group 1 (Control) (N = 20)	2.96 ± 0.34	2.96 ± 0.30	3.47 ± 0.39	0.68 ± 0.09	0.68 ± 0.09	0.80 ± 0.11
Group 2 (4.64 mg/kg) (N = 18)	2.78 ± 0.32	2.61 ± 0.52 *	3.20 ± 0.49	0.68 ± 0.08	0.64 ± 0.13	0.77 ± 0.08
Group 3 (10.0 mg/kg) (N = 11 to 15)	2.27 ± 0.33 **	2.30 ± 0.29 **	2.88 ± 0.39 **	0.58 ± 0.04 **	0.59 ± 0.05 *	0.74 ± 0.09
Group 4 (21.5 mg/kg) (n =13 or 14)	0.87 ± 0.15 **	0.88 ± 0.22 **	1.78 ± 0.67 **	0.30 ± 0.05 **	0.30 ± 0.06 **	0.39 ± 0.18 **
*: $p \leq 0.05$; **: $p \leq 0.01$ compared to the control group						

Table 23: Absolute and Relative Reproductive Organ Weight Changes in Recovery Study Males in the 52-Week Toxicology Study

	Absolute Weight			Relative Weight		
	Left Testis	**Right Testis**	**Prostate**	**Left Testis**	**Right Testis**	**Prostate**
Group 1 (Control) (n = 10)	3.02 ± 0.39	3.05 ± 0.37	3.26 ± 0.30	0.71 ± 0.11	0.71 ± 0.10	0.76 ± 0.08
Group 4 (10.0 mg/kg) (n = 7)	1.21 ± 0.38 **	1.23 ± 0.39 **	2.21 ± 0.48 **	0.40 ± 0.12 **	0.40 ± 0.12 **	0.72 ± 0.14
*: p ≤ 0.05; **: p ≤ 0.01 compared to the control group						

Histopathology

Adequate Battery
Yes; the organs and tissues noted in Table 48 were assessed for histopathology. For Groups 1 and 4 Main Study animals, and all animals found dead or euthanized for humane reasons, all of the listed organs and tissues were assessed. In addition, all organs and tissues with gross lesions from Groups 2 and 3 as well as the liver, kidney and lungs were assessed (5 μM sections, H and E staining). The liver and kidney from Groups 1 and 4 Recovery animals were also assessed. Additional slides from the adrenal glands, cervix, duodenum, epididymides, eyes with optic nerve, femur, ileum, jejunum, ovaries, pituitary gland, prostate, seminal vesicles, skin, spleen, sternum, stomach, testes, thymus, uterus and vagina were prepared from Groups 2 and 3 animals as well as Recovery animals. Bone marrow smears from all animals sacrificed due to humane reasons and all Main Study animals in Groups 1, 3, and 4 were examined.
Peer Review: No
Histological Findings
Kidneys: Severe kidney changes were present in Groups 3 and 4 with a dose relationship in incidence and severity. Histopathology included: necrosis of the papilla/medulla with secondary sclerosis, focal hyperplasia of proximal/collecting tubules, focal mononuclear cell infiltrations in connection with focal chronic interstitial nephritis and chronic nephropathy. After the 6-week recovery period all chronic changes were still present.

Eyes: Severe changes were present in the eyes of all miltefosine-treated animals. Low-dose animals (5/18 males, 9/19 females) and all mid- and high-dose animals exhibited a diffuse degeneration of the retina with complete loss of photoreceptors in the outer nuclear layer. Lens changes in the form of opacities, cataracts, homogenization of the lens nucleus, and swelling, vacuolization, and degradation of the lens fibers also occurred in a dose-dependent manner for incidence. All of the ocular histopathology persisted after 6-weeks recovery.

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Urinary bladder: a moderate to marked hyperplasia of the transitional epithelium was present in 15% of high-dose males from the Main Study.

Thymus: A higher incidence of involution occurred in low-, mid-, and high-dose males. This effect may have been related to stress. An increased incidence of thymic involution was not noted in high-dose recovery animals.

Lymph Nodes: Lymphoid atrophy occurred 10-20% of the axillary and mesenteric lymph nodes in high-dose males and females. Lymphoid atrophy persisted in Recovery animals.

Stomach: Minimal to moderate hyperplasia of chief cells in the stomach occurred in a miltefosine dose-dependent manner with an incidence of 60-80% in high-dose males and females. The incidence of hyperplasia of chief cells was reduced after the Recovery period.

Duodenum, Jejunum, Ileum: Mid- and high-dose animals demonstrated hypertrophy and hyperplasia of the mucosa with thickening and elongation of the villi in a dose-dependent manner for severity. The effect was most pronounced in the duodenum. These effects did not reverse during Recovery.

Testes: Nearly complete treatment-related atrophy of the testes occurred in mid- and high-dose males. In the high-dose males atrophy occurred with focal mineralization and diffuse or focal hyperplasia of Leydig cells. Reduced spermatogenesis and testicular atrophy was also noted in 5/20 male rats in the low-dose group. After the Recovery period some reproliferation of spermatogenic cells was noted in tubules that were not completely destroyed, but testes remained atrophied in high-dose males.

Epididymides: Epididymides were atrophied in 15% of low-dose, 60% of mid-dose, and 95% of high-dose males. Also the incidence of spermatogenic granulomas was much increased in mid-dose (15/20) and high-dose (7/20) males. These changes were also observed in high-dose Recovery males

Prostate and Seminal Vesicles: Prostate atrophy was noted for 17/20 high-dose animals respectively. Atrophic seminal vesicles were noted in 10% of low-dose, 30% of mid-dose, and 100% of high-dose males (compared to 5% of vehicle control males). These changes were also observed in high-dose Recovery males.

Ovaries/Uterus/Cervix and Vagina: The incidence of ovarian cysts inclusive of parovarian cysts was increased in mid- and high-dose females. Some high-dose females exhibited uterine mucometra, pyrometra, and metritis/perimetritis and mucosal hyperplasia. Also 10% of high-dose females had mucosal hyperplasia in cervical and vaginal tissue. The ovarian changes were largely reversed in Recovery females, but the changes in uterus, cervix and vagina were still observed.

Skin: Almost all mid- and high-dose males and females showed impaired hair growth and alopecia as well as 30% of low-dose females. The severity was dose-dependent

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with massive effects for high-dose animals. Impaire hair growth still persisted in high-dose Recovery animals

Mammary Gland: Most males in all groups experienced proliferation of mammary glands. In the affected males in the mid and high-dose groups a high percentage also exhibited atrophy of the proliferated glands. These changes persisted in Recovey animals.

Adrenal Glands: Mid- and high-dose males and females exhibited hypertrophy/hyperplasia of zona fasciculate/reticularis of the adrenal glands. High-dose males in the Recovery study no longer demonstrated this pathology, but it was still observed in 70% of Recovery females.

Pituitary Glands: Hyperplasia and hypertrophy of pars distalis cells was observed in a high percentage of mid- and high-dose males and 15% of mid-dose females. In males this effect was accompanied by testicular atrophy. The pituitary hyperplasia/hypertrophy persisted after Recovery.

Bone Marrow: Hypercellularity and an increased ratio of myeloid to erythroid cells occurred in a dose-dependent manner for incidence in mid- and high-dose animals of both genders. Hypercellularity still occurred at a higher incidence in high-dose Recovery animals compared to control animals, but the incidence was reduced compared to Main Study animals.

Neoplastic Lesions: Several neoplastic lesions were observed in animals in different Main Study dose groups including control animals (Table 24). Because of their occurrence in control animals, normal background incidence, and/or a lack of dose-response these mostly benign neoplastic lesions were considered spontaneous.

Table 24: Neoplastic Lesions Considered to be Spontaneous.

	Control	Low-dose	Mid-dose	High-dose
Axillary Lymph Node Lymphoma		1 female		
Theca-granulosa cell tumor			1 female	
Uterine papilloma	1 female	2 females	1 female	
Adenoma/fibroadenoma of mammary gland		1 female	1 female	
Pituitary gland adenoma	1 male 2 females	1 female		
Thyroid C-cell adenoma				1 female

Benign neoplastic lesions considered to be related to miltefosine administration included Leydig cell adenomas of the testes in 3/30 Group 4 males and a basal cell adenoma in 1/30 Group 4 females. Malignant neoplasms considered to be related to miltefosine administration included: a histiocytic sarcoma with widespread multi-organ metastases in one high-dose female, a squamous cell carcinoma in the uterus of another high-dose

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female, and in a third high-dose female, a malignant adenoacanthoma of the uterus with widespread metastasis.

Special Evaluation

Reflexes: No miltefosine-related changes were noted for pain, pinna or corneal reflexes.

Hearing and Dental Examinations: No miltefosine-related changes in hearing or dentition were noted.

Toxicokinetics

Blood samples were collected for toxicokinetic analysis from five animals of each group and sex. Miltefosine plasma concentrations were measured with a validated HPTLC method.

At the single measurement time-point, miltefosine plasma concentrations were as noted in Table 25 below. Based on these data, the plasma concentrations appear to increase in a roughly dose-dependent manner. The Study Report indicates that no significant differences were observed between males and females.

Table 25: Plasma Miltefosine Concentrations for Rats in the 52-Week Toxicology Study.

Group	Dose (mg/kg)	Plasma Concentration* nmol/ml
Low-Dose Group	4.64	61.00 ± 11.60
Mid-Dose Group	10.0	128.49 ± 19.41
High-Dose Group	21.5	203.96 ± 38.97
*Mean value from 10 animals (5 males plus 5 females) ± standard deviation.		

Dosing Solution Analysis

The stability of the miltefosine batch was examined every three months by the Applicant and found to be stable until at least Oct. 31, 1990. Dosing solutions were prepared fresh each day and not tested for stability. The concentrations of the dosing solutions were determined in Weeks 2, 26, and 52. According to the study report, all of the actual concentrations were within 7% of the nominal concentrations.

Study title: D-18506 (Miltefosine): 52-week oral toxicity study after repeated-administration in Beagle dogs and subsequent 6-week recovery period

Study no.: 873450
Study report location: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: Oct. 31, 1989
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: D-18506 (miltefosine), batch # E x 2136,

Reference ID: 3384901

purity of 99.31%

Key Study Findings

- Miltefosine-related toxicity primarily included clinical signs (salivation, vomiting, diarrhea, alopexia and emaciation), slightly reduced prothrombin time, male reproductive organ effects (focal mononuclear infiltration and dilatation in the prostate) and dose-dependent increases in the incidence and severity of follicular atresia and sexual cycle arrest in females.
- No retinal degeneration took place and the changes to male reproductive organs were less pronounced compared to rats.
- Follicular atresia was increased in all miltefosine dose groups compared tgo control animals, but sexual cycle arrest and follicular atresia returned to normal in high-dose Recovery females.

Methods

Doses:	0, 1, 3.16, and 10 mg/kg. The 10 mg/kg dose was reduced to 7.50 mg/kg in Week 3, 6.81 mg/kg in Week 5, and 6.19 mg/kg in Week 7 due to gastrointestinal disturbances associated with the high dose.
Frequency of dosing:	Once daily
Route of administration:	Oral gelatin capsule
Dose volume:	None
Formulation/Vehicle:	The negative control group received plain gelatin capsules
Species/Strain:	Beagle dog/ (b) (4)
Number/Sex/Group:	6/sex/group for Groups 1 and 4; 4/sex/group for Groups 2 and 3. In Groups 1 and 4, 2/6 animals/sex were used as Recovery animals.
Age:	6 months (males and females)
Weight:	Males: 10.2 to 15.0 kg; females: 9.2 to 12.9 kg
Satellite groups:	2/sex were used as Recovery animals in Groups 1 and 4.
Unique study design:	Miltefosine was administered in oral capules once daily for 52 weeks for Main Study animals. Recovery animals were maintained for an additional treatment-free 6-week period.
Deviation from study protocol:	Several deviations from the study protocol were noted, but the deviations were not considered to have altered the results or integrity of the study.

Observations and Results

Table 26: Observation Schedule for the 52-Week Dog Toxicology Study

Parameter	Schedule
Mortality	Twice daily for duration of study
Clinical Signs	Daily for duration of study

Body Weight	Once per week beginning with pre-test period
Food Consumption	Daily for duration of study and documented weekly
Reflexes	Pain, pupil, corneal, and patellar reflexes were tested once per week.
ECG	Before first administration and in Weeks 13, 26, and 52 before dosing. Also in Week 59 for Recovery animals
Heart Rate/body temperature	Once per week from pretest to Week 3 then every two weeks thereafter
Hearing and dental examinations	Before first dose and in Weeks 26, 52, and 58.
Ophthalmological Exams	Before first dose and in Weeks 39 and 51 from all animals and in Weeks 13 and 26 for Group 1 and 4 animals.
Clinical Pathology blood collection	Before first dose and in Weeks 4, 13, 26, and 52 as well as from Recovery animals in Week 59. Animals were fasted for approximately 16 hours before collection.
Urine collection	In Week 53 from all animals, in Week 59 for Recovery animals.
Toxicokinetics	Blood collection during Weeks 11 and 52 before dosing and 2, 5, and 8 hours after dosing.
Necropsy	At the end of the treatment period (Week 53) and the Recovery period (Week 59); animals were fasted overnight.

Mortality

No miltefosine-related mortality occurred during the study.

Clinical Signs

There was a dose-dependent increase in the incidence and severity of alopecia and vomiting and diarrhea including yellow feces and bloody feces. Widespread alopecia occurred in 35% of high-dose males. Additional clinical signs occurring in high-dose animals of both genders included hypokinesia, adhesion of eye lids, transient opacity of the cornea (females) and emaciation. All of the clinical signs except alopecia were fully reversible in the Recovery period.

Body Weights

High-dose males had a trend toward weight loss compared to control animals but the reduction was not statistically significant.

Feed Consumption

In individual high-dose males, intermittent periods of inappetance occurred and the high-dose was decreased in these animals until food intake normalized. During the Recovery period, food intake for the high-dose group was increased compared to controls.

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Ophthalmoscopy/Reflex Testing/Examinations of Hearing and Teeth

Miltefosine had no effect on pain, pinna, and corneal reflexes and no effect on hearing (response to loud noise) or teeth. Also no ophthalmoscopy changes considered related to miltefosine were noted.

ECG/Heart Rate/Body Temperature

Some changes in heart rate (reduced slightly in high-dose females), and body temperature (mild increases and decreases in high-dose animals of both genders) were within physiological ranges and not considered toxicologically significant. Generally electrocardiogram parameters did not change with miltefosine treatment at any measured time-point.

Hematology

The measured hematology parameters are shown in Table 46.

Most hematological parameters were not substantially or consistently altered by miltefosine treatment and the changes that did occur were of minor toxicological significance. In high-dose males, prothrombin time was slightly but significantly reduced in Weeks 4, 13, 26, and 52. A similar trend also occurred for high-dose females, but the differences from the control group were significant only in Week 4. Other hematological changes noted for high-dose males included increased RBC, and decreased MCH (also in Week 26) and MCHC in Week 4. MCHC was also reduced in mid-dose males in Week 4. In high-dose females a mild decrease of MCV was observed in Week 26 and eosinophils were decreased in Week 52.

Clinical Chemistry

The measured serum chemistry parameters are shown in Table 47.

Some slight but significant clinical chemistry changes were observed. However, often the changes were marginal, occurred at irregular time-points and/or did not occur in a dose-dependent manner. In general, the serum chemistry changes indicate a limited effect on liver enzymes by miltefosine treatment.

Low dose males had increased gamma glutamyltransferase (γ-GT) values in Weeks 4 and 13, and marginally increased creatinine kinase (Week 26 only) and total bilirubin (Week 52 only). Low-dose females also exhibited increased γ-GT values in Weeks 13, 26, and 52.

More changed parameters were apparent in the mid-dose group. For males, increased values were observed for γ-GT (Weeks 4, 13, and 26), total protein (Weeks 4, 13, 26, and 52), albumin (Weeks 26 and 52), glucose (Week 26), chloride (Week 13) and aspartate aminotransferase (ASAT) was decreased in Week 26. Mid-dose females had decreased values for ASAT in Week 4, γ-GT in Week 13, and calcium in Weeks 13 and 26.

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High-dose animals did not always present with changes consistent with the changes seen in the lower dose groups. For high-dose males, increased values were observed for glucose (Weeks 4, 13, and 26), creatinine (Week 4), cholesterol (Weeks 4, 26, and 52), and chloride (Weeks 4 and 13). Other parameters were decreased in males including ASAT (Weeks 26 and 52), alanine aminotransferase (ALAT; Weeks 4 and 26), glutamate dehydrogenase (GLDH, in Weeks 13 and 26), calcium (Week 26) and alkaline phosphatase (AKP; Week 4). In high-dose females, increased values were observed for cholesterol (Weeks 4 and 26), total bilirubin (Week 13), and inorganic phosphate (Week 52), and ASAT (Weeks 4, 13, and 26), AKP (Week 4), GLDH (Week 4), creatinine kinase (Week 13), and calcium (Weeks 4, 13, and 26) were decreased.

Urinalysis

Urinalysis consisted of measurement of bilirubin, glucose, hemoglobin/erythrocytes, ketones, leukocytes, nitrite, osmolality, pH-value, protein, and urobilinogen.

No mitefosine-related changes in any urinalysis parameters were observed.

Gross Pathology

All animals received a full gross necropsy examination of external body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents.

Gross pathology findings were limited. Reportedly, mid- and high-dose dogs of both genders exhibited areas of hairloss or thinned haircoat. A few high-dose females also had reddened intestinal mucosa.

Organ Weights

The organs that were weighed are shown in Table 48. Weights were expressed as absolute values and relative (to body weight) values.

With a few exceptions, absolute and relative organ weights were largely unaffected by miltefosine treatment. Absolute pituitary weights in high-dose males and absolute thyroid weight in high-dose females were slightly decreased. These findings had no histological correlates. Trends that did not reach significance included a slight to moderate reduction in the absolute prostate weights in mid- and high-dose males in Weeks 53 (Main Study necropsy) and 59 (Recovery necropsy), and a moderate reduction of testes weights in high-dose males in Week 59 (results based on 2 males/Recovery group). High-dose females had non-significant reductions in pituitary and ovary weights in Week 53, but weights for these organs were not reduced in Week 59. The male reproductive organ weights for Main Study and Recovery Study animals are shown below in Table 27 and Table 28 respectively.

Table 27: Male Reproductive Organ Weights (Absolute and Relative) for Main Study Animals Following 52 weeks of Dosing With Miltefosine in Dogs

	Absolute Weight			Relative Weight		
	Left Testis	Right Testis	Prostate	Left Testis	Right Testis	Prostate
Group 1	12.7 ± 5.5	13.5 ± 3.6	8.8 ± 3.0	0.08 ± 0.3	0.09 ± 0.01	0.06 ± 0.01

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(Control) (n = 4)						
Group 2 1.0 mg/kg) (n = 4)	17.1 ± 1.2	16.5 ± 0.2	8.0 ± 2.6	0.11 ± 0.02	0.11 ± 0.01	0.05 ± 0.01
Group 3 (3.16 mg/kg) (n = 4)	13.7 ± 2.6	14.1 ± 2.8	7.5 ± 1.2	0.09 ± 0.02	0.09 ± 0.02	0.05 ± 0.01
Group 4 (10.0 mg/kg) (n = 4)	12.4 ± 3.6	13.1 ± 3.4	7.6 ± 1.3	0.09 ± 0.01	0.09 ± 0.01	0.05 ± 0.01

Table 28: Male Reproductive Organ Weights (Absolute and Relative) for Recovery Animals Following 52 weeks of Dosing With Miltefosine

	Absolute Weight			**Relative Weight**		
	Left Testis	**Right Testis**	**Prostate**	**Left Testis**	**Right Testis**	**Prostate**
Group 1 (Control) (n = 2)	16.6, 16.6	16.4, 17.1	8.9, 9.9	0.08, 0.11	0.08, 0.11	0.05, 0.06
Group 4 (10.0 mg/kg) (n = 2)	6.6, 12.1	7.2, 14.1	4.7, 6.4	0.04, 0.07	0.05, 0.08	0.03, 0.04

Histopathology

Adequate Battery: Yes; the organs and tissues examined for histopathology are shown in Table 48.
Peer Review: No
Histological Findings

Microscopic examinations indicated that primary target organs for histopathology were within the genital tract and the skin. Female dogs had a dose-related increase in the incidence and severity of atretic follicles in the ovary with follicular development arrested at the secondary stage (Table 29). The arrest in development was also seen in the cycle stage of the uterus, vagina, and mammary gland with morphology compatible with anestrus or diestrus. Within the miltefosine treatment groups, 1/4, 3/4, and 3/4 animals in Groups 2, 3, and 4 respectively were affected. The absence of cyclic activity and the large number of atretic follicles returned to normal in the Recovery period. Miltefosine-treated males had a higher incidence of focal dilatation of the prostate compared to control males. Also high-dose males (1/4 in the Main Study and 1/2 in the Recovery Study) exhibited multifocal atrophy and degeneration of seminiferous tubules which was always associated with focal mononuclear infiltrates (Table 30, Table 31). Although the results suggest a treatment effect, the association with miltefosine treatment for this latter finding is not absolutely clear because similar findings are occasionally seen in control dogs. Reportedly no histopathology was found to associate with the alopecia or reddened intestinal mucosa, and no ocular histopathology was noted.

Table 29: The Incidence of Female Reproductive Organ Histopathology for Main Study Animals Following 52 weeks of Dosing With Miltefosine

	Control (n = 4)	1 mg/kg (n = 4)	3.16 mg/kg (n = 4)	10 mg/kg (n = 4)
Ovaries				
Atretic follicles	4 (1.25)	4 (2.0)	4 (2.5)	4 (3.5)
Corpera Lutea	3	2	1	
Inclusion cyst(s)	1			
Vagina/Cervix				
Proestrus/estrus	1	1		1
Metestrus	3	2	1	
Anestrus/diestrus		1	3	3
Mammary Gland				
Hyperplasia, focal		1 female		
Juvenile morphology				1 female
Dermal hyperplasia		1 male		
The average severity grade of follicular atresia is shown in parentheses.				

Table 30: The Incidence of Male Reproductive Organ Histopathology for Main Study Animals Following 52 weeks of Dosing With Miltefosine

	Control (n = 4)	1 mg/kg (n = 4)	3.16 mg/kg (n = 4)	10 mg/kg (n = 4)
Testes				
Atrophy	1			1
Hyperpl. Leydig cells	1			
Mononuclear Infiltrate, focal				1
Epididymides				
Aspermatozoaic	1			1
Arteritis, focal				1
Sperm granuloma(s)			1	1
Increased Intraepith. cyst				1
Monoclonal infiltrate, focal				1
Prostate				
Mononuclear infiltrate, focal		1	1	2
Dilatat. glands, focal	1	3	3	4

Table 31: The Incidence of Male Reproductive Organ Histopathology for Recovery Animals Following 52 weeks of Dosing With Miltefosine.

Reproductive Organs	Control (n = 2)	10 mg/kg (n = 2)
Testes		
Atrophy		2
Mononuclear Infiltrate, focal		2
Epididymides		
Aspermatozoaic		1
Arteritis, focal	1	
Mononucl. Infiltr. foc		1

Prostate		
Mononuclear infiltrate, focal		1
Dilatat glands, focal	1	2

Special Evaluation

An electron microsopic examination of the right eyes of 2 animals/sex/group from Groups 1 and 4 was performed.

No changes in retinal structures were observed.

Toxicokinetics

The toxicokinetic results indicate that miltefosine plasma concentrations at each dose did not differ significantly between genders. Absorption into plasma circulation after oral administration was relatively slow with estimated T_{max} values of 5 to 8 hours. C_{max} and AUC values were not calculated.

Table 32: Plasma Miltefosine Levels in the 52-Week Dog Toxicology Study.
(Applicant's Table)

Dose (mg/kg)	Sampling time (h)	Miltefosine plasma concentration* (nmol/ml)
3.16	0	51.47 ± 10.88
	2	55.62 ± 4.13
	5	49.69 ± 10.30
	8	99.55 ± 35.25
6.19	0	84.98 ± 16.17
	2	120.70 ± 43.57
	5	149.31 ± 50.93
	8	123.03 ± 14.15

* Mean values from 6 (3m, 3f) animals ± standard deviation

Dosing Solution Analysis

The dosing solutions were prepared daily and gelatin capsules were filled with the intended dose based on the last determined body weight.The dosing solutions were not analyzed for concentration. The Applicant indicated that the miltefosine solutions should be stable throughout the test period. A stability report is provided, but it is written in German.

7 Genetic Toxicology

Three genotoxicity studies were previously reviewed by Terry Miller, Ph.D. in the submission to DARRTS. The three studies (Ames Study, *in vitro* micronucleus assay, in vivo micronucleus test) are summarized below based on Dr. Miller's evaluation. In addition two other studies, a mammalian cell gene mutation test and an *in vivo/in vitro* unscheduled DNA synthesis assay are reviewed below.

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

Study Title: Investigations into the mutagenic effect of HPC on Salmonella Typhimurium (Study Report No.: 3000514563)

Summary: This non-GLP study was conducted at the [redacted] (b) (4) in 1988. In the study, miltefosine was found to have no mutagenic effect on bacterial strains (*Salmonella typhimurium* strains: TA 1535, TA 1537, TA 1538, TA 98, and TA 100) with or without metabolic activation. No miltefosine-associated increase in his+ revertants to twice the number of spontaneous revertants was observed. Miltefosine toxicity was determined to be ≥ 0.5 μg/plate.

7.2 *In Vitro* Assays in Mammalian Cells

Study Title: *In vitro* assessment of the clastogenic activity of D-18506 (miltefosine) in cultured human peripheral lymphocytes (Study Report No.: 3000876857; Study No.: 6057/90)

Summary: This non-GLP study was conducted by [redacted] (b) (4) in 1990. In this study miltefosine at doses of 6.25, 12.5, 25, 50, 100, and 150 μg/ml caused no detectable chromosomal aberrations or increased frequency of sister chromatid exchange in the presence and absence of S9 mixture in cultured human peripheral blood lymphocytes. In contrast the positive control agents (cyclophosphamide and mitomycin C) induced a 10-20 fold increase in the frequency of chromosomal aberrations and sister chromatid exchange in the same test system.

Study title: Mammalian Cell V79 Gene Mutation Test on D18506

Study no.:	876868 (Report No.: 3000876868)
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	April 19, 1990
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	D 18506 (Batch Ex 2136), purity not identified.

Key Study Findings

Both in the presence and absence of S9 activation, miltefosine was weakly mutagenic in the V79 mammalian cell gene mutation assay, but not in a dose-dependent manner.

Methods

Cell line:	Chinese hamster V79 cells
Concentrations in definitive study:	Without S-9 acitvation: 1, 2, 5, 7.5, 10, 15,

	20 and 30 μg/ml miltefosine; with S-9 activation, 2, 5, 10, 20, 50, 75, 100 μg/ml miltefosine.
Basis of concentration selection:	The highest concentration was one that either strongly precipitated or reduced the number of colonies in the cytotoxicity test to about 60 to 95%. In addition four or more lower concentrations are obtained by serial dilution.
Negative control:	Distilled water
Positive control:	1 μg/ml N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) without S9 mix and 20 μg/ml 7,12-Dimethylbenz(a) anthracene (DMBA) with S-9.
Formulation/Vehicle:	D 18506 (miltefosine) was dissolved in distilled water
Incubation & sampling time:	Cells were incubated with test compounds for 24 hours in the absence of S-9 activation and for 2 hours in the presence of S-9 activation.

Study Validity

The following study validation criteria were met:

1) The spontaneous mutation frequency with negative control conditions was less that 20 x 10^{-6}.
2) The positive control solutions produced a 10-fold or greater increase in mutation frequency.

Results

Without S-9 activation, doses of 10 and 20 μg/ml produced 43 and 95% cytotoxicity respectively. In the experiments without S-9 mix the negative control mutation frequencies ranged from 3.9 to 5.4 x 10^{-6}. Miltefosine at concentrations of 2, 5, 7.5, 10 and 15 μg/ml consistently increased the mutation frequency to roughly 3-4 fold negative control levels, but without a concentration-dependent effect. The positive control, MNNG, produced increased mutation frequencies roughly 500 fold in excess of the negative control frequencies.

With S-9 activation, doses of 20, 50, and 100 μg/ml produced 25, 42, and 100% cytotoxicity. The mutation frequencies associated with negative control conditions ranged from 2.2 to 6.4 x 10^{-6} which was increased roughly 200-300 fold by the positive control agent, DMBA. Miltefosine at concentrations of 2 to 20 μg/ml did not increase mutation frequencies above negative control levels but a miltefosine concentration of 50 μg/ml increased mutation frequencies roughly 3-4 fold.

Reference ID: 3384901

The results as a whole indicate that in the V79 mammalian cell gene mutation assay, miltefosine was weakly mutagenic.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study Title: D-18506: Mouse micronucleus test (single oral administration) (Study No.: 871841, Study Report No.: 3000871841)

Key Study Findings

Single oral doses of miltefosine previously shown to cause systemic toxicity (383 mg/kg in males; 464 mg/kg in females) did not produce clastogenic effects in the bone marrow of mice.

Methods: This GLP-compliant study was conducted by [redacted] (b) (4) from March, 30th 1990 to February 4th, 1991. In this study, miltefosine was administered in single oral gavage doses of 383 mg/kg (males) and 464 mg/kg (females) to 5 week old NMRI mice (n = 7/sex/time-point) , and mice were euthanized at 24, 48, and 72 hours after treatment to prepare bone marrow smears for detection of polychromatic erythrocytes (PCE). The high doses in this experiment were chosen because they were previously shown to produce significant toxicity, but were below the LD_{50} values (680 mg/kg in males and 603 mg/kg in females). Additional groups included the negative and positive control groups (n = 6/sex/time-point) receiving physiological saline or 51.1 mg/kg cyclophosphamide respectively. At least two slides per animal were prepared from the first five animals per sex, group, and time-point for analysis. One thousand PCE were scored for each animal with assessments of the number of micronucleated cells per 1000 PCE and the ratio of PCE to normochromatic erythrocytes (NCE).

Results: For all the time-points, after the single administrations of miltefosine, no significant increase in micronucleated PCE was observed in either male or female animals compared to the negative control group. A slight to moderate reduction in the PCE/NCE ratio at all sampling times in several miltefosine-treated animals was consistent with a toxic effect of miltefosine on bone marrow. Mice that were treated with a single IP dose of 51.1 mg/kg cyclophosphamide produced bone marrow with a significant increase in micronucleated PCE frequency.

7.4 Other Genetic Toxicity Studies

Study title: *In Vivo/In Vitro* Unscheduled DNA Synthesis (UDS) Assay on D-18506

Study no:	914073 (Study Report No.: 3000914073)
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 31, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Miltefosine (D-18506), Lot No.: 171260, purity of 98.14%.

Key Study Findings

Miltefosine doses of 75 and 150 mg/kg did not stimulate unscheduled DNA synthesis (UDS) in hepatocytes 2 hours or 16 hours after intragastric instillation in male Fischer rats. A miltefosine dose of 300 mg/kg also did not stimulate UDS in hepatocytes 2 hours after administration, but did produce lethality 16 hours after administration.

Methods

Doses in definitive study:	0, 75, 150, and 300 mg/kg miltefosine, 10 mg/kg 2-AAF
Frequency of dosing:	Single dose
Route of administration:	Intragastric instillation
Dose volume:	5 ml/kg body weight
Formulation/Vehicle:	Sterile deionized water
Species/Strain:	Male Fischer 344 rats
Number/Sex/Group:	4 males/group were dosed and 3 males/group were used to assess DNA repair.
Satellite groups:	none
Basis of dose selection:	Based on a range-finding study where 400 mg/kg was toxic and lethal.
Negative control:	Sterile deionized water for miltefosine, olive oil for the positive control.
Positive control:	10 mg/kg N-2-fluoenylacetamide (2-AAF)

General Procedures: Rats received intragastric instillations of miltefosine or control solutions followed by sacrifice at 2 or 16 hours after instillation. Livers from the exposed rats were perfused and hepatocytes isolated and cultured in the presence of ^{3}H-thymidine to assess DNA repair synthesis (incorporated ^{3}H-thymidine). Incorporated radioactivity was detected by autoradiography of cells prepared on slides and quantification of silver grains. Fifty randomly selected nuclei were counted per slide with 3 slides per animal and 3 animals for each dose level and exposure time. The net nuclear grain (NNG) count was determined by counting the number of grains present in each nucleus and subtracting the average number of grains detected in 3 equal sized adjacent cytoplasmic areas. For each dose of mitefosine or controls at each sacrifice time-point, the NNG counts for three slides were averaged and the standard deviation was calculated. Positive results for test article were considered to have occurred when: at least two test article concentrations yield NNG counts greater than negative control values, the increased NNG values are due to increased nuclear grain counts, a positive dose-response occurs up to toxic concentrations and at least one of the NNG counts is ≥ 5 NNG.

Study Validity

The following study validation criteria were met:

1. Negative control conditions produce NNG counts <0.
2. Positive control conditions produce significantly greater NNG counts than for negative controls and NNG values >0.

Results

Reference ID: 3384901

Animals treated with sterile deionized water in the negative control group produced hepatocytes with cell viabilities of >98% at both the 2 and 16 hour sacrifices. Mean NNG counts were approximately -20 for the cells obtained at both sacrifices. The positive control treatment with 2-AAF produced hepatocytes with >98% cell viabilities at both sacrifice time-points, and mean NNG counts ranging from 25.14 to 38.71 and 25.20 to 30.58 in cells collected two hours or 16 hours after sacrifice respectively.

Treatment with 75, 150 and 300 mg/kg miltefosine produced hepatocytes with ≥ 98% viability at both the 2 and 16 hour sacrifice time-points. At the two-hour sacrifice NNG counts associated with the 75, 150 and 300 mg/kg miltefosine doses ranged from -15.73 to -13.13, -14.52 to -10.48, and -16.59 to -11.21 respectively. Animals treated with 300 mg/kg were lethargic but survived until the two hour sacrifice. At the 16-hour sacrifice, NNG counts associated with the 75 and 150 mg/kg miltefosine doses ranged from -12.76 to -6.91, -18.22 to -14.46 respectively. All of the animals treated with 300 mg/kg and intended for the 16 hour sacrifice died before sacrifice. The available data indicates that miltefosine doses of 75, 150 and 300 mg/kg did not stimulate unscheduled DNA synthesis in hepatocytes 2 hours after dosing or 16 hours after dosing for the 75 and 150 mg/kg doses.

8 Carcinogenicity

Formal carcinogenic studies were not performed and are not required because miltefosine will be administered for no more than 28 days for the treatment of leishmaniasis. However, neoplastic lesions were observed in the 52-week rat toxicology study. Benign neoplastic lesions considered to be related to miltefosine administration included Leydig cell adenomas of the testes in 3/30 high-dose males and a basal cell adenoma in 1/30 high-dose females. Malignant neoplasms considered to be related to miltefosine administration included: a histiocytic sarcoma with widespread multi-organ metastases in one high-dose female, a squamous cell carcinoma in the uterus of another high-dose female, and in a third high-dose female, a malignant adenoacanthoma of the uterus with widespread metastasis.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

The Applicant conducted fertility studies in male and female rats and two embryo-fetal studies in rats and rabbits respectively. These studies are reviewed below.

Study title: Miltefosine (D-18506): Examination of the influence on the fertility and general reproductive performance after oral administration in male Wistar rats.

Study no.: 919528; Study Report No.: 3000919528

Reference ID: 3384901

Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 21, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Miltefosine (D-18506), lot # 171490 , purity of 100.8%

Key Study Findings

- The primary target of miltefosine appears to be the seminiferous epithelium. Atrophic changes occurred in a dose-dependent manner resulting in tubular atrophy and germ cell loss.
- At the highest dose level (21.5 mg/kg), these changes were irreversible. Changes included markedly decreased sperm number and viability, morphological sperm alterations, and persistent infertility in affected male rats.
- At the mid dose (8.25 mg/kg), effects were reduced for incidence and severity compared to high-dose animals. After the 10 week recovery period, sperm counts returned to control levels and fertility increased but sperm viability remained low (≈ 26%) showing only partial reversibility.
- The NOAEL dose was determined to 3.16 mg/kg.

Methods

Doses:	0, 3.16, 8.25, and 21.5 mg/kg
Frequency of dosing:	Daily
Dose volume:	2.15 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Ampuwa®, sterile pyrogen-free aqua ad injectabilia.
Species/Strain:	Wistar rats (HsdCpb:WU)
Number/Sex/Group:	For the Mating Trials: 20 males/group and 20/females/group/mating trial.
Satellite groups:	For sperm analysis and histopathology: 5 males/group/mating trial (dosed but not mated).
Study design:	Male Wistar rats (20 males/group/mating trial) received daily oral gavage doses of 0, 3.16, 8.25, 21.5 mg/kg miltefosine for 4 weeks prior to mating and until successful mating or up to 7 days during mating. Females (20/group/mating trial) were untreated and used for mating. Two mating trials were performed using the same 20 males/group; the first immediately after the 4-weeks of dosing with continued dosing for a maximum of 7-days; and the second 10 weeks after the four weeks of dosing without further dosing until successful mating or for a maximum

	of 7 days. Vaginal smears were used to confirm the presence of sperm or coital plugs. In addition 5 males/group/trial were dosed but not mated and sperm parameters were assessed.
Deviation from study protocol:	Only minor deviations which were not considered to have altered interpretation of the study results.

Observations and Results

Table 33: Observation Schedule

Parameter	Schedule
Mortality	Twice daily for duration of study
Clinical Signs	Daily for duration of study
Body Weight	Twice per week beginning with pre-test period
Food Consumption	Weekly for duration of study

Mortality

No mortality occurred

Clinical Signs

High-dose (21.5 mg/kg) males exhibited salivation during the last pre-cohabitation treatment week and for three weeks thereafter, and small palpable testicles first noted in the last treatment week and persisting until the end of the recovery period in Week 15. Also one mid-dose male had slightly smaller testicles several weeks after the end of treatment.

Body Weight and Food Consumption

High-dose males had slight reductions in food consumption and weight gain compared to controls during the whole treatment period.

Toxicokinetics: not performed

Dosing Solution Analysis

Dosing solutions were prepared daily and previous data indicated solutions were stable for at least 7 days. Dosing solution concentrations were analyzed by thin-layer chromatography in Week 3 of the first and second Mating Trials. The analysis indicated that the actual concentrations of the dosing solutions were within 10% of the nominal concentrations, and no miltefosine was detected in the vehicle dosing solutions.

Necropsy

Mating males were necropsied after successful mating or after 7 days in Study Week 15. In-life assessments for mating males included mating behavior. The males (5/group/trial) used for sperm assessment/histopathology were necropsied on the first day of the mating period in Weeks 5 and 15. Post-necropsy assessments for males included reproductive organ weights, sperm evaluation (number of spermatids, sperm, viability, motility, morphology), and gross pathology and histopathology assessment of all changed tissues, right testes, epididymis, seminal vesicles and prostate. Females

Reference ID: 3384901

were necropsied on Gestation Day 15. Post-necropsy assessments included: uterus weight, number of corpora lutea, number of implantation, number of early resorptions, number of late resporptions, number of dead fetuses, and number of live fetuses.

Male Mating Behavior and Fertility: Miltefosine-related effects on copulation and fertility occurred in a dose-dependent manner (Table 34 and Table 35). Four weeks of dosing with 21.5 mg/kg miltefosine (high-dose) caused a significant reduction in copulation index (12/20, 60%) which did not occur for control animals or the other miltefosine-dose groups which demonstrated ≥ 95% successful copulation in Week 5. Fertility was dramatically reduced to 1/19 (5%) for the mid-dose (8.25 mg/kg) group and 0/12 (0%) for the high-dose group compared to 15/19 (79%) for the control and low-dose (3.16 mg/kg) groups. By Week 15, after 10 weeks without treatment, mid-dose males demonstrated 100% (20/20) copulation and nearly 100% fertility (19/20). High-dose males had improved copulation in Week 15 with 18/20 (90%) copulation which was similar to control values (17/20; 85%), but fertility remained suppressed to 1/18 (5%).

Table 34: Male Mating Results for the First Mating Trial. (Applicant's Table)

Dose Group	1 Control	2 3.16 mg/kg	3 8.25 mg/kg	4 21.5 mg/kg
Animals Paired	20	20	20	20
Copulation Index	19/20 (95%)	19/20 (95%)	19/20 (95%)	12/20 (60%)
Fertility Index	15/19 (79%)	15/19 (79%)	1/19 (5%)	0/12 (0%)

Table 35: Male Mating Results for the Second Mating Trial. (Applicant's Table)

Dose Group	1 Control	2 3.16 mg/kg	3 8.25 mg/kg	4 21.5 mg/kg
Animals Paired	20	20	20	20
Copulation Index	20/20 (100%)	18/20 (90%)	20/20 (100%)	18/20 (90%)
Fertility Index	17/20 (85%)	17/18 (94%)	19/20 (95%)	1/18 (5%)

Sperm Evaluation: Miltfosine had a significant effect on the viability and number of sperm in the mid- and high-dose males (Table 36, Table 37). At 5 weeks, mid-dose males exhibited a 10% reduction in sperm count with only 2%, viable, motile sperm in epididymal preparations compared to 70% in the control group. In addition a very high 80% proportion of morphologically altered sperm occurred in the mid-dose group compared to 5.6% in control males. In the high-dose males, no viable sperm were found in epididymal preparations, and morphologically altered sperm were markedly increased. After the 10-week recovery period, all sperm parameters with the exception of the proportion of viable sperm (75% for mid-dose group and 87% for control males)

returned to near control values. In contrast, none of the sperm parameters fully recovered for the high-dose males 10 weeks after the end of dosing.

Table 36: Select Sperm Evaluations for Week 5

Parameter	Group 1 (control)	Group 2 (3.16 mg/kg)	Group 3 (8.25 mg/kg)	Group 4 (21.5 mg/kg)
Percent Sperm Viability	70 ± 39	89 ± 3	2 ± 4	0 ± 0
Percent Abnormal Sperm	5.6 ± 3.7	8.5 ± 4.3	80.8 ± 15.3	56.0 ± 28.3
Values are expressed as mean ± standard deviation				

Table 37: Select Sperm Evaluations for Week 15

Parameter	Group 1 (control)	Group 2 (3.16 mg/kg)	Group 3 (8.25 mg/kg)	Group 4 (21.5 mg/kg)
Percent Sperm Viability	87 ± 3	81 ± 6	75 ± 10	26 ± 36
Percent Abnormal Sperm	4.4 ± 3.1	4.3 ± 2.6	3.6 ± 0.7	30.7 ± 17.7
Values are expressed as mean ± standard deviation				

Male Organ Gross Pathology: Small testes were noted in 1/5 mid-dose and 4/5 high-dose males necropsied in Week 5 as well as small prostate (3/5 high-dose), small epididymis (1/5 mid-dose, 4/5 high-dose) and small seminal vesicles (3/5 high-dose). In Week 15, 24/25 high-dose males exhibited small testes. Small prostate, small epididymis, and small seminal vesicles were noted in 2/25, 20/25, and 2/25 high-dose males respectively. Single- or multiple- yellow nodules were present in the caudal epididymis of 11/25 mid-dose and 6/25 high-dose males in Week 15.

Male Reproductive Organ Weights: After 4-weeks of treatment, the absolute weights of testes, epididymis, and prostate/seminal vesicles of high-dose males were slightly to moderately decreased compared to control males. The relative weight of prostate/seminal vesicles was also slightly decreased. After 10 weeks of recovery, high-dose males still had reduced absolute weights of the male reproductive organs with greater reductions for testes and epididymis than was noted right after the 4 weeks of treatment.

Male Reproductive Organ Histopathology: Testes showed slight to massive diffuse tubular atrophy with degenerative spermatocytes and spermatogonia in 1/5 mid-dose and 5/5 high-dose animals at 5 weeks. During the 10-week recovery period, irreversible, massive tubular atrophy was detected in 13/24 high-dose animals characterized by Sertoli cell changes and complete loss of germ cells in tubular cross sections. Some tubules were mineralized. Regenerated epithelium with Sertoli cell involvement was noted in 11/24 high-dose animals suggesting a trend towards reversibility. A high proportion of all other individual animals in all groups showed focal tubular atrophy with Sertoli cell only change which could easily be differentiated from treatment-induced diffuse atrophy in Week 5.

Also in Week 5, 3/5 mid-dose animals and 5/5 high dose animals demonstrated fibrosis of the caudal epididymis, focal mononuclear cell infiltrate, apoptosis/atrophy of

epithelial cells, and accumulation of secretory fluid. In Week 15, 6/14 and 16/21 mid-dose and high-dose animals, respectively, showed similar findings with similar incidence and severity. Additionally the sperm content was decreased and immature germ cells were present in these animals. The presence of yellow nodules observed in the epididymis of affected animals appears to correlate with incidence of sperm granulomas recorded in mid- and high-dose animals.

Slight, diffuse, and reversible atrophy of prostate was noted in Week 5 in high-dose animals, and in individual low- and mid-dose animals killed in Week 15. The finding in Week 15 was considered incidental and unrelated to treatment. Also, all animals treated with miltefosine and euthanized in Week 5 demonstrated increased cellular pleomorphisms of the seminiferous epithelium associated with higher incidence of apoptosis, increased number of mitoses, cell vacuolization, and altered localization of the nucleus from a basal to central orientation. Reduction in secretory granules and cellular density were also noted with a dose-dependent increase in incidence and severity at higher doses. This finding was fully reversible during the recovery period.

Female Fertility Parameters: Low-dose miltefosine (3.16 mg/kg/day) did not affect reproductive parameters including number of corporal lutea, implantations, or resorptions with similar values to control animals after the first mating trial. The single mid-dose female that became pregnant also appeared to have normal reproductive parameters. After the second mating trial all female reproductive parameters for the low- and mid-dose groups appeared similar to controls. In the high-dose group, only one female became pregnant and this female had a marked increase in pre-implantation loss.

Study title: Miltefosine (D-18506): Examination of the influence on the fertility and general reproductive performance as well as the early embryonic development after oral administration in female Wistar rats

Study no.:	Study No.: 919934; Study Report No.: 3000919934
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 20, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Miltefosine (D-18506), Batch No.: 171490, purity of 100.8%

Key Study Findings

- Miltefosine doses ≤ 21.5 mg/kg/day did not alter the mating behavior, estrus cycle, or reproductive performance in rats.
- A miltefosine dose of 6.81 mg/kg/day (mid-dose) significantly lowered fetal weights and high-dose (21.5 mg/kg/day) dams had no fetuses.

Reference ID: 3384901

- Most of the fetuses from the mid-dose dams were dead and exhibited many visceral and skeletal malformations. Only one fetus in the low-dose group (2.5 mg/kg/day) was born dead and exhibited malformations. The Applicant indicated that the dam for this fetus was mistakenly dosed beyond the planned period.

Methods

Doses:	0, 2.15, 6.81, and 21.5 mg/kg miltefosine
Frequency of dosing:	Daily
Dose volume:	2.15 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Ampuwa® (sterile, pyrogen-free aqua ad injectabilia).
Species/Strain:	HsdCpb: WU rats
Number/Sex/Group:	7 males and 25 females/group in Groups 1-4
Satellite groups:	none
Study design:	Males were untreated. Females were treated with miltefosine daily for 4 weeks prior to mating, during the mating period, and up to Day 7 of pregnancy (day of implantation).
Deviation from study protocol:	Only minor deviations which were not considered to have altered interpretation of the study results.

Observations and Results

Table 38: Observation Schedule for the Male-Rat Fertility Study

Parameter	Schedule
Mortality	Twice daily for duration of study
Clinical Signs	Daily for duration of study
Body Weight	Twice per week beginning with pre-test period and daily during the post coitum phase of the study
Food Consumption	Weekly for duration of study
Estrus Cycle	Vaginal smears for estrus cycle assessment were prepared daily for 5 days pretest and in weeks 3 and 4 before the start of mating.
Necropsy	GD 20

Mortality

No female rats died prematurely during the study.

Clinical Signs

No miltefosine-related clinical signs were noted in the low-dose (2.15 mg/kg) and mid-dose (6.81 mg/kg) female rats. High-dose (21.5 mg/kg) females were observed to salivate with reddish, discolored saliva generally shortly after dose administration disappearing within about 4 hours. Also individual females in the high-dose group occasionally exhibited discolored urine, bloody vaginal discharge, head tilt, diuresis, pale skin, and pale feces.

Reference ID: 3384901

Body Weight

The high-dose females had reduced body weight gain compared to controls during gestation, but this was considered to be due to the lack of fetuses for the high-dose females.

Feed Consumption

A slight reduction in food intake was noted for the high-dose females during the pre-cohabitation period as well as the initial 7 days of pregnancy when dosing was continued. A slight temporary rebound of food consumption was noted for the mid- and high-dose groups in the second week of gestation when dosing was first discontinued.

Toxicokinetics: Not performed

Dosing Solution Analysis

Dosing solutions were prepared daily (previous experiments indicated stability of aqueous solutions of miltefosine for at least 7 days). Dosing solutions were analyzed for concentration by thin layer chromatography in Week 3 of both the Phase 1 and Phase 2 experiments. The results of the analysis indicated that the actual concentrations of the dosing solutions were within 10% of the nominal concentrations, and no miltefosine was found in the vehicle solutions.

Necropsy

Female animals were euthanized on Gestation Day 20. The following determinations were performed: uterus weight *in toto*, number of copora lutea (absolute, per dam, distribution), implantations (absolute, per dam, distribution), fetuses (absolute and relative alive/dead, distribution, weight, gender and external abnormalities, number of runts, skeletal and soft tissue abnormalities), and early resorptions.

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

Mating Behavior: Mating behavior was not altered by treatment with miltefosine at any dose (Table 39).

Table 39: Summary of Mating Parameters. (Applicant's Table)

Dose Group	1 Control	2 2.15 mg/kg	3 6.81 mg/kg	4 21.5 mg/kg
Animals Paired	25	25	25	25
Copulation Index	21/25 (84%)	20*/25 (80%)	25**/25 (100%)	24/25 (96%)
Fertility Index	18/21 (86%)	13*/20 (65%)	22**/25 (88%)	21/24 (88%)

* Inclusive 2 pregnant females where mating was not detected.
** Inclusive 1 pregnant female where mating was not detected.

Estrus Cycle: Estrus cycle in females was not influenced by the low- and mid-doses, but individual high-dose females had estrus cycle arrest in the metestrus or diestrus phases.

Reproductive Performance: No effect on reproductive performance was noted for the low mitefosine dose. In the mid-dose group, a marked increase in the number of embryonic and fetal resorptions and the proportion of dead fetuses was observed, but preimplantation loss was not affected. In 10/22 mid-dose liters, no live fetuses were found. In the high-dose group, no live fetuses were found. In 17/21 high-dose females, only implantation site scars were detected with the remainder (4/21) demonstrating embryonic and/or fetal resorptions.

Fetal Weights: The low dose of miltefosine did not affect fetal weights, but the weights of fetuses in the mid-dose dams were significantly lower than for control fetuses. High-dose dams had no fetuses.

External, Skeletal, and Visceral Examinations of the Fetuses: One dead fetus was detected in one litter of the low-dose group (2.15 mg/kg), and this fetus had multiple skeletal and neural malformations. The Applicant concluded that the dam for the dead fetus had been treated beyond the 7th day because mating had not been detected. In all the other low-dose fetuses, no compound-related external, skeletal or visceral malformations were observed, and the number of variations/retardations was comparable to the negative control group.

In the mid-dose (6.81 mg/kg) group, 41/80 fetuses were dead and 69/80 fetuses from 15/16 litters exhibited external abnormalities. Abnormalities included: cleft palate, domed head, anasarca, and red discolorations in the brain and/or spinal cord. Skeletal malformations included: cleft palate, dumbbell-shaped ossification of thoracic vertebral centers, markedly enlarged skullbones (exoccipital, suproccipital, parietal, interparietal, and frontal) and markedly dilated suturae. Increased skeletal variations/retardations including incomplete ossifications, holes, malpositioned/misaligned sternebrae) were also noted. Multiple visceral malformations mainly in the head and in neural tissues included: cleft palate, misshapen cerebral structures, dilated ventricles filled with brown masses, misshapen spinal cord, and misshapen and malpositioned eyes. Hypophysis and inner ear were absent in individual fetuses.

No fetuses were available for examination in high-dose dams.

9.2 Embryonic Fetal Development

Study title: Orientating Dose-Range-Finding Study to determine the dose-levels for an examination of the influence of hexadecylphophocholine in the pregnant rat and the foetus by oral administration (Segment II)

Study no.:	874282, Study Report No.: 3000874282
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)

Reference ID: 3384901

	(b) (4)
Date of study initiation:	February 28th, 1990
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hexadecylphoshocholine (miltefosine), Lot No.: PaX 8303 and PaX 8456, purity not identified.

Key Study Findings

- All of the miltefosine doses in Section 1 (6, 12, and 24 mg/kg/day) caused all of the fetuses to be resorbed.
- None of the Section 2 miltefosine doses (0.6, 1.2, 2.4 mg/kg/day) altered corpora lutea weights or the number of implantations.
- The 2.4 mg/kg/day miltefosine dose significantly increased the resorption rate, and was associated with a 34% decrease in the number of fetuses and a significantly higher post-implantation loss compared to control animals.
- One malformed fetus occurred in dams dosed with 1.2 mg/kg/day miltefosine but in dams receiving 2.4 mg/kg/day, 52/68 fetuses were malformed.
- Malformations included: underdeveloped cerebrum, hemorrhage within the skull lumina, cleft palate, and generalized edema.

Methods

Doses:	0, 6, 12, 24 mg/kg (first section); 0.6, 1.2, and 2.4 mg/kg (second section)
Frequency of dosing:	Daily from Gestation Day 6 through Gestation Day 15
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Aqua as injectabilia
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	8 females/group for both Section 1 and Section 2.
Satellite groups:	none
Study design:	Females were mated with untreated male rats until they were determined to be pregnant. . Pregnancy Day 0 was determined by vaginal smear. Pregnant female rats were then orally dosed with miltefosine from the 6th to 15th day of gestation, then sacrificed on Gestation Day 20 in conjunction with analysis of maternal and fetal parameters. In Section 1 females were dosed with 0, 6, 12, and 24 mg/kg/day miltefosine, and in Section 2 the doses were 0, 0.06, 0.12, and 0.24 mg/kg/day.
Deviation from study protocol:	Several deviations from the study protocol were noted, but none was considered to have altered

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the results or compromised the integrity of the study.

Observations and Results

Table 40: Observation Schedule for the Segment II Study in Rats

Parameter	Schedule
Mortality	Twice daily for duration of study
Clinical Signs	Twice daily for duration of study
Body Weight	Daily for duration of study
Food Consumption	Daily for duration of study
Necropsy	GD 20

Mortality

No miltefosine-related mortality in the dams occurred in Section 1 or Section 2 of the study.

Clinical Signs

No miltefosine-related clinical signs were noted for Section 1 or Section 2 of the study.

Body Weight

No changes in body weight were noted at any dose for Section 1 or Section 2 except as a result of loss of fetuses.

Feed Consumption

No changes in food consumption were noted at any dose for Section 1 or Section 2.

Toxicokinetics: not performed

Dosing Solution Analysis

Dosing solutions were prepared daily. At the time of the first dose of the Section 2 experiment, dosing solutions were collected and stored frozen for later analysis. All actual concentrations of the collected dosing solutions were reported to be within 10% of the nominal concentrations in a table, but the method of analysis was not identified.

Necropsy

Performed on Day 20

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

All of the treated dams showed only resorptions for all of the doses in Section 1. At the 6.0 mg/kg dose, fetuses were detected in most of the resorptions with body lengths ranging from 5 to 14 mm. In the 12 mg/kg dose group, fetuses were recognized in a few resorptions and their length was above 2 mm.

In the Section 2 experiment, corpora lutea weights and number of implantations were unchanged for all the doses compared to the negative control group. The lowest miltefosine dose, 0.6 mg/kg did not significantly lower the number of fetuses or increase the number of resorptions or change placental weights relative to the negative control

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group. At the 1.2 mg/kg dose, the number of fetuses and resorptions were within the normal range and comparable to the negative control group, but because of three dead fetuses, the 8.5% post-implantation loss was slightly higher than for the negative control group (5.5%). The 2.4 mg/kg group demonstrated a significant increase in the resorption rate, and much lower number of fetuses (↓34%), and the 38.9% post-implantation loss was significantly higher than for the negative control group.

Offspring (Malformations, Variations, etc.)

In the Section 1 experiment, all of the fetuses were resorbed so malformations and variations could not be assessed. In the Section 2 experiment, malformed or dead fetuses did not occur for the 0.6 mg/kg dose, and variation rates remained in the normal range for this dose. For the 1.2 mg/kg dose one fetus was malformed with generalized edema, but variations were within the normal range. In the 2.4 mg/kg group, 52/68 fetuses demonstrated malformations in the form of undeveloped cerebrum, and lumina of the skull filled with hemorrhagic fluid with a few fetuses further malformed with cleft palate and generalized edema. Also body weight for the fetuses was slightly lower than for the control group.

Study title: Orientating Dose-Range-Finding Study to determine the dose-levels for an examination of the influence of hexadecylphophocholine on the pregnant rabbit and the foetus by oral administration (Segment II)

Study no.:	874293, Study Report No.: 3000874293
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 29 , 1990
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	Hexadecylphoshocholine (miltefosine), Lot No.: PaX8456, purity not identified.

Key Study Findings

- In a pre-experiment, miltefosine doses ≥ 6 mg/kg/day caused abortions in some dams, and all fetuses were resorbed.
- Miltefosine doses ≤ 2.4 mg/kg/day did not alter the number of fetuses, the number of resorptions, or fetal and placental weights.
- Also miltefosine doses ≤ 2.4 mg/kg/day did not cause increased fetal variations or malformations.

Methods

Doses:	0, 0.6, 1.2, 2.4 mg/kg
Frequency of dosing:	Daily from Gestation Day 6 through Gestation Day 18
Dose volume:	10 ml/kg

Route of administration:	Oral gavage
Formulation/Vehicle:	Aqua as injectabilia
Species/Strain:	Himilayan rabbits
Number/Sex/Group:	4 females/group
Satellite groups:	none
Study design:	Pregnant female rabbits were orally dosed with miltefosine from the 6th day of gestation to the 18th day of gestation before sacrifice and analysis of maternal and fetal parameters on Gestation Day 20.
Deviation from study protocol:	Several deviations from the study protocol were noted, but none was considered to have altered the results or integrity of the study.

Observations and Results

Table 41: Observation Schedule

Parameter	Schedule
Mortality	Daily for duration of study
Clinical Signs	Daily for duration of study
Body Weight	Daily for duration of study
Food Consumption	Daily for duration of study
Necropsy	GD 29

Mortality

No unexpected mortality in the dams occurred.

Clinical Signs

The only reported clinical sign was an abortion on GD 26 in one high-dose (2.4 mg/kg/day) dam.

Body Weight

No changes in body weight were noted at any dose.

Feed Consumption

No changes in food consumption were noted at any dose.

Toxicokinetics: not performed

Dosing Solution Analysis

Dosing solutions were prepared fresh each day. Dosing solutions were collected on the first day of administration and stored frozen for later analysis for concentration and 24-hour stability by HPLC. The collected dosing solutions were shown to be stable after freezing for 24 hours at room temperature. The actual concentrations of the dosing solutions were always within 12% of the nominal concentrations.

Necropsy

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The necropsy was performed on Gestation Day 29. The following parameters were evaluated: corpora lutea number, implantations, fetus number (live/dead), fetal weights, placental weights, runt number, malformed fetuses, malformation rate, variation rate, resorptions (early, late, number, rate), preimplantation loss, postimplantation loss,

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

In a pre-experiment, dose of miltefosine of ≥ 6 mg/kg caused abortions in individual or several dams and all fetuses were resorbed.

For the current experiment, none of the doses, including the high dose of 2.4 mg/kg influenced the prenatal development of the rabbits. The number of fetuses and resorptions were within the normal range, fetal and placental weights did not differ significantly from the control values.

Offspring (Malformations, Variations, etc.)

Variation rates were within the normal range. The number of runts that occurred, one in the 0.6 mg/kg group and two in the 1.2 mg/kg group were considered to be in the normal range as were the number of dead fetuses, 2 for the control group, and 3, 1, and 2 for the low-, mid-, and high-dose groups respectively. One fetus each in the control, low- and mid-dose groups had malformations with all three composed of omphalocele and partially extracorporal position for the intestines, as well as cheilochisis and cleft palate in the low dose fetus.

9.3 Prenatal and Postnatal Development

A Segment III study was not conducted for miltefosine. The Applicant received permission to omit this study at the January 13, 2012 Pre-NDA meeting because of the difficulty in generating offspring in rats given the documented female fertility and embryo-fetal toxicity of miltefosine at animal doses approximating clinical doses.

10 Special Toxicology Studies

Study title: Miltefosine (D-18506): 8-week Oral toxicity study after repeated administration in juvenile rats and subsequent 6-week recovery period.

Study no.:	916266 (Study Report No.: 3000916266)
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 6, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Miltefosine, Batch # 171261, purity of 95.69%

Key Study Findings

In an 8-week toxicology study in juvenile rats, miltefosine-related toxicity occurred in the kidney, small intestine and eyes (retinal atrophy). The NOAEL dose was considered to be 1 mg/kg/day.

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Methods

Doses:	0, 1.0, 2.15, 4.64, and 10.0 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	2.15 ml/kg
Formulation/Vehicle:	Ampuwa® aua diestillata ad injectabilia
Species/Strain:	Wistar Rats/HsdCpb: WU
Number/Sex/Group:	10/sex/group for Main Study and 5/sex/group for Recovery Study
Age:	4 weeks old at the start of administration for both genders
Weight:	Males: 70.7 to 97.8 g; Females: 60.8 to 84.8 g
Satellite groups:	5/sex/group for Recovery Study
Unique study design:	Male and female juvenile rats received oral gavage doses of miltefosine for 8 weeks in the Main Study. Recovery animals were maintained for an additional 6 weeks without dosing before sacrifice.
Deviation from study protocol:	Several deviations from the study protocol were noted, but deviations were not considered to have compromised the results or integrity of the study.

Observations and Results

Table 42: Observation Schedule for the 8-Week Juvenile Rat Study

Parameter	Schedule
Mortality	Twice Daily for duration of study
Clinical Signs	Daily for duration of study
Body Weight	Once during pretest, weekly thereafter
Food Consumption	Once during pretest, weekly thereafter
Reflexes	Pain, pinna, and corneal reflexes were tested once during the pretest period and weekly thereafter
Eye, Hearing, and Dental Examinations	Once pretest and in Weeks 5 and 8
Ophthalmological Investigations	Once pretest and in Weeks 5 and 8
Blood Collection Hematology and Serum Chemistry	In Weeks 4 and 8 from Main Study animals and in Week 14 form Recovery animals
Urine Collection	Once in Week 7 before the daily dose.
Necropsy	Day for Main Study; Day for Recovery Study

Mortality

No mortality occurred during the study.

Clinical Signs

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None of the clinical signs were considered to be related to miltefosine administration

Body Weights

No miltefosine-related changes in body weight were noted.

Feed Consumption

No difference in food consumption between groups was observed.

Reflex Testing, Control of Hearing, Teeth, and Eyes

Pain, pinna and corneal reflexes were tested. The eyes were examined with focused light, audition was checked with a simple noise test, and teeth were visually inspected.

No changes were noted for reflexes, or the simple examinations of hearing, teeth, or eyes

Ophthalmoscopy

Animals were examined with a slit lamp and ophthalmoscopy was performed with a fundus camera.

The Applicant considered ophthalmoscopy findings in 2/30 high-dose (10.0 mg/kg) animals to possibly be related to miltefosine administration. One high-dose male and one high-dose female demonstrated bilateral fundus reflex starting in Week 5, and the male also exhibited bilateral thinned retinal vessels.

ECG: Not performed

Hematology

Blood was collected from the retro-orbital venous plexus of one eye under CO_2 anesthesia. The measured parameters are shown in Table 46

No miltefosine-related changes in any hematology parameters were noted by the Applicant. In high-dose females, a significant decrease in hemoglobin and hematocrit compared to controls were observed in Week 4, and hemoglobin remained decreased in Week 8. The values were within the range of historical control data, but it is unclear if the historical data encompassed values for juvenile animals.

Clinical Chemistry

The serum chemistry parameters that were measured are shown in Table 47 .

Most of the significant clinical chemistry changes occurred with miltefosine doses of 4.64 mg/kg and high-dose (10.0 mg/kg) males with fewer changes in females. A few changes occurred for males receiving 2.15 mg/kg/day including decreased serum triglycerides and increased serum chloride in Week 8 (Main Study animals) and in Week 14 (Recovery animals).

High-dose males (10.0 mg/kg) had a slight to moderated increase in AKP (Weeks 4 and 8), calcium (Weeks 4 and 8), chloride (Weeks 4 and 8), blood urea (Week 8), creatinine

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(Week 8), sodium (Week 8), ASAT (Week 8), ALAT (Week 8) and a slight to moderate decrease in triglycerides (Weeks 4, 8, 14) albumin (Week 8), inorganic phosphorus (Week 8) and potassium (Week 8). High-dose females also had slightly increased blood urea (Weeks 4 and 8), slightly increased creatinine (Week 4) and a slight decrease in inorganic phosphorus in Week 8 (Also occurred for females receiving 4.64 mg/kg/day).

Urinalysis

Urine was collected in metabolism cages. Measured urinalysis parameters included: bilirubin, glucose, hemoglobin/erythrocytes, ketones, leucocytes, nitrite, osmolality, pH-value, protein, and urobilinogen.

In high-dose animals, urate crystals were observed in the urine of 1/14 males and 3/8 females. Also in one male animal in each group receiving 2.15, 4.64 and 10 mg/kg (Groups 3, 4, and 5) ketonuria was noted as well as in one female in Groups 3 and 4.

Gross Pathology

Yellow nodules in the epididymides were observed in 3/5 Recovery males in the high-dose group. One high-dose female (Main Study) demonstrated a thickened wall in the duodenum.

Organ Weights

The organs evaluated for weight are shown in Table 48. Organ weights were expressed as absolute values and as relative to body weights.

No miltefosine-related significant changes in absolute or relative organ weight ratios were observed. A slight increase in absolute and relative right adrenal gland weights was noted in high-dose males, but the increase was considered incidental.

Histopathology

Adequate Battery. Yes, the organs and tissues that were examined for histopathology are listed in Table 48.
Peer Review: No
Histological Findings
Kidney: In both genders a reduction of brush border and tubular cell height were observed. This finding was diffusely distributed in the cortex and occurred in 4/10 high-dose males as well as 2/10 females in the 4.64 mg/kg group and 7/10 high-dose females. In 3/4 of the males exhibiting reduction of brush border, the epithelial cells of the cortical proximal tubules were reduced in height. This minimal to slight finding was reversed during recovery for both males and females.

In addition in females receiving 4.64 and 10 mg/kg/day, diffuse basophilic tubules were noted, and in a single female, focal tubular hyperplasia and nuclear crowding of collecting tubules. In the most pronounced cases in high-dose females, vacuolar changes and single cell necrosis was noted. This finding was reversible.

Eyes: Three associated retinal effects were noted. A reduction of the photoreceptor layer such that only a poor or no distinction between the outer and inner segment of

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photoreceptors was possible was noted half of the male and female rats in the group receiving 4.64 mg/kg miltefosine, and in all of the high-dose rats of both genders. The severity of the effect increased with dose with minimal to marked effects occurring in the high-dose group. Instead of reversing during the recovery period, the severity increased in both genders. Subretinal macrophages were noted in the photoreceptor region with more macrophages found in the peripheral parts of the fundus. Subretinal macrophages decreased in most recovery rats. In animals exhibiting the more pronounced photoreceptor loss (7/10 males and 8/10 females in the high-dose group), a reduction of the outer nuclear layer was evidenced by a reduced thickness and reduced cellularity. This finding was not reversible.

Small Intestine: Diffuse mucosal hyperplasia was noted in the duodenum and jejunum. Also, crypts as well as villi were increased in length in association with increased epithelial height indicating cellular hypertrophy. The severity of effects increased with dose, and effects were more prevalent in the duodenum with an incidence of 1/10 males and 2/10 females in the 4.64 mg/kg group and 9/10 males and 9/10 females in the high-dose group. In the jejunum a lower incidence in 3/10 and 2/10 high-dose males and females respectively was observed. The small intestine histopathology was absent in recovery animals.

Seminal Vesicles: Among Main Study males, the seminal vesicles for 4/10 rats in the group receiving 4.64 mg/kg miltefosine and all high-dose males demonstrated increased cellular pleomorphism, evidence of a higher rate of apoptosis, increased number of mitosis, vacuolization of individual cells and changed localization of the nucleus. The effect was reversible after the recovery period.

Epididymides: The tail portion of the epididymides had a higher incidence of sperm granulomas in 6/15 high-dose males (3/10 Main Study and 3/5 Recovery males). This finding correlated with the necropsy observation of yellow nodules.

Special Evaluation

Electron microscopy of the Eye: Three Main Study animals/sex in Groups 1-4 were used (only 2 females for Group 4) as well as 2 Recovery animals/sex for Groups 2-4.

Clear miltefosine-related morphological lesions occurred in the pigment epithelium of the outer and inner segments of the photoreceptors and in the outer nuclear layer of both male and female Main Study animals receiving 2.15 (Group 3) and 4.64 (Group 4) mg/kg body weight. These findings were partially reversed for Group 3 Recovery animals, but only minimally reduced for incidence and severity in Group 4 Recovery animals. Group 2 animals receiving 1 mg/kg/day miltefosine had retinal morphology similar to control animals with the exception of one female with a slightly enhanced degree of morphological findings in the pigment epithelium and photoreceptors.

Toxicokinetics

Toxicokinetics were examined in a separate concomitant study (No. 916277). This study involved blood collection from the same animals used in Study 916266. For toxicokinetic analysis, blood was collected in Weeks 1 and 4 from the rats in Groups 2-5 before

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administration and at 4 and 8 hours after administration and 4 hours after administration from Group 1 (vehicle control) animals. In Week 8, blood was collected from animals in Groups 2-5 before administration and at 2, 4, 8, 12, and 24 hours after administration and 4 hours after administration from animals in Group 1. The blood samples for each group and time-point were pooled separately for males and females. Miltefosine concentrations in plasma were determined using a validated HPLC/MS/MS method.

The results indicate that gender did not affect the toxicokinetics of miltefosine, but that miltefosine accumulated in plasma with time (Table 43). The AUC_{0-24} values in test Week 8 increased in an approximately dose-proportional manner (Table 44).

Table 43: Mean of the Male and Female Plasma Pools After Repeated-Oral Administration of Miltefosine in Juvenile Rats. (Applicant's Table)

$Mean_{ar}$ plasma concentrations of D-18506 [ng/ml]					
Week	Time [h]	Dose group			
		1.00 mg/kg	2.15 mg/kg	4.64 mg/kg	10.0 mg/kg
1	4	450.03	1315.22	1967.65	4013.18
	8	725.74	1747.18	2731.75	5943.76
4	0	2192.51	5657.31	11150.58	26505.68
	4	2734.59	6160.40	11106.67	29959.73
	8	2957.45	6854.85	13911.09	27380.56
8	0	3228.70	7842.51	16214.48	34411.92
	2	3527.93	7820.56	15835.08	41935.39
	4	3379.63	8062.19	17670.19	38921.62
	8	3524.90	8441.78	17055.00	39636.77
	12	3932.18	8362.09	17415.04	38652.81
	24	3089.92	8169.61	17025.92	40791.42

Table 44: The AUC_{0-24} Exposure in Plasma After Repeated-Oral Administration of Miltefosine to Juvenile Rats. (Applicant's Table)

Dose [mg/kg]	AUC_{0-24} of D-18506 in test week 8 [ng·h/ml]	
	male rats	female rats
1.00	81523.28	87516.61
2.15	187724.53	206978.78
4.64	403601.32	417580.71
10.00	919876.24	975254.84

Dosing Solution Analysis

Miltefosine concentrations in dosing solutions were determined Weeks 1, 4, and 8 of the experiment. Reportedly measurement of the actual concentrations of the dosing solutions confirmed the nominal concentrations.

Study Title: Examination on the formal pathogenesis of eye changes in rats. (Study No.: 871828; Study Report No.: 3000871828)

Methods
Albino Wistar rats and pigmented Brown-Norway-Lewis rats (24 for each strain) were administered daily administrations of 34.8 mg/kg miltefosine by oral gavage for up to 27 days. Groups of 6 albino and 6 pigmented groups received vehicle as the negative control groups. Rats of each strain were sacrificed in groups of 3 after 2, 4, 6, 10, 13, 17, 23, and 27 days and rat eyes were removed and processed for histopathology examination.

Results
Albino rats: Control animals and animals treated for two days with miltefosine did not demonstrate retinal toxicity. All animals treated for four or more days with miltefosine demonstrated retinal histopathology with severity of effects increasing with duration of dosing. Four days of dosing produced single vacuoles and focal swelling in the retinal pigment epithelial cells. Dosing for 27 days produced extremely flattened pigment epithelial cells, damaged photoreceptors with macrophages in the photoreceptor layer, loss of structures associated with the outer nuclear membrane, outer plexiform layer, and the inner nuclear layer. In addition, retinal ganglion cells were slightly swollen.

Pigmented rats: Miltefosine-associated retinal effects in pigmented rats were very similar to those seen in albino rats, and the presence of melanin did not appear to influence the retinal changes. A possible difference in the severity of changes may have occurred with pigmented rats demonstrating slightly lower severity.

Study Title: Examination on the reversibility of retina changes induced in pigmented rats by repeated oral administration of miltefosine (D-18506). (Study No.: 892855; Study Report No.: 3000892855).

Methods
Pigmented Brown-Norway Lewis rats (9 females/dosing period) received daily administrations of 21.5 mg/kg miltefosine by oral gavage for 5, 10, 15, 20, 25, and 30 days respectively. For each dosing group, 3 animals were sacrificed one day after the treatment period, 3 were sacrificed after a 4-week recovery period, and 3 after a 12-week recovery period. Nine rats were used as controls and remained untreated. After each recovery period, rat eyes were removed and processed for light and electron microscopic examination.

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Table 45: Study Design for Study No.: 892855. (Applicant's Table)

Group	Animal No.	D-18506 (mg/kg)	Duration of Admin. (Test Days)	1st Time of Sacrifice (Animal No.)	2nd Time of Sacrifice (Animal No.)	3rd Time of Sacrifice (Animal No.)
1	1-9	0	-	1-3	4-6	7-9
2	10-18	21.5	1-5	10-12	13-15	16-18
3	19-27	21.5	1-10	19-21	22-24	25-27
4	28-36	21.5	1-15	28-30	31-33	34-36
5	37-45	21.5	1-20	37-39	40-42	43-45
6	46-54	21.5	1-25	46-48	49-51	52-54
7	55-63	21.5	1-30	55-57	58-60	61-63

Results

The negative control group exhibited a baseline of retinal effects including focal flattening of retinal pigment epithelium in 1/9 animals, minimal vacuolation in the area of outer segments of photoreceptors in 3/9 animals, slight vacuolation in the area of inner segments of photoreceptors in 1/9 animals and minimal occurrence of macrophages in the photoreceptor area in 1/9 animals. In contrast, all of the animals treated with miltefosine for five or more days demonstrated retinal changes.

Animals treated for 5 days demonstrated what are considered to be early signs of retinal degeneration including slight vacuolation, focal flattening, and disorganized melanin granules in the retinal pigment epithelium, minimal vacuolation in the outer and inner segments of photoreceptors, and minimal occurrence of macrophages in the photoreceptor area. These signs were still present in all animals after 4-weeks recovery, but after 12-weeks recovery, signs of early retinal degeneration were only present in 1/3 animals (similar to control levels) suggesting reversibility of effects.

After 10, 15, 20, 25, and 30 days of dosing more severe signs of retinal degeneration were observed in all treated animals and the severity of signs occurred with the duration of dosing. After 30 days of dosing, signs of retinal degeneration included: diffuse flattening of retinal pigment epithelium, marked to massive disorganization of melanin granules, vacuolation and slight to marked destruction of inner and outer segments of photoreceptors, moderate numbers of macrophages present in the photoreceptor layer of the entire retinal and slight to moderate necrosis in the outer nuclear layer. Signs were not fully reversed after 4-weeks of recovery at any dose, but nearly full reversal appeared to occur after 12-weeks of recovery following 10 and 15 days of dosing. Full reversal of effects was not observed after 12-weeks recovery following 20, 25, or 30 days of dosing.

Electron microscopy findings are not summarized in this review.

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11 Integrated Summary and Safety Evaluation

Miltefosine is a structural analog of alkyl (lyso) phospholipids (ALP). ALPs show cytostatic and immunomodulatory effects and originally were investigated for their use in the treatment of cancer. Subsequently, ALPs were tested *in vitro* for their antileishmanial activity, and miltefosine was carried forward into antileishmanial clinical trials. The original indication was visceral leishmaniasis (VL). Subsequent clinical trials were conducted in cutaneous (CL) and mucosal leishmaniasis (ML). Miltefosine (Impavido®) has been widely studied and obtained marketing authorization in 2002 in India as an oral treatment of VL. Subsequently, marketing authorization was granted for VL and/or CL in 12 other countries including Germany (VL and CL in 2004). In 2008, Paladin Labs Inc. (Montreal, Canada) acquired Impavido~ from Aeterna Zentaris (Frankfurt, Germany), the company responsible for the discovery of miltefosine and the clinical trials until that time. The rationale for seeking an NDA in the United States is that there is a need for alternative therapies particularly an oral therapy like miltefosine for the patients with visceral leishmaniasis, and an unmet medical need for the treatment of mucosal and cutaneous leishmaniasis for which there are currently no approved therapies.

From a clinical perspective, visceral leishmaniasis is a life-threatening disease and patients are seriously ill at the beginning of treatment. Cutaneous leishmaniasis, depending on the infecting Leishmania species, is either quickly or slowly self-healing, and either restricted to a localized skin ulcer or disseminating to local lymph nodes or more rarely to the oro-nasal mucosa (mucosal disease). The mucosal disease is often localized to the oral pharynx resulting over time in life-threatening aspiration pneumonia.

Secondary Pharmacology

Secondary pharmacology studies indicated that miltefosine had no effect on the caspase 3-caspase cascade, PAF-induced edema, or PAF-induced bronchospasm. Miltefosine did have a weak bronchospastic effect on its own in guinea pigs, but the effect increased only marginally with increasing dose.

Safety Pharmacology

A number of safety pharmacology studies were performed to examine neurobehavioral, cardiovascular, respiratory, and gastrointestinal effects of miltefosine. Renal safety pharmacology studies were not performed. Among the positive safety pharmacology effects of miltefosine were antipyretic effects (no effect on normal temperature, but dose-dependent inhibition of yeast-induced fever), and antinociceptive activity. Miltefosine also increased respiratory rate by approximately 10% in cats. In cardiovascular assessments, miltefosine was negative in a hERG assay at concentrations of 3 μM, and had no direct effect in on heart rate or left ventricular contractility but markedly reduced blood pressure and cardiac output by as much as 30% at doses of 50 and 200 mg/kg administered intraduodenally to pigs. Miltefosine demonstrated dramatic gastric effects causing severely inflated stomachs, diarrhea, severe erythema of the gastric mucosa (gastric erosions) and intestinal ulcers at intragastric doses of 200 and 400 mg/kg in Sprague-Dawley rats. Miltefosine also was shown to strongly inhibit gastrointestinal transit in fasting mice at doses as low as 12.5

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mg/kg but had no effect on spasmogenic activity in isolated guinea pig ileum. Notably, intravenous administration of miltefosine was observed to cause hemolysis.

Pharmacokinetics

Nonclinical pharmacokinetic studies with miltefosine described several clear patterns. Miltefosine is widely distributed throughout many tissues with a much larger volume of distribution than the vascular compartment. Miltefosine binds well to plasma proteins (95% bound) from rats, dogs, and humans. Unlike most drugs, miltefosine distributes well to nervous tissue including the brain and spinal cord. Miltefosine has a long $t_{1/2}$ in plasma (approximately 80 hours in rats and 160 hours in dogs), but distribution studies indicate that miltefosine has a longer $t_{1/2}$ in some tissues particularly in nervous tissue. In one rat study, $t_{1/2}$ values were 47 days for spinal cord and 39 days for brain compared to approximately 16 days for blood after 21 daily doses of [^{14}C]miltefosine. Repeated-dose distribution studies indicate that miltefosine accumulates with repeated-dosing with relatively more accumulation in some tissues. After 21 days of dosing in rats with [^{14}C]miltefosine, tissue concentrations of radioactivity were generally 7-15 fold higher than after a single dose in most tissues, but higher ratios occurred in lens (68 fold), brain (50 fold), spinal cord (37 fold), optic nerve (28 fold), epididymis, contents of seminal vesicles, and testes (all about 21 fold).

In vitro studies with hepatocytes from rats, dogs, and humans revealed a low level of metabolism with choline as the only metabolite. Greater metabolism occurred *in vitro* by phospholipase D metabolism with choline again the only metabolite. *In vivo*, choline was detected in the feces and urine of rats after oral and intravenous administration. Excretion of miltefosine was slow but occurred predominantly in urine and to a lesser extent in feces.

General toxicity

In rat oral-dose studies, 13 and 52 weeks in duration, substantial toxicity occurred. In the 52-week study, mortality occurred for all dose levels including the low dose of 4.64 mg/kg/day. Clinical signs include alopecia with degeneration of hair follicles, increased secretion from the conjunctiva and nasal mucosa, mydriasis, reduced food consumption, weight loss, and later in the study, swollen testicles and red urine in the mid- (10.0 mg/kg/day) and high-dose (21.5 mg/kg/day) groups. Most clinical signs and retinal degeneration and other ophthalmic findings occurred in all dose groups but with a dose-dependent increase in incidence and severity. Miltefosine-related hematological changes included: decreased RBCs, increased reticulocytes, increased WBCs (increased segmented neutrophils) and increased platelets. Some serum chemistry changes occurring at doses ≥ 10 mg/kg may have been related to weight loss including: increased transaminases, decreased triglycerides, and decreased total protein. Other serum chemistry changes also occurring at doses ≥ 10 mg/kg appeared to result from direct toxicity to the kidney (increased BUN and creatinine, altered serum electrolytes). The direct findings correlated with urinalysis effects including increased leukocytes, protein and hemoglobin/erythrocytes. Extensive histopathology related to miltefosine administration included: chronic nephropathy, hyperplasia in mesenteric lymph node, hyperplasia of stomach chief cells, hyperplasia and hypertrophy of intestinal mucosa,

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atrophy of testes, Leydig cell hyperplasia and adenomas, atrophy of prostate, epididymides, and seminal vesicles, many spermatogenic granulomas in epididymides, ovarian cysts, hydrometra, mucometra, and pyometra of the uterus, hyperplasia of cervical and vaginal mucosa, hyperplasia and hypertrophy of pituitary gland pars distalis cells, and in the eyes corneal inflammatory changes, homogenization of the lens nucleus, swelling and vacuolization of lens fibers, and retinal degeneration. In the 8-week study, many of the same toxicities were noted but with less severity and the NOAEL for this study was the low dose of 4.64 mg/kg/day (HED of 0.742 mg/kg/day or 0.22 of the clinical dose) exclusive of alopecia and conjunctival secretions. In the 52-week study, the NOAEL was less than 4.64 mg/kg/day.

Juvenile rats exhibited more sensitivity to miltefosine toxicity compared to adults. In a 13-week toxicology study, juvenile rats (4-weeks old at the start of treatment) experienced a similar range of toxicities but at lower miltefosine doses compared to adult rats (7-9 weeks old at the start of treatment) in a study of the same duration. Retinal degeneration was noted in some juvenile animals receiving 2.15 mg/kg/day miltefosine, but not in adult rats receiving 4.64 mg/kg/day miltefosine. A number of other toxicities were noted in juvenile rats at a miltefosine dose of 4.64 mg/kg/day including reduced brush border and tubular cell height in the renal cortex, retinal degeneration, diffuse mucosal hyperplasia in the small intestine, and seminal vesicle alterations whereas the primary toxicities noted in adult rats receiving 4.64 mg/kg/day were alopecia and nasal and conjunctival secretions/red incrustations.

Two special toxicity studies were conducted to examine the formal pathogenesis and reversibility of miltefosine-induced retinal toxicity in rats. In the first study, the first lesions noted in conjunction with doses of 34.8 mg/kg/day miltefosine occurred in retinal pigment epithelium, followed by cell destruction in the outer segments of the photoreceptor layer, and progressing to loss of photoreceptors in the inner segment. In the second study examining the reversibility of retinal damage, early changes to the retinal pigment epithelium were shown to be reversible. However, with increased duration of dosing up to 30 days, complete damage to photoreceptors inclusive of nuclei and the consecutive loss of inner retinal structures was not reversible.

Electrooculogram recordings were included after oral miltefosine therapy of 150 mg/day (2.5 mg/kg/day) for up to 5 months in 25 cancer patients in order to evaluate ocular effects of miltefosine in humans. In these patients, miltefosine was shown to produce evidence of retinal impairment which was fully reversed after termination or reduction in dosing. These results suggest miltefosine can produce dose-dependent retinal impairment in humans as well as rats, but in humans treated with clinical doses of miltefosine the results appear to be reversible and less severe than those noted in rats. Based on these results patients administered miltefosine should be monitored for visual changes throughout treatment, but retinal damage should not present as a prohibitive toxicity.

In the 13-week oral-dose toxicology in Beagle dogs, the primary toxicities were nonspecific clinical symptoms including salivation, vomiting, diarrhea, and reduced food

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consumption with associated weight loss. Some high-dose dogs exhibited serious atrophy of abdominal fat. The clinical signs were largely absent at a dose of 1.0 mg/kg/day, occurred at 3.16 mg/kg/day, and were markedly severe at the high dose of 10.0 mg/kg/day. Hematologic effects which also occurred in rats included increased WBC counts in conjunction with increased numbers of segmented neutrophils and decreased lymphocytes. Unlike rats, prothrombin time was shortened. Histopathology included prostate atrophy in some high-dose dogs, alveolar histiocytosis in the lungs, pigment accumulation in Kupffer's cells, and hyperemia of the intestinal mucosa. The NOAEL for this study was considered to be 1.0 mg/kg/day which corresponds to a human equivalent dose of 540 μg/kg/day (0.2 times the clinical dose) based on body surface area comparison. No toxicokinetic measurements were performed in this study.

Clinical signs and, in particular, weight loss were too severe with the 10 mg/kg/day dose in a 52-week, oral-dose toxicology study in dogs, and the high-dose for this study was consequently lowered to 7.50 mg/kg in Week 3, 6.81 mg/kg in Week 5 and 6.19 mg/kg in Week 7 of the study. The scope of clinical signs was similar to those observed in the 13-week study with the addition of alopecia. Hematologic effects and decreased prothrombin time were similar to those observed in the 13-week study. Unlike in the 52-week toxicology study in rats, there were no serum chemistry changes considered to be of toxicological relevance. Unlike the 13-week study in dogs, prostate weights were not reduced in high-dose males, but multifocal atrophy and degeneration of seminiferous tubules associated with focal mononuclear infiltrates were observed in this group. Reduced testicular weights and testicular atrophy were noted in 2/2 high-dose Recovery males, but not Main Study animals. In general, male reproductive organ atrophy and degeneration was much less pronounced than that noted in rats, and the retinal degeneration noted in rats was not observed in dogs. A singular miltefosine-related finding of increased numbers of atretic follicles in the ovaries was observed in the 52-week study. This effect occurred at all miltefosine doses including the low dose of 1.0 mg/kg/day, but incidence and severity increased with dose and the high-dose females were morphologically in anestrus. The sexual cycle and the appearance of the follicles returned to normal after 6-weeks recovery. The NOAEL for the 52-week study exclusive of the reversible ovarian changes was considered to be the low dose of 1.0 mg/kg/day. This dose corresponds to an HED of 540 μg/kg/day (approximately 0.2 fold of the MHRD of 3.33 mg/kg/day) and toxicokinetic analysis for this dose was not performed.

Genetic toxicity

Miltefosine has been evaluated *in vitro* in Ames and DNA Amplification assays and a V79 mammalian cell gene mutation assay, *in vivo/in vitro* in an unscheduled DNA synthesis (UDS) assay and *in vivo* in a micronucleus assay. The results of the Ames and DNA amplification assays were negative for mutagenicity. The V79 mammalian cell gene mutation assay revealed a reproducible increase in mutant frequency that was not dose-dependent at concentrations of 2 to 15 μg/ml in the absence of S9 activation and at 50 μg/ml in the presence of S9 activation. In the *in vivo* micronucleus assay, miltefosine was negative for clastogenesis and results were also negative in the UDS assay. The overall results of the genetic toxicology studies suggest miltefosine has a negative potential for genetic toxicity in humans.

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Carcinogenesis
Because miltefosine is scheduled to be administered for less than 6-months, formal rodent carcinogenicity studies were not performed. However, in a 52-week oral toxicity study in rats, tumors were observed at the high-dose of 21.5 mg/kg/day miltefosine. Tumors included: benign basal cell adenoma of the skin, multiple histiocytic sarcoma, squamous cell carcinoma in the uterus and malignant adenoacanthoma in the uterus that was observed in separate females (each in 1/30 females) and testicular Leydig cell adenoma observed in 3/30 males. Toxicokinetic analysis for the 21.5 mg/kg/day dose was not performed but based on body surface area comparisons, the HED for this dose in rats is 3.44 mg/kg/day (approximately equal to the MHRD).

Reproductive and Developmental Toxicology
Very serious effects on male fertility were noted for miltefosine in rats, but these changes were greatly attenuated or absent in dogs at similar plasma exposures. In a male fertility study in rats, miltefosine produced a dose-dependent impairment of the male reproductive system in males treated for 4-weeks before mating and up to 7 days during mating. Effects that occurred at doses ≥ 8.25 mg/kg included: reduced copulation index (only at a dose of 21.5 mg/kg/day), dramatically reduced fertility, reduced sperm number and viability, increased morphologically altered sperm, and atrophied testes, prostate, and seminal vesicles. Testicular histopathology included slight to massive diffuse tubular atrophy with degenerative spermatocytes and spermatogonia. The NOAEL dose was considered to be 3.16 mg/kg. After a 10-week recovery period, the effects were reversed in rats receiving 8.25 mg/kg miltefosine, but most effects were not reversed in the rats receiving the high dose of 21.5 mg/kg. In the 52-week toxicology study, reduced spermatogenesis and testicular atrophy were noted in 5/20 rats receiving 4.64 mg/kg/day miltefosine and testicular weights remained significantly reduced after 6-weeks recovery in rats receiving the high-dose of 21.5 mg/kg/day.

In contrast, testicular atrophy was not noted in the Main Study animals in either the 13-week or 52-week toxicology studies in dogs at daily doses of ≤ 10.0 mg/kg. In the 13-week study, 2/4 male dogs in each of the mid-dose (3.16 mg/kg) and high-dose (10.0 mg/kg) groups had reduced prostate weights. A similar trend occurred in the 52-week toxicology study for the same miltefosine doses, but the difference from control values was not significant. High-dose males (1/4 in the Main study and 1/2 in the Recovery study) also exhibited multifocal atrophy and degeneration of seminiferous tubules in association with focal mononuclear infiltrates, but because this finding also occasionally occurs in control animals, the relationship to miltefosine administration is not completely clear. Testicular atrophy and reduced testicular weight was noted in 2/2 high-dose males in the Recovery Study, but not in Main Study animals.

Female fertility was affected in both rats and dogs. In a female fertility study, rats administered miltefosine for four weeks prior to mating and up to Day 7 of pregnancy were not affected for fertility index, but estrus cycle arrest in the metestrus or diestrus phases occurred with the high-dose of 21.5 mg/kg. Reproductive performance was further impacted in a dose-dependent manner at doses of 6.81 and 21.5 mg/kg in the

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form of increased numbers of embryonic and fetal resorptions and the proportion of dead fetuses. Substantial fetal visceral and skeletal malformations also occurred in these dose groups.

Fertility was not assessed in dogs, but in the 52-week toxicology study, females had a dose-related increase in the incidence and severity of atretic follicles in the ovary with follicular development arrested at the secondary stage. Similar cycle arrest was seen in the uterus, vagina, and mammary gland with morphology consistent with anestrus or diestrus. Primarily female dogs receiving 3.16 and 10 mg/kg were affected, although the sexual cycle in 1/4 females administered 1.0 mg/kg/day was also affected. After the 6-week recovery period all of the cycle effects and atretic follicles returned to normal.

In embryo fetal studies in rats and rabbits, miltefosine doses ≥ 6.0 mg/kg caused pronounced fetal resorption in dams treated during the period of organogenesis (Gestation Days 6-15 in rats and Gestation Days 6-18 in rabbits). Miltefosine was a potent teratogen in rats when administered at doses of ≥1.2 mg/kg/day. Malformations which occurred in 52/68 fetuses from dams receiving 2.4 mg/kg/day miltefosine included: undeveloped cerebrum, lumina of the skull filled with hemorrhagic fluid and in a few fetuses further malformations including cleft palate and generalized edema. In another study, a fertility study in female rats, a miltefosine dose of 6.14 mg/kg/day produced dramatic visceral (misshaped cerebral structures, cerebral ventricles filled with brown masses, misshaped spinal cord, and misshaped and malpositioned eyes, hypophysis, absent inner ear) and skeletal (cleft palate, dumbbell shaped ossification of thoracic vertebral centers, increased sizes for skull bones, and markedly dilated suturae) fetal abnormalities. In rabbits, one dam receiving a miltefosine dose of 2.4 mg/kg/day aborted and two exhibited reddened gastric mucosa. However, miltefosine doses of ≤ 2.4 mg/kg/day did not cause maternal toxicity or influence prenatal parameters (number of fetuses, number of resorptions, fetal and placental weights) increased variation rates, or cause malformations in rabbits.

Serious toxicities have been shown to occur in nonclinical toxicology studies including retinal degeneration, testicular atrophy, and dramatically impaired male fertility in rats and follicular atresia in the ovaries of female dogs at doses similar to or below the clinical dose. However, results from the many human studies suggest that the retinal degeneration effects noted in rats are attenuated or absent in humans administered the maximum recommended human dose. Less data is available regarding the male and female reproductive organ effects in humans, and it remains to be seen if these toxicities will present serious challenges to clinical administration.

12 Appendix/Attachments

Table 46: Hematology and Coagulation Parameter Table

Study No.	861344	873450	916266
Species	**rat**	**dog**	**rat**
Hemoglobin concentration	X	X	X
Hemaglobin distribution width	X		
Hematocrit	X	X	X

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Erythrocyte count	X	X	X
Platelet count	X	X	X
Plateletcrit / thrombocrit			
Mean platelet volume			
Mean corpuscular volume	X	X	X
Mean corpuscular hemoglobin	X	X	X
Mean corpuscular hemoglobin concentration	X	X	X
Red cell distribution width			
Total leukocyte count	X	X	X
Reticulocyte count	X	X	
Reticulocyte hemoglobin content			
Differential leukocyte count (Absolute and relative neutrophil, lymphocyte, monocyte, eosinophil, basophil counts)	X	X	X
Blood smear for cell morphology (if necessary for interpretation)			
Activated partial thromboplastin time (APTT)			
Prothrombin time (PT)		X	X

Table 47: Serum Chemistry Parameter Table

Study No.	**861344**	**873450**	**916266**
Species	**rat**	**dog**	**rat**
Aspartate aminotransferase	X	X	X
Alanine aminotransferase	X	X	X
Alkaline phosphatase	X	X	X
Blood urea nitrogen	X		
Urea		X	X
Creatinine	X	X	X
Creatinine kinase	X	X	X
Glucose	X	X	X
Cholesterol	X	X	X
Triglycerides	X	X	X
Total protein	X	X	X
Albumin	X	X	X
Total bilirubin	X	X	X
Sodium	X	X	X
Sorbitol dehydrogenase			
Potassium	X	X	X

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Chloride	X	X	X
Calcium	X	X	X
Inorganic phosphorus or phosphate	X	X	X
Gamma-glutamyl transferase	X	X	X
Glutamate dehydrogenase	X	X	X
Globulin			
Albumin/globulin ratio			

Table 48: Histopathology and Organ Weight Inventory Table

Study #	**861344**	**873450**	**916266**
Species	**rat**	**dog**	**rat**
Adrenals	X, *	X, *	X, *
Aorta	X	X	X
Bone Marrow smear	X	X	X
Bone (sternum, and/or femur and/or rib)	X	X	X
Brain	X, *	X, *	X, *
Bronchi, main stem	X	X	
Cecum	X	X	X
Cervix	X	X	X, *
Colon	X	X	X
Conjunctiva			
Duodenum	X	X	X
Epididymis	X	X	X
Esophagus	X	X	X
Eye	X	X	X
External ear			
Fallopian tube			
Gall bladder		X	
Gross lesions	X	X	X
Harderian gland	X	X	X
Heart	X, *	X, *	X, *
Hypophysis			
Ileum	X	X	X
Infusion site			
Jejunum	X	X	X
Joint, tibiofemoral	X	X	X
Kidneys	X, *	X, *	X, *
Lachrimal gland			

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Larynx		X	X
Liver	X, *	X, *	X, *
Lungs	X	X, *	X
Lymph nodes, inguinal			
Lymph nodes, axillary	X	X	X
Lymph nodes, mediastinal			
Lymph nodes mandibular			
Lymph nodes, mesenteric,	X	X	X
Mammary Gland	X	X	X
Muscle (biceps, femoris)			
Nasal cavity			
Nasal turbinates			
Optic nerves	X	X	X
Ovaries	X, *	X, *	X, *
Oviduct			
Pancreas	X	X	X
ParathyroidS	X	X	X
Peripheral nerve	X	X	X
Peyer’s patches			
Pharynx			
Pituitary	X, *	X, *	X, *
Prostate	X, *	X, *	X, *
Rectum	X	X	X
Salivary gland	X	X	X
Sciatic nerve			
Seminal vesicles	X, *		X, *
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X, *	X, *	X, *
Sternum			
Stomach	X	X	X
Testes	X, *	X, *	X, *
Thymus	X, *	X	X, *
Thyroid	X	X, *	X
Tongue	X	X	X
Tonsils			
Trachea	X	X	X

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Ureter			
Urinary bladder	X	X	X
Uterus	X	X	X, *
Vagina	X	X	X, *
Vertebra, Lumbar			
Zymbal gland			
X = tissue collected for histopathology; * weighed organ			

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/s/

JAMES S WILD
10/04/2013

WENDELYN J SCHMIDT
10/04/2013
I concur with Dr. Wild's assessment of the scope and interpretation of the data in this NDA.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204684 **Applicant: Paladin Therapeutics Inc.** **Stamp Date: April 19, 2013**

Drug Name: Impavido (miltefosine) **NDA Type: NME (1)**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		The absence of Segment III reproductive toxicology studies and carcinogenicity studies was approved previously.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).		X	Many of the early nonclinical studies were performed with different formulations. However, extensive clinical experience with the clinical formulation qualifies the use of the components in the clinical formulation as well as previous use of the components in other marketed products.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?		X	Not all of the pivotal pharm/tox studies were performed according to GLP regulations. Some studies were performed in the 1980s. However, these studies are considered acceptable.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	The labeling language in sections 8.1 and 13.1 will require modification
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurities have not been qualified in nonclinical studies, but they have been qualified by extensive clinical use and specification of threshold limits as stipulated in ICH guidance documents.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Abuse potential should not be a concern
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

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

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

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

None at this time.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

APPEARS THIS WAY ON ORIGINAL

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

JAMES S WILD
05/21/2013

WENDELYN J SCHMIDT
05/22/2013