CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208398Orig1s000

MICROBIOLOGY/VIROLOGY REVIEW(S)

Division of Anti-Infective Products
Clinical Microbiology Review

NDA: 208398 (SDN-001; -003; -008; -018; -019; -022; -023; -024) Original
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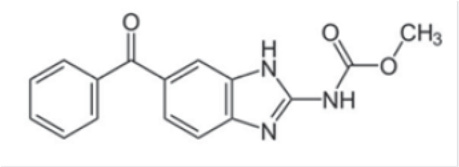
Date Completed: 10/04/2016
Reviewer: Shukal Bala, PhD

APPLICANT:
Janssen Research and Development, LLC
Janssen Pharmaceuticals, Inc.
P O Box 300
Raritan, NJ 08869

DRUG PRODUCT NAMES:
Proprietary: VERMOX™
Nonproprietary: Mebendazole; R17635
Chemical Name: methyl-5-benzoyl-1H-benzimidazol-2-yl carbamate

STRUCTURAL FORMULA:



MOLECULAR FORMULA:
$C_{16}H_{13}N_3O_3$

MOLECULAR WEIGHT:
295.3

DRUG CATEGORY:
Anthelminthic

PROPOSED INDICATION:
Treatment of (b) (4) gastrointestinal (b) (4) by *Trichuris trichiura* (whipworm); *Ascaris lumbricoides* (roundworm); and (b) (4)

PROPOSED DOSAGE FORM, ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:
Dosage form: Chewable tablets
Route of administration: Oral
Dosage: 500 mg
Duration: Single dose

DISPENSED:
Rx

RELATED DOCUMENTS:
PreIND 115,959; NDA 17-481

REMARKS AND CONCLUSIONS

The nonclinical and clinical microbiology studies submitted by the Applicant or obtained by an independent literature search, support the activity of mebendazole against *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), (b) (4) A potential for development of resistance to mebendazole exists; however, the tests to evaluate drug resistance in soil transmitted helminths infecting humans are not standardized.

RECOMMENDATIONS

From clinical microbiology perspective, this NDA is approvable pending an accepted version of the labeling.

The changes to the proposed labeling are as follows (additions marked as double-underlined and deletions as striked out):

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

(b) (4) Mebendazole ~~}~~ (b) (4) a benzimidazole (b) (4) anthelminthic (b) (4) *(12.4)]*.

12.4 Microbiology

Mechanism of Action

Mebendazole interferes with cellular tubulin formation in the helminth and causes ultrastructural degenerative changes in its intestine. As a result, its glucose uptake and the digestive and reproductive functions are disrupted, leading to immobilization, inhibition of egg production and death of the helminth.

Antimicrobial activity

(b) (4) Mebendazole is active against:
(b) (4)
Ascaris lumbricoides (b) (4)
Trichuris trichiura
(b) (4)

[redacted] **Resistance**

There is a potential for development of resistance to mebendazole. [redacted] -The mechanism of resistance to mebendazole is likely due to changes of [redacted]beta-tubulin protein, which reduces binding of mebendazole to [redacted]beta-tubulin-; however, clinical significance of this is not known.

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1. EXECUTIVE SUMMARY

The subject of this NDA is VERMOX™ (mebendazole) for the treatment of soil transmitted helminth (STH) infections caused by *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), [redacted] in children ([redacted] 1 years of age) and adults. Mebendazole is known to be effective against a broad spectrum of helminths.

Mebendazole, like other benzimidazoles, binds to different organs of the worm, reduces the uptake of glucose and other nutrients, interferes with cellular microtubule formation, alters the morphology, cause immobilization and ultrastructural degenerative changes in the intestine and other organs of the helminths leading to disruption of the digestive and reproductive functions of the parasite, inhibition of egg production, and death of the parasites. Such changes were not observed in the host cells. Most of the studies supporting the mechanism of action of mebendazole were performed with helminths that infect livestock and other animals, such as *Ascaris suum* and *Haemonchus* species.

In vitro and *ex vivo* studies show that mebendazole inhibits the maturation of eggs from the hookworms and whipworms. As a direct ovicidal effect of the drug, the eggs of the worms do not progress to the larval stage. However, there does not appear to be any effect on the infective larvae of *A. duodenale*, *N. americanus* and *Ancylostoma caninum* (dog hookworm) as the larvae incubated with mebendazole retained their ability to infect mice.

In rodents and dogs experimentally infected with different helminth species as well as naturally infected dogs, multiple doses of mebendazole were effective in reducing parasite burden. A single dose of mebendazole was compared with multiple doses of mebendazole in one study; the results showed multiple doses to be more effective in reducing parasite burden than the single dose. [redacted]

The *in vitro* and *in vivo* studies suggest a potential for development of resistance to mebendazole by helminths. Resistance appears to be due to a [redacted] in the parasite β-tubulin: an amino acid substitution from phenylalanine to tyrosine was reported. Low cure rates were reported in subjects with *T. trichiura*, [redacted] infections. However, association between mutations in the β-tubulin of helminths and clinical response in subjects with STH infections has not been evaluated.

The Applicant completed a phase 3 clinical trial (Study GAI3003) in children to support the efficacy of a single 500 mg dose of mebendazole for the treatment of *A. lumbricoides* and *T. trichiura* infections. The parasitological measurements included identification of helminthic species and egg count in fecal samples, by the Kato-Katz method. Please note that although *A. lumbricoides* and *T. trichiura* eggs can be identified by direct microscopic examination, the two hookworm species can be differentiated by coproculture and not by direct microscopic examination of the eggs; no coprocultures were performed to identify the two hookworm species. The entire thick smear was examined in a systematic way and the number of eggs counted for each of the STH, *A. lumbricoides*, *T. trichiura* and hookworms. On-site quality control (QC) was performed by experts from the [redacted] at regular

intervals on over 10% of the slides in a blinded manner. If there was discrepancy in egg count based on pre-specified criteria, reading of the slides and/or retraining of the technician was performed, under the supervision of the experts from the Swiss TPHI, and a consensus value documented on the case report form. During the pre-inspection visit, the applicant determined that the consensus value was not documented on the case report forms of nine of the 295 subjects.

To support the efficacy of mebendazole for the treatment of hookworm infection, the Applicant included several published studies based on a comprehensive literature review. The identification of helminth species and egg counts in fecal specimens were based on different methods. Hookworm species were identified by coproculture in some of the studies.

Overall, the phase 3 and published studies suggest that a single 500 mg dose of mebendazole is effective in curing and reducing egg count in patients infected with *A. lumbricoides, T. trichiura,* (b) (4)

The cure rates and egg reduction rates reported in the different published studies varied. Variability in response could be attributed to patient population such as the age of the subjects enrolled in the study [intensity of infection appears to be higher in younger children (Grade 1) compared to older children (Grade 5) and adults], previous drug exposure, and nutritional status of the subjects. The parasitological method used, number and quantity of stool specimens collected and processed for egg count, time of collection of stool specimens post-treatment varied in different studies. Daily variation in the number of excreted eggs including intermittent release of eggs by infected individuals is known. Different parasite burden at the time of initiation of treatment can lead to variability in response; for example, cure rates were lower in subjects with high intensity infection compared to those with low intensity infection.

There is a possibility of reinfection in subjects living in endemic areas; re-infection can vary in different geographic regions due to various ecological factors, levels of transmission as well as seasonal variation (e.g., prevalence can increase after the rainy season). Despite the differences in the study design, study population and procedures, the results of the published studies are comparable to those of Study GAI3003. *A. lumbricoides* appears to be more sensitive compared to *T. trichiura, A. duodenale* and *N. americanus*. Treatment with a single dose of mebendazole should be effective in decreasing the intensity and reducing transmission of STH infections.

2. INTRODUCTION AND BACKGROUND

The subject of this NDA is mebendazole for the treatment of soil transmitted helminth (STH) infections caused by *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), (b) (4) 1 years of age) and adults. STH infections are widely distributed in tropical and subtropical areas and are linked to a lack of sanitation; therefore, these infections are most common in deprived communities worldwide and are considered by the World Health Organization (WHO) as neglected tropical diseases.[1] Recent estimates by the WHO suggest that approximately 2 billion people are infected with soil-transmitted helminths worldwide.[2]

In the United States (US), VERMOX® (mebendazole) 100 mg chewable tablets were approved on 28 June 1974 (NDA 17-481) for the treatment of *T. trichiura*, *A. lumbricoides*, *Enterobius vermicularis* (pinworm), as well as *A. duodenale* and *N. americanus* in single or mixed infections. Marketing in the US was discontinued for commercial reasons in 2006; however, this NDA has not been withdrawn. Mebendazole is currently licensed in 73 countries around the world and used worldwide in approximately 60 countries for the mass treatment of single or mixed gastrointestinal infections by *E. vermicularis*, *T. trichiura*, *A. lumbricoides*, *A. duodenale*, and *N. americanus*. Mebendazole is also approved for veterinary use in the US.

The Applicant proposes to market VERMOX™ as a single chewable 500 mg tablet. For children who have difficulty chewing, VERMOX™ Chewable 500 mg tablet will be placed in a spoon and approximately 2 to 3 mL of drinking water added. This is to allow for the treatment of children down to 1 year of age, in accordance with the WHO recommendations.

The Applicant was granted orphan drug designation and a priority review of this application.

2.1. Mebendazole

Mebendazole (b) (4) Maximum plasma concentrations are generally seen within (b) (4) of drug administration. The plasma protein binding of mebendazole is (b) (4) Orally administered mebendazole is extensively metabolized primarily by the liver. Plasma concentrations of its major metabolites (hydrolyzed and reduced forms of mebendazole) are higher than those of mebendazole (Table 1).

The apparent elimination half-life of mebendazole after an oral dose ranges from 3 to 6 hours in most patients (for more details see Clinical Pharmacology review).

[1] World Health Organization, Geneva, Switzerland, 2011. Assuring safety of preventive chemotherapy interventions for the control of neglected tropical diseases (http://whqlibdoc.who.int/publications/2011/9789241502191_eng.pdf).

[2] World Health Organization (http://www.who.int/mediacentre/factsheets/fs366/en/).

Table 1: Mean (SD) capillary whole blood pharmacokinetic parameters of mebendazole and its metabolites following administration of the 500 mg chewable tablet in children 1 to 16 years of age (Study GAI3003)

A: Pharmacokinetic parameters of mebendazole

Parameter	1 to <3 years	3 to <7 years	7 to 16 years
All Helminth Species			
N	22	12	10
C_{max} (ng/mL)	210 (212)	49.9 (26.8)	34.2 (13.8)
[Range]	[31.1 – 881]	[2.26 – 101]	[15.6 – 52.1]
t_{max} (h)[a]	2.50 (1.00 – 8.00)	2.00 (0.98 – 3.00)	3.00 (1.00 – 8.00
AUC_8 (ng.h/mL)	697 (367)[b]	242 (139)[c]	182 (66.3)
[Range]	[111 – 1,437]	[102 – 467]	[88.0 – 271]
AUC_{last} (ng.h/mL)	1,320 (844)	416 (215)[d]	387 (190)
[Range]	[206 – 4,076]	[161 – 874]	[116 – 747]
Ascaris lumbricoides (large roundworm)			
N	9	3	7
C_{max} (ng/mL)	242 (250)	32.6 (26.3)	31.0 (12.4)
t_{max} (h)[a]	3.00 (1.00 – 5.00)	2.00 (1.78 – 2.60)	3.00 (1.00 – 5.00
AUC_8 (ng.h/mL)	756 (314)[e]	NR	178 (61.8)
AUC_{last} (ng.h/mL)	1,687 (1,020)	516 (32.7)	376 (141)
Trichuris trichiura (whipworm)			
N	6	8	3
C_{max} (ng/mL)	195 (112)	57.0 (27.3)	41.7 (16.4)
t_{max} (h)[a]	2.50 (1.00 – 5.00)	2.01 (0.98 – 3.00)	3.00 (2.00 – 8.00
AUC_8 (ng.h/mL)	757 (394)	256 (142)	192 (90.4)
AUC_{last} (ng.h/mL)	1,090 (476)	418 (238)	415 (317)
Ascaris lumbricoides (large roundworm) and *Trichuris trichiura* (whipworm)			
N	7	1	0
C_{max} (ng/mL)	182 (248)	44.5	NR
t_{max} (h)[a]	2.00 (1.00 – 8.00)	2.00	NR
AUC_8 (ng.h/mL)	578 (423)	133	NR
AUC_{last} (ng.h/mL)	1,045 (759)	203	NR

[a] Median (range); [b] n=21; [c] n=9; [d] n=11; [e] n=8; NR = Not reported

Source: Mod5.3.5.1\GAI3003\Tab7

B: Pharmacokinetic parameters of mebendazole, hydrolyzed mebendazole and reduced mebendazole

Parameter	1 to <3 years	3 to <7 years	7 to 16 years
Mebendazole			
N	22	12	10
C_{max} (ng/mL)	210 (212)	49.9 (26.8)	34.2 (13.8)
t_{max} (h)[a]	2.50 (1.00 – 8.00)	2.00 (0.98 – 3.00)	3.00 (1.00 – 8.00)
AUC_8 (ng.h/mL)	697 (367)[b]	242 (139)[c]	182 (66.3)
AUC_{last} (ng.h/mL)	1,320 (844)	416 (215)[d]	387 (190)
Hydrolyzed Mebendazole			
N	22	12	10
C_{max} (ng/mL)	189 (97.5)	165 (63.0)	165 (71.8)
t_{max} (h)[a]	3.00 (2.00 – 8.00)	2.01 (1.02 – 5.00)	4.00 (2.00 – 8.00)
AUC_8 (ng.h/mL)	981 (597)[b]	809 (366)[c]	936 (429)
AUC_{last} (ng.h/mL)	2,027 (1,560)	1,682 (836)	1,992 (1,200)
$MPRC_{max}$	1.97 (1.62)	7.17 (9.75)	6.15 (2.04)
$MPRAUC_8$	2.27 (1.70)[b]	4.79 (2.25)[c]	6.51 (2.34)
$MPRAUC_{last}$	2.46 (1.98)	5.67 (2.34)[d]	6.58 (2.89)
Reduced Mebendazole			
N	22	12	10
C_{max} (ng/mL)	218 (112)	184 (89.6)	139 (56.5)
t_{max} (h)[a]	3.00 (2.00 – 8.00)	3.00 (1.00 – 8.00)	5.00 (1.00 – 8.00)
AUC_8 (ng.h/mL)	1,201 (646)[b]	1,035 (461)[c]	774 (317)
AUC_{last} (ng.h/mL)	2,466 (1,630)	2,027 (921)[d]	1,630 (917)
$MPRC_{max}$	1.73 (1.13)	3.95 (0.968)	4.12 (1.12)
$MPRAUC_8$	2.10 (1.23)[b]	4.56 (0.793)[c]	4.35 (1.46)
$MPRAUC_{last}$	2.41 (1.67)	4.97 (0.723)[d]	4.28 (1.69)

[a] Median (range); [b] n=21, [c] n=9, [d] n=11; NR – Not reported

Source: Mod5.3.5.1\GAI3003\Tab8

Source: NDA Module 2.7.2 Clinical Pharmacology Summary

2.2. Biology of roundworm, whipworm and hookworm

Roundworm *(A. lumbricoides*), whipworm (*T. trichiura*), hookworms (*A. duodenale* and *N. americanus*) are long lived nematodes and are commonly known as intestinal worms. In general, the life cycle of nematodes have adult worm, egg and larval stages (Figure 1). The eggs are passed outside the body in the feces or may be deposited on the perianal skin by the female worm. Eggs can remain viable in the soil in appropriate conditions for up to two years (*A. lumbricoides* and *T. trichiura*); hookworm eggs hatch quickly but infective larvae remain viable in the soil for several weeks.

Infection is initiated through contact with eggs or larvae that have matured in warm and moist soil; in most cases the third stage larva is the infective stage. Infective larvae of hookworm penetrate the skin; matured eggs of *A. lumbricoides* and *T. trichiura* are ingested with contaminated food or water. The infective larvae develop into adult worms and parasitize the gastrointestinal tract, sometimes for years in the absence of effective treatment. Adult worms mate and produce large numbers of eggs and the prepatent period is 6 to 8 weeks (Table 2). *A. lumbricoides* produce larger number of eggs (up to 200,000 eggs/day) compared to *A. duodenale*, *N. americanus*, and *T. trichiura* (5,000 to 20,000 eggs/day).

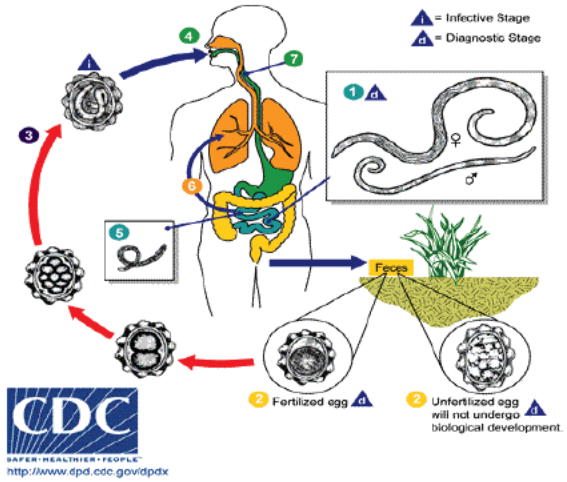
The symptoms of STH infections are nonspecific and may only be recognized in cases of heavy infection. Infection is typically most intense and debilitating in school-age children. Chronically infected children can suffer from malnutrition, physical and intellectual growth retardation, and cognitive and educational deficits.

Hookworms attach to the intestinal mucosa to feed on the blood supply, causing tissue damage and may lead to iron-deficiency and anemia. Also, because of their large size (females can be as long as 14 inches) heavy infections can cause intestinal obstruction in children that require surgery. Whipworms colonize the colon, and heavy infections may cause a dysentery-like syndrome and rectal prolapse. Hookworms and whipworms are considered to be the most refractory to treatment with existing anthelmintics.

Recent studies suggest that STH infection(s) may interfere with the development of immunity in response to vaccines and other infections such as malaria, tuberculosis, and HIV.

Figure 1: Life cycle of nematodes associated with soil transmitted infections

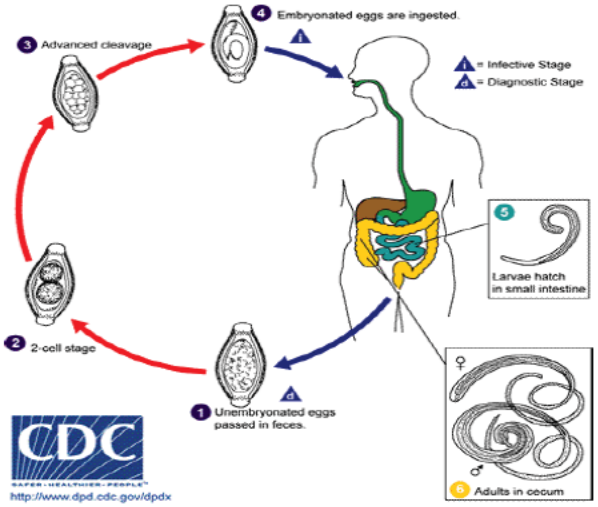
(A) *Ascaris lumbricoides*



Adult worms (1) live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces (2). Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks (3), depending on the environmental conditions (optimum: moist, warm, shaded soil). After infective eggs are swallowed (4), the larvae hatch (5), invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs (6). The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed (7). Upon reaching the small intestine, they develop into adult worms (1). Between 2 and 3 months are required from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live 1 to 2 years.

Source: http://www.cdc.gov/parasites/ascariasis/biology.html

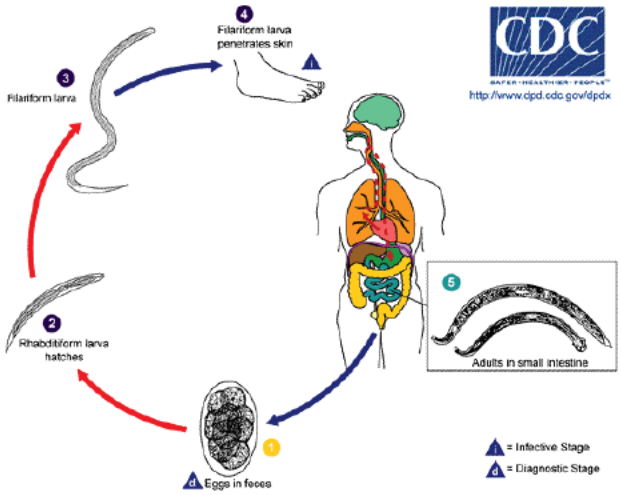
(B) *Trichuris trichiura*



The unembryonated eggs are passed with the stool (1). In the soil, the eggs develop into a 2-cell stage (2), an advanced cleavage stage (3), and then they embryonate (4); eggs become infective in 15 to 30 days. After ingestion (soil-contaminated hands or food), the eggs hatch in the small intestine, and release larvae (5) that mature and establish themselves as adults in the colon (6). The adult worms (approximately 4 cm in length) live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life span of the adults is about 1 year.

Source: http://www.cdc.gov/parasites/whipworm/biology.html

(c) Intestinal hookworms



Eggs are passed in the stool (1), and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil (2), and after 5 to 10 days (and two molts) they become filariform (third-stage) larvae that are infective (3). These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed (4). The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host (5). Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years.

Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus*, however, requires a transpulmonary migration phase.

Source: http://www.cdc.gov/parasites/hookworm/biology.html

Table 2: Some of the characteristics of soil transmitted helminths

Species	Length (mm)	Daily egg output per female worm	Location in host	Lifespan (years)
Large common roundworm				
Ascaris lumbricoides	150–400	200 000	Small intestine	1
Whipworm				
Trichuris trichiura	30–50	3000–5000	Caecum and colon	1·5–2·0
Hookworms				
Necator americanus	7–13	9000–10 000	Upper small intestine	5–7
Ancylostoma duodenale	8–13	25 000–30 000	Upper small intestine	5–7

Table 2: **Characteristics of the soil-transmitted helminths: adult worms of greatest public-health significance**

Adult hookworms of the genera *Necator* and *Ancylostoma* parasitize the upper part of the human small intestine, whereas *Ascaris* roundworms parasitize the entire small intestine and adult *Trichuris* whipworms live in the large intestine, especially the cecum. The parasites can live for several years in the human gastrointestinal tract. STH do not reproduce within the host. This feature is crucial for understanding of the epidemiology and clinical features of soil-transmitted helminth infections, as well as the approaches to their control.
Source: Bethany *et al*., 2006[3]

Diagnosis: The diagnosis can be made by microscopic examination of the eggs in the stool. Eggs of *A. lumbricoides*, *T. trichiura*, and hookworms can be distinguished (Figure 2). The eggs of the two hookworm species, *A. duodenale* and *N. americanus*, cannot be differentiated microscopically. However, the larvae of the two hookworm species can be distinguished morphologically by coproculture of eggs that allows for larvae to hatch. The distinguishing features include differences in shape of the tails and mouth parts.

Figure 2: Morphology of eggs of nematodes associated with soil transmitted infections

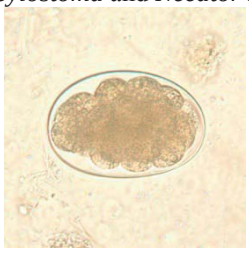
(A) *Ascaris lumbricoides*

Unfertilized and Fertilized Eggs: Females will release unfertilized eggs, which are more elongate (left) than fertilized eggs (right). Unfertilized eggs may also lack the mammillated layer.
Source: https://www.msu.edu/course/zol/316/alumscope.htm

(B) *Trichuris trichiura*

Source: http://en.wikipedia.org/wiki/Trichuris_trichiura

(C) *Ancylostoma* and *Necator species*



Source: http://www.dpd.cdc.gov/dpdx/HTML/Frames/G-L/Hookworm/body_Hookworm_mic1.htm

3. NONCLINICAL MICROBIOLOGY STUDIES

Most of the studies to evaluate the mechanism of action of mebendazole as well as its activity *in vitro* and *in vivo* were against helminthic species infecting livestock and other animals.

[3] Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diement D, and Hotez PJ. Soil transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet* (2006) 367: 1521-1533.

3.1. Mechanism of action

Most of the studies were performed using *Ascaris suum* (roundworm that causes ascariasis in pigs) and *Haemonchus* species [barber's pole worm (nematode) found in sheep, goat and cattle].

Studies *in vitro* by Barrowman *et al* (1984)[4] suggest that benzimidazoles, including mebendazole, act by interfering with the microtubule system in *A. suum*. In addition, these compounds inhibit the polymerization of tubulin into microtubules. However, the degree of binding of benzimidazoles to the tubulin extracts does not appear to be related to the activity of the compound. For example, metabolites such as albendazole sulfone and albendazole 2-amino sulfone, which are not active, were shown to bind almost as strongly as the parent drug. Mebendazole, like colchicine, binds at or near sites on tubulin containing sulphydryl groups necessary for polymerization.

Studies with *Haemonchus contortus,* show that benzimidazoles, including mebendazole, exert their anthelmintic activity by binding to β-tubulin, which interferes with the polymerization of the microtubule (Lacey and Snowdon, 1988[5]; Sangster and Gill, 1999[6]; Vermox® package insert).

3.1.1. Effect on uptake of glucose, amino acids and fatty acids

Van Den Bossche and De Nollin (1973)[7] evaluated the uptake of mebendazole as well as its effect on the uptake and/or transport of glucose, fructose, 3-O-methylglucose, glycine, proline, methionine and palmitic acid by the adult worms of *A. suum in vitro*. Worms were incubated with radiolabeled mebendazole for 24 hours in a glucose salt medium, washed and homogenized. The homogenate was processed for scintillation counting. The results show that mebendazole was detected in all organs (Table 3).

Table 3: Distribution of ^{14}C-labelled mebendazole in the different organs of *A. suum*

Organ	Radioactivity*	
	Specific activity (counts/min per g tissue)	Total activity (counts/min per total wt)
Cuticle + Muscle	4994 ± 817	16874 ± 29
Intestine	10052 ± 3005	3843 ± 1354
Reproductive system	2687 ± 517	4247 ± 361
Pseudocoelomic fluid	9382 ± 3108	18965 ± 7649

* Mean value of 4 determinations ±S.D.

[4] Barrowman M, Marriner S, and Bogan J. The binding and subsequent inhibition of tubulin polymerization in *Ascaris suum* (*in vitro*) by benzimidazole anthelmintics. *Biochem Pharm* (1984) 33 (19): 3037-3040.

[5] Lacey E and Snowdon K. A routine diagnostic assay for the detection of benzimidazole resistance in parasitic nematodes using tritiated benzimidazole carbamates. *Veterinary Parasitology* (1988) 27: 309-324.

[6] Sangster NC and Gill J. Pharmacology of anthelmintic resistance. *Parasitology Today* (1999) 15 (4): 141-146.

[7] Van Den Bossche H and De Nollin S. Effects of mebendazole on the absorption of low molecular weight nutrients by *Ascaris suum*. *Int J Parasitol* (1973) 3: 401-407.

For measuring the effect of mebendazole on uptake of nutrients, adult worms were incubated with medium containing different nutrients (radiolabeled) with and without mebendazole at 37°C for up to 72 hours in 5% CO_2. The glycogen content of the supernatant, after centrifugation of the homogenate, was determined. The results suggest that mebendazole inhibits the uptake and/or transport of glucose by *A. suum.* This inhibition is followed by a marked decrease in the glycogen content of the muscle and intestine of *Ascaris* (Table 4).

Table 4: Effect of mebendazole on the glycogen content of *Ascaris* muscle and intestine

Muscle

Additions	Mebendazole ($\times 10^{-6}$ M)	Glycogen† (mg/g muscle) 1st day	2nd day	3rd day
—	0	25·35 ± 13·62 (9)	19·26 ± 5·43 (4)	19·79 ± 5·10 (4)
	3·4	21·56 ± 4·06 $(9)_1$	21·66 ± 8·12 $(4)_1$	18·22 ± 3·47 $(4)_1$
Glucose‡	0	33·39 ± 8·69 (8)	31·23 ± 4·28 (4)	22·38 ± 5·44 (4)
	3·4	29·62 ± 7·40 $(9)_1$	13·93 ± 5·41 $(4)_3$	13·18 ± 2·32 $(4)_2$
	6·8	16·99 ± 7·39 $(4)_3$		

* *Ascaris* were incubated for 24, 48 or 72 h in a medium containing DMSO or mebendazole.
† Mean value ±S.D. followed by the number of determinations in brackets. P_1 (Mann–Whitney U-test) > 0·05; P_2 < 0·05; P_3 ≤ 0·01.

Intestine

Additions	Mebendazole ($\times 10^{-6}$ M)	Glycogen (mg/g intestine) 1st day	2nd day	3rd day
—	0	2·36 ± 1·85 (9)	2·25 ± 0·80 (4)	1·08 ± 0·99 (4)
	3·4	2·58 ± 0·93 (9)	2·05 ± 1·35 (4)	0·66 ± 0·23 (4)
Glucose	0	2·74 ± 2·80 (7)	3·67 ± 1·09 (4)	2·80 ± 1·50 (4)
	3·4	2·36 ± 1·98 (9)	1·79 ± 0·75 (4)	1·57 ± 1·02 (4)
	6·8	3·39 ± 2·04 (4)		

* *Ascaris* were incubated for 24, 48 or 72 h in a medium containing DMSO or mebendazole.
† Mean value ±S.D. followed by the number of determinations in brackets. P_1 (Mann–Whitney U-test) > 0·05; P_2 < 0·05; P_3 ≤ 0·01.

Mebendazole inhibited the uptake of proline, glycine and methionine in either the presence or absence of glucose (Table 5). The uptake of palmitic acid decreased in the presence of mebendazole when glucose was present. However, in the absence of glucose in the medium there was no significant difference.

Mebendazole did not affect the glucose uptake by the small intestine from rat (Table 5).

Table 5: Effect of mebendazole on the amino acid and fatty acid uptake by *A. suum* and rat small intestine

***A. suum* (incubated for 24 hours in a medium containing DMSO or mebendazole)**

Amino acid (proline, glycine and methionine)

Additions	Uptake (μmoles/24 h per g worm) Proline	Glycine	Methionine
DMSO	4·68 ± 1·70 (10)	3·51 ± 1·90 (13)	3·04 ± 1·02 (10)
Mebendazole	0·53 ± 0·44 (9)$_3$	0·93 ± 0·32 (13)$_3$	0·37 ± 0·10 (11)$_3$
DMSO + Glucose	8·95 ± 3·93 (13)	7·62 ± 4·86 (12)	9·37 ± 3·30 (9)
Mebendazole + glucose	0·72 ± 0·34 (10)$_3$	2·41 ± 1·21 (14)$_3$	1·57 ± 1·01 (11)$_3$

Fatty acid (palmitic acid) uptake

Additions	Palmitic acid uptake (μmoles/24 h per g worm)	P†
DMSO	0·24 ± 0·11 (3)	
Mebendazole	0·22 ± 0·10 (3)	0·87
DMSO + glucose	0·74 ± 0·40 (7)	
Mebendazole + glucose	0·22 ± 0·05 (3)	0·0039

† Mann–Whitney U-test.

Uptake of glucose by rings of rat small intestine

Mebendazole (× 10^{-5} M)	Glucose uptake* μg/30 min per g tissue
0	52·5 ± 7·0 (6)
0·34	58·1 ± 7·8 (3)
3·40	48·1 ± 8·2 (3)

* Mean value ±S.D. followed by the number of determinations in brackets.

Inhibition of glucose uptake is thought to be irreversible and appears to lead to depletion of parasite glycogen, decreased formation of ATP and ultimately cell death (Dollery, 1991[8]).

Overall, the studies suggest that mebendazole inhibits the uptake of low molecular weight nutrients by *Ascaris* but not of rat intestinal cells.

3.1.2. Effect on morphology

Borgers *et al* (1975a[9]; 1975b[10]) reported alteration in morphology of the adult worms of *A. suum* treated with mebendazole. Adult worms were collected from naturally infected pigs that were fed *ad libitum* medicated food containing 30 or 125 ppm mebendazole. The animals were sacrificed at different time intervals (6, 9, 15 or 24 hours) after initiation of treatment. The intestinal tract of *A. suum* worms is composed of a single layer of cylindrical cells which do not undergo continuous replacement. Characteristic for these epithelial cells is the presence of a large number of secretory granules, derived from the Golgi apparatus and transported through the cytoplasm to reach the terminal web area where they dissolve or fuse with the apical plasmalemma. After 6 hours of drug treatment, microtubules disappeared from the apical half of the cytoplasm and secretory granules accumulated in the Golgi areas. After 9 hours of treatment, all intestinal cells showed massive accumulation and fusion of granules. By 24 hours of treatment, cellular injury was predominant in every cell that was characterized by swelling,

[8] Dollery C (Editor). Mebendazole. In: Therapeutic Drugs, Volume 2, Edited by C. Dollery *et al*. Published by Churchill Livingstone, UK (1991) M12-M16.

[9] Borgers M, Nollin S, Verheyen A, De Brabander M, and Thienpont D. Effects of new anthelmintics on the microtubular system of parasites. *Microtubules and Microtubule Inhibitors.* Eds M Borgers and De Brabander M (1975) pp 497-508.

[10] Borgers M, Nollin S, De Brabander M, Thienpont D. Influence of the anthelmintic mebendazole on microtubules and intracellular organelle movement in nematode intestinal cells. *Am J Vet Res* (1975) 36 (8) 1153-1166.

fusion or even complete loss of the microvilli, lysis of the cytoplasm with excessive accumulation of vacuoles containing, most probably, remnants of secretory substances and degeneration of subcellular organelles. Such degenerative changes of the intestinal cells of the worm are thought to be irreversible for such a non-proliferative cell type and leads to the death of the parasite.

Similar observations were reported against other worms that include

- *Syngamus trachea* (a nematode, commonly known as grape worm that inhabits the trachea of certain birds): Infected turkeys were treated with mebendazole. The majority of microtubules in the intestine of the worms disappeared after 6 hours of treatment, whereas the microtubules in host tissues, such as intestine or in cells in the vicinity of the parasites (erythrocytes, endothelial cells, and tracheal epithelium) remained morphologically intact after 72 hours of continuous medication.
- *Taenia taeniaeformis* (a cestode that feeds solely by taking up nutrients through its syncytial tegumental surface): Infected mice were treated with a single dose of mebendazole administered either by parenteral route or orally by mixing with food (250 ppm). By electron microscopy, morphological changes were observed as early as 6 hours after initiation of treatment. There was no effect of treatment with mebendazole on microtubule distribution in the intestinal and liver parenchymal cells of the host.

Overall, the studies suggest that mebendazole causes the disintegration of the cytoplasmic microtubular system of the worms, which results in a block in the movement of subcellular organelles including a block in the transport of secretory granules from the Golgi towards the apical cell surface. The morphologic changes, after treatment with mebendazole in the absorptive organs of the three parasites living under different environmental conditions, were comparable to each other. The impact of the primary interaction of the drug with cytoplasmic microtubules is thought to be the initial cause of the subsequently observed cytoplasmic disorganization and lytic degeneration of the absorptive cells leading to the death of the parasites. Also, there is a difference in sensitivity towards this drug between the host and the parasite.

Verheyen *et al*., 1976[11] reported the effect of treatment with mebendazole in Albino Swiss mice experimentally infected with *Hymenolepis nana* (a cestode tapeworm inhabiting the small intestine of the mouse). Mebendazole was administered orally; either as a single dose of 40 mg/kg by stomach intubation or mixed with food at a concentration of 200 or 500 ppm. Two mice were necropsied at 6, 24, 48 and 72 hours after the onset of medication and a minimum of 3 worms collected from each animal and processed for morphological examination by electron microcopy. The microtubules disappeared in the early hours after the onset of treatment; however, in untreated parasites these microtubules were prominent in the Golgi areas of the nucleated region and the tiny cytoplasmic bridges interconnecting the nucleated and anucleated parts of the tegument. At later time points, there was accumulation of secretory vesicles in the Golgi areas and deterioration of the absorptive surface of the worm followed by degeneration of both nucleated and anucleated parts of the tegument of treated worms. The host tissues remain unaltered.

[11] Verheyen A, Borgers M, Vanparijs, and Thienpont D. The effects of mebendazole on the ultrastructure of cestodes. *Biochem of parasites and host-parasite relationships*. Ed H Van Den Bossche (1976) 605-618.

Comments:
Most of the studies were performed with A. suum and Haemonchus species. Overall, the studies suggest that mebendazole binds to different organs (intestine, cuticle, muscle, reproductive system and pseudocoelomic fluid) of nematodes and interferes with cellular microtubule formation, blocks glucose and other nutrient uptake, alters the morphology and cause ultrastructural degenerative changes in helminths. The digestive and reproductive functions are disrupted, leading to immobilization, inhibition of egg production, and death of the parasites. Such an action is initiated by 6 hours after drug administration.

Such changes were not observed in the host cells.

3.2. Activity in vitro/ex vivo

3.2.1. *In vitro* effect on eggs and infective larvae of hookworms

3.2.1.1. Activity against the eggs

The *in vitro* effect of mebendazole on the development of eggs of human hookworms (*A. duodenale* and *N. americanus*) and dog hookworm (*Ancylostoma caninum*) was reported (Banerjee *et al*., 1971[12]; Banerjee and Prakash, 1972[13]). Briefly, stool samples were collected from human subjects harboring an egg count of 2,803-3,300/g of feces (five cases for each hookworm species; hookworm species was identified by culture). Stool samples were also collected from four dogs naturally infected with *A. caninum* and having an egg count of 6,200-10,000/g of feces.

The activity against the egg stage was measured by incubating stool specimens (5 g) with equal volume of water containing different concentrations (25, 50, 100, 150, 200, 300 and 400 µg/mL) of mebendazole, for 24 hours. Aliquots were processed for culture by 3 different methods (slide culture, charcoal-fecal culture and the test-tube filter paper culture) and incubated at temperatures between 25 -30^{o}C. By slide culture method, cultures were examined up to Day 2 whereas by the other methods, cultures were examined up to Day 10. The authors state that the slide culture method was not reliable for evaluating the drug effect, due to extreme variability. By either the charcoal-fecal or the test-tube filter paper method, many larvae were observed from Day 5 onwards in the control cultures; however, no larvae were observed in cultures containing mebendazole at concentrations ≥50 µg/mL suggesting complete inhibition of the development of eggs to reach the larval stage.

3.2.1.2. Activity against the infective larvae

The effect of mebendazole on infective larvae was evaluated by incubating infective filariform larvae (approximately 400) obtained by the charcoal fecal culture with different concentrations of mebendazole (0, 300, 400, 500, 600, 700, 800, 900, 1000, 3000, 4000, 5000 and 6000 µg/ml) in different petri dishes (Banerjee *et al*., 1971[12]; Banerjee and Prakash, 1972[13]). The cultures

[12] Banerjee D, Mandal A, Prakash O. Mebendazole (R-17635): A new anthelmintic in the development of hookworms. *Trans Roy Soc Trop Med Hyg* (1971) 65: 685-686.

[13] Banerjee D and Prakash O. In Vitro action of a new anthelmintic, mebendazole (R-17635) on the development of hookworms. *Indian J Med Res* (1972) 60: 1-3.

were examined at 24 hours for the presence of larvae and approximately 150 larvae were harvested and used for infecting mice (CDRI strain) by the oral route. Animals were necropsied at 24 hours and liver processed to examine for the presence of infective larvae. The larvae were stated to be active in all the cultures incubated in the absence or presence of mebendazole after culture; larvae were also observed in all the livers from infected mice. No larvicidal effect was observed even at the highest mebendazole concentration (6000 µg/ml) tested.

3.2.2. *Ex vivo* effect on eggs and infective larvae of whipworm and hookworm

A direct ovicidal effect was reported by lack of progression to the larval stage of hookworms and whipworm (*T. trichiura*) eggs that were recovered from patients treated with mebendazole (Wagner and Chavarria, 1974a[14]). All patients except three were treated with 100 mg mebendazole twice daily for 4 days; 2 subjects with whipworm and one subject with hookworm infection were treated with 200 mg mebendazole twice daily for 3 days. Stool samples were collected prior to treatment; also, specimens were collected each day starting one day following administration of mebendazole until the stools were negative for eggs. The number of eggs/g feces was determined by the Stoll method. For hookworms, stool samples were incubated in petri dishes containing tap water, and daily examinations of cultures was performed up to Day 8. The number of eggs containing motile larvae or larvae that hatched from the eggs as compared to non-developed hookworm eggs was determined. Most of the larvae in the pre-treatment stool specimens hatched within 2 or 3 days of incubation. For whipworms, eggs were cultured in an aqueous solution of 2.5% potassium dichromate at room temperature (24-27^{o}C) for up to 40 days. Compared to the pre-treatment samples, a reduction in both hookworm and whipworm eggs that developed to the larval stage was observed in samples collected post-treatment (Table 6).

Similar findings were reported in another study in 14 patients with whipworm infection (Wagner and Chavarria, 1974b[15]). The experimental design was same as summarized above; it is possible that some of the subjects discussed in this study are same as those in the study[14] summarized above. The baseline egg count was not shown. A reduction in egg count (Table 7) as well as alteration in morphology of the eggs was observed as early as 24 hours after initiation of treatment. Changes in morphology include an increase in egg size, the presence of a membrane, distorted, elongated configuration; distorted shape of the polar plugs, an abnormal number of polar plugs, ranging from none to three, a marked decrease in size, and an abnormal, diffuse type of yolk material which is refractive to bile staining. It was stated that the colorless eggs without polar plugs failed to develop into the larval stage when incubated in an appropriate medium. Only a small percentage (8% and 9%) of the eggs in two of the patients was of abnormal shape but had normal yolk material and developed to the larval stage.

[14] Wagner E and Chavarria A. *In vivo* effects of a new anthelmintic mebendazole (R17635) on the eggs of *Trichuris trichiura* and hookworm. *Am J Trop Med Hyg* (1974a) 23 (2): 151-153.

[15] Wagner E and Chavarria A. Morphologically altered eggs of *Trichuris trichiura* following treatment with mebendazole. *Am J Trop Med Hyg* (1974b) 23 (2): 154-157.

Table 6: Effect on hookworm and whipworm eggs from patients before, during and after treatment with mebendazole. Results represent the numbers of eggs/g feces, and the percentage of larval development.

A: Hookworm

Patient number	Pre-treatment	Day after treatment 1	2	3	4	5
1	30,400 97.0%	—	43,100 0%	200 0%	—	negative
2	18,000 60.5%	10,800 0%	600 0%	negative	negative	
3	7,200 91.0%	16,200 85.7%	400 0%	negative	negative	

Represent 1 subject (no. 3) treated with 200 mg mebendazole twice daily for 3 days; other patients treated with 100 mg twice daily for 4 days.

B: *Trichuris trichiura*

Patient number	Pre-treatment	Day after treatment started 1	2	3	4	5	6	7
1	4,000 87.5%	6,000 22.2%	2,700 0%	1,000 0%	1,800 0%	negative		
2	115,200 92.3%	60,600 57.0%	33,600 0%	50,400 0%	15,600 0%	5,200 0%	negative	
3	66,400 96.0%	—	273,900 0%	46,000 0%	—	1,000 0%	600 —	negative
4	115,800 60.0%	73,200 27.5%	47,200 1.0%	28,000 0%	7,200 0%	2,400 0%	3,600 —	600 —*
5	22,200 98.5%	41,600 95.0%	22,000 10.0%	60,600 0%	400 0%	600 0%	negative	
6	28,800 80.3%	128,000 87.5%	19,200 15.7%	7,200 3.34%	1,200 0%	negative		

* On day 8, stool negative.

Represent 2 subjects (nos. 1 and 2) treated with 200 mg mebendazole twice daily for 3 days; other patients treated with 100 mg twice daily for 4 days.

Table 7: Percentage of atypical eggs found following mebendazole treatment

Patient no.	Day after mebendazole treatment started 1	2	3	4	5	6	7
1	23	66	100	100	100	neg	
2	11	few	few	—	93	—	100
3	few	44	—	100			
4	—	0	100	—	100	neg	
5	0	0	84	100	neg		
6	0	—	4	—	—	60	
7	0	0	0	97	100	neg	
8	0	0	0	100	neg		
9	0	0	0	0	few	87.5	
10	0	few	few	—	neg		
11	0	0	0	0	0	few	62.5
12	0	0	0	—	—	few	neg
13	0	0	0	0	0	0	0
14	0	0	0	0			

Represent 2 subjects (8, 10) treated with 200 mg mebendazole twice daily for 3 days; other patients treated with 100 mg twice daily for 4 days.

In some patients % reduction was not determined and results presented as few

Comments:

- *Overall, the in vitro and ex vivo studies suggest that mebendazole is active against the egg stage and inhibits hatching of the larvae of A. duodenale, N. americanus, A. caninum and T. trichiura. However, mebendazole may not be active against the infective larvae.*

3.3. Activity *in vivo*

The activity of mebendazole was measured against different helminth species in experimentally infected rodents and dogs (Report nos. V1127[16]; V1252[17]; V1130 [18]; Trane and Carneri, 1972[19]; V1021[20]; V970[21]; V1160[22]; V1237[23]) as well as naturally infected dogs (V783[24]; V671[25]; V672[26]; V769[27]). Most of the studies were in a small number of animals and 3 or more doses of mebendazole were administered. A single dose of mebendazole was tested in two studies (V1252[17] and V1237[23]) and shown to be effective in reducing worm burden. In one study (V1237[23]), the effect of single and multiple doses of drug was compared; treatment with multiple doses was more effective (Table 8).

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[16] Report no. V1127: Janssen Pharmaceuticals. *Ascaris suum* in rats. Prophylactic treatment with R17635.

[17] Report no. V1252: Janssen Research Products Information Service. Anthelmintic activity of mebendazole in rats. Screening test: *Strongyloides ratti*.

[18] Report no. V1130: Microbiology Laboratory of Carlo Erba Research Institute, Milan. *Trichuris muris* as a model for studies on the chemotherapy of trichuriasis.

[19] Trane F, de Carneri I. *Trichuris muris* model for studies on the chemotherapy of trichuriasis. National Congress of the Italian Parasitological Society, Bologna. (1972) 1-6. English translation.

[20] Report no. V1021: Janssen Pharmaceuticals. The anthelmintic activity of R17635 in rodents.

[21] Report no. V970: Hawthorn Park Research Laboratories Pvt Ltd. Activity of mebendazole medicated pet food against *Taenia hydatigena* in the dog.

[22] Report no. V1160: Hawthorn Park Research Laboratories Pvt Ltd. Activity of mebendazole against *Echinococcus granulosis* in the dog.

[23] Report no. V1237: Hawthorn Park Research Laboratories Pvt Ltd. Efficacy of mebendazole against *Echinococcus granulosis* in the dog.

[24] Report no. V783: Hawthorn Park Research Laboratories Pvt Ltd. Studies on the efficacy of mebendazole medicated pet food against intestinal roundworms in dogs.

[25] Report no. V671: Hawthorn Park Research Laboratories Pvt Ltd. Report to establish that a 200 mg dose of mebendazole, when administered on three occasions at intervals of 12 hours, will produce removal of ascarids and hookworms in dogs.

[26] Report no. V672: Hawthorn Park Research Laboratories Pvt Ltd. Preliminary study of the efficacy of mebendazole against whipworms in dogs.

[27] Report no. V769: Hawthorn Park Research Laboratories Pvt Ltd. The palatability and efficacy of mebendazole medicated canned food.

Table 8: Summary of experimentally and naturally infected animal studies

Helminth Species / Report	Study summary
Experimentally infected	
Ascaris suum (roundworm) Report V1127[16]	Wistar rats were infected orally with 20000 embryonated eggs of *A. suum*. Treatment with MBZ in medicated feed from Day -3 to 8 was effective in reducing lesions in the lung and the number of larvae; such an effect was dose-dependent.
Strongyloides ratii (thread worm) Report V1252[17]	Wistar rats were infected subcutaneously with 1000 infective larvae of *S. ratii*. Treatment with a single oral dose (≥10 mg/kg) of MBZ was effective in clearing worms (measured on Day 5 after treatment).
Trichuris muris (whipworm) V1130[18]; Trane and Carneri, 1972[19]	Immunocompromised CF1 mice were infected orally with 300 mature eggs of *T. muris.* MBZ, administered orally bid for 6 days, was effective in protecting mice. Such an effect was dose-dependent.
Syphacia muris (pinworm) *Nippostrongylus brasiliensis* (roundworm) *Trichinella spiralis* (porkworm) *Nematospiroides dubius (roundworm)* *Hymenolepis nana* (dwarf tapeworm) Report V1021[20]	Wistar rats were infected with *S. muris* (stage of the parasite used for infection not specified), *N. brasiliensis* (500 infective larvae), or *T. spiralis* (2000 infective larvae). Animals were treated orally or with mediated feed with either single or multiple doses of MBZ. Single dose of MBZ was effective against *S. muris* (0.63 mg/kg) and *T. spiralis* (32 and 125 ppm) but not active against *N. brasiliensis* (40 mg/kg). Multiple doses were effective against all three parasites.
	Albino mice were infected with either *N. dubius* (500 infective larvae) or *H. nana* (50 viable eggs). Treatment with a single oral dose of MBZ (10 mg/kg) was not effective against *N. dubius* infection. However, MBZ administered for 5 – 7 days was effective against *H. nana* infection.
Taenia hydatigena (tape worm) Report V970[21]	Beagle dogs were infected with 10 cysticerci of *T. hydatigena*. Treatment with MBZ (administered with food; 20 mg/kg, bid for 5 days) initiated 34 days post-infection was effective in removing established infection.
Echinococcus granulosis (tape worm) Report V1160[22]	Beagle dogs were infected with hydatid cysts (8 times spread over 7 weeks) of *E. granulosis*. Treatment with MBZ (200 mg, bid, for 5 days) initiated 22 days after the last dose of cyst was effective in removing worms.
Echinococcus granulosis (tape worm) Report V1237[23]	Beagle dogs were artificially infected with 9000 protocsolices of *E. granulosis*. Time of initiation of treatment not specified. Oral treatment with MBZ single dose [200 mg/kg or 100 mg/kg (with MORPHA)] or multiple doses (200 mg/kg or 100 mg/kg, bid, for 5 days) were effective in removing worms. Multiple doses were more effective.
Naturally infected	
Ancylostoma caninum (hookworm) *Trichuris vulpis* (whipworm) *Toxocara canis* (roundworm) *Uncinaria stenocephala* (northern hookworm) Report V783[24]	Mongrel dogs infected with one or more of the helminth species were treated with MBZ (10 mg/kg; bid for two or three days) with food. Worm burden was reduced in dogs treated with MBZ.
Toxocara canis (roundworm) *A. caninum* (hookworm) Report V671[25]	Beagle dogs infected with one or more of the helminths were treated orally with three doses (200 mg) of MBZ, 12 hours apart. Six days following treatment, the dogs were slaughtered and their intestines removed. The contents of the intestines were processed for worm count. The gut was opened and examined for the presence of parasites adhering to the wall. No eggs were detected in treated dogs. Two of the control dogs lost their infections (eggs and worms) during the course of the trial. At autopsy, hookworms were detected in one of the treated dogs and all the control animals; Ascarids were detected in 2 of the 4 control animals.
Trichuris vulpis (whipworm) Report V672[26]	Mongrel dogs were treated orally with either three doses (200 mg each) or 5 doses (200 mg each) of MBZ with food, 12 hours apart. On Day 7, the content of cecum, colon and proximal four inches of the ileum examined for the parasites. The lining of the wall was examined for any adhering parasites. No worms were found in two of the 3 dogs treated with 5 doses of MBZ; of the 3 dogs treated with 3 doses of MBZ, worms were present in one dog. Worms were present in both of the untreated dogs.
Ancylostoma caninum (hookworm) *Trichuris vulpis* (whipworm) Report V769[27]	Beagle dogs infected with one or more of the helminthes were treated with MBZ (200 mg bid) for three or five feeds. Fecal egg count reduced to zero, seven days post treatment. Some animals showed the presence of parasite eggs in their feces 14 days post treatment. Although no animal showed the presence of ascarid eggs on pre-treatment egg count, four animals shed a small number of ascarid worms following treatment.

MBZ=mebendazole

Rodriguez-Caabeiro *et al*., 1987[28] reported the activity of (b) (4) administered intraperitoneally, in mice infected with the nematode *Trichinella spiralis* (GM-1 strain). Briefly, the mice were infected with the larvae and treatment initiated at different time intervals (Day 1 and 6) post-infection (PI). The effect on the parenteral phase was determined by counting the number of muscle stage larvae, appearing after pepsin digestion, at Day 30 PI. (b) (4)

In another experiment, the intestinal population was suppressed by methyridine treatment (60 mg/kg) at Day 9 PI; this was to ensure that the drug action was only directed against emigrant larvae and not against intestinal adults and embryos. (b) (4)

In another experiment, the effect on encysted larvae was measured by initiating treatment with (b) (4) Days 45, 46 and 47 PI. Mice were euthanized on either Day 5 or 15 after the last dose of mebendazole. (b) (4)

Table 9: Effects of different (b) (4) mebendazole 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 MBZ polymorph (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.

Polymorph form C (b) (4).

Comments:
Activity of single or multiple doses of mebendazole was measured in rodents and dogs experimentally infected with different helminth species as well as naturally infected dogs. Overall, the studies show that mebendazole was effective in reducing parasite burden; such an effect was dose-dependent. A single and multiple doses of mebendazole were compared in one study; the results showed multiple doses to be more effective in reducing parasite burden than the single dose.

(b) (4)

[28] Rodriguez-Caabeiro F, Criado-Fornelioa A, Jimenez-Gonzaleza A, and Guzmanb L. Experimental chemotherapy and toxicity in mice of (b) (4). *Chemotherapy* (1987) 33: 266-271.

3.4. Drug Resistance

3.4.1. *In vitro*

Development of resistance to mebendazole and other benzimidazoles by helminths infecting livestock and other animals has been reported. For example, Sangster *et al* (1985)[29] reported a differential inhibitory effect of thiabendazole, another benzimidazole, on microtubule-dependent acetylcholinesterase secretion by the worm of a susceptible and a resistant strain of *Trichostrongylus colubriformis*; inhibitory effect was more on the susceptible strain compared to the resistant strain. Also, inhibition of colchicine (known to bind the same site as benzimidazoles) binding by mebendazole was to a greater extent with the extracts from worms of the susceptible strain compared to the resistant strain suggesting either reduced affinity of tubulin for the drug or lower tubulin content in the resistant strain compared to the susceptible strain.

Roos *et al* (1995)[30] reported development of resistance by *H. contortus* to mebendazole and thiabendazole, *in vitro*, under increasing drug concentrations. Resistance appears to be associated with mutation in the *β-tubulin* gene, which is highly polymorphic; there was a loss of alleles at the locus of *β-tubulin* isotype 1 and isotype 2. Alteration in isotype 1 is thought to be correlated with a conserved mutation (Phe being replaced by Tyr) at amino acid 200 (Kwa *et al*., 1994[31]).

Kerboeuf *et al* (1999[32]) reported that P-glycoproteins (P-gp), known to be important in drug transport for pumping drugs out of cells, may play a role in benzimidazole resistance in *H. contortus*.

3.4.2. *In vivo*

3.4.2.1. Helminth species infecting livestock

In helminths infecting livestock, resistance was evaluated by fecal egg count reduction (FECRT) and egg hatch assay (Le Jambre *et al*., 1976[33]; Lacey *et al*., 1987[34]; Lacey and Snowdon, 1988[5]; Roos *et al* 1995[30]; Geerts and Gryseels, 2000[35]). Both high and low treatment frequencies of

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[29] Sangster NC, Prichard RK, and Lacey E. Tubulin and benzimidazole-resistance in *Trichostrongylus colubriformis* (Nematoda). *J Parasit* (1985) 71 (5): 645-651.

[30] Roos MH, Kwa MSG, and Grant WN. New genetic and practical implications of selection for anthelmintic resistance in parasitic nematodes. *Parasitol Today* (1995) 11**:**148–150.

[31] Kwa MSG, Veenstra JG, and Roos MH. Benzimidazole resistance in *Haemonchus contortus* is correlated with a conserved mutation at amino acid 200 in b-tubulin isotype 1. *Mol Biochem Parasitol* (1994) 63: 299-303.

[32] Kerboeuf D, Chambrier P, Le Vern Y, and Aycardi J. Flow cytometry analysis of drug transport mechanisms in *Haemonchus contortus* susceptible or resistant to anthelmintics. *Parasitol Res* (1999) 85:118–123.

[33] Le Jambre L, Southcott W, Dash K. Resistance of selected lines of *Haemonchus contortus* to thiabendazole, morantel tartrate and levamisole. *Int J Parasitology* (1976) 6: 217-222.

[34] Lacey E, Snowdon L, Eagleson GK, and Smith EF. Further investigations of the primary mechanism of benzimidazole resistance in *Haemonchus contortus*. *Int J Parasitol* (1987) 17 (8): 1421-1429.

[35] Geerts S and Gryseels B. Drug resistance in human helminthes: Current situation and lessons from livestock. *Clin Microb Rev* (2000) 13 (2): 201-222.

drug administration are considered to contribute to the selection of resistant or tolerant strains especially when the same drug is used over many years (Geerts and Gryseels, 2000[35]).
The egg hatch assay (EHA) assesses the ability of drugs at given concentrations to inhibit the embryonation and hatching of freshly-collected nematode eggs. The results are expressed as the dose required for inhibiting 50% of the eggs (ED_{50}). However, the results vary due to variables that include the parasite strain and incubation time. The use of these assays to assess resistance in humans has not been standardized.

3.4.2.2. In soil transmitted helminth species infecting humans

Low cure rates (CRs), especially in subjects infested with whipworms (*T. trichiura*) or (b) (4) have been reported in some of the studies (Albonico *et al*., 1994a ; 1994b ; 2002a ; 2003[39]; Charoenlarp *et al*., 1993[40]; De Clercq *et al*.1997[41]; Namwanje *et al*., 2011[42]; Schutte, 1989 [43]; Soukhathammavong *et al*., 2012[44]; Speich *et al*., 2015[45]). It is unclear whether low CRs are due to the development of resistance due to

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[36] Albonico M, Renganathan E, Bosman A, Kisumku UM, Kassim S, Alawi L and Savioli L. Efficacy of a single dose of mebendazole on prevalence and intensity of soil-transmitted nematodes in Zanzibar. *Trop Geogr Med* (1994a) 46 (3): 142-146.

[37] Albonico M, Smith PG, Hall A, Chwaya HM, Alawi KS, and Savioli L. A randomized controlled trial comparing mebendazole and albendazole against *Ascaris*, *Trichuris* and hookworm infections. *Trans Roy Soc Trop Med Hyg* (1994b) 88: 585-589.

[38] Albonico M, Bickle Q, Haji H, Ramsan M, Khatib KJ, Montresor A, Savioli L, and Taylor M. Evaluation of the efficacy of pyrantel-oxantel for the treatment of soil-transmitted nematode infections. *Trans Roy Soc Trop Med Hyg* (2002a) 96: 685-690.

[39] Albonico M, Bickle Q, Ramsan M, Montresor A, Savioli L, and Taylor M. Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. *Bull World Health Organ* (2003) 81(5): 343-352.

[40] Charoenlarp P, Waikagul J, Muennoo C, Srinophakun S, and Kitayaporn D. Efficacy of single-dose mebendazole, (b) (4) the treatment of hookworm and *Trichuris* infections. *Southeast Asian J Trop Med Publ Health* (1993) 24 (4):712-716.

[41] De Clercq D, Sacko M, Behnke J, Gilbert F, Dorny P, and Vercruysse J. Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. *Am J Trop Med Hyg* (1997) 57 (1): 25-30.

[42] Namwanje H, Kabatereine NB, and Olsen A. Efficacy of single and double doses of albendazole and mebendazole alone and in combination in the treatment of Trichuris trichiura in school-age children in Uganda. *Trans R Soc Trop Med Hyg* (2011) 105(10):586-590.

[43]Schutte. Report on mebendazole (Vermox) 500 mg trial in northern Kwazulu. *Unpublished internal report N 64704/1* (1989).

[44] Soukhathammavong PA, Sayasone S, Phongluxa K, Xayaseng V, Utzinger J, Vounatsou P, Hatz C, Akkhavong K, Keiser J, and Odermatt P . Low efficacy of single-dose albendazole and mebendazole against hookworm and effect on concomitant helminth infection in Lao PDR. *PLoS Negl Trop Dis* (2012) 6 (1):e1417.

[45] Speich B, Ali SM, Ame SM, Bogoch II, Alles R, Huwyler J, Albonico M, Hattendorf J, and Utzinger J, and Keiser J. Efficacy and safety of albendazole plus ivermectin, albendazole plus mebendazole, albendazole plus oxantel pamoate, and mebendazole alone against Trichuris trichiura and concomitant soil-transmitted helminth infections: a four-arm, randomised controlled trial. *Lancet Infect Dis* (2015) 15 (3): 277-284.

prior treatments or the parasites are intrinsically resistant or tolerant to mebendazole and other benzimidazoles (cross-resistance) or the subjects were re-infected. For example, one study (De Clercq *et al*., 1997[41]), reported low CRs in patients with hookworm infections that were treated with mebendazole, in Mali, although there was no previous history of intense treatment or clinical suspicion of drug resistance in that region (Geerts and Gryseels, 2000[35]). The failures could be due to re-infection in an endemic area as well as the maturation of prepatent infections (mebendazole does not appear to affect immature forms of the parasite) as the egg reduction (ERRs) and egg counts were determined at week 4 after treatment. Another study (Sacko *et al*., 1999[46]) by the same group conducted in the same region reported higher CRs (b) (4)

One study (van der Werff *et al*., 2014[47]), reported CRs and ERRs in subjects administered mebendazole (500 mg) as part of a mass drug administration (MDA) program for *A. lumbricoides*, *T. trichiura*, *N. americanus*, and *A. duodenale*. The CRs and ERRs at about Month 6 after the first dose and after about 3 years (subjects received mebendazole every 6 months) were similar (for more details see section 4.2).

As stated above, the EHA works on nematode species in which eggs hatch rapidly and has been used in veterinary medicine to detect drug resistance. In one clinical study (De Clercq *et al*., 1997[41]), EHA was performed on some of the *N. americanus* eggs recovered from subjects that failed mebendazole treatment; results were compared with the eggs from hamsters infected with a laboratory strain of the parasite that had not been exposed to anthelmintics for more than 100 generations. The Mali strain of *N. americanus* was stated to be almost twice as resistant to benzimidazoles compared to the laboratory reference strain; it should be noted that this is based on the criteria (thiabendazole $ED_{50} \geq 0.1$ µg/mL) used to evaluate the sensitivity of nematodes affecting domestic animals (ruminants such as sheep and cattle) to benzimidazoles. Another study (Albonico *et al*., 2005[48]) reported no change in ED_{50} values in the samples collected from children in Standard 1 (never received any mebendazole treatment) and Standard 5 (had received a total of 13 rounds of mebendazole treatment) living on Pemba Island within Zanzibar. The results suggest that a drug-resistant worm population had not built up within treated individuals, and that periodic treatment did not select for widespread benzimidazoles resistance, at least at the threshold detectable by the EHA. These results should be interpreted with caution as this technique is not standardized for human hookworms or other STH and the clinical relevance of such findings is not known. Factors such as strain differences, processing of the field samples, and delays during transport are known to affect the results.

[46] Sacko M, De Clercq D, Behnke J.M, Gilbert FS, Dorny P, and Vercruysse J. Comparison of the efficacy of mebendazole, albendazole and pyrantel in treatment of human hookworm infections in the Southern Region of Mali, West Africa. *Trans R Soc Trop Med Hyg* (1999) 93**:**195–203.

[47] van der Werff SD, Vereecken K, van der Laan K, Ponce MC, Diaz JR, Nunez FA, Rivero LR, Gorbea MB, Polman K. Impact of periodic selective mebendazole treatment on soil transmitted helminth infections in Cuban schoolchildren. *Trop Med Int Health* (2014) 19(6):706-718.

[48] Albonico M, Wright V, Ramsan M, Haji HJ, Taylor M, Savioli L, and Bickle Q. Development of the egg hatch assay for detection of anthelminthic resistance in human hookworms. *Int J Parasitol* (2005) 35: 803-811.

Efforts are being made to evaluate mechanism of resistance for benzimidazoles, including mebendazole, against STH infecting humans. A study by Schwenkenbecher *et al* (2007)[49] reported (b) (4), by real-time PCR, in codons 167-200 of the hookworm (*A. duodenale* and *N. americanus*) isolates collected from school children in Pemba Island who had demonstrated a reduced response to treatment with mebendazole. Overall, the study suggests changes in codons 167 and 200 of *β-tubulin* isotype-1 may not be responsible for resistance to benzimidazoles.

Diawara *et al*., 2009[50] evaluated the genomic sequences of the *β-tubulin* gene around codon 200 in *A. lumbricoides* and *T. trichiura* by a pyrosequencing assay. DNA of individual adult worms and pooled eggs from the field were processed to determine the occurrence and frequency of (b) (4)) in areas that were known to be either naïve (Kenya) to benzimidazole (albendazole or mebendazole) treatment or areas (Uganda, Zanzibar, and Panama) where benzimidazole MDA had been implemented. All *A. lumbricoides* samples from Kenya, Panama, Uganda or Zanzibar were sensitive (TTC) and did not carry the resistant (TAC) mutation at (b) (4) the *β-tubulin* gene. However, for the *T. trichiura* pooled egg samples from Kenya and Panama, both mixed TTC/TAC and TAC alone, in different pools, were reported. These results should be interpreted with caution as a correlation with clinical and parasitological response was not evaluated. Also, the clinical relevance of such finding is not known.

Comments:
Overall, the in vitro and animal studies suggest a potential for development of resistance to mebendazole by helminths. Resistance appears to be due to a (b) (4) *Although low CRs have been reported in subjects with STH infections, however, an association between mutations in the β-tubulin gene and clinical or parasitological response has not been established.*

MDA, 2 times a year for about 5 to 6 years, have been shown to be effective in improving CRs and reducing egg count. Also, CRs were similar in children who had never received any mebendazole treatment (Standard 1) and those who had received a total of 13 rounds of treatment (Standard 5) on Pemba Island within Zanzibar. The subjects living in endemic areas are likely to be reinfected and may reach the pretreatment level of infection rapidly depending on ecological and other factors.

The tests to detect mebendazole resistance for STH infecting humans have not been standardized.

4. CLINICAL MICROBIOLOGY

The Applicant conducted a phase 3 clinical trial (Study GAI3003) in children to support the efficacy of a single dose of mebendazole for the treatment of *A. lumbricoides*, and *T. trichiura*

[49] Schwenkenbecher JM, Albonico M, Bickle Q, and Kaplan RM. Characterization of beta-tubulin genes in hookworms and investigation of resistance-associated mutations using real-time PCR. *Mol & Biochemical Parasitol* (2007) 156: 167-174.

[50] Diawara A, Drake LJ, Suswillo RR, Kihara J, Bundy DAP, Scott ME, Halpenny C, Stothard JR, and Prichard RK. Assays to detect β-tubulin (b) (4) in *Trichuris trichiura* and *Ascaris lumbricoides*. *PLoS Neglected Tropical Diseases* (2009) 3 (3): e397.

infection. (b) (4) [redacted]

4.1. Efficacy of a single dose of mebendazole

4.1.1. Phase 3 study GAI3003

This was a phase 3, randomized, double-blind, parallel-group, placebo-controlled, multi-center [3 centers: 2 centers (Gondar and Jimma sites) in Ethiopia and 1 center (Kigali) in Rwanda] trial to evaluate the efficacy and safety of a single dose of mebendazole (500 mg, chewable tablets) for the treatment of *A. lumbricoides* and *T. trichiura* infections in 295 children between 1 and 16 years of age. Sufficient number of subjects were screened to ensure that at least 50 subjects with positive egg counts for *A. lumbricoides* and 200 subjects with positive egg counts for *T. trichiura* were enrolled. If a subject was infected with hookworm only, the subject was not enrolled in the study but was referred through the appropriate health care system for further evaluation, treatment, and follow-up. However, the subject was enrolled in the study in the event of multiple STH infections where hookworm was one of them.

Primary objective

Compare the efficacy and safety of mebendazole with placebo in the treatment of *A. lumbricoides* and *T. trichiura*.

Secondary objective

Assess the systemic exposure to mebendazole following oral dosing.

Exploratory objective

Compare the efficacy and safety of mebendazole to placebo in the treatment of hookworm infections.

Primary efficacy endpoint

The two primary efficacy endpoints were the CRs for *A. lumbricoides* and *T. trichiura* at the end of the double-blind treatment period i.e., Day 19.

Note: Cure was based on a post-treatment egg count of zero in subjects who had a positive egg count for that STH at baseline.

Secondary efficacy endpoint

Egg count reduction (ER) for *A. lumbricoides* and *T. trichiura* at the end of the double-blind period i.e., Day 19.

Note: The baseline value for efficacy analyses was based on the average positive egg count for that STH from the screening visit.

(b) (4) [redacted]

Inclusion criteria

- Subjects, male or female, who were 1 to 16 years old, inclusive, and lived in a high STH prevalence area.
- Female subjects who were ≥9 years had a negative urine pregnancy test at screening or at the time of randomization.
- Subject must have been an otherwise healthy child, based on medical history, physical examination, vital signs, hemoglobin, and concomitant medications.
- Subjects ≥3 years of age must have had teeth and be able to chew.
- Subject must have been available to return to the study site for all visits, including the follow-up visit.
- Parent(s)/guardians of subjects (or their legally-accepted representatives) must have signed an informed consent document indicating that they understand the purpose of and procedures required for the study and were willing to have their child participate in the study.
- Children 6 years of age and older were asked to agree to their participation using language appropriate to their level of understanding. Assent was documented.

Exclusion criteria

- Had active diarrhea (defined as the passage of 3 or more loose or liquid stools per day) at screening or at the time of randomization.
- Had a medical disorder causing difficulty in chewing or swallowing.
- Had a history of clinically significant liver or renal insufficiency; cardiac, vascular, pulmonary, gastrointestinal, endocrine, neurologic (e.g., convulsions), hematologic (e.g., anemia), rheumatologic, psychiatric, or metabolic disturbances that, in the opinion of the investigator, rendered the candidate not suitable for mebendazole treatment.
- Subjects with any acute medical condition that, in the opinion of the investigator, rendered the candidate not suitable for participation in this trial. Subjects with such an acute medical condition were referred through the appropriate health care system for further evaluation, follow-up, and care.
- Had significant anemia (<8 g/dL). Subjects with significant anemia were referred through the appropriate health care system for further evaluation, follow-up, and care.
- Had significant wasting [greater than 2 standard deviations below the mean WHO Child Growth Standards for weight-for-height or body mass index (BMI)[51]]. Subjects with significant wasting were referred through the appropriate health care system for further evaluation, follow-up, and care.
- Had a known hypersensitivity to mebendazole, any inert ingredients in the chewable formulation, or other medications in the benzimidazole class (e.g., albendazole).
- Had preplanned surgery/procedures that would interfere with the conduct of the study during the course of study.
- Had received an investigational drug (including vaccines) or used an investigational medical device within 30 days before the planned start of treatment, or was currently enrolled in an investigational study.
- Had taken any form of medication containing mebendazole or any other treatment for STH infection within 30 days of entry into the study.

[51] If at any Visit, a subject had significant wasting (greater than 2 standard deviations below the mean World Health Organization (WHO) Child Growth Standards for weight-for-height or BMI), this subject was excluded from the study and referred through the appropriate health care system for further evaluation, follow-up, and care.

- Employees of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator.

Study design and measurements

The subjects were randomized and administered a single dose (500 mg) of mebendazole or a matching placebo on Day 1 (Visit 2). The study was unblinded on Day 19±2 (Visit 3), at which time all subjects were administered another dose (500 mg) of mebendazole. The subjects were examined for pharmacokinetic and parasitological measurements at baseline and post-treatment (Figure 3 and Table 10).

Figure 3: Overall study design - Study GAI3003

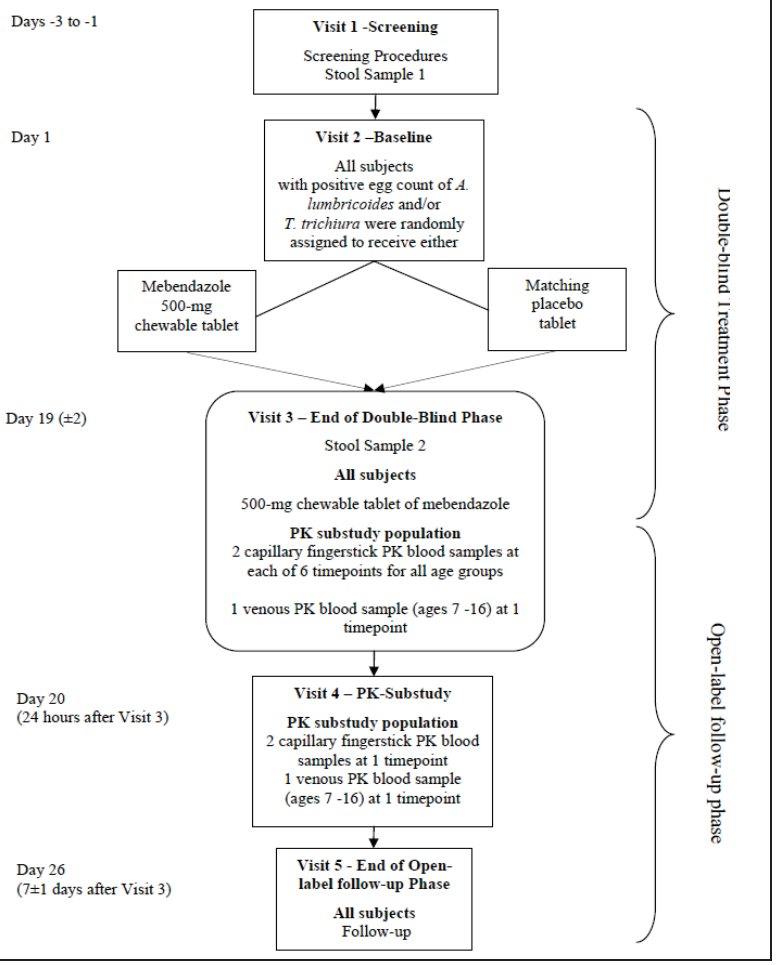

Table 10: Time and events schedule

Phase	Screening	Treatment			Posttreatment	
Period	Screening	Baseline	Double-Blind	PK Visit	Follow-up	Early Withdrawal[a]
Visit	1[b]	2[c]	3[c,d]	4 (PK subset only)	5 (All subjects)	
Day	-3 to -1	1	19[e]	20[f]	26[g]	
Screening/Administrative						
Informed consent	X					
Informed assent[h]	X					
Inclusion/exclusion criteria	X					
Medical history	X					
Urine pregnancy test[i]	X		X			
Hemoglobin[j]	X					
Study Drug						
Randomization		X				
Drug Administration		X[k]	X[l]			
Study Drug Accountability		X	X			
Stool sample collection for egg count analysis	X[m]		X[n]			X[o]
Safety						
Physical examination	X		X		X	X
Height	X					
Weight	X		X		X	X
BMI	X		X		X	X
Vital signs[p]	X	X	X	X	X	X
Blood samples for PK analysis			X[q,r]	X[s]		
Adverse event monitoring[t]	X	X	X	X	X	X
Concomitant therapy[u]	X	X	X	X	X	X

[a]Every effort will be made to perform final assessments for all subjects who withdraw from the study at any time before Visit 3.
[b]Only subjects with a positive egg count for any STH will be enrolled in the study.
[c]Subjects will be observed 3 hours post-treatment for safety evaluations.
[d]Subjects who come for Visit 3 and have their stool sample collected will be considered as "completers" for the study efficacy analysis.
[e]Day listed is an approximation. Actual day is Day 19±2 days.
[f]Day listed is an approximation. Actual day occurs 1 day after Visit 3.
[g]Day listed is an approximation. Actual day occurs 7±1 days after Visit 3.h For children ≥6 years old.
[h]For children ≥6 years old.
[i]Female subjects who are ≥9 years old will have a urine pregnancy. If needed, the investigator may conduct additional pregnancy tests to confirm the absence of pregnancy at any time during the study. If at any time the results of the pregnancy test are positive, study drug administration will be discontinued, the subject will be followed for safety, and the pregnancy outcome will be assessed.
[j]Fingerstick testing using Hemocue machine.
[k]Subject will receive either a 500 mg chewable tablet of mebendazole or matching placebo tablet after all screening assessments are performed and results reviewed.
[l]All subjects will receive a 500 mg chewable tablet of mebendazole in the open-label manner.
[m]Two Kato-Katz smears will be performed on the stool sample. Only subjects with an average positive egg count for either STH will return for Visit 2.
[n]Stool collection will occur before 2nd dose of 500 mg mebendazole chewable tablet. Two Kato-Katz smears will be performed on the stool sample.
[o]Two Kato-Katz smears will be performed on the stool sample.
[p]Include heart rate, respiration rate, body temperature, and blood pressure.
[q]Capillary blood samples (approximately 0.5 mL) for PK analysis will be collected at the following timepoints: predose, 1, 2, 3, 5, and 8 hours post-treatment.
[r]One venous blood sample will be taken at the 3-hour timepoint post-treatment from each subject ages 7 to 16 years.
[s]The last PK blood sample will be obtained 24 hours post-treatment.
[t]Adverse events will be assessed by direct observations of the investigator, or reported by the subject, parent, or guardian.
[u]Will be recorded from time the ICF is signed throughout the follow-up visit after the last dose of the study drug administration.

Parasitological measurements

Stool samples were collected from subjects to evaluate STH infections and egg count. The egg counts were assessed by the Kato-Katz thick smear method. Briefly, stools samples were forced through the nylon screen to remove excess debris and the hole in the Kato-Katz template filled with the filtered sample. A glycerol-malachite green soaked cellophane clipping was placed on the stool aliquot sample on the microscope slide. The hole in the template was filled up to the rim and the surface smoothened while pressing the template onto the microscope slide. Two slides were prepared from each stool sample at each visit. Thick smears were cleared for 30-60 minutes, kept at room temperature, direct sunlight was avoided, and smears examined immediately under a microscope, especially for hookworm, using 40X (only by experienced technicians and if samples are clear) or 100X magnification. The entire thick smear was examined in a systematic way and the number of eggs counted for each of the STH, *A. lumbricoides*, *T. trichiura* and hookworms. The presence of eggs from additional species was documented in the STH Result Form (only qualitative diagnosis).

Quality control (QC) was performed by a senior technician on over 10% of the slides in a blinded manner. In addition, on-site QC was performed at regular intervals by Peter Steinmann and/or Benjamin Speich from the (b) (4) Upon completion of stool collection and slide reading, all slides were to be forwarded to the (b) (4) If discrepancies were within the pre-specified tolerance margin, no further action was required; the pre-specified criteria for no change needed were as follows:

- No difference in the presence/absence of *Ascaris lumbricoides* and *Trichuris trichiura*.
- Egg counts were +/-10 eggs for counts ≤100 eggs or +/-20% for counts >100 eggs (for each species separately).

Any discrepancies detected, outside the pre-specified criteria, were discussed with the technician and re-reading and retraining was implemented under the supervision of the Swiss TPHI expert; a consensus value of the egg count was documented on case report forms. It appears that discrepancies reported were more for the Kigali site in Rwanda compared to the two sites in Ethiopia.

Note: In communication dated September 23, 2016, the Applicant stated that all Kato-Katz thick smear slides remained at the clinical investigator sites and were not sent to the Swiss TPHI.

Results

Of the 295 subjects enrolled, 278 subjects (94.2%) completed the double-blind phase and continued into the open-label phase of the study. *A. lumbricoides* and *T. trichiura* infections were reported in 167 and 243 subjects, respectively; 115 subjects were infected with both the worms. Thirteen subjects also had hookworm infection. The majority of the study population had light or moderate STH.

Round worm (A. lumbricoides)

Of the 167 randomized subjects with *A. lumbricoides* infection, 86 subjects were in the mebendazole arm and 81 in the placebo group. Mebendazole was effective in reducing egg count by 97.9% and 83.8% of the subjects were cured i.e., no eggs found at Day 19; in the placebo group,11.1% of the subjects were cured and egg count was reduced by 19.2% (Table 11). CRs and ERRs in subjects with either *A. lumbricoides* infection alone or mixed with other STHs were similar (Tables 11 and 12).

Whipworm (T. trichiura)

Of the 243 subjects with *T. trichiura* infection, 124 subjects were in the mebendazole group and 119 in the placebo group. Mebendazole was effective in reducing egg count by 59.7% and 33.9% of the subjects were cured; in the placebo group, 7.6% in subjects were cured and egg count was reduced by 10.5% (Table 11). CRs and ERRs in subjects with either *T. trichiura* infection alone or mixed with other STHs were similar (Tables 11 and 12).

(b) (4)

During the pre-inspection visits and subsequent analyses, it was noted by the Applicant that although re-readings of slides was performed and the new consensus values of egg count determined, case report forms were not updated with the new consensus values for nine subjects. If the original egg count values are replaced by the new consensus values for the 9 subjects, the CRs and ERRs change slightly (Tables 13 and 14). However, the overall conclusions do not change. The results show that mebendazole is effective in curing and /or reducing egg count in patients with *A. lumbricoides* and *T. trichiura* infection.

Comments

Overall, the phase 3 study suggests that a single dose of mebendazole is effective in curing and reducing egg count in patients with either of the STHs; CRs and ERRs were similar in subjects with either single or mixed STH infections. A. lumbricoides appears to be more sensitive compared to T. trichiura. (b) (4)

Table 12: Parasitological observations before and after treatment with mebendazole in patients with roundworm (*A. lumbricoides*), whipworm *(T. trichiura),* and/or hookworm (species not identified) mixed infections at baseline

Soil Transmitted Helminth	Placebo				Mebendazole			
	Egg count		CR n (%)	ERR%[1]	Egg count		CR n (%)	ERR%[1]
	Pre-treatment	Post-treatment			Pre-treatment	Post-treatment		
A. lumbricoides + T. trichiura (n=109)								
A. lumbricoides								
Mean±SD	19321.7±22772.9	13743.6±24142.1	9 (18.0)	28.9	18756.0±23371.2	512.6±2788.7	48 (81.4)	97.3
Median	14555.4	6432.0			10176.0	0		
(Range)	(48-90804)	(0-143040)			(168-117384)	(0-20064)		
T. trichiura								
Mean±SD	604.1±824.3	528.5±957.4	6 (12.0)	12.5	928.3±1668.02	362.6±1440.9	22 (37.3)	60.9
Median	377.6	180.0			216.0	41.6		
(Range)	(60-4992)	(0-5496)			(12-8808)	(0-10536)		
N	50	47			59	56		
A. lumbricoides + T. trichiura + Hookworm (species not identified) (n=6)								
A. lumbricoides								
Mean±SD	10011.0±4609.8	7521.0±4888.3	0 (0)	24.9	11016.0±780.7	0.0±0.0	2 (100)	100.0
Median	10175.0	6766.8			11002.2	0.0		
(Range)	(4212-15468)	(2376-13944)			(10464-11568)	(0-0)		
T. trichiura								
Mean±SD	1134.0±1031.8	891.0±908.2	0 (0)	21.4	678.0±670.34	102.0±25.5	0 (0)	85.0
Median	884.0	488.3			484.8	100.4		
(Range)	(168-2592)	(240-2184)			(204-1152)	(84-120)		
Hookworm								
Mean±SD	120.0±120.0	147.0±117.3	0 (0)	22.5	102.0±127.3	0.0±0.0	2 (100)	100.0
Median	60	113.8			48.0	0.0		
(Range)	(60-300)	(36-276)			(12-192)	(0-0)		
N	4	4			2	2		

[1] Egg reduction rates based on arithmetic mean

Table 13: Study 3003 - Parasitological observations before and after treatment with mebendazole in patients with *A. lumbricoides*, *T. trichiura*, and hookworms (species not identified) as single or mixed infections – Based on updated case report forms

Soil Transmitted Helminth	Placebo				Mebendazole			
	Egg count		CR n (%)	ERR%[1]	Egg count		CR n (%)	ERR%[1]
	Pre-treatment	Post-treatment			Pre-treatment	Post-treatment		
Irrespective of single or mixed STH infections at baseline								
A. lumbricoides (n=168)								
Mean±SD	16663.5±20663.9	137201.1±23160.7	9 (11.0)	17.7	17610.1±23476.8	366.4±2325.3	73 (84.9)*	97.9*
Median	9923.3	5932.9			9389.6	0.0		
(Range)	(36-90804)	(0-143040)			(48-117384)	(0-20064)		
N	82	76			86	81		
T. trichiura (n=243)								
Mean±SD	584.5±930.1	523.0±1020.9	9 (7.6)	10.5	647.8±1256.2	260.8±1042.9	41 (33.1)*	59.7*
Median	264.0	210.0			168.0	53.7		
(Range)	12-5916	0-7704			12-8808	0-10536		
N	119	112			124	118		
Hookworm - species not identified (n=13)								
Mean±SD	548.0±753.6	452.0±671.57	2 (22.2)	17.5	102.0±74.9	0.0±0.0	4 (100)	100
Median	240.0	216.0			102.0	0.0		
(Range)	(48-2316)	(0-2064)			(12-192)	(0-0)		
N	9	9			4	4		
Single STH infection only at baseline								
A. lumbricoides (n=49)								
Mean±SD	14596.8±17851.7	15369.9±24280	0 (0)	5.3	16035.5±25253.0	45.3±212.4	21 (87.5)	99.7
Median	9252.0	4440.0			7236.5	0.0		
(Range)	(36-83292)	(432-97260)			(48-112764)	(0-996)		
N	25	23			24	22		
T. trichiura (n=123)								
Mean±SD	555.1±1021.5	504.6±1110.72	3 (4.9)	9.1	388.1±616.8	164.1±450.7	19 (30.6)	57.7
Median	209.9	185.9			137.9	48.0		
(Range)	(12-5916)	(0-7704)			(12-2760)	(0-3360)		
N	61	58			62	59		

*p<0.001 based on the Cochran–Mantel–Haenszel (CMH) test, controlling the effect of site. [1] Egg reduction rates based on arithmetic mean
Note: Subject with missing stool sample at Visit 3 was considered not cured.

Table 14: Parasitological observations before and after treatment with mebendazole in patients with roundworm (*A. lumbricoides*), whipworm *(T. trichiura),* and/or hookworm (species not identified) mixed infections at baseline – Based on updated case report forms

Soil Transmitted Helminth	Placebo				Mebendazole			
	Egg count		CR n (%)	ERR%[1]	Egg count		CR n (%)	ERR%[1]
	Pre-treatment	Post-treatment			Pre-treatment	Post-treatment		
A. lumbricoides + T. trichiura (n=110)								
A. lumbricoides								
Mean±SD	18799.3±22753.3	13765.3±24130.3	9 (17.6)	26.8	18756.0±23371.2	512.1±2788.8	49 (83.1)	97.3
Median	11796.0	6432.0			10176.0	0		
(Range)	(48-90804)	(0-143040)			(168-117384)	(0-20064)		
T. trichiura								
Mean±SD	595.5±818.3	528.5±957.4	6 (11.8)	11.3	928.3±1668.02	362.6±14440.9	22 (37.3)	65.9
Median	360	180.0			216.0	41.6		
(Range)	(60-4992)	(0-5496)			(12-8808)	(0-10536)		
N	51	47			59	56		
A. lumbricoides + T. trichiura + Hookworm (species not identified) (n=6)								
A. lumbricoides								
Mean±SD	10011.0±4609.8	7521.0±4888.3	0 (0)	24.9	11016.0±780.7	0.0±0.0	2 (100)	100.0
Median	10175.0	6766.8			11002.2	0.0		
(Range)	(4212-15468)	(2376-13944)			(10464-11568)	(0-0)		
T. trichiura								
Mean±SD	1134.0±1031.8	891.0±908.2	0 (0)	21.4	678.0±670.34	102.0±25.5	0 (0)	85.0
Median	884.0	488.3			484.8	100.4		
(Range)	(168-2592)	(240-2184)			(204-1152)	(84-120)		
Hookworm								
Mean±SD	120.0±120.0	147.0±117.3	0 (0)	22.5	102.0±127.3	0.0±0.0	2 (100)	100.0
Median	60	113.8			48.0	0.0		
(Range)	(60-300)	(36-276)			(12-192)	(0-0)		
N	4	4			2	2		

[1]Egg reduction rates based on arithmetic mean

4.1.2. Published studies

The applicant performed a comprehensive literature review, through November 2015 and included relevant studies to support the efficacy of a single 500 mg dose of mebendazole (any formulation or that of the Applicant) for the treatment of *Ascaris, Trichuris* (b) (4) . Specific search terms included for MEDLINE search, were as follows:

- Mebendazole (focus on 500 mg dose, VERMOX).
- Soil-transmitted helminth (STH, nematodes, *A. lumbricoides, T. trichiura,* (b) (4) whipworm, roundworm, (b) (4)
- Efficacy, effectiveness, cure, egg reduction rate (ERR), fecal egg count reduction (FECR).

An evaluation of the efficacy from the published studies is limited by differences in study design, definitions, formulations, number of fecal samples collected and processed for parasitological examination, parasitological methods used for identifying STH species and enumerating egg count, geographic location, time from treatment to assessment, intensity of infection, and other factors that could influence CRs and ERRs. The focus was on studies in which a single 500 mg dose of mebendazole was used for the treatment of at least one STH infection in humans and results reported as CRs and/or ERRs; the definitions for CRs and ERRs were same as that for the clinical trial summarized above in section 4.1.1. ERRs results were shown as individual means, medians, group geometric means, or group arithmetic means.

4.1.2.1. *Ascaris lumbricoides*

Efficacy of mebendazole (single 500 mg dose) in patients with *A. lumbricoides* infection, alone or as mixed infections, was reported in 22 studies (Abadi, 1985[52]; Albonico *et al*., 1994a[36]; 1994b[37]; 2002a[38], 2002b[53]; 2003[39]; Cauwenbergh, 1985[54]; Evans *et al*., 1987[55]; Gorodner, 1987[56]; Jongsuksuntigul *et al*., 1993[57]; Knopp *et al*., 2010[58]; Larocque *et al*., 2006[59]; Levecke *et*

[52] Abadi K. Single dose mebendazole therapy for soil-transmitted nematodes. *Am J Trop Med Hyg* (1985) 34 (1): 129-133.

[53] Albonico M, Ramsan M, Wright V, Jape K, Haji HJ, Taylor M, Saviolo L, and Bickle Q. Soil-transmitted nematode infections and mebendazole treatment in Mafia Island schoolchildren. *Ann Trop Med Parasitol* (2002b) 96 (7): 717-726.

[54] Cauwenbergh G. The effect of single dose mebendazole on the egg reduction rates (ERR) and cure rates (CR) in patients with *Ascaris*-, *Trichuris*- and hookworm infestations. Unpublished internal report – Serial number R17635/51, February 1985.

[55] Evans AC, Hollmann AW, and Du Preez L. Mebendazole 500 mg for single-dose treatment of nematode infestation. *S Afr Med J* (1987) 72: 665-667.

[56] Gorodner JO. Treatment of intestinal parasite infections with a single dose of mebendazole (translation from Spanish). Unpublished internal report 59167 2 (1987).

[57] Jongsuksuntigul P, Jeradit C, Pornpattanakul S, and Charanasri U. A comparative study on the efficacy of albendazole and mebendazole in the treatment of Ascariasis, hookworm infection and Trichuriasis. *Southeast Asian J Trop Med Publ Health* (1993) 24 (4):724-9.

al., 2014[60]; Lubis *et al*., 2012[61]; Luoba *et al*., 2005[62]; Schutte, 1989[43]; Sorensen *et al*., 1996[63]; Soukhathammavong *et al*., 2012[44]; Speich *et al*., 2015[45]; Staudacher *et al*., 2014[64]; Steinmann *et al*., 2011[65]; van der Werff *et al*., 2014[47]). These studies were conducted in different countries that include Indonesia, Tanzania, Malaysia, Brazil, Philippines, Bangladesh, Germany, Thailand, Mexico, USA, Sri Lanka, South Africa, Argentina, Peru, Cambodia, Cameroon, Ethiopia, Vietnam, Western Kenya, Rwanda, China, or Cuba (Table 15). The number of subjects treated with a single dose of mebendazole varied from 9 to 1209 in different studies. Of the 22 studies, 11 were randomized, 3 were blinded, and 4 were placebo-controlled.

Different parasitological methods used for detection and quantitation of eggs include formalin-ether concentration (2 studies), Barthelmi and Wills (1 study), flotation/McMaster (1 study), or Kato-Katz (18 studies; 1 using cardboard templates; 1 using cellophane; others not specified) techniques. PCR was used in one study; the authors state that the PCR assay used also detects *A. suum* (Staudacher *et al*., 2014[64]); details of the method and performance characteristics of the assay were not available for review. The number of fecal specimens processed at each time as well as the number of smears from each fecal sample varied from one to two. Follow-up time post-treatment varied between Day 7 and Day 76.

Of the 4,403 subjects treated with a single dose of mebendazole, 3,194 subjects were evaluated for CRs and 4027 subjects were evaluated for ERRs; 389 subjects were administered placebo.

[58] Knopp S, Mohammed KA, Speich B, Hattenndorf J, Khamis S, Khamis AN, Stothard JR, Rollinson D, Marti H, and Utzinger J. Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial. *Clin Infect Dis* (2010) 51(12):1420-1428.

(b) (4)

[redacted]

[60] Levecke B, Montresor A, Albonico M, Montresor A, Albonico M, Ame SM, Behnke JM, Bethony JM, Noumedem CD, Engels D, Guillard B, Kotze AC, Krolewiecki AJ, McCarthy JS, Mekonnen Z, Periago MV, Sopheak H, Tchuem-Tchuente LA, Duong TT, Huong NT, Zeynudin A, and Vercruysse J. Assessment of anthelmintic efficacy of mebendazole in school children in six countries where soil-transmitted helminths are endemic. *PLoS Negl Trop Dis* (2014) 8(10): e3204.

[61] Lubis IN, Pasaribu S, and Lubis CP. Current status of the efficacy and effectiveness of albendazole and mebendazole for the treatment of Ascaris lumbricoides in North-Western Indonesia. *Asian Pac J Trop Med* (2012) 5(8):605-609.

[62] Luoba AI, Geissler PW, Estambale B, Ouma JH, Alusala D, ayah R, Mwaniki D, Magnussen P, and Friis H. Earth-eating and reinfection with intestinal helminths among pregnant and lactating women in western Kenya. *Trop Med Int Health* (2005) 10 (3):220-227.

[63] Sorensen E, Ismail M, Amarasinghe DKC, and Hettiarachchi I. The efficacy of three anthelminthic drugs given in a single dose. *Ceylon Med J* (1996) 41: 42-45.

[64] Staudacher O, Heimer J, Steiner F, Kayonga Y, Havugimana JM, Ignatius R, Musemakweri A, Ngaba F, Harms G, Gahutu J-B, and Mockenhaupt FP. Soil-transmitted helminths in southern highland Rwanda: associated factors and effectiveness of school-based preventive chemotherapy. *Trop Med Int Health* (2014) 19(7): 812-824.

[65] Steinmann P, Utzinger J, Du ZW, Jiang J-Y, Chen J-X, Hattendorf J, Zhou H, and Zhou X-N. Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp: a randomized controlled trial. *PLos ONE* (2011) 6(9): e25003.

The mebendazole CRs varied between 72.5% and 100%; ERRs varied between 89.8% and 100%. Both CRs and ERRs were higher in mebendazole treated subjects compared to the placebo group of patients in three studies; in one study, the CRs were not reported for the placebo group (Table 15).

In one study (van der Werff *et al*., 2014[47]), MDA with a single dose (500 mg) of mebendazole was performed two times a year and follow-up performed at Months 6 and 36. The CR was 76.9% and ERR 98.0% at about Month 6 (n=78) after the first dose; CR was 78.2% and ERR 98.7% after about 3 years in patients that received mebendazole 500 mg every 6 months (n=55).

The CRs and ERRs of the Janssen and the local/generic products appear to be comparable.

Table 15: Summary of the efficacy studies for single dose mebendazole for *A. lumbricoides* based on published studies

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
Abadi, 1985[52] Ujung Pandang and South-Sulawesi, Indonesia	Double blind, placebo-controlled trial	2-70	Kato-Katz (modified using cardboard templates). Single fecal sample at baseline and about 2-4 weeks after treatment. Cure based on at least two consecutive negative fecal examinations.	MBZ 500 mg SD (61)	93.4	99	
				Placebo (44)	0	6	
Albonico *et al.*, 1994a[36] Pemba Island, Zanzibar, Tanzania	Open-label, multi-site trial	All ages (Adults and children)	Kato-Katz (cellophane thick smear technique – WHO 1993). Follow-up at Month 1 post-treatment. Quality control performed on 10% of the slides.	MBZ 500 mg SD; 250 mg for <2 year old children (250)	93.2	89.8	Level of transmission of *T. trichiura* was greater than those of hookworms and *A. lumbricoides*. Passage of worms was reported in 15.5% of subjects, but only a few cases complained of worm expulsion via the mouth and the nose (0.8%).
Albonico *et al.*, 1994b[37] Pemba Island, Zanzibar, Tanzania	Randomized single-blind, controlled trial	6-12	Kato-Katz (WHO 1993); follow-up18 to 31 days after treatment. Quality control performed on 15% of the slides.	MBZ 500 mg SD (730)	97.8	99.3	Janssen product
				ABZ 400 mg SD (818)	98.9	99.6	
	Controlled trial			MBZ 500 mg SD (139)	97.8	99.6	Janssen product
				MBZ 500 mg SD (147)	96.6	99.5	Pharmamed product
Albonico *et al.*, 2002a[38] Pemba Island, Zanzibar, Tanzania	Randomized, placebo-controlled trial	6-9	Kato-Katz (WHO, 1994). Follow-up 21 to 24 days after treatment. Stool samples were processed to assess egg counts within 6 hours. All laboratory investigations were blinded; the technicians examining the slides were unaware of the treatment regimen of the patients. Quality control: 10% of each batch of Kato–Katz smears (selected at random) were all read independently by two technicians; the whole batch was re-examined if the mean egg counts determined by the technicians differed by >10%.	MBZ 500 mg SD (107)	98.0	96.1	
				Pyrantel-oxantel 10 mg (100)	96.3	95.1	
				Placebo (103)	27.9	18.1	
Albonico *et al.*, 2002b[53] Mafia Island, Tanzania	Open label, single arm trial	6-18	Kato-Katz method. Follow-up 21 to 24 days after treatment. Quality-control: 10% of each batch of Kato–Katz smears were read independently by two technicians; the whole batch was re-examined if the mean egg counts differed by >10%.	MBZ 500 mg SD (16)	100	97.1	

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
Albonico *et al.*, 2003[39] Pemba Island, Zanzibar, Tanzania	Randomized, single center, placebo-controlled trial	7-18	Kato-Katz method. Follow-up at Day 21 post-treatment. Quality control performed on 10% of the slides.	MBZ 500 mg SD (236)	96.5	99.0	
				Levamisole 40 or 80 mg (210)	91.2	98.5	
				MBZ 500 mg + levamisole 40 or 80 mg (216)	98.5	99.1	
				Placebo (242)	22.5	33.9	
Cauwenbergh, 1985[54] International (Malaysia, Brazil, Philippines, Bangladesh, Germany, Indonesia, Thailand, Mexico, U.S.A. and Sri Lanka)	Multicenter controlled trial	1-50	Kato-Katz method. Follow-up at one to 3 weeks post-treatment.	MBZ 100 mg SD (7)	100	100	
				MBZ 200 mg SD (104)	100	100	
				MBZ 500 mg SD (61)	93.0	99.0	
				MBZ 600 mg SD (157)	95.0	99.0	
Evans *et al.*, 1987[55] Karino, Hazyview and Brondal areas; South Africa	Open label, single arm trial	5-16	Formalin-ether concentration technique using 1 g stool sample. Two stool samples collected at baseline as well as at Day 14 post-treatment	MBZ 500 mg SD (147)	93.2	NR	
Gorodner, 1987[56] Provinces of Chaco and Corrientes, Argentina	Multi-center controlled trial	5-60	C Barthelemi and Willis' techniques and the counting of eggs/g of feces using the method of Stoll and modified by the author. Follow-up at 15-30 days post-treatment	MBZ 500 mg SD (9)	89.0	NR	
				MBZ 500 mg x 3 days (9)	89.0	NR	Only patients with *Ascaris* infection at baseline enrolled
				Pyrantel pamoate; 250, 500 or 750 mg (9)	100	NR	
Jongsuksuntigul *et al.*, 1993[57] Pattani Province, Southern part of Thailand	Randomized, single site, controlled trial	3-80	Kato-Katz method. Follow-up at Day 14 post-treatment. The laboratory technicians were blind to the respective treatment of each patient group.	MBZ 300 mg SD (14)	100	100	Original MBZ product
				MBZ 300 mg SD (12)	50.0	87.3	Local (generic) MBZ product
				MBZ 500 mg SD (17)	100	100	Original MBZ product
				ABZ 400 mg SD (13)	100	100	
Knopp *et al.*, 2010[58] Zanzibar, Tanzania	Randomized controlled trial	Not specified Grades 1-7 children (Mean 11 years)	Kato-Katz method. Follow-up between Day 22-39 after treatment. 2 consecutive stool samples collected. Duplicate smears from each sample were processed and eggs examined.	MBZ 500 mg SD + Placebo (18)	77.8	99.8	
				MBZ 500 mg SD + Ivermectin 200 µg/kg (18)	100	100	
				ABZ 400 mg SD +	100	100	

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
				Placebo (14)			
				ABZ 400 mg SD + Ivermectin 200 µg/kg (14)	92.9	99.9	
Larocque *et al.*, 2006[59] Peruvian Amazon region, Peru	Randomized, double-blind, placebo-controlled trial	18-44 (Only pregnant women enrolled)	Kato-Katz. Follow-up at approximately Day 76.	MBZ 500 mg SD + Iron (302)	72.5	98.3	Known to be malaria endemic region; only 2 women were smear positive.
				Placebo + Iron	ND	ND	
Levecke *et al.*, 2014[60] International (Brazil, Cambodia, Cameroon, Ethiopia, United Republic of Tanzania, and Vietnam)	Open label, multi-center, single arm trial	4-18	Flotation technique (McMaster egg counting method). Follow-up between Days 7 and 15 post-treatment.	MBZ 500 mg SD (1209)	NR	97.6	Vermox product
Lubis *et al.*, 2012[61] Island of Sumatera (North-Western part), Indonesia	Randomized controlled trial	Not specified (Primary school children)	Kato-Katz method. Follow-up at week 1.	MBZ 500 mg SD (106)	100	100	Patients with either *Ascaris* alone or mixed infections with *Ascaris* + other STH infection enrolled. Results limited to *Ascaris*.
				ABZ 400 mg SD (123)	96.7	99.3	
Luoba *et al.*, 2005[62] Nyanza Province, Western Kenya	Open label, longitudinal trial	14-47 (Pregnant women only)	Kato-Katz cellophane method. Stool samples collected on 2 consecutive days and duplicate smears prepared. Follow-up time not specified	MBZ 500 mg SD (135)	79.0	NR	Vermox product
Schutte, 1989[43] South Africa	Open label, single arm, 2 center trial	5-18	Formalin-ether concentration technique. All fecal samples were fixed and preserved in 4% formalin; all examinations conducted in the RIDTE laboratory in Durban. Two baseline samples (2 weeks apart) and follow-up at 2 weeks post-treatment.	MBZ 500 mg SD (226)	88.1	97.1	
Sorensen *et al.*, 1996[63] Sri Lanka	Randomized controlled trial	3-15	Kato-Katz technique. Follow-up at week 3 post-treatment. Laboratory technicians were blinded to the treatment group. All stool samples were coded and kept cold during transportation and storage.	MBZ 500 mg SD (84)	97.6	99.7	Janssen product
				MBZ 500 mg SD (95)	95.8	98.0	Local- 2
				ABZ 400 mg SD (71)	97.2	99.6	
Soukhathammavong *et al.*, 2012[44] Laos	Randomized, open label, 2 arm trial	6-12	Kato-Katz quadruplicate thick smears derived from two stool samples at each time point. Follow-up stool samples at Days 21–23 post-treatment	MBZ 500 mg SD (30)	93.3	100	Vermox product. All patients were with hookworm infection + other helminthes.
				ABZ 400 mg SD (28)	92.9	100	
Speich *et al.*,	Randomized	6-14	Kato-Katz technique; two stool samples on	MBZ 500 mg SD (44)	95.5	100	

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
2015[45] Pemba Island, Tanzania	controlled trial		consecutive days. Time of assessment following treatment not specified.	MBZ 500 mg SD + ABZ 400 mg SD (40)	97.5	99.9	
				ABZ 400 mg SD + oxantel pamoate 20 mg/kg (47)	97.9	99.9	
				ABZ 400 mg SD + ivermectin 200 µg/kg (50)	98.0	100	
Staudacher *et al.*, 2014[64] Rwanda	Open label, single arm, 2 center trial	5-17	Kato-Katz technique and PCR assay. Follow-up at 2 weeks post-treatment. A single stool sample was considered positive for STHs when either the Kato-Katz smear or the *Ascaris*-specific PCR assay yielded a positive result; a positive PCR result for *Ascaris* but a negative one by microscopy was considered to reflect a submicroscopic infection.	MBZ 500 mg SD (85)	100	NR	PCR assay used in the present study also detects *Ascaris suum*. Only *Ascaris* patients enrolled; 11 subjects had *T. trichiura* and 2 hookworm infection. All subjects were cured.
Steinmann *et al.*, 2011[65] China	Community based randomized controlled trial	≥5	2 stool samples collected before and 3-4 weeks after treatment. First, samples were visually inspected for adult *A. lumbricoides* and *Taenia* spp. proglottids. Second, two 41.7 mg Kato-Katz thick smears were prepared from each sample. Depending on the ambient temperature and considering over-clearance of hookworm eggs, slides were read within 30–90 min of preparation. At least 5% of the daily diagnoses were cross-checked by the principal investigator.	MBZ 500 mg SD (71)	93.0	>99.9	
				MBZ 500 mg x 3 days (72)	93.1	>99.9	
				ABZ 400 mg SD (78)	96.1	>99.9	
				ABZ 500 mg x 3 days (63)	96.8	>99.9	
van der Werff *et al.*, 2014[47] Cuba	Cohort from a MDA study	5-14	Kato-Katz method. Follow-up at approximately Month 6 or 36 months. Duplicate smears from each stool sample examined.	MBZ 500 mg SD (78)	76.9	98.0	Follow-up ~6 months
				MBZ 500 mg SD administered 2 times/year (55)	78.2	98.7	Follow-up ~36 months

* Number of subjects secreting eggs at baseline
SD= single dose; MBZ=mebendazole; ABZ=albendazole; NR=not reported; ND=not done; [redacted] 2 Pages have been . Epg=egg per gram; MDA=mass drug administration

4.1.2.2. *Trichuris trichiura*

Efficacy of mebendazole (single 500 mg dose) in patients with *T. trichiura* infection, alone or mixed infections, was reported in 24 studies (Abadi, 1985[52]; Albonico *et al*., 1994a[36], 1994b[37], 2002a[38], 2002b[53], 2003[39], Cauwenbergh, 1985[54]; Charoenlarp *et al*., 1993[40]; Evans *et al*., 1987[55]; Jackson *et al*., 1998[66]; Jongsuksuntigul *et al*., 1993[57]; Khieu *et al*., 2013[67]; Knopp *et al*., 2010[58]; Larocque *et al*., 2006[59]; Levecke *et al*., 2014[60]; Luoba *et al*., 2005[62]; Mekonnen *et al*., 2013[68]; Namwanje *et al*., 2011[42]; Schutte, 1989[43]; Sorensen *et al*., 1996[63]; Soukhathammavong *et al*., 2012[44]; Speich *et al*., 2015[45]; Steinmann *et al*., 2011[65]; van der Werff *et al*., 2014[47]). Of these, 19 studies were same as those summarized above for *A. lumbricoides*. The studies were conducted in different countries that include Indonesia, Tanzania, Malaysia, Brazil, Philippines, Bangladesh, Germany, Thailand, Mexico, USA, Sri Lanka, South Africa, Peru, Cambodia, Cameroon, Ethiopia, Vietnam, Western Kenya, China, Cuba, or Uganda (Table 16). Of the 24 studies, 12 were randomized trials, 4 were blinded, and 5 were placebo-controlled. The number of subjects treated with a single dose of mebendazole varied from 7 to 1095 in different trials.

Different parasitological methods used for the detection of eggs and egg count include formalin-ether concentration (3 studies), flotation/McMaster (2 studies), or Kato-Katz (19 studies; 1 using cardboard templates; 1 using cellophane; others not specified) techniques. Like for the studies summarized above for *A. lumbricoides*, the number of fecal specimens processed at each time as well as the number of smears from each fecal sample varied from one to two. Follow-up time post-treatment varied between 7 and 76 days.

Of the 5,819 subjects treated with mebendazole, 4,599 subjects were evaluated for CRs and 5,571 subjects were evaluated for ERRs; 719 subjects (4 studies) were administered placebo. The mebendazole CRs varied between 8.4% and 100%; ERRs varied between 31.6% and 93.0%. Both CRs and ERRs were higher in mebendazole treated subjects compared to the placebo group of patients in four studies; in one study, the CRs were not reported for the placebo group (Table 16). Unlike *A. lumbricoides* infested subjects, considerable variability in efficacy was reported in subjects with *T. trichiura* infections treated with a single 500 mg dose of mebendazole.

In one study (van der Werff *et al*., 2014[47]), MDA with a single dose (500 mg) of mebendazole was performed two times a year and follow-up performed at Month 6 and 36 (van der Werff *et al*., 2014[47]). The CR was 67.4% and ERR 85.0% at Month 6 (n=132) after the first dose; CR was 89.7% and ERR 97.7% after about 3 years in patients that received mebendazole every 6 months (n=107).

[66]Jackson TFHG, Epstein SR, Gouws E, and Cheetham RF. A comparison of mebendazole and albendazole in treating children with Trichuris trichiura infection in Durban, South Africa. *S Afr Med J* (1998) 88 (7):880-883.

[67] Khieu V, Schar F, Marti HS, Sayasone S, Duong S, Muth S, and Odermatt P. Diagnosis, treatment and risk factors of *Strongyloides stercoralis* in schoolchildren in Cambodia. *PLOS Neglect Trop Dis* (2013) 7 (2): e2035.

[68] Mekonnen Z, Levecke B, Boulet G, Bogers JP, and Vercruysse J. Efficacy of different albendazole and mebendazole regimens against heavy-intensity *Trichuris trichiura* infections in school children, Jimma Town, Ethiopia. *Pathog Glob Health* (2013) 107 (4): 207-209.

Three studies reported CRs and/or ERRs after treatment with mebendazole, either a single dose or for 2 to 3 days; 2 to 3 days of treatment with mebendazole was more effective than the single dose.

The CRs and ERRS of the Janssen and the local/generic products appear to be comparable.

Table 16: Summary of the efficacy studies for single dose mebendazole for *T. trichiura* based on published studies

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
Abadi, 1985[52] Ujung Pandang and South-Sulawesi, Indonesia	Double blind, placebo-controlled trial	2-70	Kato-Katz (modified using cardboard templates). Single fecal sample at baseline and about 2-4 weeks after treatment. Cure based on at least two consecutive negative fecal examinations.	MBZ 500 mg SD (67)	77.6	92.8	Three subjects passed 1-2 *Ascaris* worms through the mouth on the day following treatment
				Placebo (38)	0	10.7	
Albonico *et al.*, 1994a[36] Pemba Island, Zanzibar, Tanzania	Open-label, multi-site trial	All ages (Adults and children)	Kato-Katz (cellophane thick smear technique – WHO 1993). Follow-up at Month 1 post-treatment. Quality control performed on 10% of the slides.	MBZ 500 mg SD, 250 mg SD for <2 year old children (379)	25.6	47.0	Level of transmission of *T. trichiura* was greater than those of hookworms and *A. lumbricoides*. Passage of worms was reported in 15.5% of subjects; few subjects complained of worm expulsion via the mouth and the nose (0.8%).
Albonico *et al.*, 1994b[37] Pemba Island, Zanzibar, Tanzania	Randomized single-blind, controlled trial	6-12	Kato-Katz (WHO 1993); Follow-up between Days18 - 31 post-treatment. Quality control performed on 15% of the slides.	MBZ 500 mg SD (1095)	14.2	81.6	Janssen product
				ABZ 400 mg SD (1138)	10.5	73.3	
	Controlled trial			MBZ 500 mg SD (190)	12.1	81.8	Janssen product
				MBZ 500 mg SD (207)	9.2	77.9	Pharmamed product
Albonico *et al.*, 2002a[38] Pemba Island, Zanzibar, Tanzania	Randomized, placebo-controlled trial	6-9	Kato-Katz (WHO, 1994). Stool samples were processed to assess egg counts within 6 hours. Follow-up 21 to 24 days after treatment. All laboratory investigations were blinded; the technicians examining the slides were unaware of the treatment regimen of the patients. Quality control: 10% of each batch of Kato–Katz smears (selected at random) were all read independently by two technicians; the whole batch was re-examined if the mean egg counts determined by the technicians differed by >10%.	MBZ 500 mg SD (404)	25.2	83.6	
				Pyrantel-oxantel 10 mg (382)	38.2	86.9	
				Placebo (369)	11.7	21.2	
Albonico *et al.*, 2002b[53] Mafia Island, Tanzania	Open label, single arm trial	6-18	Kato-Katz method. Follow-up between Day 21 and 24 days post-treatment. Quality-control: 10% of each batch of Kato–Katz smears were read independently by two technicians; the whole batch was re-examined if the mean egg counts differed by >10%.	MBZ 500 mg SD (145)	50.3	61.4	

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
Albonico *et al.*, 2003[39] Pemba Island, Zanzibar, Tanzania	Randomized, single center, placebo-controlled trial	7-18	Kato-Katz method. Follow-up at Day 21 post-treatment. Quality control performed on 10% of the slides.	MBZ 500 mg SD (236)	22.9	81.0	
				Levamisole 40 or 80 mg (210)	9.6	41.5	
				MBZ 500 mg + levamisole 40 or 80 mg (216)	22.9	85.0	
				Placebo (242)	4.8	18.3	
Cauwenbergh, 1985[54] International (Malaysia, Brazil, Philippines, Bangladesh, Germany, Indonesia, Thailand, Mexico, U.S.A. and Sri Lanka)	Multicenter controlled trial	1-50	Kato-Katz method. Follow-up at one to 3 weeks post-treatment	MBZ 100 mg SD (21)	92.0	83.0	
				MBZ 200 mg SD (113)	33.0	83.0	
				MBZ 500 mg SD (67)	78.0	93.0	
				MBZ 600 mg SD (117)	52.0	74.0	
Charoenlarp *et al.*, 1993[40] Nakhon Si Thammarat Province, Southern Thailand	Randomized, placebo-controlled trial	6-14	Kato-Katz method. Children with >500 eggs/g of feces selected for the study. Two fecal samples collected at baseline (1 week apart) and post treatment (week 3 and 4). The cure was based on two negative egg counts. All patients had hookworm infection	MBZ 300 mg SD (79)	11.4	20	GPO[1] product (polymorph A)
				MBZ 300 mg SD (76)	22.4	34.3	Other domestic product (polymorph A)
				MBZ 300 mg SD (83)	26.5	65.9	GPO[1] product (polymorph C)
				MBZ 300 mg SD (83)	42.2	90.5	Janssen product (polymorph C)
				MBZ 500 mg SD (71)	38.0	82.6	Janssen product (polymorph C)
				MBZ 100 mg x 3 days (83)	85.5	100	Janssen product (polymorph C)
				Placebo (70)	18.6	-11.7	
Evans *et al.*, 1987[55] Karino, Hazyview and Brondal areas; South Africa	Open label, single arm trial	5-16	Formalin-ether concentration technique using 1 g stool sample. Two stool samples collected at baseline as well as at Day 14 post-treatment	MBZ 500 mg SD (7)	100	NR	
Jackson *et al.*, 1998[66] Durban area of	Randomized, single-blind controlled trial	2-12	Formalin-ether concentration method. Ova counted in fecal specimens before and at Day 10 post-treatment.	MBZ 500 mg SD (42)	NR	72.0 (Range: 33-89)	Two shelters for abandoned and orphaned children. Results based on median values.

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
KwaZulu-Natal, South Africa			Patients With *T. trichiura* moderate infection (5-70 ova per coverslip on low power) enrolled.	ABZ 400 mg SD (40)	NR	44.0 (Range: 7-72)	
Jongsuksuntigul *et al.*, 1993[57] Pattani Province, Southern part of Thailand	Randomized, single site, controlled trial	3-80	Kato-Katz method.. Follow-up at Day 14 post-treatment. The laboratory technicians were blinded to the respective treatment of each patient group.	MBZ 300 mg SD (30)	43.3	77.9	Original MBZ product
				MBZ 300 mg SD (33)	0	28.3	Local (generic) MBZ product
				MBZ 500 mg SD (37)	70.3	89.9	Original MBZ product
				ABZ 400 mg SD (43)	67.4	87.4	Original
Khieu *et al.*, 2013[67] Cambodia	Open label, single arm trial	6-19	Kato-Katz thick smear examination and Baermann technique performed. Follow-up at week 3.	MBZ 500 mg SD (38)	97.4	NR	
Knopp *et al.*, 2010[58] Zanzibar, Tanzania	Randomized controlled trial	Not specified (Mean 11 years: Grades 1-7 children)	Kato-Katz method. Follow-up between 22-39 days after treatment. 2 consecutive stool samples collected. Duplicate smears from each sample were processed and eggs examined.	MBZ 500 mg SD + Placebo (138)	18.8	66.7	
				MBZ 500 mg SD + Ivermectin 200 µg/kg (138)	55.1	96.7	
				ABZ 400 mg SD + Placebo (132)	9.8	40.3	
				ABZ 400 mg SD + Ivermectin 200 µg/kg (140)	37.9	91.1	
Larocque *et al.*, 2006[59] Peruvian Amazon region, Peru	Randomized, double-blind, placebo-controlled trial	18-44 (Only pregnant women enrolled)	Kato-Katz method. Follow-up at approximately Day 76.	MBZ 500 mg SD + Iron (391)	39.1	92.9	Known to be malaria endemic region; only 2 women were smear positive.
				Placebo + Iron	ND	ND	
Levecke *et al.*, 2014[60] International (Brazil, Cambodia, Cameroon, Ethiopia, United Republic of Tanzania, and Vietnam)	Open label, multi-center, single arm trial	4-18	Flotation technique (McMaster egg counting method). Follow-up between Day 7 and 15 post-treatment	MBZ 500 mg SD (1075)	NR	63.1	Vermox product
Luoba *et al.*, 2005[62] Nyanza Province, Western Kenya	Open label, longitudinal trial	14-47 (Pregnant women only)	Kato-Katz cellophane method. Stool samples collected on 2 consecutive days and duplicate smears prepared. Follow-up time not specified	MBZ 500 mg SD (203)	70	NR	Vermox product
Mekonnen *et al.*, 2013[68]	Randomized controlled trial	Not specified (School	McMaster egg counting method; single stool samples examined at baseline and Day 14	MBZ 500 mg SD (103)	NR	60.0	ERRs based on arithmetic mean at BL and post-treatment

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
Jimma Town, Ethiopia		children – Grades 2 to 8)	post-treatment. Heavy-intensity trichuriasis (mean fecal egg counts approximately 863-1590 eggs/g of stool.	MBZ 500 mg x 2 days (90)	NR	87.1	
				ABZ 400 mg SD (102)	NR	29.3	
				ABZ 400 mg x 2 days (90)	NR	73.5	
Namwanje *et al.*, 2011[42] Uganda	Randomized controlled trial	5-14	Kato-Katz thick smear method. One stool sample was collected from each child, and four slides of 41.7 mg each were prepared from different parts of the sample. Children were followed-up weekly (Days 7, 14, 21, and 28) for 1 month. All children present in the five schools who were in classes one to six (aged 5-14 years) were screened for *T. trichiura* infection, and 611 infected subjects with ≥ 100 *T. trichiura* eggs/g of feces were selected and randomized to different treatment groups.	MBZ 500 mg SD (98)	20.4	66.7	Cure rates of the different drugs and drug combinations as measured at different times of follow-up varied between 25.6% and 57.5% at Day 7. Cure rates were significantly higher using double doses compared with single doses. Cure rates measured at Day 7 were significantly higher than on Days 14 and 21 after treatment. The results represent 28 day CRs and ERRs. Double doses versus a single dose significantly increased the CRs.
				MBZ 500 mg X 2 days (93)	41.9	90.2	
				ABZ 400 mg SD (91)	15.4	54.9	
				ABZ 400 mg x 2 doses (8 hours apart) (76)	43.4	89.3	
				MBZ 500 mg + ALB 400 mg (83)	54.2	94.3	
				MBZ 500 mg + ALB 400 mg (8 hours apart) (92)	56.5	95.9	
Schutte, 1989[43] South Africa	Open label, single arm, 2 center trial	5-18	Formalin-ether concentration technique. All fecal samples were fixed and preserved in 4% formalin; all examinations conducted in the RIDTE laboratory in Durban. Two baseline samples (2 weeks apart) and follow-up at 2 weeks post-treatment.	MBZ 500 mg SD (283)	33.2	77.3	
Sorensen *et al.*, 1996[63] Sri Lanka	Randomized controlled trial	3-15	Kato-Katz technique. Laboratory technicians were blinded to the treatment arm. All stool samples were coded and kept cold during transportation and storage. Follow-up at week 3 post-treatment.	MBZ 500 mg SD (88)	26.1	61.6	Janssen product
				MBZ 500 mg SD (110)	29.1	31.6	Local [redacted] 2
				ABZ 400 mg SD (84)	26.2	50.3	
Soukhathammavong *et al.*, 2012[44] Laos	Randomized, open label, 2 arm trial	6-12	Kato-Katz quadruplicate thick smears derived from two stool samples at each time point. Follow-up stool samples at Days 21–23 post-treatment	MBZ 500 mg SD (43)	27.6	66.0	Vermox product. All patients were infected with hookworm(s) + other helminths.
				ABZ 400 mg SD (39)	33.3	67.0	
Speich *et al.*, 2015[45] Pemba Island, Tanzania	Randomized controlled trial	6-14	Kato-Katz method; Two stool samples on consecutive days. Time of assessment following treatment not specified.	MBZ 500 mg SD (107)	8.4	58.5	
				MBZ 500 mg SD + ABZ 400 mg SD (107)	8.4	51.6	

Reference / Location	Study Design	Age Range (Years)	Parasitological Method / Number of Stool Samples	Treatment Group (n)*	Parasitological Findings		Comments
					Cure rate (%)	ERR (%)	
				ABZ 400 mg SD + oxantel pamoate 20 mg/kg (108)	68.5	99.2	
				ABZ 400 mg SD + ivermectin 200 µg/kg (109)	27.5	94.5	
Steinmann *et al.*, 2011[45] China	Community based randomized controlled trial	≥5	2 stool samples collected before and between Week 3-4 post-treatment. First, samples were visually inspected for adult *A. lumbricoides* and *Taenia* spp. proglottids. Second, two 41.7 mg Kato-Katz thick smears were prepared from each sample. Depending on the ambient temperature and considering over-clearance of hookworm eggs, slides were read within 30–90 min of preparation. At least 5% of the daily diagnoses were cross-checked by the principal investigator.	MBZ 500 mg SD (63)	39.7	82.5	
				MBZ 500 mg x 3 days (58)	70.7	97.3	
				ABZ 400 mg SD (65)	33.8	76.7	
				ABZ 500 mg x 3 days (48)	56.2	94.0	
·an der Werff *et* ., 2014[47] uba	Cohort from a MDA study	5-14	Kato-Katz method. Follow-up at approximately Month 6 or 36 months. Duplicate smears from each stool sample examined.	MBZ 500 mg SD (132)	67.4	85	Follow-up ~6 months
				MBZ 500 mg SD administered 2 times/year (107)	89.7	97.7	Follow-up ~36 months

* Number of subjects secreting eggs at baseline; [1]GPO=Government Pharmaceutical Organization
SD= single dose; MBZ=mebendazole; ABZ=albendazole; NR=not reported; ND=not done [redacted] . Epg=egg per gram; MDA=mass drug administration

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4.1.2.3. *Necator americanus* and *Ancylostoma duodenale*

Efficacy of mebendazole (single 500 mg dose) in patients with hookworm *(N. americanus* and *A. duodenale)* infection, alone or with mixed infections, was reported in 24 studies (Abadi, 1985[52]; Albonico *et al*., 1994a[36], 1994b[37]; 2002a[38], 2002b[53], 2003[39]; Cauwenbergh, 1985[54]; Charoenlarp *et al*., 1993[40]; De Clercq *et al*., 1997[41]; Evans *et al*., 1987[55]; Flohr *et al*., 2007[69]; Jongsuksuntigul *et al*., 1993[57]; Khieu *et al*., 2013[67]; Knopp *et al*., 2010[58]; Larocque *et al*., 2006[59]; Levecke *et al*., 2014[60]; Luoba *et al*., 2005[62]; Sacko *et al*., 1999[46]; Schutte, 1989[43]; Sorensen *et al*., 1996[63]; Soukhathammavong *et al*., 2012[44]; Speich *et al*., 2015[45]; Steinmann *et al*., 2011[65]; van der Werff *et al*., 2014[47]). Of the 24 studies, 19 were same as those summarized above for *A. lumbricoides* as well as *T. trichiura*; 2 studies were same as those for *T. trichiura*. The studies were conducted in different countries that include Indonesia, Tanzania, Malaysia, Brazil, Philippines, Bangladesh, Germany, Thailand, Mexico, USA, Sri Lanka, South Africa, Peru, Cambodia, Cameroon, Ethiopia, Vietnam, Western Kenya, China, Cuba, Mali, or Uganda (Table 17). Of the 24 studies, 14 were randomized trials, 4 were blinded, and 8 were placebo-controlled. Of the 8 placebo-controlled studies, 7 reported results for placebo. The number of subjects treated with a single dose of mebendazole varied from 34 to 1011 in different trials.

Different parasitological methods used for detection and quantitation of eggs include formalin-ether concentration (2 studies), flotation/McMaster (2 studies), or Kato-Katz (20 studies; 1 using cardboard templates; 1 using cellophane; others not specified) techniques. Like for the studies summarized above for *A. lumbricoides* and *T. trichiura*, the number of fecal specimens processed at each time as well as the number of smears from each fecal sample varied from one to two. In one study (Flohr *et al*., 2007[69]), both Kato-Katz and salt flotation method was used; also, in one part of the study, McMaster salt flotation was compared with ether sedimentation method. The results showed sensitivity of the salt flotation method was similar to the formalin-ether sedimentation method. Follow-up time varied between 7 and 76 days.

Of the 5628 subjects treated for hookworm infections with mebendazole, 4729 subjects were evaluated for CRs and 5001 subjects were evaluated for ERRs; 974 subjects (from 7 studies) were administered placebo. The CRs varied between 2.9% and 91.1%; ERRs varied between 6.5% and 98.3%. Both CRs and ERRs were higher in mebendazole treated subjects compared to the placebo group of patients in 6 studies; in one study (De Clerq *et al*., 1997[41]), the CRs in mebendazole treated subjects were not different from the placebo group (Table 17).

In one study (van der Werff *et al*., 2014[47]), MDA with a single dose (500 mg) of mebendazole was performed two times a year and follow-up performed at month 6 and 36. The CR was 44.4% and ERR 63.9% at Month 6 (n=117) after the first dose; CR was 70% and ER 93.6% after about 3 years in patients that received mebendazole 500 mg every 6 months (n=100).

Two studies (Charoenlarp *et al*., 1993[40] Steinmann *et al*., 2011[65]) reported CRs and/or ERRs after single dose or 3 days of mebendazole. Treatment with mebendazole for 3 days, even at a lower dose, was more effective in reducing egg count compared to the single dose.

[69] Flohr C, Tuyen LN, Lewis S, Minh TT, Campbell J, Britton J, Williams H, Hien TT, Farrar J, and Quinnell RJ. Low efficacy of mebendazole against hookworm in Vietnam: two randomized controlled trials. *Am J Trop Med Hyg* 2007;76(4):732-36.

The CRs and ERRs of the Janssen and the local/generic products appear to be comparable.

The hookworm species was identified by coproculture in 5 studies (Abadi, 1985[52]; Albonico *et al*., 2002b[53]; Charoenlarp *et al*., 1993[40]; De Clerq *et al*., 1997[41]; Laroque *et al*., 2006[59]). *N. americanus* was identified in all the studies; *A. duodenale* was identified in 2 studies (Charoenlarp *et al*., 1993[40] and Abadi, 1985[52]) in a small number of subjects (1.5% and 11%, respectively). The CRs or ERRs by hookworm species were reported in one study (Abadi, 1985[52]); of the 45 subjects with hookworm infection, 88.9% were infected with *N. americanus* alone and 11.1% had mixed infections of *N. americanus* and *A. duodenale.* CR was 90% in subjects with necatoriasis; all the five cases with mixed hookworm infections were cured. However, the number of cases was limited. CRs were not reported by species in any of the other studies. Mebendazole appears to be equally effective against both hookworm species.

Comments:

- *Overall, the published studies suggest that a single dose (500 mg) of mebendazole is effective in curing and reducing egg count in patients with A. lumbricoides, T. trichiura, and N. americanus and A. duodenale infections. There was a wide range of variability in CRs and ERRs reported in different studies. Such a variability in response could be due to*
  - *study design that includes patient population including the age of the subjects; for example, intensity of infection appears to be higher in younger children such as those in Grade 1compared to older children and adults.*
  - *daily variation in the numbers of nematode eggs excreted by infected individuals.*
  - *parasitological methods used, number and quantity of stool specimens collected and processed for egg count, as well as the time of collection of stool specimens post-treatment.*
  - *different parasite burden at the time of initiation of treatment.*
  - *previous drug exposure.*
  - *variability in the possibility of re-infection due to different levels of transmission in different geographic regions and seasonal variation e.g., a doubling / increase of intensity after the rainy season.*
  - *nutritional status of the subjects.*

  Despite the differences in the studies, the results are comparable with those of the phase 3 study GAI3003.
- *A. lumbricoides appears to be more sensitive to mebendazole compared to T. trichiura, and N. americanus and A. duodenale.*
- *Hookworm species was identified by coproculture in some of the studies. Mebendazole appears to be equally effective against both hookworm species.*
- *CRs were similar in placebo-controlled vs non-controlled studies.*
- *The efficacy of the Janssen product appears to be comparable to other products (generic/local) used in some of the studies.*
- *The CRs and ERRs in subjects receiving mebendazole 500 mg over time as part of a MDA program were comparable at about Month 6 after the first dose to those after about 3 years that received mebendazole 500 mg every 6 months.*

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4.2. Effect of parasitic load on response to mebendazole treatment

In few studies the effect of treatment with mebendazole on CRs and ERRs was reported in subjects with different parasite burden, prior to treatment. Abadi (1985)[52] reported lower CRs in subjects with heavy infections compared to those with light infections (Table 18). ERRs do not appear to be altered by the intensity of infection (Table 18).

Table 18: Efficacy of single dose of 500 mg of mebendazole based on the severity of infection

A: Ascariasis

Severity of ascariasis according to pretreatment EPG*	No. of cases			Mean EPG*		
	Before treatment	After treatment	% cure	Before treatment	After treatment	% egg reduction
Light (≤49,999)	45	2	95.5	16,583	101	99.3
Moderate (50,000–99,999)	9	1	99.8	78,600	800	99.0
Heavy (≥100,000)	7	1	85.7	120,150	1,150	99.1
Total group	61	4	93.4	215,333	2,051	99.0
Placebo group	44	44	—	249,643	234,790	6.0

* EPG, eggs/g feces.

B: Trichuriasis

Severity of trichuriasis according to pretreatment EPG*	No. of cases			Mean EPG*		
	Before treatment	After treatment	% cure	Before treatment	After treatment	% egg reduction
Light (≤4,999)	29	4	86.2	1,900	106	94.4
Moderate (5,000–24,999)	31	8	74.1	6,180	459	92.5
Heavy (≥25,000)	7	3	57.1	26,345	1,880	92.8
Total group	67	15	77.6	34,425	2,445	92.8
Placebo group	38	38	—	21,875	19,552	10.7

* EPG, eggs/g feces.

(b) (4)

[redacted]

Evans *et al* (1987) [55] reported a trend towards a lower CR in a small number of subjects with moderate intensity of hookworm infection compared to low intensity infection (Table 19).

Table 19: Effect of single-dose treatment of *Ascaris,* hookworm and *Trichuris* infections with 500 mg mebendazole

	No. treated	No. cured	No. of refractory cases	EPG rates in refractory cases			
				No. decreased or unchanged	Range of decrease (%)	No. increased	Range of increase (%)
A. lumbricoides							
(147 cases; 69,7%)							
Light (< 49 999)*	**147†**	**137 (93,2%)**	**10 (6,8%)**	**6**	**17,9-83,0**	**4**	**19,5-758,8**
[redacted]							
T. trichiura							
(7 cases; 3,3%)							
Light (< 4999)*	**7†**	**7 (100%)**	—				

(b) (4)

Classification described by Abadi.
†Category based on average of 2 pretreatment egg counts; only 1 post-treatment count made.

Similar findings were reported by Albonico *et al* (2003)[39] in patients with [redacted] (b) (4) The authors stated that the efficacy of mebendazole was not

influenced by the STH species (b) (4)) present as single or multiple infections.

Table 20: Effect of treatment with mebendazole on CRs and ERRs in patients with different intensities of infection

Causative pathogen	Cure rates (%)		Significant	ERRs (%)		Significant
	Light*	Heavy*		Light*	Heavy*	
A. lumbricoides	97.0	93.3	No (p=0.07)	Not specified	Not specified	Not applicable
T. trichiura	19.3	8.3	No (p=0.1)	81.4	93.8	Yes (p<0.05)
Hookworm	16.3	8.4	Yes (p<0.05)	73.2	91.3	Yes (p<0.01)

*Egg counts (eggs/g) used to describe intensity of infection was based on the following criteria:

Causative pathogen	Intensity of infection (egg count per gram)		
	Light	Moderate	Heavy
A. lumbricoides	1–4999	5000–9999	⩾10 000
T. trichiura	1–999	1000–9999	⩾10 000
Hookworm	1–1999	2000–3999	⩾ 4000

CRs and ERRs in patients with moderate infection were not included in the publication.
Note: The study enrolled children in the first and fifth grades of primary schools. The authors stated that the Standard 5 children had received 15 rounds of treatment with mebendazole; however, the children in Standard 1 who had never been treated. The egg counts at baseline were significantly lower in Standard 5 children compared to Standard 1 children.

Levecke *et al* (2014)[60] stated that the efficacy of mebendazole was dependent on the intensity of *A. lumbricoides* and *T. trichiura* infection; efficacy decreased with increasing intensity.

Comments:
Overall, the studies suggest higher parasite burden at the time of initiation of treatment may be associated with low CRs.

5. INTERPRETIVE CRITERIA/BREAKPOINTS

Standardized tests to detect resistance to mebendazole by the STHs are not available (for more details see section 3.4 above).

6. THE LABELING

6.1. Applicant's version of the microbiology section of the labeling

12.1 Mechanism of Action

(b) (4)

12.4 Microbiology

Mechanism of Action

Mebendazole interferes with cellular tubulin formation in the helminth and causes ultrastructural degenerative changes in its intestine. As a result, its glucose uptake and the digestive and reproductive functions are disrupted, leading to immobilization, inhibition of egg production and death of the helminth.

Mebendazole is active against:
Trichuris trichiura (b) (4)
Ascaris lumbricoides (b) (4)
(b) (4)

[redacted] Resistance [redacted]

6.2. Comments

- [redacted] *The activity against different helminth studies should be summarized under a separate subheading 'Antimicrobial activity'.*
- *Few edits are recommended for clarity and accuracy.*

6.3. FDA's version of the labeling

12.1 Mechanism of Action

[redacted] Mebendazole [redacted] a benzimidazole, is an anthelminthic [redacted] *Microbiology (12.4)]*.

12.4 Microbiology

Mechanism of Action

Mebendazole interferes with cellular tubulin formation in the helminth and causes ultrastructural degenerative changes in its intestine. As a result, its glucose uptake and the digestive and reproductive functions are disrupted, leading to immobilization, inhibition of egg production and death of the helminth.

Antimicrobial activity

[redacted] Mebendazole is active against:

[redacted]

Ascaris lumbricoides [redacted]

Trichuris trichiura

[redacted]

[redacted] **-Resistance**

There is a potential for development of resistance to mebendazole. [redacted] The mechanism of resistance to mebendazole is likely due to changes of [redacted] beta-tubulin protein, which reduces binding of mebendazole to [redacted] beta-tubulin; however, clinical significance of this is not known.

[See appended electronic signature page]
Shukal Bala, PhD
Microbiologist, DAIP

CONCURRENCE:
DAIP/Acting Microbiology Team Leader/ Lynette Berkeley PhD

CC:
NDA # 208398
DAIP/PM/Alison Rodgers

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/s/

SHUKAL BALA
10/04/2016

LYNETTE Y BERKELEY
10/04/2016