CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	209570
Link to EDR	EDR Link
Submission Date	12/29/2016
Submission Type	Priority; NME
Brand Name (Proposed)	(b) (4)
Generic Name	Benznidazole
Dosage Form and Strength	Tablet, 100 mg and 12.5 mg
Route of Administration	Oral
Proposed Indication	Treatment of Chagas disease
Applicant	Chemo Research S.L.
Associated IND	IND 118976
OCP Review Team	Abhay Joshi, Ph.D.; Yongheng Zhang, Ph.D.; Luning (Ada) Zhuang, Ph.D.; Jeffry Florian, Ph.D.
OCP Final Signatory	John Lazor, Pharm.D. *Office of Clinical Pharmacology Division IV Director*

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1. EXECUTIVE SUMMARY

This is an original NDA submitted by Chemo Research, SL on 12/29/2016 seeking approval of [(b) (4)] (Benznidazole tablets, 100 mg and 12.5 mg) for the treatment of Chagas disease, which is a zoonosis caused by the parasite *Trypanosoma cruzi (T. cruzi)*. Benznidazole (BNZ) has been in use as a treatment of *T. cruzi* infection since the 1970s. BNZ is not approved in the US, however, the drug can be obtained from the Center for Disease Control and Prevention (CDC) under an investigational protocol for compassionate use.

In this NME 505(b)(2) submission, the Applicant has provided publications and subject level data from previously completed placebo-controlled trials, analyses of prospective and retrospective observational studies, reviews of BNZ use in the treatment of Chagas disease, and published case study reports. The Applicant has also provided reports from in vitro studies that assessed the role of hepatic enzymes and transporters in BNZ disposition as well as from in vitro studies that evaluated BNZ's drug interaction potential.

From the submitted publications and clinical trials, the Applicant has identified six key studies, which are presented in Section 4.6.6, that are supporting this application. For the key studies, the Applicant has obtained access to datasets and has performed analyses to obtain information on the efficacy of BNZ in the treatment of Chagas disease. From the key studies, two published studies (Sosa-Estani et al., 1998 and De Andrade et al., 1996) are of primary interest as they provided the pivotal evidence of effectiveness and safety. The formulation used in these two randomized trials was the 100 mg BNZ tablets known as the Radanil formulation, which was manufactured by Hoffman-LaRoche. Given that the Radanil formulation is no longer available and none of the to-be-marketed (TBM) formulations (i.e., 100 mg and 12.5 mg tablets) was used in the clinical trials from which the supportive evidence of effectiveness is based upon, the Applicant has provided an exposure bridge. The exposure bridge is relying on the cross-study comparison of the BNZ bioavailability from the Radanil 100 mg tablet, which was reported in 1979, and the single dose administration of the two TBM tablet formulations, which were evaluated by the Applicant in the two submitted clinical pharmacology studies. Therefore, the primary focus of the review is evaluation of the adequacy of provided exposure bridge between the TBM formulations and Radanil formulation.

In addition, the two aforementioned published studies (Sosa-Estani and De Andrade) evaluated the BNZ treatment in pediatric patients who were 6 – 12 years of age. From a separate study (Altcheh et al., 2014), which evaluated the Radanil formulation in pediatric patients aged 2 – 12 years of age, BNZ exposure data are also available. Therefore, the clinical pharmacology team utilized the available information to derive BNZ dosing recommendation for pediatrics 2 – 6 years of age. Given the limited information regarding the exposure-response efficacy and safety relationships of BNZ treatment in Chagas disease, the aim of the analyses was to identify a BNZ dosage regimen that is expected to provide matching BNZ systemic exposures in pediatrics 2 – 6 years of age to the reported exposures in pediatrics 6 – 12 years of age. Based on the results from the pharmacometrics analyses, the team recommends a weight-based dosing regimen for pediatric patients (2 - 12 years of age).

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1.1   Recommendations

The Office of Clinical Pharmacology has reviewed the information provided by the Applicant in NDA 209570 and considers the information to be acceptable from a clinical pharmacology perspective.

The application is approvable from a clinical pharmacology perspective, provided that (1) an agreement is reached between the Applicant and the Agency on the Post-Marketing Requirements and Commitments listed in this review, (2) an agreement is reached on the dosing regimen, and 3) acceptable findings from the pending clinical site inspection report for Studies LPR1747-101 and LPR1747-102.

Table 1.1-1: Summary of OCP's Recommendations & Comments on Key Review Issues

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Two randomized trials provide pivotal evidence of effectiveness. These trials were conducted in children (6 - 12 years) with Chagas disease using Radanil formulation[i].
General dosing instructions	The Applicant's proposed dosing in _____ (b) (4) children is based on the dosing utilized in literature studies provided as part of this submission. The proposed dosing from the applicant is _____ (b) (4) _____ (b) (4) The clinical pharmacology review team recommends different dosing based on the available pediatric information with the Radanil formulation. For the children 2 to 12 years of age, the recommended dose is 5 - 8 mg/kg/d. Given that the safety/effectiveness data are only available for pediatrics 6 – 12 years and exposure data available for pediatrics 2 – 12 years, the clinical pharmacology review team limited the dosing recommendations to pediatric patients from 2 to 12 years of age. A dosing instruction table below is provided to guide physician and patients on this weight-based dosing. <table><tr><td>Body weight range (kg)</td><td>BID Dose (mg)*</td></tr><tr><td>< 15</td><td>50</td></tr><tr><td>15 - < 20</td><td>62.5</td></tr><tr><td>20 - < 30</td><td>75</td></tr><tr><td>30 - < 40</td><td>100</td></tr><tr><td>40 - < 60</td><td>150</td></tr><tr><td>≥ 60</td><td>200</td></tr></table>

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[i] Two randomized trials that provide pivotal evidence of effectiveness are two published studies: Sosa-Estani et al., 1998 and De Andrade et al., 1996. Roche formulation was used in these studies; however, in De Andrade 1996 study, the 100 mg BNZ tablets (Roche, Brazil) were reformulated into 50 mg tablets by the Goiás State Chemical Industry for prescription to children. No information is available on the methodology of reformulation.

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	* The dose should be administered twice a day approximately 12 hours apart for 60 days
Dosing in patient subgroups (intrinsic and extrinsic factors)	A mass balance/ADME study for BNZ in humans is pending. Therefore, BNZ treatment should not be recommended in Chagas disease patients with impaired renal and or hepatic function(s)
Labeling	Recommendations to be conveyed to the Applicant are presented in Section 2.4
Exposure bridge between the TBM and clinical trial formulations	The TBM BNZ formulations were not used in the clinical trials that provide the pivotal evidence of effectiveness. The exposure bridge relies on a cross-study PK comparison between a historical 1979 PK study using Radanil formulation and two PK studies using the TBM formulations

1.2   Post-Marketing Requirements and Commitments

PMC or PMR	Key Issue(s) to be Addressed	Rationale	Time Line
☒PMR ☐PMC	A mass balance/ADME study for BNZ in humans is pending	Lack of information on the ADME properties of BNZ in humans	4Q 2017 – 1Q 2018
		(b) (4)	To be decided
			To be decided

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2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1   Pharmacology and Clinical Pharmacokinetics

Benznidazole (BNZ) is a nitroimidazole antiparasitic agent. The following is a summary of the clinical pharmacokinetics of BNZ:

Absorption: Absolute bioavailability has not been determined. The observed mean T_{max} following a single dose of ▨▨(b) (4)▨▨ 100 mg tablet in healthy subjects ranged between 2 - 4 hours. No significant food effect was observed on the systemic exposure to BNZ from ▨▨(b) (4)▨▨ 100 mg tablet.

Distribution: BNZ plasma protein binding is approximately 44 % and BNZ is a substrate for P-glycoprotein (P-gp). Body weight was identified as significant covariate on apparent volume of distribution (Vd/F).

Metabolism & Excretion: The mean elimination half-life of BNZ following a single dose in healthy subjects was about 12 - 13 hours. Body weight was identified as significant covariate on apparent clearance (CL/F).

2.2   Dosing and Therapeutic Individualization

2.2.1   General dosing

The Applicant's proposed BNZ dosing regimen for ▨▨▨▨▨▨(b) (4)▨▨▨▨▨▨ ▨▨▨▨▨▨(b) (4)▨▨▨▨▨▨ The Applicant's proposed BNZ dosing regimen for ▨▨▨▨▨▨(b) (4)▨▨▨▨▨▨ ▨▨▨(b) (4)▨▨▨.

The clinical pharmacology review team recommends ▨▨(b) (4)▨▨ dosing ▨▨▨(b) (4)▨▨▨ based on the available efficacy (6 – 12 years) and pharmacokinetic (2 – 12 years) data in pediatric patients where the Radanil formulation was utilized. Firstly, as there is no information for bridging between the ▨▨▨▨▨▨(b) (4)▨▨▨▨▨▨ ▨▨▨▨▨▨(b) (4)▨▨▨▨▨▨ Second, as there was only efficacy data in pediatrics 6 – 12 years and exposure data in pediatrics 2 – 12 years, the clinical pharmacology review team limited dosing recommendations to such patients ▨▨▨(b) (4)▨▨▨ ▨▨▨(b) (4)▨▨▨ Given the limited information regarding the exposure-response efficacy and safety relationships of BNZ treatment in Chagas disease, dosing in pediatrics 2 – 6 years was based on matching BNZ systemic exposures to the exposures in pediatrics 6 – 12 years. Based on the findings, the team recommends a weight-based dosing regimen for pediatric patients (2 - 12 years of age). Finally, a slightly wider dosing range is proposed based on the study data and dosing by body weight tiers are provided to assist clinicians in prescribing. This information is summarized above in Table 1.1-1 (Section 1.1).

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2.2.2   Therapeutic individualization

Other than weight based dosing for a specific age range, no therapeutic individualization is proposed. Since the ADME information of BNZ in humans is not yet available, BNZ dosing will not be recommended for Chagas disease patients with renal and or hepatic impairment.

2.3   Outstanding Issues

The PMR/PMC list in Section 1.2 describes the outstanding issues.

2.4   Summary of Labeling Recommendations

The following Clinical Pharmacology pertinent labeling recommendations/concerns will be shared with the Applicant:

Section 2 Dosage and Administration

- Once an agreement is reached with the Applicant on a weight-based dosing regimen for pediatric patients (2-12 years of age), pertinent changes to this section will be recommended.

Section 8.6 Use in Patients with Hepatic Impairment

- Use of ⬚ (b) (4) is not recommended for patients with hepatic impairment

Section 8.7 Use in Patients with Renal Impairment

- Use of ⬚ (b) (4) is not recommended for patients with renal impairment

Section 12.2 Pharmacodynamics

- (b) (4)

Section 12.3 Pharmacokinetics

- Specific revisions to the format and content of this section will be recommended.
- There were no PK studies or analyses in various subpopulations. Therefore, as recommended in the FDA guidance [ii], lack of PK information from these populations will be recommended to be presented in one or more summary sentences instead of listing all of the subpopulations under different subheadings.
- Inclusion of the information on BNZ as a potential CYP enzyme/transporter substrate, inducer, and inhibitor will be recommended.

[ii] Clinical Pharmacology Section of Labeling for Human Prescription Drug and Biological Products — Content and Format Guidance for Industry, Dec 2016.

Reference ID: 4108775

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

The Applicant is seeking approval of benznidazole (BNZ) for the treatment of Chagas disease via the 505(b)(2) pathway. BNZ is not approved by FDA. However, it has been used in the treatment of *T. cruzi* infection since the 1970s. In US, the drug can be obtained from the Center for Disease Control and Prevention (CDC) under an investigational protocol. BNZ is one of two Chagas disease treatments included in the WHO Essential Medicines List. The other listed treatment is nifurtimox, which is also not approved in US but can be obtained from CDC. In this application, the proposed BNZ dosing regimen for

(b) (4)

(b) (4) The BNZ dosing recommendation by WHO/Médecins Sans Frontières[iii] is 5 - 7mg/kg/day and up to 10 mg/kg/day for infants and infants/neonates by 1 year old, and the duration of treatment is not shorter than 30 days and could be up to 60 days. This product has been granted an Orphan Product Designation by FDA.

In support of the application, the Applicant has provided publications on placebo-controlled trials, analyses of prospective and retrospective observational studies, reviews of BNZ use in the treatment of Chagas disease, and published case study reports. The Applicant has also provided reports from in vitro studies that assessed the role of hepatic enzymes and transporters in the BNZ disposition as well as from in vitro studies that evaluated the BNZ's drug interaction potential. From the submitted publications and clinical trials, the Applicant has identified six key studies, which are presented in Section 4.6.6, that are supporting this application. For these key studies, the Applicant has obtained access to datasets and has performed analyses to obtain information on the efficacy of BNZ in the treatment of Chagas disease. In these studies, various BNZ formulations were evaluated including the Hoffman-LaRoche formulation: Radanil®. Per the summary provided by the Applicant , the Radanil formulations (100 mg and 50 mg benznidazole tablets) were registered by Roche (Hoffman-LaRoche) in Brazil, Argentina, Bolivia, Uruguay, Peru, Nicaragua, and Japan in the 1970s as Radanil®, Ragonil®, or Rochagan®. However, after donating all commercial rights and the technology to manufacture BNZ to the Brazilian government as a "generic" version of Roche's product, Roche withdrew its registration. The latter BNZ formulation was manufactured by Pernambuco State Pharmaceutical Laboratory (Laboratório Farmacêutico do Estado de Pernambuco; LAFEPE), which is a public laboratory in Brazil. This formulation is referred as LAFEPE product.

[iii] http://www.who.int/selection_medicines/committees/expert/19/applications/MSF_Comments.pdf?ua=1 Page 15

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3.2   General Pharmacological and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	The postulated mechanism of action for BNZ activity is via a parasite type I nitroreductase. This enzyme is absent in mammalian cells, therefore, the 2-electron reduction of nitroheterocyclic compounds results in the production of toxic metabolites within the parasite without significant generation of superoxide[iv].
QT Prolongation	In a safety pharmacology study, a concentration-dependent inhibition of hERG tail current in stably transfected HEK-293 cells was reported with an average inhibition of 17.0 ± 2.7% at 100 µM (~26 µg/mL), which is above the expected concentrations from the proposed BNZ regimen.
Absorption (Single dose, Healthy Volunteers)	
Relative Bioavailability	The within study comparisons shows comparable pharmacokinetics of BNZ from three different formulations: 100 mg tablet in a slurry, 100mg tablet intact, and 8 x 12.5mg tablets in a slurry
T_{max}	2 - 4 hours (In Healthy subjects)
Food-Effect	Not significant (In Healthy subjects)
Distribution	
Protein Binding	44%[iv]
Substrate transporter systems [in vitro]	BNZ is a substrate for P-gp and is also reported to increase the expression of P-gp as well as expression of multidrug resistance-associated protein 2 (MRP2). BNZ inhibits OAT3 transporter (IC_{50} of 34 µM, i.e. ~9 µg/mL).
Permeation	BNZ is reported to readily pass the blood brain barrier, crosses the placenta, and binds to fetal tissues[iv].
Metabolism & Excretion	
Inhibition	In study with human hepatocytes, the IC_{50} values of BNZ against CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4 -mediated metabolism were > 50 µM (~13 µg/mL).
Induction	In human hepatocytes, no induction of CYP enzymes 1A2, 2B6, and 3A4 occurred with BNZ at concentrations up to 100 µM.
Mean Terminal Elimination half-life	In healthy subjects, mean elimination half-life for BNZ was about 11 -13 hours. Limited BNZ PK data are available from patients. The available data indicate that BNZ PK could be different in patients with chronic Chagas disease. In adult patients with chronic Chagas disease, the mean elimination half-life is reported as high as 36 hours by *Soy, Aldasoro et al. 2015* following BNZ treatment at 2.5 mg/kg/12 h for 8 weeks, with a maximum dosage of 400 mg/day (Abarax formulation, Elea Laboratory, Argentina).
Primary excretion pathways	Results from the mass-balance/ADME study are pending. The Applicant plans to submit this information by 4Q 2017 – 1Q 2018.

[iv] This information is derived by the Applicant from the literature and the Reviewer was not able to verify it further.

3.3 Clinical Pharmacology Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

From a Clinical Pharmacology perspective, the pivotal evidence of effectiveness and safety is derived from the two published studies (Sosa-Estani et al., 1998 and De Andrade et al., 1996). These studies provide pivotal evidence to support effectiveness and safety for children 6 to 12 years of age, based on serology results. The formulation used in these two randomized trials was the 100 mg BNZ tablets known as the Radanil formulation, which was manufactured by Hoffman-LaRoche. Given that the Radanil formulation is no longer available and none of the to-be-marketed (TBM) formulations (i.e., 100 mg and 12.5 mg tablets) was used in the clinical trials from which the evidence of effectiveness is based upon, the Applicant has provided an exposure bridge. The clinical pharmacology review team recommends dosing in children from 2 - 12 years of age, based on the available efficacy data (6 – 12 years; Sosa-Estani 1998 and De Andrade 1996) and pharmacokinetic (2 – 12 years; Altcheh et al., 2014) data in pediatric patients where the Radanil formulation was utilized.

Collectively, the available clinical pharmacology information provides supportive evidence of effectiveness with respect to the following two aspects:

1) Exposure bridge between the TBM and clinical trial formulations
2) Dosing recommendations in children from 2 to 12 years of age

1) Exposure bridge between the TBM and clinical trial formulations

Neither of the ____ (b) (4) tablets (the TBM formulations) was used in clinical trials from which the pivotal evidence of effectiveness is based upon. The Applicant is proposing an exposure bridge between the TBM formulation and Radanil, which was used in the cited literature submitted in support of this 505(b)(2) NDA. Additional details are provided in Section 4.1.

The Applicant has conducted two clinical pharmacology studies with the TBM formulations in healthy volunteers, i.e., LPRI 747/101 and LPRI 747/102. In addition, the Applicant has provided a cross-study comparison report that compared the BNZ exposure between clinical trial formulation Radanil (100 mg BNZ tablet) and the TBM 100 mg BNZ tablet. Specifically, the report compared the BNZ exposure parameters: $AUC_{0-\infty}$ and C_{max} from Study LPRI 747/101 to the parameter estimates for Radanil published by Raaflaub and Ziegler, 1979. The $AUC_{0-\infty}$ and C_{max} estimates were found comparable between the TBM 100 mg BNZ formulation and Radanil (Table 1).

Table 1: Statistical comparison of PK parameters from studies conducted with the TBM 100 mg BNZ (Test) formulation and with Radanil (Adapted from LPRI747/101-DOC006, Table 3)

Parameters	% Point Estimates (90% CI)
Test fasting / Radanil	
$AUC_{0-\infty}$	89.53 % (77.38 – 103.58)
C_{max}	97.39 % (86.17 – 110.06)
Test fed / Radanil	
$AUC_{0-\infty}$	89.11 % (75.33 – 105.41)
C_{max}	88.17 % (76.97 – 100.99)

With respect to the exposure/PK link between the TBM formulations: 12.5 mg BNZ tablet and 100 mg BNZ tablet, the Applicant has conducted Study LPRI 747-102 in healthy subjects, which evaluated the pharmacokinetics of BNZ given as three different formulations, : a 100 mg tablet in a slurry, a 100 mg tablet taken whole, and 8 x 12.5 mg tablets. The $AUC_{0-\infty}$ and Cmax estimates were comparable between all three treatment groups (Table 2). These within study comparisons demonstrated comparable BNZ pharmacokinetic profiles amongst the TBM formulations.

Table 2: Statistical comparison of PK parameters of two TBM BNZ formulations (100 mg tablets and 12.5 mg tablets) administered intact or as a slurry under fasting conditions. (Adapted from LPRI747/102, TT 7)

BENZNIDAZOLE (n=18)			
Variable	method	point estimates	confidence intervals
AUC(0-t) (ratio Test 1 (A) / Test 1 (B))	ANOVA-log	104.57%	99.32% - 110.09%
Cmax (ratio Test 1 (A) / Test 1 (B))	ANOVA-log	102.17%	97.11% - 107.48%
AUC(0-t) (ratio Test 1 (A) / Test 2 (C))	ANOVA-log	99.78%	94.37% - 105.51%
Cmax (ratio Test 1 (A) / Test 2 (C))	ANOVA-log	101.42%	97.93% - 105.03%
AUC(0-t) (ratio Test 1 (B) / Test 2 (C))	ANOVA-log	95.43%	91.57% - 99.45%
Cmax (ratio Test 1 (B) / Test 2 (C))	ANOVA-log	99.27%	95.94% - 102.72%

Treatment A: 1 BNZ 100 mg Tablet Intact
Treatment B: 1 BNZ 100 mg Tablet slurry
Treatment C: 8 BNZ 12.5 mg Tablets slurry

Test 1: BNZ 100 mg Tablet
Test 2: BNZ 12.5 mg Tablet

The findings from within and between study comparisons (Table 1 and Table 2) demonstrated comparable BNZ pharmacokinetic profiles amongst the TBM formulations and Radanil. In addition, the population PK analysis showed no significant impact of the formulation on the PK of BNZ from TBM formulation and Radanil (See Section 4.2 for details).

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Overall, the available clinical pharmacology information supports the exposure bridge between the TBM and clinical trial formulation, i.e., Radanil formulation.

2) Dosing Recommendations in Children between 2 to 12 Years of Age

Based on the pharmacometrics analyses of the available information, the review team recommends a weight-based dosing regimen (i.e., 5-8 mg/kg/d) for pediatric patients (2-12 years of age). Please see Section 4.3 for the rationale and details for the pharmacometrics analyses.

In addition, to translate this weight-based dosing regimen to a practical dosing instruction for physicians and patients, a dosing instruction table (Table 3) is provided by the review team, based on the following considerations:

1) The dosing instruction should be easy to follow and practical, given the availability of both 100 mg (scored) and 12.5 mg tablets.
2) The recommended daily dose ranges from 5 to 8 mg/kg/d. Pediatrics with lower body weight (< 20 kg) are permitted slightly higher mg/kg dosing based on the proposed dosing. This is partly due to restrictions from available dosing strengths but the slightly higher mg/kg doses may also better result in comparable exposures to heavier pediatrics. This is because the body weight normalized BNZ clearance increases with decreasing body weight such that flat mg/kg dosing would result in lower exposures at lower body weights (See Sections 3.3.3 and 4.4 for details).

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Table 3: Dosing instruction recommended for children from 2 to 12 years of age, 5 - 8 mg/kg/d

Body weight range (kg)	BID Dose (mg)*	Maximum daily dose (mg/kg)	Minimum daily dose (mg/kg)
< 15	50	-	6.7
15 - < 20	62.5	8.3	6.3
20 - < 30	75	7.5	5.0
30 - < 40	100	6.7	5.0
40 - < 60	150	7.5	5.0
≥ 60	200	6.7	-

* To be administered twice a day approximately 12 hours apart for 60 days.

It is noteworthy that with the above-mentioned dosing instruction, for children with body weight 11 kg (reported 5th percentile weight for 2 year old) or lower, the BNZ daily dosing would be higher than 9 mg/kg/day. Safety information at dosing of 9 mg/kg/day is limited. However, based on the Anthropometric Reference Data[v], most children who are 2 years or older are expected to weigh greater than 11 kg. For 2 years old, the 5th percentile for the weight is reported as 11.3 kg and 10.9 kg for a male and female child, respectively.

3.3.2   Is the proposed general dosing regimen appropriate for the general patient population for which the indication is being sought?

No, the clinical pharmacology review team does not agree with the proposed dosing regimen in the general patient population. The proposed dosing for ⬛ (b) (4)

⬛ (b) (4)

⬛ (b) (4) The clinical pharmacology review team recommends ⬛ (b) (4) dosing ⬛ (b) (4) based on the available efficacy (6 – 12 years) and pharmacokinetic (2 – 12 years) data in pediatric patients where the Radanil formulation was utilized. This information is summarized above in Table 3 and the rationale for the proposed dosing is provided in Section 3.3.1.

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[v] Fryar CD, Gu Q, Ogden CL. Anthropometric reference data for children and adults: United States, 2007–2010. National Center for Health Statistics. Vital Health Stat 11(252). 2012.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

Yes. At least one intrinsic factor, i.e., body weight has been identified as a significant covariate on clearance (CL/F) of BNZ (Figure 1). Therefore, a body weight based BNZ dosing is required. However, the relationship between body weight normalized CL/F and body weight shows the body weight normalized CL/F in pediatrics is relatively higher than that in adults (Figure 2). Therefore, pediatrics at lower body weights may require slightly higher mg/kg dosing to have comparable exposures to older pediatrics _____ (b) (4). Please see Table 3 in Section 3.3.1 for the proposed dosing table based on body weight. Please see the Section 4.4 for details on the population PK analysis.

Figure 1 Relationship between CL/F and body weight for BNZ based on Raaflaut 1979, 101, 102, and Altcheh 2014 studies
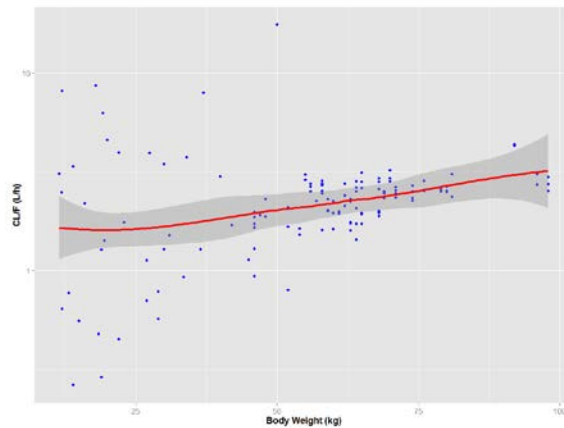
Source: Reviewer's analysis

Figure 2 Relationship between body weight normalized CL/F and body weight for BNZ based on Raaflaut 1979, 101, 102, and Altcheh 2014 studies
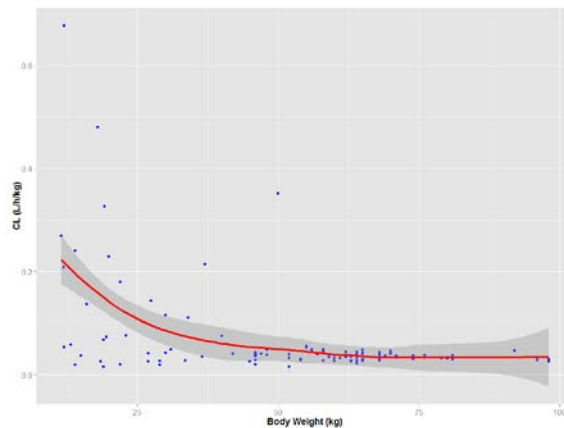
Source: Reviewer's analysis

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

With respect to food-drug interaction, there were no significant differences observed in the BNZ PK parameter estimates when 100 mg BNZ tablet was administered with food and without food.

With respect to clinically relevant drug-drug interactions, the Applicant has provided reports from in vitro studies that assessed the role of hepatic enzymes and transporters in the BNZ disposition as well as from in vitro studies that evaluated BNZ's drug interaction potential. These results are summarized in Section 3.2. However, information on BNZ drug disposition and excretion pathways in humans is not yet available as findings from a mass-balance/ADME study are pending. The Applicant plans to submit this information from the mass-balance/ADME study by 4Q 2017 – 1Q 2018.

(b) (4)

(b) (4)

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4. APPENDICES

4.1 Exposure bridge between the TBM and clinical trial formulations

The Applicant has provided a cross-study comparison report that compared the BNZ exposure between clinical trial formulation Radanil (100 mg BNZ tablet) and the TBM 100 mg BNZ tablet. Specifically, the report compared the BNZ exposure parameters: $AUC_{0-\infty}$ and C_{max} from Study LPRI 747/101 to the parameter estimates for Radanil published by Raaflaub and Ziegler, 1979. LPRI 747/101 was a food effect study that contained fed and fasted arms in a crossover design in 9 male and 9 female healthy volunteers. The Raaflaub and Ziegler study contained only one study group, in which the drug was administered with a light breakfast in six female healthy volunteers. The detailed information of this report and the relevant studies are given in Section 4.6.2 – Section 4.6.5.

The $AUC_{0-\infty}$ and C_{max} estimates were found comparable between the TBM 100 mg BNZ formulation and Radanil (Table 1). Since all of the study subjects in the Raaflaub and Ziegler study were female, the comparative statistical analysis was done by the Applicant using the data from the female subjects enrolled in LPRI 747/101 study and the findings are presented in Table 2.

Table 1: Statistical comparison of PK parameters from studies conducted with the TBM 100 mg BNZ (Test) formulation and with Radanil *(Adapted from LPRI747/101-DOC006, Table 3)*

Parameters	% Point Estimates (90% CI)
Test fasting / Radanil	
$AUC_{0-\infty}$	89.53 % (77.38 – 103.58)
C_{max}	97.39 % (86.17 – 110.06)
Test fed / Radanil	
$AUC_{0-\infty}$	89.11 % (75.33 – 105.41)
C_{max}	88.17 % (76.97 – 100.99)

Table 2: Statistical comparison of PK parameters from studies conducted with the TBM 100 mg BNZ (Test) formulation and with Radanil – Females only *(Adapted from LPRI747/101-DOC006, Table 4)*

Parameters	% Point Estimates (90% CI)
Test fasting / Radanil	
$AUC_{0-\infty}$	91.85 % (77.42 – 108.97)
C_{max}	109.16 % (98.51 – 120.96)
Test fed / Radanil	
$AUC_{0-\infty}$	95.18 % (77.32 – 117.15)
C_{max}	99.01 % (87.77 – 111.69)

The findings reported in Table 1 show that the lower bounds of 90 % CI for AUC ratios were less than 80 %, which suggests that the TBM formulation may have a slightly lower (BNZ) bioavailability than Radanil formulation. Given that the pharmacometrics analyses using either adult data or both the pediatric and

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adult data identified body weight as a significant covariate on CL/F and Vd/F of BNZ, the above-mentioned analysis was performed with using body weight as a regressor during the comparison of the least squares means of the test (TBM 100 mg BNZ formulation) and reference formulations (Radanil formulation). Inclusion of body weight into the analysis moved the point estimates of AUC ratios towards 1 (Table 3). Moreover, the population PK analysis showed that no significant impact of the formulation was identified on the PK of BNZ. A consistent result was seen for the ratio of AUC and C_{max} between the TBM formulation and Radanil (See Section 4.2).

Overall, the reviewer concludes that the systemic exposure to BNZ is comparable following administration of the TBM formulation and Radanil formulation. The noted bioavailability differences between the test and reference formulations could be attributable to the following inherent limitations with the cross-study comparison:

1) The differences in the analytical methods used
2) The small subject number (especially in the Radanil study)
3) The lack of information with regard to the food effect on BNZ bioavailability from the Radanil tablet

There is a difference in fed/fasted conditions of the two studies. In the Raaflaub and Ziegler (1979) study, the Radanil tablet was administered with light breakfast, whereas, Study LPRI 747/101 included two treatments arms; one treatment arm received the assigned treatment on an empty stomach and the other treatment arm received the assigned treatment with a high-fat breakfast. There is no information available on the food effect upon the bioavailability of BNZ from the Radanil tablet; therefore, a direct comparison between these two studies may not be ideal due to the differences in fed/fasted conditions.

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Table 3: Statistical comparison of PK parameters from studies conducted with the TBM 100 mg BNZ (Test) formulation and with Radanil – With body weight as a regressor *(Source: Reviewer's independent analysis)*

Parameter Estimates		% Point Estimates (90% CI) (Test/Radanil(Ref))	
		Without Body Weight Adjustment	Body Weight Adjustment (p<0.05)
Cmax (ng/ml)	Fasting	97 (85 - 111)	102 (92 - 113)
	Fed	88 (77 - 101)	92 (83 - 103)
AUC (ng*hr/ml)	Fasting	93 (80 - 109)	97 (84 - 112)
	Fed	93 (79 - 108)	96 (83 - 111)

With respect to the exposure/PK link between the TBM formulations: 12.5 mg BNZ tablet and 100 mg BNZ tablet, the Applicant has conducted Study LPRI 747-102 in healthy subjects, which evaluated the pharmacokinetics of BNZ given as three different formulations: a 100 mg tablet in a slurry, a 100 mg tablet taken whole, and 8 x 12.5 mg tablets. The $AUC_{0-\infty}$ and C_{max} estimates were comparable between all three treatment groups (Table 4). These within study comparisons demonstrated comparable BNZ pharmacokinetic profiles amongst the TBM formulations.

Table 4: Statistical comparison of PK parameters of two TBM BNZ formulations (100 mg tablets and 12.5 mg tablets) administered intact or as a slurry under fasting conditions. *(Adapted from LPRI747/102, TT 7)*

BENZNIDAZOLE (n=18)			
Variable	method	point estimates	confidence intervals
AUC(0-t) (ratio Test 1 (A) / Test 1 (B))	ANOVA-log	104.57%	99.32% - 110.09%
Cmax (ratio Test 1 (A) / Test 1 (B))	ANOVA-log	102.17%	97.11% - 107.48%
AUC(0-t) (ratio Test 1 (A) / Test 2 (C))	ANOVA-log	99.78%	94.37% - 105.51%
Cmax (ratio Test 1 (A) / Test 2 (C))	ANOVA-log	101.42%	97.93% - 105.03%
AUC(0-t) (ratio Test 1 (B) / Test 2 (C))	ANOVA-log	95.43%	91.57% - 99.45%
Cmax (ratio Test 1 (B) / Test 2 (C))	ANOVA-log	99.27%	95.94% - 102.72%

Treatment A: 1 BNZ 100 mg Tablet Intact
Treatment B: 1 BNZ 100 mg Tablet slurry
Treatment C: 8 BNZ 12.5 mg Tablets slurry

Test 1: BNZ 100 mg Tablet
Test 2: BNZ 12.5 mg Tablet

Overall, based on the available information and the additional assessments (The detailed information on the assessment of the relevant studies are given in Section 4.6.2 – Section 4.6.5), the systemic exposure to BNZ is expected to be comparable following administration of the TBM 100 mg BNZ formulation and Radanil formulation.

4.2 Additional analyses to support a link between the TBM formulation and Radanil

Applicant conducted two PK studies (Study 101 and 102) using the TBM formulations in healthy volunteers and aimed to bridge the PK between Radanil and the TBM formulations by merging historical PK data and newly derived PK data. As it is a cross-study comparison, the demographic information was not well controlled, thus, the two formulations failed to meet the traditional bioequivalence criteria based on a direct comparison. To investigate whether the two formulations had significant impact on PK of benznidazole, a population PK model was developed by Reviewer based on PK data of Radanil and the TBM formulations (Section 4.4.2).

The population PK model based on Raaflaub 1979, 101 and 102 studies confirmed that body weight had a significant influence on the exposure of benznidazole; thus, the higher exposures in Raaflaub 1979 study may have resulted from the lower body weights in the study (58.5 kg) compared to that of healthy volunteers in Study 101 and 102 (63.2 and 69.8 kg, respectively). The exposure (AUC) in Raaflaub 1979 and 101 studies were adjusted using the following equation based on the result of the population PK model:

$$AUC_{adj} = AUC_i \times (\frac{WT}{64})^{0.703}$$

AUC_i is the estimated AUC based on population PK model using dose divided by estimated CL/F for each subject. The adjusted AUC (AUC_{adj}) under two formulations from Raaflaub 1979 and 101 studies were compared assuming parallel study design. The calculation method[vi] was as follows: 1) log transformed AUC_{adj} values; 2) calculated the mean and standard error of log(AUC_{adj}) for two formulation groups; 3) the point estimate was the exponential of the difference between mean log(AUC_{adj}) values from two formulation groups and the 90% confidence interval was calculated similarly based on mean and standard error values assuming t distribution.

The results in Table 1 show that the exposure was comparable between TBM formulation and Radanil after adjustment based on body weight.

[vi] *The method of bioequivalent analysis for parallel study was kindly provided by Yuzhuo Pan, a Reviewer from Division of Bioequivalence II, Office of Generic Drugs, FDA*

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Table 1 Exposure comparison between the TBM formulations from Study 101 relative to the Radanil formulation from Raaflaub 1979 study after adjustment based on body weight

	Point estimate	90% CI
AUC_{0-inf} (fasting/Radanil)	0.93	(0.81, 1.05)
AUC_{0-inf} (fed/Radanil)	0.94	(0.79, 1.12)

Source: Reviewer's independent analysis

An alternative approach based on a population PK approach was also utilized to compare both AUC and Cmax for two formulations. In this approach, separate absorption rates (ka1 for Radanil and ka2 for TBM formulation) and a relative bioavailability F for TBM formulation (F1 for TBM formulation, bioavailability for Radanil was assumed to be 1) were added to population PK model.

The parameter estimates are listed in Table 2.

Table 2 Parameter estimates for population PK model based on Raaflaub 1979, 101 and 102 studies with a separate Ka and relative bioavailability F

Parameter	Estimate (RSD)	Inter-subject variability
CL/F (L/h)	2.20 (4.7%)	17.2%
V/F (L)	35.5 (4.0%)	9.5%
Ka1 (h^{-1})	1.57 (54.6%)	73.3%
Ka2 (h^{-1})	1.08 (12.3%)	
F (TBM formulation)	1.04 (3.8%)	
WT on CL/F	0.703 (26.6%)	
WT on Vd/F	0.840 (12.5%)	
Residual		
Additive	0.033 (2.2%)	

Source: Reviewer's independent analysis

Then the point estimates of Cmax and AUC ratio for two formulations were calculated using the analytical solutions shown below and PK parameter estimates from Table 2 assuming body weight was normalized to 64 kg. The standard error of Cmax and AUC ratio was calculated by the delta method using the estimates of variance-covariance matrix from NONMEM output. The results are shown in Table 3.

$$AUC = \frac{F \times DOSE}{CL}$$

$$Cmax = \frac{F \times DOSE \times Ka}{Vd \times \left(Ka - \frac{CL}{Vd}\right)} \left(e^{-\frac{CL}{Vd} \times \left(\frac{\log\left(\frac{Ka}{CL} \times Vd\right)}{Ka - \frac{CL}{Vd}} \right)} - e^{-Ka \times \left(\frac{\log\left(\frac{Ka}{CL} \times Vd\right)}{Ka - \frac{CL}{Vd}} \right)} \right)$$

Table 3 Exposure comparisons for Radanil and TBM formulations based on population PK model

	Point estimates	90% CI
AUC (TBM/Radanil)	0.963	(0.893, 1.038)
Cmax (TBM/Radanil)	0.925	(0.809, 1.057)

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Source: Reviewer's independent analysis

The results demonstrated that the two formulations would meet traditional bioequivalence criteria of 80-125% if body weight was normalized. Therefore, the effectiveness data from publications using the formulation of Radanil can be used to support the TBM formulation.

It is worth noting that this was a cross-study comparison where the PK studies were conducted by different research groups over multiple decades. In addition, drug concentrations were determined by different analytical methods and no cross-validation of the analytical methods are available. Finally, the current conclusion assumes that no other known or unknown factors would impact the PK of benznidazole. Despite these assumptions and the relatively small sample size in Raaflaub 1979 and 101, the analysis suggests that the TBM formulation would have, at most, 11% lower AUC than the Radanil formulation.

4.3   Dosing in pediatrics 2-6 years of age

The Applicant is proposing ⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛ (b) (4)
⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛ (b) (4), the pivotal efficacy studies (Sosa-Estani et al., 1998 [1] and De Andrade et al., 1996 [2]) only support the dose of 5-7.5 mg/kg/day BID in pediatric population of 6-12 years of age. ⬛⬛⬛⬛⬛⬛⬛⬛⬛ (b) (4)
⬛⬛⬛⬛⬛⬛⬛⬛ (b) (4) In a separate study (Altcheh et al., 2014 [3]), which evaluated the Radanil formulation in pediatric patients aged 2-12 years of age, BNZ exposure data are available. Per the Review team's request to identify a BNZ dosage regimen appropriate for pediatric patients 2-6 years of age, the clinical pharmacology review team performed additional pharmacometrics analyses relying on the above-mentioned information.

Given the limited information on exposure-response relationship with respect to efficacy and safety of the BNZ treatment in Chagas disease, exposure matching was used to determine the dose in pediatric population of 2-6 years of age.

The reviewer developed a population PK model based on data in adults and pediatrics. This population PK model (Section 4.4.2) was used to simulate the exposure (steady state AUC, Cmax, and Cmin) based on the following dosing regimen, 5-8 mg/kg/d in two divided doses separated by 12 h for pediatric patients 2-12 years of age with body weight range of 10-75 kg based on CDC growth Chart. The simulation dataset including 500 subjects was created and 100 simulations were run. The results are shown in Figure 1.

Figure 1 Simulated exposure vs body weight based on FDA's dosing regimen
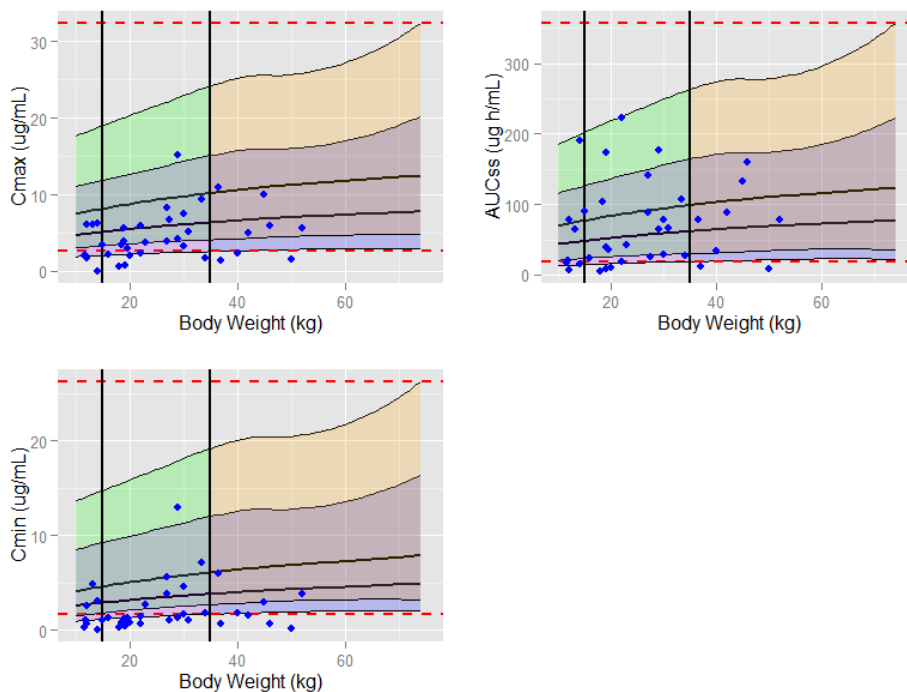
Purple band = the 95% prediction interval for Cmax, AUC, and Cmin at steady state with a dose of 5 mg/kg for pediatric population of 2-6 years old;

Green band = the 95% prediction interval for Cmax, AUC, and Cmin at steady state with a dose of 8 mg/kg for pediatric population of 2-6 years old;

Blue band = the 95% prediction interval for Cmax, AUC, and Cmin at steady state with a dose of 5 mg/kg, respectively, for pediatric population of 6-12 years old;

Yellow band = the 95% prediction interval for Cmax, AUC, and Cmin at steady state with a dose of 8 mg/kg, respectively, for pediatric population of 6-12 years old;

Black vertical lines = the body weight range of 15-35 kg which is 5% and 95% percentile of body weight for pediatric population of 6 years of age, respectively (The upper bound of 95% percentile of body weight corresponding to age of 6 years is 35 kg according to the CDC growth chart; For illustration purpose, the vertical 35 Kg black line is used as the arbitrary cut-off to divide the 2-6 years old and 6-12 years old);

Red dashed lines = the 95% prediction interval of exposure for pediatric population of 6-12 years of age.

Blue dots = the observed exposure from Altcheh 2014 study (Cmax dots used the maximum observed concentration for each subject, Cmin dots used the minimum observed concentration for each subject, and AUC plot used the AUC values calculated using dose divided by estimated CL/F for each subject);

Source: Reviewer's independent analysis

The exposure from pediatric population of 6-12 years of age was used as a reference. The observed exposure in Altcheh 2014 study for pediatric patients with a dose of 5-8 mg/kg/day BID was also plotted. Most of the observed AUC values in pediatric population from Altcheh 2014 study were covered by the simulated AUC. Most of the observed Cmax and Cmin values appeared to be over-predicted by the simulation. The possible explanation is that highest and lowest concentrations were used as observed Cmax and Cmin for each subject, which may not be real Cmax and Cmin as sparse samplings were taken and PK samples were derived from different time windows.

It can been seen from Figure 1 that the simulated exposure (AUC, Cmax, and Cmin) in pediatric population of 2-6 years of age was relatively lower than that in pediatric population of 6-12 years of age, but the exposure was still within a similar range of that in pediatric population of 6-12 years of age. Based on the simulation, the FDA recommended dose of 5-8 mg/kg/d appears to be acceptable from Pharmacometrics perspective. Moreover, the recommended dose also aligns well with CDC and WHO guidance.

Based on the analyses, the review team recommends a weight-based dosing regimen (i.e., 5 - 8 mg/kg/d) for pediatric patients (2 - 12 years of age).

The recommended dosing of 5 - 8 mg/kg/d in pediatric patients of 2-12 years of age is projected to provide comparable BNZ exposure in pediatric patients of 2-12 years of age as those reported in Altcheh 2014, in which the sparse PK samples were taken and the treatment response was evaluated by qPCR. At the end of treatment (~60 days), all 37 patients had negative qPCR results. However, it is noteworthy that the relationship between the negative PCR results and clinical benefit (seroconversion) is not yet known. A similar dosing in patients with 6-12 years of age was evaluated in pivotal evidence of efficacy reported in two published studies (Sosa-Estani et al., 1998 and De Andrade et al., 1996). Therefore, the current recommended dosing in pediatric patients of 2-6 years of age would provide exposure matching to the exposures reported in pediatric population of two pivotal studies.

4.4   Population PK Analysis

4.4.1   Applicant's population PK analysis

A population PK model for benznidazole was developed by Dr. Altcheh *et al* [1] using pooled data from healthy adult subjects (Raaflaub 1979 study), adult patients with Chagas disease (Raaflaub 1980 study), and pediatric patients with Chagas disease (Altcheh 2014 study).

Raaflaub 1979 study: The study was published in 1979 by Dr. Raaflaub *et al* [4]. A total of 6 healthy female subjects were enrolled. One tablet Radanil containing 100 mg benznidazole together with two cups of tea and two slices of unbuttered toast was given to each subject after an overnight fasting. Blood was sampled (10 mL) before and 0.5, 1, 2, 3, 4, 6, 12, 24, 35, 48, and 72 h after administration of the tablet. The concentrations of benznidazole were determined using differential pulse polarography established by Dr. Brooks *et al* [5].

Raaflaub 1980 study: The study was published in 1980 by Dr. Raaflaub [6]. A total of 8 adult patients (4 males and 4 females) with Chagas disease were enrolled. Radanil tablets, each containing 100 mg of benznidazole, were administered. The treatment started with 3 mg/kg per day and increased progressively to 5 mg/kg and 7 mg/kg during the first week. From the 8th day to the end of the treatment (30 days), a full dose of 7 mg/kg per day was given twice daily together with a cup of tea or warm water before breakfast or evening meal. The numbers of tablets given to patients for the full dose are listed as follows:

- Subjects weighting 40-45 kg: 2 x 1.5 tablets per day (daily dose of 300 mg)
- Subjects weighting 45-55 kg: 2 x 1.75 tablets per day (daily dose of 350 mg)
- Subjects weighting 55-65 kg: 2 x 2 tablets per day (daily dose of 400 mg)
- Subjects weighting 65-80 kg: 2 x 2.5 tablets per day (daily dose of 500 mg)

Blood was sampled (10 mL) at 3 h and 12 h after administration of the tablet on Day 10, 15, 20, and 25. Five additional samples were taken at 12, 24, 36, 48, and 60 h after administration of the last tablet (Day 30). The concentrations of benznidazole were determined using the same analytical method as that applied in Raaflaub 1979 study.

Altcheh 2014 study: The study was published in 2014 by Dr. Altcheh *et al* [1]. A total of 37 pediatric patients (19 males and 18 females) with Chagas disease was included in the PK study. Patients were treated with benznidazole (Radanil) 100 mg tablets with a dose of 5-8 mg/kg/day BID for 60 days. Tablet was fractioned by a hospital pharmacist in an individualized manner. At least 3 blood samples per child were obtained at a random time within pre-specified windows:

- 0-2 h, 2-6 h and 6-12 h post dose for patients taking the first dose (11 subjects)
- Trough, 0-2 h, and 2-6 h post dose for patients at steady state on day 3-59 (21 subjects)
- 12-18 h, 18-24 h and 24-36 h post dose for patients receiving the last dose (5 subjects)

In total, thirty-four patients provided 3 samples, two patients provided 5 samples, one patient provided 4 samples and one patient provided 1 sample. In the three patients with more than 3 samples, the extra samples were opportunistically obtained from leftover blood taken for routine laboratory tests. The

concentrations of benznidazole were determined using HPLC established by Dr. Marson *et al* in 2013 [5, 6].

The demographics for Raaflaub 1979, Raaflaub 1980, and Altcheh 2014 studies are listed in Table 1.

Table 1 Demographics for adults and children in Raaflaub 1979, Raaflaub 1980 and Altcheh 2014 studies

	Raaflaub 1979 study	Raaflaub 1980 study	Altcheh 2014 study
No of patients (N)	6	8	37
Age, years (mean, range)	23.2 (22-24)	40.9 (32-60)	7.3 (2.1-12.6)
WT, kg (mean, range)	58.5 (47.0-63.0)	53.1 (39.0-60.0)	27.2 (11.5-64.0)
Gender			
Male (N, %)	0	4 (50%)	19 (51%)
Female (N, %)	6 (100%)	4 (50%)	18 (49%)

Source: Summarized by Reviewer based on publications for Raaflaub 1979, Raaflaub 1980 and Altcheh 2014 studies.

A one compartment model with a first-order absorption best described the benznidazole PK data. Covariate analysis showed that patients' weight was a statistically significant covariate on CL/F and V/F and age was a statistically significant covariate for CL/F. Final parameter estimates, with 95% CI, are listed in Table 2.

Table 2 Population PK parameter estimates

Parameter	Median [95% CI]	Interindividual variability [95% CI]
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Source: Published by Dr. Altcheh et al [1], Page 4, Table 2

Goodness-of-fit diagnostic plots results suggested acceptable model performance (Figure 1). The plot of predictions vs observations over time showed conformity of final model predictions with the observed data (Figure 2).

Figure 1 Goodness of fit plot for final model

Predicted (benznidazole mg/L)
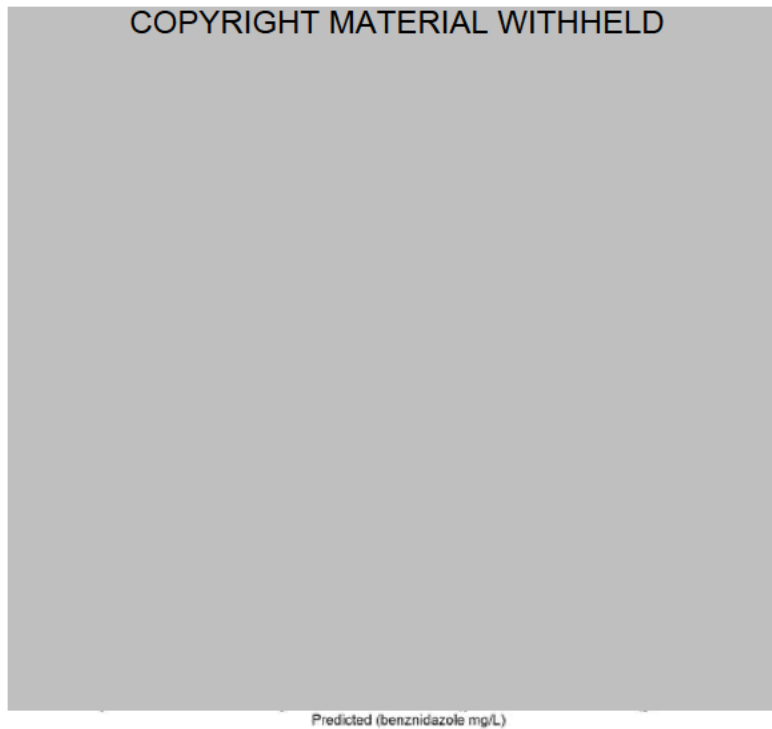
Source: Published by Dr. Altcheh et al [1], Page 6, Figure 3

Figure 2 Predictions vs observations over time plot for benznidazole concentration in plasma vs time after each dose for final model

Time after dose (hs)
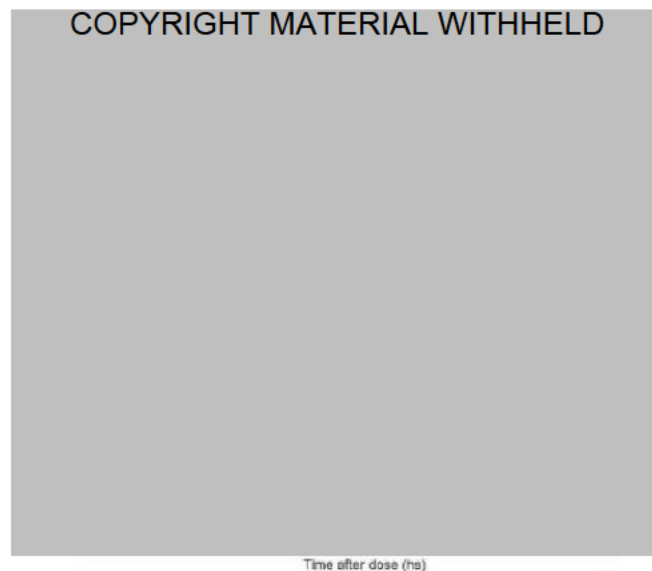
Source: Published by Dr. Altcheh et al [1], Page 5, Figure 2

Individual weight-corrected estimates for CL/F were significantly higher for children than for adults. Individual weight-corrected CL/F vs age are depicted in Figure 3.

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Figure 3 Individual predicted CL/F data for patients

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Source: Published by Dr. Altcheh et al [1], Page 7, Figure 4

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4.4.2 Reviewer's population PK analysis

Two separate population PK analysis were conducted by the Reviewer. In the first analysis, the reviewer included PK data of Radanil from Raaflaub 1979 and 1980 studies and PK data of the TBM formulation from Study 101 and 102. A brief summary of PK data from Study 101 and 102 is shown below. The intent of this additional population PK analysis was to investigate whether the two formulations had significant impact on BNZ PK as described in Section 4.2:

Study 101: The study was a two-period, randomized, crossover study conducted by Applicant to assess the effect of food on benznidazole PK for the TBM formulation. A total of 18 healthy female subjects were enrolled. One TBM tablet (100 mg) was given to subjects either under fasting conditions with 240 mL tap water in one study period or under fed conditions (30 minutes after the start of the standard high-fat breakfast) with 240 mL tap water in another study period. Blood was sampled before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24, 48, and 72 h after administration of the tablet. The concentrations of benznidazole were determined using LC/MS-MS.

Study 102: The study was a three-period, randomized, crossover study conducted by Applicant to assess the relative bioavailability of two TBM tablets containing 100 mg or 12.5 mg benznidazole administered intact or as a slurry under fasting condition. A total of 18 healthy subjects 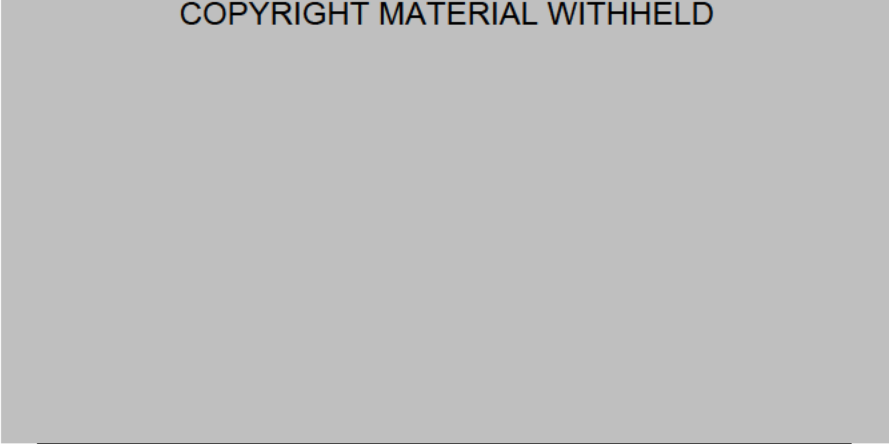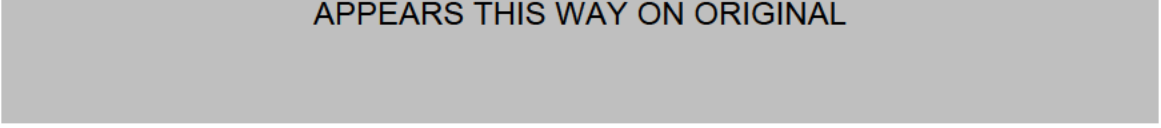were enrolled. One TBM tablet containing 100 mg benznidazole was given intact or crushed to form a slurry in water to the subjects under fasting conditions, or eight TBM tablets, each containing 12.5 mg benznidazole were given crushed as a slurry in water to the subjects under fasting conditions. Blood was sampled before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24, 48, and 72 h after administration of the tablet. The concentrations of benznidazole were determined using LC/MS-MS.

The demographics for Study 101 and 102 are listed in Table 3.

Table 3 Demographics for healthy volunteers in Study 101 and 102

	Study 101	Study 102
No of patients (N)	18	18
Age, years (mean, range)	37.6 (21-51)	39.8 (32-60)
WT, kg (mean, range)	63.2 (46.0-81.0)	69.8 (39.0-60.0)
Gender		
Male (N, %)	9 (50%)	10 (56%)
Female (N, %)	9 (50%)	8 (44%)

Source: Reviewer's independent analysis

A one compartment model with first-order absorption was used to characterize the PK of benznidazole. The initial population PK model was established based on adult data from Raaflaub 1979, Raaflaub 1980, 101 and 102 studies. Body weight was identified as a significant covariate on clearance (CL/F) and volume of distribution (Vd). Even though the impact of body weight on PK of benznidazole had been considered in the model, the population model appeared unable to describe the PK data of benznidazole in adult patients from Raaflaub 1980 study. As shown below in Figure 4, the population

predicted values from Raaflaub 1980 study were biased (under predicted) relative to observed concentrations.

Figure 4 Goodness-of-fit for population PK model based on Raaflaub 1979, Raaflaub 1980, 101 and 102 studies
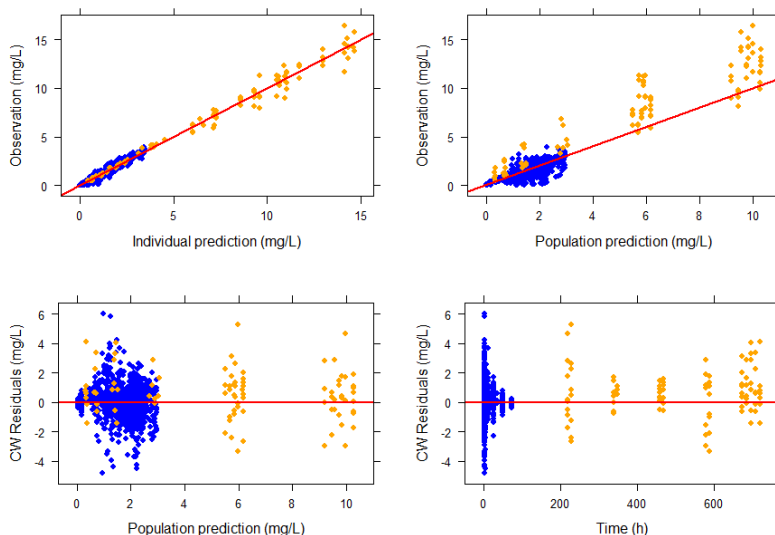
The blue dots represent the PK data from Raaflaub *1979, 101 and 102; the orange dots represent the PK data from Raaflaub 1980 study*

Source: Reviewer's independent analysis

It should be noted that Raaflaub 1980 study was the only PK study with steady state concentration data while the other three studies were single dose studies. As the objective of the reviewer's population PK analysis was to evaluate the influence of the formulation (TBM formulation vs Radanil) on benznidazole PK, Raaflaub 1980 study was excluded from the population PK analysis. First, it is the only study with steady state concentrations, and the difference in exposure could be a result of non-linear or time-dependent (auto-inhibition) metabolism that would not have been identified from the single dose healthy volunteer studies. In addition, Raaflaub 1980 study was the only PK study in patients with Chagas disease. As such, the difference in PK between studies may be related to disease, which would not be present in the three PK studies conducted in healthy volunteers.

The new population PK model based on Raaflaub 1979, 101, and 102 studies was able to describe the PK of benznidazole in adults with the two formulations. Body weight was identified as a significant covariate on CL/F and Vd/F and was included on these terms as a power function. Other than body weight, no other demographics or formulation factors were identified as a significant covariate for CL/F, Vd/F, and Ka. Figure 5, Figure 6, and Figure 7 show the relationship between model parameters and covariate candidates (body weight, age, sex, and formulation) after adding body weight on CL/F and Vd/F. It should be noted that the addition of body weight as a parameter on CL/F and Vd/F results in no

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trend between inter-individual variability and body weight. The parameter vs formulation plots illustrate that no significant difference of PK parameters was observed between the two different formulations.

Figure 5 Relationship between inter-subject variability of CL/F and covariates after including covariates in the model
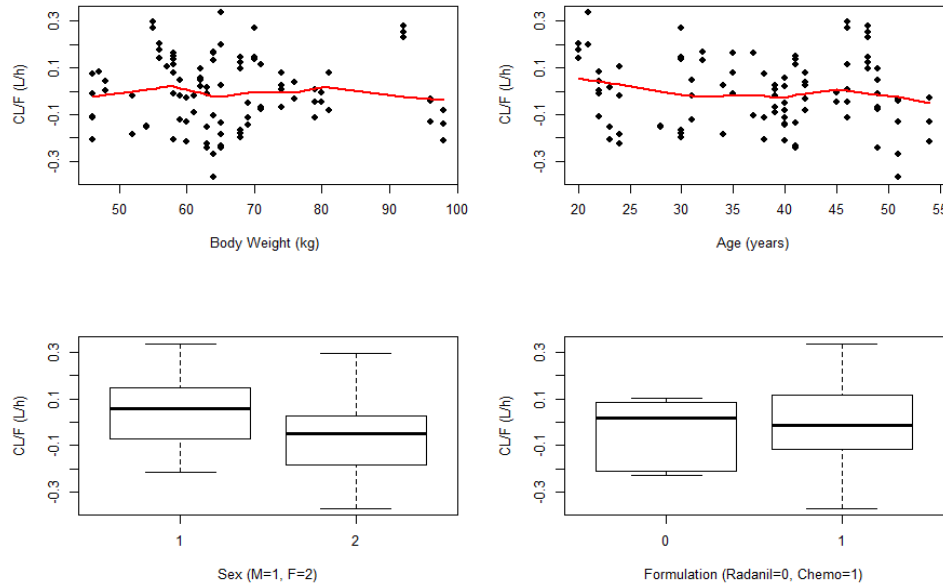
Source: Reviewer's independent analysis

Figure 6 Relationship between inter-subject variability of Vd/F and covariates after including covariates in the model
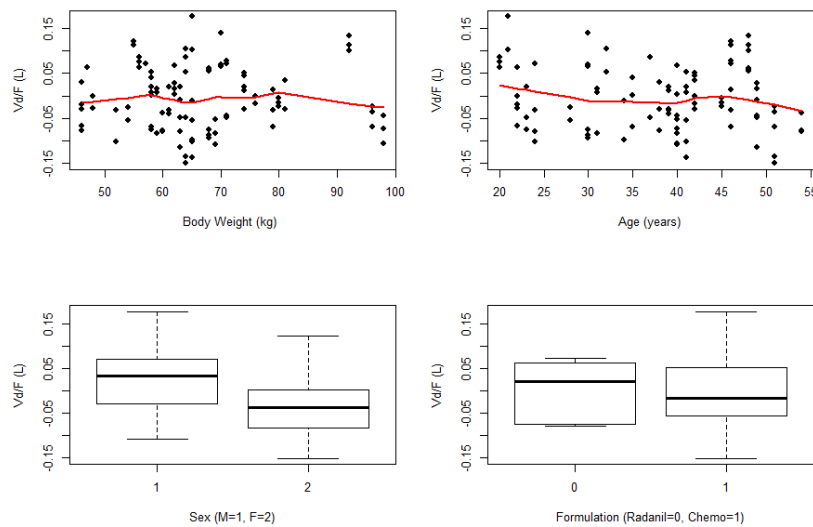
Source: Reviewer's independent analysis

Figure 7 Relationship between inter-subject variability of Ka and covariates after including covariates in the model
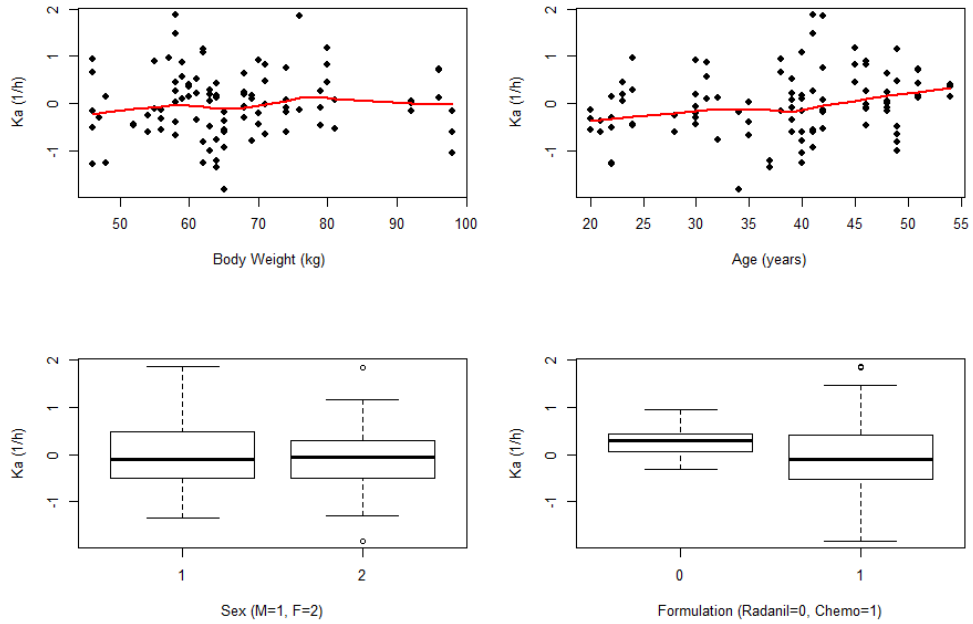
Source: Reviewer's independent analysis

The parameter estimates are listed in Table 4. The goodness-of-fit plots are shown in Figure 8. The plots suggest that the model predictions had a good agreement with observations.

Table 4 Parameter estimates for population PK model based on Raaflaub 1979, 101 and 102 studies

Parameter	Estimate (RSD)	Inter-subject variability
CL/F (L/h)	2.28 (2.4%)	19.1%
V/F (L)	37.3 (1.1%)	7.8%
Ka (h^{-1})	1.13 (11.3%)	72.2%
WT on CL/F	0.703 (19.9%)	
WT on Vd/F	0.831 (7.5%)	
Residual		
Additive	0.033 (3.7%)	

Source: Reviewer's independent analysis

Figure 8 Goodness-of-fit for population PK model based on Raaflaub 1979, 101 and 102 studies
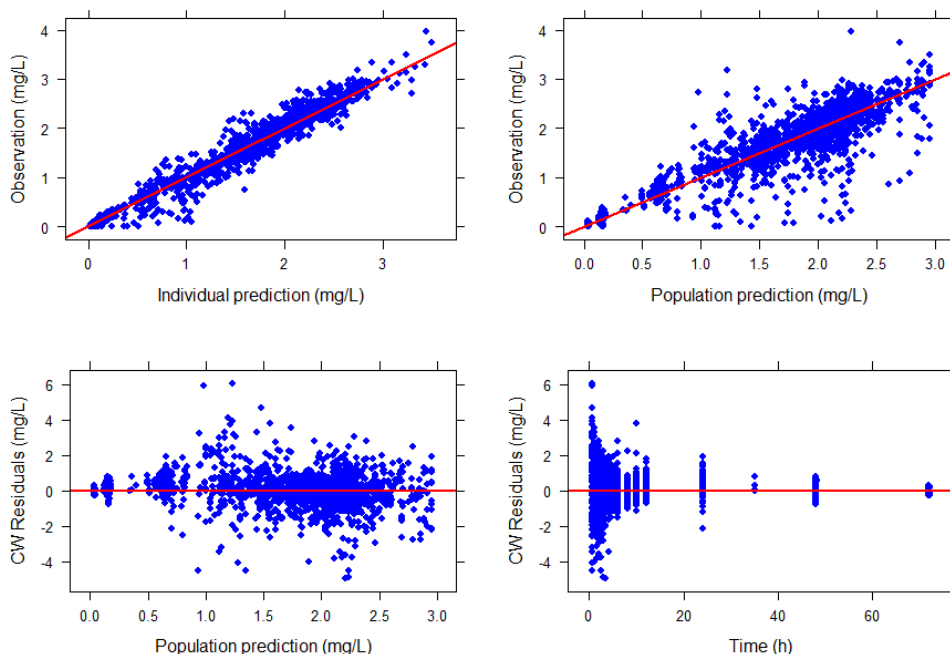
Source: Reviewer's independent analysis

The second population PK analysis conducted by the reviewer was to develop a combined adult and pediatric model that could be used to inform dosing in pediatrics. In this analysis data from Study 101 and 102 as well as the data from the Applicant's population PK analysis (excluding Study 1980) were combined for the reviewer's analysis. Observations were considered as outliers when their conditional weighted residuals were higher than 5 mg/L. At first, the impact of body weight on CL/F and Vd/F were estimated and found to be significant, but the allometric scaling values were estimated to be 0.404 and 0.817 for CL/F and Vd/F, respectively. While the allometric coefficient for Vd/F is relatively similar to that from the population PK model using only the adult data, the coefficient for CL/F was substantially lower. This value would suggest that pediatric exposures would substantially drop off at lower body weights. It was considered that the estimate may have been influenced by sparse sampling and variability from the pediatric data. As a result, the coefficient values for body weight on CL/F and Vd/F were fixed to 0.703 and 0.831, respectively, which were derived from previous population PK analysis results using only single dose adult data with intensive sampling. The final parameter estimates from the combined adult and pediatric population PK analysis are listed in Table 5. The goodness-of-fit plots are shown in Figure 9.

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Table 5 Parameter estimates for population PK model based on Raaflaub 1979, 101, 102, and Altcheh 2014 studies

Parameter	Estimate (RSD)	Inter-subject variability
CL/F (L/h)	2.57 (7.7%)	60.7%
V/F (L)	37.1 (3.3%)	22.9%
Ka (h^{-1})	0.925 (11.1%)	97.3%
WT on CL/F	0.703 FIX	
WT on Vd/F	0.831 FIX	
Residual		
Additive	0.192 (0.9%)	

Source: Reviewer's independent analysis

Figure 9 Goodness-of-fit for population PK model based on Raaflaub 1979, 101, 102, and Altcheh 2014 studies
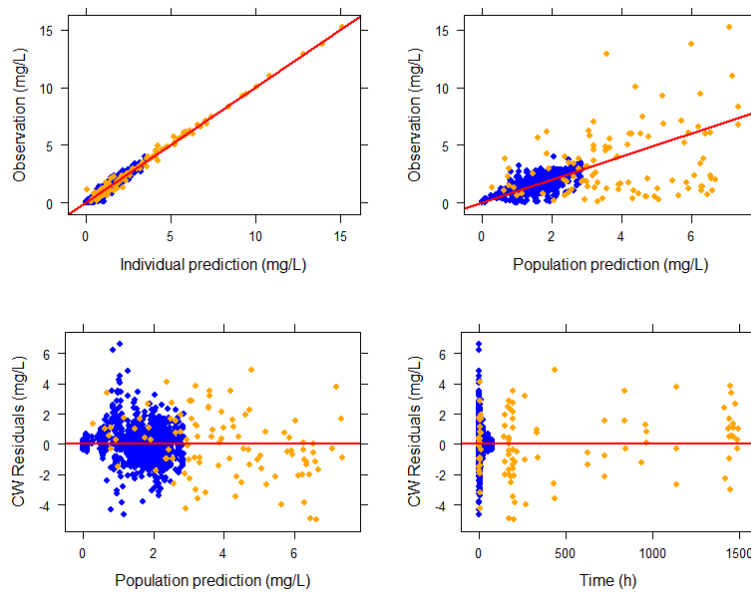
The blue dots represent the PK data from Raaflaub 1979, 101 and 102 studies; the orange dots represent the PK data from Altcheh 2014 study

Source: Reviewer's independent analysis

The goodness-of-fit plot shows the final population model can fairly characterize pediatric PK data. To take a closer look at the model performance on pediatric PK data, particularly, the goodness-of-fit plot for Altcheh 2014 study separated by different sampling time frame including PK samples after first dose, on-treatment and after last dose (Figure 10). The results clearly elucidated that the model was unable to describe the PK data well after the last dose; that most of observed PK data after last dose were

under-predicted. The sparse sampling and possible uncertainty in the dosing records in the pediatric population are major reasons contributing to the observed large inter-subject variability.

Figure 10 Goodness of fit for Altcheh 2014 study separated by sampling time frame
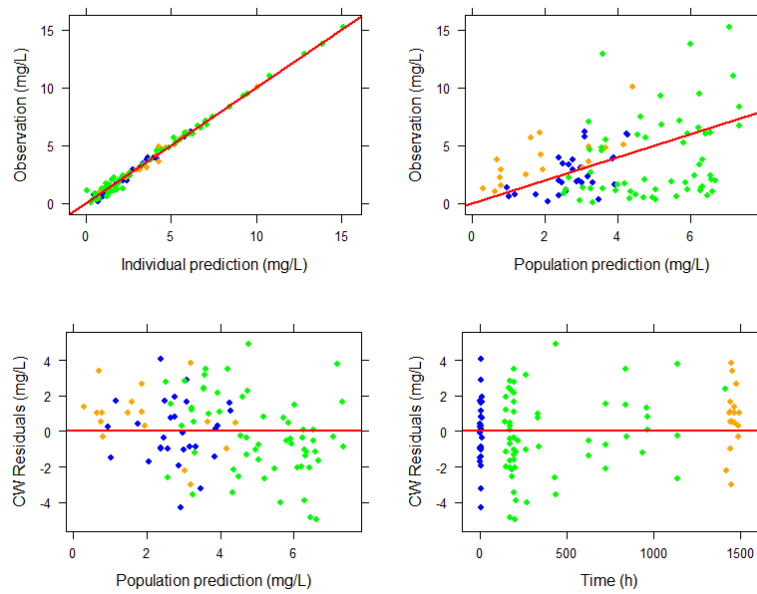
The blue dots represent the PK samples after first dose; the green dots represent PK samples at steady state on Day 3-59; the orange dots represent the PK samples after last dose from Altcheh 2014 study

Source: Reviewer's independent analysis

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4.5   Summary of Bio-Analytical Method Validation

The quantification of benznidazole (BNZ) in human EDTA plasma was done by a LC/MS/MS method (Analytical site: (b) (4)   In support of the analytical method, the Applicant has submitted two reports: 14 (b) (4)-2453V and 14 (b) (4)-2453V01. Report 14 (b) (4)-2453V provides the information on assay performance whereas Report 14 (b) (4)-2453V01 contains the information on the long-term stability of analyte: BNZ in matrix, stock solution, working solutions, and internal standard.

The key validation parameters from both reports are summarized in Table 1.

Table 1: Method validation & stability parameters for the LC/MS/MS method used to quantify BNZ in human plasma

Information from Report 14 (b) (4)-2453V (Dec 2014-Jan 2015)	
Linearity	r ≥ 0.9990
Calibration Curve Range*	50.25 to 3015.00 ng/mL
Between-Run Accuracy#	QC % nominal conc. 94.89 to 102.17 % (LLQC,QC1,QC2,QC3)
Between-Run Precision	QC coefficients of variation 1.95 to 5.64 % (LLQC,QC1,QC2,QC3)
Within-Run Accuracy	QC % nominal conc. 94.60 to 101.78 % (LLQC,QC1,QC2,QC3,ULQC)
Within-Run Precision	QC coefficients of variation 1.57 to 2.45 % (LLQC,QC1,QC2,QC3,ULQC)
Lower Limit of Quantitation (LLOQ)	50.25 ng/mL with a signal to noise ratio of 52
Recovery of Analyte	QC means 96.13, 101.56 and 97.61 % (QC1,QC2,QC3)
Recovery of Internal Standard	100.57%
Sample Collection and Handling Stability at 4°C	% change after 61 minutes in human EDTA K2 whole blood and 181 minutes in human EDTA K2 plasma partitioned over erythrocytes 4.52 % and 2.43 % (QC1,QC3)
Post-Preparative Stability at Room Temperature	Mean % change after 138 hours 6.12 and 8.25 % (QC1,QC3)
Freeze and Thaw Stability at -20°C	Mean % changes after 4 cycles 0.95 and 3.20 % (QC1,QC3)
Freeze and Thaw Stability at -80°C	Mean % changes after 4 cycles -0.10 and 3.47 % (QC1,QC3)
Short-Term Stability of Analyte in Matrix at Room Temperature	Mean % changes after 49 hours -9.68% and -4.66% (QC1,QC3)
Information from Report 14 (b) (4)-2453V01 (Jul 2015)	
Long-Term Stability of Analyte in Matrix at -20°C	Mean % change after 182 days: -0.63 and 0.06 % (QC1,QC3)
Long-Term Stability of Analyte in Matrix at -80°C	Mean % change after 182 days: -0.67 and -0.18 % (QC1,QC3)

* Calibration standard concentrations (ng/mL): 50.25, 100.5, 301.5,603,1206,1809,2412,3015
\# QC concentrations (ng/mL): LLQC=50.17, QC1=150.5, QC2=1505, QC3= 2257.5, ULQC=3010

Reviewer's assessment:

Based on the reported validation parameters in Table 1, the bio-analytical method used for the quantification of BNZ appears acceptable.

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4.6   Review of Individual Study Reports

4.6.1   In Vitro Studies

205598 – 505538

Title:

Interspecies comparison of in vitro metabolism of benznidazole in mouse, rat, rabbit and human hepatocytes

Test Facility:

(b) (4)

Objective:

To determine the metabolic stability and metabolite profiles of BNZ using hepatocyte suspension from male CD1 mouse, Wistar rat, New Zealand white rabbit and human (pool of 10 donors).

Methods:

For each species, duplicate incubations were performed as follows:

- In 200 µL buffer containing $1x10^6$ hepatocytes/mL, 10 µM BNZ was incubated for five different time periods (1±1, 30±1, 60±1, 90±1, and 120±1 minutes)
- In 500 µL buffer containing $0.5x10^6$ hepatocytes/mL, 50 µM phenacetin (as Phase I metabolic activity control) was incubated for two different time periods (1±1 and 120±1 minutes)
- In 500 µL buffer containing $0.5x10^6$ hepatocytes/mL, 50 µM 7-hydroxycoumarin (as Phase II metabolic activity control) was incubated for two different time periods (1±1 and 120±1 minutes)

A Trypan blue dye exclusion test was performed in all incubated samples to assess hepatocyte viability at the end of the different incubation times.

All incubated samples were subjected to LC-PDA-MS analysis. Using PDA chromatograms and MS data, the formation of metabolites was evaluated.

Results:

For all species except the rabbit, the viability of hepatocytes after incubation was above 50% of the initial viability. For rabbit hepatocytes, the viability after 120 minutes of incubation was slightly below 50% (46 and 48% of initial viability, duplicate incubations). However, as the rabbit hepatocyte batches

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metabolized the activity controls (phenacetin and 7-hydroxycoumarin), it was considered that despite the observed lower viability, the hepatocytes had acceptable enzymatic activity.

For each species, the metabolic activity of the hepatocytes was confirmed by the measurement of phenacetin metabolism (for CYP dependent enzymatic/Phase I) and 7-hydroxycoumarin metabolism (for Phase II enzymatic activity).

BNZ was found chemically stable under the tested conditions and all $t_{1/2}$ values were above 120 minutes (Table 1 and Table 2). BNZ incubations with mouse or rabbit hepatocytes for 120±1 minutes produced metabolic degradation of 7.3 and 11.7%, respectively; whereas no metabolic degradation was observed in rat or human hepatocytes.

Six BNZ metabolites were (partly) identified from the incubations with hepatocytes from four different species. Metabolic reactions observed included nitro reduction, oxidation, and glutathione conjugation. No human-specific metabolite was detected. The identification and presence of metabolites detected in the hepatocyte incubations with different species are presented in Table 3. The proposed BNZ metabolic pathway is shown in Figure 1.

Table 1: BNZ metabolic stability in incubations with hepatocytes from four different species (source: Report 505538, Table 9)

Species	Remaining percentage Benznidazole (mean)				
	1±1 min	30±1 min	60±1 min	90±1 min	120±1 min
Mouse	100[1]	103	91.9	95.7	92.7
Rat	100[1]	103	103	101	105
Rabbit	100[1]	85.1	82.7	84.8	88.3
Human	100[1]	101	107	111	105

[1] Set at 100%
[2] based on a single measurement because the duplicate was rejected

Table 2: Estimated in vitro $t_{1/2}$ and in vitro intrinsic clearance values from BNZ incubations with hepatocytes from four different species (source: Report 505538, Table 10)

Species	$t_{1/2}$ (min)			$CL'_{int, in\ vitro}$ (mL/min/kg)		
	Series 1	Series 2	Mean	Series 1	Series 2	Mean
Mouse	>120	>120	>120	3.16[1]	1.16[1]	2.16[1]
Rat	>120	>120	>120	0[1]	0[1]	0[1]
Rabbit	>120	>120	>120	1.51[1]	3.35[1]	2.43[1]
Human	>120	>120	>120	0[1]	0[1]	0[1]

[1] Estimated values obtained by extrapolation

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Table 3: Possible BNZ metabolites detected in hepatocyte samples after two hour incubation (source: Report 505538, Table 11)

[M+H]⁺ m/z	Rt (min)[1]	Mass Shift[2]	Metabolic reaction	Detected in[3]
231.124	10.6-10.7	-29.974	Nitro reduction	M, R, RB, H
536.192	10.8	+275.093	Glutathione conjugation	M, R
536.192	11.4	+275.093	Glutathione conjugation	M
536.192	11.8	+275.093	Glutathione conjugation	M
277.093	13.8-13.9	+15.995	Oxidation	M, R, RB
277.093	17.5	+15.995	Oxidation	RB
261.098	20.2-20.4	0	=Parent compound	M, R, RB, H

Rt=retention time; min=minutes;
[1] Range of retention time mentioned as observed in different runs and different species
[2] Mass shift compared to parent compound
[3] M=mouse, R=rat, RB=rabbit, H=human

Figure 1: Proposed metabolic reactions for BNZ in incubations with mouse, rat, rabbit, and human hepatocytes (source: Report 505538, Figure 20)

M=mouse, R=rat, RB=rabbit, H=human

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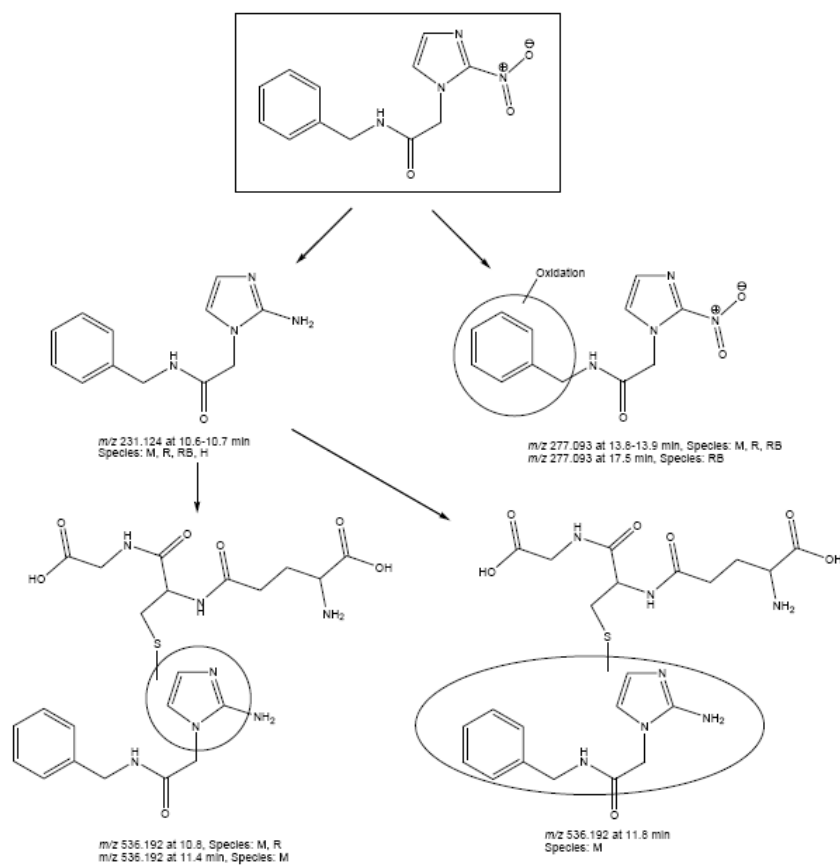
Applicant's Conclusion:

- BNZ showed high metabolic stability
- No human-specific metabolite was detected
- Six metabolites of BNZ were found in incubations with hepatocytes in suspension of 4 different species

Reviewer's Assessment:

In this study, the maximum reported decrease in BNZ concentrations is 12% from the incubations with rabbit hepatocytes, whereas, no changes in BNZ concentrations are reported from the incubations with human hepatocytes. In addition, the in vitro clearance of BNZ is estimated to be negligible. Therefore, the Applicant's conclusion that BNZ has high metabolic stability appears appropriate. In addition, from six metabolites that were identified in incubations with the hepatocytes from four different species, one metabolite was detected from the incubations human hepatocytes. This metabolite was also identified in incubations with other three species; therefore, the Applicant's conclusion that no human-specific metabolite was detected appears valid.

205598 – 513489

Title:

Characterization of human cytochrome p450 isoenzymes involved in the in vitro metabolism of benznidazole

Test Facility:

(b) (4)

Objective:

To identify the human cytochrome P450 (CYP) enzymes involved in the in vitro metabolism of BNZ.

Methods:

Experiment 1:

To determine Phase I metabolites of BNZ in presence of human liver microsomes (HLM), pilot incubations were performed at 0.25 mg/mL protein concentration with 10 µM BNZ for 30 minutes. The following incubation samples were prepared in duplicate:

    (1) 295 µL 0.1 M potassium phosphate buffer pH 7.4
    (2) 100 µL 5 mM NADPH in 0.1 M potassium phosphate buffer pH 7.4

(3) 100 µL 1.25 mg/mL pooled HLM in 0.1 M potassium phosphate buffer pH 7.4

The samples were pre-warmed for 5±1 minutes at 37±1°C in a water bath. 5 µL of BNZ spiking solution or 5 µL solvent (in blank incubations) were added to each incubation sample. After 30±1 minutes of incubation, the samples were subjected to protein precipitation followed by centrifugation. Subsequently, samples were subjected to LC-PDA-MS analysis.

Zero-time incubation samples for BNZ were prepared as described above, except, 200 µL acetonitrile was added to the incubation immediately. Next, 5 µL of BNZ spiking solution or 5 µL solvent (in blank incubations) were added to each incubation sample that was followed by centrifugation. Subsequently, samples were subjected to LC-PDA-MS analysis.

The incubated samples were evaluated for BNZ degradation/metabolism and metabolite formation.

Experiment 2

Since, no quantifiable BNZ metabolism was observed in Experiment 1, the following incubation samples were prepared:

(1) 295 µL 0.1 M potassium phosphate buffer pH 7.4
(2) 50 µL 5 mM NADPH in 0.1 M potassium phosphate buffer pH 7.4
(3) 50 µL 100 mM glucose-6-phosphate and 100 U/mL glucose-6-phosphate dehydrogenase in 0.1 M potassium phosphate buffer pH 7.4
(4) 100 µL 1.25 and 2.5 mg/mL pooled HLM in 0.1 M potassium phosphate buffer pH 7.4

The samples were pre-warmed for 5±1 minutes at 37±1°C in a water bath. 5 µL of BNZ spiking solution or 5 µL solvent (in blank incubations) were added to each incubation sample. After 30±1 and 60±1 minutes of incubation, the samples were subjected to protein precipitation followed by centrifugation. Subsequently, samples were subjected to LC-PDA-MS analysis.

Zero-time incubation samples for BNZ were prepared as described above, except, 200 µL acetonitrile were added in the incubation immediately. Next, 5 µL of BNZ spiking solution or 5 µL solvent (in blank incubations) were added to each incubation sample that was followed by centrifugation. Subsequently, samples were subjected to LC-PDA-MS analysis.

Experiment 3

To confirm the adequate metabolic activity of the microsomal batch used and the high metabolic stability of BNZ, the following incubation samples were prepared in parallel with the controls (to assess the metabolic activity of the microsome batch):

(1) 295 µL 0.1 M potassium phosphate buffer pH 7.4
(2) 50 µL 5 mM NADPH in 0.1 M potassium phosphate buffer pH 7.4
(3) 50 µL 100 mM glucose-6-phosphate and 100 U/mL glucose-6-phosphate dehydrogenase in 0.1 M potassium phosphate buffer pH 7.4
(4) 100 µL 1.25 and 2.5 mg/mL pooled HLM in 0.1 M potassium phosphate buffer pH 7.4

The samples were pre-warmed for 5±1 minutes at 37±1°C in a water bath. 5 µL of BNZ spiking solution or 5 µL solvent (in blank incubations) were added to each incubation sample. After 30±1 and 60±1 minutes of incubation, the samples were subjected to protein precipitation followed by centrifugation. Subsequently, samples were subjected to LC-PDA-MS analysis.

Zero-time incubation samples for BNZ were prepared as described above, except, 200 µL acetonitrile was added to the incubation immediately. Next, 5 µL of BNZ spiking solution or 5 µL solvent (in blank incubations) were added to each incubation sample that was followed by centrifugation. Subsequently, samples were subjected to LC-PDA-MS analysis.

As BNZ was hardly metabolized by human liver microsomes, the study was discontinued and the additional planned assessments were cancelled.

Results:

Results from Experiment 1, Experiment 2, and Experiment 3 are reported in Table 1, Table 2, and Table 3, respectively. The results of the metabolic activity experiment are summarized in Table 4. None of the experiments showed significant decrease in BNZ concentration, i.e., over 97% remaining after 60 minutes incubation. During the Experiment 2, two metabolites were detected. A metabolite with m/z 277.093 (oxidation), which might originate from Phase I (microsomal metabolism) and was also observed in the project 505538. Another metabolite with m/z 380.102 (S-cysteine), which might originate from Phase II, was deemed not relevant for this study. The concentration level of metabolite with m/z 277.093 was too low for reliable quantification of BNZ metabolism.

Table 1: Extent of BNZ metabolism - Experiment 1 (source: Report 513489, Table 3)

Benznidazole concentration	Protein concentration (mg/mL)	Replicate	MS peak area Benznidazole		% remaining after 30 min incubation
			t=0 min	t=30 min	
10 µM	0.25	1	147968358	148336505	98
		2	156198281	151245937	
		Average	152083320	149791221	

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Table 2: Extent of BNZ metabolism - Experiment 2 (source: Report 513489, Table 4)

Benznidazole concentration	Protein concentration (mg/mL)	Replicate	MS peak area Benznidazole		
			t=0 min	t=30 min	t=60 min
10 µM	0.25	1	172500331	167958670	162069815
		2	172048373	165181082	162882255
		Average	172274352	166569876	162476035
%remaining			100%[1]	97%	94%
10 µM	0.5	1	163434604	159593441	164931891
		2	170309859	165343071	168293998
		Average	166872231	162468256	166612944
%remaining			100%[1]	97%	100%

[1] Set at 100%

Table 3: Extent of BNZ metabolism - Experiment 3 (source: Report 513489, Table 7)

Benznidazole concentration	Protein concentration (mg/mL)	Replicate	Peak area Benznidazole	
			t=0 min	t=60 min
10 µM	0.25	1	261698371	247243227
		2	243763521	244797626
		average	252730946	246020427
%remaining			100%[1]	97%
10 µM	0.5	1	240033080	234763550
		2	237546771	265011927
		average	238789925	249887739
%remaining			100%[1]	105%

[1] Set at 100%

Table 4: Extent of phenacetin (control) metabolism - Experiment 3 (source: Report 513489, Appendix 4 - Table 11)

Species	Time point of sampling (min)	Phenacetin peak area	% remaining	Rate of phenacetin metabolism (pmol/min/mg protein)[1]	Maximum metabolism rate at 100% metabolism in pmol/min/mg protein (theoretical)[2]
Human	1	324	100	0	12000
	10	319	98	185	

[1] Calculated according to the following formula: [(100-% remaining)/100] * maximum metabolism rate

[2] Based on the presence of 30 µM substrate in the incubations, incubation for 10 minutes, protein concentration of 0.25 mg per mL and an incubation volume of 0.5 mL.

Applicant's Conclusion:

- The microsome batch used metabolized phenacetin, indicating that microsomes were metabolically viable

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- BNZ was hardly metabolized by human liver microsomes
- From the two metabolites detected, a metabolite with m/z 277.093 (oxidation), which might originate from Phase I (microsomal metabolism), was detected at very low level and the quantification was not reliable
- The second metabolite with m/z 380.102 (S-cysteine) which might originate from Phase II, not expected microsomal metabolism, and therefore was deemed not relevant for this study

Reviewer's Assessment:

The length of the experiment that provided information on the extent of phenacetin (control) metabolism (Table 4) appears inadequate. In Experiment 3, the extent of BNZ metabolism was measured after 60 minutes whereas the extent of phenacetin metabolism was measured after 10 minutes. At 10 minutes only 2% phenacetin was metabolized. Therefore, to this Reviewer, these findings cannot adequately support that the microsome batch used in this study was metabolically viable. Based on the pending mass balance/ADME study results for BNZ in humans, the need of repetition of this study will be reassessed.

205598 – 513491

Title:

In vitro determination of the inhibitory properties (reversible or time dependent) of benznidazole for the human cytochrome P450 isoenzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4 using human liver microsomes

Test Facility:

(b) (4)

Objective:

To determine in vitro whether BNZ inhibits the activity of the human CYP isoenzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 towards model substrates using human liver microsomes.

Methods:

The study consisted of two different parts: (1) determination of the reversible inhibitory properties of BNZ and (2) determination of the time dependent inhibition of BNZ. In Table 1 the CYP isoenzymes, substrates, and positive control inhibitors are summarized.

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Table 1: Percentages of substrate remaining in control incubations compared to zero time incubation (reversible inhibition) (source: Report 513491, Table 2)

Human CYP isoenzyme	Substrate	Metabolite	Positive control inhibitor(s) (reversible)	Positive control inhibitor (time dependent)
CYP1A2	Phenacetin	Acetaminophen	Fluvoxamine	Furafylline
CYP2B6	Bupropion	Hydroxybupropion	Ticlopidine	Thiotepa
CYP2C8	Paclitaxel	6α-hydroxypaclitaxel	Ketoconazole	Isoniazid
CYP2C9	Diclofenac	4'-hydroxydiclofenac	Sulfaphenazole	Tienilic acid
CYP2C19	(S)-mephenytoin	(S)-4'-hydroxymephenytoin	Tranylcypromine	S-fluoxetine
CYP2D6	Bufuralol	1'-hydroxybufuralol	Quinidine	Paroxetine
CYP3A4	Midazolam	1'-hydroxymidazolam	Ketoconazole	Mifepristone
	Testosterone	6β-hydroxytestosterone	Ketoconazole	Mifepristone

Incubation conditions that were used in the study are given in Table 2. Incubations to determine inhibition were performed at 37°C in duplicate at a concentration range of 0.03 to 100 µM BNZ. For all the control inhibitors, the IC_{50} values were within the historic standard ranges (Table 3).

Table 2: Incubation conditions for the determination of CYP inhibition by BNZ (source: Report 513491, Table 5)

Human CYP isoenzyme	Potassium phosphate buffer pH 7.4 (M)	NADPH in buffer (mM)	HLM[1] (mg/mL)	Incubation time (min)	Substrate		Total volume of incubation mixture (µL)
					Nature	Concentration (µM)	
CYP1A2	0.1	0.5[2]	0.05	30	phenacetin	30	250
CYP2B6	0.1	1[2,3,4]	0.1	20	bupropion	30	250
CYP2C8	0.025	1	0.25	10	paclitaxel	6	500
CYP2C9	0.025	1	0.1	10	diclofenac	10	250
CYP2C19	0.025	0.5[2]	0.25	30	(S)-mephenytoin	10	250
CYP2D6	0.1	1	0.1	10	bufuralol	7.5	250
CYP3A4	0.1	1	0.05	5	midazolam	2	250
	0.1	1	0.1	10	testosterone	50	250

[1] HLM: human liver microsomes
[2] In addition, 10 mM glucose-6-phosphate and 10 U/mL glucose-6-phosphate dehydrogenase were added
[3] Instead of NADPH, NADP+ was added.
[4] In addition, 3.3 mM $MgCl_2$ was added.

Table 3: Positive control inhibitors and reference IC$_{50}$ values (reversible inhibition)

(source: Report 513491, Table 3)

Human CYP isoenzyme	Positive control inhibitor	Concentrations used in the study (µM)	IC$_{50}$ historical control values (µM)[a]	
			min	max
CYP1A2	Fluvoxamine	0.003, 0.01, 0.03, 0.1, 0.3, 1, 3	0.02	0.34
CYP2B6	Ticlopidine	0.02, 0.06, 0.2, 0.6, 2, 6, 20	0.02	0.26
CYP2C8	Ketoconazole	0.1, 0.3, 1, 3, 10, 30, 50	3.3	23
CYP2C9	Sulfaphenazole	0.01, 0.03, 0.1, 0.3, 1, 3, 10	0.35	3.0
CYP2C19	Tranylcypromine	0.1, 0.3, 1, 3, 10, 30, 100	1.1	24
CYP2D6	Quinidine	0.01, 0.03, 0.1, 0.3, 1, 3, 10	0.02	0.14
CYP3A4 (substrate testosterone)	Ketoconazole	0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1	0.01	0.08
CYP3A4 (substrate midazolam)			0.01	0.07

[a] based on values obtained at ▨ (b) (4) from 2005- 2014

Incubations to determine possible time dependent inhibition were performed using the same conditions as for the reversible inhibition, with the additional pre-incubation of BNZ with the microsomal mixture for 30 minutes. After pre-incubation the reaction was started by the addition of substrate. The positive controls that were used in time dependent inhibitors are listed in Table 4.

Table 4: Concentrations of positive control inhibitors used for time dependent inhibition

(source: Report 513491, Table 4)

Human CYP isoenzyme	Positive control inhibitor (time dependent)	Concentration (µM)
CYP1A2	Furafyllline	5
CYP2B6	Thiotepa	5
CYP2C8	Isoniazid	200
CYP2C9	Tienilic acid	1
CYP2C19	S-Fluoxetine	30
CYP2D6	Paroxetine	0.5
CYP3A4	Mifepristone (testosterone)	1
	Mifepristone (midazolam)	1

Metabolite formation in incubation samples was analyzed by LC-MS.

Results:

Percentages of substrate remaining in control incubations (compared to zero time incubation) for reversible and time dependent inhibition study are provided in Table 5 and Table 6, respectively. The IC$_{50}$ values from the reversible and time dependent inhibition experiments for BNZ are summarized in Table 7. The IC$_{50}$ of pre-incubated samples versus co-incubated samples showed a >2-fold shift for CYP2C19, indicating that a time dependent mechanism might play a role in CYP2C19 inhibition.

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Table 5: Percentages of substrate remaining in control incubations compared to zero time incubation (reversible inhibition) (source: Report 513491, Table 25)

CYP	substrate	Control inhibitor[1]		Benznidazole control incubations[1]	
		series 1 (%)	series 2 (%)	series 1 (%)	series 2 (%)
1A2	Phenacetin	97	100	98	96
2B6	Bupropion	87	93	90	93
2C8	Paclitaxel	78	90	84	90
2C9	Diclofenac	80	86	79	87
2C19	(S)-Mephenytoin	102	114	100	113
2D6	Bufuralol	99	99	98	101
3A4	Midazolam	84	86	86	88
3A4	Testosterone	93	91	95	93

[1] The percentage of remaining substrate at the end of incubation is shown

Table 6: Percentages of substrate remaining in control incubations compared to zero time incubation (time dependent inhibition) (source: Report 513491, Table 26)

CYP	substrate	Control inhibitor[1]		Benznidazole control incubations[1]	
		series 1 (%)	series 2 (%)	series 1 (%)	series 2 (%)
1A2	Phenacetin	95	94	96	95
2B6	Bupropion	88	91	91	90
2C8	Paclitaxel	93	84	90	80
2C9	Diclofenac	80	58	81	83
2C19	(S)-Mephenytoin	97	106	99	98
2D6	Bufuralol	99	97	98	97
3A4	Midazolam	91	90	87	87
3A4	Testosterone	90	90	90	92

[1] The percentage of remaining substrate at the end of incubation is shown

Table 7: Summary of IC_{50} values of BNZ for CYP reversible and time dependent inhibition

(source: Report 513491, Table 1)

CYP isoform	Substrate	Reversible inhibition	Time dependent inhibition	IC_{50} shift
		IC_{50} Benznidazole (μM)	IC_{50} Benznidazole (μM)	
1A2	Phenacetin	No inhibition	>100	n.a.
2B6	Bupropion	>100	>100	n.a.
2C8	Paclitaxel	No inhibition	>100	n.a.
2C9	Diclofenac	No inhibition	No inhibition	n.a.
2C19	(S)-mephenytoin	>100	51	>2
2D6	Bufuralol	No inhibition	No inhibition	n.a.
3A4	Midazolam	No inhibition	>100	n.a.
3A4	Testosterone	>100	>100	n.a.

n.a.: not applicable

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Applicant's Conclusion:

- BNZ appeared to inhibit CYP2C19 activity
- BNZ is probably a time dependent inhibitor of CYP1A2, CYP2B6 and CYP3A4 at higher concentrations

Reviewer's Assessment:

Based on the reported shift in the BNZ IC_{50} values for CYP2C19, the Applicant's conclusions that BNZ may inhibit CYP2C19 appear appropriate. Also, >2-fold shift in IC_{50} values suggests time dependent inhibition of CYP2C19. In addition, given that the extent of time dependent inhibition for CYP1A2, CYP2B6, and CYP3A4 was higher than their respective extent of reversible inhibition at the concentrations of 30 μM and 100 μM, the Applicant's conclusion that BNZ is probably a time dependent inhibitor of CYP1A2, CYP2B6 and CYP3A4 at higher concentrations appears reasonable. Due to the pending mass-balance/ADME study, no relevant label statements are to be made at present.

205598 – 513492

Title:

Induction towards CYP 1A2, 2B6 and 3A4 by benznidazole in human cryopreserved primary hepatocytes from three donors

Test Facility:

(b) (4)

Objective:

The aim of this study was to test the capacity of BNZ to induce human cytochrome P450 (CYP) enzymes 1A2, 2B6, and 3A4 in cryopreserved human hepatocytes from three donors.

Methods:

After seeding cells from human hepatocytes from three donors (identified as GNA, FOS, and SVL), hepatocytes were exposed to BNZ or positive control inducers in 100 μl of InVitroGRO™ medium at five different BNZ concentrations. Experiment conditions are presented in Table 1. The cell culture medium

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was replaced after 24 hours. Hepatocytes from one donor were exposed to chlorpromazine, which was used as cytotoxicity control.

Table 1: BNZ and control compound concentrations and exposure conditions (source: Report 513492, Table 1)

Benznidazole	0.1, 0.3, 1, 3, 10, 30 and 100 µM
Control inducers	1000 µM Phenobarbital (CYP2B6 and CYP3A4), 10 µM Rifampicin (CYP3A4 and CYP2B6), 50 µM Omeprazole (CYP1A2)
Cytotoxicity positive control	Chlorpromazine (50 µM)
Replicate incubations	Three
Solvent content in incubation	0.5% (v/v) DMSO used in all incubations
Duration of test compound exposure	48 hr; fresh medium (containing positive control, vehicle, or study compound) replaced after 24 hr
Incubation conditions	37°C under 5% CO_2

After the 48h exposure/incubations, the hepatocytes were washed with phosphate buffered saline. Then the samples were prepared for qPCR analyses as per the process summarized in Table 2. The resulting cDNA was diluted 5-fold with sterile-filtered H_2O. Single plex qPCR analysis was performed as summarized in Table 3.

Table 2: Sample preparation for qPCR analysis (source: Report 513492, Table 2)

RNA extraction	Purelink Pro 96 RNA purification kit (Invitrogen / Thermo Scientific Inc.) according to manufacturer's protocol
DNAse treatment	Purelink DNAse set (Invitrogen/ Thermo Scientific Inc.)
cDNA synthesis	High-Capacity cDNA Reverse Transcription Kit with Rnase Inhibitor (Applied Biosystems/ Thermo Scientific Inc.) according to manufacturer protocol Amount of RNA/reaction: 10 µl (corresponding to 200-500 ng RNA) Total reaction volume: 20 µl
Reverse transcriptase	MultiScribe™ MuLV
Storage of cDNA	−20°C until analysis

Table 3: Real-time quantitative PCR analysis conditions (source: Report 513492, Table 3)

Instrumentation	Quantstudio™ 6 Flex Real-Time PCR System with fast 96-well block		
Target gene assay	CYP1A2 Hs_00167927_m1(Life Technologies) CYP2B6 Hs_03044634_m1(Life Technologies) CYP3A4 Hs_00604506_m1 (Life Technologies)		
Reference gene assay	GAPDH Hs02758991_g1 (Life Technologies) B2M Hs00984230_m1 (Life Technologies)		
Chemistry used	TaqMan™ probe chemistry		
Dye label reporter	FAM (CYP1A2, CYP2B6, CYP3A4), VIC (GAPDH, B2M), ROX (internal reference dye)		
Dye label quencher	non-fluorescent		
Software	Quantstudio™ Real-Time PCR software v1.0		
Real Time PCR cycling conditions			
Time	Temperature	Repeats	
20 sec (hold)	95 °C	Stage 1: 1	
1 sec (cycle)	95 °C	Stage 2: 40	
20 sec (cycle)	60 °C	Stage 2: 40	
Master mix +H2O/reaction	16 µl		
cDNA/reaction	4 µl		
Total Volume	20 µl		

In addition, the cytotoxicity of BNZ was assayed in the hepatocytes, from the samples of one hepatocyte donor after 24 h exposure by measuring membrane integrity (LDH leakage) and visual inspection.

Omeprazole, rifampicin, and phenobarbital were included as positive controls for induction of CYP expression. Measurement of LDH leakage and visual inspection of the cells during incubations did not indicate overt cytotoxicity by BNZ within 24 hours at the tested concentration range.

Results:

Results of relative CYP1A2, CYP2B6, and CYP3A4 mRNA-levels in the presence of BNZ and control inducers are reported in Table 4, Table 5, and Table 6, respectively. The incubations with BNZ showed minor increases in CYP1A2 mRNA levels (up to 2.5 fold / 29 % maximum induction by positive control), CYP2B6 (up to 3.2 fold / 35 % maximum induction by positive control) and CYP3A4 (up to 4.2 fold / 11 % maximum induction by positive control). However, near maximum increases were observed at the lowest concentration (0.1 µM) and no clear increase in enzyme mRNA occurred at increasing BNZ concentrations. Therefore EC_{50} and E_{max} values were not calculated. Visual inspection of the cells treated with BNZ for 48 hours revealed no morphological modifications. LDH leakage at 24 h incubation with BNZ concentrations up to 100 µM did not indicate cytotoxicity.

Table 5: Relative CYP1A2 mRNA-levels in the presence of BNZ and control inducers

(source: Report 513492, Table 4)

RNA results	Fold induction (mean±SD)			% of maximum positive control (mean)		
Hepatocyte donor	GNA	FOS	SVL	GNA	FOS	SVL
0.5% DMSO	1.00±0.15	1.00±0.05	1.00±0.05	0%	0%	0%
50 µM Omeprazole	6.30±2.14*	33.9±1.18	10.4±0.96	100%	100%	100%
1000 µM Phenobarbital	3.06±0.63	2.17±0.36	2.33±0.30	39%	4%	14%
10 µM Rifampicin	1.38±0.30	0.84±0.18	1.11±0.26	7%	0%	1%
0.1 µM Benznidazole	1.89±0.12	1.31±0.08	1.90±0.33	17%	1%	9%
0.3 µM Benznidazole	2.02±0.43	1.26±0.11	1.78±0.27	19%	1%	8%
1 µM Benznidazole	2.19 ±0.58	1.54±0.14	1.98±0.51	22%	2%	10%
3 µM Benznidazole	2.53 ±0.98	1.28±0.25	2.26±0.69	29%	1%	13%
10 µM Benznidazole	2.03 ±0.52	1.17±0.12	1.98±0.38	19%	1%	10%
30 µM Benznidazole	2.50 ±0.84	1.31±0.09	1.85±0.31	28%	1%	9%
100 µM Benznidazole	1.56 ±0.42	1.62±0.13	1.69±0.33	11%	2%	7%

* high variability between 3 replicates

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Table 6: Relative CYP2B6 mRNA-levels in the presence of BNZ and control inducers

(source: Report 513492, Table 5

RNA results	Hepatocyte donor (mean±SD)			% of maximum positive control (mean)		
Hepatocyte donor	GNA	FOS	SVL	GNA	FOS	SVL
0.5% DMSO	1.00±0.22	1.00±0.06	1.00±0.04	0%	0%	0%
50 µM Omeprazole	1.18±0.67	2.54±0.16	1.26±0.10	3%	15%	6%
1000 µM Phenobarbital	7.38±1.15	11.6±2.92	5.24±1.29	100%	100%	100%
10 µM Rifampicin	5.88±1.00	7.58±1.58	4.17±1.44	76%	62%	75%
0.1 µM Benznidazole	2.65±0.32	1.12±0.15	1.85±0.57	26%	1%	20%
0.3 µM Benznidazole	2.39±0.38	1.48±0.10	2.12±0.30	22%	4%	26%
1 µM Benznidazole	2.52±0.64	2.17±0.22	2.15±0.83	24%	11%	27%
3 µM Benznidazole	3.23±1.15	1.98±0.69	2.44±0.96	35%	9%	34%
10 µM Benznidazole	2.54±0.80	1.04±0.20	2.32±0.66	24%	0%	31%
30 µM Benznidazole	2.83±1.36	1.42±0.24	2.45±0.41	29%	4%	34%
100 µM Benznidazole	2.07±0.55	1.97±0.13	1.59±0.59	17%	9%	14%

Table 7: Relative CYP3A4 mRNA-levels in the presence of BNZ and control inducers

(source: Report 513492, Table 6)

RNA results	Hepatocyte donor			% of maximum positive control (mean)		
Hepatocyte donor	GNA	FOS	SVL	GNA	FOS	SVL
0.5% DMSO	1.00±0.52	1.00±0.10	1.00±0.16	0%	0%	0%
50 µM Omeprazole	3.16±1.20	2.34±0.50	3.20±0.49	8%	21%	18%
1000 µM_Phenobarbital	28.5±4.35	7.37±0.97	13.3±2.16	100%	100%	100%
10 µM Rifampicin	26.9±5.04	7.19±1.45	11.3±2.30	94%	97%	84%
0.1 µM Benznidazole	1.62±0.27	1.02±0.07	1.26±0.06	2%	0%	2%
0.3 µM Benznidazole	2.48±0.57	0.82±0.15	1.03±0.17	5%	-3%	0%
1 µM Benznidazole	2.45±0.83	1.37±0.23	1.47±0.18	5%	6%	4%
3 µM Benznidazole	4.15±2.31	0.90±0.19	1.24±0.50	11%	-2%	2%
10 µM Benznidazole	1.29±0.22	1.00±0.15	1.30±0.10	1%	0%	2%
30 µM Benznidazole	2.17±0.81	0.87±0.16	0.99±0.20	4%	-2%	0%
100 µM Benznidazole	2.18±0.62	1.62±0.17	0.95±0.19	4%	10%	0%

Applicant's Conclusion:

- Measurement of LDH leakage after 24 hours exposure and visual inspection of the cells during 48 hour incubations did not indicate overt cytotoxicity by BNZ at the tested concentration range.

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- Minor increases (2.5 - 4.5 folds) in mRNA of CYP1A2, CYP2B6 and CYP3A4 were observed upon exposure of BNZ in one of the three tested donors. However, clear concentration dependence was not observed.
- The observed increases in CYP mRNA-levels in BNZ treated hepatocytes were minimal when compared to the increases induced by the positive control agents and did not exceed 40% threshold (compared to % induction caused by a positive control inducer). The increases in CYP mRNA-levels observed in BNZ treated hepatocytes were not consistently observed in all donors and were not proportional to the tested concentrations, therefore their biological significance is questionable.

Reviewer's Assessment:

The Applicant has used a threshold of 40% increase for determining if the increase in CYP mRNA-levels in BNZ treated hepatocytes is significant. Based on the 40% threshold value, the Applicant's conclusions appear valid, i.e., the observed increases in CYP mRNA-levels in BNZ treated hepatocytes were minimal when compared to the increases induced by the positive control agents and did not exceed 40% threshold (compared to % induction caused by a positive control inducer). In addition, despite the observed high variability in the CYP mRNA-levels, increases in expression levels were not proportional to the tested BNZ concentrations (Table 7).

205598 – 513493

Title:

Determination of benznidazole as a possible substrate or inhibitor of P-glycoprotein using the bi-directional transport assay in Caco-2 cells

Test Facility:

(b) (4)

Objective:

To determine if BNZ was a possible substrate or inhibitor of P-glycoprotein (P-gp) using Caco-2 cell monolayers.

Methods:

(1) BNZ as a P-gp substrate

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Caco-2 cells were exposed to 1, 10, and 100 µM BNZ either at the apical or at the basolateral side. Transport from the apical side to the basolateral side (A→B) and from the basolateral side to the apical side (B→A) in the presence and absence of cyclosporin A or quinidine (reference inhibitors) were studied for BNZ in triplicate wells. Vinblastine was used as a positive control substrate. As a control for monolayer integrity, a compound with low permeability (Atenolol) and a compound with high permeability (Propranolol) were used as reference. The integrity of the Caco-2 monolayer was checked by measuring the transepithelial electrical resistance (TEER) prior to start of the experiment and by measuring the permeability of Lucifer yellow (marker for paracellular permeability) as post-experiment permeability control. In addition, the permeability of the reference items atenolol (low permeability, paracellular transport) and propranolol (high permeability, passive transcellular transport) was measured. The reference item vinblastine was included as a positive control P-gp substrate.

The cells with no inhibitors were treated as follows: after 30, 60, and 120 minutes of incubation (at 37 ± 1.0°C and 5.0 ± 0.5% CO_2), a 100 µL sample was drawn from the receiver compartment, which was immediately replaced with transport buffer. At the end of the experiment, samples from both the donor and the receiver compartments were analyzed to determine recovery. The BNZ concentrations in the samples were determined by HPLC-UV.

The cells containing one of the inhibitors (cyclosporin A or quinidine) were treated as follows: After 30 minutes, the inhibitor solution of the donor compartment was replaced with a corresponding inhibitor solution containing 100 µM BNZ. After 120 minutes of incubation (at 37.0 ± 1.0°C and 5.0 ± 0.5% CO_2), a 100 µL sample was drawn from the donor and receiver compartment of the transwells. The concentration of BNZ in the samples was determined by HPLC-UV.

(2) BNZ as an inhibitor of P-gp

For the P-gp inhibition assay, caco-2 cells were exposed to 100 µM BNZ and cyclosporin A (positive control inhibitor) at both the basolateral and the apical side for 30 minutes. After that, the transport of vinblastine (known P-gp substrate) was determined. Transport from the apical side to the basolateral side (A→B) and from the basolateral side to the apical side (B→A) in the presence and absence of test item or control inhibitor was studied in triplicate wells.

Results:

Results of the BNZ permeability are summarized in Table 1. The efflux ratio of BNZ in the presence of the two P-gp selective inhibitors cyclosporin A and quinidine was reduced by 50% as compared to the efflux ratio in the absence of these inhibitors, indicating that it is likely that BNZ is a P-gp substrate. The efflux ratio of vinblastine did not decrease when co-incubated with BNZ at concentration of 100 µM (Table 2) indicating that BNZ is most likely not an inhibitor of the P-gp transport.

Table 1: Summarized results of the BNZ permeability (source: Report 513493, Table 1)

Inhibitor	Benznidazole C_0 (µM)	$P_{appA \rightarrow B}$ (nm/s±SD)	$P_{appB \rightarrow A}$ (nm/s±SD)	Efflux ratio (R_E)
None	1	208 ± 31	363 ± 33	1.7
None	10	185 ± 18	338 ± 10	1.8
None	100	172 ± 28	352 ± 25	2.0
Cyclosporin A	100	152 ± 16	145 ± 13	1.0
Quinidine	100	164 ± 3	164 ± 1	1.0

Papp: apparent permeability; A: apical side, B basolateral side, R_E efflux ratio, C_0 initial concentration; SD: standard deviation

Table 2: Permeability of vinblastine in the presence of 100 µM BNZ (source: Report 513493, Table 2)

Concentration Benznidazole (µM)	$P_{appA \rightarrow B}$ (nm/s)	$P_{appB \rightarrow A}$ (nm/s)	$R_E^{1)}$	Inhibition (%)
0	20.2	68.7	3.4	0
100	16.0	77.4	4.9	$0^{2)}$

[1)] $R_E = P_{app\ B \rightarrow A} / P_{app\ A \rightarrow B}$
[2)] Actual value -42.8%, reported as 0

Applicant's Conclusion:

P-gp does participate in the process of membrane permeation of BNZ and therefore BNZ is a substrate for P-gp but not an inhibitor of the P-gp transport.

Reviewer's Assessment:

Based on study results (Table 1 and Table 2), the Applicant's conclusions with respect to the potential of BNZ as a P-gp substrate or inhibitor appear valid. It is noteworthy that the Lucifer yellow transport was in the range $12*10^{-6}$ to $19*10^{-6}$ cm/s which is above the Applicant's acceptability criteria ($P_{app} <1x10^{-6}$ cm/s). However, citing the fact that TEER values and the permeability of the controls (atenolol, propranolol and vinblastine) are all within the acceptability criteria, it was considered by the Applicant that the higher permeability of Lucifer yellow have no influence on the integrity of the results. In principle, the Applicant's rationale appears reasonable; however, the deviation of more than 10-fold from the normal cut-off raises the concern on the integrity of the Caco-2 monolayer used in this study and no further explanation or potential reasons were given by the Applicant for these discrepancies. Due to the questionable results, no relevant label statements are to be made at present.

Title:

An in vitro investigation to assess if benznidazole is an inhibitor of the uptake transporters (OATP1b1*1a, OATP1B3, OAT1, OAT3, OCT1, and OCT2)

Test Facility:

(b) (4)

Objective:

To evaluate any inhibitory effect of BNZ on the human drug uptake transporters OATP1B1 *1a, OATP1B3, OAT1, OAT3, OCT1 and OCT2.

Methods:

This study used control HEK293 (Untransfected) cells or HEK293 cells expressing the relevant transporter (Transfected) cultured in suspension. The information on the cells, test items, and experimental design is presented in Table 1. HEK293 cells were used after 24-hour culture at 37°C under 5% CO_2. Incubations were performed in the presence of a probe substrate for each transporter and any inhibitory effects of test compound on the uptake of probe substrates were determined. The transporters, their respective probe substrates (positive control) and known inhibitors used in this study are shown in Table 2.

Table 1: Experimental design (source: Report EXL/REP/01, Table 1)

Cells	Test item	Experimental design
Untransfected HEK 293 cells	Benznidazole at eight concentrations – 0.1 to 200 µM Specific probe substrate at one concentration tested with test compound or known inhibitor at eight concentrations	Each concentration of benznidazole or specific inhibitor is tested in triplicate (in 3 wells) for one incubation time
HEK 293 cells transfected with specific transport gene	Benznidazole at eight concentrations – 0.1 to 200 µM Specific probe substrate at one concentration tested with test compound or known inhibitor at eight concentrations	

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Table 2: **Incubation conditions for uptake transporter inhibition assays** (source: Report EXL/REP/01, Table 3)

Transporter	Probe substrate	Concentration (µM)	Known Inhibitors (concentration tested)	Incubation times (minutes)
OATP1B1*1a	Estradiol-β-glucuronide (E17βGluc)	0.5	Cyclosporine A (0.1 - 30 µM)	5
OATP1B3	Cholecystokinin (CCK-8)	0.002	Cyclosporine A (0.1 - 50 µM)	5
OAT1	p-Aminohippuric acid (PAH)	2.3	Probenecid (0.1 - 100 µM)	5
OAT3	Estrone-3-sulphate (E3S)	0.52	Probenecid (0.1 - 100 µM)	5
OCT1	Metformin	10	Quinidine (0.1 - 100 µM)	10
OCT2	Metformin	50	Quinidine (1 - 500 µM)	10

Incubations were carried out at 37°C in Hanks' Balanced Salt Solution buffer containing 10 mM HEPES, pH 7.4 , with incubations initiated by the addition of test compound (or reference inhibitor) and probe substrate. The effect of BNZ (or reference inhibitor) on the uptake of probe substrates were also investigated in parental cells as a control.

At the specified times, incubations were terminated and cells were lysed. Cell lysates were mixed with scintillant prior to analysis of samples on a scintillation counter. Cell lysate/scintillant mixes from assays investigating OCT1 and OCT2, were mixed gently at room temperature for 10 minutes prior to liquid scintillation analysis. In addition, the protein concentration in each lysate was measured. Disintegration per minute (dpm) values obtained from pametal HEK293 cells were subtracted from the dpm obtained with HEK293 cells expressing the transporter and the results were reported as percentage inhibition of probe substrate uptake relative to the solvent control, following normalization of protein concentration.

Results:

The submitted results are summarized in the Table 3 below.

Table 3: Percent inhibition and reported IC$_{50}$ values

Transporter	Compound ID	Concentration Range (µM)	Mean IC$_{50}$ (µM)
OATP1B1*1a	BNZ	0 -200	>200
	Cyclosporin A	0 - 30	2.2
OATP1B3	BNZ	0 -200	>200
	Cyclopsorin A	0-50	0.23
OAT1	BNZ	0 -200	>200
	Probenecid	0-100	16
OAT3	BNZ	0 -200	34
	Probenecid	0-100	3.4
OCT1	BNZ	0 -200	>200
	Verapamil	0-100	3.2
OCT2	BNZ	0 -200	>200
	Verapamil	0-500	7.1

Applicant's Conclusion:

BNZ produced moderate inhibition of the transporter OAT3 (with an IC_{50} of 34 µM {~9 µg/mL}). BNZ had no inhibitory activity towards the transporters OATP1B1 *1a, OATP1B3, OAT1, OCT1 and OCT2 at concentrations up to 200 µM.

Reviewer's Assessment:

IC_{50} value for the BNZ's inhibition potential for all the transporter - except for OAT3 - tested in this study is estimated to be >200 µM, which is above the expected range of clinical concentrations following the proposed BNZ dosing. For OAT3 estimated mean IC_{50} is 34 µM (i.e.,~9 µg/mL}, which is within the expected range of clinical concentrations following the proposed BNZ dosing. Therefore, the Applicant's conclusion that BNZ may inhibit OAT3 appears appropriate. One of the PMC recommended for this application is that the based on pending mass balance/ADME study, additional Clinical Pharmacology study(ies) may be required (e.g. Patients with renal and/or hepatic impairment, in vitro studies, in vivo DDI studies). For example, if renal active secretion is found to be >25%, additional in vivo DDI studies may be needed.

EXL-REP-02

Title:

An in vitro Investigation to assess if Benznidazole is a substrate of the uptake transporters (OATP1B1*1a, OATPB13, OAT1, OAT3, OCT1 and OCT2)

Test Facility:

(b) (4)

Objective:

To evaluate if BNZ is a substrate of the human drug uptake transporters OATP1B1*1a, OATP1B3, OAT1, OAT3, OCT1, OCT2 using cultures of HEK293 cells expressing the relevant transporter.

Methods:

HEK293 cells transfected with each transporter gene and untransfected cells were exposed to BNZ at 1 or 10 µM. The information on the cells, test items, and experimental design is presented in Table 1. HEK293 cells were used after 24-hour culture at 37 °C under 5% CO_2. For each tested transporter, HEK293 (transfected and untransfected) cells were incubated with BNZ at 1 and 10 µM for two periods of time. In addition, for each tested transporter, a positive control assay was conducted concomitantly to confirm the effectiveness of the assay design (Table 2). For each positive control assay, the uptake of

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a specific probe substrate was measured with and without a known inhibitor of the tested transporter. The probe substrate and the known inhibitor were incubated each at a single concentration and for two periods of time.

Table 1: Experimental design (source: Report EXL/REP/02, Table 1)

Cells	Test item	Experimental design
Untransfected HEK 293 cells	Benznidazole at 1 and 10 µM Specific probe substrate at one concentration tested with and without a known inhibitor at one concentration	Each concentration of benznidazole or specific inhibitor is tested in triplicate (in 3 wells) using two incubation times
HEK 293 cells transfected with specific transport gene	Benznidazole at 1 and 10 µM Specific probe substrate at one concentration tested with and without a known inhibitor at one concentration	

Table 2: Incubation conditions for uptake transporter inhibition assays (source: Report EXL/REP/02, Table 3)

Transporter	Probe substrate (concentration used)	Known inhibitor (concentration tested)	Incubation time
OATP1B1*1a	Estrone-3-Sulphate (1 µM)	Cyclosporine A (10 µM)	5 & 20 min
OATP1B3	Atorvastatin (1 µM)	Cyclosporine A (10 µM)	5 & 20 min
OAT1	p-Aminohippuric Acid (5 µM)	Probenecid (100 µM)	5 & 20 min
OAT3	Estrone-3-Sulphate (1 µM)	Probenecid (100 µM)	5 & 20 min
OCT1	1-Methyl-4-phenylpyridinium iodide (10 µM)	Quinidine (100 µM)	10 & 20 min
OCT2	Metformin (10 µM)	Quinidine (100 µM)	10 & 20 min

At the specified times, incubations were terminated and cells were lysed. Cells lysates were analyzed by LC-MS/MS. The amounts of BNZ or known substrates present in the cell lysates were quantified. In addition, the protein concentration in each lysate was measured.

Results:

The mean uptake ratios for all the transporters are summarized in the Table 3.

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Table 3: Reported mean uptake ratios of test and control compounds by transporters

Transporter	Test Item	Incubation time (minutes)	Concentration (µM)	Mean uptake ratio
OATP1B1*1a	BNZ	5	1	1.1
		5	10	0.99
		20	1	0.86
		20	10	0.85
	E3S (1 µM)	5	1	2.3
		20	1	2.7
	E3S (1 µM) + Cyclosporin A (10 µM)	5	1,10	1.3
		20	1,10	0.58
OATP1B3	BNZ	5	1	0.98
		5	10	1.3
		20	1	1.5
		20	10	1.9
	Atorvastatin (1 µM)	5	1	2.3
		20	1	3.7
	Atorvastatin (1 µM) + Cyclosporin A (10 µM)	5	1,10	1.4
		20	1,10	2
OAT1	BNZ	5	1	0.9
		5	10	0.54
		20	1	1.6
		20	10	1.2
	PAH (5 µM)	5	5	24
		20	5	63
	PAH (5 µM) + Probenecid (100 µM)	5	5,100	1.2
		20	5,100	2.4
OAT3	BNZ	5	1	0.77
		5	10	1.4
		20	1	0.95
		20	10	0.69
	E3S (1 µM)	5	1	5.8
		20	1	9.9
	E3S (1 µM) + Probenecid (100 µM)	5	1,100	0.79
		20	1,100	0.82
OCT1	BNZ	10	1	0.92
		10	10	1.1
		20	1	0.9
		20	10	0.85
	MPP+ (10 µM)	10	10	12
		20	10	13
	MPP+ (10 µM) + Quinidine (100µM)	10	10,100	>4.1
		20	10,100	3.1
OCT2	BNZ	10	1	0.81
		10	10	0.78
		20	1	0.57
		20	10	0.57
	Metformin (10 µM)	10	10	4.1
		20	10	2.3
	Metformin (10 µM) + Quinidine (100µM)	10	10,100	0.34
		20	10,100	0.51

Applicant's Conclusion:

The findings did not show that BNZ is a substrate of uptake transporters OATP1B1*1a, OATP1B3, OAT1, OAT3, OCT1 and OCT2 at any of the tested concentrations.

Reviewer's Assessment:

For all the controls used in this study, mean uptake ratio was > 2 (Table 3), whereas with the BNZ, mean update ratio was < 2 (Table 3). Therefore, the Applicant's conclusions appear reasonable . It is noteworthy that the maximum BNZ concentration tested was 10 μM, i.e., ~3.6μg/mL, which is likely below the maximum clinical concentrations expected from the proposed BNZ dosing. However, one of the PMC recommended for this application (Refer to Section 1.2) is that the based on pending mass balance/ADME study, additional Clinical Pharmacology study(ies) may be required (e.g. Patients with renal and/or hepatic impairment, in vitro studies, in vivo DDI studies). For example, if renal active, hepatic, or biliary secretion is found to be >25%, additional in vivo DDI studies may be needed.

EXL-REP-03

Title:

An in vitro investigation to assess if benznidazole is an inhibitor of BCRP efflux transporter

Test Facility:

(b) (4)

Objective:

To evaluate if BNZ is an inhibitor of the Breast Cancer Resistance Protein (BCRP) human efflux transporter

Methods:

This study used Maclin Darby Canine Kidney (MDCK) cell line transfected with the human BCRP gene or untransfected (wild type). MDCK-wild type (MDCK-WT) and MDCK-BCRP cells were used in confluent monolayers after an eight day culture at 37°C. Sodium butyrate (1 mM) was added on the day prior to experiment to induce BCRP. The information on the cells, test items, and experimental design is presented in Table 1.

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Table 1: Experimental design (source: Report EXL/REP/03, Table 1)

Cells used	MDCK-wild type (MDCK-WT) and MDCK-BCRP
Control compounds used	Prazosin (known BCRP substrate, positive control) and Propranolol (permeability and BCRP negative control) at 10 μM final concentration
Known BCRP inhibitors	Ko143 (1 μM), Elacridar (10 μM) and Cyclosporin A (10 μM)
Assay buffer	25mM HEPES in HBSS + 2g/mL glucose, pH 7.4
Incubation time (h)	1
Assay conditions	37 ^{0}C, 5% CO_2
Number of replicates	2

Propranolol (as permeability control) and prazosin (BCRP substrate) were added to donor compartment during the assessment of both apical to basolateral (A->B) and basolateral to apical (B->A) transfer. Permeation of control compounds were assessed in duplicate in the absence and presence of 200 μM BNZ and known BCRP inhibitors (Ko143 (1 μM), Elacridar (10 μM) and Cyclosporin A (10 μM)). Lucifer Yellow at 50 μM was used to assess viability. Incubations were performed at 37 °C, with samples removed from the donor chamber at time zero and at one hour and were analyzed by LC-MS/MS for propranolol and prazosin together with analytical internal standard. Assessment of BNZ inhibition was performed in both wild type MDCK and MDCK-BCRP cells as described above.

Samples were also analyzed using a fluorescence plate reader and the P_{app} of Lucifer Yellow (LY) determined. As LY cannot freely permeate lipophilic barriers, a high degree of LY transport indicates poor integrity of the cell monolayer and wells with a LY P_{app} > 10 x 10^{-6} cm/s were rejected. None of the wells reported LY P_{app} > 10 X 10^{-6} cm/s.

Results:

The results of the bi-directional MDCK wild-type and MDCK-BCRP permeation study are shown in Tables 2 and 3, respectively. The reported efflux and effective efflux ratios are given in Table 4.

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Table 2: Mean recovery results of the bi-directional MDCK-WT permeation study

(source: Report EXL/REP/03, Table 6)

Compound	Inhibitor (concentration µM)	A→B Recovery (%)		B→A Recovery (%)		Mean Recovery (%)	
		Replicates		Replicates		A→B	B→A
		1	2	1	2		
Propranolol (negative control)	- inhibitor	90	85	91	101	88	96
	+ Ko143 (1 µM)	74	71	87	75	73	81
	+ Elacridar (10 µM)	79	95	101	91	87	96
	+ Cyclosporin A (10 µM)	76	87	91	96	82	93
	+ Benznidazole (200 µM)	79	88	88	102	83	95
Prazosin (positive control)	- inhibitor	73	81	99	107	77	103
	+ Ko143 (1 µM)	83	86	105	114	84	109
	+ Elacridar (10 µM)	82	93	105	93	87	99
	+ Cyclosporin A (10 µM)	72	69	100	81	70	90
	+ Benznidazole (200 µM)	76	72	101	104	74	103

Table 3: Mean recovery results of the bi-directional MDCK-BCRP permeation study (source: Report EXL/REP/03, Table 9)

Compound	Inhibitor (concentration µM)	A→B Recovery (%)		B→A Recovery (%)		Mean Recovery (%)	
		Replicates		Replicates		A→B	B→A
		1	2	1	2		
Propranolol (negative control)	- inhibitor	84	96	85	106	90	95
	+ Ko143 (1 µM)	73	96	94	104	85	99
	+ Elacridar (10 µM)	102	112	93	109	107	101
	+ Cyclosporin A (10 µM)	103	94	96	120	98	108
	+ Benznidazole (200 µM)	83	83	84	102	83	93
Prazosin (positive control)	- inhibitor	119	110	71	93	114	82
	+ Ko143 (1 µM)	98	92	74	98	95	86
	+ Elacridar (10 µM)	115	116	77	95	115	86
	+ Cyclosporin A (10 µM)	98	103	72	103	101	87
	+ Benznidazole (200 µM)	118	108	72	103	113	88

Table 4: Efflux ratios and effective efflux ratios (source: Report EXL/REP/03, Table 11)

Compound	Inhibitor (concentration µM)	MDCK-WT Efflux ratio			MDCK-BCRP Efflux ratio			Mean effective efflux ratio
		Replicates		Mean	Replicates		Mean	
		1	2		1	2		
Propranolol (negative control)	- inhibitor	0.70	0.77	0.73	0.77	0.84	0.81	1.1
	+ Ko143 (1 µM)	0.72	0.83	0.77	0.87	0.67	0.75	0.97
	+ Elacridar (10 µM)	0.87	0.65	0.75	0.64	0.75	0.70	0.92
	+ Cyclosporin A (10 µM)	0.78	0.77	0.78	0.63	0.93	0.77	0.99
	+ Benznidazole (200 µM)	0.70	0.72	0.71	0.71	0.84	0.77	1.1
Prazosin (positive control)	- inhibitor	1.2	1.1	1.2	12	16	14	12
	+ Ko143 (1 µM)	0.61	0.66	0.63	0.52	0.69	0.61	0.96
	+ Elacridar (10 µM)	0.66	0.56	0.61	2.5	3.3	2.9	4.8
	+ Cyclosporin A (10 µM)	0.70	0.71	0.70	7.7	8.4	8.1	11
	+ Benznidazole (200 µM)	0.82	0.89	0.85	12	17	14	17

Applicant's Conclusion:

The findings showed that at 200 µM BNZ did not inhibit the BCRP efflux transporter as no change in prazosin efflux was observed. The permeability and efflux of prazosin in the presence and in the absence of BNZ were comparable.

Reviewer's Assessment:

See the Reviewer's Assessment under study EXL-REP-04.

EXL-REP-04

Title:

An in vitro Investigation to assess if benznidazole is a substrate of the MDCK-BCRP efflux transporter

Test Facility:

(b) (4)

Objective:

To evaluate if BNZ is a substrate of the human Breast Cancer Resistance Protein (BCRP) efflux transporter.

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Methods:

This study used Maclin Darby Canine Kidney (MDCK) cell line transfected with the human BCRP gene or untransfected (wild type). MDCK-wild type (MDCK-WT) and MDCK-BCRP cells were used in confluent monolayers after an eight day culture at 37°C. Sodium butyrate (1 mM) was added on the day prior to experiment to induce BCRP. The information on the cells, test items, and experimental design is presented in Table 1.

Table 1: Experimental design (source: Report EXL/REP/04, Table 1)

Cells used	MDCK-Wild type (MDCK-WT) and MDCK-BCRP
Control compounds used	Prazosin (known BCRP substrate, positive control) and Propranolol (permeability and permeability negative control at 10 μM final concentration
Known BCRP inhibitors	Ko143 (1 μM), Elacridar (10 μM) and Cyclosporin A (10 μM)
Assay buffer	25mM HEPES in HBSS + 2g/mL glucose, pH 7.4
Incubation time (hr)	1
Assay conditions	37 °C, 5% CO_2
Number of replicates	3

BNZ, propranolol (as permeability control), and prazosin (BCRP substrate) were added (all at the concentration of 10 μM) to donor compartment during the assessment of both apical to basolateral (A->B) and basolateral to apical (B->A) transfer. Permeation of control compounds were assessed in triplicate in the absence and presence of known BCRP inhibitors (Ko143 (1 μM), Elacridar (10 μM) and cyclosporin A (10 μM)). Lucifer Yellow at 50 μM was used to assess viability. Incubations were performed at 37 °C, with samples removed from the donor chamber at time zero and at one hour and were analyzed by LC-MS/MS for propranolol and prazosin as appropriate together with analytical internal standard. Assessment of BNZ inhibition was performed in both wild type MDCK and MDCK-BCRP cells as described above.

Samples were also analyzed using a fluorescence plate reader and the P_{app} of Lucifer Yellow (LY) determined. As LY cannot freely permeate lipophilic barriers, a high degree of LY transport indicates poor integrity of the cell monolayer and wells with a LY $P_{app} > 10 \times 10^{-6}$ cm/s were rejected. None of the wells reported LY $P_{app} > 10 \times 10^{-6}$ cm/s.

Results:

The results of the bi-directional MDCK wild-type and MDCK-BCRP permeation study are shown in Tables 2 and 3, respectively. The reported efflux and effective efflux ratios are given in Table 4.

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Table 2: Mean recovery results of the bi-directional MDCK wild-type permeation study

(source: Report EXL/REP/04, Table 6)

Compound	Inhibitor (concentration µM)	A→B Recovery (%)			B→A Recovery (%)			Mean Recovery (%)	
		Replicates			Replicates			A→B	B→A
		1	2	3	1	2	3		
Benznidazole	- inhibitor	107	106	112	91	80	82	108	84
	+ Ko143 (1 µM)	98	105	103	86	94	84	102	88
	+ Elacridar (10 µM)	104	108	109	87	89	83	107	86
	+ Cyclosporin A (10 µM)	107	105	100	94	92	91	104	93
Propranolol	- inhibitor	107	111	107	103	96	87	108	95
	+ Ko143 (1 µM)	88	96	99	89	86	83	94	86
	+ Elacridar (10 µM)	107	105	111	92	85	86	108	88
	+ Cyclosporin A (10 µM)	117	96	109	103	96	80	107	93
Prazosin	- inhibitor	82	81	87	82	94	89	83	88
	+ Ko143 (1 µM)	87	88	97	89	93	92	91	91
	+ Elacridar (10 µM)	90	86	84	97	89	91	86	92
	+ Cyclosporin A (10 µM)	77	74	83	99	86	85	78	90

Table 3: Mean recovery results of the bi-directional MDCK-BCRP permeation study

(source: Report EXL/REP/04, Table 9)

Compound	Inhibitor (concentration µM)	A→B Recovery (%)			B→A Recovery (%)			Mean Recovery (%)	
		Replicates			Replicates			A→B	B→A
		1	2	3	1	2	3		
Benznidazole	- inhibitor	111	100	99	96	76	94	104	89
	+ Ko143 (1 µM)	100	109	109	82	83	90	106	85
	+ Elacridar (10 µM)	100	95	86	86	93	90	94	90
	+ Cyclosporin A (10 µM)	105	104	104	96	96	86	104	93
Propranolol	- inhibitor	116	121	132	82	87	94	123	88
	+ Ko143 (1 µM)	110	110	116	96	88	94	112	92
	+ Elacridar (10 µM)	111	116	120	85	92	92	116	90
	+ Cyclosporin A (10 µM)	110	124	135	88	101	89	123	93
Prazosin	- inhibitor	108	101	107	95	114	95	105	101
	+ Ko143 (1 µM)	98	92	97	100	88	90	96	93
	+ Elacridar (10 µM)	105	110	102	94	103	108	106	102
	+ Cyclosporin A (10 µM)	102	98	112	91	99	94	104	95

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Table 4: Efflux ratios and effective efflux ratios (source: Report EXL/REP/04, Table 11)

Compound	Inhibitor (concentration μM)	MDCK-WT efflux ratio				MDCK-BCRP efflux ratio				Mean Effective efflux ratio
		Replicates			Mean	Replicates			Mean	
		1	2	3		1	2	3		
Benznidazole	– inhibitor	0.87	0.77	0.78	0.80	1.5	1.4	1.8	1.5	1.9
	+ Ko143 (1 μM)	0.84	0.73	0.86	0.81	1.1	1.1	1.3	1.1	1.4
	+ Elacridar (10 μM)	0.80	0.97	0.97	0.91	1.4	1.4	1.4	1.4	1.6
	+ Cyclosporin A (10 μM)	0.74	0.69	0.76	0.73	1.2	1.2	1.2	1.2	1.7
Propranolol	– inhibitor	0.68	0.65	0.63	0.65	0.68	0.64	0.66	0.66	1.0
	+ Ko143 (1 μM)	0.80	0.67	0.73	0.73	0.68	0.73	0.72	0.71	0.97
	+ Elacridar (10 μM)	0.80	0.68	0.62	0.70	0.68	0.90	0.73	0.77	1.1
	+ Cyclosporin A (10 μM)	0.60	0.82	0.75	0.71	0.66	0.69	0.61	0.65	0.91
Prazosin	– inhibitor	0.83	0.85	0.72	0.80	27	34	29	30	38
	+ Ko143 (1 μM)	0.83	0.90	0.79	0.84	0.75	0.85	0.80	0.80	0.95
	+ Elacridar (10 μM)	0.56	0.63	0.73	0.63	7.9	7.5	7.2	7.5	12
	+ Cyclosporin A (10 μM)	0.81	0.77	0.77	0.78	13	14	12	13	17

Applicant's Conclusion:

BNZ is not a substrate of BCRP efflux transporter. Also, BNZ transport was unaffected in the presence of known BCRP inhibitors.

Reviewer's Assessment:

In both Study EXL-REP-04 and Study EXL-REP-03, wells with a Lucifer Yellow P_{app} > 10 X 10^{-6} cm/s were rejected. This threshold for LY P_{app} is higher than the threshold of $0.4 – 1 \times 10^{-6}$ cm/s adopted in the literature (e.g. Wang, Strab et al. 2008 & Ma, Qin et al. 2015). Therefore, an information request was sent to the Applicant seeking rationale for the selected LY P_{app} threshold value utilized in the submitted reports EXL-REP-03 and EXL-REP-04. The Applicant responded that the LY P_{app} acceptance criterion, i.e., P_{app} > 10 X 10^{-6} cm/s, was generated in house and is suitable for application to the experiments reported in reports EXL-REP-03 and EXL-REP-04. The acceptance criterion was based on the twenty data points generated over seven separate experiments. Based on these data LY P_{app} of <10 x 10^{-6} cm/s was selected as the acceptance criteria. A LY P_{app} value of 10 x 10^{-6} cm/s is approximately 3 standard deviations from the average LY data obtained and also resulted in an atenolol P_{app} within 3 standard deviations of the mean. In the Reviewer's opinion, usage of 3 standard deviations in determining the cut-off to evaluate the integrity of cell monolayer appears to be too liberal, therefore, we cannot fully validate the results of the two studies. No relevant label statements are to be made at present.

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Title:

Assessment of potential CYP3A4 inhibition by twenty four compounds using a substrate specific interaction approach in human liver microsomes.

Test Facility:

(b) (4)

Objective:

The aim of this study was to assess the potential inhibitory effects of twenty four compounds against CYP3A4 utilizing a substrate-specific interaction approach in human liver microsomes.

Methods:

The substrate specific CYP450 inhibition study relies on the formation of a metabolite that is mediated by a specific CYP450 isoform using human liver microsomes. In this study, 1'-hydroxylation of midazolam was monitored to assess specific interaction with CYP3A4. Ketoconazole, a known inhibitor of CYP3A4, was included in the assay as a positive control.

Multiple concentrations (0.04 to 30 µM) of each test compound were incubated (37°C) for 4 minutes, concomitantly with 2.5 µM midazolam (a specific substrate for CYP3A4) in human liver microsomes at a protein concentration of 0.4 mg/mL. The reaction was initiated by the addition of an NADPH-regenerating system and the samples were quenched by the addition of acetonitrile. The concentration of the specific metabolite(s) was measured by LC-MS.

The IC_{50} of each test compound (and positive control) was assessed according to the percent reduction in the extent of formation of 1'-hydroxy midazolam, noting that the maximal metabolite formation occurs in the absence of inhibitor. The IC_{50} was deemed to be the concentration at which there was a 50% reduction in the amount of metabolite formed, relative to the maximal extent of formation.

Results:

From the provided results from 24 compounds, only BNZ relevant results are summarized below.

IC_{50} value for BNZ, which is denoted as EPL-BS0063 in this study report, was determined to be > 30µM.

Applicant's Conclusion:

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BNZ has low potential to inhibit the in vivo CYP-mediated metabolism for concomitantly administered drugs which are CYP3A4 substrates.

Reviewer's Assessment:

IC_{50} value for the BNZ's inhibition potential of CYP3A4 is estimated to be > 30μM (the highest concentration tested), i.e., > ~7 μg/mL, which is within the range of expected clinical concentrations following the proposed BNZ dosing. However, the finding from Study 205598 – 513491, which tested BNZ at higher concentrations, indicated that BNZ would not inhibit CYP3A4 at 100 μM , i.e., IC_{50} of >100 μM (>26 μg/mL), which is towards the higher end of the possible clinical concentrations expected from the proposed BNZ dosing. Nevertheless, the reviewer concludes that BNZ inhibition potential on the metabolism of CYP3A4 substrates is likely to be low.

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4.7   In vivo study - LPRI 747/101

Title:

A study to evaluate the food effect of a new formulation containing 100 mg benznidazole [BNZ 100 mg Tablet (Test) administered with and without high-fat breakfast]. A monocentric, open, randomized, single dose, two-period crossover trial in healthy volunteers (EDR Link)

Trial Information:

Study center:	Bio-analytical Laboratory
Bed space for short term stay at Diagnostic & Consultative Centre 'Ascendent' Ltd. 47 Bacho Kiro str. 1202 Sofia, Bulgaria	(b) (4)
Period: 06/16/2015 – 06/30/2015	Sample analysis period: (b) (4)

Primary Objective:

To assess the food effect on BNZ bioavailability of the TBM formulation, i.e., 100 mg BNZ tablet after oral administration under fasting and fed conditions.

Test Preparation:

TBM formulation: Benznidazole 100 mg tablet (Manufacturer: Laboratories Liconsa S.A., Spain, Batch # DG1501528)

Study Design:

This was a monocentric, open, randomized, single-dose, two-period, crossover trial in healthy volunteers. Out of 20 screened volunteers, 18 were randomized to receive treatments and all subjects completed the study. Enrolled subjects were randomly assigned to 1 of 2 possible treatment sequences to ensure that they received both of the following treatments with a washout period of at least 7 days:

- Single dose of 1 tablet (BNZ 100 mg tablet, the test preparation) under fasting conditions
- Single dose of 1 tablet (the test preparation) under fed conditions (i.e. 30 minutes after the start of the standard high-fat breakfast)

For both the treatment groups, based on the treatment assignment, the volunteer fasted from food and beverages other than water, from 9 pm on the evening before dosing either until lunchtime on the following day approximately 6 hours post-dose OR until the standard high-fat breakfast on the following day. For this study, the high-fat meal (breakfast) consisted 2 eggs fried in 10 g butter, 2 strips of bacon, 2 slices of toast with 10 g of butter, 113 g hashed brown potatoes with 10 g butter, and 240 mL milk.

Demographic Information:

The demographic data of the subjects that received treatments (per protocol) are summarized in the table below.

(n=18)	Mean ± SD	Min – Max
Age [years]	37.6 ± 10.5	21.0 - 51.0
Height [cm]	166.2 ± 7.5	150.0 - 176.0
Weight [kg]	63.2 ± 9.5	46.0 - 81.0
BMI [kg/m^2]	22.8 ± 2.5	18.9 - 28.4
male : female	9 : 9	

Sample Collection and Bioanalysis:

During both the study periods, blood samples were collected for BNZ PK analysis at pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24, 48, and 72 hours post-dose.

In total 648 plasma samples were analyzed to determine BNZ concentrations using a validated bioanalytical method, with the quantification range of 50.25-3015 ng/mL. Following is the information about the submitted bio-analytical report.

Bio-analytical report: 14 [(b) (4)]-2415 (See Section 4.5 for Bio-analytical method validation)

Study Samples Analysis	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable (150.5 - 2257.5 ng/mL)	☒ Yes ☐ No
	Chromatograms provided (Only for six subjects)	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred samples analysis is acceptable (In 64/65 samples %change <20%)	☒ Yes ☐ No
	Overall performance acceptable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected[vii]	☐ Yes ☒ No

Pharmacokinetic Assessments

Primary endpoints: $AUC_{(0-t)}$ and C_{max}
Secondary endpoint: T_{max}
Additional endpoints: $AUC_{(0-\infty)}$, AUC_{res}, MRT, and $t_{1/2}$

[vii] The Division of New Drug Bioequivalence Evaluation (DNDBE) within the Office of Study Integrity and Surveillance (OSIS) recommended that data should be accepted without an on-site inspection. The rationale for this decision was an OSIS's recent inspection of the site, outcome of which was classified as No Action Indicated (NAI).

Bioavailability Assessment:

To assess the primary endpoints, $AUC_{(0-t)}$ and C_{max} of BNZ were derived for both the treatment groups. Ratios of these estimates between both the treatment groups and 90% confidence intervals (CIs) for the ratios were calculated by the parametric method: ANOVA-log. The T_{max} of BNZ was assessed descriptively only.

Safety Assessments:

Safety assessments included clinical and laboratory examinations (at the beginning and at the end of the trial) and monitoring of adverse events and/or adverse drug reactions.

Pharmacokinetic Results:

Mean BNZ plasma concentration time-curves for both the treatment groups are presented in Figure 1. Descriptive statistics of pharmacokinetic parameter estimates are presented in Table 1.

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Figure 1: Mean BNZ plasma concentration-time profiles following administration of the TBM 100 mg BNZ tablets (with and without food) in linear and semi-logarithmic scale

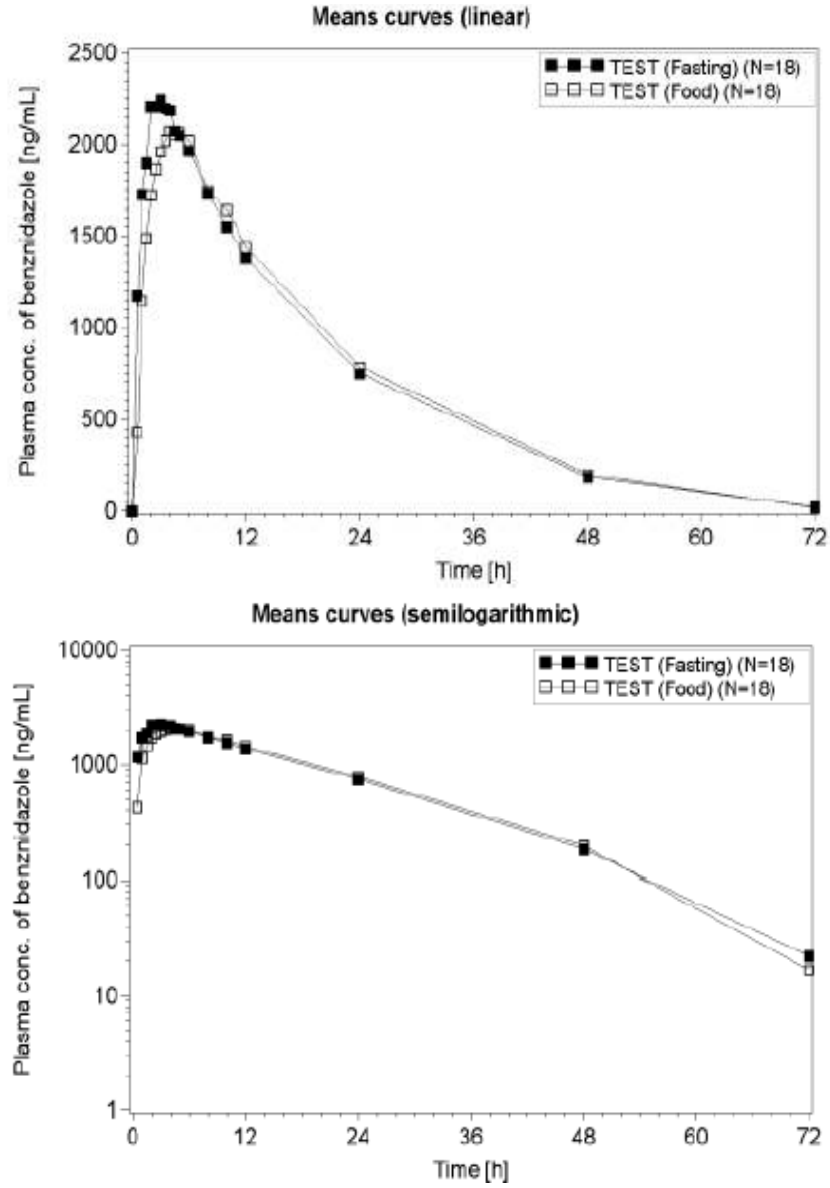*(source: Clinical Study Report LPRI 747/101, TF 7, and TF 8)*

Table 1: Pharmacokinetic parameter estimates following administration of the TBM 100 mg BNZ tablets with and without food (source: Clinical Study Report LPRI 747/101, TT6)

BENZNIDAZOLE

TEST (after food intake)

Variable	geom. mean	arithm. mean	SD	CV	range	median	N
AUC(0-t) [ng*h/mL]	45097.18	46210.18	11003.88	23.8	33789.80 - 70998.82	42417.79	18
AUC(0-∞) [ng*h/mL]	47772.47	48930.46	11456.72	23.4	34852.97 - 73190.84	45394.15	18
AUCres [%]	4.97	5.57	2.47	44.3	1.75 - 10.41	5.23	18
Cmax [ng/mL]	2228.62	2264.69	420.07	18.5	1623.94 - 2996.02	2182.12	18
tmax [h]	-	3.722	2.116	56.8	1.000 - 10.000	3.250	18
MRT [h]	18.333	18.493	2.375	12.8	12.202 - 21.566	19.202	18
t½ [h]	12.247	12.354	1.594	12.9	8.190 - 14.776	12.535	18

TEST (under fasting conditions)

Variable	geom. mean	arithm. mean	SD	CV	range	median	N
AUC(0-t) [ng*h/mL]	45272.54	46211.58	9769.13	21.1	30324.81 - 66594.07	44648.03	18
AUC(0-∞) [ng*h/mL]	47994.56	48817.96	9519.64	19.5	37046.30 - 68756.63	46829.06	18
AUCres [%]	4.95	5.61	3.50	62.5	1.94 - 18.14	4.95	18
Cmax [ng/mL]	2461.69	2493.53	414.98	16.6	1910.23 - 3342.30	2516.78	18
tmax [h]	-	2.140	1.082	50.6	0.500 - 4.000	2.000	18
MRT [h]	17.661	17.797	2.291	12.9	13.880 - 22.076	17.199	18
t½ [h]	12.179	12.270	1.550	12.6	9.558 - 14.910	11.937	18

AUCres Residual area in percent calculated as:

$$AUC_{res} = \frac{AUC_{(0-\infty)} - AUC_{(0-t)}}{AUC_{(0-\infty)}} \times 100$$

Bioavailability Results

The results from the statistical evaluation of the relative bioavailability of BNZ from the test preparation with and without food are presented in Table 2. The evaluation of relative bioavailability was based on an ANOVA-log method for both the exposure parameters, i.e., $AUC_{(0-t)}$ and C_{max} of BNZ.

Table 2: Summary statistics of the relative bioavailability assessment by ANOVA-log method: parameters estimate ratios with 90% CI (Adapted from: Clinical Study Report LPRI 747/101, TT7)

BENZNIDAZOLE (n=18)

Variable	method	point estimates	confidence intervals
AUC(0-t) (ratio test with food / test without food)	ANOVA-log	99.61%	93.95% - 105.62%
Cmax (ratio test with food / test without food)	ANOVA-log	90.53%	86.75% - 94.48%

Safety Results

 The treatments were well tolerated in both the treatment groups. In total, six non-serious adverse events (AEs) were recorded in 3 volunteers. 5 AEs were in the treatment arm with fasting condition and 1 AE was reported in the treatment arm with the fed condition. No clinically significant abnormalities or changes were observed during the study.

Applicant's Conclusion:

The point estimates revealed no food effect on BNZ bioavailability from the test formulation, i.e., no significant differences were observed in the bioavailability of BNZ from the test formulation when administered with food and without food and the exposure parameter estimates for BNZ were within the bioequivalence acceptance ranges.

Reviewer's Assessment:

The comparison of exposure parameters estimates, i.e., $AUC_{(0-t)}$ and C_{max}, showed no significant food effect on the BNZ bioavailability from the TBM 100 mg BNZ tablet under fasted and fed conditions. The Applicant's conclusions appear valid. The observed median T_{max} was 2 hour (range = 0.5 – 4 hour) and 3.25 hours (range = 1 – 10 hours) under fasted and fed conditions, respectively. In the individual concentrations data of two subjects (Subject 8 and Subject 10), the reported BNZ plasma concentrations were above the upper limit of quantification (ULOQ, i.e. 3015 ng/mL) of the bio-analytical method that utilized at one time point/subject. Therefore, the statistical analysis reported in Table 2 was re-evaluated after omitting BNZ concentration data from these two subjects from both the treatment arms. The results from the "ULOQ adjusted" statistical evaluation of the relative bioavailability of BNZ from the test preparation with and without food are presented in Table 3. Findings from the ULOQ adjusted analysis were similar to the findings from the Applicant's analysis except for the point estimates for C_{max} (and CIs), which were moderately high. However, this does not affect the conclusions of this study.

Table 3: Summary statistics of the relative bioavailability assessment by ANOVA-log method: parameters estimate ratios with 90% CI: ULOQ adjusted

Parameters	% Point Estimates (90% CI) (Fasting/Food)
C_{max} (ng/ml)	110 (104 - 116)
AUC_{last} (ng*hr/ml)	99 (93 - 105)

4.7.1   In vivo study - LPRI 747/102

Title:

A study to evaluate the relative bioavailability of two formulations containing benznidazole (100 mg tablets and 12.5 mg tablets) administered intact or as a slurry under fasting conditions. A monocentric, open, randomized, single dose, three-period crossover trial in healthy volunteers (EDR Link)

Trial Information:

Study center:	Bio-analytical Laboratory
Bed space for short term stay at Diagnostic & Consultative Centre 'Ascendent' Ltd. 47 Bacho Kiro str. 1202 Sofia, Bulgaria	(b) (4)
Period: 07/26/2016 – 08/17/2016	Sample analysis period: 08/23/2016 – 08/30/2016

Primary Objectives:

- To assess the relative bioavailability of the 100 mg BNZ tablet and a slurry prepared in water from the 100 mg BNZ tablet
- To assess the relative bioavailability of the 100 mg BNZ tablet and a slurry prepared in water from eight 12.5 mg BNZ tablets

Test Preparations:

TBM formulation: Benznidazole 100 mg tablet (Manufacturer: Laboratories Liconsa S.A., Spain, Batch # DG1501748-A)
TBM formulation: Benznidazole 12.5 mg tablet (Manufacturer: Laboratories Liconsa S.A., Spain, Batch # DG1501724-A)

Study Design:

This was a monocentric, open, randomized, single-dose, three-period, crossover trial in healthy volunteers with a planned wash-out period of at least 7 days between the subsequent study periods. Out of 26 screened volunteers, 18 were randomized to receive treatments and all subjects completed the study. Enrolled subjects were randomly assigned to 1 of 3 possible treatment sequences to ensure that they received all of the following treatments under fasting condition with a washout period of 7 days:

- Test A: Single dose of 1 intact tablet (BNZ 100 mg tablet) under fasting condition
- Test B: Single dose of a slurry made by crushing 1 tablet (BNZ 100 mg tablet) in water

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- Test C: Single dose of a slurry made by crushing 8 tablets (BNZ 12.5 mg tablet) in water

For all the treatment groups, the volunteers were fasted from food and beverages other than water, from 9 p.m. on the evening before dosing and until lunchtime on the following day.

Demographic Information:

The demographic data of the subjects that received treatments (per protocol) are summarized in the table below.

(n=18)	Mean ± SD	Min – Max
Age [years]	39.8 ± 8.5	20.0 - 54.0
Height [cm]	170.1 ± 8.1	153.0 - 182.0
Weight [kg]	69.8 ± 14.4	46.0 - 98.0
BMI [kg/m^2]	23.9 ± 3.4	18.9 - 29.6
male : female	10 : 8	

Sample Collection and Bioanalysis:

During the study periods, blood samples were collected for BNZ PK analysis at pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24, 48, and 72 hours post-dose.

In total 971 plasma samples were analyzed to determine concentrations of BNZ using a validated assay method, with the quantification range of 50-3000 ng/mL. One sample was missing from the analysis. The missing sample was the 48 hr sample from subject 5 during period 1. Following is the information about the submitted bio-analytical report.

Bio-analytical report: 16 [(b) (4)]-2747 (See Section 4.5 for Bio-analytical method validation)

Study Samples Analysis	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable (150.25 – 2253.8 ng/mL)	☒ Yes ☐ No
	Chromatograms provided (Only for six subjects)	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred samples analysis is acceptable (In 98/98 samples %change <20%)	☒ Yes ☐ No
	Overall performance acceptable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected[viii]	☐ Yes ☒ No

[viii] The Division of New Drug Bioequivalence Evaluation (DNDBE) within the Office of Study Integrity and Surveillance (OSIS) recommended that data should be accepted without an on-site inspection. The rationale for this decision was an OSIS's recent inspection of the site, outcome of which was classified as No Action Indicated (NAI).

Pharmacokinetic Assessments

Primary endpoints: $AUC_{(0-t)}$ and C_{max}
Secondary endpoint: T_{max}
Additional endpoints: $AUC_{(0-\infty)}$, AUC_{res}, MRT, and $t_{1/2}$

Bioavailability Assessment:
To assess the primary endpoints, $AUC_{(0-t)}$ and C_{max} of BNZ were derived for all three the treatment groups. Ratios of these estimates between the treatment groups and 90% confidence intervals (CIs) for the ratios were calculated by the parametric method: ANOVA-log. The T_{max} of BNZ was assessed descriptively only.

Safety Assessments:

Safety assessments included clinical and laboratory examinations (at the beginning and at the end of the trial and monitoring of adverse events and/or adverse drug reactions.

Pharmacokinetic Results:

Mean BNZ plasma concentration time-curves from all the treatment groups are presented in Figure 1. Descriptive statistics of pharmacokinetic parameter estimates are presented in Table 1.

Figure 1: Mean BNZ plasma concentration-time profiles in linear and semi-logarithmic scale *(source: Clinical Study Report LPRI 747/102, TF 7 and TF 8)*
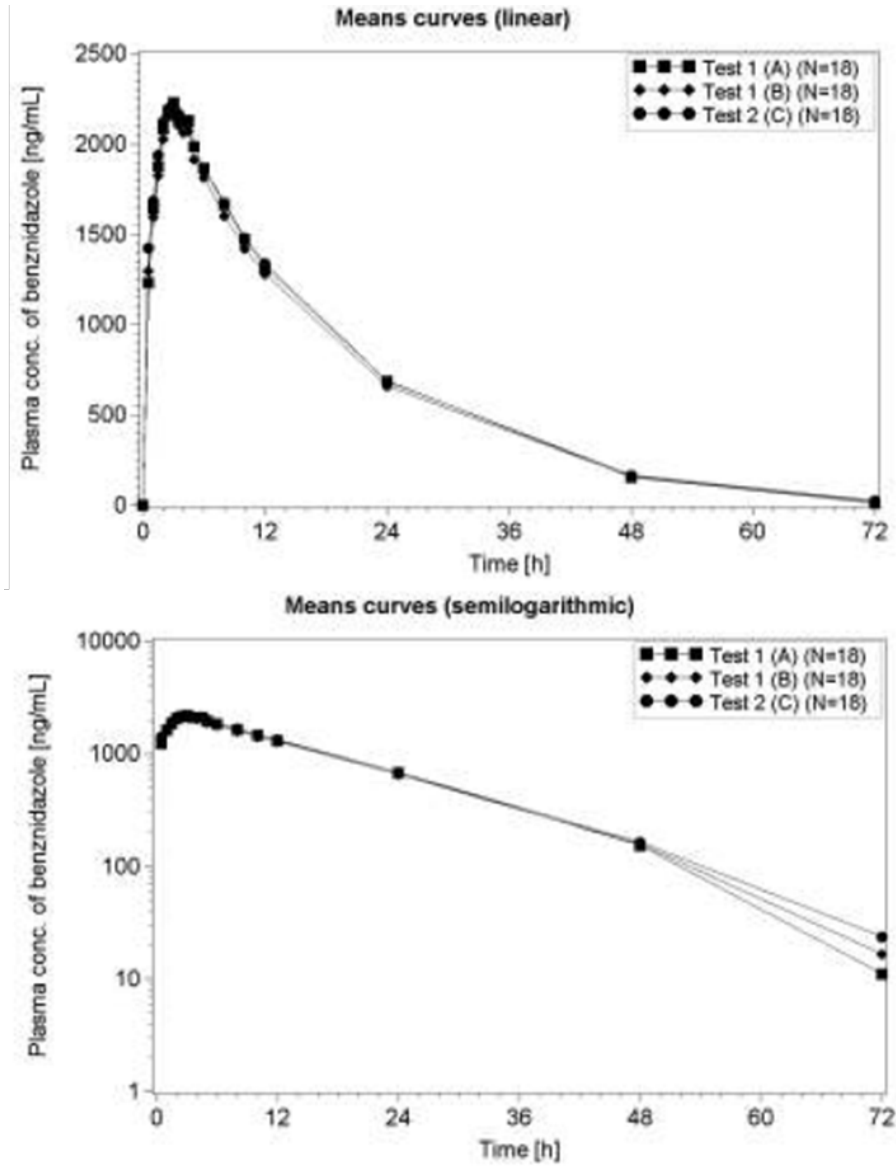
Table 1: Pharmacokinetic parameters estimates (source: Clinical Study Report LPRI 747/102, TT6)

BENZNIDAZOLE

TEST 1 (A) (1 intact tablet of Benznidazole 100 mg)

Variable	geom. mean	arithm. mean	SD	CV	range	median	N
AUC(0-t) [ng*h/mL]	42646.00	43521.41	8973.56	20.6	26254.89 - 61826.45	41951.69	18
AUC(0-∞) [ng*h/mL]	44724.28	45609.12	9281.04	20.3	27735.64 - 65555.85	43961.99	18
AUCres [%]	4.16	4.63	2.06	44.6	1.49 - 8.13	4.36	18
Cmax [ng/mL]	2388.88	2429.84	490.06	20.2	1775.27 - 3955.95	2452.14	18
tmax [h]	-	2.306	0.877	38.0	1.000- 4.000	2.000	18
MRT [h]	16.731	16.896	2.371	14.0	11.566 - 20.884	16.681	18
t½ [h]	11.458	11.588	1.732	14.9	7.694 - 14.156	11.568	18

TEST 1 (B) (1 tablet of Benznidazole 100 mg as a slurry)

Variable	geom. mean	arithm. mean	SD	CV	range	median	N
AUC(0-t) [ng*h/mL]	40783.92	41827.73	9591.11	22.9	25566.07 - 57052.71	40759.38	18
AUC(0-∞) [ng*h/mL]	43338.21	44293.37	9373.20	21.2	27039.55 - 58715.65	43093.03	18
AUCres [%]	4.97	5.83	3.65	62.7	2.40 - 15.01	4.65	18
Cmax [ng/mL]	2338.23	2374.26	419.58	17.7	1574.69 - 3173.76	2472.42	18
tmax [h]	-	2.333	0.907	38.9	0.500 - 4.000	2.000	18
MRT [h]	16.772	17.005	2.854	16.8	12.395 - 20.855	17.264	18
t½ [h]	11.518	11.707	2.106	18.0	7.648 - 14.494	11.820	18

TEST 2 (C) (8 tablets of Benznidazole 12.5 mg as a slurry)

Variable	geom. mean	arithm. mean	SD	CV	range	median	N
AUC(0-t) [ng*h/mL]	42738.59	44125.71	11789.08	26.7	25991.21 - 73948.16	41191.09	18
AUC(0-∞) [ng*h/mL]	45052.41	46321.75	11505.49	24.8	27141.40 - 75468.33	43267.74	18
AUCres [%]	4.33	5.09	3.06	60.2	2.01 - 11.66	3.89	18
Cmax [ng/mL]	2355.41	2388.59	422.56	17.7	1754.40 - 3495.54	2311.55	18
tmax [h]	-	2.280	0.929	40.8	1.000 - 4.500	2.000	18
MRT [h]	16.787	17.080	3.107	18.2	10.597 - 21.624	17.308	18
t½ [h]	11.528	11.739	2.183	18.6	7.534 - 15.015	12.012	18

AUCres Residual area in percent calculated as:

$$AUC_{res} = \frac{AUC_{(0-\infty)} - AUC_{(0-t)}}{AUC_{(0-\infty)}} \times 100$$

Bioavailability Results

The results from the statistical evaluation of the relative bioavailability across all three treatment groups are presented in Table 2 below. The evaluation of relative bioavailability was based on an ANOVA-log method for both exposure parameters, i.e., $AUC_{(0-t)}$ and C_{max} of BNZ.

Table 2: Summary statistics of the relative bioavailability assessment by ANOVA-log method: parameters estimate ratios with 90% CI (Adapted from: Clinical Study Report LPRI 747/102, TT7)

BENZNIDAZOLE (n=18)			
Variable	method	point estimates	confidence intervals
AUC(0-t) (ratio Test 1 (A) / Test 1 (B))	ANOVA-log	104.57%	99.32% - 110.09%
Cmax (ratio Test 1 (A) / Test 1 (B))	ANOVA-log	102.17%	97.11% - 107.48%
AUC(0-t) (ratio Test 1 (A) / Test 2 (C))	ANOVA-log	99.78%	94.37% - 105.51%
Cmax (ratio Test 1 (A) / Test 2 (C))	ANOVA-log	101.42%	97.93% - 105.03%
AUC(0-t) (ratio Test 1 (B) / Test 2 (C))	ANOVA-log	95.43%	91.57% - 99.45%
Cmax (ratio Test 1 (B) / Test 2 (C))	ANOVA-log	99.27%	95.94% - 102.72%

Treatment A: 1 BNZ 100 mg Tablet Intact
Treatment B: 1 BNZ 100 mg Tablet slurry
Treatment C: 8 BNZ 12.5 mg Tablets slurry

Test 1: BNZ 100 mg Tablet
Test 2: BNZ 12.5 mg Tablet

Safety Results

The treatments were well tolerated in both the study arms. No adverse events or clinically important laboratory changes were reported.

Applicant's Conclusion:

Based on the study results, the Applicant concluded that there is no considerable difference between both the test products and their different preparations as an intact tablet or as slurry in water.

Reviewer's Assessment:

The Applicant's conclusions appear valid based on study results reported in Table 2. However, similar to Study LPRI 747/101, it was noted from the provided individual BNZ concentration data that two subjects (Subject 5 and Subject 15) had BNZ plasma concentrations above the upper limit of quantification (ULOQ, i.e. 3000 ng/mL) of the bio-analytical method,. Therefore, the statistical analysis reported in Table 2 was re-evaluated after omitting the BNZ concentration data from these two subjects from all three treatment arms. The results from the "ULOQ adjusted" statistical evaluation of the relative bioavailability of BNZ from the three treatments are presented in Table 3. Findings from the ULOQ adjusted analysis were similar to the findings from the Applicant's analysis and do not affect the conclusions of this study.

Table 3: Summary statistics of the relative bioavailability assessment by ANOVA-log method: parameters estimate ratios with 90% CI: ULOQ adjusted analysis *(Source: Reviewer's independent analysis)*

Parameters	% Point Estimates (90% CI)
Test A / Test B	
AUC_{last} (ng*hr/ml)	103 (98 - 107)
C_{max} (ng/ml)	102 (98 - 105)
Test A / Test C	
AUC_{last} (ng*hr/ml)	99 (95 - 103)
C_{max} (ng/ml)	101 (98 - 105)
Test B / Test C	
AUC_{last} (ng*hr/ml)	97 (93 - 101)
C_{max} (ng/ml)	100 (97 - 103)

4.7.2 In vivo study - Raaflaub et. al. 1979

Title:

Single-dose Pharmacokinetics of the Trypanosomicide Benznidazole in Man

Publication Information:

By: J. Raaflaub and W. H. Ziegler
Pharma Research Department and Department Clinical Investigation and Development of F. Hoffmann-La Roche & Co. Ltd., Basel (Switzerland), *Arzneimittel-Forschung, 29(10): 1611-1614.*

Primary Objectives:

To assess PK of the single-dose pharmacokinetics of N-benzyl-2-niotro-1-imidazole acetamide (BNZ , Radanil®) following administration of 1 tablet Radanil (100 mg)

Test Preparations:

Radanil tablet (100 mg of BNZ, F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland)

Study Design:

This was a monocentric, open, single-dose study conducted in six healthy volunteers. The treatment was administered with two cups of tea and two slices of unbuttered toast.

Demographic Information:

The demographic data of the subjects that received treatments are summarized in the table below.

(n=6)	Mean ± SD	Min – Max
Age [years]	23.2 ± 0.8	22 - 24
Height	NA	NA
Weight [kg]	58.5 ± 6.3	47 - 63
BMI [kg/m2]	NA	NA
male : female	No male, 6 female	

Sample Collection and Bioanalysis:

During the study periods, blood samples were collected for BNZ PK analysis at pre-dose and at 0.5, 1, 2, 3, 4, 6, 12, 24, 35, 48, and 72 hours post-dose.

A total of 68 plasma samples were analyzed to determine BNZ concentrations using an assay method that is postulated to be linear within the range of 0.05 - 20 µg/mL. From the planned 72 samples, four samples were missing. From subject 1 and 2, two samples were missing, i.e., 35 hour and 72 hour samples. The samples were analyzed with differential pulse polarography technique. The plasma samples were subjected to liquid-liquid extraction and the reported recovery from the separate experiment is 78.2 ± 3.94 (SD). No additional information is available with respect to the bio-analytical method validation.

Pharmacokinetic Results:

Mean as well as individual (for four out of six enrolled subjects) BNZ plasma concentration time-curves are reproduced in Figure 1. Individual parameter estimates as well as descriptive statistics of pharmacokinetic parameter estimates are reproduced in Table 1.

Relative Bioavailability Results

In this study, the relative bioavailability, i.e., the bioavailability of the drug tablet relative to an oral solution, for Radanil formulation was evaluated in three subjects. The solution was made by dissolving100 mg of BNZ tablet in 10 ml of a mixture of polyethyleneglycol (65%), and propyleneglycol (35%), warm water (q.s. 100 ml) prior to use. The values of relative bioavailability in the three tested subjects were determined by comparison of the areas under curve from time 0 to ∞ and the values are reported in Table 1. The average value was 92%.

Figure 1: Mean and individual BNZ plasma concentration-time profiles *(source: Adapted from Raaflaub et.al. 1979, Figure 2 and Figure 1)*

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Benznidazole plasma levels in the subjects (4/6 subjects)

*Biopharmaceutics and Relevant Pharmacokinetics, Drug Intell. Publications, 1st ed., Hamilton/Ill. (1971)

Table 1: Pharmacokinetic parameter estimates for Radanil tablets *(source: Adapted from Raaflaub et.al. 1979, Table 2)*

Subject	F. B.	U. C.	P. H.	S. S.	C. M.	B. L.	Average

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Reviewer's Assessment:

The information summarized above is from a literature published by Raaflaub and Ziegler (1979). The reviewer concludes that the results from the study are likely to be valid with the caveat that no bio-analytical method validation report is available for review.

4.7.3   In vivo cross-study comparison report - LPRI747-101-DOC006

Title:

A historical comparison of bioavailability between Hoffman-Roche formulation (Radanil®) and new 100 mg tablet formulation

Study Rationale:

The Radanil formulation is discontinued and the Applicant has performed a comparison of the TBM 100 mg BNZ formulation (a.k.a. Exeltis formulation) against the published PK data from Radanil formulation. This statistical report compares the PK parameters obtained from Study LPRI 747/101 to the data from Radanil published by Raaflaub and Ziegler, 1979.

Primary Objectives:

To compare the BNZ exposure in healthy volunteers following administration of the TBM BNZ 100 mg formulation and Hoffman-Roche formulation (Radanil, BNZ 100 mg). BNZ $AUC_{(0-\infty)}$ and C_{max} were the endpoints that were compared .

Comparison Methodology:

Comparison of the PK parameters from the TBM BNZ formulation and the Radanil formulation was done by using a linear normal model (ANOVA) with log-transformed $AUC_{(0-\infty)}$ and C_{max} as dependent variables and formulation as fixed factors. From this model, the mean difference in log-transformed endpoints, i.e., $AUC_{(0-\infty)}$ between the two studies was estimated and back-transformed to the original scale and presented as a ratio, together with corresponding two-sided 90% confidence intervals (CIs).

The PK endpoints $AUC_{(0-\infty)}$ and C_{max} from LPRI 747/101 were converted from ng*h/mL and ng/mL to µg*h/mL and µg/mL respectively by multiplying by a conversion factor of 0.001, in order to have the same units as used in the Radanil study. The PK endpoints from the Radanil study were taken from the table in the Raaflaub and Ziegler (1979) publication. The $AUC_{(0-\infty)}$ endpoint was not given in the publication but was derived here as Dose/K/(V/F). The PK parameters of all six enrolled subjects are given in Table 1.

In addition, there was a difference between the enrolled subjects between Study LPRI747/101 and the Radanil study. Study LPRI747/101 enrolled both males and females, whereas in the Radanil study, all subjects were female. Therefore, the Applicant also included the consideration of possible gender differences in their analysis.

Table 1: Pharmacokinetic parameter estimates following oral administration of a single Radanil tablet as obtained/derived from Raaflaub et.al. 1979 (source: LPRI747-101-DOC006, Table 3 – page 5)

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
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Results of Statistical Comparison:

The results from statistical comparison of the $AUC_{(0-\infty)}$ and C_{max} from the TBM 100 mg BNZ formulation and Radanil are provided in Table 2. A similar comparison was done by utilizing the PK data from females subjects enrolled in LPRI 747/101 and results are provided in Table 3.

Table 2: Statistical comparison of PK parameters from studies conducted with the TBM 100 mg BNZ formulation and with Radanil (Adapted from: LPRI747-101-DOC006, Table 3 - page 6)

Ratio test	Point estimates	90% Confidence Intervals
$AUC_{0-\infty}$ (Exeltis* fasting/Radanil®)	89.53 %	[77.38 ; 103.58]
C_{max} (Exeltis fasting/Radanil®)	97.39 %	[86.17 ; 110.06]
$AUC_{0-\infty}$ (Exeltis fed/Radanil®)	89.11 %	[75.33 ; 105.41]
C_{max} (Exeltis fed/Radanil®)	88.17 %	[76.97 ; 100.99]

Separate statistical analyses were conducted with the data from the fed and the fasted groups in Study LPRI747/101.
*Exeltis formulation = To-be-marketed 100 mg BNZ formulation

Table 3: Statistical comparison of PK parameters from studies conducted with the TBM 100 mg BNZ formulation and with Radanil: Females only (Adapted from: LPRI747-101-DOC006, Table 4)

Ratio test	Point estimates	90% Confidence Intervals
$AUC_{0-\infty}$ (Exeltis* fasting/Radanil®)	91.85 %	[77.42 ; 108.97]
C_{max} (Exeltis fasting/Radanil®)	109.16 %	[98.51 ; 120.96]
$AUC_{0-\infty}$ (Exeltis fed/Radanil®)	95.18 %	[77.32 ; 117.15]
C_{max} (Exeltis fed/Radanil®)	99.01 %	[87.77 ; 111.69]

Separate statistical analyses were conducted with the data from the fed and the fasted groups in Study LPRI747/101.
*Exeltis formulation = To-be-marketed 100 mg BNZ formulation

Applicant's Conclusions:

Despite the different analytical methods used, the small subject number (especially in the Radanil 1979 study), and the slightly different fed/fasted conditions of the two studies, systemic exposure to BNZ exposure from the two formulations is approximately similar. The similarity hold if the results from the Radanil study (all female subjects) are compared with (1) all subjects (male and female) from LPRI 747/101 study or (2) only the female subjects from LPRI 747/101 study.

The results of this report indicate that the two formulations, i.e., Radanil and the TBM 100 mg BNZ formulation, are comparable from a pharmacokinetic perspective, allowing bridging Exeltis formulation/TBM formulation to other formulations used in the literature.

Reviewer's Assessment:

Overall, the Applicant's conclusions appear valid based on findings of this report.

The findings reported in Table 3 showed that the point estimate ratios were towards to the lower end of 0.8-1.25 range, suggesting that the TBM formulation may have a lower BNZ bioavailability than Radanil formulation. Given that the pharmacometrics analyses identified body weight as a significant covariate on CL/F and Vd/F of BNZ, the above-mentioned analysis was performed using body weight as a regressor during the comparison of the least squares means of the test (TBM 100 mg formulation) and reference formulations (Radanil formulation). Inclusion of body weight into the analysis moved the point estimates slightly towards 1 and associated CIs within the range of 0.8 -1.25 (Table 3). However, the following factors could also contribute to the potential differences in bioavailability between the test and reference formulations:

1) *The differences in the analytical methods used;*
2) *The small subject number (especially in the Radanil study);*

3) The lack of information with regard to the food effect on BNZ bioavailability from the Radanil tablet

There is a difference in fed/fasted conditions of the two studies. In the Raaflaub and Ziegler (1979) study, the Radanil tablet was administered with light breakfast, whereas, Study LPRI 747/101 included two treatments arms; one treatment arm received the assigned treatment on an empty stomach and the other treatment arm received the assigned treatment with a high-fat breakfast. There is no information available on the food effect upon the bioavailability of BNZ from the Radanil tablet; therefore, a direct comparison between these two studies may not be ideal due to the differences in fed/fasted conditions.

Table 3: Statistical comparison of PK parameters from studies conducted with the test and Radanil formulations – With and without body weight as a regressor *(Source: Reviewer's independent analysis)*

Parameter Estimates		% Point Estimates (90% CI) (Test/Radanil(Ref))	
		Without Body Weight Adjustment	Body Weight Adjustment (p<0.05)
Cmax (ng/ml)	Fasting	97 (85 - 111)	102 (92 - 113)
	Fed	88 (77 - 101)	92 (83 - 103)
AUC (ng*hr/ml)	Fasting	93 (80 - 109)	97 (84 - 112)
	Fed	93 (79 - 108)	96 (83 - 111)

4.7.4   List of Applicant denoted key studies

Study Code	Description	Formulation Used
DNDi-CD-PEDBZ-001	Population PK study of BNZ in children with Chagas' disease. *ClinicalTrials.gov Identifier: NCT01549236*	Pharmaceutical Laboratory in the State of Pernambuco – LAFEPE (12.5 mg or 100 mg tablet)
DNDi-CH-E1224-001	Phase II randomized, multicenter, placebo-controlled, safety and efficacy study to evaluate three oral E1224 dosing regimens and BNZ for the treatment of adult patients with chronic indeterminate Chagas disease. *ClinicalTrials.gov Identifier: NCT01489228*	LAFEPE (100 mg tablet)
Sosa-Estani et al., 1998	Efficacy of chemotherapy with BNZ in children in the indeterminate phase of Chagas' disease.	Radanil®; Roche, Olivos, Argentina
De Andrade et al., 1996	Randomized trial of efficacy of BNZ in treatment of early Trypanosoma Cruzi infection	Roche (100 mg tablet after reformulation to 50 mg tablet)
Molina et al., 2014	Randomized Trial of posaconazole and BNZ for chronic Chagas' disease. *ClinicalTrials.gov*	*NA*

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	Identifier: NCT01162967	
Altcheh et al., 2014	Population PK study of BNZ in pediatric Chagas disease suggests efficacy despite lower plasma concentrations than in adults *ClinicalTrials.gov Identifier: NCT00699387*	Radanil; Roche, Sao Paulo, Brazil (100 mg tablet)

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