CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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:	208398
Supporting document/s:	SDN#1, original submission
Applicant's letter date:	4/19/2016
CDER stamp date:	4/19/2016
Product:	Vermox (mebendazole) chewable tablets
Indication:	For the treatment of single or mixed gastrointestina (b) (4) by *Trichuris trichiura* (whipworm); *Ascaris lumbricoides* (large roundworm); and (b) (4)
Applicant:	Janssen Pharmaceuticals, Inc.
Review Division:	DAIP
Reviewer:	Amy Nostrandt
Supervisor/Team Leader:	Wendelyn Schmidt
Division Director:	Sumati Nambiar
Project Manager:	Alison Rodgers

Template Version: September 1, 2010

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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

The current application is for a new dosing form and regimen of the previously approved anthelmintic, mebendazole. The previously approved product was a 100 mg tablet that was administered daily for three consecutive days. The current product is a chewable tablet (that can be partially dissolved prior to administration) that facilitates administration to younger patients, and is a 500 mg tablet to be administered as a single dose.

1.2 Brief Discussion of Nonclinical Findings

Safety pharmacology

Three studies were performed to address potential effects on the cardiovascular system. In a HERG assay, at the highest concentration tested (10 µM, 2953 ng/ml), mebendazole only caused a 5.4% reduction of the membrane K+ current (IKr). In a study in isolated Langendorff-perfused female rabbit hearts, no relevant electrophysiological effects were observed at concentrations up to 10 µM. In a study in anaesthetized guinea pigs, mebendazole (0.16 to 1.25 mg/kg IV) had no statistically significant effects on the parameters examined.

No safety pharmacology studies of the respiratory or central nervous systems were performed. A series of experiments evaluating ex vivo or in vivo alimentary tract effects concluded that mebendazole has no anticholinesterase activity, which could have some bearing in CNS safety.

General toxicology

No general toxicology studies were submitted to this application. The Applicant's summary indicates that NOAEL doses of mebendazole were 10 mg/kg (HED = 1.7 mg/kg) in a 13-week rat study and approximately 2.5 mg/kg/day (HED = 1.25 mg/kg) in a 13-week dog study. Single doses were said to be well-tolerated in multiple species.

Genetic toxicology

Potential for mutation was evaluated in two Ames assays, both of which were considered to be negative under the conditions of the tests. In the mouse lymphoma assay, mebendazole was considered to be mutagenic in the absence of S9 when tested using continuous (24 hour) incubation.

In an in vitro micronucleus assay, mebendazole induced aneuploidy via non-disjunction in cultured human peripheral blood lymphocytes.

Two in vivo mouse micronucleus assays were submitted. In the first, no increase in the number of micronucleated polychromatic erythrocytes (PCEs) was reported. In the second study, mebendazole induced micronuclei in PCEs in the bone marrow of treated mice and fluorescence in-situ hybridization (FISH) evaluation of two samples demonstrated the effect to be due to an aneugenic mechanism. This non-disjunction in dividing cells may be related to the proposed pharmacological mechanism of interference with microtubule function.

Carcinogenicity

No carcinogenicity studies were submitted to this application. The Applicant's summary states that 22- and 24-month studies in mice and rats, respectively, were negative for carcinogenicity.

Developmental and reproductive toxicology

No effects on either male or female fertility in rats were reported when males were treated for at least 60 days prior to mating or when females were treated for 14 days prior to mating and through Day 21 of gestation with doses up to 40 mg/kg/day (HED = 6.7 mg/kg) in food.

Six studies of embryofetal toxicity, in which dams were dosed during the period of organogenesis, were submitted. Two studies in rabbits and one in hamsters appeared to be negative or had an equivocal finding. In two rat studies (including one experiment with single doses administered on specific gestation days) and one mouse study, mebendazole was embryotoxic and teratogenic. Findings included embryo-fetal death (at doses of 10 mg/kg and above in rats and mice; as high as 100% at doses with maternal toxicity) skeletal anomalies involving ribs, limbs (especially hindlimbs), tail, skull , vertebrae and sternum, soft or mixed tissue anomalies including exencephaly, facial cleft / cleft palate, anophthtalmia/ small/ displaced eyes, hydrocephalus, gastroschisis, coelosomy, enlarged atrio-ventricular valve, and unilateral renal and adrenal agenesis. Signs consistent with delayed growth, such as decreased fetal body weights and incomplete ossification, were seen at doses of 10 mg/kg/day and higher. In rats, the findings at the low dose, 2.5 mg/kg/day (HED = 0.42 mg/kg), administered through the period of organogenesis, were similar to control.

In a peri- and postnatal development study, rats were administered 0, 5, 10, 20, and 40 mg/kg in food daily from GD 16 through a 3-week lactation period. There was a dose-related trend toward reduced maternal body weight gain, likely significant at the 40 mg/kg dose. The percent live fetuses was reduced at 40 mg/kg to 61%. All live pups in that group died within the first four days after birth. Pup birth weights were decreased at 20 and 40 mg/kg. No abnormalities were reported, but evaluation was not complete.

1.3 Recommendations

1.3.1 Approvability

The product is approvable from a pharmacology/toxicology perspective. There is a history of clinical use of this dose using this polymorph that can support the safety of this drug product.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Based on review of literature, treatment with mebendazole during pregnancy was not associated with a significant risk of increasing major congenital defects. However, in a cross-sectional study, fetuses exposed to mebendazole during the first trimester of pregnancy, 2.5% had major congenital abnormalities, defined as structural or functional defects that require surgical or medical intervention.

Mebendazole was embryotoxic and teratogenic in pregnant rats at single oral doses as low as 10 mg/kg (approximately 0.2-fold the MRHD, based on mg/m^2).

The estimated background risk in the U.S. general population of major birth defects is 2-4% and of miscarriage is 15-20% of clinically recognized pregnancies.

Clinical Considerations

Data

Human Data

The possible risks associated with prescribing VERMOX™ Chewable during pregnancy, particularly during the first trimester, should be weighed against the expected therapeutic benefits.

Animal Data

Embryo-fetal developmental toxicity studies in rats revealed no adverse effects on dams or their progeny at doses up to 2.5 mg/kg/day. Dosing at ≥ 10 mg/kg/day resulted in maternal toxicity as evidenced by a lowered body weight gain and a decreased number of pregnancies at termination (b) (4) At 10 mg/kg/day, ~~an~~ increased embryo(b) (4) fetal resorption (which was 100% at 40 mg/kg/day), ~~a~~ decreased pup weight and ~~an~~ increased incidence of ~~skeletal~~ malformations (primarily skeletal) were observed. Mebendazole was also embryotoxic and teratogenic in pregnant rats at single oral doses as low as 10 mg/kg (approximately 0.2-fold the MRHD, based on mg/m^2).

In embryo-fetal developmental toxicity studies in mice, doses of 10 mg/kg/day and higher resulted in (b) (4) (b) (4) decreased body weight gain at 10 and 40 mg/kg/day and a higher mortality rate at 40 mg/kg/day (b) (4). At doses of 10 mg/kg/day (approximately 0.1-fold the MRHD, based on mg/m^2) and higher, embryo(b) (4)-fetal resorption increased (100% at 40 mg/kg) and fetal ~~abnormalities~~ malformations were present. Dosing of hamsters and rabbits did not result in embryotoxicity or teratogenicity at doses up to 40 mg/kg/day (0.6 to 1.6-fold the MRHD, based on mg/m^2).

In a peri- and post-natal toxicity study in rats, mebendazole did not adversely affect dams or their progeny at 20 mg/kg/day. At 40 mg/kg (0.8-fold the MRHD, based on mg/m^2), a reduction of the number of live pups was observed and there was no survival at weaning. [redacted] (b) (4)

*Reviewer's comments: The approved label for Vermox 100 mg tablets includes the single sentence regarding the single dose embryo-fetal toxicity study in rats, which is repeated here with the parenthetical comparison to the current clinical dose. The current submission includes a study report for an embryo-fetal study in rats at repeated doses of 2.5, 10, and 40 mg/kg, but a rat study including a 5 mg/kg dose was not originally provided. In SDN #14, another rat study did include animals treated with 5 mg/kg as a single dose on GD 7, 8, 9, or 10. Since there is no study of animals treated throughout organogenesis with 5 mg/kg mebendazole, it is recommended that the first sentence read "*up to 2.5 mg/kg/day."

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In carcinogenicity tests of mebendazole in mice and rats, no carcinogenic effects were seen at doses as high as 40 mg/kg (0.4 to 0.8-fold the MRHD, based on mg/m^2, respectively) given daily over two years. No mutagenic activity was observed with mebendazole in a bacterial reverse gene mutation test. Mebendazole was mutagenic in the absence of S-9 when tested using a continuous (24 hour) treatment incubation period in the mouse lymphoma thymidine kinase assay. Mebendazole was aneugenic *in vitro* in mammalian somatic cells [redacted] (b) (4) In the ~~I~~*in vivo* mouse micronucleus assay, orally administered mebendazole induced an increased frequency of micronucleated polychromatic erythrocytes with evidence suggestive of aneugenicity. [redacted] (b) (4)

Doses up to 40 mg/kg in rats (0.8-fold the MRHD, based on mg/m^2), given to males for 60 days and to females for 14 days prior to gestation, had no effect upon fetuses and offspring. [redacted] (b) (4)

Reviewer's comments: The in vitro genetic toxicity tests are for hazard identification only. The concentration considered to be a threshold by the Applicant was determined in an in vitro study that only looked at two chromosomes in cultures derived from a single individual. The threshold was defined as the lowest concentration at which a significant effect was seen, not the no-effect level, leaving a gap between those concentrations in which effects were unknown. Inclusion of this information infers a safety level that cannot be evaluated in patients and is not clinically useful.

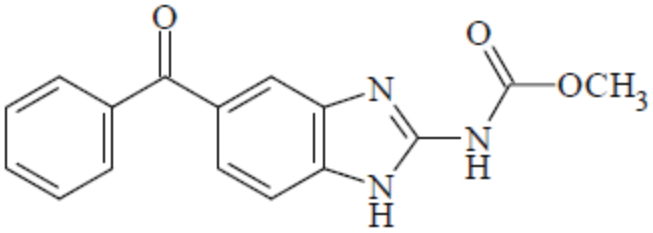
The approved label for Vermox 100 mg tablets described the fertility study as being conducted in mice, not rats. The Applicant submitted the study on which this statement is based in SDN#14, and it was performed in rats. The report did not identify any confirmed maternal toxicity. There was one death at

the highest dose, but the report does not indicate that the death was treatment-related.

2 Drug Information

2.1 Drug

CAS Registry Number
31431-39-7
Generic Name
Mebendazole
Code Name
HJP Product Code: (b) (4)
R 17635
Chemical Name
methyl (5-benzoyl-(b) (4)-benzimidazol-2(b) (4) carbamate
Molecular Formula/Molecular Weight
C16H13N3O3
MW = 295.30
Structure or Biochemical Description



Pharmacologic Class
Anthelmintic

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 115,959 for mebendazole 500 mg chewable tablets
NDA 17-481 for mebendazole 100 mg tablets

2.3 Drug Formulation

The formulation is shown in the Applicant's table below. Mebendazole in this formulation is polymorph C.

Table 1: Composition of the Drug Product

Component	Quality Reference[a]	Function	Quantity/Unit Dose (mg/tablet)
Mebendazole[b]	Company Specification	Active	500.00
Povidone	USP-NF/Ph.Eur.	(b) (4)	(b) (4)
Purified water[c]	USP-NF/Ph.Eur		
Microcrystalline Cellulose	USP-NF/Ph.Eur		
Crospovidone	USP-NF/Ph.Eur		
Sucralose	USP-NF/Ph.Eur.		
Strawberry flavor[d]	Company Specification		
Magnesium stearate	USP-NF/Ph.Eur		
Total tablet weight			

[a] Where multiple compendia are listed, the compendium specific to the applicable region of the submission is applied
[b] Refer to supplier's DMF (b) (4)
[c] Removed during processing
[d] Refer to supplier's DMF (b) (4)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The Applicant states that VERMOX™ CHEWABLE (mebendazole chewable tablets) 500 mg is an anthelmintic indicated for the treatment of single or mixed gastrointestina (b) (4) by *Trichuris trichiura* (whipworm); *Ascaris lumbricoides* (large roundworm); and (b) (4)
It is intended for adult and pediatric (b) (4) 1 year of age) patients, and will be administered as a single VERMOX™ CHEWABLE 500 mg tablet.

2.7 Regulatory Background

NDA 17-481 was approved in 1974.

3 Studies Submitted

3.1 Studies Reviewed

Safety Pharmacology

1. **Preliminary report EDMS-PSDB-2468622: Effects of mebendazole (JNJ-108329-AAA; R017635) on the membrane K+ current IKr in HERG-transfected HEK293 cells compared to astemizole**
2. **Study no. CCS161 (HPC32): Electrophysiological effects of mebendazole on isolated, Langendorff-perfused female rabbit hearts [Anthelmintic]**
3. **Study no. CPF937: Effects of mebendazole on cardio-hemodynamic and cardio-electrophysiological parameters in combination with the**

determination of the compound concentration in plasma, heart and lung tissue in anesthetized guinea-pigs: doses 0.16, 0.32, 0.64 and 1.25 mg/kg intravenously

4. **Internal report: Comparative study of mebendazole and eserine** (Document no. EDMS-ERI-109314177)

Genetic Toxicology

1. **Study title: In vitro mutagenicity study of R17635, lot BF 801**
2. **Study title: In vitro mutagenicity of mebendazole (R 17635) by the Ames Salmonella/microsomal activation assay**
3. **CLE Study no. 1073/19: Mebendazole: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the MicrotitreR Fluctuation Technique**
4. **CLE Study no. 1073/18: Mebendazole: Induction of Micronuclei in Cultured Human Peripheral Blood Lymphocytes**
5. **Study no. l557 (85.08.14): Micronucleus Test in Mice**
6. **Study no. 4785 (CLE Study no. 1073/17): Mebendazole: Induction of Micronuclei in the Bone Marrow of Treated Mice**

Developmental and Reproductive Toxicology (from the original NDA and SDN #14)

1. **Study no. 298: Effects of oral R 17635 on Male and Female Fertility in Rats**
2. **Study no. 495: Potential of oral R 17635 for Embryotoxicity and Teratogenic Effects in Rats after oral administration once daily by gavage**
3. **Study no. 443: Teratology Study (Segment II) of TELMIN® (Mebendazole, PM-JR-17635) in CRL:COBS-CD-l (ICR)BR Outbred Albino Mice**
4. **Study no. 448: Teratology Study (Segment II) of TELMIN® (Mebendazole, PM-JR-17635) in Lakeview Golden Hamsters**
5. **Study no. 311: Potential of oral R 17635 for embryotoxicity and teratogenic effects in rabbits**
6. **Study no. 386: Potential of oral R 17635 for embryotoxicity and teratogenic effects in rabbits**
7. **Study no. 269: Potential of oral R 17635 for embryotoxicity and teratogenic effects in rats**
8. **Study no. 297: Effects of R 17635 in rats after oral administration during the peri- and postnatal period**

3.2 Studies Not Reviewed

Literature references submitted are not reviewed. Pharmacokinetics studies are not reviewed, with the exception of summaries of reviews of two dog studies previously submitted to IND 115,959. The report for Experiment no. 517, the dominant lethal mutation test, was provided, but was not reviewed.

3.3 Previous Reviews Referenced

Nonclinical general toxicology studies of mebendazole were originally reviewed under NDA 17-481 in 1974. The nonclinical reviews from that time are not well detailed, but presumably support the information that was in the label for that product at that time.

4 Pharmacology

4.1 Primary Pharmacology

The Applicant's nonclinical overview indicates that mebendazole is a broad spectrum benzimidazole anthelmintic with activity against nematode and cestode species. It states that the mechanism of action is by interference with cytoplasmic microtubule function in nematode intestinal cells, resulting in degenerative changes which lead to decreased digestion and absorption of nutrients, leading to the death of the parasite.

4.2 Secondary Pharmacology

Not applicable

4.3 Safety Pharmacology

Cardiovascular system

1. Preliminary report EDMS-PSDB-2468622: Effects of mebendazole (JNJ-108329-AAA; R017635) on the membrane K+ current IKr in HERG-transfected HEK293 cells compared to astemizole

(no GLP compliance statement; signature date March 12, 2003)

Whole-cell voltage clamp experiments were performed using HEK293 cells stably expressing the HERG potassium channel in order to assess the effect of mebendazole on IKr current. Astemizole was used as the positive control. Both mebendazole and astemizole were dissolved in DMSO to obtain stock solutions of 10 mM to 3 mM and 30 µM to 1 µM, respectively. The final DMSO concentration was 0.1 %. Patch-clamp experiments were performed, and whole cell currents were recorded at room temperature. Current signals were amplified and digitized by an EPC-9 patch-clamp amplifier, stored and analyzed using Pulse/Pulsefit software (HEKA), DataAccess (Bruxton) and Igor (Wavemetrics). After establishing the whole-cell configuration and a 5 min equilibrium period the following stimulation protocol was applied:

HEK293 cells expressing HERG were clamped at a holding potential of -80 mV. Cells were depolarized by a pre-pulse for 500 ms to –60 mV to determine leak current, then depolarized from the holding potential of –80 mV to +60 mV for 2 seconds followed by a 6 second repolarization back to -40 mV. The peak of the tail current at –40 mV was analyzed and corrected for current run-down and leak current. Pulse cycling rate was 15 seconds. Test pulses were given for 5 minutes to quantify the HERG current under control conditions. Perfusion was switched from solvent control solution to drug-containing solution. The effect of the drug was evaluated after 5 minutes of drug application. One to three concentrations of the drug (applied cumulatively) were tested per cell.

The concentration-dependent effects of mebendazole on the HERG mediated K+ current are presented in the Applicant's table below (3 x 10-6 M: 2.5 % reduction in current; 10-5 M: 5.4 % reduction). Higher concentrations could not be tested due to solubility limits of the compound. Astemizole reduced the peak current amplitude of the HERG-mediated K+ tail current (3 x 10-9 M: 31.4 % reduction in current; 10-8 M: 74.2 % reduction; 3 x 10-8 M: 92.6 % reduction).

Mebendazole was considered to have no relevant effect on the membrane K+ current (IKr) in HERG-transfected HEK293 cells at the concentrations tested as measured by the whole-cell voltage clamp technique. The positive control, astemizole reduced the membrane K+ current (IKr) to a significant extent at lower (nanomolar) concentrations.

Table 1: Inhibition of HERG-mediated K^+ current in HEK293 cells by increasing concentrations of cumulatively applied mebendazole (JNJ-108329-AAA; R017635) compared to astemizole (JNJ-120432-AAA)

		Mean inhibition (%) ± SEM	
	Conc. (M)	Test drug	Solvent control
Mebendazole	3×10^{-6}	2.5 ± 1.0 (n = 4)	2.5 ± 1.8 (n = 4)
	1×10^{-5}	5.4 ± 1.3 (n = 5)	5.2 ± 2.1 (n = 5)
Astemizole	3×10^{-9}	31.4 ± 6.9 (n = 5)	
	1×10^{-8}	74.2 ± 10.2 (n = 5)	
	3×10^{-8}	92.6 ± 4.7 (n = 5)	

The application states that the highest concentration tested (10 µM, 2953 ng/ml) of mebendazole would be 140-fold higher than the free drug Cmax exposure (21 ng/ml) in the highest exposure group (1 - <3 years old) in the recently completed Phase 3 clinical trial in pediatric subjects using the new chewable tablet formulation.

2. Study no. CCS161 (HPC32): Electrophysiological effects of mebendazole on isolated, Langendorff-perfused female rabbit hearts [Anthelmintic]

(Studies conducted between December 2000 and January 2003 at Johnson & Johnson Pharmaceutical Research & Development, Division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (Belgium); no GLP compliance statement)

In two pilot studies (n=1 each), no major effects were seen in one study, while effects were considered "borderline" at 10 µM in the other, leading the investigator to conclude that there could be problems at higher concentrations. Due to the inconsistency in these two studies, the current study of isolated cardiac tissue electrophysiology was performed.

In the current study, the electrophysiological effects of mebendazole (JNJ-108329-AAA, n = 6) and vehicle (0.1% DMSO, n = 6) in isolated Langendorff-perfused, female albino rabbit hearts were investigated.
Reviewer's comment: It is notable that mebendazole concentrations greater than 10 µM were not investigated, although concentrations higher than10 µM were thought to be of potential concern in the pilot study.

For each heart preparation, the bundle of His was dissected, and two stimulating electrodes were sutured in proximity to the distal His-bundle. A recording electrode was placed in the left ventricular sub-endocardium of the septum. An epicardial recording electrode and reference electrode were positioned on the left ventricular epicardium. The heart was paced at 1.5 times threshold stimulation current. If automaticity and escape cycle length were >1000 ms, threshold stimulation current < 300 µA, coronary flow > 17 ml/min, ectopic rate < 8 beats/min, and the cardiac activation time < 60 ms, then the preparation was stimulated until instability of the last 20 trains became less than 10 ms. Only preparations that met these criteria were used.

After recording baseline values, mebendazole (n = 6) at 0.1, 0.3, 1, 3, and 10 µM (± 30 min for each concentration) was continuously infused into the medium. In the vehicle control group, matching volumes of 0.1% DMSO were used (n = 6). The following electrophysiological parameters were measured:

- action potential duration (APD) at 30%, 60% and 90% repolarization (APD30, APD60 and APD90; in ms),
- instability index of the APD (instability in ms was obtained by calculating the difference between the upper and lower quartile estimates of APD60 over 3 min during the control period and each concentration of vehicle or drug perfusion),
- triangulation of the action potential (difference between APD30 and APD90; in ms),
- an index of proarrhythmia (number of ectopic beats, excluding ventricular tachycardia or fibrillation, during the control period and the last 10 min of each drug perfusion; in extra beats/min over the pacing rate of 60 beats/min),
- reverse-use dependence of the action potential (median value of the last 20 APD60 measurements minus the median value of the first 10 APD60 measurements at 1 Hz: reverse-use; in ms),
- coronary flow (in ml/min), and
- intraventricular conduction time (IVC as a percentage of the control value; values reported in data tables are in ms).

The incidence of early after-depolarizations (EADs), torsades de pointes (TdPs), ventricular tachycardia (VT), and ventricular fibrillation (VF), if present, was also recorded.

Data on electrophysiological parameters (only the APD at 60% repolarization appears to have been analyzed) and coronary flow were sampled at baseline and after each 15-min perfusion period of vehicle or test article at 1 Hz (except for IVC, which was measured at 300 ms cycle length electrical stimulation). The index of proarrhythmia was measured over the last 10 min of 30 min perfusion with each concentration of the compound or vehicle. All values (actual units and percentage changes from baseline values) are expressed as median (minimum and maximum) in the Applicant's tables below.

Table 1: Effects of vehicle (0.1% DMSO) on the electrophysiological parameters and coronary flow, expressed in actual units, in isolated, Langendorff perfused rabbit hearts (n = 6).

Parameters						
min -->	0	10	20	30	40	50
M -->	Baseline	1×10^{-7}	3×10^{-7}	1×10^{-6}	3×10^{-6}	1×10^{-5}
APD_{60} (ms)	208 193/232	209 187/231	204 181/241	208 174/221	207 176/241	213 183/257
Triangulation (ms)	74 58/85	72 53/84	72 60/81	71 45/87	71 39/81	63 40/69
Instability (ms)	6 2/10	6 4/13	4 3/8	7 2/15	4 2/7	4 3/5
Reverse Use (ms)	-3 -4/-2	-4 -7/-1	-4 -6/-2	-3 -5/-1	-5 -6/-2	-5 -7/-2
Proarrhythmia (extra beats/min)	7 1/12	5 1/35	6 0/10	4 1/9	6 1/23	3 2/6
Coronary Flow (ml/min)	25 21/31	26 22/30	24 21/29	25 21/28	23 20/28	23 20/28
I V C (ms)	99 83/104	100 88/105	100 88/109	103 88/105	103 88/106	103 88/108

Values are median (minimum/maximum).

Table 2: Effects of mebendazole on the electrophysiological parameters and coronary flow, expressed in actual units, in isolated, Langendorff-perfused rabbit hearts (n = 6).

Parameters						
min -->	0	10	20	30	40	50
M -->	Baseline	1×10^{-7}	3×10^{-7}	1×10^{-6}	3×10^{-6}	1×10^{-5}
APD_{60} (ms)	217 208/230	232 210/239	233 207/242	231 202/245	229 195/236	228 191/237
Triangulation (ms)	79 63/105	83 58/92	75 55/103	75 57/87	74 53/92	72 53/86
Instability (ms)	3 2/7	4 3/9	5 2/7	5 2/5	6 2/8	6 3/14
Reverse Use (ms)	-3 -5/-1	-2 -3/1	-3 -4/-2	-3 -5/-1	-3 -4/-1	-3 -5/-1
Proarrhythmia (extra beats/min)	2 0/8	2 0/15	1 0/5	2 0/21	4 0/10	3 0/9
Coronary Flow (ml/min)	23 14/31	22 14/26	20 13/23	21 13/23	20 13/23	19 13/22
I V C (ms)	101 100/106	102 101/103	102 100/103	103 100/106	104 100/106	104 99/112

Values are median (minimum/maximum).

Mebendazole, at increasing concentrations of 0.1, 0.3, 1, 3 and 10 μM (n = 6) did not cause physiologically relevant changes in the duration of the action potential at 60%

repolarization (APD60), triangulation, instability, the index of proarrhythmia, reverse-use dependence of the action potential, coronary flow, or intraventricular conduction time (IVC), relative to vehicle (n = 6). At 0.3 µM, APD60 was statistically significantly increased relative to vehicle (+5% *versus* -4% with vehicle; $p < 0.05$). However, since there was no dose-dependence, and the values were within the range of historical controls, this change was not considered to be a relevant compound-related finding.

Mebendazole at concentrations from 0.1 to 10 µM did not elicit early after-depolarizations (EADs), torsades de pointes (TdPs), ventricular tachycardia (VT; unlike TdPs) and ventricular fibrillation (VF).

The study concluded that mebendazole (JNJ-108329-AAA), at concentrations of 0.1, 0.3, 1, 3 and 10 µM, did not cause any significant or physiologically relevant changes in the measured electrophysiological parameters, the index of proarrhythmia, coronary flow, or intra-ventricular conduction time (IVC), and did not elicit severe arrhythmias including EADs, TdPs, VT and VF.

3. Study no. CPF937: Effects of mebendazole on cardio-hemodynamic and cardio-electrophysiological parameters in combination with the determination of the compound concentration in plasma, heart and lung tissue in anesthetized guinea-pigs: doses 0.16, 0.32, 0.64 and 1.25 mg/kg intravenously

(Study conducted in 2003 by Janssen Research & Development, Division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (Belgium); original study report EDMS-PDSB-2889474 issued August 2003; current revised report completed in January 2012; no GLP compliance statement)

The test article, mebendazole (JNJ-108329-AAA-9547104; R017635) was dissolved at concentrations of 0.32, 0.64 and 1.28 mg/ml in solutions containing hydroxypropyl-beta-cyclodextrin (20%), HCl, NaOH, mannitol and pyrogen-free water (pH = 4.16 to 4.40; 270 to 303 mOsmol/kg. A similar solution without compound was also prepared (pH = 4.90; 309 mOsmol/kg) and used as the vehicle control. Formulation analysis was said to demonstrate that mebendazole concentrations were 103% to 105% of the target concentrations and that the test articles were stable for the duration of the study.

Fourteen female Dunkin Hartley guinea-pigs (437 to 579 grams body weight) were randomized to treatment with mebendazole or vehicle. They were anesthetized with sodium pentobarbital and placed on an electrical heating pad (temperature 34°C). Tracheotomies were performed to facilitate ventilation of the animals with room air. Catheters were inserted into a carotid artery for monitoring the arterial blood pressure and into a jugular vein for intravenous administration of test article or vehicle. Needle electrodes were attached for the recording of the ECG (leads I, II and III). Mean arterial blood pressure (BPm in kPa) was recorded using a pressure transducer attached to the arterial catheter. ECG recordings were used to calculate heart rate (HR in beats/min) and the duration of the PQ, RR and QT intervals (in ms), and QRS duration (in ms) were measured. Corrected QT interval (QTc in ms) was calculated using Bazett's formula (QTc = QT/ RR).

After instrumentation and a stabilization period of approximately 30 minutes, baseline control values were recorded. Animals were then dosed with mebendazole at escalating doses of 0.16, 0.32, 0.64 and 1.25 mg/kg IV (n=7), or with corresponding volumes of the vehicle (0.5, 0.5, 0.5 and 1 ml/kg (n=7), administered at 15-min intervals to the same animal (total dose = 2.37 mg/kg IV over 45 minutes). The different doses of compound or vehicle were each injected as a bolus over 1 to 3 seconds. After each injection the catheter was flushed with 0.1 ml of saline. Data on hemodynamic and ECG parameters were recorded before the first administration and at 5 min after each intravenous injection of vehicle or test article. The incidence of cardiac conduction disturbances was recorded in vehicle- and compound-dosed animals, but none were reported.

Median and extremes (min/max) of the values during the control period, and at 5 min after each intravenous injection of vehicle or compound, are presented in Applicant's tables below:

Table 1: Effects of vehicle on heart rate (HR), mean arterial blood pressure (BPm) and ECG parameters in anesthetized guinea-pigs (data expressed as actual values)

Parameters	Solvent i.v.; n = 7				
	0 min	5 min	20 min	35 min	50 min
	Baseline	0.16 mg/kgeq	0.32 mg/kgeq	0.64 mg/kgeq	1.25 mg/kgeq
HR (b/min)	231 (191/254)	224 (189/245)	210 (187/230)	207 (180/223)	201 (163/218)
BPm (kPa)	3.7 (3.1/4.6)	3.7 (3.4/4.9)	3.9 (3.4/4.8)	4.0 (3.3/4.8)	3.7 (3.2/4.9)
PQ-interval (ms)	58 (49/64)	58 (50/64)	58 (51/65)	60 (52/66)	61 (56/67)
QRS-duration (ms)	49 (45/54)	49 (46/53)	48 (44/55)	49 (47/51)	49 (46/52)
QT-interval (ms)	195 (178/240)	195 (184/244)	201 (192/245)	203 (196/259)	209 (203/277)
QTcB-interval (ms)	379 (350/431)	380 (353/434)	388 (365/437)	390 (366/450)	393 (368/456)

Values are median (min/max).
eq = equivalent: the volume of the vehicle is the same as the volume of the compound.

Table 2: Effects of mebendazole on heart rate (HR), mean arterial blood pressure (BPm) and ECG parameters in anesthetized guinea-pigs (data expressed as actual values)

Parameters	Mebendazole i.v. ; n = 7				
	0 min	5 min	20 min	35 min	50 min
	Baseline	0.16 mg/kg	0.32 mg/kg	0.64 mg/kg	1.25 mg/kg
HR (b/min)	211 (193/233)	213 (186/232)	202 (180/227)	194 (168/226)	187 (167/224)
BPm (kPa)	4.3 (2.8/4.6)	4.4 (3.4/5.1)	4.4 (3.6/5.1)	4.3 (3.7/5)	4.7 (4/5.4)
PQ-interval (ms)	58 (56/70)	58 (56/71)	61 (59/74)	63 (60/74)	66 (61/78)
QRS-duration (ms)	49 (45/55)	50 (44/53)	49 (44/54)	51 (45/55)	49 (44/53)
QT-interval (ms)	204 (189/244)	207 (190/243)	219 (194/249)	223 (195/257)	224 (196/262)
QTcB-interval (ms)	388 (372/433)	393 (373/431)	397 (379/429)	401 (379/434)	402 (379/439)

Values are median (min/max).

Percentage changes relative to the corresponding baseline values were calculated for vehicle and test article-treated groups. Statistical comparison of the changes relative to the corresponding baseline values (in actual units) observed in the group dosed with mebendazole with those values in the vehicle control was performed using the Wilcoxon-Mann-Whitney test

The study report stated that guinea pigs administered mebendazole at doses of 0.16 to 1.25 mg/kg IV (total dose 2.37 mg/kg IV; n = 7) had no statistically significant differences in heart rate, mean arterial blood pressure, the duration of the QRS, QT and QTcB intervals, and no induced changes in ECG morphology, relative to vehicle controls (n = 7).

A statistically significant difference was seen for the duration of the PQ interval at 0.32 and 0.64 mg/kg mebendazole. This change was not considered to be relevant, since a dose-related change at the higher dose was not observed.

Reviewer's comment: Heart rate slowed and QT/QTc increased with time and dose in both vehicle- and mebendazole-treated groups. The magnitude of the change appeared greater in the mebendazole-treated group. It is unclear whether prolongation was related to heart rate or to test article effects.

An arterial blood sample (4 ml) was taken from the animals dosed with mebendazole 5 minutes after the intravenous injection of the highest dose. Plasma was separated and frozen until analysis. The animals were euthanized and heart and lungs were removed. Heart and lung tissue samples were stored frozen until they were analyzed for drug concentrations.

The median plasma level of mebendazole at 5 min after the intravenous injection of 1.25 mg/kg was 1,390 ng/ml. The median concentration of mebendazole in heart and lung tissue was 638 ng/g and 1320 ng/g, respectively. The median ratio of the compound concentration in heart tissue *to* plasma concentration was 0.39. The median ratio of the compound concentration in lung tissue *to* plasma concentration was 1.01.

Reviewer's comments: Biological relevance of QT values should have been taken into account. Increases of 10 ms or more were seen in treated animals and could be clinically relevant.

Nevertheless, mebendazole was administered IV in this study, and exposures should have been much higher than after single oral dosing. With lower oral bioavailability, QT interval may be unaffected.

Digestive system:

4. Internal report: Comparative study of mebendazole and eserine

(Document no. EDMS-ERI-109314177)

This document summarizes results for in vitro studies on isolated guinea pig ileum and rabitt duodenum exposed to a range of concentrations of R 17 635 (mebendazole) and limited in vivo observations of rats and mice administered oral doses of R 17 635. The comparator, eserine is a plant-derived slowly reversible cholinesterase inhibitor.

The document states that there was no potentiation of cholinergic effects on isolated guinea pig ileum at concentrations of 0.63 to 40 mg/L of R 17 635. It states that acetylcholine- and methacholine-induced contractions were potentiated by eserine at concentrations of 0.04 mg/L and higher.

The document states that no increase of intestinal tone was observed in rabbit duodenum in the presence of R 17 635 at concentrations up to 10 mg/L. An increase in tone was induced by eserine at concentrations of 0.04 mg/L and higher.

In rats or mice administered oral doses of R 17 635 up to 160 mg/kg, the document states that "no eserine-tremors were observed."

The signatory scientist concluded that mebendazole is "devoid of anticholinesterase activity."

Other:

No safety pharmacology studies of CNS or respiratory effects were provided. The application states that in vivo toxicology studies in rats, mice and dogs did not reveal any adverse effect on general behavior, body temperature, cardiovascular or respiratory parameters.

(b) (4) proposed labeling for this product include warning for the risk of convulsions in children administered mebendazole.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Two pharmacokinetics studies in were performed in dogs and reviewed under IND 115,959. Results are summarized below.

1. Study no. FK10471

After a single oral 7 mg/kg dose of mebendazole to male beagle dogs (N=6, 1-week washout between doses), peak plasma concentrations were generally observed between 0.5 and 1.5 hours after dosing, and high concentrations were still

(b) (4)

(b) (4)

Table 2: Mean (SD) Plasma Pharmacokinetic Parameters Following a Single 7 mg/kg Oral Gavage Dose of Mebendazole in 4 Different Polymorph Suspensions (N=6/Treatment)

Parameter	(b) (4)
C_{max} (ng/mL)	
t_{max} (h)	
$t_{1/2}$ (h)	
AUC_{0-24h} (ng·h/mL)	
$AUC_{0-\infty}$ (ng·h/mL)	

Note: The dogs (N = 6) were fasted for approximately 21 hours prior to dosing and were fed approximately 2 hours after dosing. Dogs were washed out for at least 1 week between doses. Blood samples were removed at predose (0.0 hour), 0.5, 1, 1.5, 2, 4, 7 and 24 hours postdose.

[a] N=3; [b] N=2; [c] N=5; [d] N=1

NC = not calculated; SD = standard deviation; SLS = sodium lauryl sulfate

(b) (4)

2. Study no. FK10505:

A study was performed to compare the pharmacokinetic profiles of four different tablet formulations of mebendazole (polymorph C), including the proposed drug product, in 6 male Beagle dogs after a single oral dose of 500 mg/dog. Each tablet formulation was disintegrated in water prior to administration. Dogs were washed out for at least 1 week between doses. Analysis was limited to data from 5 dogs/treatment, since one male (animal no. 3M) was an outlier in all 4 phases of the study.

In nearly all animals, plasma concentrations were higher at the 24 hour sampling time than at the 7 hour sampling time, and in some cases, higher than the initial Tmax,

suggesting persistence in drug absorption in the intestinal tract, e.g. enterohepatic recirculation. Based on the mean $AUC_{0-24\,h}$ values, the order of exposure of the different tablets was Non-chewable Tablet Current Donation program > New Chewable Tablet+SLS > Chewable Tablet > New Chewable Tablet. However, variability was again high, and none of the formulations was clearly absorbed to a higher extent than another. The addition of SLS to the new chewable formulation did not appear to have a major impact on absorption.

Table 3: Summary of Mean (SD) Pharmacokinetic Parameters of Mebendazole in the Dog (n=5/treatment) After a Single 500-mg Tablet Dose of Mebendazole

	Non-Chewable Tablet (current donation program)	Current Chewable Tablet	New Proposed Chewable Tablet	
			without SLS	+ SLS
C_{max} (ng/mL)	35.3 (12.0)	23.4 (4.44)	17.0 (5.04)	22.7 (7.44)
t_{max} (h)	5.20	9.90	9.60	1.40
$t_{1/2}$ (h)	2.[31a]	2.072	3.35	4.14
AUC_{0-24h} (ng.h/mL)	305 (46)	221 (92.1)	202 (69.6)	245 (106)
AUC_{0-31h} (ng.h/mL)	343 (39)	258 (115)	222 (76.6)	271 (116)
$AUC_{0-\infty}$ (ng.h/mL)	357 (43)b	294 (111)2	228 (77.9)	283 (129)
% Non-Chewable (Frel)[c]	100	64	59	85

Note: Dogs were washed out for at least 1 week between doses. 500 mg tablets were admixed with water in a syringe prior to dose administration. The contents of the syringe were then administered by oral gavage followed by a 5-mL water rinse of the syringe to each dog. Blood samples were removed at predose (0.0 hour), 0.5, 1, 1.5, 2, 4, 7, 23, 24, 26 and 31 hours postdose.

[a] n = 3; [b] n = 4;

[c] = Frel (%) analysis was performed by comparing the AUC_{0-24h} values of each tablet to the Non-Chewable Tablet. The mean AUC_{0-24h} (SD) values have been changed since the Briefing Book, but overall %Frel analysis has not changed.

n = number (subset of sample); SD = standard deviation; SLS = sodium lauryl sulfate

The following summary of absorption, distribution, metabolism and excretion of mebendazole following oral administration to rodents and dogs is found in the Applicant's nonclinical overview:

Absorption

"Low dissolution of mebendazole in the gastrointestinal tract explains the relatively poor oral bioavailability (<20%) in rats and dogs. Peak mebendazole concentrations in the plasma are attained within 2 to 4 hours in rats, and within 24 hours after oral administration, the majority of the administered dose was found in the gastrointestinal tract and/or feces as unchanged drug.

Mebendazole is chemically available in 3 different (b) (4) with a potential for different pharmacokinetic properties. (b) (4) was used in the nonclinical toxicity studies submitted to NDA 17-481, and was the active pharmaceutical ingredient (API) in the original approved 100-mg chewable tablet. However, (b) (4) is used for the new 500-mg chewable tablet. Therefore, a PK study was recently conducted to compare the bioavailability of the (b) (4).

(b) (4)

[redacted]

Using the polymorph C form of the API, the 500-mg solid oral tablet was compared to the previous 500-mg and the new 500-mg chewable tablets in a relative bioavailability study in dogs. All formulations showed substantial inter-animal variability and mean mebendazole plasma concentration-time profiles; however, the previous 500-mg chewable tablet and the new chewable tablet had lower bioavailability than the solid oral tablet. The dog may not be the best animal model for evaluating mebendazole tablet formulations for predicting PK in humans since these results are not consistent with the human PK data."

Distribution

"After oral administration to rodents and dogs, mebendazole remains largely in the gastrointestinal tract because of its poor absorption. In the gastrointestinal tract, mebendazole is present primarily as unchanged parent compound (>80% of dose). The very low amount of absorbed mebendazole is rapidly metabolized. Mebendazole and its metabolites are distributed to tissues, including highly perfused organs such as lungs, kidney, liver and gonads. Although metabolism of mebendazole does not occur in gastrointestinal tract, the intestinal microflora are capable of hydrolyzing the conjugated glucuronides excreted in bile. The hydrolyzed aglycones may be subject to enterohepatic recirculation. The elimination half-life of mebendazole related metabolites from the plasma is between 2 to 3 hours in rats."

Metabolism

"Mebendazole is metabolized via ketone reduction and carbamate hydrolysis followed by glucuronide or sulfate conjugations. After oral administration, mebendazole is metabolized in the liver and the resulting metabolites excreted in urine and bile. Hydrolyzed mebendazole (H-MBZ) is the predominant metabolite in urine, whereas glucuronide and sulfate conjugates of reduced (R-MBZ), hydrolyzed and reduced-hydrolyzed metabolites (RH-MBZ) are found in bile. Plasma concentrations of mebendazole in rat and dog were not well studied, however, relatively high mebendazole exposure was observed in the mouse. Due to relatively low concentrations observed in the systemic circulation, H-MBZ and R-MBZ were not quantified in rat or dog plasma. In mice, plasma H-MBZ and R-MBZ were quantified at

concentrations slightly lower than mebendazole. Overall, the metabolic pathways in nonclinical species and humans are similar.

H-MBZ does not have pharmacological activity because the presence of a carbamate group in the 2-position of benzimidazole is essential for binding to β-tubulin. Therefore, hydrolysis of the carbamate function abolishes pharmacological activity. R-MBZ maintains a much lower pharmacological activity compared to mebendazole. Reduction of the carbonyl moiety of mebendazole to R-MBZ resulted in a 9- to 11-fold loss of activity in the tubulin binding assay."

Excretion

"Mebendazole is poorly absorbed; more than 90% of the orally administered dose is excreted in the feces. Following a single-dose, relatively higher levels of mebendazole related metabolites were excreted in bile than in urine. However, urinary and biliary excretion may be equally important in a multiple dosing regimen. The elimination half-life for mebendazole and related metabolites in the bile is dose-dependent, ranging from 5 to 9 hours in rats. A great majority (≥90%) of the mebendazole related metabolites are excreted from treated animals within 48 hours after oral administration."

6 General Toxicology

No general toxicology studies were submitted for review. The descriptions of data in the sections below are derived from the Applicant's nonclinical overview.

6.1 Single-Dose Toxicity

"Acute single-dose toxicity was tested in 12 different animal species (mice, rats, guinea pigs, pheasants, chickens, cats, rabbits, dogs, horses, sheep, cattle, and pigs). Mebendazole was well tolerated with very low oral toxicity which may be related to the low solubility and poor bioavailability."

6.2 Repeat-Dose Toxicity

"Mebendazole was administered to rats in the diet for 13 weeks (0, 10, 40, 160 mg/100 g food; ~10, 40, 160 mg/kg/day). No effects were observed at 10 mg/kg. At 40 mg/kg, some slight changes occurred: the hair coat became rough during the first weeks of the experiment, 2 male rats died and in some males small sized gonads and a relative liver weight increase were found. Histologically, some mild testicular changes were detected. At 160 mg/kg a clear toxicity was found: the food consumption dropped during the first weeks of the experiment, followed by a significant weight decrease. The hair coat was rough, the albumin level was subnormal in males, whereas females showed an increased alkaline phosphatase level. Four males and 7 females died. The gross pathology and the histopathology were the same as with 40 mg/kg but much more pronounced. Additionally, signs of a chronic liver stimulation and an inhibition of spermatogenesis were found. Overall, the effects were clearly drug- and dose-related. All these results can be explained by the poor general condition as a result of a low food intake and chronic hepatic stimulation. The important role of the liver in the metabolism of mebendazole has been confirmed by the pharmacokinetic studies.

Young beagle dogs were treated orally 6-days a week for 13 weeks with doses of 0, 2.5, 10 and 0.63 mg/kg weeks 1-7 followed by 40 mg/kg weeks 8-13. No deaths occurred during 13-weeks of mebendazole administration and there were no toxic effects. Liver weight slightly increased at 10 and 40 mg/kg; however, this finding was not associated with histological changes. Young beagle dogs were treated orally 6-days a week for 24 months (0, 2.5, 10, 40 mg/kg). No mebendazole-related deaths or toxicity were noted during the study."

A published study comparing toxicity after administration of different mebendazole polymorphs in mice was previously submitted and reviewed under IND 115,959. The review is reproduced here.

1. Rodriguez-Caabeiro, F et al. Experimental chemotherapy and toxicity in mice of three mebendazole polymorphic forms. Chemotherapy 33:266-271 (1987).

The paper submitted describes experiments in mice to determine the LD_{50} values after oral (p.o.) and intraperitoneal (i.p.) administration and the anthelmintic effect of these polymorphic forms on the enteral and parenteral phases of the nematode *Trichinella spiralis.*

Test animals were male and female Swiss mice, weighing 15-25 g. The individual polymorphic forms of the test article mebendazole were suspended in 0.5%(w/ v) methylcellulose 1500. Dose volumes ranged from 5-40 mL/kg.

For LD_{50} determination, groups of 5 male and 5 female mice were randomly assigned to treatments. Groups were dosed either p.o. or i.p. with the polymorphic form tested, while another group only received the vehicle. The observation period was 15 days. Results are shown in the authors' table below:

(b) (4)

A series of four experiments were performed to evaluate the effect on multiple life stages of the parasite, *Trichinella spiralis*. In this study the GM-I strain of *T. spiralis*, which was isolated from a wildcat in Spain in 1963, was used. Each experiment was performed on groups of 8 female mice. Groups were dosed with the respective polymorphic form while the control group received the vehicle. The efficacy treatment of

the parenteral phase was determined by counting the number of muscle stage larvae appearing after pepsin digestion of infested mice at 30 days postinfestation (p.i.). Although not specifically stated, it is assumed that dosing was by the oral route, and that evaluation of anthelmintic efficacy of the enteral phases of the parasite was performed by fecal egg counts. Results are shown in the authors' table below:

Table IV. Effects of MBZ polymorphic forms on the preadult, adult, emigrant, and encysted larvae stages of *T. spiralis*

Stage	Dosage (mg/kg)	Day(s) of drug administration (days p.i.)	Day(s) of recovery of the adults or larvae (days p.i.)	Percentage of reduction in the number of adults or larvae (b) (4) (b) (4)
Preadult	30	1	8	
Adult	40	6	8	
	60	6	8	
Emigrant larvae	40	13, 14, 15	30	
	60	13, 14, 15	30	
Encysted larvae	60	45 ,46, 47	52	
	60	45, 46, 47	62	

* $p < 0.01$ with respect to control values; ** $p < 0.005$ with respect to control values.

In general, (b) (4)

The authors concluded that (b) (4) and the authors recommended the use of polymorph C for oral treatment with mebendazole in mice, (b) (4)

7 Genetic Toxicology

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

1. Study title: In vitro mutagenicity study of R17635, lot BF 801

Study no.:	Not provided
Study report location:	Section 4.2.3.3.1 of electronic submission
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	Not provided
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R17635, lot BF 801, purity not provided

Key Study Findings

R17635 (mebendazole) was considered to be non-mutagenic in the S. typhimurium strains tested.

Methods

Strains:	S.typhimurium strains TA 1530, TA 1535, TA 1538, TA 98, TA 100, TA 1537 and TA 97.
Concentrations in definitive study:	0, 10, 50, 100, 250, 500, 1000, and 2000 µg/plate in the absence and presence of S9 (Aroclor 1254-induced) 0, 50, 100, 250, 500, and 1000 µg/plate in the presence of S9 (phenobarbital-induced) in strains TA1530, TA1535, TA 1537, and TA1538 only
Basis of concentration selection:	Preliminary "spot test" in the presence and absence of S9 using discs containing 100, 500, and 1000 µg per disc. Mutagenicity results were negative in all strains tested
Negative control:	Unclear, but probably vehicle
Positive control:	NaN_3 (0.1 µg/plate) in the absence of S9 for strains TA 100, TA 1530, and TA 1535 Nitrofluorene (0.5 µg/plate) in the absence of S9 for strains TA 98, TA 1538, and TA 1537 2-amino-anthracine (1 µg/plate) in the presence of S9 for all strains
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 hours at 37ºC in the dark

Study Validity

Criteria for acceptance and positive results were not provided

Results

The results of all assays using either the "spot test" or the plate incorporation method were negative.

2. Study title: In vitro mutagenicity of mebendazole (R 17635) by the Ames Salmonella/microsomal activation assay

Study no.:	Not provided
Study report location:	Section 4.2.3.3.1 of electronic submission
Conducting laboratory and location:	(b) (4) [redacted] S
Date of study initiation:	6/01/1982
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Mebendazole (R 17635), lot BF801, purity not provided

Key Study Findings

The report concluded that mebendazole "exerts no direct or indirect mutagenic activity towards strain TA98 and TA100" under the conditions of the experiment.

Methods

Strains:	S. typhimurium strains TA98 and TA100
Concentrations in definitive study:	0, 0.01, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 mg/plate
Basis of concentration selection:	Not specified
Negative control:	Not specified, presumably solvent
Positive control:	2-nitrofluorene (2-NF) Benzo (a) pyrene (B(a)P) 2-aminoanthracene (2-AA) Methyl methanesulfonate (MMS)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 hours at 37ºC in the dark

The report states that this study is to amend the "previous report of May 27, 1982," but it is unclear whether or not this refers to the above study. Assays were performed using S9 liver post-mitochondrial fractions from rats pre-treated with different compounds (Aroclor 1254, 500 mg/kg; 3-methylcholanthrene, 2 x 40 mg/kg; phenobarbitone 0.1% w/v in drinking water for 7 days). Three concentrations of S9 mix derived from each chemical induction were used in assays with S9 (25, 50, and 150 µL/plate).

The classical Ames assay was compared to a modified procedure referenced in the report which modifies the agar/medium components. Assays were carried out in duplicate.

Study Validity

Criteria for study acceptance or for a positive result were not provided.

Results

Comparison of positive controls 2-NF, MMS, and 2-AA, in the absence of S9, using the two methods revealed higher numbers of revertant colonies, particularly at higher tested concentrations. The same appeared to be true for B(a)P in the presence of Aroclor-induced S9. The report stated that both bacterial strains were more sensitive to 2-AA and B(a)P when tested using the modified procedure.

Mebendazole, tested using the modified procedure, did not appear to result in an increased number of revertant colonies in the absence of S9 in either bacterial strain. There also did not appear to be any significant increase in the number of revertant colonies over control in the presence of S9, regardless of the chemical used to induce S9 or the concentration of S9 in the plate.

The report states that mebendazole "exerts no direct or indirect mutagenic activity towards strain TA98 and TA100" under the conditions of the experiment. No apparent differences in results were observed in the presence of S9 fractions induced by different compounds, nor were the results affected by changing S9 concentration.

7.2 *In Vitro* Assays in Mammalian Cells

3. Study title: Mebendazole: Mutation at the Thymidine Kinase *(tk)* Locus of Mouse Lymphoma L5178Y Cells (MLA) using the MicrotitreR Fluctuation Technique

Study no.:	CLE Study Number 1073/19
Study report location:	Section 4.2.3.3.1 of electronic submission
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	6 November 1998
GLP compliance:	Yes, OECD
QA statement:	Yes
Drug, lot #, and % purity:	Mebendazole, batch number ZR017635EXA804, purity 100%

Key Study Findings

Statistically significant increases in mutant frequency were obtained following 24-hour incubation with mebendazole in the absence of S9 metabolic activation, but not following 3-hour incubation in the absence of S9 or following incubations in the presence of S9. It was concluded that mebendazole is mutagenic in the absence of S-9 when tested using a continuous (24 hour) treatment incubation period.

Methods

Cell line:	L5178Y TK +/- mouse lymphoma cells
Concentrations in definitive study:	For Experiment 1: Eight concentrations were chosen ranging from 0.25 to 40 µg/mL in the absence and presence of S-9 (3 hour

	incubation). Ten concentrations were chosen ranging from 0.002 to 1 µg/mL in the absence of S-9 (24 hour incubation). For Experiment 2: Nine concentrations were tested in the absence of S-9 (24 hour incubation), ranging from 0.025 to 0.25 µg/mL, and eight concentrations were tested in the presence of S-9 (3 hour incubation), ranging from 1.25 to 20 µg/mL. For Experiment 3: Nine concentrations were tested in the absence and presence of S-9, ranging from 11 to 19 µg/mL (3 hour incubation).
Basis of concentration selection:	In a range-finding cytotoxicity experiment, cells were treated by 3-hour incubation in the presence of S9 and 3- and 24-hour incubations in the absence of S9. For 3 hour incubation, the treatment range was from 2.5 to 80 µg/mL (limit of solubility). "Extreme toxicity" was reported at the top 2 concentrations; the highest concentration with >10% relative survival was 20 µg/mL (53 and 58% relative survival in absence and presence of S9, respectively). For the 24-hour incubation, the treatment range was from 0.3125 to 80 µg/mL. "High toxicity" was reported at all concentrations.
Negative control:	DMSO diluted 100-fold in the cell culture medium
Positive control:	4-nitroquinoline 1-oxide (without S9) and benzo(a)pyrene (with S9)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 hours in the presence and absence of S9 metabolic activation, and 24 hours in the absence of S9

Study Validity

The assay was considered valid if all the following criteria were met:

1. The mutant frequencies in the negative (solvent) control cultures fell within the normal range (above 60 mutants per 106 viable cells but not more than three times the historical mean value)

2. At least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value)
3. The plating efficiencies of the negative controls from the mutation experiments were between the range of 60 % to 140 % on Day 0 and 70 % to 130 % on Day 2.

The test article was considered to be mutagenic if all the following criteria were met:

1. The assay was valid
2. The mutant frequency at one or more concentrations was significantly greater than that of the negative control ($p < 0.05$)
3. There was a significant dose-relationship as indicated by the linear trend analysis ($p < 0.05$).

Negative (solvent) and positive control treatments were included in each mutation experiment in the absence and presence of S-9. Mutant frequencies in negative control cultures were reported to have fallen within normal ranges, and clear increases in mutation were induced by the positive control chemicals 4-nitroquinoline I-oxide (without S-9) and benzo(a)pyrene (with S-9). The study was accepted as valid.

Results

Cytotoxicity assay:

Precipitate was observed at the top two concentrations tested (40 and 80 µg/mL) at the time of addition of the test article to the cultures and following the treatment incubation period. F ollowing the treatment incubation period, precipitate was also observed at 20 µg/mL. "Extreme toxicity" was observed at the top two concentrations tested in the absence and presence of S9 (40 and 80 µg/mL). The report stated that the top concentration tested that yielded > 10% relative survival was 20 µg/mL (53.02% relative survival in the absence of S9 and 58.27% relative survival in the presence of S9)

Experiment 1:

"High toxicity" was reported at the top concentrations tested, under all treatment conditions. For 3 hour incubations, the top concentration analyzed was 10 µg/mL, which yielded 90.34% and 93.14% relative survival (0.89 and 0.91 relative total growth (RTG)) in the absence and presence of S9, respectively. For the 24 hour incubation, the top concentration analyzed was 0.13 µg/mL which yielded 52.17% relative survival (0.84 RTG) in the absence of S9.

Experiment 2:

"High toxicity" was reported at the top concentrations tested, under both treatment conditions. The top concentrations analyzed were 0.25 µg/mL in the absence of S-9, which yielded 4.69% relative survival (0.14 RTG) and 15 µg/mL in the presence of S-9, which yielded 85.42 % relative survival (0.90 RTG).

The report states that, due to the steep dose-response curve, it was not possible to achieve a concentration yielding approximately 10 % to 20 % relative survival for the 3 hour treatments in Experiment 1 or 2. A third experiment was performed to attempt to meet this requirement.
Reviewer's comment: Generally, the RTG should be used as the measure of cytotoxicity for purposes of dose selection and data evaluation. In most instances, higher concentrations should have been analyzed.

Experiment 3:

"High toxicity" was reported at all concentrations tested in the absence of S9 and at the top concentrations tested in the presence of S9. The top concentrations analyzed were 11 µg/mL in the absence of S9, which yielded 99.08% relative survival (0.18 RTG) and 13 µg/mL in the presence of S-9, which yielded 66.29% relative survival (1.31 RTG).

Results of the mutation experiments are summarized in the Applicant's tables below:

TABLE 3

Mebendazole: summary of results

Experiment 1 (3 hour treatment, - / + S-9)

Treatment (μg/mL)		-S-9 %RS	-S-9 RTG	-S-9 MF§		Treatment (μg/mL)		+S-9 %RS	+S-9 RTG	+S-9 MF§	
0		100.00	1.00	88.62		0		100.00	1.00	103.34	
0.25		97.01	0.80	118.34	NS	0.25		86.25	0.96	75.21	NS
5		108.48	0.95	87.01	NS	5		88.20	0.91	93.68	NS
10		90.34	0.89	101.83	NS	10		93.14	0.91	89.43	NS
20	X	60.66	0.00			20	X	59.70	0.00		
25	$	18.60				25	$	16.70			
30	$	8.67				30	$	7.76			
35	$	5.90				35	$	3.86			
40	$	2.39				40	$	3.13			
Linear trend				NS		Linear trend				NS	
NQO						BP					
0.05		80.90	0.70	489.73		2		67.21	0.56	684.33	
0.1		95.24	0.84	405.85		3		37.28	0.24	970.77	

Experiment 1 (24 hour treatment, - S-9)

Treatment (μg/mL)		-S-9 %RS	-S-9 RTG	-S-9 MF§	
0		100.00	1.00	134.07	
0.002	$	103.34			
0.0039	$	106.07			
0.0078	$	101.80			
0.016		100.10	1.17	98.15	NS
0.031		89.48	1.17	99.55	NS
0.063		94.43	1.14	87.46	NS
0.13		52.17	0.84	108.64	NS
0.25	X	2.96	0.12	175.99	
0.5	X	2.61	0.11	106.88	
1	X	2.32	0.12	112.83	
Linear trend				NS	
NQO					
0.02		85.49	0.93	469.29	
0.04		51.65	1.29	539.74	

§ 5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS Percent relative survival adjusted by post treatment cell counts
$ Not plated for viability / 5-TFT resistance
X Treatment excluded from final test statistics due to excessive toxicity
NS Not significant

Experiment 2 (24 hour treatment - S-9, 3 hour treatment + S-9)

Treatment (μg/mL)		-S-9 %RS	RTG	MF§		Treatment (μg/mL)		+S-9 %RS	RTG	MF§	
0		100.00	1.00	64.05		0		100.00	1.00	82.69	
0.025	$	89.01				1.25	$	99.15			
0.05	$	75.74				2.5		102.10	0.89	102.09	NS
0.1		72.78	0.82	80.40	NS	5		111.50	1.06	74.76	NS
0.125		50.76	0.81	75.30	NS	10		75.84	1.01	82.95	NS
0.15		39.91	0.57	106.68	*	12.5		100.03	1.10	62.06	NS
0.175		23.76	0.36	167.21	*	15		85.42	0.90	83.88	NS
0.2		11.44	0.27	151.05	*	17.5	$	76.41			
0.225		4.32	0.18	151.03	*	20	$	76.51			
0.25		4.69	0.14	170.77	*						
Linear trend				***		Linear trend				NS	
NQO						BP					
0.02		66.74	0.99	240.05		2		76.04	0.67	602.00	
0.04		56.69	1.00	430.08		3		30.34	0.31	861.22	

Experiment 3 (3 hour treatment - / + S-9)

Treatment (μg/mL)		-S-9 %RS	RTG	MF§		Treatment (μg/mL)		+S-9 %RS	RTG	MF§	
0		100.00	1.00	125.78		0		100.00	1.00	132.08	
11	$$	99.08	0.18	154.57	NS	11		84.90	1.12	91.39	NS
12	$	65.61				12		86.53	1.16	100.10	NS
13	$	71.23				13		66.29	1.31	101.18	NS
14	$	51.44				14	X	0.00	1.19	100.53	
15	$	56.65				15	X	0.00	0.00		
16	$	50.80				16	$	0.00			
17	$	49.91				17	$	0.00			
18	$	47.44				18	$	0.00			
19	$	43.65				19	$	0.00			
Linear trend				NS		Linear trend				NS	
NQO						BP					
0.05		65.39	0.76	472.94		2		79.51	0.64	828.83	
0.1		57.30	0.48	547.11		3		31.41	0.37	959.83	

§	5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS	Percent relative survival adjusted by post treatment cell counts
$	Not plated for viability / 5-TFT resistance
$$	Treatment has high heterogeneity, but is included in analysis
X	Treatment excluded from final test statistics due to excessive toxicity
NS	Not significant
*	Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level
*, **, ***	Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively

In the experiments tested using three hour treatment incubation, no statistically significant increases in mutant frequency were reported following treatment with mebendazole at any concentration level analyzed in the absence of S9 in Experiments 1 or 3 or in the presence of S9 in Experiments 1, 2 or 3.

In Experiment 1 (24 hour incubation) in the absence of S9, no statistically significant increases in mutant frequency were observed.

Reviewer's comment: This may be due to the unusually high mutant frequency reported for the control. Additionally, if RTG had been used to determine which concentrations were analyzed, a higher concentration may have had a statistically significant increase in mutant frequency.

In Experiment 2 (24 hour incubation) in the absence of S9, statistically significant increases in mutant frequency were observed at the top five concentrations analyzed (0.15 to 0.25 µg/mL), and a linear trend was confirmed. The report suggested that mebendazole may be an aneugen, but no analysis of chromosome number was performed in this study to confirm that hypothesis. The report states that increases in both small and large colonies were observed at mebendazole concentrations that induced a statistically significant increase in mutant frequency, but that the majority of colonies were small at the highest concentrations tested, suggestive of gross chromosomal abnormalities rather than point mutations.

4. Study title: Mebendazole: Induction of Micronuclei in Cultured Human Peripheral Blood Lymphocytes

Study no.:	CLE Study no. 1073/18
Study report location:	Section 4.2.3.3.1 of electronic submission
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	17 November 1998
GLP compliance:	Yes, OECD
QA statement:	Yes
Drug, lot #, and % purity:	Mebendazole, batch no. ZR017635EXA804, purity 100%

Key Study Findings

Mebendazole induced aneuploidy via non-disjunction in cultured human peripheral blood lymphocytes.

Methods

Cell line:	Human lymphocyte cultures from female human donors (1 donor per experiment)
Concentrations in definitive study:	Experiment 1: 2.403 to 1000 ng/mL (15 concentrations with a 0.65-fold interval between dose levels) Experiment 2: 10 to 250 ng/mL (17

	concentrations)
Basis of concentration selection:	In an initial trial (data not provided), doses of 1.582 to 80 µg/mL (limit of solubility) were described as severely toxic.
Negative control:	Untreated and solvent in Experiment 1 Solvent in Experiment 2
Positive control:	Carbendazim (both experiments) and 4-nitroquinolilne-1-oxide (NQO; Experiment 1 only)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Twenty-four hours after the start of culture, cells were treated with the solvent or test article. After an additional 20 hours, Cytochalasin B was added to give a final concentration of 6 µg/mL (Cytochalasin B inhibits cell division but not nuclear division, resulting in the formation of binucleate cells. Micronuclei are only counted in binucleate cells as a true measurement of their induction.). Cultures were harvested 72 hours after initiation (48 hours continuous treatment). *Reviewer's comment: There is no mention in the report of a metabolic activation system in these experiments.*

Experiment 1 consisted of Phases 1-3. Experiment 2 was comprised of Phase 4. Phase 1 investigated the ability of the test article to induce micronuclei using Giemsa staining. In Phase 2, micronuclei were examined to determine whether or not they contained centromeres ("centromere-positive or centromere-negative") using a pan-centromeric fluorescent probe in order to determine whether induction was via an aneugenic or clastogenic mechanism. Because evidence of aneugenic potential was seen, the incidence of non-disjunction in this experiment was determined in Phase 3 using chromosome specific centromere probes in order to select concentrations for Phase 4.

Phase 4 was an investigation to determine the threshold of action of the aneugenicity of the test compound. Fluorescence in situ hybridization (FISH) was used to investigate the threshold of effect for chromosome 17 and the X chromosome.

Phase 1

Slides from 2 solvent control cultures, 2 positive controls and 2 cultures at each of the test article doses were stained with Giemsa. Slides were examined for proportions of binucleate and mononucleate cells. The top dose for analysis was to be one at which a minimum of 50 % reduction in the fraction of binucleate cells occurred (number of binucleate/number of mono- plus binucleate) as compared with solvent controls. Slides from two solvent control cultures and two positive controls were taken

for analysis of micronuclei along with slides from two cultures receiving the selected top dose and an additional two lower doses. Where possible, 1000 binucleate cells from each replicate (2000 per dose level) were analyzed for micronuclei.

Phase 2

Positive effects in the micronucleus assay were further investigated using FISH. Slides were prepared from cultures treated with test article at a concentration that resulted in a significantly increased frequency of micronucleated binucleate cells, and from one dose of positive control cultures (both carbendazim and NQO) for examination using FISH.

Slides were analyzed under fluorescence microscopy. As many micronuclei as possible, present in binucleate cells (to a maximum of 200 per dose level), were analyzed for the presence of a fluorescent signal. Micronuclei were classified as centromere positive if one or more probe signals were present. The proportion of cells with micronuclei for each treatment condition was compared with the proportion in solvent controls using Fisher's exact test. Probability values of $p \leq 0.05$ were accepted as significant.

Phases 3 and 4

"Centromere positive" effects apparent following analysis of the data from Phase 2 were further investigated using FISH with chromosome-specific centromeric probes. Slides from the selected treatments in Experiment 2 and from solvent and carbendazim-treated positive controls were analyzed. Seven concentrations were selected to define the no-effect level and threshold of action. Slides were prepared and examined by fluorescence microscopy. Where possible, five hundred binucleate cells in Experiment 1 (Phase 3) and 2000 cells per dose level (1000/culture, where possible) in Experiment 2 (Phase 4) were analyzed for centromeric signals. Hypo-, hyper- and polyploid cells were recorded. Any cell with 1, 3 or more centromeres (2 would be expected for each chromosome) in one or both of the nuclei were recorded. The presence of micronuclei in binucleate cells was noted.

The report states that a signal lost from a nucleus as a result of chemically-induced aneuploidy may be seen in an accompanying micronucleus, and that probing with chromosome specific centromeric probes investigates the level of nondisjunction. It goes on to state that non-disjunction, or abnormal chromosome segregation not resulting in loss of chromosomes into micronuclei, is a more sensitive measure of aneuploidy than micronucleus induction, and thus can be used to determine a threshold level.

Study Validity

For Giemsa stained preparations, the assay was considered valid if the following criteria were met:

1. the binomial dispersion test demonstrates acceptable heterogeneity between replicate cultures, and
2. the frequency of cells with micronuclei in solvent controls falls within the normal range, and

3. the positive control chemicals induce statistically significant increases in the proportion of cells with micronuclei.

A test chemical was considered to have clear clastogenic potential in this assay if:
1. a statistically significant increase in the proportion of cells with micronuclei occurs at one or more concentrations, and
2. the incidence of micronucleated cells at such data points exceeds the normal range.

Both positive controls were stated to have given "satisfactory responses in terms of quality and quantity of preparations and frequency of micronuclei.

For fluorescence in situ hybridization (FISH) experiments, the assay was considered valid if the following criteria were met:
1. the positive control chemicals induce statistically significant increases in the proportion of cells with micronuclei, and
2. the centromere labelled control cultures confirm the labelling of all centromeres in the pan-centromeric probe.

A test chemical was considered to have a clear potential to induce aneuploidy if a statistically significant proportion of the micronuclei in binucleate cells contain a fluorescent centromere label.

Results

Phase 1:

Test article dose levels for micronucleus analysis were selected by evaluating the effect of mebendazole on the ratio between binucleate and mono- plus binucleate cells. The frequency of micro nucleated binucleate cells was analysed at three dose levels, 178.5, 422.5, and 650 ng/mL (*Reviewer's comment: There was an additional set of replicates treated with 274.6 ng/mL that was not selected for evaluation)*. The highest concentration chosen for analysis, 650 ng/mL, induced an approximately 81 % reduction in this ratio.

The report states that acceptable numbers of binucleate cells with micronuclei were reported in solvent control cultures. The positive control chemicals NQO and carbendazim induced statistically significant increases in the proportion of cells with micronuclei.

Treatment of cultures with mebendazole resulted in frequencies of cells with micronuclei which were significantly increased above levels seen in concurrent solvent control cultures at the higher two concentrations analyzed, 422.5 and 650 ng/mL. Frequencies of micronucleated cells in these treated cultures exceeded the historical negative control range.

Phase 2:

Analysis of cells treated with 422.5 ng/mL mebendazole using a pan-centromeric probe revealed that the majority (77%) of the micronuclei observed carried a fluorescent

signal indicating that their origin was from whole chromosomes rather than acentric fragments; i.e. indicating aneugenicity. Controls (carbendazim, an aneugen, at a concentration of 1.25 µg/mL, and NQO, a clastogen, at a concentration of 2.5 µg/mL) were said to demonstrate correct labelling profiles, although not in all replicates (*Reviewer's comment: This may limit the reliablility of the study*).

Phase 3:

Cells from cultures receiving 2.403, 20.71 and 178.5 ng/mL were analyzed using fluorescent probes specific for chromosomes X and 17. These data showed that mebendazole caused non-disjunction and that clear increases over controls were seen at 178.5 ng/mL but not at 2.403 or 20.71 ng/mL. These data were used to select an appropriate range of concentrations for Experiment 2.

Phase 4 (Experiment 2):

In this experiment, the report indicates that the positive control, carbendazim, did not induce significant increases in the proportion of cells with micronuclei. It also states that it did, nevertheless, induce non-disjunction.

The report states that non-disjunction was observed for both of the investigated chromosomes (X and 17). An attempt was made to define threshold of action (the lowest concentration at which there was a statistically significant increase) and a highest "no-effect" concentration (the highest concentration at which there was no statistically significant increase).

The no-effect level for non-disjunction for chromosome 17 was determined to be 85 ng/mL, and for chromosome X, it was determined to be 115 ng/mL. The corresponding threshold doses, based on the Applicant's definitions, were 115 ng/mL for chromosome 17 and 130 ng/mL for chromosome X (*Reviewer's comment: This definition of "threshold" does not allow for the possibility of effects at concentrations closer to the no-effect level*).

Reviewer's comment: Given that these data are derived from lymphocyte cultures from a single individual and examine potential effects on only two of the entire complement of chromosomes, the values derived do not provide clinically useful information.

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

5. Study title: Micronucleus test in mice

Study no:	Experiment no. 1557 (listed in electronic submission as no. 1577)
Study report location:	Electronic submission, Section 4.2.3.3.2
Conducting laboratory and location:	Janssen Pharmaceutica N.V. Beerse, Belgium
Date of study initiation:	Report date 85.08.14 Proposed starting date July 3, 1985
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Mebendazole (R 17635), batch no. V 2401

"The test article has been synthesized in our own laboratories and passed the specifications of the chemical quality control department."
Certificate of analysis indicates % assay (base) 100.2% and % assay (acid) 99.0%

Key Study Findings

Bone marrow was examined for micronucleated polychromatic erythrocytes (PCEs) 30 hours after single oral administration of 2.5, 10, or 40 mg/kg to mice. No increase in the number of micronucleated PCEs was reported. Positive control samples from mice treated with 40 mg/kg cyclophosphamide PO revealed an increase in the number of micronucleated PCEs.

Methods

Doses in definitive study:	2.5, 10, or 40 mg/kg mebendazole
Frequency of dosing:	Single administration, 30 hours prior to sacrifice and preparation of bone marrow
Route of administration:	Oral (presumably by gavage, but this is not stated)
Dose volume:	0.1 mL per 10 g body weight
Formulation/Vehicle:	Tween 80 and water for mebendazole treatments 1 equivalent 0.4M tartaric acid for positive control
Species/Strain:	Albino Swiss mice
Number/Sex/Group:	5/sex/group
Satellite groups:	None
Basis of dose selection:	Not stated
Negative control:	Undosed
Positive control:	Cyclophosphamide 40 mg/kg

Study Validity

A total of 1000 polychromatic erythrocytes (PCEs) were counted per animal, and the number of micronucleated PCEs was recorded. The number of micronucleated normochromatic erythrocytes (NCEs) was also recorded in the fields containing the 1000 PCEs. The ratio of PCEs to NCEs + PCEs was determined for each animal by counting a total of 1000 erythrocytes.

The initial counts for some animals revealed a slightly higher incidence of PCEs than the historical background 0.0-0.3% range. Therefore 5000 PCEs were counted for these animals, and the medians used for statistical analysis. Recounts of PCEs in these animals resulted in medians that fell within the reference range for most of the animals. For two low dose and one high dose animals, the median of 5 countings was still slightly greater than the reference range, but was not considered to be significant.

The percent micronucleated PCEs in the negative control group fell within the normal reference range of 0.0-0.3%.

Results

The positive control induced a significant ($p \leq 0.001$) increase in the number of micronucleated PCEs and a significant decrease ($p \leq 0.01$) in the proportion of PCE to PCE + NCE.

The test article, at single doses of 2.5, 10, or 40 mg/kg PO did not result in an increase in the frequency of micronucleated PCEs. In female mice at all three doses and in male mice at the low and mid-doses, the proportion of PCE to PCE + NCE in treated groups was comparable to negative controls. In high dose male mice, a decrease in the ratio of PCE to PCE + NCE was observed ($p \leq 0.01$), but this was not observed when data for males and females were combined. It is unclear whether or not mebendazole was sufficiently bioavailable by the oral route to sufficiently expose bone marrow for a potential effect.

The study was considered to be negative.

6. Study title: Mebendazole: Induction of micronuclei in the bone marrow of treated mice

Study no:	1073/17-D5140
Study report location:	Electronic submission, Section 4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6 November 1998
GLP compliance:	Yes, UK/OECD
QA statement:	Yes
Drug, lot #, and % purity:	Mebendazole, (b) (4) lot no. ZR017635EXA804, purity 100%

Key Study Findings

Mebendazole induced micronuclei in PCEs in the bone marrow of treated mice. Based on FISH evaluation of two samples from treated mice, this effect was due to an aneugenic mechanism. Further experimentation led the Applicant to conclude that there was a threshold dose for this effect that fell between 10 and 40 mg/kg (HED = 0.83 – 3.3 mg/kg), although the degree of variability in response and in systemic exposure make this difficult to determine conclusively.

Methods

Doses in definitive study:	Experiment 1: 0, 500, 1000, and 2000 mg/kg Experiment 2: 2.5, 10, 40, 80, and 320 mg/kg
Frequency of dosing:	Single dose in the early morning (The report states that absorption is increased after feeding.)
Route of administration:	Oral, by gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	1% (w/v) methyl cellulose
Species/Strain:	CD-1 mice
Number/Sex/Group:	Experiment 1: 8 males per group Experiment 2: 7 males per group
Satellite groups:	For plasma pharmacokinetics analysis, there were 3 control animals and 12 high dose animals in Experiment 1 and 3 per group in the vehicle and test article-treated groups in Experiment 2. In Experiment 1, samples were taken by cardiac puncture at 0 (undosed), 2, 4, 6, and 8 hours post-dose from 3 animals per time point. In Experiment 2, samples were taken by cardiac puncture at 6 hours post-dose from all satellite animals. Plasma was separated and frozen at < -10ºC until analysis.
Basis of dose selection:	Toxicity range-finding study at single doses of 1000, 1500, or 2000 mg/kg to 3/sex/group; observations over 2 days following administration included clinical signs and body weights. No clear signs of toxicity were observed.
Negative control:	Vehicle
Positive control:	Cyclophosphamide in saline (2 mg/mL), 40 mg/kg

Study Validity

The assay was to be considered valid if the following criteria were met:

1. the incidence of micronucleated PCE in vehicle control groups falls within or close to the historical vehicle control range, and
2. at least seven animals out of each group are available for analysis, and
3. the positive control chemical (CPA) induces a statistically significant increase in the frequency of micronucleated PCE.

In both experiments, all of the above criteria were met. Both experiments were therefore considered to be valid.

Formulation analysis revealed that dosing solutions for Experiment 1 all fell within 10% of nominal concentrations, as did the high dose solution for Experiment 2. The

dosing solutions for the lower concentrations were lower in concentration than expected, and were approximately 20% lower than nominal concentraions.

The test article would be considered to be positive in this assay if:

1. a statistically significant increase in the frequency of micronucleated PCE occurs at least at one dose, and
2. the frequency of micronucleated PCE at such a point exceeds the historical vehicle control range.

Results

In the first experiment, intended to investigate the ability of mebendazole to induce micronuclei, oral gavage doses of 0, 500, 1000, and 2000 mg/kg were administered. Animals were killed 24 hours after dosing by CO_2 asphyxiation followed by cervical dislocation. Bone marrow from both femurs was flushed out for slide preparation and Giemsa staining. Relative proportions of PCE and NCE were determined for a total of at least 1000 cells. Counting continued until at least 2000 PCE had been observed and the PCE containing micronuclei were recorded. PCE/NCE ratios were calculated to determine if there were decreases at any dose that might indicate bone marrow toxicity (and confirmation of bone marrow exposure). Numbers of micronucleated PCE in vehicle controls were compared to historical controls and those in treated groups compared to concurrent controls. Mebendazole-treated animals had significantly lower group mean PCE to NCE ratios and significantly increased frequencies of micronucleated PCEs relative to concurrent controls. The dose-response relationship was not clear, most likely due to variability in exposure. The report stated that these frequencies were outside of the historical control range.

Fluorescence in situ hybridization (FISH), using a probe specific for mouse centromeres, was performed on 2 slides from animals treated with 500 mg/kg and from one positive control animal. Most micronuclei in the two mebendazole samples contained a centromere (89-92%), indicating that the micronuclei were formed by aneuploidy from whole chromosomes rather than from acentric fragments. Only 30% of positive control micronuclei contained a centromere.

The table below from the study report summarizes results of plasma analysis of samples from Experiment 1. Large inter-animal variability was demonstrated, making it difficult to define a time course.

Experiment 1

Treatment (mg/kg)	Group	Sampling time (hours)	Mebendazole (ng/mL)	
			Mean	±SD
Untreated	6	0	LOQ	-
2000	7	2	1937.26	91.52
2000	8	4	3466.56	695.47
2000	9	6	2866.55	551.19
2000	10	8	3617.73	1272.4

In Experiment 2, a wider range of doses was examined in an attempt to define a threshold of effect. No statistically significant increase in micronucleated PCEs was seen at 2.5 or 10 mg/kg, although the frequency at the lowest dose was more than twice that of vehicle control, and the frequency at these two doses was not dose-related and highly variable. Statistically significant increases in micronucleated PCEs were seen at 40, 80, and 320 mg/kg. The study concluded that a threshold for the aneugenic mechanism in this experiment was between 10 and 40 mg/kg, but the degree of variability make it difficult to conclusively determine this.

The table below from the study report summarizes results of plasma analysis of samples from Experiment 2. Large inter-animal variability was demonstrated again in this part of the study. Exposure at the lowest two doses appeared to be less than dose proportional, but this may have been influenced by the variability; larger sample sizes might have been needed to draw more definitive conclusions.

Experiment 2

Treatment (mg/kg)	Group	Sampling time (hours)	Mebendazole(ng/mL)	
			Mean	±SD
Vehicle	8	6	LOQ	-
2.5	9	6	11.22	-
10	10	6	34.32	15.35
40	11	6	362.65	152.75
80	12	6	742.72	436.62
320	13	6	1404.07	442.47

7.4 Other Genetic Toxicity Studies

A [redacted] (b) (4) test was performed and submitted to NDA 17-381. It was submitted to the current NDA, but was not reviewed. This test is no longer considered to be an option for the genetic toxicology test battery and does not provide information that can be included in the labeling.

8 Carcinogenicity

Carcinogenicity reports were not submitted to this NDA. The following is derived from the Applicant's summaries:

"Carcinogenicity was evaluated in two life-time studies, one in rats for 24 months duration and one in mice for 22 months duration. Animals in both studies received test material via the diet at dosage levels of 10, 20 or 40 mg/kg/day. Results showed no evidence of carcinogenicity in either study. However, the interpretation of the carcinogenicity results is limited by the absence of measures of systemic exposure in these studies. The 40 mg/kg/day mebendazole dose level is 0.4-fold and 0.8-fold the maximum recommended human dose on a mg/m^2 basis (500 mg; 60 kg adult) for mice and rats, respectively."

Reviewer's comment: It should be noted that many patients will be children weighing far less than 60 kg, but receiving the same 500 mg dose.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

1. Study title: Effects of oral R 17635 on Male and Female Fertility in Rats

Study no.:	298
Study report location:	SDN#14, section 4.2.3.5.1
Conducting laboratory and location:	"Janssen Pharmaceutica, Research Laboratoria"
Date of study initiation:	Not provided; report date is "70.05.11"
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R 17635, batch no. A 01/1 "The compound was synthesized in our own laboratories and passed the specifications for purity."

Key Study Findings

No effects on either male or female fertility were reported when males were treated for at least 60 days prior to mating or when females were treated for 14 days prior to mating and through Day 21 of gestation.

Methods

Doses:	5, 10, 20, and 40 mg/kg
Frequency of dosing:	Daily, in food
Dose volume:	Not applicable
Route of administration:	Oral, in food as 5, 10, 20, and 40 mg/100g food
Formulation/Vehicle:	Not specified
Species/Strain:	Wistar rats
Number/Sex/Group:	20
Satellite groups:	None
Study design:	Dosed females were treated for 14 days before cohabitation with untreated males and then through GD 21. Dosed males were treated for 60 days until mating with untreated females. Females were sacrificed in GD 22.
Deviation from study protocol:	None reported

Observations and Results

Mortality

During the pre-mating dosing period, one 40 mg/kg female died before mating. Of the treated males, four 5 mg/kg animals died and two males dosed with 20 mg/kg died.

Clinical Signs

None reported

Body Weight

Body weight was recorded on the 1st, 7th, 14th, and 21st day after insemination. Dosed females gained more weight over the course of the study than did un-dosed females. There was no clear dose response in weight gain among dosed females.

Feed Consumption

The report states that dosed females consumed more feed than did un-dosed females, consistent with observations on body weight.

Toxicokinetics

Not performed

Dosing Solution Analysis

Not reported

Necropsy

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

No difference between percent pregnancies in dosed and undosed groups. In treated groups 100% of females were pregnant.

With regard to the time between the beginning of cohabitation and confirmation of mating, females in the groups dosed with test article became pregnant after 2-3 days (median). Un-dosed females that were mated to treated males became pregnant within 10 days.

Offspring

There were no differences reported between dosed and un-dosed females in the average number of implantations (10.4-13.3 implantation sites per dam), litter size (10.1-12.7), number of live (93.5-98.7%), dead (0%), and resorbed (1.3-6.5%) fetuses. No abnormalities were reported for fetuses either from treated females mated with untreated males or untreated females mated with treated males.

9.2 Embryonic Fetal Development

2. Study title: Potential of oral R 17635 for Embryotoxicity and Teratogenic Effects in Rats after oral administration once daily by gavage

Study no.:	495
Study report location:	Electronic submission section 4.2.3.5.2
Conducting laboratory and location:	Janssen Pharmaceutics Research Laboratories; location not provided
Date of study initiation:	Not provided; report date is “73.10.06”
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R17635, batch no.SM 16096; “passed specifications for purity”

Key Study Findings

Mated rats were administered the test article by gavage at doses of 0, 2.5, 10, and 40 mg/kg/day from gestation days (GD) 6 through 15. Teratogenicity and embryofetal lethality were noted at the mid- and high doses. At 10 mg/kg/day, decreased live pups, increased resorptions, and decreased pup weight were found. Malformations, mostly skeletal, were present in 10 of the 18 mid-dose litters. At 40 mg/kg, 100% of pups were resorbed, and 12 of 20 dams died prior to sacrifice. Dams in both groups had decreased body weight gain and decreased food consumption. Findings at the low dose, 2.5 mg/kg/day, were similar to control.

Methods

Doses:	0, 2.5, 10, and 40 mg/kg
Frequency of dosing:	Once daily
Dose volume:	1mL per 100 g body weight
Route of administration:	Oral, by gavage
Formulation/Vehicle:	A stock concentration of 10% mebendazole in Keltrol, propylene glycol, Aerosol OTP, Plasdone W, methylparaben, sodium citrate 5.5 H_2O, citric acid, Antifoam C 10% dilution, and purified water was diluted in water to obtain the desired concentration for dosing formulations.
Species/Strain:	Wistar rats
Number/Sex/Group:	20 mated females per group
Satellite groups:	None
Study design:	Animals were dosed from gestation Day (GD) 6 to GD 15. Sacrifice and caesarean section were on GD 22.
Deviation from study protocol:	No deviations were reported.

Observations and Results

Mortality

All control and low dose animals survived until termination. One 10 mg/kg/day female died on GD 14; the cause of death was not determined. Twelve of the twenty 40 mg/kg/day females died on one of GD 11-19. One died due to pneumonia, but the cause of death was undetermined for the remaining eleven animals.

Clinical Signs

No clinical signs were reported.

Body Weight

The rats were weighed weekly, starting on the first day of pregnancy. There was a dose-related decrease in weight gain that was statistically significant at 10 and 40 mgkg during the third week of pregnancy. One high dose dam had a body weight loss of 38 g.

Feed Consumption

Food consumption was decreased in treated groups in a dose-related manner. The decrease in food consumption in the 10 and 40 mg/kg groups persisted after the treatment period until the end of the study.

Toxicokinetics

Not performed

Dosing Solution Analysis

No information is provided.

Necropsy

At the end of the study, all control and low dose animals were pregnant, while 94.7% of the remaining 10 mg/kg animals (18/19) and 87.5% of the remaining 40 mg/kg animals (7/8) were pregnant.

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

Uteri were examined for number and distribution of dead and live fetuses in each uterine horn, presence of empty implantation sites, and resorbing fetuses.

There were no meaningful differences in the mean number of implantation sites between controls and treatment groups: 11.7, 11.8, 10.1, and 11.1, in the 0, 2.5, 10, and 40 mg/kg groups, respectively.

The percentage of live fetuses in each group were 99.6%, 99.1%, 73.1%, and 0% in the 0, 2.5, 10, and 40 mg/kg groups, respectively, while the percentage of dead fetuses was 0% in all groups.

The percentage of resorbed fetuses in each group were 0.4%, 0.9%, 26.9%, and 100% in the 0, 2.5, 10, and 40 mg/kg groups, respectively.

Average litter size was 11.7 in the low dose and control groups, but was 7.4 in the 10 mg/kg/group.

Offspring (Malformations, Variations, etc.)

All fetuses were examined macroscopically and radiographically. One third of fetuses from each litter were randomized for dissection, and two thirds were randomized for clearing and bone staining with alizarin.

Average body weight was 5.4 g for control pups and 5.1 g for low dose pups. At 10 mg/kg, the average pup weight was 3.7 g. No pups were delivered in the high dose group.

Developmental anomalies in the control group consisted of one pup in one litter with absent metatarsal bones and one pup in one litter with wavy ribs. One pup in one low dose litter also had wavy ribs. In the mid-dose group, pups in ten litters had anomalies, including rib anomalies (wavy, bifurcated, fused, or deformed) in 7 litters, deformed thoracic bones (presumably thoracic vertebrae) in 6 litters, tail anomalies (absent, short, or deformed) in 2 litters, scoliosis in 2 litters, coelosomy (not defined) in 2 litters, deformed hind legs in 1 litter, and absence of the upper jaw in 1 litter. There were no offspring to be examined in the high dose group.

The conclusion of the report describes two previous experiments in rats (report no. 269, not provided in this submission). Doses of 0.63, 2.5, 5, 10, 40, or 160 mg/kg were administered either through the diet from GD 6 through GD 15, or given as a single dose on GD 7, 8, 9, or 10. It states that results of these experiments were similar to those in this study: increased numbers of resorptions and developmental abnormalities were noted at doses of 10 mg/kg and higher. This study was provided in SDN #14 and is reviewed at the end of this section.

3. Study title: Teratology Study (Segment II) of TELMIN® (Mebendazole, PM-JR-17635) in CRL:COBS-CD-I (ICR)BR Outbred Albino Mice

Study no.:	443
Study report location:	Electronic submission section 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not provided
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Mebendazole, lot # and purity not provided

Key Study Findings

Mebendazole was teratogenic at 10.0 mg/kg/day and embryolethal at 40.0 mg/kg/day when administered orally to mice on Days 6 through 15 of gestation. Observed fetal anomalies attributed to treatment included tail anomalies (kinked or looped and/or short or "stub" tails), exencephaly, varied degrees of hydrocephalus, displaced and/or small eyes, vertebral, rib and sternal fusions, bifurcated ribs, and a 14th pair of ribs. Findings at lower incidence attributed to treatment included cleft palate, gastroschisis, enlarged atrio-ventricular valve, unilateral renal and adrenal

agenesis and incomplete ossification in various vertebrae. These fetal anomalies were significantly more frequent in 10.0 mg/kg/day group litters. In addition, developmental alterations considered to be indicative of delayed growth were seen at higher incidence in the 10 mg/kg/day litters and correlated with smaller fetal body weights in this group. No teratogenic effects were reported at doses of 2.5 or 5.0 mg/kg/day, but fetal body weights were slightly decreased and there was a slight decrease in ossification centers in hindpaw phalanges at 5 mg/kg/day.

Methods

Doses:	0 (vehicle), 2.5, 5.0, 10.0 and 40.0 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral, by gavage
Formulation/Vehicle:	0.5% tragacanth
Species/Strain:	Female CRL:COBS-CD-l (ICR)BR outbred albino mice
Number/Sex/Group:	54 female mice in the control group and 28 female mice/treated group)
Satellite groups:	Female mice were bred and assigned to a control or treatment group. Day zero of pregnancy was the day a vaginal plug was observed ..
Study design:	Treatment was administered on Days 6 through 15 of presumed pregnancy
Deviation from study protocol:	None reported

Observations and Results

Mortality

Eight pregnant mice in the 40.0 mg/kg/day group died during GD 14-18; one aborted before dying. No gross lesions were reported on necropsy, but these deaths were considered to be drug-related, since these mice lost weight during the treatment interval, and five of the eight mice became emaciated and exhibited pilo-erection and ptosis. Gaseous distention of the gastrointestinal tract in one of the affected animals was observed at necropsy.

Clinical Signs

Behavior and clinical signs were recorded daily during treatment. Signs attributed to treatment were seen at the high dose (40.0 mg/kg/day) and included emaciation, piloerection, and ptosis. Effects were first observed after five treatments (Day 11 of presumed pregnancy) and persisted. No other drug-related signs were reported.

Body Weight

Body weight was recorded on GD 0, 6-15, and 18. Mice administered 10 and 40 mg/kg/day gained significantly less weight (P=0. 001) than control mice, while mice at the 5 mg/kg/day dose had slightly less weight gain than controls.

The report indicates that these differences could have been due to direct effects on dams, increased resorption rates, lower fetal weights, or a combination of the above. (These data excluded dams which aborted, died, or delivered before caesarean section on Day 18.)

Feed Consumption

Not measured

Toxicokinetics

Not performed

Dosing Solution Analysis

Not provided

Necropsy

Gross necropsies were performed only on females that died during the study in order to determine the cause of death. No gross lesions were reported in these animals.

Female mice were killed by CO_2 asphyxiation immediately before Caesarean sectioning on Day 18 of gestation.

At termination, 51/54 (94.4%), 27/28 (96.4%), 27/28 (96.4%), 26/28 (92.8%), and 26/28 (92.8%) of mice in the control, 2.5, 5.0, 10.0 and 40.0 mg/kg/day groups were pregnant.

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

The number of fetuses, their uterine placement, and numbers of live and dead fetuses, early and late resorptions, and corpora lutea were recorded.

Caesarean sectioning observations were based on 51, 27, 27, 26 and 26 pregnant 0 (vehicle), 2.5, 5.0, 10.0 and 40.0 mg/kg/day group mice. Eight 40.0 mg/kg/day dosage group mice died; since early resorptions were present at all implantation sites with the exception of the mouse which aborted before dying, dead mice were included in Caesarean sectioning observations. Two 40:0 mg/kg/day dosage group mice aborted, including the one that died. Six control, one 5.0, and one 10.0 mg/kg/day group mice delivered partial or entire litters on GD 18 prior to scheduled Caesarean sectioning. Mice which aborted were excluded from all except corpora lutea and implantation data.

Another high dose animal that survived until termination had10 early resorptions and two aborted implantation sites.

Of the animals that survived and were still pregnant at GD 18, there was a significant increase in resorptions at 10 and 40 mg/kg/day. One or more resorptions occurred in 37.3, 59.3, 37.0, 80.8 (P = 0.001) and 100.0 (P = 0.001) percent of 0 (vehicle), 2.5, 5.0, 10.0 and 40.0 mg/kg/day groups. The average number of

resorptions per pregnant mouse in these same respective dose groups was 0.6, 0.7, 0.5, 2.4 and 12.7 (significant at 10 and 40 mg/kg/day, P = 0.001), of which 0.57, 0.48, 0.37, 2.12 and 12.67 were early (significant at 10 and 40 mg/kg/day, P = 0.001) and 0, 0.26, 0.15, 0.27, and 0 were late (significant at 2.5 and 5 mg/kg/day, P = 0.01, and at 10 mg/kg/day, P = 0.001). One dead fetus occurred in one litter each in the control, 2.5 and 5.0 mg/kg/day groups and in two 10.0 mg/kg/day dosage group litters. Average live litter size was 12.3, 12.9, 12.5, 10.7 and 0 fetuses for 0 (vehicle), 2.5, 5.0, 10.0 and 40.0 mg/kg/day dosage group mice, respectively (statistically different at 10 mg/kg, P = 0.01, and 40 mg/kg, P = .001).

The number and distribution of corpora lutea and implantations were similar between groups, as was the gender ratio.

Offspring (Malformations, Variations, etc.)

Each fetus was weighed and examined for external anomalies. Two-thirds of each litter was cleared, stained with alizarin red-S, and examined for skeletal anomalies. One-third of each litter was examined for visceral anomalies

Average live fetal weight per litter was 1.39, 1.38, 1.33 (P = 0.01) and 1.11 (P=0.001) grams in 0 (vehicle), 2.5, 5.0 and 10.0 mg/kg/day dosage groups respectively. At the 40.0 mg/kg/day dosage there were no live fetuses available for weight measurement.

External, visceral, and skeletal evaluations were based on 51, 27, 27, and 26 (25 for skeletal evaluation) litters in control, 2.5, 5, and 10 mg/kg/day groups, respectively. Five (9.8%), two (7.4%), five (18.5%) and 12 (46.2%) litters in the 0 (vehicle), 2.5, 5.0 and 10.0 mg/kg/day dosage groups, respectively, had one or more fetuses with a gross anomaly, with the overall incidence at 10 mg/kg/day being statistically significant (P=0.001). There were no fetuses available for evaluation in the 40 mg/kg/day group.

Observed fetal anomalies attributed to mebendazole treatment and statistically significant at the 10.0 mg/kg/day dosage level included tail anomalies (kinked or looped and/or short or "stub" tails, in 11 litters only at 10 mg/kg/day), exencephaly (seen in 3, 1, 1, and 5 litters in 0, 2.5, 5, and 10 mg/kg groups, respectively), cleft palate (in three 10 mg/kg litters on external and/or visceral examination), gastroschisis (rare anomaly seen in one 10 mg/kg litter on external evaluation and in a second on visceral evaluation), various degrees of hydrocephalus (dilated brain ventricles in 2 control, two 2.5 mg/kg, one 5 mg/kg, and eleven 10 mg/kg litters), and displaced and/or small eyes (three 10 mg/kg litters). One fetus in one 10 mg/kg litter had an enlarged atrio-ventricular valve, unilateral renal and adrenal agenesis, and gastroschisis, all considered to be treatment-related. Skeletal anomalies included vertebral, rib and sternal fusions (in 16, 10, and 10 litters in the 10 mg/kg group, respectively, compared to sternal fusion in 2 control litters), bifurcated ribs (two 10 mg/kg litters), and a 14th pair of ribs (average number of pairs of ribs at 10 mg/kg was 13.5, compared to 13.2 in control, 2.5, and 5 mg/kg groups), the latter correlated with a significant increase in the average number of thoracic vertebrae and a decrease in the average number of lumbar vertebrae in the 10 mg/kg group. Two 10 mg/kg litters had incomplete ossification of one or more vertebrae.

Minor skeletal variation, considered to be correctable with growth, was significantly more frequent (P=0.001) in the 10 mg/kg/day group, and correlated with smaller fetal body weights in this group. Twenty-six (51.0%), 16 (59.3%) 11 (40.7%)

and 24 (96.0%) of the 0 (vehicle), 2.5, 5.0 and 10.0 mg/kg/day group litters had one or more fetuses with skeletal variations. These included incomplete ossification of the skull or ribs, and sternal variations consisting of asymmetric or split centers or an extra ossification center or process.

The average number of fetal ossification centers was significantly decreased (P=0.05 to 0.001) in the 10 mg/kg/day group litters for caudal vertebrae, sternal centers, tarsals, and metatarsals. It was also slightly decreased for hindpaw phalanges in the 5 and 10 mg/kg/day group litters.

A number of fetal variations were considered to be unrelated to treatment. They were considered to be minor, common in mice, of low incidence, and/or were not dose-related in incidence.

4. Study title: Teratology Study (Segment II) of TELMIN® (Mebendazole, PM-JR-17635) in Lakeview Golden Hamsters

Study no.:	448
Study report location:	Electronic submission section 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not provided
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Mebendazole, drug batch not provided; dose group formulations were batch nos. K-1307-B, K-1303-B, K-1304-B, K-1305-B, and K-1306-B for the vehicle, 2.5, 5.0, 10, and 40 mg/kg groups, respectively; purity not provided.

Key Study Findings

Mebendazole was not considered to be teratogenic in hamsters at doses up to 40 mg/kg/day. However, total litter loss was observed in one 10 mg/kg dam, and one fetus in one 5 mg/kg litter had amelia of all four limbs, that could be consistent with findings in the rat. High dose dams had slightly increased resorptions and slightly smaller live litter size. Without toxicokinetic data, it is unclear if mebendazole is sufficiently bioavailable in the hamster or if exposure is too variable for evaluation.

Methods

Doses:	0 (vehicle), 2.5, 5.0, 10, and 40 mg/kg
Frequency of dosing:	Daily on Days 6 through 10 of presumed pregnancy
Dose volume:	10 mL/kg
Route of administration:	Oral, by gavage
Formulation/Vehicle:	0.5% aqueous tragacanth suspension
Species/Strain:	Golden hamsters
Number/Sex/Group:	52 mated females in the control group

	26 mated females in each of the four treated groups
Satellite groups:	None
Study design:	The day after mating was designated Day 0 of pregnancy. Dams were sacrificed on GD 15.
Deviation from study protocol:	No deviations were reported.

Observations and Results

Mortality

None

Clinical Signs

Behavior and physical signs were recorded during the treatment period. Ptosis was observed in two high dose animals at 30-90 minutes post-dose on 1or 4 occasions during the first five days of treatment.

One 10 mg/kg female had bloody vaginal discharge on GD 15. On Caesarean section, this animal had 11 dead term fetuses and one early resorption in a hemorrhagic uterus.

Body Weight

Body weight was recorded on GD 0, 6-10, and 15. Weight gain was lower at 10 and 40 mg/kg/day during treatment, but 10 mg/kg animals recovered by GD 15. High dose dams had significantly lower weight gain that persisted to the end of the study.

Feed Consumption

Not reported

Toxicokinetics

Not performed

Dosing Solution Analysis

Not provided

Necropsy

Dams were killed with CO_2 on GD 15 and subjected to a gross examination. At termination, 50/52, 24/26, 25/26, 22/26 and 24/26 animals at 0, 2.5, 5, 10, and 40 mg/kg/day were pregnant.

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

The number of fetuses, uterine placement, live and dead fetuses, early and late resorptions and number of corpora lutea were evaluated.

The report states that there were no significant differences between vehicle and drug-treated values for number and distribution of corpora lutea, implantations, resorptions, live and dead fetuses, fetal body weights, and sex ratios. There was one late resorption in one high dose litter; remaining resorptions were early. Resorptions

were found in 30-44% of hamsters across groups, with the fewest in the control group and the highest in the 5 mg/kg and the 40 mg/kg groups. Similarly, the mean number of resorptions and the percent of implantation sites resorbing were highest in in the 5 mg/kg and the 40 mg/kg groups. Live litter size was said to be similar across control and treated groups, but was slightly lower in the high dose group (mean 10.5 vs. 11.4 in controls).

Reviewer's comment: The one 10 mg/kg animal with total litter loss appears to have been excluded from calculations.

Offspring (Malformations, Variations, etc.)

All fetuses were weighed and examined for external anomalies. One third of each litter was examined for visceral alterations and two-thirds of each litter was cleared, stained with alizarin red-S, and examined for skeletal anomalies.

No gross external, visceral, or skeletal anomalies were reported that were considered to be due to treatment. Findings included:

- One 2.5 mg/kg fetus in one litter had small body size and a hemorrhagic area on the back attributed to trauma.
- One 5 mg/kg fetus had small body size and amelia of all four limbs. This was the only fetus with major skeletal malformations, but this was not attributed to treatment due to absence of similar lesions at higher doses.
  Reviewer's comment: These findings are similar to those seen in the rat. It is unclear whether or not this is a sporadic finding, or if it could be treatment-related and the lack of dose-response could be related to individual variability in bioavailability of the drug. All of the teratogenicity studies might have been more reliable if dosing was systemic rather than oral.
- Hemorrhagic areas on the head were seen in 0-3 litters per group with no dose-relationship
- One 10 mg/kg fetus had slightly dilated kidneys

Other skeletal variations were considered to be more consistent with growth retardation than with teratogenic activity of the drug.

5. Study title: Potential of oral R 17635 for embryotoxicity and teratogenic effects in rabbits

Study no.:	311
Study report location:	SDN #14, section 4.2.3.5.2
Conducting laboratory and location:	Janssen Pharmaceutica, Research Laboratoria
Date of study initiation:	Not provided, report date "71.06.25"
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R 17635, batch no. A 01/1 "The compound was synthesized in our own laboratories and passed the

specifications for purity."

Key Study Findings

In controls and in treated animals, there was a low rate of pregnancy, that appeared to be typical of the rabbit colony. Anomalies were limited to single incidences of skeletal anomalies in the three highest dose groups (2.5, 10, and 40 mg/kg; HED = 0.83, 3.3, and 13.3 mg/kg, respectively).

Methods

Doses:	0, 0.63, 2.5, 10, and 40 mg/kg
Frequency of dosing:	Daily
Dose volume:	0.5 mL/kg
Route of administration:	Oral, by gavage
Formulation/Vehicle:	Microcrystalline suspension containing 1.25, 5, 20, and 80 mg/mL in water Control animals received isotonic NaCl.
Species/Strain:	Belgian hare
Number/Sex/Group:	30 mated females in control, 10, and 40 mg/kg groups, 12 in the 0.63 and 2.5 mg/kg groups
Satellite groups:	None
Study design:	Females were fertilized by artificial insemination with 3-4 million sperm in a 0.25 mL diluted semen sample following administration of chorionic gonadotropin (300 IU/kg). Dosing was on gestation Days 6-18. Dams were sacrificed on the 28th day after insemination.
Deviation from study protocol:	None reported

Observations and Results

Mortality

All control animals survived. In treated groups, 1/12, 1/12, 1/30, and 4/29 animals in the 0.63, 2.5, 10, and 40 mg/kg groups, respectively, died during the study. The 0.63 mg/kg animal died on GD20 from pneumonia. The 2.5 mg/kg animal died on GD5 from mucoid enteritis. The 10 mg/kg animal died on GD11, but not cause of death was identified. At the high dose, one animal died at GD5 of enteritis, two animals died on GD 12 and 15, respectively from pneumonia, and one animal died on GD15 and was reported to be autolyzed. The GD 15 death was a pregnant dam with two resorbed fetuses, while the other early decedents were not pregnant.

Clinical Signs

Not reported

Body Weight

Body weight was recorded on the day of insemination, daily from GD 6-18, and on the day of sacrifice. All pregnant does were reported to have gained weight, although the two highest groups gained less than controls. However, there was no statistical comparison, and no clear dose-response.

Feed Consumption

Not recorded

Toxicokinetics

Not performed

Dosing Solution Analysis

Not reported

Necropsy

The percentage of pregnancies in the experimental groups was 30% in the control group, 25% in the 0. 63 mg/kg group, 67% in the 2. 5 mg/kg group, 23% in the 10 mg/kg group, and 34% in the 40 mg/kg group. Data from the pregnant females that died before sacrifice on GD 28 were included in these calculations.

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

There did not appear to be a treatment effect on the percent of live, dead and resorbed fetuses. Resorptions were reported to be highest in the control and in the 2. 5 mg/kg dosed groups, 24 and 26%, respectively. At 0.63, 10, and 40 mg/kg, 5% of the fetuses were resorbed. Stillborn fetuses were only noted in the 40 mg/kg group, but the Sponsor considered the incidence (7%) to be within normal limits.

Offspring (Malformations, Variations, etc.)

Fetuses were weighed and examined grossly. Radiographic examination was performed on all fetuses. One third of the fetuses were examined for visceral anomalies. Two thirds were retained for clearing and bone staining with alizarin.

No visceral anomalies were reported. Findings were limited to single gross (and skeletal) anomalies in one fetus in one litter in each of the 2.5, 10, and 40 mg/kg groups. At 2.5 mg/kg, the finding was duplicas posterior (not defined), and at 10 and 40 mg/kg, the finding was absence of the tail.

6. Study title: Potential of oral R 17635 for embryotoxicity and teratogenic effects in rabbits

Study no.:	386
Study report location:	SDN #14, section 4.2.3.5.2
Conducting laboratory and location:	Janssen Pharmaceutica, Research Laboratoria
Date of study initiation:	Not provided Report date is "71.12.10"
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R 17635, batch no. SM 12758 "The compound was synthesized in our own laboratories and passed the specifications for purity."

Key Study Findings

This study was considered to be negative for embryotoxic and teratogenic effects.

Methods

Doses:	0, 10, and 40 mg/kg
Frequency of dosing:	Daily
Dose volume:	0.4 mL/kg
Route of administration:	Oral by gavage
Formulation/Vehicle:	Microcrystalline suspension of 25 and 100 mg/mL of R 17635 in water Control animals received isotonic NaCl.
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	20
Satellite groups:	None
Study design:	Females were fertilized by artificial insemination with 3-4 million sperm in a 0.25 mL diluted semen sample following administration of chorionic gonadotropin (300 IU/kg). Dosing was on gestation Days 6-18. Dams were sacrificed on the 28th day after insemination.
Deviation from study protocol:	None reported

Observations and Results

Mortality

One high dose female died on GD 24, but the cause of death was not determined. The animal was pregnant with seven normal fetuses. Survival

percentages were 100% for the control and 10 mg/kg groups and 95% for the 40 mg/kg group.

Clinical Signs

Not reported

Body Weight

Body weight was recorded on the day of insemination, daily from GD 6-18, and on the day of sacrifice. All pregnant does gained weight except for one control female and one high dose female that lost 100 and 200 g, respectively. At sacrifice, one low dose and one high dose animal weighed the same as their initial weight.

Average weight gain was 224 g in the 10 mg/kg group and 277 g in the 40 mg/kg group. In the controls, average weight gain was 332 g.

Feed Consumption

Not recorded

Toxicokinetics

Not performed

Dosing Solution Analysis

Not reported

Necropsy

In the control group, 70% of does were pregnant at termination, while 85% of 10 mg/kg does and 80% of 40 mg/kg does were pregnant (including the one doe that died during the study).

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

Average litter size was 5.6 in the control group, 5.4 in the 10 mg/kg group, and 4.5 in the 40 mg/kg group, demonstrating a trend toward smaller litters with increasing dose. The percentages of live (82.8, 88.4, and 88.2% in the 0, 10, and 40 mg/kg groups, respectively), dead (1.1, 0, and 0% in the 0, 10, and 40 mg/kg groups, respectively), and resorbed (16.1, 11.6, and 11.8% in the 0, 10, and 40 mg/kg groups, respectively) fetuses were considered to be comparable for all groups.

Offspring (Malformations, Variations, etc.)

Fetuses were weighed and examined grossly. Radiographic examination was performed on all fetuses. One third of the fetuses were examined for visceral anomalies. Two thirds were retained for clearing and bone staining with alizarin.

Average birth weights for live pups was 43.2, 42.2, and 42.8 g for the control, 10, and 40 mg/kg groups, respectively.

Anomalies reported included one control fetus with fused ribs, one 10 mg/kg fetus with exencephaly and fused ribs, a second 10 mg/kg fetus in a second litter with coelosomy, and one 40 mg/kg fetus with hydrophthalmus. The report stated that all of these findings have been seen in control fetuses by the performing laboratory. Litter incidences were not provided, but the report indicates that the overall total fetal incidence of anomalies was 1.6%, while it was 1.2% in the historical control database.

Therefore, the report concluded that these findings did not indicate an embryotoxic or teratogenic effect under the conditions of the experiment.

7. Study title: Potential of oral R 17635 for embryotoxicity and teratogenic effects in rats

Study no.:	269
Study report location:	SDN #14, section 4.2.3.5.2
Conducting laboratory and location:	Janssen Pharmaceutica, Research Laboratoria
Date of study initiation:	Not provided; revised report date is "70.01.20"
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R 17635, batch no. A 01/1 "The compound was synthesized in our own laboratories and passed the specifications for purity."

Key Study Findings

This study was comprised of two experiments in rats. In one, pregnant rats were dosed with 0, 2.5, 10, 40, or 160 mg/kg/day administered in food from GD 6-15. Dose-related decrease in weight gain was seen in dams, with one 40 mg/kg dam and two 160 mg/kg dams exhibiting weight loss. At necropsy on GD 22, only 25% of 160 mg/kg dams were pregnant. At 40 mg/kg, litter size, percent live fetuses, and mean fetal body weight were decreased, and the percent total resorptions was increased. At 160 mg/kg, the resorption rate was 100%. Anomalies of potential concern were seen in litters from dams treated with 40 mg/kg. These included three fetuses in one litter with a deformed tail and one fetus in a second litter with exencephaly and coelosomy (not defined). No fetuses were available for evaluation from the 160 mg/kg group

In the second experiment, single doses of 0.63, 2.5, 5, 10, and 40 mg/kg were administered by gavage to pregnant rats on GD 7, 8, 9, or 10. Decreased weight gain was seen in dams administered 10 mg/kg on GD 10 and in dams administered 40 mg/kg on GD9 or 10. At necropsy, the percent of pregnant dams in the 40 mg/kg groups were lower than controls and were lowest in animals treated on GD 10. When 10 mg/kg was administered on GD 9 or 10, resorption rates were increased. At 40 mg/kg, litter size, number of live fetuses, and fetal birth weights were decreased, and resorption rates were increased. These effects were most pronounced when mebendazole was administered on GD 9 or 10. Fetal anomalies were seen as low as 2.5 mg/kg, with increased incidence when treatment was on GD 9 or 10. These consisted of absent or malformed tail, malformed hind legs, abnormal ribs, exencephaly, facial cleft, exencephaly, anophthtalmia,kyphosis, scoliosis, and/or coelosomy.

Methods

Doses: Approximately 0, 2.5, 10, 40, and 160 mg/kg/day

	when administered with food 0.63, 2.5, 5, 10, and 40 mg/kg single doses by gavage
Frequency of dosing:	Daily (in food) or single dose (gavage)
Dose volume:	1 mL per 100 g body weight for gavage solutions
Route of administration:	Oral, mixed with food for animals treated on GD6-15 as 0, 2.5, 10, 40, and 160 mg/100g food, By gavage for animals administered a single dose on a particular gestation day
Formulation/Vehicle:	For gavage, microcrystalline suspension in water
Species/Strain:	Wistar rats
Number/Sex/Group:	20 mated females per group
Satellite groups:	None
Study design:	Animals receiving doses in food were treated from GD 6-15. Animals receiving single doses were administered their respective doses by gavage on GD 7, 8, 9, or 10. Sacrifice and Caesarean section were on GD 22.
Deviation from study protocol:	None reported

Observations and Results

Mortality

Among the groups treated in food for the period of organogenesis, one 160 mg/kg/day female (out of 20) died on GD 21, but was not pregnant. Cause of death was not addressed in the report.

Among groups administered a single dose of the test article, one 2.5 mg/kg female (out of 20) dosed on GD 8 died on GD 21. This animal was pregnant with 9 normal fetuses and was found to have ulcerative gastritis. One 10 mg/kg animal dosed on GD 10 (out of 20) died on GD 21. This animal was pregnant, but had one dead fetus and 11 resorptions; cause of death was not addressed.

The report states that another 10 mg/kg female, this one dosed on GD 8, died on GD 7 and was not pregnant. Tabulated data do not mention the date of death, so it is unclear when it actually died, or if it died on GD 7 and was never actually dosed.

Clinical Signs

Not reported

Body Weight

Body weight was recorded on the 1st, 7th, 14th, and 21st day of pregnancy.

For all groups treated over the period of organogenesis, positive mean weight gain was observed, but there was a clear dose-related decrease in weight gain (Weight

gain was 79.7, 76.0, 69.1, 42.1, and 16.6 g at 0, 2.5, 10, 40, and 160 mg/kg/day, respectively). One 40 mg/kg/day dam lost 7 g, and two 160 mg/kg day dams lost 3 and 15 mg/kg, respectively. Individual body weight data were not provided, so it is unclear whether or not these animals were pregnant.

For groups administered a single dose on GD 7, 8, 9, or 10, weight gain was similar to controls at 0.63, 2.5, or 5 mg/kg. At 10 mg/kg, when administered on GD 10, mean body weight gain was 49.4 g, approximately 20 g less than dams administered this dose on GD 7, 8, or 9. One of these animals lost 5 g body weight. At 40 mg/kg, mean weight gain in animals treated on GD 9 or 10 was approximately half that of those treated on GD 7 or 8 (26.1 and 17.5 vs. 45.2 and 49.5 g, respectively). However one 40 mg/kg dam treated on GD 7 lost 12g of body weight, which explains the lower mean gain for that dosing day relative to GD 8.

Feed Consumption

The report does not indicate how or when food consumption was recorded, but states that consumption was comparable between all groups except the highest; mean food consumption at 160 mg/kg/day was slightly decreased (504g during pregnancy relative to 542 g in controls).

Toxicokinetics

Not performed

Dosing Solution Analysis

Not provided

Necropsy

Among animals treated in food throughout the period of organogenesis, the percentage of animals that were pregnant at termination was 90% for controls, 95% for 2.5 and 10 mg/kg animals, and 100% for 40 mg/kg animals. At 160 mg/kg/day, only 25% of animals (5 of 20) were pregnant.

Among animals administered single doses, 89-100% of animals at doses of 0.63, 2.5, 5, and 10 mg/kg were pregnant at termination. The percent of pregnancies at 40 mg/kg were lower than controls: 80% of animals treated on GD 7, 60% of animals treated on GD 8, 85% of animals treated on GD 9, and 50% of animals treated on GD 10.

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

No significant differences in the mean number of implantation sites among groups were reported.

For animals treated in food throughout the period of organogenesis, no difference was reported for litter size, and numbers of live, dead and resorbed fetuses between controls and the groups dosed at 2.5 and 10 mg/kg. At 40 mg/kg, litter size was decreased (mean of 4.4 as compared with 10.7 in the control group), the percent live fetuses was decreased (41.6% as compared with 96. 5% in the control group), body weight of live fetuses was decreased (4.1 g versus 5.2 g in the control group), and the percent total resorptions was increased to 58.4% as compared with 3.5% in the control group. At 160 mg/kg, the resorption rate was 100%.

For animals administered single doses, no difference was reported for litter size, and number of live, dead, and resorbed fetuses between controls and the groups dosed at 0.63, 2.5, and 5 mg/kg. However, when 10 mg/kg was administered on GD 9 or 10, resorption rates were increased, to 9.3 and 27.5%, respectively, as compared to 3.5% in the controls. At 40 mg/kg, litter size, number of live fetuses, and fetal birth weights were decreased, and resorption rates were increased. These effects were more pronounced when R 17635 was administered on GD 9 or 10. The percent of total resorptions after a single dose of 40 mg/kg was 36.9% (GD 7), 54.2% (GD 8), 79.6% (GD 9), and 97.2% (GD 10).

Offspring (Malformations, Variations, etc.)

All fetuses were examined for external anomalies. All fetuses from treated litters (not controls) underwent radiographic examination. One third of the fetuses of each litter were dissected for examination for visceral anomalies, and two thirds underwent clearing and bone staining with alizarin.

No anomalies were noted in the control group.

Among animals treated in food throughout the period of organogenesis, findings at 2.5 mg/kg were limited to 4 fetuses in one litter (out of 19) with wavy ribs. No anomalies were recorded at 10 mg/kg. At 40 mg/kg, three fetuses out of one litter (out of 20) had a deformed tail, and 1 fetus in a second litter had exencephaly and coelosomy. All 160 mg/kg fetuses had been resorbed, so no anomalies were noted.

Among animals administered single doses, at the 0.63 mg/kg dose, wavy ribs (this is generally a non-specific finding, and the report did not attach any significance to it in any dose group.) were noted in two fetuses in two litters following dosing on GD 7 and in another fetus in one litter following dosing on GD 8. At 2.5 mg/kg, one fetus from a dam dosed on GD 7 had wavy ribs, and no abnormalities were noted after dosing on GD 8 or 9. After dosing with 2.5 mg/kg on GD 10, one fetus in one litter had wavy ribs, while another fetus in another litter had an absent tail (The latter finding is consistent with previous test article-related findings and showed dose-related incidence here.). At 5 mg/kg, no anomalies were noted after dosing on GD 7, and two fetuses in two litters were found with wavy ribs after dosing on GD 8. After dosing with 5 mg/kg on GD 9, one litter contained four fetuses with wavy ribs and one fetus with no tail. After dosing with 5 mg/kg on GD 10, two fetuses had rib abnormalities (fused ribs, abnormal ribs), one had no tail, and another had a malformed tail (litter incidence of malformations was 3 out of 19) In litters from dams dosed with 10 mg/kg, on GD 7 or 8, findings were limited to one fetus from a GD8 litter with wavy ribs. After dosing with 10 mg/kg on GD 9, 3 out of 20 litters had developmental anomalies: one fetus in one litter with bifurcated ribs, one fetus in another litter with coelosomy and short tail, and three fetuses in a third litter with exencephaly, one of which also had malformed hindlegs and tail, kyphosis, hydrops, and coelosomy. For dams treated with 10 mg/kg on GD 10, 4 of 19 litters had developmental anomalies: one litter with 6 fetuses with malformed ribs, two fetuses in another litter with exencephaly and a third fetus in that litter with malformed hindlegs and tail, kyphosis and scoliosis, a third litter with one fetus with exencephaly, absent tail and some ribs, and scoliosis, another fetus with exencephaly and malformed ribs, and a third fetus with encephalomeningocele and facial cleft. At 40 mg/kg administered on GD 7, one fetus in one litter had retarded ossification and another fetus in a second litter

had ectopia cordis, No abnormalities were seen in litters from dams dose on GD 8 or GD 9 with 40 mg/kg. After dosing with 40 mg/kg on GD 10, one fetus (out of a total of 3 fetuses in the group) had malformed hind legs, short tail, rachischisis, exencephaly, anophthtalmia, and protrusion of the tongue, and a second fetus from another litter had coelosomy and hydrops.

9.3 Prenatal and Postnatal Development

8. Study title: Effects of R 17635 in rats after oral administration during the peri- and postnatal period

Study no.:	297
Study report location:	SDN #14, Section 4.2.3.5.3
Conducting laboratory and location:	Janssen Pharmaceutica, Research Laboratoria
Date of study initiation:	Not provided; report date is 70.05.11
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R 17635, batch no. A 01/1 "The compound was synthesized in our own laboratories and passed the specifications for purity."

Key Study Findings

Rats were administered 0, 5, 10, 20, and 40 mg/kg in food Daily from GD 16 through a 3-week lactation period. There was a dose-related trend toward reduced body weight gain, likely significant at the 40 mg/kg dose. The percent live fetuses was reduced at 40 mg/kg to 61%. All live pups in that group died within the first four days after birth. Pup birth weights were decreased at 20 and 40 mg/kg. No abnormalities were reported, but evaluation was not complete.

Methods

Doses:	0, 5, 10, 20, and 40 mg/100g food, yielding approximately 0, 5, 10, 20, and 40 mg/kg
Frequency of dosing:	Daily from GD 16 through a 3-week lactation period
Dose volume:	N/A
Route of administration:	Oral in food
Formulation/Vehicle:	N/A
Species/Strain:	Wistar rats
Number/Sex/Group:	20 mated females per dose group
Satellite groups:	None
Study design:	If parturition did not occur on the expected date, dams were sacrificed on GD 24 and evaluated as in an embryo-fetal development study. Pups were evaluated macroscopically and radiographically after delivery (live and dead animals).
Deviation from study protocol:	None reported

Observations and Results

F_0 Dams

Survival:	All animals survived.
Clinical signs:	Not recorded
Body weight:	Body weights were recorded on GD 1, 7, 14, and 21, on the day of parturition, and on Days 14 and 21 after parturition. All groups gained weight during the third week of pregnancy, however there was a trend toward reduced mean gain with increased dose (83.8, 77.6, 71.1, and 41.2 g in pregnant rats at the 5, 10, 20, and 40 mg/kg doses, respectively). The control group mean weight gain was 71.8 g; gain in the 40 mg/kg group was likely significantly lower than control.
Feed consumption:	Food consumption was comparable among groups prior to treatment and during treatment during the final trimester of the pregnancy. During lactation, mean food consumption was greater than control in the 5 (1508 g), 10 (1504 g) and 20 (1403) mg/kg groups. During that period, mean food consumption in the 40 mg/kg group (1130 g) was slightly less than controls (1214 g).
Uterine content:	Litter size was similar across groups, but was slightly lower than control in the high dose group (mean size was 9.4, 9.8, 10.2, 10.1, and 8.4 in the control, 5, 10, 20, and 40 mg/kg groups, respectively). The percent of live fetuses at birth was 99.4, 94.7, 98.9, and 92.4 in the control, 5, 10, and 20 mg/kg groups, respectively. At the high dose, 39% of fetuses were dead at birth or within 12 hours of birth; percent live fetuses was 61%. This appears to correlate with lower body weight gain and lower food consumption in dams at the high dose. Due to cannibalism in 5 (of 19) high dose litters, complete records could not be made. Cannibalism was also reported in one control and one 20 mg/kg litter.
Necropsy observation:	90-95% of the females (18 or 19 out of 20) were pregnant in all groups.
Toxicokinetics:	Not performed
Dosing Solution Analysis	Not reported
Other:	Average duration of gestation was 23.3, 23.1, 23.0, 23.2, and 23.8 days in the control, 5, 10, 20 and 40 mg/kg groups, respectively.

F_1 Generation

Survival:	All pups born to 40 mg/kg dams died within the first four days after birth. At weaning at 3 weeks, control survival was low (34.8%), but was considered to be normal in the 5, 10, and 20 mg/kg groups (61, 51.1, and 58.2%, respectively).
Clinical signs:	Not reported
Body weight:	Pups were weighed 8-12 hours after birth, and at postnatal Days (PNDs) 14 and 21. Birth weights of control, 5, and 10 mg/kg pups were 6.1-6.2 g. Birth weights were lower at 20 mg/kg (5.6 g) and 40 mg/kg (5.2 g). At two weeks, control mean pup body weights were slightly lower than mean pup weights in the 5, 10, and 20 mg/kg groups, but were comparable at 3 weeks. Surviving pups were said to have had normal body weight gains over the entire 3 week neonatal period that was comparable between control and treated groups.
Feed consumption:	Not reported
Physical development:	No abnormalities were reported.
Neurological assessment:	Not performed
Reproduction:	Not performed

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

The Applicant's nonclinical overview indicates that mebendazole is a broad spectrum benzimidazole anthelmintic with activity against nematode and cestode species. It states that the mechanism of action is by interference with cytoplasmic microtubule function in nematode intestinal cells, resulting in degenerative changes which lead to decreased digestion and absorption of nutrients, leading to the death of the parasite.

Safety pharmacology

Three studies were performed to address potential effects on the cardiovascular system. In the first study, mebendazole was investigated for potential cardiovascular and electrophysiological effects in vitro in whole-cell voltage clamp experiments using HEK293 cells stably expressing the HERG potassium channel. A concentration-dependent effect on HERG mediated potassium current was identified, but, at the highest concentration tested (10 µM, 2953 ng/ml), mebendazole only caused a 5.4%

reduction of the membrane K+ current (IKr). In a second study, no relevant electrophysiological effects were observed in isolated Langendorff-perfused female rabbit hearts at concentrations up to 10 µM.

The application states that plasma protein binding of mebendazole is 90 to 95%. Taking that into consideration, it states that the 10 µM concentration is 140-fold higher than the free drug Cmax exposure (21 ng/ml) in the highest exposure group (1 - <3 years old) in the recently completed Phase 3 clinical trial in pediatric subjects using the new chewable tablet formulation.

In the third study, in anaesthetized guinea pigs, mebendazole (0.16 to 1.25 mg/kg IV) had no statistically significant effect on heart rate, mean arterial blood pressure, and electrocardiographic parameters(PQ, QRS, QT and QTcBazett), and did not induce any changes in ECG morphology. Heart rate slowed and QT/QTc increased with time and dose in both treated and control animals; it is unclear whether QT changes in treated animals were related to heart rate or were an effect of mebendazole. QT increases of 10 ms or more were seen in treated animals, but exposures should have been much higher after IV administration than they will be in patients after oral administration. The median plasma level of mebendazole at 5 min after the intravenous injection of 1.25 mg/kg was 1,390 ng/ml. The median concentration of mebendazole in heart and lung tissue was 638 ng/g and 1320 ng/g, respectively. The median ratio of the compound concentration in heart tissue to plasma concentration was 0.39. The median ratio of the compound concentration in lung tissue *to* plasma concentration was 1.01.

No safety pharmacology studies of the respiratory system were performed. The application states that no respiratory clinical signs were noted in nonclinical studies.

No safety pharmacology studies of the central nervous system were performed. The application states that in vivo toxicology studies in rats, mice and dogs did not reveal any adverse effect on general behavior. (b) (4) the proposed labeling for this new drug product include warning for the (b) (4) risk of convulsions in children administered mebendazole.

Experiments were conducted in isolated guinea pig ileum at mebendazole concentrations up to 40 mg/L, in isolated rabbit duodenum at mebendazole concentrations up to 10 mg/L, and in rats or mice administered oral doses of R 17 635 up to 160 mg/kg. The positive control, eserine, a cholinesterase inhibitor, was reported to potentiate acetylcholine- and methacholine-induced contractions in guinea pig ileum, to result in increased intestinal tone in rabbit duodenum, and to produce tremors in vivo after oral administration. The report concluded that mebendazole is “devoid of anticholinesterase activity.”

General toxicology

No general toxicology studies were submitted to this application. The Applicant’s summary indicates that mebendazole was well-tolerated in single dose studies in multiple species, but does not specify doses or other details.

A 13 week study in rats of mebendazole administered in food (0, 10, 40, 160 mg/100 g food; ~10, 40, 160 mg/kg/day) is described. The apparent NOAEL was 10 mg/kg (HED = 1.7 mg/kg). Findings at higher doses were described as clearly drug- and dose-related. These included deaths, decreased food consumption and body

weight, decreased albumin, increased alkaline phosphatase, small testicles correlating histologically to inhibition of spermatogenesis, and increased liver weight correlating histologically to signs of chronic liver stimulation

A study of young beagle dogs treated orally 6 days per week for 13 weeks with doses of 0, 2.5, 10 and 0.63 mg/kg over Weeks 1-7 followed by 40 mg/kg over Weeks 8-13. Findings were reported to be limited to liver weight increases at 10 and 40 mg/kg without histological correlates. Assuming 2.5 mg/kg/day to be the NOAEL, the HED would be 1.25 mg/kg. In another study in young beagle dogs treated orally 6 days per week for 24 months (0, 2.5, 10, 40 mg/kg), no mebendazole-related deaths or toxicity were noted.

Genetic toxicology

Potential for mutation was evaluated in two Ames assays, both of which were considered to be negative under the conditions of the tests. In the mouse lymphoma assay, mebendazole was considered to be mutagenic in the absence of S9 when tested using continuous (24 hour) incubation.

In an in vitro micronucleus assay, mebendazole induced aneuploidy via non-disjunction in cultured human peripheral blood lymphocytes.

Two in vivo mouse micronucleus assays were submitted. In the first, bone marrow was examined for micronucleated polychromatic erythrocytes (PCEs) 30 hours after single oral administration of 2.5, 10, or 40 mg/kg to mice. No increase in the number of micronucleated PCEs was reported. Positive control samples from mice treated with 40 mg/kg cyclophosphamide PO revealed an increase in the number of micronucleated PCEs.

Mebendazole induced micronuclei in PCEs in the bone marrow of treated mice in a second study. Based on fluorescence in-situ hybridization (FISH) evaluation of two samples from treated mice, this effect was shown to be due to an aneugenic mechanism. Further experimentation led the Applicant to conclude that there was a threshold dose for this effect that fell between 10 and 40 mg/kg (HED = 0.83 – 3.3 mg/kg) in this experiment, but the degree of variability make it difficult to conclusively determine this. There was also a great deal of variability in systemic exposure. It is possible that non-disjunction in dividing cells may be related to the proposed pharmacological mechanism of interference with microtubule function.

Carcinogenicity

No carcinogenicity studies were submitted to this application. The Applicant's summary states that 22- and 24-month studies in mice and rats, respectively, of mebendazole administered in the diet at doses of 10, 20 or 40 mg/kg/day revealed no evidence of carcinogenicity. Although there was no measurement of systemic exposure, the 40 mg/kg/day mebendazole dose level would be 0.4-fold and 0.8-fold the maximum recommended human dose on a mg/m^2 basis (500 mg; 60 kg adult) for mice and rats, respectively.

Developmental and reproductive toxicology

No effects on either male or female fertility in rats were reported when males were treated for at least 60 days prior to mating or when females were treated for 14

days prior to mating and through Day 21 of gestation with doses up to 40 mg/kg/day (HED = 6.7 mg/kg) in food.

Six studies of embryofetal toxicity, in which dams were dosed during the period of organogenesis, were submitted:

Two studies were performed in rats. In the first, mated rats were administered the test article by gavage at doses of 0, 2.5, 10, and 40 mg/kg/day from gestation days (GD) 6 through 15. Teratogenicity and embryofetal lethality were noted at the mid- and high doses. At 10 mg/kg/day, decreased live pups, increased resorptions, and decreased pup weight were found. Malformations, mostly skeletal, were present in 10 of the 18 mid-dose litters. At 40 mg/kg, 100% of pups were resorbed, and 12 of 20 dams died prior to sacrifice. Dams in both groups had decreased body weight gain and decreased food consumption. Findings at the low dose, 2.5 mg/kg/day (HED = 0.42 mg/kg), were similar to control.

A second study in rats was comprised of two experiments. In one, pregnant rats were dosed with 0, 2.5, 10, 40, or 160 mg/kg/day administered in food from GD 6-15. Dose-related decrease in weight gain was seen in dams, with one 40 mg/kg dam and two 160 mg/kg dams exhibiting weight loss. At necropsy on GD 22, only 25% of 160 mg/kg dams were pregnant. At 40 mg/kg, litter size, percent live fetuses, and mean fetal body weight were decreased, and the percent total resorptions was increased. At 160 mg/kg, the resorption rate was 100%. Anomalies of potential concern were seen in litters from dams treated with 40 mg/kg. These included three fetuses in one litter with a deformed tail and one fetus in a second litter with exencephaly and coelosomy (not defined). No fetuses were available for evaluation from the 160 mg/kg group

In the second experiment in rats, single doses of 0.63, 2.5, 5, 10, and 40 mg/kg were administered by gavage to pregnant rats on GD 7, 8, 9, or 10. Decreased weight gain was seen in dams administered 10 mg/kg on GD 10 and in dams administered 40 mg/kg on GD9 or 10. At necropsy, the percent of pregnant dams in the 40 mg/kg groups were lower than controls and were lowest in animals treated on GD 10. When 10 mg/kg was administered on GD 9 or 10, resorption rates were increased. At 40 mg/kg, litter size, number of live fetuses, and fetal birth weights were decreased, and resorption rates were increased. These effects were most pronounced when mebendazole was administered on GD 9 or 10. Fetal anomalies were seen as low as 2.5 mg/kg, with increased incidence when treatment was on GD 9 or 10. These consisted of absent or malformed tail, malformed hind legs, abnormal ribs, exencephaly, facial cleft, anophthtalmia,kyphosis, scoliosis, and/or coelosomy.

Mebendazole was teratogenic at 10.0 mg/kg/day and embryolethal at 40.0 mg/kg/day when administered orally to mice on Days 6 through 15 of gestation. Observed fetal anomalies attributed to treatment included tail anomalies (kinked or looped and/or short or "stub" tails), exencephaly, varied degrees of hydrocephalus, displaced and/or small eyes, vertebral, rib and sternal fusions, bifurcated ribs, and a 14th pair of ribs. Findings at lower incidence attributed to treatment included cleft palate, gastroschisis, enlarged atrio-ventricular valve, unilateral renal and adrenal agenesis and incomplete ossification in various vertebrae. These fetal anomalies were significantly more frequent in 10.0 mg/kg/day group litters. In addition, developmental alterations considered to be indicative of delayed growth were seen at higher incidence

in the 10 mg/kg/day litters and correlated with smaller fetal body weights in this group. No teratogenic effects were reported at doses of 2.5 or 5.0 mg/kg/day, but fetal body weights were slightly decreased and there was a slight decrease in ossification centers in hindpaw phalanges at 5 mg/kg/day.

In contrast, mebendazole was not considered to be teratogenic in a study in hamsters at doses up to 40 mg/kg/day. However, total litter loss was observed in one 10 mg/kg dam, and one fetus in one 5 mg/kg litter had amelia of all four limbs, that could be consistent with findings in the rat. High dose dams had slightly increased resorptions and slightly smaller live litter size. Without toxicokinetic data, it is unclear if mebendazole is sufficiently bioavailable in the hamster or if exposure is too variable for evaluation.

Two studies of embryofetal development were conducted in rabbits. In the first, there was a low rate of pregnancy in controls and in treated animals that appeared to be typical of the rabbit colony. Anomalies were limited to single incidences of skeletal anomalies in the three highest dose groups (2.5, 10, and 40 mg/kg; HED = 0.83, 3.3, and 13.3 mg/kg, respectively). The second rabbit study was considered to be negative for embryotoxic and teratogenic effects at doses up to 40 mg/kg.

In a peri- and postnatal development study, rats were administered 0, 5, 10, 20, and 40 mg/kg in food daily from GD 16 through a 3-week lactation period. There was a dose-related trend toward reduced maternal body weight gain, likely significant at the 40 mg/kg dose. The percent live fetuses was reduced at 40 mg/kg to 61%. All live pups in that group died within the first four days after birth. Pup birth weights were decreased at 20 and 40 mg/kg. No abnormalities were reported, but evaluation was not complete.

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/s/

AMY C NOSTRANDT
09/23/2016

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