

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**21-818 and 21-498/S-003**

**MICROBIOLOGY REVIEW(S)**

**MICROBIOLOGY REVIEW**  
**DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)**

**NDA #:** 21-498 and 21-818      **REVIEWER** : Kalavati Suvarna  
**CORRESPONDENCE DATE** : 12-17-04  
**CDER RECEIPT DATE** : 12-20-04  
**REVIEW ASSIGN DATE** : 01-04-05  
**REVIEW COMPLETE DATE** : 04-28-05

**SPONSOR:** Romark Laboratories Inc.  
6200 Courtney Campbell Causeway  
Suite 880  
Tampa, FL 33607

**SUBMISSION REVIEWED:** N-000 (AZ), SE5-003

**DRUG CATEGORY:** Anti-parasitic

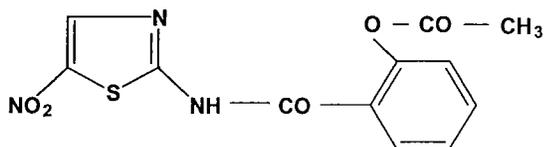
**INDICATION:** Treatment of diarrhea caused by *Cryptosporidium parvum*

**DOSAGE FORM:** Tablets

**PRODUCT NAMES:**

- a. **PROPRIETARY:** Alinia<sup>®</sup>
- b. **NONPROPRIETARY:** Nitazoxanide; CAS: 55981-09-4
- c. **CHEMICAL:** 2-(acetolyloxy)-N-(5-nitro-2-thiazolyl) benzamide

**STRUCTURAL FORMULA:**



Molecular weight: 307.2  
Empirical formula: C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>5</sub>S

**SUPPORTING DOCUMENTS:** NDA # 20-871, 21-497, 21-498, 21-818; IND # 48,620,  
— , 58,895, — ; Type II DMF # — , DMF — DMF — DMF —

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**TABLE OF CONTENTS**

1. EXECUTIVE SUMMARY .....	3
2. INTRODUCTION AND BACKGROUND .....	4
2.1. Biology of <i>Cryptosporidium parvum</i> .....	4
2.2. Pathogenesis of cryptosporidial infection .....	4
3. PRECLINICAL MICROBIOLOGY .....	4
4. CLINICAL MICROBIOLOGY .....	5
4.1. Study RM01-3010 .....	5
4.2. Study RM-NTZ-98-002 .....	9
4.3. Interpretive criteria .....	10
5. CONCLUSIONS .....	10
6. LABEL .....	11
6.1. Sponsor's proposed label .....	11
6.2. Comments .....	11
7. RECOMMENDATIONS .....	11

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**1. EXECUTIVE SUMMARY:**

The sponsor is seeking approval of nitazoxanide tablets (500 mg BID for 3 days) for the treatment of diarrhea due to *Cryptosporidium parvum* in immunocompetent adults. In 2002, the nitazoxanide oral suspension was approved for the treatment of cryptosporidial diarrhea and giardiasis in children. In 2004, nitazoxanide tablets and suspension were approved for the treatment of giardiasis in adults. However, there was insufficient evidence to support approval of the tablet formulation for treatment of cryptosporidial diarrhea in adults.

The clinical study (RM01-3010) was conducted in Egypt to determine the safety and efficacy of nitazoxanide in the treatment of diarrhea due to *C. parvum* in adults. The parasitological outcome (presence of oocysts) was assessed microscopically in unconcentrated stool samples using 3 different staining procedures (Iodine, Ziehl-Neelsen or Immunofluorescence), and in concentrated stool samples stained with iodine, at baseline, EOT, and 11 to 14 days after discontinuation of treatment. However, the oocysts were not quantified. In this study, the sensitivity of the Ziehl-Neelsen stain was greater (94%) compared to the immunofluorescence stain (51%). Resolution of diarrhea and absence of oocysts in 2 stool samples by Ziehl-Neelsen staining was observed in 92% (24/26) patients treated with nitazoxanide tablets compared to 86% (25/29) patients treated with nitazoxanide suspension, and 35% (8/23) patients treated with placebo, at 4 to 7 days after discontinuation of therapy. The clinical outcome correlated with the parasitological outcome. Also, clinical outcome was sustained in patients who had follow-up evaluation at 11-14 days after discontinuation of therapy.

Study RM-NTZ-98-002 submitted to the original NDA compared the efficacy of nitazoxanide tablets to placebo in patients with cryptosporidiosis. This study was also conducted in Egypt. The parasitological outcome (presence of oocysts) was assessed microscopically in unconcentrated stool samples stained with Ziehl-Neelsen's stain at baseline and EOT. Immunofluorescence staining for detection of oocysts was limited to 2 patients. Nitazoxanide showed resolution of diarrhea in 71% (15/21) patients treated with tablet compared to 43% (9/21) patients who received placebo. However, the parasitological outcome did not correlate with clinical outcome. The reason for the differences in the overall response in the 2 studies is unclear.

Overall, the clinical efficacy of nitazoxanide tablets was similar to nitazoxanide suspension and greater than placebo.

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## **2. INTRODUCTION AND BACKGROUND:**

The subject of this NDA is nitazoxanide. Nitazoxanide oral suspension is approved for the treatment of diarrhea due to *Cryptosporidium parvum* in immunocompetent children (NDA# 21-498). Nitazoxanide tablet and suspension formulations are approved for the treatment of diarrhea due to *Giardia lamblia* in adults and children (NDA# 21-497 and 21-498). However, there was insufficient evidence to support approval of the tablet formulation of nitazoxanide for the treatment of immunocompetent adults with cryptosporidiosis (NDA# 21-497 and 21-818). The sponsor is seeking approval of nitazoxanide tablet formulation (500 mg BID x 3 days) for the treatment of cryptosporidial diarrhea in immunocompetent adults based on a second clinical trial conducted in Egypt.

### **2.1. Biology of *Cryptosporidium parvum*:**

*Cryptosporidium* is an intracellular parasite present in the gastrointestinal and respiratory tract. The infection is caused by ingestion of oocysts. The oocyst contains four sporozoites within a membrane. Upon ingestion, the sporozoites excyst from the oocyst and invade the epithelial cells and become enveloped in a parasitophorous vacuole. Sporozoites undergo maturation into type I meronts, which release merozoites. The merozoite stage can undergo asexual replication and reinvade the host cells or form type II meronts by sexual replication. The type II meronts release the macrogametocytes or microgametocytes that fertilize to give rise to a zygote. The zygote can develop into oocyst that may either rupture releasing sporozoites *in vivo* or shed via the feces. Therefore, the presence of oocyst(s) in the stool samples may be intermittent.

### **2.2. Pathogenesis of cryptosporidial infection:**

The major clinical manifestation observed with cryptosporidial infection is diarrhea. The diarrheal infection can lead to malabsorption. However, the mechanism by which *Cryptosporidium* causes diarrhea is not known. The severity of the infection and ultimate pathology is influenced by the immune status of the host. Cryptosporidial diarrhea is self-limiting in immunocompetent individuals but may be life threatening in AIDS or immunocompromised patients.

## **3. PRECLINICAL MICROBIOLOGY:**

No new information was included in this submission. Studies describing the mechanism of action, activity of nitazoxanide *in vitro* and *in vivo* against *C. parvum* were reviewed earlier [please see microbiology reviews dated 06-01-98 (NDA 20-871, N-000), and 11-06-02 (NDA 21-497 and 21-498, N-000)]. Nitazoxanide and its metabolite, tizoxanide, were active *in vitro* in inhibiting the growth of the sporozoites and oocyst stages of *C. parvum*.

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#### 4. CLINICAL MICROBIOLOGY:

The safety and efficacy of nitazoxanide for the treatment of cryptosporidiosis in adults was evaluated in studies RM01-3010 (new study) and RM-NTZ-98-002 (previously submitted to the original NDA).

##### 4. 1. Study RM01-3010:

Study RM01-3010 was a phase III, multi-center, double-blind, randomized, placebo controlled study to determine the safety and efficacy of nitazoxanide (tablet and oral suspension formulations) for the treatment of diarrhea due to *C. parvum* in adults. A total of 90 immunocompetent patients ( $\geq 12$  years of age) with diarrhea ( $\geq 3$  bowel movements/day with or without other symptoms) and a positive diagnosis of *C. parvum* were randomized (1:1:1) to receive either nitazoxanide tablets (500 mg BID for 3 days), nitazoxanide oral suspension [25 ml (500 mg) BID for 3 days], or placebo tablets (BID for 3 days) with food. Patients receiving drugs with anti-protozoal activity within 2 weeks of enrollment were excluded. One stool sample was collected at baseline and two stool samples were collected at the end of therapy (4 to 7 days after discontinuation of therapy) for detection of *C. parvum* oocysts. The detection of *C. parvum* oocysts was based on microscopic examination of unconcentrated stool sample stained with iodine or Ziehl-Neelson (ZNN) stain and concentrated stool sample stained with iodine. The test results were confirmed by immunofluorescence assay (—, FDA approved) using unconcentrated stool sample. If the sponsor was unable to conduct the immunofluorescence assay, the microscopic examinations alone were used for determining the parasitological response. Patients with diarrhea due to other pathogenic bacteria or protozoa (*G. lamblia* and *Entamoeba histolytica*) were excluded. The following methods were used to exclude patients with diarrhea due to pathogens other than *C. parvum*: bacterial culture, microscopic examination of stool for protozoa other than *C. parvum*, immunofluorescence assay for *G. lamblia*, and the Baermann concentration test for *Strongyloides stercoralis*.

The clinical responses were defined as:

**Well:** patient had no symptoms, no watery stools,  $\leq 2$  soft stools, and had no hematochezia within the 24 hours prior to evaluation or no symptoms and no unformed stools within the 48 hours prior to evaluation;

**Continuing illness:** patient passed watery stools,  $> 2$  soft stools per 24 hours or documentation of hematochezia or enteric symptoms plus any number of soft or watery stools during the 48 hours prior to evaluation;

**Clinical treatment failure:** patient showed clinical deterioration or worsening of symptoms after 24 hours of treatment.



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A total of 90 patients were enrolled from 2 study sites (Benha and Alexandria) in Egypt. Sensitivity of the different methods used to identify baseline *C. parvum* oocysts was compared. Baseline stool samples (both unconcentrated and concentrated) stained with iodine from all 90 patients were negative (Table 1). The sensitivity of ZNN was greater (94%; 85/90) compared to DFA (51%; 46/90) for identification of *C. parvum* using unconcentrated stool samples. The sensitivity of the DFA observed in this study (51%) was lower than that reported in the package insert (92%) by the manufacturer of the DFA kit. Please note that quality control data using the DFA kit in the laboratory where the clinical trial samples were tested were not included for review. The reason for the lower sensitivity of DFA is unclear.

Table 1: Analysis of the sensitivity of the different methods used to detect *C. parvum* oocysts in a baseline stool sample from all patients enrolled in study RM01-3010.

UC/ZNN	UC/DFA		UC/I		C/I	
	+	-	+	-	+	-
+	45	40	0	85	0	85
-	1	4	0	5	0	5

UC/ZNN = unconcentrated stool sample stained with Ziehl-Neelsen stain;

UC/DFA = unconcentrated stool sample stained using an immunofluorescence assay kit;

UC/I = iodine stained unconcentrated stool sample;

C/I = iodine stained concentrated stool sample;

+ = presence of oocysts;      - = absence of oocysts;

The modified intent-to-treat (MITT) population consisted of all patients randomized to the study excluding patients who did not have *C. parvum* oocysts and other identified causes of diarrhea. Of the 90 patients enrolled in the study, 4 patients (#65, #71, #99, #110) were excluded by the sponsor from the modified intent-to-treat (MITT) population as they did not have *C. parvum* oocysts at baseline. Please note that the sponsor has included patients who had *Blastocystis hominis*, and/or *Hymenolepis nana* in addition to *C. parvum* in the baseline stool sample in their analysis of efficacy. Although asymptomatic carriers of *B. hominis* and *H. nana* cysts have been reported in Egypt, these pathogens can cause diarrhea. For the purpose of this review, 8 additional patients who had *C. parvum* mixed with *B. hominis* and *H. nana* at baseline were excluded. The 78 patients analyzed included 26 patients treated with NTZ tablets, 29 with NTZ oral suspension, and 23 with placebo (Table 2).

On days 4 to 7 after discontinuation of therapy (test of cure/visit 2), absence of oocysts in 2 stool samples by ZNN staining and resolution of diarrhea was observed in 24 (92%) of the 26 patients treated with NTZ tablets. Of the 2 patients who showed persistence of oocysts, one showed resolution of diarrhea and was also positive for oocysts by DFA (Tables 2 and 3). Of the 29 patients treated with NTZ oral suspension, one patient did not return for evaluation and was considered to be a clinical and parasitological failure as per protocol design. Resolution of diarrhea and absence of oocysts in the stool samples were observed in 86% (25/29) of the patients (Table 2). The 3 remaining patients continued to have diarrhea. Absence of oocysts was observed in 1 of the 3 patients by ZNN staining. One patient was positive for oocysts by both ZNN and DFA (Table 3). Placebo was less effective than NTZ. Resolution of diarrhea and eradication of oocysts was observed in 35% (8/23) patients (Table 2). Two patients with resolution of diarrhea continued to shed oocysts. The patients were positive for oocysts by

ZNN and DFA staining. Of the remaining 13 patients who continued to have diarrhea, 2 showed eradication of oocysts.

Table 2: Parasitological and clinical response of patients with cryptosporidiosis from Egypt at visit 2 (end of therapy).

Treatment group	Parasitological and clinical responses			Patients with eradication of oocysts N (%)	Patients with clinical well response N (%)	Patients clinically well and showing eradication of oocysts N (%)
	Oocysts Eradicated by ZNN and DFA (CR)	Oocysts Persisted (CR)				
		ZNN alone	ZNN + DFA			
500 mg NTZ tablet BID 3 days (n = 26)	24 (24 well, 0 CI)	1 (1 CI)	1 (1 well)	24 (92)	25 (96)	24 (92)
500 mg NTZ oral suspension BID 3 days (n = 29)	26 (25 well, 1 CI)	2* (2 CI)	1 (1 CI)	26 (90)	25 (86)	25 (86)
Placebo BID 3 days (n = 23)	10 (8 well, 2 CI)	5 (5 CI)	8 (2 well, 6 CI)	10 (43)	10 (43)	8 (35)

NTZ = Nitazoxanide; CR = clinical response; N = number of subjects; CI = continuing illness;  
\* one patient did not return for end of therapy evaluation and was considered to be a failure (continuing illness with persistence of oocysts) by sponsor

Table 3: Correlation of the ZNN and DFA staining method in the post-treatment stool samples obtained at visit 2 (4 to 7 days after discontinuation of therapy) or visit 3 (11 to 14 days after discontinuation of therapy).

**Tablets (n = 26):**

ZNN	DFA	
	+	-
+	1*	1*
-	0	24

ZNN	DFA	
	+	-
+	1	0
-	0	25

\* patient had positive result in one of the 2 stool samples

**Suspension (n = 29, one patient did not return for follow-up and was considered as failure by sponsor):**

ZNN	DFA	
	+	-
+	1*	1
-	0	26

ZNN	DFA	
	+	-
+	0	1
-	0	27

\* Patient had 2 stool samples positive by ZNN and 1 stool sample positive by DFA

**Placebo (n = 23):**

ZNN	DFA	
	+	-
+	8 <sup>#</sup>	5 <sup>^</sup>
-	0	10

ZNN	DFA	
	+	-
+	4	3
-	0	15

<sup>#</sup> for 7 patients, positive result in 1 of 2 stool samples

<sup>^</sup> for 3 patients, positive result in 1 of 2 stool samples

\* data not available for 1 patient

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At 11 to 14 days after discontinuation of therapy, 60 clinically well patients (NTZ tablets, n = 25; NTZ suspension, n = 25; and placebo, n = 10) returned for follow-up of parasitological and/or clinical evaluation (Table 4). All 25 patients treated with NTZ tablets continued to be free of oocysts based on evaluation of 1 stool sample by ZNN staining. However, only 11 patients had follow-up clinical evaluations and all 11 continued to be free of diarrhea. Of the 25 patients treated with NTZ suspension, follow-up clinical and parasitological evaluations were performed in 13 patients, while parasitological evaluation alone was performed in the remaining 12 patients (Table 4). All 13 patients were clinically well at 11 to 14 days after discontinuation of therapy; however, 1 showed shedding of oocysts by ZNN staining. Of the 12 patients who had parasitological evaluation only, all were free of oocysts at 11-14 days after discontinuation of therapy. Of the 10 patients in the placebo group, follow-up clinical and parasitological evaluations were performed in 3 patients (Table 4). All 3 patients continued to be well; however, 1 patient showed shedding of oocysts in the stool sample. The remaining 7 patients had only parasitological evaluation at follow-up. Shedding of oocysts was detected by ZNN staining in 1 patient who had previously showed absence of oocysts at EOT.

Overall, the parasitological response correlated with the clinical response at 4 to 7 and 11 to 14 days after discontinuation of therapy.

Table 4: Evaluation of sustained parasitological and clinical response at follow-up (11-14 days after discontinuation of therapy) in the 60 patients with cryptosporidiosis who were clinically well at end of therapy.

Treatment group	Day 4-7		Day 11-14	
	Clinical well	Clinical well	Absence of oocysts by ZNN*	
	absence of oocysts	absence of oocysts by ZNN		
<i>NTZ Tablet</i>	24/25	11/11	14/14	
<i>NTZ Suspension</i>	25/25	12/13	12/12	
<i>Placebo</i>	8/10	2/3 <sup>#</sup>	6/7	

NTZ = Nitazoxanide;

ZNN = Ziehl-Neelsen stain

\* No clinical evaluation performed. The data was obtained prior to protocol amendment.

<sup>#</sup> One of the 3 patients showed presence of oocysts using both ZNN and DFA staining

#### 4.2. Study RM-NTZ-98-002:

Study RM-NTZ-98-002 was a randomized, double-blind, placebo controlled study that determined the safety and efficacy of nitazoxanide tablets in 50 adults with cryptosporidial diarrhea in Egypt [please see microbiology review dated 11-06-02 (NDA 21-497 and 21-498, N-000)]. Eradication of oocysts was observed in 12/21 (57%) patients and resolution of diarrhea was observed in 7 of the 12 patients. Placebo treatment eradicated oocysts in 6/21 (28%) patients, and in 3 of the 6 patients, diarrhea was resolved. Parasitological evaluation in this study was limited to microscopic examination of a small amount of unconcentrated stool sample after ZNN staining. Quantitation of oocysts was not done uniformly for all patients. DFA staining of unconcentrated stool sample was performed in only 2 of the 21 patients.

Overall, nitazoxanide tablet and suspension were more effective than placebo in resolving diarrhea (Table 5).

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Table 5: Overall parasitological and clinical outcome of patients treated with nitazoxanide (NTZ) for cryptosporidiosis.

Study	NTZ tablet n/N (%)			NTZ suspension n/N (%)			Placebo n/N (%)		
	CR	PR	CR + PR	CR	PR	CR + PR	CR	PR	CR + PR
RM01-3010	25/26 (96%)	24/26 (92%)	24/26 (92%)	25/29 (86%)	26/29 (90%)	25/29 (86%)	10/23 (43%)	10/23 (43%)	8/23 (35%)
RM-NTZ-98-002	15/21 (71%)	12/21 (57%)	7/21 (33%)	-	-	-	9/21 (43%)	6/21 (28%)	3/21 (14%)

n = number of subjects with observed effect

N = total number of subjects

CR = clinical well

PR = eradication of oocysts

**4.3. Interpretive criteria:**

There are no standardized methods for measuring *in vitro* susceptibility of antiprotozoal drugs against *C. parvum* and no interpretive criteria have been established.

**5. CONCLUSIONS:**

The sponsor is seeking approval of nitazoxanide tablets and suspension for the treatment of diarrhea due to *C. parvum* in adults. The sponsor has proposed a dose of 500 mg nitazoxanide BID for 3 days for treatment of adults with cryptosporidial diarrhea.

Study RM01-3010 conducted in Egypt evaluated the safety and efficacy of nitazoxanide tablets or suspension for the treatment of cryptosporidiosis in adults. Resolution of diarrhea and absence of oocysts in 2 stool samples at EOT were observed in 92% (24/26) patients treated with nitazoxanide tablets and 86% (25/29) patients treated with nitazoxanide suspension compared to 35% (8/23) patients treated with placebo. The clinical outcome correlated with the parasitological response as measured by ZNN staining of unconcentrated stool samples. At 11-14 days after discontinuation, 1 patient treated with nitazoxanide suspension and 2 patients treated with placebo showed shedding of oocysts. However, all patients who had clinical evaluation continued to be well.

Study RM-NTZ-98-002 submitted to the original NDA was also conducted in Egypt and compared the efficacy of nitazoxanide tablets to placebo in adults with cryptosporidiosis. Nitazoxanide showed resolution of diarrhea in 71% (15/21) patients treated with tablet compared to 43% (9/21) patients who received placebo. However, the parasitological outcome did not correlate with clinical outcome. The reason for the variation in results in the 2 studies is unclear.

Overall, nitazoxanide tablets and suspension were effective in the treatment of cryptosporidial diarrhea.

## 6. LABEL:

### 6.1. Sponsor's proposed label:

#### MICROBIOLOGY

##### Mechanism of action

The antiprotozoal activity of nitazoxanide is believed to be due to interference with the pyruvate:ferredoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction which is essential to anaerobic energy metabolism. Studies have shown that the PFOR enzyme from *Giardia lamblia* directly reduces nitazoxanide by transfer of electrons in the absence of ferredoxin. The DNA-derived PFOR protein sequence of *Cryptosporidium parvum* appears to be similar to that of *Giardia lamblia*. Interference with the PFOR enzyme-dependent electron transfer reaction may not be the only pathway by which nitazoxanide exhibits antiprotozoal activity.

##### Activity *in vitro*

Nitazoxanide and its metabolite, tizoxanide, are active *in vitro* in inhibiting the growth of (i) sporozoites and oocysts of *Cryptosporidium parvum* and (ii) trophozoites of *Giardia lamblia*.

##### Drug Resistance

A potential for development of resistance by *Cryptosporidium parvum* or *Giardia lamblia* to nitazoxanide has not been examined.

##### Susceptibility Tests:

For protozoa such as *Cryptosporidium parvum* and *Giardia lamblia*, standardized tests for use in clinical microbiology laboratories are not available.

## INDICATIONS AND USAGE

### Diarrhea caused by *Giardia lamblia* or *Cryptosporidium parvum*:

Alinia for Oral Suspension (patients 1 year of age and older) and Alinia Tablets (patients 12 years and older) are indicated for the treatment of diarrhea caused by *Giardia lamblia* or *Cryptosporidium parvum*.

Alinia for Oral Suspension and Alinia Tablets have not been shown to be superior to placebo for the treatment of diarrhea caused by *Cryptosporidium parvum* in HIV-infected or immunodeficient patients (see **CLINICAL STUDIES**).

### 6.2. Comments:

There are no changes to the Microbiology section of the label.

## 7. RECOMMENDATIONS:

This NDA is recommended for approval with respect to Microbiology for the treatment of cryptosporidiosis. There are no changes to the Microbiology section of the label.

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Kalavati Suvarna  
Microbiologist, HFD-590

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**CONCURRENCES:**

HFD-590/Deputy Dir. \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_  
HFD-590/Micro TL \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

CC:

HFD-590/Original IND

HFD-590/Division File

HFD-590/MO

HFD-590/Pharm

HFD-590/Chem

HFD-590/Review Micro

HFD-590/CSO/MillerK

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/s/

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Kalavati Suvarna  
6/3/05 12:23:27 PM  
MICROBIOLOGIST

Shukal Bala  
6/3/05 03:21:21 PM  
MICROBIOLOGIST

**MICROBIOLOGY REVIEW**  
**DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)**

**NDA #:** 21-497 and 21-818

**REVIEWER** : Kalavati Suvarna  
**CORRESPONDENCE DATE** : 01-28-04, 4-26-04  
**CDER RECEIPT DATE** : 01-29-04; 4-27-04  
**REVIEW ASSIGN DATE** : 02-05-04; 5-16-04  
**REVIEW COMPLETE DATE** : 06-18-04

**SPONSOR:** Romark Laboratories Inc.  
6200 Courtney Campbell Causeway  
Suite 880  
Tampa, FL 33607

**SUBMISSION REVIEWED:** N-000 (AZ, BM)

**DRUG CATEGORY:** Anti-parasitic

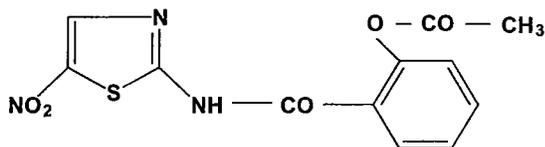
**INDICATION:** Treatment of diarrhea caused by *Giardia lamblia* and *Cryptosporidium parvum*

**DOSAGE FORM:** Tablets

**PRODUCT NAMES:**

- a. **PROPRIETARY:** Alinia<sup>®</sup>
- b. **NONPROPRIETARY:** Nitazoxanide; CAS: 55981-09-4
- c. **CHEMICAL:** 2-(acetolyloxy)-N-(5-nitro-2-thiazolyl) benzamide

**STRUCTURAL FORMULA:**



Molecular weight: 307.2  
Empirical formula: C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>5</sub>S

**SUPPORTING DOCUMENTS:** NDA # 20-871; IND # 48,620, 58,895,  
Type II DMF # DMF, DMF, DMF

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**TABLE OF CONTENTS**

1. EXECUTIVE SUMMARY ..... 3

2. INTRODUCTION AND BACKGROUND ..... 4

    2.1. Biology of *Giardia lamblia* .....5

    2.2. Pathogenesis of Giardiasis .....5

    2.3. Biology of *Cryptosporidium parvum* .....5

    2.4. Pathogenesis of cryptosporidial infection.....5

3. PRECLINICAL MICROBIOLOGY ..... 6

4. CLINICAL MICROBIOLOGY ..... 6

    4.1. Giardiasis .....6

        4.2.1. Study RM01-3011 .....6

    4.2. Cryptosporidial diarrhea .....18

        4.2.1. Study RM-NTZ-98-002 .....21

        4.2.2. Comparison of the pathophysiology of cryptosporidial diarrhea and giardiasis .....21

5. CONCLUSIONS ..... 22

6. LABEL ..... 24

    6.1. Sponsor’s proposed label .....24

    6.2. Comments .....25

7. RECOMMENDATIONS ..... 25

8. REFERENCES ..... 27

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**1. EXECUTIVE SUMMARY:**

The sponsor is seeking approval of nitazoxanide tablets (500 mg nitazoxanide tablets b.i.d. for 3 days) for the treatment of diarrhea due to *Giardia lamblia* and *Cryptosporidium parvum* in immunocompetent adults. In 2002, the nitazoxanide oral suspension was approved for the treatment of cryptosporidial diarrhea and giardiasis in children. However, the tablet formulation was not approved due to lack of efficacy in adults.

The clinical study (RM01-3011) was conducted in Peru and Egypt to determine the safety and efficacy of nitazoxanide in the treatment of diarrhea due to *G. lamblia* in adults. Resolution of diarrhea was observed in 85% (46/54) patients treated with nitazoxanide tablets compared to 83% (45/54) patients treated with nitazoxanide suspension, and 44% (12/27) patients treated with placebo, 4 to 7 days after discontinuation of therapy.

The sponsor used microscopic examination of unconcentrated stool stained by iodine or immunofluorescence, and concentrated stool samples stained with iodine, to assess presence of *G. lamblia* cysts in stool samples, at baseline and 4 to 7 days after discontinuation of treatment. The processing of the stool samples at the two sites appears to be similar. However, the Peru site determined the actual cyst counts and the Egypt site used a semi-quantitative grading system and approximated the counts to number of cysts per high power field. Based on absence or presence of cysts in stool sample, the percentage of patients that were parasitologically eradicated in the nitazoxanide tablet arm was 55.5% (30/54) compared to 48% (26/54) in the nitazoxanide suspension, and 18.5% (5/27) in the placebo arm. The clinical (94%) and parasitological (94%) outcome were better (94%) in patients treated with nitazoxanide tablets at the Egypt site, where cysts counts at baseline were low, compared to the Peru site (clinical outcome = 80.5%, parasitological outcome = 64%) where cyst counts at baseline were high.

Resolution of diarrhea and eradication of cysts were observed in 28 patients in the nitazoxanide tablet arm, 26 patients in the nitazoxanide suspension arm, and 4 patients in the placebo arm, at 4 to 7 days after discontinuation of treatment. These patients were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. Shedding of cysts was observed in some of these patients at 12 to 14 days after discontinuation of therapy [nitazoxanide tablet arm = 7/28 (25%), nitazoxanide suspension arm = 6/26 (23%) and placebo arm = 2/4 (50%)]. The clinical outcome was not measured at this time point. Most of these patients were from the Peru site. The sponsor has stated that Peru being a hyper-endemic area compared to Egypt, the recurrence of cysts was more likely due to re-infection rather than relapse. However, information supporting the basis for higher endemicity of *G. lamblia* in Peru compared to Egypt was not included. The differences in parasitological response in the Egypt and Peru site may also be due to host factors or differences in the virulence of the *G. lamblia* isolates at the two sites.

The effect of nitazoxanide on reduction of cysts could not be evaluated with certainty, due to limitations of the detection method, differences in methods used for quantification of cysts at the two sites, expression of the quantitative result as cysts per high power field using actual counts (Peru site) or approximated counts obtained using a semi-quantitative method (Egypt site), variability in the consistency and number of stools passed within a 24 hour period by

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patients at the two sites. Several factors effect the detection of cysts in stool samples such as specimen collection and transport method, addition of stool preservatives, age of the stool (fresh versus 24 hour old), consistency, number of stools examined, presence of debris, clarity of smears prepared from concentrated stool sediments, presence of background fluorescence, and expertise of the examiner. Hanson and Cartwright (2001)<sup>1</sup> described the sensitivity of unconcentrated and concentrated stool for the detection of cysts to be 66-70%, when cyst counts were low. However, the stain used for testing was not specified. It is known that sensitivity of the assay can vary with the type of stain used. Because of these limitations and intermittent shedding of cysts observed in patients with giardiasis, the parasitological outcome of the patients based on reduction in cysts counts alone should be interpreted with caution.

Overall, the clinical efficacy of nitazoxanide tablets was similar to nitazoxanide suspension and greater than placebo.

The sponsor is seeking the indication "Treatment of cryptosporidiosis diarrhea" based on (a) *in vitro* activity of nitazoxanide against the sporozoite stage of *C. parvum*, (b) activity of nitazoxanide against *C. parvum* in animal studies, (c) clinical studies (2 in children and 1 in adult) submitted in the original NDA, (d) similarities between the pathophysiology of *C. parvum* and *G. lamblia*, and (e) the clinical study demonstrating efficacy of nitazoxanide tablets in the treatment of giardiasis. Although, the pathophysiology of the disease due to the two pathogens appears to be similar, *C. parvum* can invade epithelial cells of the small intestine unlike *G. lamblia*. Additionally, there is no regulatory precedence that allows for efficacy in giardiasis to support efficacy in cryptosporidial diarrhea. The sponsor is currently conducting a study to evaluate efficacy of nitazoxanide tablets in the treatment of cryptosporidial diarrhea in adults. Additional data from this clinical study would be necessary to adequately determine the efficacy of nitazoxanide tablets in adults.

## 2. INTRODUCTION AND BACKGROUND:

The subject of this NDA is nitazoxanide. Nitazoxanide oral suspension is approved for the treatment of diarrhea due to *Giardia lamblia* and *Cryptosporidium parvum* in immunocompetent children (NDA# 21-498). However, the tablet formulation of nitazoxanide failed to show adequate efficacy in immunocompetent adults with giardiasis or cryptosporidiosis (original NDA# 21-497).

The sponsor is seeking approval of nitazoxanide tablet formulation for the treatment of giardiasis and cryptosporidial diarrhea in immunocompetent adults. The sponsor has proposed 500 mg nitazoxanide tablets b.i.d. for 3 days for treatment of adults with giardiasis and cryptosporidial diarrhea. The efficacy of nitazoxanide tablets for giardiasis indication in adults is supported by a clinical study. The study to evaluate the safety and efficacy of nitazoxanide tablets for the treatment of cryptosporidial diarrhea is ongoing (only small number of patients have been enrolled thus far). However, the sponsor is requesting a waiver of the clinical study based on (a) *in vitro* activity of nitazoxanide against the sporozoite stage of *C. parvum*, (b) activity of nitazoxanide against *C. parvum* in animal studies, (c) clinical studies in adults and children with cryptosporidial diarrhea submitted to the original NDA, (d) the study

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demonstrating efficacy of nitazoxanide tablets in the treatment of giardiasis, and (e) similarities between the pathophysiology of *C. parvum* and *G. lamblia*.

### **2.1. Biology of *Giardia lamblia*:**

*Giardia lamblia* is a flagellated protozoan found in intestinal tract of humans. *Giardia duodenalis* or *Giardia intestinalis* are alternate names for *G. lamblia*. Infection is caused by ingestion of contaminated food or water containing *G. lamblia* cysts. Following ingestion, the cysts pass through the stomach to the small intestine where they excyst to give rise to trophozoites. The trophozoites attach to the epithelial cells in the duodenum and bile duct of the host, divide by longitudinal fission and encyst on reaching the colon. *G. lamblia* does not invade epithelial cells like *C. parvum*. In cases of severe infection, trophozoites are more commonly observed than cysts in diarrheic stool samples.

### **2.2. Pathogenesis of Giardiasis:**

The major clinical manifestations of *Giardia lamblia* infection are diarrhea and malabsorption. Although changes in the villi of the intestine have been observed, the mechanism by which *Giardia* causes diarrhea is not known. The host immune response plays an important role in protection from the infection. In addition to the immune status of the host, the severity and duration of the infection can be affected by the number of cysts ingested and the virulence of the *Giardia* strain.

### **2.3. Biology of *Cryptosporidium parvum*:**

*Cryptosporidium* is an intracellular parasite present in the gastrointestinal and respiratory tract. The infection is caused by ingestion of oocysts. The oocyst contains four sporozoites within a membrane. Upon ingestion, the sporozoites excyst from the oocyst and invade the epithelial cells and become enveloped in a parasitophorous vacuole. Sporozoites undergo maturation into type I meronts, which release merozoites. The merozoite stage can undergo asexual replication and reinvade the host cells or form type II meronts by sexual replication. The type II meronts release the macrogametocytes or microgametocytes that fertilize to give rise to a zygote. The zygote can develop into oocyst that may either rupture releasing sporozoites *in vivo* or shed via the feces. Therefore, the presence of oocyst(s) in the stool samples may be intermittent.

### **2.4. Pathogenesis of cryptosporidial infection:**

The major clinical manifestation observed with cryptosporidial infection is diarrhea. The diarrheal infection can lead to malabsorption. However, the mechanism by which *Cryptosporidium* causes diarrhea is not known. The severity of the infection and ultimate pathology is influenced by the immune status of the host. Cryptosporidial diarrhea is self-limiting in immunocompetent individuals but may be life threatening in AIDS or immunocompromised patients.

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### 3. PRECLINICAL MICROBIOLOGY:

No new information was included in this submission. Studies describing the mechanism of action, activity of nitazoxanide *in vitro* and *in vivo* against *G. lamblia* and *C. parvum* were reviewed earlier [please see microbiology reviews dated 06-01-98 (NDA 20-871, N-000), and 11-06-02 (NDA 21-497 and 21-498, N-000)]. Nitazoxanide and its metabolite, tizoxanide, were active *in vitro* in inhibiting the growth of trophozoites of *G. lamblia* and the sporozoites and oocyst stages of *C. parvum*.

### 4. CLINICAL MICROBIOLOGY:

#### 4.1. Giardiasis:

The study RM01-3011 conducted to examine the safety and efficacy of nitazoxanide tablets in the treatment of giardiasis in adults from Peru and Egypt is described in detail below. In addition, study RM-NTZ-98-001 included in the original submission (NDA 21-497/21-498, N-000) provides supportive evidence for the efficacy of nitazoxanide tablets in adults [for details please see microbiology review dated 11-06-02 (NDA 21-497/21-498, N-000)].

#### 4.2.1. Study RM01-3011:

Study RM01-3011 was a phase III, multi-center, double-blind, randomized, placebo controlled study to determine the safety and efficacy of nitazoxanide for the treatment of diarrhea due to *G. lamblia* in adults. A total of 135 immunocompetent patients ( $\geq 12$  years of age) who showed presence of *G. lamblia* cysts in the baseline stool sample and had diarrhea ( $\geq 3$  bowel movements/day and one or more enteric symptoms) were eligible to participate in the study. The patients were randomized (2:2:1) to receive either nitazoxanide tablets (500 mg b.i.d. for 3 days), nitazoxanide oral suspension [25 ml (500 mg) b.i.d. for 3 days], or placebo tablets (b.i.d. for 3 days) with food. Patients with diarrhea due to pathogens other than *Giardia* such as *C. parvum*, *E. histolytica* or bacteria, and those receiving drugs with anti-protozoal activity within 2 weeks of study entry were excluded. The following methods were used to exclude patients with diarrhea due to pathogens other than *G. lamblia*: bacterial culture, microscopic examination of stool for protozoa other than *G. lamblia*, acid fast staining or immunofluorescence assay for *C. parvum*, and the Baermann concentration test for *Strongyloides stercoralis*.

The *G. lamblia* cysts were detected by microscopic examination of concentrated stool samples after iodine staining and unconcentrated stool after iodine and immunofluorescence staining ( , a FDA approved immunofluorescence assay kit). The methods used at the Peru and Egypt sites are described below:

**Unconcentrated stool sample:** At the Peru site, approximately 2 mg of stool sample (size of a match stick head) was mixed with a drop of saline and a drop of 1% Lugol's iodine and the entire smear examined microscopically at 400 x magnification. The number of cysts per field was recorded.

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The FDA approved immunofluorescence assay (IFA) kit manufactured by \_\_\_\_\_, was also used to detect *G. lamblia* cysts in unconcentrated stool samples. The testing was performed at \_\_\_\_\_ However, the cysts in stool samples from all patients were not quantified.

At the Egypt site, the method for detecting cysts in iodine stained unconcentrated stool was identical to that at the Peru site except that the cyst count was done differently. Although, a grading system was used to quantify cysts: 1+ = one or fewer cysts per field, 2+ = 2-5 cysts per field, 3+ = 5-10 cysts per field and 4+ = >10 cysts per field, the data were expressed as number of cysts per field.

The unconcentrated stool samples were also stained by immunofluorescence using the same kit and tested at the same laboratory in \_\_\_\_\_ as described for the Peru site.

**Concentrated stool sample:** At the Peru site, 1-1.5 gm of stool sample was mixed with 8 ml of 10% formalin to form a suspension. The suspension was strained through a sieve, mixed with 2 ml ether, and centrifuged at 2,500 rpm for 5 minutes. A drop of the sediment was used to prepare a smear and the entire smear examined microscopically at 400 x magnification. The number of cysts per field were recorded.

At the Egypt site, the method used for concentration was similar to the one in Peru except the exact amount of stool sample examined was not specified. The protocol called for sufficient stool sample that would result in 0.5-1.0 ml of fecal sediment. A drop of the sediment was used to prepare a smear. The entire smear was examined and the grading system used was same as the one described above for cyst quantification in unconcentrated stool sample at the Egypt site. The data were expressed as number of cysts per field.

Patients underwent clinical and parasitological evaluations at baseline and 7-10 days after initiation of therapy. For patients who responded to treatment clinically, an additional parasitological evaluation was performed at 14-17 days after initiation of therapy. However, clinical outcome was not measured.

One stool sample was examined at screening as well as baseline. Two stool samples were collected at 7 to 10 days after initiation of therapy while one stool sample was collected at follow-up (14 to 17 days after initiation of therapy).

The clinical responses were defined as:

**Well:** patient had no symptoms, no watery stools,  $\leq 2$  soft stools, and had no hematochezia within the 24 hours prior to evaluation or no symptoms and no unformed stools within the 48 hours prior to evaluation;

**Continuing illness:** patient passed watery stools, more than 2 soft stools per 24 hours or documentation of hematochezia or enteric symptoms plus any number of soft or watery stools during the 48 hours prior to evaluation;

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**Clinical treatment failure:** clinical deterioration or worsening of symptoms after 24 hours of treatment.

The parasitological responses were defined as:

**Eradication:** Absence of cysts or trophozoites of *G. lamblia* in either of the 2 stool samples collected between days 7 and 10 after initiation of therapy;

**Persistence:** Presence of cysts or trophozoites of *G. lamblia* in at least 1 stool sample collected between days 7 and 10 after initiation of therapy.

The primary endpoint of the study was resolution of clinical symptoms of giardiasis. The secondary endpoints of the study were (a) eradication of *G. lamblia* cysts from 2 stool samples collected at 7 to 10 days after initiation of therapy, and (b) time to last unformed stool. Although, time to last unformed stool was one of the secondary endpoints, data for this endpoint were not collected.

The sponsor has stated that 4,278 patients were screened at the Peru site and 593 at the Egypt site. However, only 90 and 45 patients were enrolled at the Peru, and Egypt sites, respectively. The reasons for not enrolling the remaining patients were as follows:

<u>Reason for not being enrolled at Peru</u>	<u>No. of Subjects</u>
No <i>Giardia</i> cysts or trophozoites observed in stool sample at screening	4,092
Subjects declined participation in the study prior to enrollment	28
<i>Giardia</i> cysts or trophozoites observed in stool sample at screening, but not at baseline	28
Clinical symptoms did not satisfy inclusion criteria	25
Mixed infections (4 <i>Hymenolepis nana</i> , 1 <i>Strongyloides stercoralis</i> , 1 <i>Fasciola hepatica</i> + <i>Taenia</i> )	6
Younger than 12 years of age	5
Enrollment terminated at the study site due to completion of study	2
Concomitant therapy incompatible with study requirements	1
Pregnancy	1
<b>TOTAL</b>	<b>4,188</b>

<u>Reason for not being enrolled at Egypt</u>	<u>No. of Subjects</u>
No <i>Giardia</i> cysts or trophozoites observed in stool sample at screening	523
<i>Giardia</i> cysts or trophozoites observed in stool sample by microscopic exam but not by immunofluorescence assay	10
<i>Giardia</i> cysts or trophozoites observed in stool sample by immunofluorescence assay but not by microscopic exam	5
Concomitant therapy incompatible with study requirements	4
Subjects declined participation in the study prior to enrollment	4
Mixed infections with other pathogens (1 <i>Blastocystis hominis</i> + <i>Hymenolepis nana</i> , 1 <i>H. nana</i> )	2
<b>TOTAL</b>	<b>548</b>

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All the 135 patients (90 at the Peru site and 45 at the Egypt site) that were enrolled showed presence of cysts at screening and baseline by one of the 3 methods (iodine stained unconcentrated stool, immunofluorescence stained unconcentrated stool, and iodine stained concentrated stool). However, all 3 methods were not used consistently at screening and baseline, either at the Peru or Egypt sites (Table 1).

At the **Peru site**, the unconcentrated and concentrated stools stained with iodine were used to detect and quantify cysts at screening and baseline in the 90 patients. Of the 90 patients, 89 were positive for cysts using either unconcentrated or concentrated stool stained with iodine. As expected, the cyst counts were lower using unconcentrated stool sample (median cyst count = 2.5 cysts per high power field and range = 0.5 to 20 cysts per high power field) compared to concentrated stool (median cyst count = 5 cysts per high power field and range = 0.5 to 80 cysts per high power field).

Immunofluorescence stained unconcentrated stool sample were examined at baseline but not at screening. A stool sample from 85 of the 90 patients was examined at baseline using this method. Of the 85 patients, 66 (77%) were positive for cysts. Of the 19 patients that were negative by immunofluorescence, 18 were positive by iodine staining using unconcentrated stool. The reason for such a discrepancy is unclear. As immunofluorescence testing was performed in France, it is possible that the stools were not preserved properly for transport or stools were examined after long period of storage. This could affect the morphology of the cyst, thereby effecting detection by immunofluorescence. No cyst quantification was performed in patients that were positive by immunofluorescence.

At the **Egypt site**, all 3 methods were used to detect cysts. However, quantification of cysts was performed using the unconcentrated stool stained with immunofluorescence and concentrated stool stained with iodine at screening and baseline in the 45 patients. Of the 45 patients, 30 (same patients) were positive for cysts using either unconcentrated or concentrated stool stained with iodine at baseline. Using iodine stained concentrated stool sample, the cyst counts were lower than at the Peru site (median count = 1 cysts per high power field; cyst count range = 0 to 10 cysts per high power field).

Immunofluorescence stained unconcentrated stool sample were examined at baseline and screening. All patients were positive for cysts by this method including the 15 patients that were negative using iodine stained unconcentrated or concentrated stool. Most (13/15) of the patients that were negative at baseline using iodine stained stool samples, were positive at screening using all 3 methods. The data for these 15 patients suggests that (a) immunofluorescence staining improves the detection of cysts in unconcentrated stool when counts are low, and (b) cysts are shed intermittently, therefore, examination of 2 or more samples improves the sensitivity of detection of cysts.

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Table 1: Baseline characteristics of stool examinations for patients enrolled in study RM01-3011 at the Egypt and Peru sites.

Baseline characteristics of stool examination	Peru			Egypt		
	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA
Number of patients positive for cysts	89/90	90/90	66/85	30/45	30/45	45/45
Number of patients negative for cysts	1/90	0/90	19/85	15/45 <sup>#</sup>	15/45 <sup>#</sup>	0/45
Number of patients in whom the cysts were quantified	81/90 <sup>a</sup>	89/90 <sup>b</sup>	19/85 <sup>*</sup>	15/45 <sup>*</sup>	40/45 <sup>c</sup>	45/45
Median cyst counts per high power field (Range)	2.5 (0-20)	5 (0.5 – 80)	ND*	ND*	1 (0-10)	1 (1-10)

UC/I = unconcentrated stool sample stained with iodine;

C/I = concentrated stool sample stained with iodine;

UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit;

<sup>#</sup> 13 of the 15 patients positive for cysts at screening by all 3 methods;

<sup>\*</sup> quantification performed only on unconcentrated stool samples (stained by iodine or immunofluorescence) and did not show presence of cysts. The reason for quantifying samples which were negative is unclear.

<sup>a</sup> cyst quantification not performed for 9 of the 89 iodine stained unconcentrated stool samples that were positive for cysts;

<sup>b</sup> cyst quantification not performed for 1 of the 90 iodine stained concentrated stool samples that was positive for cysts;

<sup>c</sup> cyst quantification not performed for 5 of the 30 iodine stained concentrated stool samples that were positive for cysts;

Yellow highlight = number of patients in whom cysts were quantified at the Egypt and Peru sites using iodine stained unconcentrated stool sample, and immunofluorescence stained unconcentrated stool sample, respectively;

Red = number of patients in whom the baseline cyst counts were obtained using iodine stained concentrated stool sample.

In summary, the iodine stained concentrated stool sample was used consistently to detect and quantify cysts at baseline, at both sites (shown in red, Table 1).

Of the 135 patients enrolled in the study, 54 (36 in Peru and 18 in Egypt) received nitazoxanide tablets, 54 (36 in Peru and 18 in Egypt) received nitazoxanide oral suspension, and 27 (18 in Peru and 9 in Egypt) received placebo. The results of the study at the Peru and Egypt site are discussed separately because (a) there were slight differences in the method used for quantification of cysts in the stool sample obtained from patients at the two sites, and (b) differences in the baseline cyst count were noted (shown in red, Table 1). Additionally, the sponsor has stated that Peru was hyper-endemic for *G. lamblia* while Egypt was non hyper-endemic. However, the basis of determining endemicity is unclear.

The clinical and parasitological response of patients treated with nitazoxanide or placebo in **Peru** is summarized in Table 2. The individual patient data for the nitazoxanide tablet, nitazoxanide suspension, and placebo treatment groups are shown in Tables 3, 4, and 5, respectively. Overall, the clinical outcome of patients treated with nitazoxanide tablets (29/36; 80.5%) was similar to that of nitazoxanide suspension (29/36; 80.5%) and greater than placebo (9/18; 50%) at the Peru site. Based on absence of cysts in 2 unconcentrated (stained with iodine and immunofluorescence) and concentrated (iodine stained) stool samples, the parasitological outcome appears to be greater in patients treated with nitazoxanide tablets (13/36; 36%) or suspension (11/36; 30.5%) than placebo (3/18; 17%).

Resolution of diarrhea and eradication of cysts was observed in 12 patients in the nitazoxanide tablet arm, 11 patients in the nitazoxanide suspension arm, and 3 patients in the placebo arm, at 4 to 7 days after discontinuation of treatment. These patients were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. Shedding of cysts was observed in 7/12 patients in the nitazoxanide tablet arm (shaded; Table 3), 5/11 patients in nitazoxanide

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suspension arm (shaded; Table 4), and 2/3 patients in the placebo arm (shaded; Table 5). However, clinical outcome was not measured. The sponsor has proposed that the parasitological response observed at 12-14 days after discontinuation of therapy at the Peru site may be due to (1) failure to eliminate all of the cysts with a single course of treatment in patients with heavy colonization, or (2) re-infection by ingestion of cysts during treatment and follow-up, as the area is hyper-endemic for *G. lamblia*. In the absence of clinical evaluation, the parasitological findings should be interpreted with caution.

Table 2: Parasitological and clinical response of patients with giardiasis at the Peru site.

Treatment group	Parasitological and clinical responses		Patients with eradication of cysts N (%)	Patients with clinical well response N (%)	Patients clinical well and showing eradication of cysts N (%)
	Cysts Eradicated (CR)	Cysts Persisted (CR)			
500 mg NTZ tablet BID 3 days (n = 36)	13 (12 well, 1 CI)	23 (17 well, 6 CI)	13 (36)	29 (80.5)	12 (33)
500 mg NTZ oral suspension BID 3 days (n = 36)	11 (11 well, 0 CI)	25 (18 well, 7 CI)	11 (30.5)	29 (80.5)	11 (30.5)
Placebo BID 3 days (n = 18)	3 (3 well, 0 CI)	15 (6 well, 9 CI)	3 (17)	9 (50)	3 (17)

NTZ = Nitazoxanide;  
CI = continuing illness.

CR = clinical response;

N = number of subjects;

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Table 3: Parasitological data for patients receiving nitazoxanide tablets for treatment of giardiasis at the Peru site.

PID	Cysts (count per high power field) in stool sample at screening			Cysts (count per high power field) in baseline stool sample			Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy stool sample 1			Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Clinical response	Parasitological response	Day 14 parasitological response	
	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA				
NTZ tablets: 500 mg BID x 3 days																
23	+(ND)	+(ND)	ND	+(15)	+(80)	+(ND)	+(ND)	+(ND)	+(1)	+(12)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	ND	PERISTENCE
25	+(ND)	-(0)	ND	+(ND)	+(1)	-(0)	-(0)	+(ND)	+(ND)	+(1)	ND	ND	PERISTENCE	PERISTENCE	+(7)	PERISTENCE
26	+(1)	+(2)	ND	+(1)	+(1)	+(ND)	ND	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	+(1)	PERISTENCE
30	+(ND)	+(2.5)	ND	+(1)	+(2)	+(ND)	+(ND)	+(ND)	+(ND)	+(2.5)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	-(0)	ERADICATION
33	+(1.5)	+(5)	ND	+(ND)	+(3.5)	+(ND)	+(ND)	+(ND)	+(ND)	+(3.5)	ND	ND	PERISTENCE	PERISTENCE	ND	ERADICATION
35	+(ND)	+(5)	ND	+(ND)	+(5)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	+(9)	PERISTENCE
36	+(ND)	+(2.5)	ND	+(ND)	+(1.5)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	+(ND)	PERISTENCE
37	+(ND)	+(2.5)	ND	+(ND)	+(1.5)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	PERISTENCE	PERISTENCE	+(4.5)	PERISTENCE
42	+(2.5)	+(5.5)	ND	+(1.5)	+(5)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	PERISTENCE	PERISTENCE	+(1.5)	PERISTENCE
45	+(2.5)	+(4.5)	ND	+(1.5)	+(2.5)	-(0)	-(0)	-(0)	-(0)	-(0)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(3)	PERISTENCE
48	+(2.5)	+(4.5)	ND	+(2.5)	+(5)	+(ND)	+(3)	+(7)	+(3.5)	+(10)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(2.5)	PERISTENCE
50	+(5)	+(17.5)	ND	+(9)	+(18)	+(ND)	-(0)	-(0)	-(0)	-(0)	ND	ND	PERISTENCE	PERISTENCE	+(3)	PERISTENCE
51	+(1.5)	+(2)	ND	+(1)	+(2)	+(ND)	-(0)	-(0)	-(0)	-(0)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	-(0)	PERISTENCE
55	+(1)	+(2)	ND	+(1)	+(2)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	-(1)	PERISTENCE
58	+(3.5)	+(6.5)	ND	+(4)	+(8)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	-(0)	ERADICATION
60	+(2.5)	+(7)	ND	+(4)	+(8)	+(ND)	+(3)	+(2.5)	+(2.5)	+(7)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	No follow-up	ERADICATION
61	+(1.5)	+(5)	ND	+(1)	+(2)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	PERISTENCE	PERISTENCE	+(5)	PERISTENCE
62	+(6)	+(8)	ND	+(3)	+(6)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	+(2)	PERISTENCE
67	+(4)	+(6)	ND	+(15)	+(20)	+(ND)	+(3)	+(7)	+(3)	+(7)	-(0)	-(0)	PERISTENCE	PERISTENCE	+(4)	PERISTENCE
70	+(3)	+(7)	ND	+(3)	+(5)	+(ND)	+(1)	+(1)	+(1)	+(1)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(3)	PERISTENCE
73	+(4)	+(5)	ND	+(2)	+(4)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	PERISTENCE	PERISTENCE	+(1)	PERISTENCE
75	+(2)	+(4)	ND	+(1)	+(2)	+(ND)	+(1)	+(1)	+(1)	+(1)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	-(0)	PERISTENCE
76	+(2)	+(5)	ND	+(2)	+(4)	+(ND)	-(0)	-(0)	-(0)	-(0)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(1)	PERISTENCE
80	+(1)	+(1)	ND	+(1)	+(1)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	-(0)	ERADICATION
83	+(2)	+(3)	ND	+(2)	+(3)	+(ND)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	-(0)	ERADICATION
85	-(0)	+(1)	ND	+(3)	+(4)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	-(0)	ERADICATION
86	+(4)	+(9)	ND	+(3)	+(8)	+(ND)	+(6)	+(13)	+(7)	+(14)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	ND	PERISTENCE
87	+(2)	+(4)	ND	+(4)	+(6)	+(ND)	+(4)	Lost to follow-up	+(7)	+(14)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	ND	PERISTENCE
92	+(3)	+(8)	ND	+(4)	+(10)	-(0)	+(2)	+(3)	+(4)	+(9)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(2)	PERISTENCE
95	+(5)	+(10)	ND	+(3)	+(5)	+(ND)	+(2)	+(3)	+(2)	+(5)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(7)	PERISTENCE
98	+(1)	+(4)	ND	+(2)	+(4)	+(ND)	+(2)	+(4)	+(3)	+(5)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(5)	PERISTENCE
100	+(3)	+(16)	ND	+(16)	+(25)	+(ND)	+(4)	+(12)	+(8)	+(15)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(2)	PERISTENCE
101	+(2)	+(6)	ND	+(3)	+(11)	+(ND)	+(3)	+(7)	+(2)	+(5)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(20)	PERISTENCE
105	+(1)	+(1)	ND	+(2)	+(5)	ND	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	+(1)	PERISTENCE
108	+(3)	+(10)	ND	+(5)	+(12)	ND	+(1)	+(1)	+(4)	+(5)	+(ND)	+(ND)	PERISTENCE	PERISTENCE	+(8)	PERISTENCE
110	+(3)	+(5)	ND	+(4)	+(5)	ND	-(0)	-(0)	-(0)	-(0)	-(0)	-(0)	ERADICATION	ERADICATION	-(0)	PERISTENCE

NTZ = nitazoxanide;  
 UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit;  
 + = presence of cysts;  
 Shaded rows represent patients who shed cysts after initial eradication

UC/I = unconcentrated stool sample stained with iodine;  
 C/I = concentrated stool sample stained with iodine;  
 PID = patient identification number;  
 CI = continuing illness;  
 ND = not done;

Table 4: Parasitological data for patients receiving nitazoxanide oral suspension for treatment of giardiasis at the Peru site.

PID	Cysts (count per high power field) in stool sample at screening				Cysts (count per high power field) in baseline stool sample				Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy stool sample 1				Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy stool sample 2				Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy	Day 14 parasitological response
	UC/I	C/I	UC/IFA	IFA	UC/I	C/I	UC/IFA	IFA	UC/I	C/I	UC/IFA	IFA	UC/I	C/I	UC/IFA	IFA				
NTZ suspension: 500 mg BID x 3 days																				
21	+	(ND)	+	(6)	+	(1)	+	(1)	+	(1)	+	(1)	+	(5)	+	(18)	ND	PERISTENCE	CI	No follow-up
22	+	(ND)	+	(25)	+	(ND)	+	(1)	+	(ND)	+	(ND)	+	(0)	+	(3.5)	ND	PERISTENCE	WELL	Lost to follow-up
27	-	(0)	+	(1)	+	(ND)	+	(0)	+	(0)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
29	+	(17.5)	+	(30)	+	(ND)	+	(0)	+	(ND)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
32	+	(ND)	+	(ND)	+	(0)	+	(0)	+	(0)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
34	+	(ND)	+	(3)	+	(ND)	+	(6.5)	+	(ND)	+	(7)	+	(ND)	+	(2.5)	+	PERISTENCE	WELL	Lost to follow-up
38	+	(ND)	+	(2.5)	+	(ND)	+	(5)	+	(ND)	+	(0)	+	(ND)	+	(0)	+	PERISTENCE	WELL	Lost to follow-up
39	+	(ND)	+	(4)	+	(ND)	+	(3)	+	(ND)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
41	+	(2.5)	+	(5)	+	(ND)	+	(3.5)	+	(ND)	+	(1.5)	+	(ND)	+	(3.5)	+	ERADICATION	WELL	Lost to follow-up
44	-	(0)	+	(1.5)	+	(ND)	+	(2.5)	+	(0)	+	(1.5)	+	(ND)	+	(1)	+	PERISTENCE	WELL	Lost to follow-up
46	+	(1.5)	+	(2.5)	+	(0)	+	(0.5)	+	(0)	+	(0)	+	(0)	+	(1)	+	PERISTENCE	CI	Lost to follow-up
47	+	(1)	+	(1.5)	+	(ND)	+	(4.5)	+	(ND)	+	(2)	+	(ND)	+	(1)	+	PERISTENCE	WELL	Lost to follow-up
52	+	(1.5)	+	(3.5)	+	(ND)	+	(1)	+	(ND)	+	(3)	+	(ND)	+	(4)	+	PERISTENCE	WELL	Lost to follow-up
54	+	(1)	+	(2)	+	(ND)	+	(3)	+	(0)	+	(6)	+	(ND)	+	(2)	+	PERISTENCE	CI	Lost to follow-up
57	+	(1)	+	(2)	+	(ND)	+	(2)	+	(ND)	+	(1)	+	(ND)	+	(1)	+	PERISTENCE	WELL	Lost to follow-up
59	+	(1)	+	(2)	+	(ND)	+	(2)	+	(ND)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
63	+	(1)	+	(3)	+	(ND)	+	(1)	+	(0)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
64	+	(4)	+	(8)	+	(ND)	+	(5)	+	(ND)	+	(2)	+	(ND)	+	(4)	+	PERISTENCE	CI	Lost to follow-up
66	+	(1)	+	(2)	+	(ND)	+	(1)	+	(ND)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
69	+	(3)	+	(8)	+	(ND)	+	(3)	+	(ND)	+	(4)	+	(ND)	+	(1)	+	PERISTENCE	WELL	Lost to follow-up
71	+	(3)	+	(7)	+	(ND)	+	(2)	+	(0)	+	(5)	+	(ND)	+	(2)	+	PERISTENCE	WELL	Lost to follow-up
72	+	(3)	+	(5)	+	(ND)	+	(1)	+	(ND)	+	(0)	+	(0)	+	(1)	+	PERISTENCE	WELL	Lost to follow-up
77	+	(1)	+	(4)	+	(ND)	+	(2)	+	(ND)	+	(0)	+	(ND)	+	(1)	+	PERISTENCE	WELL	Lost to follow-up
79	+	(1)	+	(2)	+	(ND)	+	(1)	+	(ND)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
82	+	(2)	+	(5)	+	(ND)	+	(2)	+	(ND)	+	(0)	+	(0)	+	(0)	+	PERISTENCE	CI	Lost to follow-up
84	+	(1)	+	(2)	+	(ND)	+	(2)	+	(0)	+	(1)	+	(ND)	+	(1)	+	PERISTENCE	WELL	Lost to follow-up
88	+	(1)	+	(3)	+	(ND)	+	(1)	+	(ND)	+	(0)	+	(0)	+	(0)	+	PERISTENCE	WELL	Lost to follow-up
89	+	(3)	+	(5)	+	(ND)	+	(3)	+	(0)	+	(7)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
91	+	(1)	+	(3)	+	(ND)	+	(4)	+	(ND)	+	(8)	+	(ND)	+	(0)	+	PERISTENCE	CI	Lost to follow-up
94	+	(6)	+	(13)	+	(ND)	+	(3)	+	(ND)	+	(3)	+	(ND)	+	(0)	+	PERISTENCE	WELL	Lost to follow-up
96	+	(1)	+	(2)	+	(ND)	+	(1)	+	(ND)	+	(0)	+	(0)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
97	+	(1)	+	(13)	+	(ND)	+	(5)	+	(ND)	+	(0)	+	(ND)	+	(0)	+	ERADICATION	WELL	Lost to follow-up
102	+	(3)	+	(9)	+	(ND)	+	(5)	+	(ND)	+	(14)	+	(ND)	+	(3)	+	PERISTENCE	WELL	Lost to follow-up
104	+	(3)	+	(5)	+	(ND)	+	(3)	+	(ND)	+	(7)	+	(ND)	+	(3)	+	PERISTENCE	WELL	Lost to follow-up
107	+	(3)	+	(9)	+	(ND)	+	(2)	+	(ND)	+	(4)	+	(ND)	+	(3)	+	PERISTENCE	WELL	Lost to follow-up
109	+	(2)	+	(6)	+	(ND)	+	(6)	+	(ND)	+	(15)	+	(ND)	+	(5)	+	PERISTENCE	CI	Lost to follow-up

NTZ = nitazoxanide;  
UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit;  
+ = presence of cysts;  
- = absence of cysts;  
Shaded rows represent patients who shed cysts after initial eradication

UC/I = unconcentrated stool sample stained with iodine;  
C/I = concentrated stool sample stained with iodine;  
PID = patient identification number;  
CI = continuing illness.

ND = not done;

Table 5: Parasitological data for patients receiving placebo for treatment of giardiasis at the Peru site.

PID	Cysts (count per high power field) in stool sample at screening			Cysts (count per high power field) in baseline stool sample			Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy stool sample 1			Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy stool sample 2			Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Day 14 parasitological response	
	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA			UC/I	C/I	UC/IFA		
	Placebo tablets BID x 3 days:																		
24	+ (ND)	+ (ND)	ND	+ (1)	+ (1)	+ (ND)	+ (1)	+ (5.5)	+ (ND)	+ (ND)	+ (ND)	+ (3.5)	ND	PERSISTENCE	CI	No follow-up	- (0)	ND	ERADICATION
28	+ (1)	+ (1)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	ND	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	ND	ERADICATION
31	+ (ND)	+ (17.5)	ND	+ (5)	+ (17.5)	+ (ND)	+ (ND)	+ (4.5)	ND	+ (ND)	+ (ND)	+ (6.5)	ND	PERSISTENCE	CI	No follow-up	- (0)	ND	ERADICATION
40	+ (ND)	+ (4)	ND	+ (5)	+ (6.5)	+ (ND)	+ (4.5)	+ (6.5)	+ (ND)	+ (4.5)	+ (ND)	+ (5.5)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
43	+ (3)	+ (5.5)	ND	+ (2.5)	+ (6.5)	+ (ND)	- (0)	- (0)	ND	+ (1.5)	+ (ND)	+ (3)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
49	+ (6.5)	+ (11)	ND	+ (7.5)	+ (16.5)	+ (ND)	+ (1.5)	+ (2)	+ (ND)	+ (1.5)	+ (3)	+ (3)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
53	+ (2)	+ (3.5)	ND	+ (5.5)	+ (7)	+ (ND)	+ (3)	+ (5)	- (0)	+ (2)	+ (5)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (4)	+	+ (ND)	PERSISTENCE
56	+ (5)	+ (12.5)	ND	+ (6)	+ (12)	+ (ND)	+ (3)	+ (7)	+ (ND)	+ (2)	+ (10)	+ (10)	+ (ND)	PERSISTENCE	WELL	+ (3)	+ (6)	+ (ND)	PERSISTENCE
65	+ (1)	+ (2)	ND	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	+ (1)	+ (1)	+ (1)	- (0)	PERSISTENCE	WELL	+ (1)	+ (1)	- (0)	PERSISTENCE
68	+ (2)	+ (5)	ND	+ (3)	+ (6)	- (0)	+ (1)	+ (91)	- (0)	+ (3)	+ (7)	+ (7)	+ (ND)	PERSISTENCE	WELL	+ (3)	+ (4)	- (0)	PERSISTENCE
74	+ (2)	+ (4)	ND	+ (2)	+ (3)	+ (ND)	+ (1)	+ (2)	+ (ND)	+ (2)	+ (3)	+ (3)	+ (ND)	PERSISTENCE	CI	+ (1)	+ (1)	+ (ND)	PERSISTENCE
78	+ (3)	+ (4)	ND	+ (2)	+ (5)	+ (ND)	+ (2)	+ (4)	+ (ND)	- (0)	+ (1)	+ (1)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
81	+ (1)	+ (2)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (1)	+ (1)	- (0)	PERSISTENCE
90	+ (3)	+ (8)	ND	+ (4)	+ (12)	+ (ND)	+ (6)	+ (10)	+ (ND)	+ (2)	+ (5)	+ (5)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
93	+ (9)	+ (20)	ND	+ (2)	+ (15)	- (0)	+ (2)	+ (4)	+ (ND)	+ (3)	+ (4)	+ (4)	+ (ND)	PERSISTENCE	WELL	+ (2)	+ (3)	+ (ND)	PERSISTENCE
99	+ (4)	+ (5)	ND	+ (3)	+ (5)	+ (ND)	- (0)	- (0)	ND	- (0)	- (0)	- (0)	ND	ERADICATION	WELL	+ (1)	+ (1)	ND	PERSISTENCE
103	+ (2.5)	+ (40)	ND	+ (20)	+ (38)	ND	+ (7)	+ (13)	+ (ND)	+ (14)	+ (20)	+ (20)	+ (ND)	PERSISTENCE	CI	+ (20)	+	ND	PERSISTENCE
106	- (ND)	+ (1)	ND	+ (3)	+ (7)	+ (ND)	+ (3)	+ (6)	+ (ND)	+ (3)	+ (5)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (4)	+ (9)	ND	PERSISTENCE

UC/I = unconcentrated stool sample stained with iodine;

UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit;

PID = patient identification number; ND = not done;

+ = presence of cysts; - = absence of cysts.

Shaded rows represent patients who shed cysts after initial eradication

C/I = concentrated stool sample stained with iodine;

CI = continuing illness;

CI = continuing illness;

CI = continuing illness;

CI = continuing illness;

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The clinical and parasitological response of patients treated with nitazoxanide or placebo in Egypt is shown in Table 6. The individual patient data for the nitazoxanide tablet or suspension, and placebo treatment groups are shown in Tables 7 and 8. Overall, the clinical outcome of patients treated with nitazoxanide tablets (17/18; 94%) was similar to that of nitazoxanide suspension (16/18; 89%) and greater than placebo (3/9; 43%) at the Egypt site. Based on absence of cysts in 2 unconcentrated (stained with iodine and immunofluorescence) and concentrated (iodine stained) stool samples, the parasitological outcome was better in patients treated with nitazoxanide tablets (17/18; 94%) or suspension (15/18; 83%) than placebo (2/9; 22%).

Resolution of diarrhea and eradication of cysts was observed in 16 patients in the nitazoxanide tablet arm, 15 patients in the nitazoxanide suspension arm, and 1 patient in the placebo arm, at 4 to 7 days after discontinuation of treatment. These patients were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. The 16 patients treated with nitazoxanide tablets and 1 treated with placebo continued to be free of cysts while shedding of cysts was observed in 1 of the 16 patients treated with nitazoxanide suspension (shaded rows, Table 7). The clinical outcome was not measured.

Table 6: Parasitological and clinical response of patients with giardiasis at the Egypt site.

Treatment group	Parasitological and clinical responses		Patients with eradication of cysts N (%)	Patients with clinical well response N (%)	Patients clinical well and showing eradication of cysts N (%)
	Cysts Eradicated (CR)	Cysts Persisted (CR)			
500 mg NTZ tablet BID 3 days (n = 18)	17 (16 well, 1 CI)	1 (1 well, 0 CI)	17 (94)	17 (94)	16 (89)
500 mg NTZ oral suspension BID 3 days (n = 18)	15 (15 well, 0 CI)	3 (1 well, 2 CI)	15 (83)	16 (89)	15 (83)
Placebo BID 3 days (n = 9)	2 (1 well, 1 CI)	7 (2 well, 5 CI)	2 (22)	3 (43)	1 (11)

NTZ = Nitazoxanide;  
CI = continuing illness.

CR = clinical response;

N = number of subjects;

Table 7: Parasitological data for patients receiving nitazoxanide tablets or suspension for treatment of giardiasis at the Egypt site.

PID	Cysts (count per high power field) in stool sample at screening				Cysts (count per high power field) in baseline stool sample				Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy				Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Day 14 parasitological response					
	UC/I		C/I		UC/IFA		C/I		UC/IFA		C/I				UC/IFA								
	UC/I	C/I	UC/IFA	C/I	UC/IFA	C/I	UC/IFA	C/I	UC/IFA	UC/IFA	C/I	UC/IFA			UC/IFA	C/I	UC/IFA						
NTZ tablets: 500 mg BID x 3 days																							
1	+	(1)	+	(1)	-	(0)	+	(1)	-	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
5	+	(ND)	+	(1)	-	(0)	+	(3.5)	-	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
8	+	(ND)	+	(1)	+	(ND)	+	(ND)	+	(ND)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
10	+	(ND)	+	(3.5)	+	(1)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
11	+	(ND)	+	(7.5)	+	(10)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
12	+	(ND)	+	(3.5)	+	(3.5)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
17	+	(ND)	+	(ND)	+	(7.5)	+	(3.5)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
20	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(7.5)	-	(0)	-	(0)	ERADICATION	WELL	Lost to follow-up	-	(0)	-	(0)	ERADICATION	
123	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(1)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
125	+	(ND)	+	(1)	+	(ND)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
126	+	(ND)	+	(1)	+	(ND)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
130	-	(0)	-	(0)	+	(ND)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
133	+	(ND)	+	(1)	+	(ND)	+	(7.5)	+	(7.5)	-	(0)	-	(0)	ERADICATION	CI	-	(0)	-	(0)	ERADICATION		
135	+	(ND)	+	(1)	+	(1)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
142	+	(ND)	+	(1)	+	(1)	+	(ND)	+	(3.5)	+	(ND)	+	(1)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
145	+	(ND)	+	(1)	+	(3.5)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
148	+	(ND)	+	(3.5)	+	(3.5)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
150	+	(ND)	+	(3.5)	+	(3.5)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	ERADICATION		
NTZ suspension: 500 mg BID x 3 days																							
2	-	(0)	-	(0)	+	(1)	+	(0)	+	(1)	+	(ND)	+	(3.5)	PERSISTENCE	WELL	+	(ND)	+	(10)	+	(10)	PERSISTENCE
4	+	(ND)	+	(3.5)	+	(3.5)	+	(0)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
7	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(1)	-	(0)	-	(0)	ERADICATION	WELL	+	(ND)	+	(10)	+	(10)	PERSISTENCE
9	+	(ND)	+	(3.5)	+	(3.5)	+	(3.5)	+	(ND)	+	(ND)	+	(1)	ERADICATION	CI	-	(0)	-	(0)	-	(0)	ERADICATION
13	-	(ND)	+	(1)	+	(3.5)	+	(0)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
14	+	(ND)	+	(ND)	+	(1)	+	(1)	+	(1)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
16	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(3.5)	+	(ND)	+	(1)	ERADICATION	CI	+	(ND)	+	(10)	+	(10)	PERSISTENCE
19	+	(ND)	+	(1)	+	(0)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
121	+	(ND)	+	(1)	+	(0)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
122	+	(ND)	+	(1)	+	(ND)	+	(3.5)	+	(3.5)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
127	+	(ND)	+	(1)	+	(0)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
129	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(1)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
132	+	(ND)	+	(ND)	+	(1)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
134	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
141	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
144	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(0)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
146	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(3.5)	+	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION
147	+	(ND)	+	(1)	+	(ND)	+	(1)	+	(1)	-	(0)	-	(0)	ERADICATION	WELL	-	(0)	-	(0)	-	(0)	ERADICATION

NTZ = nitazoxanide; PID = patient identification number; UC/I = iodine stained unconcentrated stool sample; C/I = iodine stained concentrated stool sample; UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit; + = presence of cysts; - = absence of cysts; ND = not done; CI = continuing illness; Shaded row represents the patient who shed cysts after initial eradication

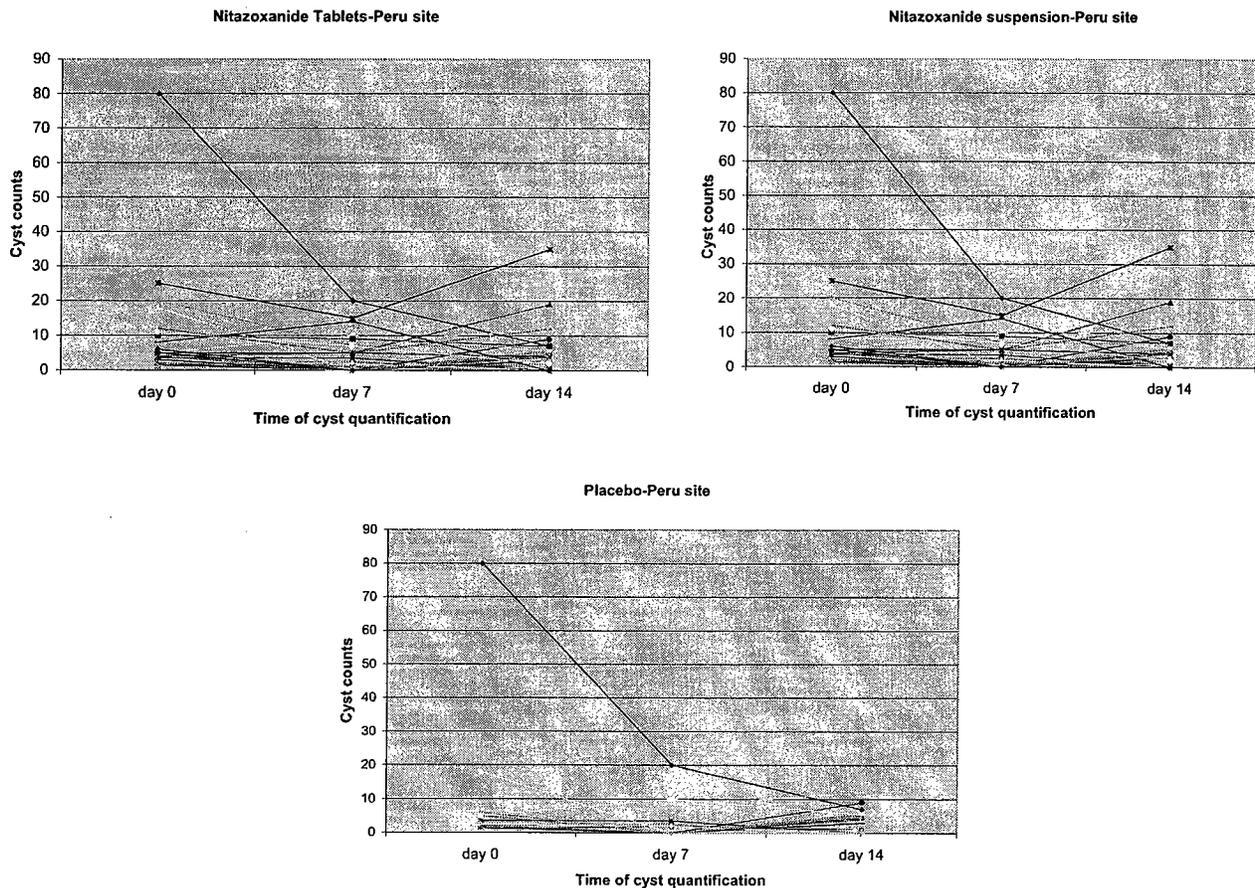


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At the Peru and Egypt sites, quantification of cysts per high power field was done using iodine stained concentrated stool samples. The other two methods were not used consistently for all patients at both sites, at the different time points. For analysis of the reduction in cyst counts, the highest cyst count in either of the 2 concentrated stool samples stained with iodine on day 7 was used.

At the Peru site, the reduction in cyst counts in the concentrated stool samples stained with iodine in the 3 groups was similar at days 4 to 7 after discontinuation of therapy (Figure 1). Additionally, a trend towards increase in cyst counts at 12-14 days after discontinuation of therapy was observed in some patients. However, in the absence of clinical outcome, changes in the parasite counts should be interpreted with caution.

Figure 1: Reduction in *G. lamblia* cyst count (using the concentrated stool samples stained with iodine) in the nitazoxanide (tablet or suspension) and placebo treated groups at the Peru site.



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At the Egypt site, the baseline cyst counts of the patients were low (median count = 1 cyst per high power field). Therefore, no meaningful conclusions could be drawn regarding reduction in cyst counts (cyst count on day 7 – cyst count on day 0) for nitazoxanide or placebo treated patients.

Various factors effect detection of cysts in stools such as specimen collection, transport, addition of stool preservatives, age of the stool (fresh versus 24 hours old), consistency, number of stool samples examined, presence of debris in the stool sample, clarity of concentrated stools sediment, and background fluorescence in fluorescent stained stool samples. The limit of detection for iodine stained unconcentrated or concentrated stool can be altered by number of factors such as quality of smears, time spent on smear examination, and expertise of the examiner in addition to the factors described above. However, the sensitivity of unconcentrated and concentrated stool (staining method not specified) for cyst detection was stated to be 66 - 70% when the cyst counts were low<sup>1</sup>. The limit of detection of cysts using the IFA assay kit has not been determined using fecal samples seeded with *Giardia* cysts. However, the sensitivity and specificity of the IFA assay compared to iodine stained stool samples was stated to be 95% (IFA kit package insert). Because of the limitation of the detection methods used to detect cysts, differences in the methods used to quantify cysts at the two sites (actual counts at the Peru site versus approximated counts at the Egypt site), consistency of the stool samples, and number of stool passed by the patient in a 24 hour period, it is difficult to estimate the actual parasite count.

The effect of nitazoxanide on cyst eradication and parasitological outcome should be interpreted with caution due to the following limitations: (a) differences in virulence of different *G. lamblia* strains, (b) resolution of diarrhea over time in an immunocompetent host, (c) inability to differentiate re-infection from relapse, (d) inability of current methods to detect cysts, when counts are low, and (e) intermittent shedding of cysts,.

In summary, nitazoxanide tablet and suspension were more effective than placebo in resolving diarrhea (Table 9).

Table 9. Parasitological and clinical outcome of all patients in study RM01-3011.

Treatment group	Parasitological and clinical responses		Patients with eradication of cysts n (%)	Patients with clinical well response n (%)	Patients clinical well and showing eradication of cysts n (%)	Patients that shed cysts after initial eradication n/N (%)
	Cysts Eradicated (CR)	Cysts Persisted (CR)				
Placebo BID 3 days (N = 27)	5 (4 well, 1 CI)	22 (8 well, 14 CI)	5 (18.5)	12 (44)	4 (14.8)	7/48(13)
500 mg NTZ tablet BID 3 days (N = 54)	30 (28 well, 2 CI)	24 (18 well, 6 CI)	30 (55.5)	46 (85)	28 (52)	6/49 (12)
500 mg NTZ oral suspension BID 3 days (N = 54)	26 (26 well, 0 CI)	28 (19 well, 9 CI)	26 (48)	45 (83)	26 (48)	3/25(12)

NTZ = Nitazoxanide;  
CI = continuing illness.

N = number of patients;

n = number of patients showing response

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## 4.2. Cryptosporidial diarrhea:

A phase III, randomized, multicenter, placebo controlled study (RM01-3010) to determine the efficacy of nitazoxanide tablets for the treatment of diarrhea due to *C. parvum* in immunocompetent adults is ongoing. The sponsor has been able to enroll only 9 patients until now. Data from these patients were not included for review. However, the sponsor believes that the support for the efficacy of nitazoxanide tablets for treatment of diarrhea due to *C. parvum* in adults could be based on the efficacy of nitazoxanide tablets in the treatment of giardiasis, similarities between infection due to the *C. parvum* and *G. lamblia*, and clinical efficacy based on data from studies submitted to the original NDA. Please note that children under 5 years of age appear to be more susceptible to *C. parvum* infection than adults, possibly reflecting increased fecal-oral transmission or lack of immunity in this population<sup>2</sup>. Therefore, efficacy of nitazoxanide in children may not correspond to efficacy of nitazoxanide in adults. The clinical study RM-NTZ-98-002 (submitted to the original NDA) that evaluated the efficacy of nitazoxanide tablets in adults with cryptosporidial diarrhea and similarities/dissimilarities between cryptosporidial diarrhea and giardiasis are summarized below.

### 4.2.1. Study RM-NTZ-98-002:

Study RM-NTZ-98-002 (submitted to the original NDA) was a randomized, double-blind, placebo controlled study that determined the safety and efficacy of nitazoxanide tablets in 50 adults with cryptosporidial diarrhea [please see microbiology review dated 11-06-02 (NDA 21-497 and 21-498, N-000)]. Eradication of oocysts was observed in 12/21(57%) patients and resolution of diarrhea was observed in 7 of the 12 patients. Placebo treatment eradicated oocysts in 6/21 (28%) patients, and 3 of the 6 resolved diarrhea. However, parasitological evaluation in this study was limited to microscopic examination of a small amount of stool sample after acid-fast staining. Based on the small number of patients, nitazoxanide tablets appear to be effective in the treatment of cryptosporidial diarrhea in adults. However, data from additional patients from the ongoing study (RM01-3001) in which oocysts will be detected and quantified by 2 different methods, acid-fast staining and immunofluorescence assay, in 2 stool samples would provide a better measure of oocyst levels in the stool and efficacy of nitazoxanide tablets in treatment of adults with cryptosporidial diarrhea.

### 4.2.2. Comparison of the pathophysiology of cryptosporidial diarrhea and giardiasis:

The similarities and differences between cryptosporidial diarrhea and giardiasis are summarized in Table 10. Although, the infection route and disease appear to be similar, *C. parvum* can invade epithelial cells of the small intestine while *G. lamblia* is only known to adhere to epithelial cells.

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Table 10: Summary of similarities and differences between cryptosporidial diarrhea and giardiasis.

Characteristics	Cryptosporidial diarrhea	Giardiasis
Causative agent <sup>2,4</sup>	<i>C. parvum</i>	<i>G. lamblia</i>
Route of infection <sup>2,4</sup>	Ingestion of contaminated food or water	Ingestion of contaminated food or water
Infective stage <sup>2,4</sup>	Oocyst	Cyst
Invasion of epithelial cells of the small intestine <sup>2,4</sup>	Observed in humans	Not observed in humans
Effect on intestinal ion transport and glucose absorption <sup>5,6</sup>	Observed in animals	Observed in animals
Histology of biopsy samples from humans <sup>2,3</sup>	Changes in villi, crypt hyperplasia, epithelial cell damage and infiltration of lamina propria by lymphocytes, neutrophils and macrophages	Similar to that seen with <i>C. parvum</i> infection except infiltration of lamina propria only by lymphocytes and neutrophils
Activation of nuclear factor-kB <sup>7</sup>	Observed <i>in vitro</i> using human biliary epithelial cells; may play a role in survival of pathogen within host	Not known

## 5. CONCLUSIONS:

The sponsor is seeking approval of nitazoxanide tablets for the treatment of diarrhea due to *G. lamblia* and *C. parvum* in adults. The sponsor has proposed 500 mg nitazoxanide tablets b.i.d. for 3 days for treatment of adults with giardiasis and cryptosporidial diarrhea.

**Giardiasis:** A total of 54 adults with giardiasis were treated with nitazoxanide tablets in a phase III, randomized, double-blind, placebo controlled study conducted in Peru and Egypt. Resolution of diarrhea was observed in 85% (46/54) patients treated with nitazoxanide tablets compared to 83% (45/54) treated with nitazoxanide suspension and 44% (12/27) treated with placebo.

The parasitological outcome was based on qualitative data, i.e., presence or absence of cysts in 2 stool samples at 4 to 7 days after discontinuation of therapy. The sponsor also quantified the cysts in the unconcentrated stool sample stained with iodine or immunofluorescence, and concentrated stool sample stained with iodine. The method for detecting the cysts at the two sites, Peru and Egypt, appear to be similar. However, the quantification of the cysts was done differently. The actual cyst counts were determined at Peru, while a semi-quantitative grading system was used and cyst counts per high power field approximated at Egypt. The cyst counts were reported as number of cysts per high power field rather than per weight or volume of stool. Based on qualitative results, the percentage of patients that showed absence of *G. lamblia* cysts in the nitazoxanide tablet arm was 55.5% (30/54) compared to 48% (26/54) in the nitazoxanide suspension arm, and 18.5% (5/27) in the placebo arm. The clinical (94%) and parasitological (94%) outcome were better (94%) in patients treated with nitazoxanide tablets

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at the Egypt site, where cysts counts at baseline were low, compared to the Peru site (clinical outcome = 80.5%, parasitological outcome = 64%), where cyst counts at baseline were high.

Patients (nitazoxanide tablets arm, n = 28; nitazoxanide suspension arm, n = 26; and placebo arm, n = 4) who had resolution of diarrhea and eradication of cysts at 4 to 7 days after discontinuation of therapy were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. Shedding of cysts was observed in 25% (7/28) patients in the nitazoxanide arm, 23% (6/26) patients in the nitazoxanide suspension arm, and 50% (2/4) patients in the placebo arm, at the 12 to 14 day follow-up. The clinical outcome was not measured at this time point. Most of these patients were from the Peru site. The sponsor has stated that Peru being a hyper-endemic area compared to Egypt, the recurrence of cysts was more likely due to re-infection. However, no information was included to support the basis of higher endemicity of *G. lamblia* in Peru compared to Egypt. Another explanation provided by the sponsor was that the dose of nitazoxanide was inadequate for complete elimination of cysts from patients. However, it is also possible that the patient relapsed. At the present time, there are no standardized methods to differentiating re-infection from relapse. The differences in the parasitological response may also have been influenced by differences in the strain of *G. lamblia* causing infection in these two sites, and the ability of immunocompetent host to resolve diarrhea spontaneously over time.

Quantification of cysts was done uniformly at baseline and post-treatment using only iodine stained concentrated stools at the two sites. The baseline cyst counts at the Peru site were high (median count = 5 per high power field) compared to Egypt (median count = 1 per high power field). The reduction of cyst count at the Peru site for the nitazoxanide tablet or suspension and the placebo arms were similar. No meaningful conclusions could be made about reduction in cyst counts at the Egypt site, as the baseline cyst counts were low. The effect of nitazoxanide on reduction of cysts could not be evaluated with certainty, due to limitations of the detection method, differences in methods used for quantification of cysts at the two sites, measurement of cysts in a small volume of stool, and variability in the consistency and number of stools passed by the patients. In the absence of 24 hour collection of stool sample, the cyst count per high power field (Peru site) or approximated counts obtained using a semi-quantitative method (Egypt site) from a small aliquot of sample, irrespective of whether the stool is formed or unformed, may not be useful.

Several factors effect cyst detection in stool samples such as specimen collection, transport, addition of stool preservatives, age of the stool (fresh versus 24 hour old), consistency, number of stools examined, presence of debris, clarity of smears prepared using a concentrated stool sediment, presence of background fluorescence, when using a fluorescence assay and expertise of the slide examiner. The limit of detection of cysts using iodine or immunofluorescence stained unconcentrated, and iodine stained concentrated stool have not been determined. However, a report from the literature suggests that the sensitivity of the unconcentrated and concentrated stool for detection of cysts in patients with giardiasis is 66-70%, when cyst counts are low. The sensitivity of the immunofluorescence assay used in this study is similar to iodine stained stool samples. Because of the inability of current diagnostic methods

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to detect cysts, when the counts are low and intermittent shedding of cysts in patients with giardiasis, the parasitological outcome should be interpreted with caution.

Overall, the clinical efficacy of nitazoxanide tablets was similar to nitazoxanide suspension and greater than placebo.

**Cryptosporidial diarrhea:** Study RM01-3010 to evaluate the efficacy of nitazoxanide tablets in adults with cryptosporidiosis is ongoing and only 9 patients have been enrolled. The data from these patients were not included. However, the sponsor is seeking the indication for treatment of diarrhea due to *C. parvum* based on (a) the clinical study evaluating efficacy of nitazoxanide tablets compared to nitazoxanide suspension (an approved product for treatment of cryptosporidiosis and giardiasis in children) and placebo for the treatment of diarrhea due to *G. lamblia*, (b) similarities in the pathophysiology of the disease due to the two pathogens, (c) *in vitro* activity of nitazoxanide against the sporozoite stage of *C. parvum*, (d) activity of nitazoxanide against *C. parvum* in animal studies, and (e) clinical studies in adults and children with cryptosporidial diarrhea submitted to the original NDA. Although, the pathophysiology of the disease due to the two pathogens appears to be similar, *C. parvum* can invade epithelial tissue unlike *G. lamblia*. Additionally, there is no regulatory precedence that allows for efficacy in giardiasis to support efficacy in cryptosporidiosis. Therefore, data from a well controlled clinical study would be necessary to determine the efficacy of nitazoxanide tablets in adults with cryptosporidial diarrhea.

## 6. LABEL:

### 6.1. Sponsor's proposed label:

#### MICROBIOLOGY

##### Mechanism of action

The antiprotozoal activity of nitazoxanide is believed to be due to interference with the pyruvate:ferredoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction which is essential to anaerobic energy metabolism. Studies have shown that the PFOR enzyme from *Giardia lamblia* directly reduces nitazoxanide by transfer of electrons in the absence of ferredoxin. The DNA-derived PFOR protein sequence of *Cryptosporidium parvum* appears to be similar to that of *Giardia lamblia*. Interference with the PFOR enzyme-dependent electron transfer reaction may not be the only pathway by which nitazoxanide exhibits antiprotozoal activity.

##### Activity *in vitro*

Nitazoxanide and its metabolite, tizoxanide, are active *in vitro* in inhibiting the growth of (i) sporozoites and oocysts of *Cryptosporidium parvum* and (ii) trophozoites of *Giardia lamblia*.

##### Drug Resistance

A potential for development of resistance by *Cryptosporidium parvum* or *Giardia lamblia* to nitazoxanide has not been examined.

##### Susceptibility Tests:

For protozoa such as *Cryptosporidium parvum* and *Giardia lamblia*, standardized tests for use in clinical microbiology laboratories are not available.

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**INDICATIONS AND USAGE**

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**6.2. Comments:**

The division and sponsor agreed that the label for nitazoxanide tablets and suspension could be combined. There are no changes to the Microbiology section of the label.

**7. RECOMMENDATIONS:**

This NDA is recommended for approval with respect to Microbiology for the treatment of giardiasis and is approvable for the treatment of cryptosporidiosis. Additional data from a well controlled clinical study would be necessary to determine the efficacy of nitazoxanide tablets for the treatment of cryptosporidiosis in adults. There are no changes to the Microbiology section of the label.

The following recommendation should be considered for future drug development.

1. If future clinical studies are conducted, please consider performing parasitological evaluations using at least 2 stool samples at different time points such as at baseline, end of therapy, 2 and 4 weeks post-therapy. Attempts should be made to correlate parasitological outcome with clinical outcome at each time point.

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Kalavati Suvarna  
Microbiologist, HFD-590

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**CONCURRENCES:**

HFD-590/Deputy Dir. \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

HFD-590/Micro TL \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

CC:

HFD-590/Original IND

HFD-590/Division File

HFD-590/MO

HFD-590/Pharm

HFD-590/Chem

HFD-590/Review Micro

HFD-590/CSO/MillerK

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**8. REFERENCES:**

- (1) Hanson KL, Cartwright CP. Use of an enzyme immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia lamblia*. J Clin Microbiol. 2001;39:474-477.
- (2) Ungar BLP. *Cryptosporidium*. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. Pennsylvania: 2000:2903-2915.
- (3) Hart CA. Cryptosporidiosis. In: Gilles HM, ed. Protozoal diseases. New York: Oxford University Press Inc; 1999:592-606.
- (4) Hill DR. *Giardia lamblia*. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. Pennsylvania: 2000:2888-2894.
- (5) Guarino A, Canani RB, Casola A *et al*. Human intestinal cryptosporidiosis: secretory diarrhea and enterotoxin activity in Caco-2 cells. J Infect Dis. 1995;171:976-983.
- (6) Buret A, Hardin JA, Olson ME, Gall DG. Pathophysiology of small intestinal malabsorption in gerbils infected with *Giardia lamblia*. Gastroenterology. 1992;103:506-513.
- (7) Chen XM, Levine SA, Splinter PL *et al*. *Cryptosporidium parvum* activates nuclear factor kappaB in biliary epithelia preventing epithelial cell apoptosis. Gastroenterology. 2001;120:1774-1783.

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/s/  
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Kalavati Suvarna  
7/19/04 02:27:53 PM  
MICROBIOLOGIST

Shukal Bala  
7/19/04 02:33:43 PM  
MICROBIOLOGIST

Steve Hundley  
7/19/04 03:48:54 PM  
PHARMACOLOGIST

**MICROBIOLOGY REVIEW**  
**DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)**

NDA #: 21-497 and  
21-498

REVIEWER : Kalavati Suvarna  
CORRESPONDENCE DATE : 05-29-02; 07-22-02; 08-30-02  
CDER RECEIPT DATE : 05-30-02; 07-24-02; 08-30-02  
REVIEW ASSIGN DATE : 06-04-02; 07-31-02; 08-30-02  
REVIEW COMPLETE DATE : 11-06-02

SPONSOR: Romark Laboratories Inc.  
6200 Courtney Campbell Causeway  
Suite 880  
Tampa, FL 33607

SUBMISSION REVIEWED: N-000, BZ, BI

DRUG CATEGORY: Anti-parasitic

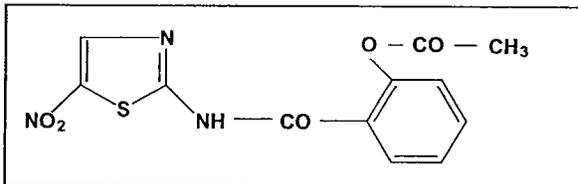
INDICATION: Treatment of diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*

DOSAGE FORM: Tablets and oral suspension

**PRODUCT NAMES:**

- a. PROPRIETARY: None
- b. NONPROPRIETARY: Nitazoxanide  
CAS: 55981-09-4
- c. CHEMICAL: 2-(acetolyloxy)-N-(5-nitro-2-thiazolyl) benzamide

**STRUCTURAL FORMULA:**



Molecular weight: 307.2  
Empirical formula: C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>5</sub>S

SUPPORTING DOCUMENTS: NDA # 20-871; IND # 48,620, 58,895,  
Type II DMF : DMF DMF DMF

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**TABLE OF CONTENTS:**

1. INTRODUCTION AND BACKGROUND.....	4
A. <i>Cryptosporidium parvum</i> .....	5
1.1. Biology of <i>Cryptosporidium parvum</i> .....	5
1.2. Pathogenesis of cryptosporidial infection.....	5
2. MECHANISM OF ACTION.....	5
3. ACTIVITY <i>IN VITRO</i> .....	6
3.1. Activity of nitazoxanide and its metabolite against a human strain.....	6
3.2. Activity of nitazoxanide against clinical isolates.....	13
3.3. Activity of nitazoxanide and its metabolites against a bovine isolate.....	15
3.4. Effect of protein binding on the activity of nitazoxanide.....	21
4. ACTIVITY <i>IN VIVO</i> .....	21
4.1. Suckling mice.....	21
4.2. Scid mice.....	23
4.3. Immunosuppressed rats.....	27
4.4. Gnotobiotic piglets.....	29
5. CLINICAL MICROBIOLOGY.....	32
5.1. Diagnosis of <i>Cryptosporidium parvum</i> infection.....	32
5.2. Clinical studies.....	32
B. <i>Giardia lamblia</i> :.....	44
1.1. Biology of <i>Giardia lamblia</i> .....	44
1.2. Pathogenesis of Giardiasis.....	45
2. MECHANISM OF ACTION.....	45
3. ACTIVITY <i>IN VITRO</i> .....	45
4. ACTIVITY <i>IN VIVO</i> .....	47
5. CLINICAL MICROBIOLOGY.....	48
5.1. Diagnosis of Giardiasis.....	48
5.2. Clinical studies.....	49
C. Protozoa (other than <i>Cryptosporidium</i> and <i>Giardia</i> ), Helminths and Bacteria.....	54
2. MECHANISM OF ACTION.....	54
2.1. Protozoa.....	54
2.2. Anaerobic and microaerophilic bacteria.....	55
3. ACTIVITY <i>IN VITRO</i> AND <i>IN VIVO</i> .....	61
3.1. <i>Trichomonas vaginalis</i> .....	61
3.1.1. <i>In vitro</i> .....	61
3.1.2. <i>In vivo</i> .....	62
3.2. <i>Entamoeba histolytica</i> .....	63
3.2.1. <i>In vitro</i> .....	63
3.2.2. <i>In vivo</i> .....	64

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3.3. <i>Microsporidium</i> .....	64
3.3.1. <i>In vitro</i> .....	64
3.3.2. <i>In vivo</i> .....	64
3.4. Trematodes.....	65
3.4.1. <i>In vitro</i> .....	65
3.4.2. <i>In vivo</i> .....	65
3.5. Nematodes and Cestodes.....	65
3.5.1. <i>In vitro</i> .....	65
3.5.2. <i>In vivo</i> .....	65
3.6. Bacteria.....	68
3.6.1. <i>In vitro</i> .....	68
3.6.2. <i>In vivo</i> .....	69
D. Effect of nitazoxanide on the inflammatory responses.....	69
E. Drug Resistance.....	72
F. CONCLUSIONS.....	73
G. THE LABEL.....	78
1. Sponsor's proposed label for tablets and oral suspension.....	78
2. Comments.....	79
3. FDA's proposed label.....	81
H. RECOMMENDATIONS.....	83
I. REFERENCES.....	85

## 1. INTRODUCTION AND BACKGROUND:

The subject of this NDA is Nitazoxanide for (a) the treatment of diarrhea due to *Cryptosporidium parvum* and (b) the treatment of diarrhea due to *Giardia lamblia*.

The sponsor has proposed the following doses of nitazoxanide for the treatment of cryptosporidial diarrhea and giardiasis: 500 mg tablets b.i.d for 3 days in adults, 200 mg oral suspension b.i.d for 3 days in children ages 4 - 11 years, and 100 mg oral suspension b.i.d for 3 days in children ages 1 - 3 years.

Nitazoxanide is registered in Latin America for the treatment of a wide range of parasitic infections including *C. parvum* and *G. lamblia*. It is approved for veterinary use (for the treatment of helminthic infections in cats and dogs) in Switzerland and France. In the United States, the drug was the subject of NDA 20-871 (submitted in 1998) for the treatment of cryptosporidial diarrhea in AIDS patients but was not approved.

There is no approved therapy for the treatment of cryptosporidiosis in the United States. Furazolidone and Quinacrine have been approved for treatment of giardiasis in the United States. In addition to these drugs, several drugs such as metronidazole, albendazole, and paromomycin, although not approved are available in the United States for the treatment of giardiasis.

Nitazoxanide is a nitro-thiazolyl with a salicylic acid amide moiety. It is soluble in DMSO and in aqueous media at alkaline pH. The drug is highly unstable and is rapidly metabolized to various metabolites (2 major and 5 minor metabolites have been identified). The two major metabolites are tizoxanide and tizoxanide glucuronide. Following oral administration, nitazoxanide is hydrolyzed to tizoxanide (desacetyl nitazoxanide) that undergoes glucuronidation to form tizoxanide glucuronide. The time to maximum concentration ( $T_{max}$ ) for both metabolites (tizoxanide and tizoxanide glucuronide) was  $\leq 4.5$  hours after administration of a single oral dose of nitazoxanide [500 mg to healthy adults ( $\geq 12$  years), 100 mg to children ( $\leq 3$  years) or 200 mg to children (4 - 11 years)]. The maximum plasma concentration ( $C_{max}$ ) and the area under the concentration versus time curve (AUC) for both metabolites were about 3-fold higher in adults than children (Table 1). Both nitazoxanide and tizoxanide were shown to exhibit high protein binding ( $> 99\%$ ). The protein binding property of tizoxanide glucuronide was not examined.

Table 1: Pharmacokinetic parameters of tizoxanide and tizoxanide glucuronide.

Population	Dose (mg)	Tizoxanide			Tizoxanide glucuronide		
		$C_{max}$ ( $\mu\text{g/ml}$ )	$T_{max}$ (hours)	AUC ( $\mu\text{g.h/ml}$ )	$C_{max}$ ( $\mu\text{g/ml}$ )	$T_{max}$ (hours)	AUC ( $\mu\text{g.h/ml}$ )
Adults	500	10.4	3.0	41.8	10.4	4.5	64.7
12-17 years	500	91.2	4.0	39.5	7.27	4.0	46.5
4-11 years	200	3.00	2.0	13.5	2.84	4.0	16.9
12-47 months	100	3.11	3.5	11.7	3.64	4.0	19.0

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The sponsor has examined the mechanism of action of nitazoxanide and its activity *in vitro* and/or in animal models against protozoa (*Cryptosporidium*, *Giardia*, and others), helminths (nematodes, cestodes, and trematodes), and bacteria (anaerobic and aerobic gram positive and negative bacteria). However, in this review only studies examining the activity against *Cryptosporidium* and *Giardia* (the infective agents for the indication under consideration) are discussed in detail. The activity against other parasites and bacteria are summarized briefly.

#### **A. *Cryptosporidium parvum*:**

##### **1.1. Biology of *Cryptosporidium parvum*:**

*Cryptosporidium* is an intracellular parasite present in the gastrointestinal and respiratory tract. The infection is caused by ingestion of oocysts. The oocyst contains four sporozoites within a membrane. Upon ingestion, the sporozoites excyst from the oocyst and invade the epithelial cells and become enveloped in a parasitophorous vacuole. Sporozoites undergo maturation into type I meronts, which release merozoites. The merozoite stage can undergo asexual replication and reinvade the host cells or form type II meronts by sexual replication. The Type II meronts release the macrogametocytes or microgametocytes that fertilize to give rise to a zygote. The zygote can develop into oocyst that may either rupture releasing sporozoites *in vivo* or is shed via the feces. Therefore, the presence of oocyst(s) in the stool samples may be intermittent.

The mechanism by which oocysts rupture allowing sporozoites to invade the mammalian cells is not known. Studies conducted *in vitro* show that oocysts can excyst spontaneously. However, the excystation of oocysts can be enhanced by exposure to acids, bile salts or enzymes. Thus, the exposure of oocysts to acids or enzymes in the gastrointestinal tract may play a role in the rupture of the oocyst cell wall.

##### **1.2. Pathogenesis of cryptosporidial infection:**

The major clinical manifestation observed with cryptosporidial infection is diarrhea. However, the mechanism by which *Cryptosporidium* causes diarrhea is not known. The severity of the infection and ultimate pathology is influenced by the immune status of the host. Cryptosporidial diarrhea is self-limiting in immunocompetent individuals but may be life threatening in AIDS or immunocompromised patients.

#### **2. MECHANISM OF ACTION:**

The survival of protozoa, that lack mitochondria, under anaerobic conditions depend on the presence of the enzyme pyruvate:ferredoxin oxidoreductase (PFOR). The enzyme PFOR is involved in carbon metabolism and oxidizes pyruvate to acetylCoA using ferredoxin as an electron acceptor *in vivo*. Nitazoxanide can act as an alternate electron acceptor for this enzyme and be activated. The activated product has not been identified, however, it is thought to play a role in the mechanism by which nitazoxanide exhibits activity against protozoa by generation of a toxic radical.

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Genome analyses of *C. parvum* revealed the presence of a gene that encodes a PFOR like protein. In report RM01-0401<sup>1</sup>, the *C. parvum* DNA derived PFOR peptide sequence was compared to peptide sequences from other organisms. The percent similarity between the peptide sequence of *C. parvum* PFOR and that of *Giardia lamblia*, *Entamoeba histolytica*, *Trichomonas vaginalis* and *Clostridium pasteurianum* was 31%, 49%, 43%, and 51%, respectively. However, the effect of nitazoxanide on the activity of *C. parvum* PFOR enzyme was not measured.

### 3. ACTIVITY IN VITRO:

The *in vitro* activity of nitazoxanide against *C. parvum* was measured using different cell lines such as Madin-Darby bovine kidney (MDBK-F5D2), human adenocarcinoma ileocecal (HCT-8) or human lung carcinoma (A-549) cells infected with oocysts or sporozoites. The sponsor has submitted 3 published and 2 unpublished studies in support of the activity of nitazoxanide and its metabolites against *C. parvum in vitro*.

#### 3.1. Activity of nitazoxanide and its metabolite against a human strain:

In study ROM-022<sup>2</sup>, five experiments were conducted (all in the same laboratory) to determine the *in vitro* activity of nitazoxanide and/or its metabolite against the GCH1 strain of *C. parvum* [these reports were reviewed previously NDA# 20-871 (N-000), microbiology review dated 06-01-98]. All of these experiments were conducted using MDBK-F5D2 cells as the feeder layer and GCH1 oocysts at a concentration of  $5 \times 10^4$  per well in DMEM with 5% fetal bovine serum. The cultures were incubated at 37°C for 24 hours and/or 48 hours with different concentrations of nitazoxanide (dissolved in DMSO; final concentration 0.025-0.5%) or paromomycin (dissolved in water/medium). The drugs were added at the time of initiation of infection in culture. The anti-cryptosporidial effect of nitazoxanide was measured by immunofluorescence using anti-*C. parvum* sporozoite rabbit serum. The anti-sporozoite antiserum raised in rabbits reacted with all developmental forms except the oocyst wall. The toxic effects of the drug on the uninfected mammalian (MDBK) cells was determined by measuring the absorbance of the supernatant at 490 nm after incubation of the cells in the presence of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and phenazine methosulfate (PMS) for 2 hours in the dark. The results of the five experiments are shown below:

Table 2A: *C. parvum* Oocysts Assay (24 hours) - Experiment #17 (final DMSO concentration = 0.5%)

#### Trial 1 - 24hr

Compound	Conc.	Mean ( $\pm$ SD)*	Percent Toxicity	Percent Inhibition
Infected Media	0	983.5 ( $\pm$ 128.2)	0	0
Paromomycin	2mg/ml	482 ( $\pm$ 47.1)	23.8	51
NTZ	100 $\mu$ g/ml	Lost	88.1	NA**
	10 $\mu$ g/ml	55.5 ( $\pm$ 13.5)	65.1	94.4
	1 $\mu$ g/ml	224.5 ( $\pm$ 28.5)	8.3	77.2
	0.1 $\mu$ g/ml	474.5 ( $\pm$ 29.3)	19.3	51.8

\* Parasite Count/10 fields

\*\* Not available due to toxicity

Table 2B: *C. parvum* Oocysts Assay (48 hours) - Experiment #17

Trial 2 - 48hr

Compound	Conc.	Mean ( $\pm$ SD)*	Percent Toxicity	Percent Inhibition
Infected Media	0	2231.25 (+90.03)	0	0
Paromomycin	2mg/ml	580 (+33.42)	40.8	74.01
NTZ	20 $\mu$ g/ml	68.75 (+13.77)	92.87	96.92
	2 $\mu$ g/ml	113.75 (+21.36)	24.93	94.90
	0.2 $\mu$ g/ml	1020 (+158.48)	16.56	54.29
	0.02 $\mu$ g/ml	1041 (+191.46)	21.23	53.33

\* Parasite Count/10 fields

Table 3: *C. parvum* Oocysts Assay (24 hours)- Experiment #19

Combined Drugs vs. *C. parvum* Oocysts

Paromomycin (mg/ml) (water base)	NTZ ( $\mu$ g/ml) (DMSO base)	Par/10	$\pm$ SD	Tox/OD $\pm$ SD	%Inhib	%Tox	Score
0	0	928.50	$\pm$ 79.32	1.187 $\pm$ 0.023	0	0	0
2	0	270.00	$\pm$ 12.65	1.023 $\pm$ 0.006	70.92	13.82	1
1	0	373.00	$\pm$ 83.66	1.118 $\pm$ 0.066	59.83	5.82	1
0.5	0	490.50	$\pm$ 98.36	NA* NA	47.17	NA	NA
0.25	0	599.00	$\pm$ 74.13	NA NA	35.49	NA	NA
0	0	779.50	$\pm$ 63.08	1.049 $\pm$ 0.066	0	0	0
0	20	88.50	$\pm$ 15.86	0.329 $\pm$ 0.074	88.65	68.62	3
0	10	110.0	$\pm$ 16.57	0.633 $\pm$ 0.093	85.89	39.68	2
0	5	72.50	$\pm$ 22.23	1.071 $\pm$ 0.052	90.70	0	0
0	2.5	168.50	$\pm$ 16.60	1.131 $\pm$ 0.294	78.38	0	0
2	20	52.00	$\pm$ 18.11	0.532 $\pm$ 0.101	93.33	49.26	2
2	10	84.50	$\pm$ 12.37	0.610 $\pm$ 0.066	89.16	41.87	2
2	5	91.00	$\pm$ 25.32	0.901 $\pm$ 0.152	88.33	14.07	1
2	2.5	87.50	$\pm$ 2.52	1.011 $\pm$ 0.156	88.77	3.58	0
1	20	84.50	$\pm$ 36.93	0.601 $\pm$ 0.041	89.16	42.68	2
1	10	75.00	$\pm$ 15.56	0.645 $\pm$ 0.049	90.38	38.48	2
1	5	88.50	$\pm$ 25.16	0.811 $\pm$ 0.045	88.65	22.70	1
1	2.5	135.50	$\pm$ 19.49	1.030 $\pm$ 0.021	82.62	1.76	0
0.5	20	137.00	$\pm$ 27.25	0.350 $\pm$ 0.034	82.42	66.62	3
0.5	10	83.33	$\pm$ 14.05	0.611 $\pm$ 0.008	89.31	41.73	2
0.51	5	95.50	$\pm$ 22.71	0.912 $\pm$ 0.104	87.75	13.07	1
0.5	2.5	116.50	$\pm$ 25.68	1.021 $\pm$ 0.052	85.05	2.58	0
0.25	20	70.67	$\pm$ 12.86	0.349 $\pm$ 0.073	90.93	66.71	3
0.25	10	65.50	$\pm$ 37.07	0.647 $\pm$ 0.062	91.60	38.29	2
0.25	5	102.00	$\pm$ 37.63	0.896 $\pm$ 0.007	86.91	14.56	1
0.25	2.5	126.00	$\pm$ 19.66	1.082 $\pm$ 0.075	83.84	0	0

Par/10 = Parasite counts per 10 high power fields

%Inhib = Percent Inhibition of parasite infection

%Tox = Percent toxicity to cells by the drug

\*NA - Information not available

Table 4A: *C. parvum* Oocysts Assay (48 hours) Experiment #28- submitted as part of Experiment #29A

Drugs	Conc.	Parasite ± SD	Tox/OD ± SD	%Inhib	%Tox	Score
Aq. Media	0	1218.4 ±210.22	1.013 ±.024	0	0	0
Paromomycin	2mg/ml	219.08 ±70.69	.873 ±.016	82.02	13.82	1
	1	279.17 ±100.80	1.061 ±.061	77.09	≤ 0	0
	0.5	309.83 ±77.92	.874 ±.158	74.57	13.72	1
	0.25	485.67 ±94.33	.697 ±.006	60.14	31.19	2
0.25% DMSO Media	0	824.92 ±173.73	.928 ±.071	0	0	0
Nitazoxanide	100 <sub>ug/ml</sub>	LOST NA*	.515 ±.107	NA	NA	4
	10	43.42 ±14.69	.201 ±.023	94.74	78.34	4
	1	120.00 ±40.25	.922 ±.017	85.45	.65	0
	0.1	782.75 ±251.45	.824 ±.086	5.11	11.21	1

Table 4B: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite ± SD	Tox/OD ± SD	% Inhib	%Tox	Score
Aqueous Media	0	895.13 ±248.28	1.753 ±.068	0	0	0
Fresh Paromomycin	2000	265.00 ±63.44	1.527 ±.250	70.40	12.92	1
0.25% DMSO Media	0	678.50 ±114.69	1.741 ±.194	0	0	0
Fresh Nitazoxanide	100	LOST NA	.243 ±.037	NA	86.04	4
	10	52.50 ±15.88	.246 ±.012	92.26	85.87	4
	1	479.67 ±94.98	1.718 ±.261	29.30	1.32	0
	0.1	549.00 ±145.22	1.834 ±.274	19.09	≤0	0

Table 4C: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite ± SD	Tox/OD ± SD	% Inhib	%Tox	Score
Aqueous Media	0	709.89 ±343.85	1.544 ±.066	0	0	0
Fresh Paromomycin	2000	174.50 ±58.49	1.188 ±.030	75.42	23.03	1
0.25% DMSO Med	0	535.58 ±242.96	1.479 ±.041	0	0	0
11 Day Old Nitazoxanide	100	LOST NA	.479 ±.001	NA	67.60	3
	10	46.78 ±21.66	.230 ±.016	91.27	84.41	4
	1	118.17 ±63.16	1.420 ±.013	77.94	3.99	0
	0.1	405.33 ±142.79	1.515 ±.086	24.32	≤0	0

Table 4D: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite	+ SD	Tox/OD	+ SD	% Inhib	%Tox	Score
Aqueous Media	0	406.33	±115.38	1.698	±.248	0	0	0
Fresh Paromomycin	2000	146.83	±50.71	1.455	±.130	63.86	14.32	1
0.25% DMSO Med	0	370.91	±118.02	1.474	±.064	0	0	0
17 Day Old Nitazoxanide	100	LOST	NA	.749	±.008	NA	49.20	2
	10	32.44	±16.84	.324	±.008	91.25	78.05	4
	1	56.00	±11.97	1.693	±.056	84.90	≤0	0
	0.1	344.67	±43.87	1.389	±.126	7.07	5.73	0

Table 4E: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite	+ SD	Tox/OD	+ SD	% Inhib	%Tox	Score
Aqueous Media	0	1218.42	±210.22	1.013	±.024	0	0	0
Fresh Paromomycin	2000	219.08	±70.69	.873	±.016	82.02	13.82	1
0.25% DMSO Media	0	824.92	±173.73	.928	±.071	0	0	0
Fresh Nitazoxanide	100	LOST	NA	.515	±.107	NA	44.56	2
	10	43.42	±14.69	.201	±.023	94.74	78.34	4
	1	120.00	±40.25	.922	±.017	85.45	0.65	0
	0.1	782.75	±251.45	.824	±.086	5.11	11.21	1

Note: It appeared that the data shown in Table #4A and #4E are the same. However, the sponsor stated that "while the results appear to be similar, they are not all the same". Given that all of the values reported in both tables were identical out to two or three decimal points it is unclear what data were new or different. Also, the results in Table 4B were stated to be anomalous.

Table 5: *C. parvum* Oocysts Assay (48 hours)-Experiment #30

*C. parvum* Oocysts Assay (48 hr.)

Drugs	Conc.	Parasite	+ SD	Tox/OD	+ SD	%Inhib	%Tox	Score
Aqueous Media	0	681.58	±271.02	2.024	±.018	0	0	0
Paromomycin	2000	115.75	±44.65	1.219	±.009	83.02	39.79	2
0.025% DMSO Media	0	628.50	±171.94	1.799	±.145	0	0	0
NTZ	10	11.75	±7.33	.413	±.013	98.13	77.07	4
	1	39.67	±13.13	1.618	±.326	93.69	10.09	1
	0.1	643.42	±229.73	1.878	±.154	≤0	≤0	0
	0.01	714.33	±194.79	1.617	±.072	≤0	10.12	1
New NTZdes	10	13.75	±6.66	.337	±.005	97.81	81.27	4
	1	39.92	±13.49	1.710	±.033	93.65	4.97	0
	0.1	649.86	±152.19	1.506	±.119	≤0	16.29	1
	0.01	749.33	±139.49	1.721	±.144	≤0	4.36	0

Conc. - µg/ml  
 Parasite - Mean parasite count/field (12 fields analyzed)  
 %Inhib - Percent Inhibition of parasite infection  
 %Tox - Percent toxicity to cells by the drug

Table 6: *C. parvum* Oocysts Assay (48 hours)-Experiment #31

*C. parvum* Oocysts Assay (48 hr.)

Drugs 1	Drug 2	Parasite ± SD	Tox/OD ± SD	%Inhib	%Tox	Score
Aqueous Media	-----	611.58 ±160.21	1.616 ±.019	0	0	0
Paromomycin 1mg/ml	-----	180.50 ±64.27	1.324 ±.073	70.49	18.13	1
0.01% DMSO Media	-----	694.92 ±163.42	1.612 ±.113	0	0	0
Nitazoxanide (des) 0.75µg/ml	-----	237.50 ±134.48	1.356 ±.123	65.82	15.89	1
HL 2945 10µM	-----	134.08 ±49.56	1.240 ±.122	80.71	23.05	1
HL 2945 5µM	-----	692.67 ±256.21	1.232 ±.141	0.32	23.55	1
HL 2945 10µM	Paromomycin 1mg/ml	233.89 ±176.79	1.192 ±.147	66.34	26.03	2
HL 2945 5µM	Paromomycin 1mg/ml	393.60 ±214.53	1.217 ±.163	43.36	24.48	1
HL 2945 10µM	NTZdes 0.75µg/ml	126.42 ±56.37	1.218 ±.179	81.81	24.42	1
HL 2945 5µM	NTZdes 0.75µg/ml	139.11 ±50.61	1.224 ±.101	79.98	24.01	1

Parasite - Mean parasite count/field (12 fields analyzed)  
 %Inhib - Percent Inhibition of parasite infection  
 %Tox - Percent toxicity to cells by the drug

The results of the *in vitro* studies (shown in Tables 2 to 6 and summarized in Table 7) indicate that at 24 hours, nitazoxanide at concentrations between 1 and 5 µg/ml inhibited parasite count by 77 to 91% with ≤ 8.35% toxicity to MDBK cells. These data are based on a single experiment. At 48 hours of incubation, the data collected from 5 different experiments (conducted in the same laboratory) show that nitazoxanide at a concentration of 1 µg/ml inhibited parasite number from 29 to 85% with minimal toxicity to uninfected feeder cells. Higher concentrations (≥ 10 µg/ml) of the drug were highly cytotoxic (≥ 40%).

The results in Table 4B (which show comparatively lower inhibition of the parasite i.e. 29%) were stated to be anomalous but the reasons for that are unclear. The parasite count in the untreated cultures was within the range observed in other experiments. Also, paromomycin, which was used for comparison of drug activity, showed results comparable to that observed in other experiments.

Table 7: Summary of results shown in Tables 2 to 6.

Experiment #	Parasite count in untreated control	Drug concentration $\mu\text{g/ml}$	% inhibition	% toxicity
<b>After 24 hours incubation with NTZ</b>				
# 17 and # 19	984 $\pm$ 128; 780 $\pm$ 63	0	0	0
		100	NA	88.1
		<u>20</u>	<u>89</u>	<u>69</u>
		10	94.4	65.1
		<u>10</u>	<u>86</u>	<u>40</u>
		<u>5</u>	<u>91</u>	<u>0</u>
		<u>2.5</u>	<u>78</u>	<u>0</u>
		1	77.2	8.3
		0.1	51.8	19.3
<b>After 48 hours incubation with NTZ</b>				
# 17, # 29A, and # 30	2231 $\pm$ 90; <u>825 <math>\pm</math> 174; 679 <math>\pm</math> 115; 825 <math>\pm</math> 174; 536 <math>\pm</math> 243; 371 <math>\pm</math> 118;</u> 629 $\pm$ 172	0	0	0
		<u>100</u>	<u>NA</u> <u>NA<sup>a</sup></u> <u>NA<sup>a</sup></u> <u>NA<sup>b</sup></u> <u>NA<sup>c</sup></u>	<u>NA</u> <u>86<sup>a</sup></u> <u>45<sup>a</sup></u> <u>68<sup>b</sup></u> <u>49<sup>c</sup></u>
		20	97	93
		<u>10</u>	<u>95</u> <u>93<sup>a</sup></u> <u>95<sup>a</sup></u> <u>91<sup>b</sup></u> <u>91<sup>c</sup></u>	<u>78</u> <u>86<sup>a</sup></u> <u>78<sup>a</sup></u> <u>84<sup>b</sup></u> <u>78<sup>c</sup></u>
		<u>10</u>	<u>98</u>	<u>77</u>
		2	95	25
		<u>1</u>	<u>85</u> <u>29<sup>a</sup></u> <u>85<sup>a</sup></u> <u>78<sup>b</sup></u> <u>85<sup>c</sup></u>	<u>0.7</u> <u>1<sup>a</sup></u> <u>0.7<sup>a</sup></u> <u>4<sup>b</sup></u> <u>0<sup>c</sup></u>
		1	94	10
		0.2	54	17
		<u>0.1</u>	<u>5</u> <u>19<sup>a</sup></u> <u>5<sup>a</sup></u> <u>24<sup>b</sup></u> <u>7<sup>c</sup></u>	<u>11</u> <u>0<sup>a</sup></u> <u>11<sup>a</sup></u> <u>0<sup>b</sup></u> <u>6<sup>c</sup></u>
		0.1	0	0
		0.02	53	21
		<u>0.01</u>	<u>0</u>	<u>4</u>
<b>After 48 hours incubation with NTZdes</b>				
# 30 and # 31	629 $\pm$ 172; 694 $\pm$ 92	0	0	0
		<u>10</u>	<u>98</u>	<u>81</u>
		<u>1</u>	<u>94</u>	<u>5</u>
		<u>0.75</u>	<u>66</u>	<u>16</u>
		<u>0.1</u>	<u>0</u>	<u>16</u>
		<u>0.01</u>	<u>0</u>	<u>4</u>

NTZ = nitazoxanide, NTZdes = Nitazoxanide desacetyl

<sup>a</sup> fresh NTZ (nitazoxanide)

<sup>b</sup> 11 day old NTZ

<sup>c</sup> 17 day old NTZ

Results of different experiments are represented as without, single, double or thick underline.

Bold numbers appeared to be same except for toxicity result with 100 $\mu\text{g/ml}$  NTZ; sponsor has stated that they are from different experiments.

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In another study by the same group of investigators<sup>3</sup>, the *in vitro* activity of nitazoxanide against the same strain of *C. parvum* was examined. The experimental design was the same as that described above. The cultures were incubated for 48 hours in the presence or absence of drug and the cytotoxic effect of the drug on uninfected MDBK cells was determined using the CellTiter 96 aqueous cell proliferation assay kit containing the same reagents (MTS and PMS) as in the previous study (see page 6). However, the incubation period with the reagents was longer (4 hours) than in the previous study (2 hours). The activity of nitazoxanide (1 µg/ml and 10 µg/ml) and paromomycin (2 mg/ml) was within the range of activity of the two drugs observed in the previous study (Tables 7 and 8). In this single experiment, minimal cytotoxicity (11%) was observed at 1 µg/ml nitazoxanide and no toxicity was observed at 10 µg/ml of nitazoxanide or 2 mg/ml of paromomycin. The reason(s) for this discrepancy are unclear.

Table 8: Dose responses for inhibition by paromomycin (PRM) and nitazoxanide (NTZ) of *C. parvum* forms in cell cultures.

Medium or drug(s)	Concn	Parasite count <sup>b</sup>	Growth inhibition		Toxicity <sup>c</sup>	
			%	Score	%	Score
Medium		1,416.4 ± 302	NA <sup>d</sup>	NA	0.0	0
Medium + DMSO		1,231.7 ± 281	NA	NA	1.06	0
PRM	3.2 mM (2 mg/ml)	256.4 ± 64.8	81.9	3	-1.7	0
	1.6 mM (1 mg/ml)	293.6 ± 96.7	79.3	3	7.7	1
	0.8 mM (0.5 mg/ml)	398.3 ± 87.1	71.9	3	2.3	0
	0.4 mM (0.25 mg/ml)	453.9 ± 75	68	2	-7.5	0
NTZ	325 µM (100 µg/ml)	ND <sup>e</sup>	ND	ND	74.1	3
	32.5 µM (10 µg/ml)	87.3 ± 20.1	93	4	-25	0
	3.25 µM (1 µg/ml)	695 ± 173	44	1	11.3	1
	0.325 µM (0.1 µg/ml)	1,105 ± 127	10.3	0	18	1
PRM-NTZ <sup>f</sup>	0.4 mM/3.25 µM	422.4 ± 65	70	2	16	1
	0.2 mM/3.25 µM	619 ± 158	47	1	12.2	1
Medium <sup>g</sup>		ND	NA	NA	20	1
Lysate <sup>h</sup>		ND	NA	NA	17	1

<sup>a</sup> The dose responses were evaluated after all parasites and treatments were applied to the MDBK cells and incubated for 48 h.

<sup>b</sup> Mean number of parasites per field ± standard deviation. Values were determined by counting parasites in 16 fields per well for a total of 4 wells per treatment.

<sup>c</sup> Toxicity values were determined with uninfected cells.

<sup>d</sup> NA, not applicable.

<sup>e</sup> ND, not determined.

<sup>f</sup> Combined treatment with PRM and NTZ.

<sup>g</sup> Toxicity values were determined for cells infected with  $3 \times 10^4$  oocysts/well.

<sup>h</sup> Toxicity values were determined for cells exposed to  $3 \times 10^6$  oocyst equivalents per well.

It should be noted that in all of these studies, parasite count was determined either at 24 or 48 hours of incubation. Later time points were not tested. The enumeration of parasites does not include oocyst(s) present in the culture since the anti-sporozoite polyclonal serum used for performing parasite count does not detect the oocyst wall. The medium was not supplemented with bile salts, or any other factors, nor were the oocysts pretreated with any agent that could maximize the rupture of oocyst wall and release the sporozoites for initiating infection of mammalian cells. Some investigators have reported a reduction or disappearance of oocyst(s) within 24 hours of infection *in vitro* suggesting that the majority of the oocysts were ruptured. However, such an excystation of the oocyst could be influenced by the culture conditions (e.g., the cell lines used as a feeder layer, components of the media, age/viability/infectivity of the oocyst, pretreatment of the oocyst to enhance the excystation of the oocyst, etc.). Also, while the polyclonal serum used for identifying parasites in the cultures was stated to react with all other developmental stages (except oocyst) of the *Cryptosporidium* parasite including trophozoite and

Cryptaz

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merozoite forms, there is no information available to show which developmental forms were actually present in cultures within the time frame tested. Based on literature reports using other cell lines such as Caco-2 or colonic epithelial cells, studies have shown that asexual forms develop between 24 to 48 hours but the development of macrogametes occurs after extended periods of incubation (3 to 5 days).

### 3.2. Activity of nitazoxanide against clinical isolates:

The activity of nitazoxanide was measured *in vitro* against clinical isolates, collected at different timepoints from a single patient with AIDS (CD4 count = 55/mm<sup>3</sup>; and viral load = 104,472 copies/ml plasma)<sup>4</sup>. The patient had failed treatment with paromomycin (500 mg bid for 25 days), azithromycin (1200 mg bid for 27 days) and nitazoxanide (1000 mg bid for 28 days). The isolates Cp 98-1, Cp 98-3, Cp 98-7, Cp 98-8 and Cp 98-10 were collected after treatment of the patient with paromomycin or azithromycin while isolates Cp 99-2 and Cp 99-4 were collected after the patient was treated with nitazoxanide (Table 9). The oocysts from stool samples (stored in 2.5% potassium dichromate for 6 months) were sterilized with sodium hypochlorite, washed and resuspended in Dulbecco's modified eagles medium (DMEM). Excystation was achieved by incubating the oocysts in 0.25% trypsin and 0.75% sodium taurocholate for 60 minutes at 37°C and the sporozoites were collected by centrifugation at 200 x g for 20 minutes. Sporozoites (10<sup>5</sup>) were then added to a monolayer of A-549 feeder cells in DMEM with 10% fetal calf serum and incubated at 37°C for 4 hours in 5% CO<sub>2</sub>. Non-invasive sporozoites and residual oocysts were removed by washing with fresh medium. Different concentrations of the drugs (nitazoxanide, paromomycin and azithromycin) were added to the cultures and incubation continued for an additional 48 hours. Meront and microgamont stages of the parasite were observed within 48 hours of infecting A-549 cells with sporozoites. The number of meronts and gamonts per 50 oil immersion fields was determined. The toxic effect of the drug on the uninfected A-549 cells was examined by the trypan blue dye-exclusion assay. The activity of nitazoxanide against the different isolates obtained before and after treatment of the patient with nitazoxanide was similar (Table 9). Nitazoxanide at 1 µg/ml (low concentration) and 10 µg/ml (intermediate concentration) reduced meronts and gamonts by about 20% and 45%, respectively. The reduction in meronts and gamonts was about 65% at 100 µg/ml nitazoxanide, however, at this concentration the drug was stated to be cytotoxic to the cell line. The raw data on drug toxicity to the cell line were not included. It is of note, however, that studies by another group of investigators<sup>2</sup> showed nitazoxanide at 10 µg/ml to cause about 40% cytotoxicity to MDBK-F5D2 cells (for details see pages 6-11). The activity of nitazoxanide against the isolates was similar to paromomycin. Azithromycin was less effective. No correlation was observed between the clinical outcome and the *in vitro* activity of nitazoxanide against the small number of isolates tested from a single patient.

Table 9: Inhibitory effect of low, intermediate, and high concentrations of different drugs on *Cryptosporidium parvum*, expressed as percent reduction in the number of parasites.

Antimicrobial agent	Strain	Percent reduction in <i>C. parvum</i>			
		Low concentration <sup>a</sup>	Intermediate concentration <sup>a</sup>	High concentration <sup>a</sup>	
Paromomycin	Cp 98-1	18.8	42.8	63.4	
	Cp 98-3	17.9	40.9	64.0	
	Cp 98-7	19.0	41.0	58.3	
	Cp 98-8	16.9	45.6	61.7	
	Cp 98-10	17.3	39.7	56.9	
	Cp 99-2	18.4	42.6	60.8	
	Cp 99-4	17.6	40.7	61.1	
	Azithromycin	Cp 98-1	5.7	15.3	26.5
		Cp 98-3	6.0	16.5	27.8
		Cp 98-7	4.8	17.3	29.5
Cp 98-8		4.6	14.8	24.8	
Cp 98-10		5.1	13.5	26.0	
Cp 99-2		5.3	15.6	25.3	
Cp 99-4		5.8	14.5	30.1	
Nitazoxanide		Cp 98-1	19.0	44.3	67.2
		Cp 98-3	20.1	41.5	65.0
		Cp 98-7	18.7	50.2	68.4
	Cp 98-8	20.9	43.6	60.1	
	Cp 98-10	19.4	47.2	66.3	
	Cp 99-2	17.3	44.0	64.8	
	Cp 99-4	22.6	42.8	65.0	

<sup>a</sup> Low, intermediate, and high concentrations were defined as 0.05 mg/l, 0.5 mg/l, and 1 mg/l for paromomycin; 1 mg/l, 4 mg/l, and 8 mg/l for azithromycin; 1 mg/l, 10 mg/l, and 100 mg/l for nitazoxanide

In another study<sup>5</sup>, the *in vitro* activity of nitazoxanide alone or in combination with azithromycin and rifabutin against *C. parvum* isolates (obtained from 4 AIDS patients) was examined using A-549 feeder cells. Drugs were dissolved in methanol/acetone. The experimental conditions, the method used for excystation of oocysts, and the method for determination of toxicity were same as that described in the previous study. However, the inoculum size was 10 fold lower ( $10^4$  sporozoites). The number of meronts and gamonts per 50 oil immersion field was determined. The results in Table 10 show that nitazoxanide (8  $\mu$ g/ml) decreased the parasite counts by 50%, rifabutin (8  $\mu$ g/ml) and azithromycin (8  $\mu$ g/ml) by 23% and 25%, respectively. The toxicity to A-549 cells ranged from -8.9 to 11.2% at the different concentrations ( $\leq 8 \mu$ g/ml) of the 3 drugs. The activity of nitazoxanide in combination with azithromycin or rifabutin was better than the activity of either drug alone, suggesting an additive effect. The toxicity to A-549 cells was  $\leq 8.4\%$  when the drugs were used in combination. The raw data showing the anti-parasitic activity and the toxicity to A-549 cells in the presence of the different drugs alone or in combination were not included.

Table 10: Inhibitory effects of nitazoxanide in combination with azithromycin and rifabutin on *C. parvum* in A-549 cells: parasite count and percentage reduction versus control plates without antimicrobials.

Drug (mg.L)	Parasite count <sup>a</sup> (% reduction) with nitazoxanide (mg/L)			
	0	0.5	2	8
<b>Azithromycin</b>				
control	40.8 (0.0)	36.0 (11.8)	24.1 (41.0)	18.2 (55.4)
0.5	39.1 (4.2)	34.2 (16.2)	22.7 (44.4)	16.4 (59.9)
2	35.3 (13.5)	30.3 (25.8)	18.3 (55.2)	12.5 (69.4)
8	30.4 (25.5)	26.4 (35.3)	13.6 (66.7)	6.6 (83.9)
<b>Rifabutin</b>				
control	42.9 (0.0)	38.3 (10.8)	25.1 (41.5)	19.3 (55.1)
0.5	40.3 (6.1)	36.0 (16.1)	22.5 (47.6)	17.6 (59.0)
2	38.2 (11.0)	33.1 (22.9)	21.0 (51.1)	15.2 (64.6)
8	33.1 (22.9)	29.5 (31.3)	15.4 (64.2)	8.7 (79.8)

<sup>a</sup>Each value is based on the mean count of three experiments from each isolate.

Number of parasites in the control group ranged from 29.4 to 52.8 (mean 41.1 per 50 oil immersion fields).

### 3.3. Activity of nitazoxanide and its metabolites against a bovine isolate:

The activity of nitazoxanide and its metabolites was measured against the development of *C. parvum* sporozoites using HCT-8 cells as feeder layer in Eagle's modified Dulbecco's medium (BHK21) containing 20% fetal calf serum<sup>6</sup>. Calf oocysts were purified from fecal samples (stored for less than 3 months in potassium dichromate solution) by layering over discontinuous sucrose gradient. The oocysts were washed, treated with sodium hypochlorite for cell surface sterilization, and washed again prior to incubation with 1.5% taurocholic acid at 37°C for 90 minutes for excystation of oocysts. The sporozoites released were separated from non-excysted oocysts and empty shells by filtration using a filter. Sporozoites (1.5 to 2 x 10<sup>5</sup>) suspended in BHK21 medium were added to HCT-8 cell monolayers within 15 minutes of isolation and the cultures incubated for 2 hours at 37°C. The cultures were then supplemented with para-aminobenzoic acid, ascorbic acid, fetal calf serum, etc. Nitazoxanide and its metabolites (tizoxanide and tizoxanide glucuronide) were added at different time intervals after inoculation of sporozoites to the cell culture (0, 2, and 18 hours, see Table 11). The cultures were incubated for up to 46 hours in the presence or absence of drug. The number of parasites was measured in 20 microscopic fields (at X1250 magnification; epifluorescence microscope) by immunofluorescence assay using hyperimmune sera (raised in rats by immunization with the sporozoites in complete/incomplete Freund's adjuvant). It was stated that the antiserum cross-reacts with all stages of *C. parvum* including oocysts.

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Table 11: Time of adding the agents and contact duration in culture for the enzyme immunoassay.

Time of adding agents (hrs from time of adding sporozoites)	Contact duration in culture (hrs)	Corresponding parasite stage(s)
0	2	sporozoite
+2	4	meront with 8 nuclei (asexual stage)
+18	4	meront with 4 nuclei and other sexual stages
+2	46	complete parasite development (asexual and sexual stages)

The sponsor has enumerated the number of trophozoites, meronts (4 or 8 nuclei) and other sexual stages of *C. parvum* in the presence or absence of nitazoxanide and its metabolites (Tables 12 to 14). It would have been useful to confirm the different stages by morphological identification. It appears that the different stages of the parasite enumerated were based on the times indicated in Table 11. The sponsor has stated that photographs depicting the morphology of the different stages after drug treatment were not taken. The number of parasites referred to by the sponsor as sexual meronts (4 nuclei) observed in 18 to 22 hour old control cultures (i.e., drug added at 18 hours and cultures incubated for 4 hours) was relatively small (Tables 12 to 14). In 2-hour and 18-hour old cultures exposed to nitazoxanide ( $\geq 10 \mu\text{g/ml}$ ) for 4 hours, a reduction in the number of parasites was observed (Table 12). The reduction in parasite counts was 88%, when the 2-hour old cultures were exposed to  $10 \mu\text{g/ml}$  nitazoxanide for 46 hours (Table 12). The raw data for the drug toxicity at the different drug concentrations were not included. The sponsor has stated that at concentrations of 10 to  $50 \mu\text{g/ml}$  nitazoxanide, the toxicity to HCT-8 cells ranged from 14% to 39% using the trypan blue exclusion and nitroblue tetrazolium chloride monohydrate reduction assays.

The activity of tizoxanide was similar to nitazoxanide (Table 13), while tizoxanide glucuronide was less effective (Table 14). However, fragilation and/or peeling of HCT-8 cells were observed at tizoxanide and tizoxanide glucuronide concentrations of 10 and  $50 \mu\text{g/ml}$ . No other drugs were used for comparison.

Table 12: Immunofluorescent evaluation of stage dependent anticryptosporidial activity of nitazoxanide on *Cryptosporidium parvum* development on HCT-8 cells<sup>1</sup>.

Hours of incubation with NTZ	Number (mean $\pm$ S.D.) of parasites/20 microscopic fields				
	0 - 2 hours* (2 hours)	2 - 6 hours* (4 hours)	18 - 22 hours* (4 hours)		2 - 48 hours* (46 hours)
	Trophozoites	Meronts 8N	Meronts 4N	Other sexual stages (gametocytes, gametes and oocysts)	All parasite stages
NTZ concentration ( $\mu\text{g/ml}$ )					
0	82.0 $\pm$ 17.0	60.0 $\pm$ 8.5	14.0 $\pm$ 2.8	106.0 $\pm$ 14.1	262.0 $\pm$ 42.4
10	22.0 $\pm$ 2.3	2.3 $\pm$ 1.7	0.5 $\pm$ 1.0	8.0 $\pm$ 6.7	32.0 $\pm$ 10.3
30	28.0 $\pm$ 0.0	4.0 $\pm$ 5.7	0.0 $\pm$ 0.0	13.0 $\pm$ 7.1	45.0 $\pm$ 12.7
50	7.0 $\pm$ 9.9	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	7.0 $\pm$ 9.9	14.0 $\pm$ 19.8

<sup>1</sup> Pooled results of 2 experiments (with each experiment conducted in duplicate for the  $10 \mu\text{g/ml}$  concentration).

\* The time of addition of the drug (duration of drug exposure).

NTZ = nitazoxanide.

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Table 13: Immunofluorescent evaluation of stage dependent anticryptosporidial activity of tizoxanide on *Cryptosporidium parvum* development on HCT-8 cells<sup>2</sup>.

Hours of incubation with TZ	Number (mean ± S.D.) of parasites/20 microscopic fields				
	0 - 2 hours* (2 hours)	2 - 6 hours* (4 hours)	18 - 22 hours* (4 hours)		2 - 48 hours* (46 hours)
	Trophozoites	Meronts 8N	Meronts 4N	Other sexual stages (gametocytes, gametes and oocysts)	All parasite stages
TZ concentration (µg/ml)					
0	124.7 ± 20.4	14.7 ± 13.6	2.0 ± 2.0	120.0 ± 13.1	261.3 ± 28.0
10	67.58 ± 30.8	5.5 ± 6.4	0.5 ± 1.0	36.0 ± 15.7	109.5 ± 25.9
20	20.7 ± 23.7	3.3 ± 3.1	0.0 ± 0.0	5.3 ± 9.2	29.3 ± 22.5
30	8.0 ± 5.7	7.0 ± 4.2	0.0 ± 0.0	0.0 ± 0.0	15.0 ± 1.4
50	12.0 ± 8.7	3.3 ± 3.1	0.0 ± 0.0	0.0 ± 0.0	15.3 ± 11.5

<sup>2</sup> Pooled results of 3 experiments (with one experiment conducted in duplicate for the 10 µg/ml concentration in lieu of the 30 µg/ml concentration).

\* The time of addition of the drug (duration of drug exposure).

TZ = tizoxanide.

Table 14: Immunofluorescent evaluation of stage dependent anticryptosporidial activity of tizoxanide glucuronide on *Cryptosporidium parvum* development on HCT-8 cells<sup>3</sup>.

Hours of incubation with TZg	Number (mean ± S.D.) of parasites/20 microscopic fields				
	0 - 2 hours* (2 hours)	2 - 6 hours* (4 hours)	18 - 22 hours* (4 hours)		2 - 48 hours* (46 hours)
	Trophozoites	Meronts 8N	Meronts 4N	Other sexual stages (gametocytes, gametes and oocysts)	All parasite stages
TZg concentration (µg/ml)					
0	197.5 ± 34.5	27.0 ± 17.1	11.0 ± 13.1	291.0 ± 44.3	526.5 ± 88.4
10	223.0 ± 4.2	34.0 ± 0.0	9.0 ± 7.1	301.0 ± 77.8	567.0 ± 66.5
20	114.0	8.0	4.0	232.0	358.0
30	72.0	4.0	0.0	144.0	220.0
50	44.0 ± 14.0	10.0 ± 5.7	4.0 ± 0.0	76.0 ± 17.0	134.0 ± 36.8

<sup>3</sup>Pooled results of 4 experiments (with the 10 µg/ml and 50 µg/ml concentrations tested in only two of the experiments, and the 20 µg/ml and 30 µg/ml concentrations tested in only one experiment).

\* The time of addition of the drug (duration of drug exposure).

TZg = tizoxanide glucuronide.

In summary, the results show that at 46 hours, nitazoxanide (10 µg/ml) reduced parasite counts in 2-hour old cultures by 88% with 14% toxicity to HCT-8 cells. Exposure of 2-hour old and 18-hour old cultures to nitazoxanide (10 µg/ml) for 4 hours also resulted in a similar reduction in parasite counts. Concentrations of nitazoxanide above 10 µg/ml were cytotoxic (36% - 39%). The concentration of tizoxanide and tizoxanide glucuronide (10 to 50 µg/ml) that reduced parasite counts in 2-hour and 18-hour old cultures also caused fragilation and peeling of HCT-8

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cells. In the absence of complete information, these results show a decrease in parasite counts when nitazoxanide (10 µg/ml) was added to 2-hour and 18-hour old cultures for 4 or 46 hours. However, the effect of the drugs on the different stages is unclear.

In another experiment, the effect of nitazoxanide and its metabolites on cryptosporidial growth was measured using an enzyme immunoassay (EIA)<sup>6,7</sup>. The experimental conditions and the antiserum used for detection of the parasite were same as in the immunofluorescence assay. The percentage inhibition (I) was calculated as follows:

$$I = \frac{(\text{optical density in infected wells containing drug}) - (\text{optical density in uninfected wells containing drug})}{(\text{optical density in infected wells without drug}) - (\text{optical density in uninfected wells without drug})} \times 100$$

The results in Figures 1 to 4 show inhibition of *C. parvum* at different time points in the presence of different concentrations (10 - 50 µg/ml) of nitazoxanide or its metabolites. Please note that at these concentrations, toxicity to HCT-8 cells ranged between 14 - 39% with nitazoxanide and fragilation/peeling of HCT-8 cells was observed with tizoxanide and tizoxanide glucuronide. The results in Figure 1 show 70% inhibition of parasites (sporozoite stage) after exposure of cultures to nitazoxanide (10 µg/ml) for 2 hours. The metabolites, tizoxanide and tizoxanide glucuronide, were less effective against the sporozoites (about 50% and 10% inhibition at a concentration of 10 µg/ml, respectively). Parasite inhibition was lower (40 - 60%) when 2-hour old cultures were exposed to tizoxanide glucuronide and nitazoxanide (10 µg/ml) for 4 hours (Figure 2). The sponsor has stated that asexual meront stages were observed at this time point. Under similar conditions, tizoxanide was less active (5% parasite inhibition; Figure 2). The activity of nitazoxanide and its metabolites seems to be better when the cultures (2-hour old) were exposed to the drug for 46 hours (Figure 4) compared to 4 hours (Figure 2). Exposure of 18-hour old cultures of *C. parvum* (sponsor has stated that the sexual stages are observed at this time point; Figure 3) to nitazoxanide (10 µg/ml) for 4 hours inhibited parasites by about 30% compared to tizoxanide (50%) and tizoxanide glucuronide (98%). Please note that in the previous study examining activity of nitazoxanide in sporozoite infected A-549 cells, the meront and gamont stages were observed at 48 hours post-infection. Although, the sponsor has stated that nitazoxanide is active against the different stages (asexual meronts and sexual stages), it is unclear if the drug has an effect on these different stages as the morphology of the stages of the parasite at the different time points was not described nor were the photographs taken.

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Figure 1: Effects of nitazoxanide, tizoxanide and tizoxanide glucuronide on *Cryptosporidium parvum* sporozoites in HCT-8 cells. Agents were added in cultures at the time of incubation of  $1.5$  to  $2 \times 10^5$  sporozoites per well, and left in culture for 2 hours. Results of EIA detection, performed after a further 46 hour incubation, are expressed as mean ( $\pm 1$  SD) inhibition percentages. Pooled data from 5 independent experiments.

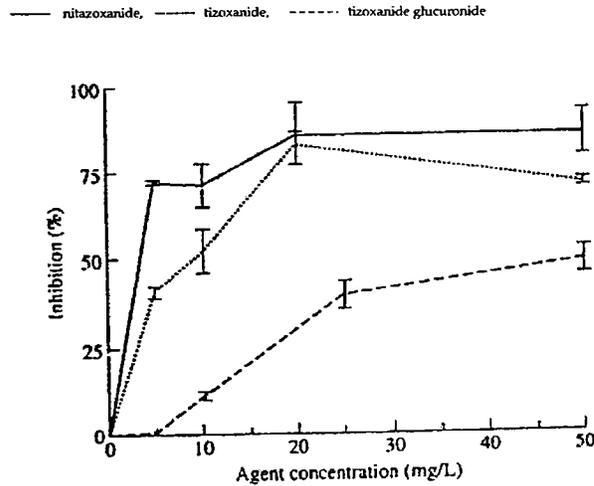


Figure 2: Effects of nitazoxanide, tizoxanide and tizoxanide glucuronide on the asexual development of *Cryptosporidium parvum* in HCT-8 cells. Agents were added in cultures 2 hours after addition of  $1.5$  to  $2 \times 10^5$  sporozoites per well, and left in culture for 4 hours. Results of EIA detection, performed after a further 46 hour incubation, are expressed as mean ( $\pm 1$  SD) inhibition percentages. Pooled data from 3 independent experiments.

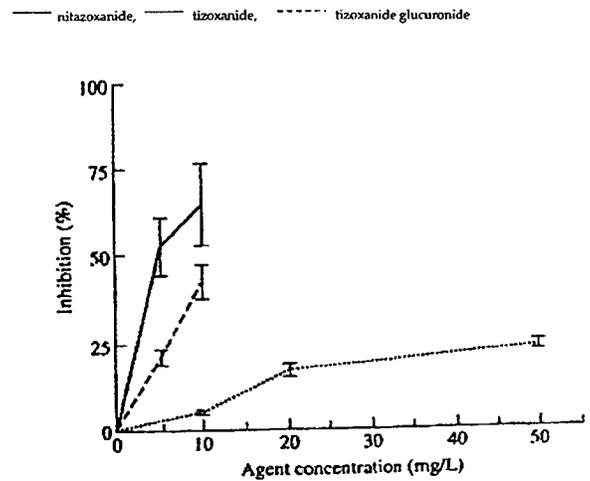


Figure 3: Effects of nitazoxanide, tizoxanide and tizoxanide glucuronide on the sexual development of *Cryptosporidium parvum* in HCT-8 cells. Agents were added in cultures 18 hours after addition of  $1.5$  to  $2 \times 10^5$  sporozoites per well, and left in culture for 4 hours. Results of EIA detection, performed after a further 46 hour incubation, are expressed as mean ( $\pm 1$  SD) inhibition percentages. Pooled data from 5 independent experiments.

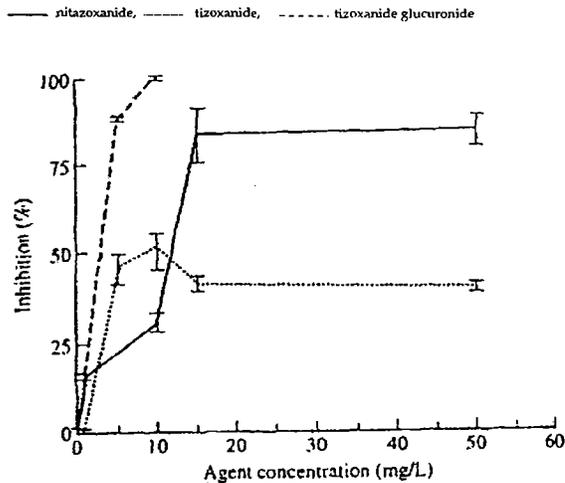
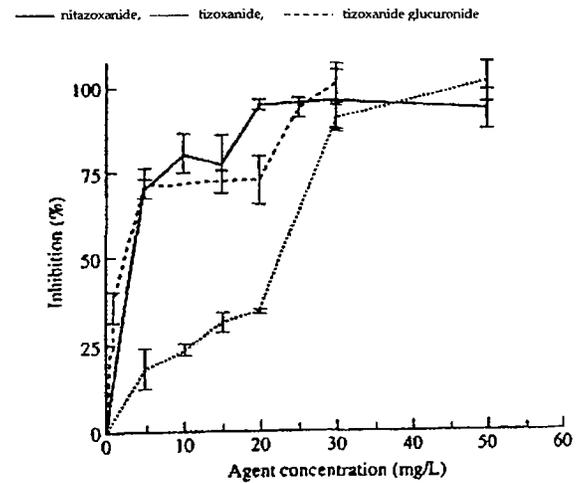


Figure 4: Effects of nitazoxanide, tizoxanide and tizoxanide glucuronide on asexual and sexual stage development of *Cryptosporidium parvum* in HCT-8 cells. Agents were added in cultures 2 hours after addition of  $1.5$  to  $2 \times 10^5$  sporozoites per well, and left in culture for 46 hours. Results of EIA detection are expressed as mean ( $\pm 1$  SD) inhibition percentages. Pooled data from 20 independent experiments.



Based on the above results, the concentration of the drug required to inhibit the different stages of the parasite by 50% (IC<sub>50s</sub>) was determined. The nitazoxanide IC<sub>50</sub> against asexual and sexual stages of the parasite was <12 µg/ml (Table 15). The IC<sub>50</sub> values for tizoxanide was comparable to nitazoxanide against the sporozoite and sexual stages and higher (> 10 fold) against the asexual meront stages (Table 15). Tizoxanide glucuronide was the most active agent against the sexual stages but least effective against the sporozoite stage (Table 15).

Table 15: EIA evaluation of IC<sub>50</sub> (mg/L) of nitazoxanide, tizoxanide, and tizoxanide glucuronide on sporozoite, asexual and sexual stages and complete development of *Cryptosporidium parvum* in HCT-8 cells. Mean values from 6 wells carried out in triplicate.

Agent	IC <sub>50</sub> (mg/L)			
	Sporozoite stage	Asexual stages	Sexual stages	Complete development
Nitazoxanide	5.8	4.7	11.8	1.2
Tizoxanide	8.6	>50	9	22.6
Tizoxanide-glucuronide	45.2	11.7	2.8	2.2

The inhibitory effect of nitazoxanide (10 µg/ml) measured by the two methods i.e., immunofluorescence and EIA were similar (88% by the immunofluorescence assay and 75-85% by EIA; Table 12 and Figure 4). However, a difference in the inhibitory effects of tizoxanide and tizoxanide glucuronide were observed using the two methods. Tizoxanide at a concentration of 10 µg/ml showed 58% inhibition of parasite by immunofluorescence (Table 13) and about 25% inhibition by EIA (Figure 4). In the case of tizoxanide glucuronide, no effect was observed at 10 µg/ml by immunofluorescence (Table 14) and about 70% parasite inhibition was observed by EIA (Figure 4). The sponsor has stated that this difference was possibly due to interference of the color in absorbance measurements at 405 nm by tizoxanide and tizoxanide glucuronide (yellow) or due to effect of different mass of the various stages on the optical density values obtained by EIA. Based on literature review, Nomarski interference contrast microscopy and electron microscopy methods are used for morphological identification of the different stages of *C. parvum* in cell culture. It would have been worthwhile to measure the activity of the drug against different stages of the parasite by these methods.

In another experiment, the effect of nitazoxanide against the different stages of *C. parvum* was examined using electron microscopy. For this, HCT-8 cells grown on tissue culture inserts (— µm pore size) were infected with sporozoites (inoculum same as in previous experiments). Two hours after infection, nitazoxanide (10 µg/ml) was added and the incubation continued for 46 additional hours. Thin sections from the inserts were processed for electron microscopic examination. The results in Table 16 show the number of parasites per 45-46 mm of tissue culture insert. In this single experiment, the number of zygotes in cultures in the presence of nitazoxanide was reduced compared to control cultures without drug (Table 16). However, 2 macrogametes were observed in the nitazoxanide treated cultures but not in control cultures. The number of parasites counted in the cultures (in the presence and absence of drug) is too small to conclusively state the effect of nitazoxanide on the different stages of the parasite. At 10 µg/ml nitazoxanide, the toxicity to HCT-8 cells was 14%. The metabolites and other comparator drugs were not used in this experiment.

Table 16: Transmission electron microscopy counting of *Cryptosporidium parvum* stages in cultures in the presence of nitazoxanide (10 mg/L) for 46 hours.

Culture condition	<i>Cryptosporidium parvum</i> stages (number of parasites/tissue culture insert length)				
	zygote	macrogamete	microgamete	meront 8N	meront 4N
<i>Cryptosporidium parvum</i> infected culture (control)	5	0	1	1	1
<i>Cryptosporidium parvum</i> infected culture with nitazoxanide (10mg/L)	0	2	0	1	0

### 3.4. Effect of protein binding on the activity of nitazoxanide:

Nitazoxanide and tizoxanide were both shown to exhibit high protein binding (> 99%). All the *in vitro* experiments were conducted in the presence of 5% to 20% fetal calf or bovine serum. The specific effect of protein binding on *in vitro* activity was not examined.

## 4. ACTIVITY *IN VIVO*:

The activity of nitazoxanide against *C. parvum* was examined in several animal models such as the (a) suckling mice, (b) scid mice, (c) immunosuppressed rat, and (d) gnotobiotic piglets.

### 4.1. Suckling mice:

Two studies examined the activity of nitazoxanide against *C. parvum* in suckling mice. In one study<sup>8</sup>, 2-day old normal (immunocompetent) suckling mice were infected with *C. parvum* oocysts ( $10^5$ : obtained from infected calves) by the oral route [this study is the same as reviewed previously, NDA# 20-871 (N-000), microbiology review dated 06-01-98]. Oocysts were observed in the rectal swabs obtained on day 2 of infection. Treatment with nitazoxanide (1.3 mg b.i.d. for 7 days) by the oral route was initiated 3 days post-infection. Oocyst count in rectal swabs was evaluated daily for up to 7 days after discontinuation of treatment. The results (expressed as the number of oocyst per 100 oil immersion fields) show that treatment of infected mice with nitazoxanide decreased the oocyst count compared to the untreated control animals (Table 17). No vehicle treated mice were used for comparison of drug activity. Also, no attempts were made to measure the activity of the drug in tissues obtained from different parts of the intestine.

Table 17: Efficacy of nitazoxanide against *Cryptosporidium* infection in experimentally infected mice.

Mice No.	No. of oocyst detected per oil immersion field							
	At 3rd day of treatment		At last day of treatment		At 3rd day post-treatment		At 7th day post-treatment	
	Control group	Treated group	Control group	Treated group	Control group	Treated group	Control group	Treated group
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
Total	35	8.0	4.2	0.0	30	0.0	10	0.0
Mean	3.5	0.8	4.2	0.0	3.0	0.0	1.0	0.0
Efficacy		60%		100%		100%		100%

Note: Treatment started 2 days after the beginning of oocyst shedding.  
 Microscopic examination of oocysts after Ziehl-Neelsen staining.

In another study in suckling mice<sup>9</sup>, the activity of 2 different formulations of nitazoxanide was examined against a bovine strain of *C. parvum* (strain AUCp1). Mice (16-24 per group) were infected with 1 to 2 x 10<sup>5</sup> oocysts by oral gavage. Treatment with 2 different formulations (oral and injectible) of nitazoxanide (100 or 150 mg) by the oral route was initiated 4 hours after infection and continued for 5 additional days. Paromomycin was used as the comparator. Vehicle (water or 1% DMSO) treated animals were used as controls. The number of oocysts in the tissue (pylorus to rectum) was determined at the end of treatment i.e., day 6. The results in Table 18 show the calculated mucosal oocyst levels in treated animals (expressed as mean percentage oocyst counts ± SE compared to controls). The oocysts counts for each of the treatment groups were not included. A 50 to 74% reduction in the mucosal oocyst counts was observed in animals treated with 100 mg/kg of the two nitazoxanide formulations (Table 18). The authors have stated that the powder formulation was obtained from Romark laboratories and contained 70.8% active nitazoxanide while the injectible formulation was obtained from Blue Ridge Pharmaceuticals and contained 20% active nitazoxanide. Additional details on the two formulations were not provided. Although, the powder formulation contained a higher concentration of active nitazoxanide, it was not as effective as the injectible formulation in reducing oocyst counts in the intestinal tissues when administered orally. This could be due to poor bioavailability of the powder formulation. At a higher dose of nitazoxanide (150 mg/kg), the reduction in mucosal oocysts counts was about 95%. The sponsor has stated in the footnote of Table 18 that this dose was moderately toxic and only 14 out of 25 mice survived treatment. Paromomycin (50 mg/kg) was more effective in reducing oocysts counts than nitazoxanide. The shedding of oocysts in the stool was not examined.

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Table 18: Efficacy of NTZ and paromomycin against *C. parvum* in the neonatal mouse model.

Compound	Dose (mg/kg of body weight) <sup>a</sup>	Formulation	Oocyst level (% of control) <sup>b</sup> (mean ± SE)
NTZ	100	Powder	42.3 ± 4.6 <sup>c</sup>
	100	Injectible	26.0 ± 4.5 <sup>c</sup>
	150 <sup>d</sup>	Injectible	4.3 ± 1.0 <sup>c</sup>
Paromomycin	50	Powder	1.2 ± 0.6 <sup>c</sup>

<sup>a</sup>Mice were treated at a constant dose rate daily for 6 days.

<sup>b</sup>Mean numbers of oocysts in treated mice are expressed as percentages of the mean number of oocysts recovered from control mice (taken as 100%).

<sup>c</sup>Treated mice and control mice were significantly different ( $p \leq 0.05$ ).

<sup>d</sup>This dosage appeared moderately toxic; 14 of 25 mice survived the treatment period.

#### 4.2. Scid mice:

The activity of nitazoxanide and desacetyl nitazoaxanide alone or in combination with paromomycin against *C. parvum* was determined using a scid mouse model [this study is the same as reviewed previously, NDA# 20-871 (N-000), microbiology review dated 06-01-98]<sup>10</sup>. Mice were injected intraperitoneally with antibodies to interferon gamma and 2 hours later infected with *C. parvum* oocysts ( $10^7$ ) by the oral route. Six days post-infection, different doses (50, 100 or 200 mg/kg) of nitazoxanide (lot# 12049) were administered twice daily for 10 days by oral gavage. Paromomycin was used as a positive control. Body weight of the mice was not altered by infection or treatment. Mice were examined for the presence of oocyst in the fecal samples at multiple time points during the course of the study. The presence of parasites in the tissues, including the pyloric region of the stomach, mid section of the small intestine, ileum, cecum, proximal colon, and liver/gall bladder was determined on day 20 of challenge (i.e., 5 days after discontinuation of treatment). The results in Figure 5 indicate that in animals treated with nitazoxanide at 100 mg/kg from days 6 to 16 of infection, oocyst shedding decreased in comparison to the vehicle treated mice ( $p < 0.001$ ). The extent of mucosal infection (Figure 6) was also low in mice treated with 100 mg/kg nitazoxanide ( $p = 0.0002$ ). In this study the anticryptosporidial activity of nitazoxanide was comparable to paromomycin.

Figure 5: Oocyst shedding of 6 groups (7 mice each) of weaned male C.B-17 SCID mice infected with  $10^7$  oocysts of the GCH1 isolate. The experimental drug, Phavic-1 (nitazoxanide) was dissolved in DMSO and treatments were administered as follows:

- Group 1 = 200 mg/kg/day Phavic-1,
- Group 2 = 100 mg/kg/day Phavic-1,
- Group 3 = 50 mg/kg/day Phavic-1,
- Group 4 = 200 mg/kg/day Phavic-1 (uninfected control to determine toxicity of drug),
- Group 5 = 2000 mg/kg/day Paromomycin (positive control),
- Group 6 = DMSO (vehicle control).

All treatments were administered orally in two divided doses/day for 10 days.  
 The mice were maintained for an additional 5 days following the cessation of treatment.

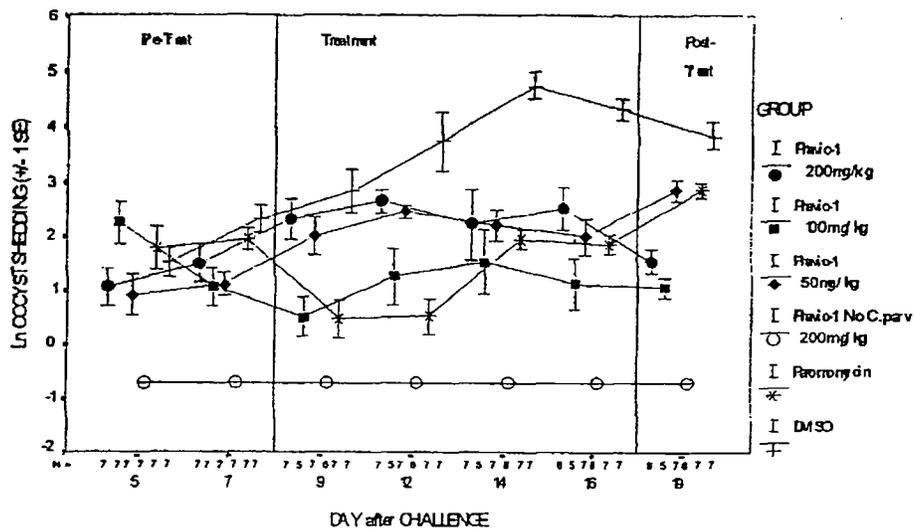
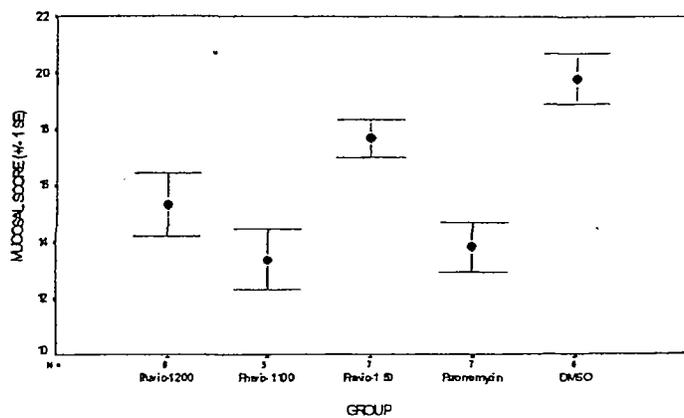


Figure 6. Extent of mucosal infection on day 20 of infection, expressed as a mean total score of 5 intestinal sites (pyloric region of the stomach, mid-small intestine, ileum, cecum and proximal colon) for the different treatment groups described in Figure 5.



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The anti-cryptosporidial activity of nitazoxanide in the groups of animals treated with either 200 mg/kg or 50 mg/kg was statistically similar. The sponsor stated that this could be due to the fact that 50 mg/kg was an insufficient dose and 200 mg/kg could cause subclinical toxicity by reducing or eliminating normal gut flora.

Another experiment conducted in scid mice used the same design as the first one but a different lot of nitazoxanide (lot # 001). The study was also published by Theodas *et al.*, (1998)<sup>3</sup> and has been reviewed earlier [NDA# 20-871 (N-000), microbiology review dated 06-01-98]. Paromomycin alone or in combination with nitazoxanide was effective in reducing the parasite load during the period of drug administration (Figures 7 and 8). However, oocyst count increased in all the groups after discontinuation of treatment (Figure 7). Unlike the first experiment, nitazoxanide was not effective in reducing the shedding of oocyst in the stool (Figure 7) nor in decreasing the extent of mucosal infection (Figure 8) at the doses tested i.e., 200 and 100 mg/kg. The reason for this variability in activity between the two different experiments is unclear. Although differences in the particle size were observed between the 2 lots, the specifications of the lot #001 were within the range of those observed among the various lots used in the clinical trials (for details see chemistry review to the original NDA# 20-871)

Figure 7: Oocyst shedding of 7 groups (7 mice each) of weaned male C.B-17 SCID mice infected with  $10^7$  oocysts of the GCH1 isolate. The experimental drug, Phavic-1 (nitazoxanide) was dissolved in 100% DMSO and administered orally in two divided doses of 30  $\mu$ l each per day. Paromomycin was dissolved in drinking water to a concentration of 10 mg/ml. Treatments were administered as follows:

Group 1 = 200 mg/kg/day Phavic-1,

Group 2 = 100 mg/kg/day Phavic-1,

Group 3 = 200 mg/kg/day Phavic-1 + 2500 mg/kg/day paromomycin,

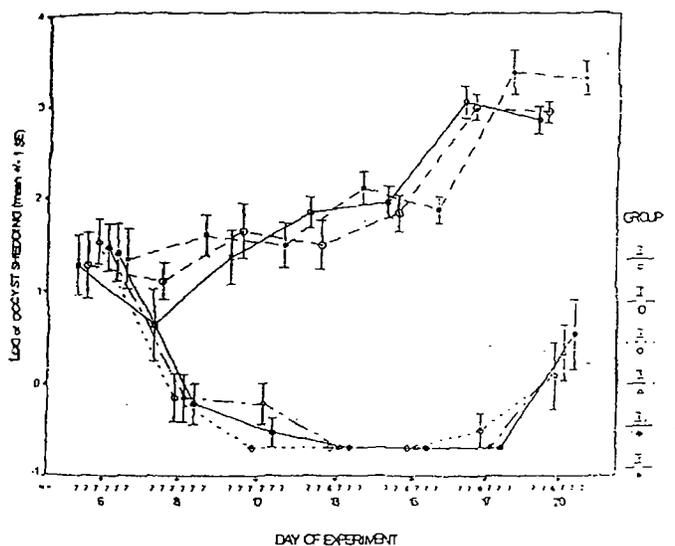
Group 4 = 100 mg/kg/day Phavic-1 + 2500 mg/kg/day paromomycin,

Group 5 = 2500 mg/kg/day Paromomycin (positive control),

Group 6 = 200 mg/kg/day Phavic-1 + 2500 mg/kg/day paromomycin (uninfected control for determining toxicity)

Group 7 = 30  $\mu$ l DMSO orally twice a day (vehicle control).

The mice were maintained for an additional 5 days following the cessation of treatment



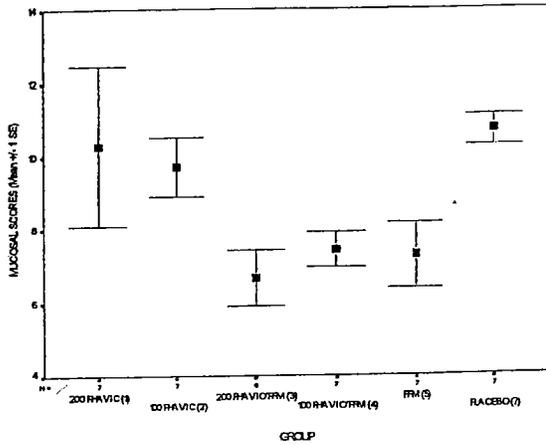


Figure 8: Extent of mucosal infection on day 20 of infection, expressed as a mean score of 5 intestinal sites (pyloric region of the stomach, mid-small intestine, ileum, cecum and proximal colon) for the different treatment groups described in Figure 7.

In another experiment, the activity of desacetyl nitazoxanide alone or in combination with paromomycin against *C. parvum* was examined. The experimental design was similar to that described above except that nitazoxanide (the parent compound) was not used for comparison. The results in Figures 9 and 10 show that desacetyl nitazoxanide does not exhibit any activity against *C. parvum* as measured by presence of oocysts in the stool as well as the mucosal scores. Paromomycin, however, significantly decreased the shedding of oocyst in the stool as well as the mucosal scores in the gastro-intestinal tract. The activity of desacetyl nitazoxanide in combination with paromomycin against *C. parvum* was similar to paromomycin alone.

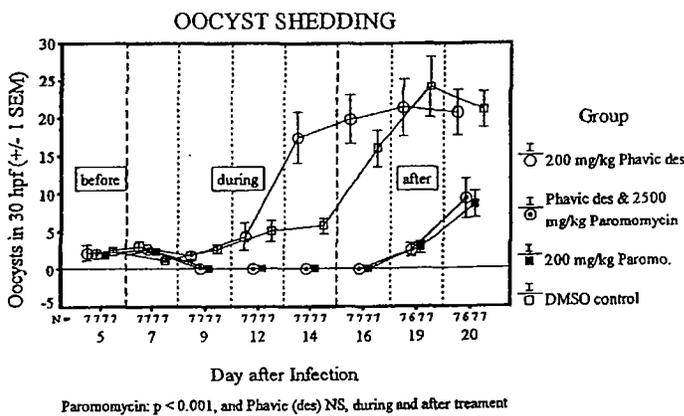


Figure 9: Oocyst shedding of 4 groups (7 mice each) of weaned male C.B-17 SCID mice infected with  $10^7$  oocysts of the GCH1 isolate. Treatments were administered as follows:

- Group 1 = 200 mg/kg/day Nitazoxanide (des),
- Group 2 = 200 mg/kg/day Nitazoxanide (des) + 2500 mg/kg/day paromomycin,
- Group 3 = 2500 mg/kg/day paromomycin dissolved in drinking water,
- Group 4 = 30  $\mu$ l DMSO orally twice a day (vehicle control).

The mice were maintained for an additional 5 days following the cessation of treatment

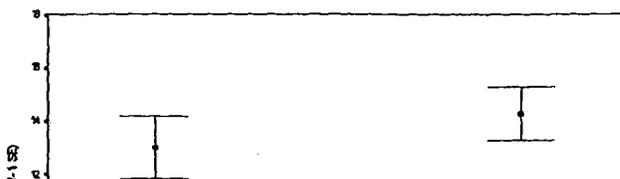


Figure 10: Extent of mucosal infection on day 20 of infection, expressed as a mean score of 5 intestinal sites (pyloric region of the stomach, mid-small intestine, ileum, cecum and proximal colon) for the different treatment groups described in Figure 9.

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#### 4.3. Immunosuppressed rats:

In immunosuppressed rats, the activity of nitazoxanide against *C. parvum* was compared to paromomycin and sinefungin<sup>11</sup>. The rats were immunosuppressed by subcutaneous administration of 25 mg hydrocortisone acetate twice a week for 5 weeks before infection and immunosuppression maintained for 3 additional weeks. Rats were infected with  $10^5$  oocysts (obtained from infected calves) by oral gavage. On day 7 post-infection, the drugs (dissolved in 5% DMSO) were administered orally three times daily for 8 days. Vehicle-treated animals were used as controls. The fecal samples collected at different time points for up to 21 days post-infection were examined using a phase contrast microscope (after carbolfuchsin staining) and the oocyst counted per 10 microscopic fields (X400 magnification). The percentage inhibition of oocyst in drug treated animals was compared to the control groups. The results in Table 19 show that the oocyst counts in the control animals decreased with time, suggesting that the infection was cured spontaneously despite immunosuppression. In rats treated with nitazoxanide (100 mg and 200 mg), a 2-fold reduction in oocyst counts was observed compared to vehicle-treated control animals. The activity of paromomycin was similar to nitazoxanide while sinefungin was 2-fold more active. The oocyst counts increased in the sinefungin and paromomycin treated animals after discontinuation of treatment while no increase was observed in the nitazoxanide treated group. The mean oocyst counts for all treatment groups on days 16 to 21 (i.e., 1 to 6 days after discontinuation of drug treatment) appear to be similar to the control group (Table 19). However, the sponsor has stated that the oocyst counts in nitazoxanide treated animals and controls were significantly different.

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Table 19: Mean number of oocysts shed per 10 microscopic fields (x 400 magnification) by treatment group.

Drug (mg/kg)	Period									
	D7	D8-9	D10-11	D12-13	D14-15	D16-17	D18-19	D20-21	D8-15 (during Rx)	D16-21 (After Rx)
<b>NTZ 50</b>										
Mean	83.55	38	33.33	26.6	12.45	9.1	5.45	4.2	27.6	6.19
SD	(52.9)	(19.3)	(19.9)	(22.4)	(12.5)	(7.9)	(5.46)	(4)	(20.9)	(6.4)
% inhibition	0%	31.7%	0%	24.4%	44.1%	16.5%	48%	50%	24.2%	37.9%
P*	.1805	.2338	.6228	.1641	.0351	.8148	.0184	.0419	.0444	.0110
<b>NTZ 100</b>										
Mean	87.45	28.2	15.2	14.45	10.65	6.65	3.3	2.75	17.12	4.23
SD	(56.1)	(27.1)	(12.5)	(11.9)	(11)	(8)	(2.9)	(1.86)	(18.0)	(5.3)
% inhibition	0%	49.3%	50.6%	58.9%	52.2%	39%	68.5%	67.2%	53.0%	57.6%
P*	.1595	.0053	.0038	.0020	.0158	.2577	.0006	.0050	<.0001	<.0001
<b>NTZ 200</b>										
Mean	81.85	28	19.45	10.3	5.15	7.75	3.9	2	15.73	4.55
SD	(42.6)	(18.4)	(15.4)	(7.6)	(4.4)	(7.18)	(3)	(11.4)	(15.3)	(5.1)
% inhibition	0%	49.3%	36.8%	70.7%	76.9%	28.9%	2.8%	76.2%	56.8%	54.4%
P*	.1016	.0186	.0523	.0005	.0002	.5187	.0044	.0009	<.0001	.0002
<b>Paromomycin 100</b>										
Mean	81.25	61	20	1.76	3.9	5.87	6.72	9.35	21.67	8.15
SD	(32.5)	(49.7)	(22.3)	(2)	(5.5)	(10)	(10.9)	(11.5)	(34.7)	(9.7)
% inhibition	0%	0%	35%	95%	82.5%	46.1%	36%	0%	40.5%	18.3%
P*	.2298	.6701	.2904	.0093	.0153	.1861	.7014	.8313	.0035	.2310
<b>Sinefungin 10</b>										
Mean	79.25	15.75	13.75	1.77	1.05	1.7	3.1	9.65	8.08	4.82
SD	(91.6)	(19.1)	(17.6)	(3.1)	(1.96)	(3.2)	(5.9)	(18.9)	(13.7)	(11.1)
% inhibition	0%	71.7%	55.3%	94.9%	95.3%	84.4%	70.4%	0%	77.8%	51.7%
P*	.7862	.0364	.1042	.0051	.0049	.0150	.0331	.1865	<.0001	.0006
<b>Controls</b>										
Mean	60.9	55.7	30.78	35.2	22.3	10.9	10.5	8.4	36.4	9.97
SD	(38.3)	(41)	(16.9)	(21.4)	(16.3)	(9.7)	(7)	(6.9)	(28.7)	(8.0)

\* Mean oocysts shed for the treatment group compared to mean oocysts shed for controls, non-parametric Wilcoxon rank sum test

The ileum tissue from nitazoxanide (different doses) treated animals only was examined for the presence of parasite after hematoxylin-eosin staining. A 2 to 4 fold decrease in mucosal infection was observed in nitazoxanide treated animals compared to controls (Table 20). The variability in the number of parasites among the different rats within a group was not shown. Paromomycin and sinefungin treated animals were not used for comparison of drug activity.

Table 20: Extent of mucosal infection in sample of rats from the nitazoxanide-treated and untreated control groups presented in comparison with oocysts shedding on days 20-21.

Dose (mg/kg/day)	Mean no. of oocysts/10 fields days 20-21	Inhibition %	No. of parasites in 10 villi of the ileum	Inhibition %
NTZ 50	4.20	50%	22	46%
NTZ 100	2.75	67%	17	59%
NTZ 200	2.00	76%	10	76%
Untreated control	8.4	-	41	-

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4.4. Gnotobiotic piglets:

The effect of nitazoxanide treatment was examined in gnotobiotic piglets (24 hour old, derived from 2 litters) infected with the GCH1 strain of *C. parvum* [this study was reviewed previously NDA# 20-871 (N-000), microbiology review dated 06-01-98]<sup>12</sup>. The piglets were inoculated orally with 2 x 10<sup>7</sup> oocysts. At 56 hours post-infection, the animals were treated with nitazoxanide (125 mg/kg b.i.d. for 11 days) by the oral route (mixed with milk). Activity of nitazoxanide was compared with paromomycin (250 mg/kg bid for 11 days) or vehicle administered by the same route. Fecal oocyst counts and extent of mucosal infection in 12 intestinal sections (pylori of stomach, duodenum, 7 other small intestinal sections, terminal ileum, cecum, and colon) were determined. Body weights were not significantly altered in animals from either of the groups, however, results in Table 21 show paromomycin to be more effective than nitazoxanide with respect to reduction in oocyst or mucosal scores. Nitazoxanide decreased the mucosal scores by about 50% (Figure 13). However, due to the high variability in the oocyst score there appears to be no significant difference in nitazoxanide treated and vehicle treated groups at these time points (Figure 14). While the sponsor has analyzed the cumulative oocyst shedding over a period of 13 days and has shown a decrease in the number of oocysts in the group treated with nitazoxanide compared to infected controls (p = 0.0039; Figures 15 and 16), the statistical significance of the difference between the nitazoxanide treated vs vehicle treated animals at different time points was not determined. The incidence of diarrhea also appears to be same in the 2 groups. The sponsor has stated that all the infected animals irrespective of treatment, exhibited watery diarrhea which was white gray in color. Diarrhea was also observed in uninfected piglets treated with nitazoxanide although the appearance of the diarrhea (pasty and yellow in color) was different. The diarrhea resolved in piglets administered paromomycin.

Table 21: Oocyst shedding and extent of mucosal infection in *C. parvum* infected gnotobiotic piglets.

Fig #	Days after challenge												Mean Mucosal Scores (SD)
	2*	3	4	5	6	7	8	9	10	11	12	13	
1. Placb.	0/d	0/d	1/d	3/d	4/d	3/d	2/d	2/d	2/d	2/d	2/d	3/d	16
2. Placb.	0/d	0/d	4/d	4/d	3/d	3/d	3/d	4/d	3/d	2/d	2/d	3/d	15
3. Placb.	1/d	2/d	5/d	3/d	2/d	2/d	4/d	3/d	5/d	4/d	2/d	3/d	10
4. Placb.	1/	2/d	2/d	3/d	2/d	3/d	5/d	3/d	4/d	3/d	3/d	3/d	6
5. Placb.	0/d	1/d	2/d	1/d	3/d	2/d	2/d						N/A 11(±4.6)
6. NTZ	0/d	1/d	1/d	4/d	3/d	2/d	2/d	3/d	3/d	3/d	2/d	2/d	7
7. NTZ	0/	2/d	2/d	4/d	3/d	2/d	3/d	2/d	1/d	2/d	1/d	1/d	3
8. NTZ	0/	1/d	2/d	3/d	2/d	1/d	3/d	1/d	1/d	1/d	1/d	1/d	5
9. NTZ	1/d	3/d	2/d	2/d	2/d	2/d	3/d	2/d#					N/A
10. NTZ	0/d	0/d	2/d	1/d	2/d	2/d	3/d#						N/A 5(±2)
11. PRM	0/d	1/d	2/d	2/d	0/d	1/d	1/d	0/1	0/1	0/1	0/1	0/1	0
12. PRM	1/d	2/d	2/d	2/d	1/d	0/d	0/1	1/1	0/1	0/1	0/1	0/1	1
13. PRM	0/	1/d	2/d	1/d	0/d	0/d	0/d						N/A 0.5(±.2)
14. Cont.	0/	0/	0/	0/d*	N/A								
15. Cont.	0/	0/	0/	0/d*	N/A								
16. Cont.	0/	0/	0/	0/d*	0/d*	0/d*	0/d*						N/A

Placebo = milk  
NTZ = nitazoxanide  
PRM = paromomycin  
Control = uninfected control treated with 250 mg/kg NTZ

\* Onset of treatment 56 hours after challenge coinciding with the onset of diarrhea in more than 50% of piglets.

Oocyst shedding: 0 = no oocysts detected in fecal smear; 1 = ≤10 detected in the entire smear; 2 = ≤25; 3 = ≤50;

4 = ≤100; and 5 = ≥100.

d = watery white-grey diarrhea (representing maldigestion and malabsorption); d\* drug-related yellow diarrhea (normal digestion); 1 = loose feces (higher water content than normal).

# piglet euthanized because of poor health associated with diarrhea.

Figure 13: Individual mucosal scores of piglets in varying treatment groups. Despite the small numbers, mucosal scores were significantly related to group ( $p = 0.0355$ ).

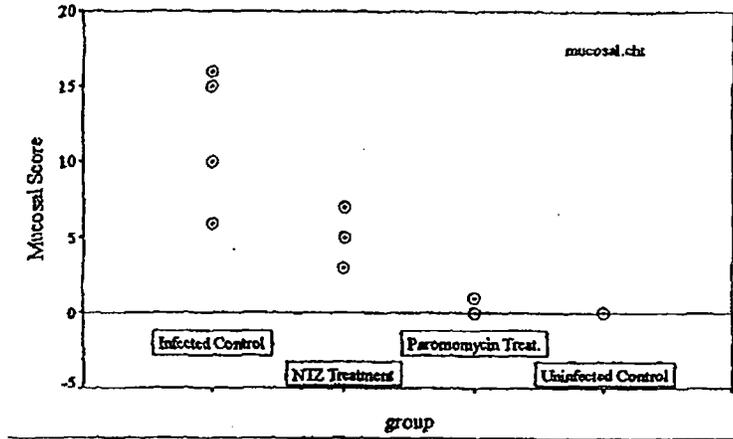


Figure 14: Effect of nitazoxanide on oocyst score in gnotobiotic piglets.

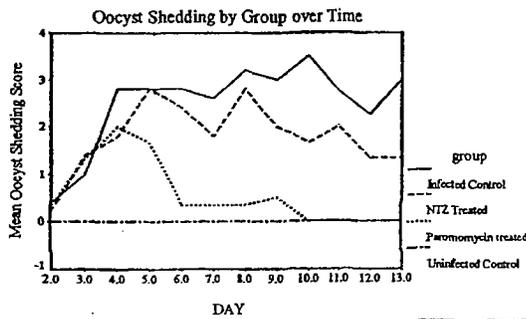
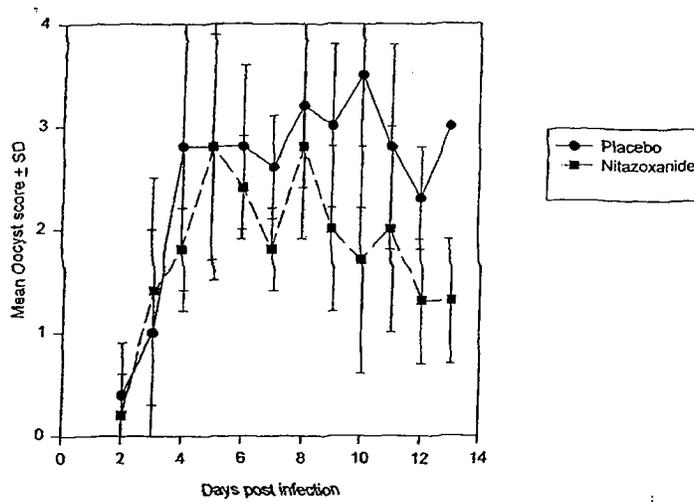
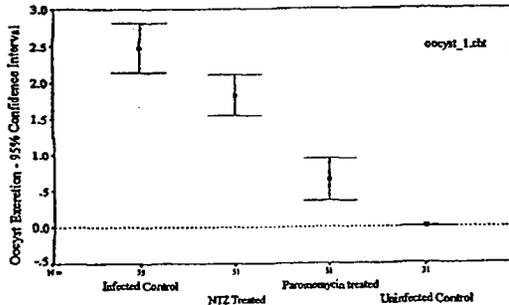


Figure 15: The mean daily oocyst excretion score by group.

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Figure 16: Overall mean oocyst excretion score in each group. Each group is significantly different from each of the others. These differences are detectable despite inclusion of the score from the first few days of treatment, when little effect of treatment would have been expected.



groups	Mann-Whitney U- Wilcoxon Rank Sum test
control uninfected and paromomycin treated	p < 0.0001
paromomycin vs. nitazoxanide treated	< 0.0001
nitazoxanide treatment vs. infected control	= 0.0039
paromomycin vs. infected control	< 0.0001

In another study in gnotobiotic piglets<sup>3</sup>, the effect of nitazoxanide on oocyst shedding was examined using the same experimental design as the previous study except that two different doses of nitazoxanide (62.5 or 125 mg b.i.d for 11 days) were tested. The results show that the oocyst scores in animals treated with nitazoxanide (125 or 250 mg) were similar to that of untreated animals (Figure 17). The activity of paromomycin against *C. parvum* was same as that observed in the previous study. It was stated that nitazoxanide induced diarrhea in these animals whereas paromomycin was effective in resolving diarrhea.

Figure 17: Fecal oocyst excretion scores of infected piglets treated with nitazoxanide at either 125 (o) or 250 ( ) mg/kg/day, placebo (■) or paromomycin at 500 mg/kg/day (▲). In multiple regression analysis, the oocyst excretion score was found to be significantly related to treatment group, with highest scores being observed in the placebo group, followed by the lower-dose nitazoxanide group and then the higher dose nitazoxanide group, and the lowest scores being observed in the paromomycin group ( F = 42.507; p < 0.001). Further comparison revealed that during days 7 through 14, the scores for the piglets treated with nitazoxanide 250 mg/kg/day were significantly lower than those for the placebo treated piglets ( Z = -3.258; p = 0.001, two tailed Wilcoxon signed rank test). In contrast, subgroup comparison of the infected placebo-treated control piglets and piglets treated with nitazoxanide at 125 mg/kg/day did not reveal any significant difference. Values are means ± standard errors of the means (SEM).

