Population pharmacokinetic-pharmacodynamic model of oxfendazole in healthy adults in a multiple ascending dose and food effect study and target attainment analysis

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Running title: Oxfendazole popPKPD model and target attainment analysis

ABSTRACT

Oxfendazole is a potent veterinary antiparasitic drug undergoing development for human use to treat multiple parasitic infections. Results from two recently completed Phase I clinical trials conducted in healthy adults showed that the pharmacokinetics of oxfendazole is nonlinear, affected by food, and, after the administration of repeated doses, appeared to mildly affect hemoglobin concentrations. To facilitate oxfendazole dose optimization for its use in patient populations, the relationship among oxfendazole dose, pharmacokinetics and hemoglobin concentration was quantitatively characterized using population pharmacokinetic-pharmacodynamic modeling. In fasting subjects, oxfendazole pharmacokinetics was well described by a one-compartment model with first-order absorption and elimination. The change in oxfendazole pharmacokinetics when administered following a fatty meal was captured by an absorption model with one transit compartment and increased bioavailability. The effect of oxfendazole exposure on hemoglobin concentration in healthy adults was characterized by a lifespan indirect response model in which oxfendazole has positive but minor inhibitory effect on red blood cell synthesis. Further simulation indicated that oxfendazole has a low risk of posing a safety concern regarding hemoglobin concentration, even at a high oxfendazole dose of 60 mg/kg once daily. The final model was further used to perform comprehensive target attainment simulations for whipworm infection and filariasis at various dose regimens and target attainment criteria. The results of our modeling work, when adopted appropriately, have the potential to greatly facilitate oxfendazole dose regimen optimization in patient populations with different types of parasitic infections.

1 INTRODUCTION

Neglected tropical diseases are a diverse set of infections caused by bacteria, viruses, protozoa, and metazoan that affect more than 1.5 billion people worldwide(1). Even though some neglected tropical diseases have been effectively controlled and almost eradicated, there remain multiple difficult-to-treat infections, which include neurocysticercosis, trichuriasis, echinococcosis, fascioliasis, and filariasis. Although these diseases are currently treated by the benzimidazole anthelmintic drugs albendazole, mebendazole, or triclabendazole, these treatments are not optimal due to their low efficacy(2-5), drug resistance(6), and/or unfavorable pharmacokinetics (e.g. short and greatly variable half-life)(7-9).

Oxfendazole is a potent benzimidazole anthelmintic marketed to treat lungworm and enteric helminths in animals(10). A single oral low dose of oxfendazole effectively reduced worm burden in *Trichuris suis* infected pigs(11, 12), a surrogate model of human whipworm infection. In filaria infected mice, orally or subcutaneously administered oxfendazole demonstrated up to 100% macrofilaricidal efficacy at a dosing regimen of 25 mg/kg daily for 5 days(13). The preclinical efficacy of oxfendazole in the treatment of neurocysticercosis, echinococcosis, and fascioliasis were presented in detail in a recent review(14). These results suggest that oxfendazole is a potential candidate for the treatment of multiple parasitic infections in humans. This is further supported by the favorable safety(10, 15) and pharmacokinetic profiles of oxfendazole in preclinical species. Oxfendazole exposure and half-life were greater than or comparable to those of albendazole and mebendazole in dogs(16), sheep(17), pigs(18, 19), and rats(15, 20).

Previously, the safety, tolerability, and pharmacokinetics of oxfendazole and its metabolites in healthy adults were evaluated following oral administration of single ascending oxfendazole

doses between 0.5 and 60 mg/kg(21). In that study, oxfendazole was the major moiety detected in plasma, followed by oxfendazole sulfone and fenbendazole(21). The same relative systemic exposure to parent drug and metabolites was observed in pigs(22), supporting their use as an appropriate preclinical model for oxfendazole toxicity and efficacy. In a disposition study of oxfendazole in *T. suis* infected pigs, the oxfendazole concentration in whipworm tissue was highly correlated to the drug's concentration in pig plasma(22), suggesting that plasma is a significant route for oxfendazole access to whipworm. Further, in humans, the dose-normalized exposure of oxfendazole was 27 times higher than that of albendazole and 538 times higher than that of mebendazole(23-25). Taken together, these data suggest that oxfendazole has an advantageous pharmacokinetic profile compared to those of albendazole and mebendazole for the treatment of whipworm infection in humans.

Given the encouraging results from the single ascending dose oxfendazole clinical trial, we recently completed and published the results of the second clinical trial assessing oxfendazole safety, tolerability, and pharmacokinetics in healthy adults following the administration of multiple ascending doses from 3 to 15 mg/kg (ClinicalTrials.gov ID: NCT03035760)(23). In healthy adults, oxfendazole absorption was rapid with a time to maximum concentration (T_{max}) of ~2 h. Oxfendazole elimination half-life (9.21 – 11.8 h) was consistent across dose groups and, following the administration of multiple doses at 24 h intervals, oxfendazole plasma levels reached steady state on Day 3 with little accumulation (accumulation ratio: 0.970 – 1.27). Oxfendazole exhibited substantial nonlinear pharmacokinetics with a less than dose-proportional increase in plasma exposure with escalating doses, most likely due to oxfendazole's low solubility, which caused a decrease a bioavailability with increasing doses. When oxfendazole was administered following a high-fat breakfast, oxfendazole's peak concentration (C_{max})

increased 1.49 times and the T_{max} increased by 6.88 h compared to those in the fasted state. A mild decrease in hemoglobin concentration with increasing oxfendazole dose was observed, although hemoglobin concentrations remained within the normal range for most subjects.

Because oxfendazole concentration in plasma is related to both efficacy and potential hematologic effects, an insight into the correlation between oxfendazole dose and exposure is important for dose optimization. However, oxfendazole nonlinear pharmacokinetics makes correlation of oxfendazole dose to exposure difficult. Therefore, in this study, we performed a secondary analysis of oxfendazole pharmacokinetics and its effect on hemoglobin concentration using the population pharmacokinetic-pharmacodynamic (popPK/PD) modeling approach. Because a popPK/PD model describes the underlying system, once developed, the model can be used to predict oxfendazole PK/PD with new dosing regimens that have not been evaluated in humans, as well as applied to other efficacy endpoints. Thus, we have applied the developed popPK/PD model of oxfendazole to predict 1) the change in hemoglobin concentration following multiple ascending doses from 3 to 60 mg/kg, and 2) the probability of target attainment of oxfendazole in treating whipworm infection and filariasis in humans.

2 RESULTS

2.1 Population pharmacokinetic-pharmacodynamic model

2.1.1 Structural model

Pharmacokinetic model

Structural pharmacokinetic models for oxfendazole in the fasted state and fed state are presented in **Figure 1A** and **Figure 1B**, respectively. The prolonged absorption of oxfendazole in the fed state was best captured by an absorption model with 1 transit compartment.

Pharmacodynamic model

Two models were examined to characterize the decrease in hemoglobin concentration with multiple ascending oxfendazole doses: 1) the basic indirect response model with inhibition of hemoglobin synthesis and 2) the lifespan indirect response model with inhibition of red blood cell synthesis. The two models performed similarly well based on Akaike Information Criterion (AIC), parameter feasibility and precision, and goodness-of-fit plots. The more mechanistic lifespan model (**Figure 1A**) was chosen as the final structural pharmacodynamic model because it is known that red blood cells are cleared after an average of 120 days in adults (i.e., they have relatively consistent lifespan). In this model, oxfendazole's inhibitory effect on hemoglobin synthesis was linearly correlated to oxfendazole concentration in plasma.

2.1.2 Stochastic model

Inter-individual and inter-occasion variability of all pharmacokinetic parameters were investigated. Inter-individual variability in transit rate constant (k_{TR}), absorption rate constant (k_a), oxfendazole apparent volume of distribution (V_{OXF}), oxfendazole apparent clearance

131 (CL_{OXF}), and hemoglobin synthesis rate constant (k_{in}), and inter-occasion variability in V_{OXF} and
132 CL_{OXF} were significant.

133 **2.1.3 Covariate model**

134 None of the evaluated covariates had significant impact on oxfendazole pharmacokinetics. Sex
135 significantly affected k_{in}, with k_{in} in females being 91.3% (95% confidence interval: 90.8 –
136 91.8%) of that in males (**Table 1**).

137 **2.1.4 Model evaluation**

138 Model evaluation based on goodness-of-fit plots indicated no systemic bias in terms of
139 oxfendazole plasma concentration (**Supplementary Figure S1**) or hemoglobin concentration
140 (**Supplementary Figure S2**). **Figure 2** presents the time course of population predicted
141 oxfendazole concentrations versus the mean observed oxfendazole concentrations following the
142 administration of multiple ascending doses (upper panel) or a single dose in fasted and fed states
143 (lower panel). The change in population predicted hemoglobin concentration versus change in
144 observed hemoglobin concentration after the administration of multiple oxfendazole doses is
145 presented in **Figure 3**. Overall, there was good agreement between model predicted and
146 observed data under all dosing regimens and conditions, indicating that the model was sufficient
147 at capturing oxfendazole PK/PD.

148 Final estimates of model parameters are summarized in **Table 1**. Relative standard error (%RSE)
149 was <30% for all PK/PD parameters, suggesting that all PK/PD parameters were estimated with
150 good precision. The estimated variance of inter-individual variability in k_a, k_{TR}, and V_{OXF} had
151 %RSE of more than 50%. However, removal of these inter-individual variability terms
152 negatively impacted model performance. Condition numbers (ratio of the highest to the lowest
153 eigenvalue) were 269.3 for the final pharmacokinetic model and 1.6 for the final

pharmacodynamic model. Condition numbers being less than 1000 indicate that the model is not ill-conditioned or over-parameterized.

Model predictive performance was evaluated using prediction-corrected visual predictive check as presented in **Figure 4** for all dose groups and in **Supplemental Figure S4** and **S5** for each dose group. Based on these figures, the observed 5^{th}, 50^{th} and 95^{th} percentiles were within 95% confidence interval of the respective simulated percentiles, suggesting that the final popPK/PD model for oxfendazole and its effect on hemoglobin concentrations had good predictive performance.

2.2 Simulation

2.2.1 Simulation of exposure-response on safety

To investigate the maximal decrease in hemoglobin concentrations following the administration of multiple ascending oxfendazole doses, hemoglobin concentrations in healthy subjects were simulated following the administration of multiple ascending oxfendazole doses at 3, 7.5, 15, 30, and 60 mg/kg once daily for 5 days and compared to the normal range and toxicity grade 1 – 3 ranges of hemoglobin in adults. Among the simulated doses, 3 – 15 mg/kg were evaluated in oxfendazole multiple ascending dose trial, and 30 – 60 mg/kg doses were evaluated in the single ascending dose trial. The simulation did not include doses higher than 60 mg/kg because oxfendazole exposure is saturated due to dose-limited bioavailability(21).

Simulated hemoglobin concentrations following the administration of multiple ascending doses of oxfendazole to males and females are presented in **Figure 5**. According to the lifespan model, baseline hemoglobin concentration is a product of T_R and k_{in}. Because inter-individual variability in T_R was fixed to 0, the variability in simulated hemoglobin concentration at baseline was due to inter-individual variability of k_{in} (**Table 1**). Although the magnitude of the decrease in

hemoglobin concentration increased with ascending oxfendazole doses (**Figure 5**), the median hemoglobin concentration remained in the normal range at all dose levels.

2.2.2 Simulation of exposure-response on efficacy

Target attainment analysis for whipworm infection – approach #1

In the first approach to target attainment analysis for whipworm infection, an IC_{50} = 480 ng/mL was estimated as the concentration of oxfendazole in plasma that resulted in 50% inhibition of tubulin assembly in whipworm. (Detailed derivation of this concentration is described in the **Methods** section.)

If 100% of adult whipworms are eliminated from the body when peak concentration at steady state ($C_{max,ss}$) is equal to IC_{50}, 100% of the patients would be cured even at the lowest simulated dose of 0.5 mg/kg (**Figure 6**). If targeted $C_{max,ss} = 5*IC_{50}$, 90% target attainment was achieved at doses ≥7.5 mg/kg. At targeted $C_{max,ss} = 10*IC_{50}$, 90% target attainment was achieved at doses ≥50 mg/kg. At targeted $C_{max,ss} \geq 15*IC_{50}$, probability of target attainment dropped below 70% at all doses 0.5 – 60 mg/kg.

Because whipworm elimination might depend on how long the worm is exposed to oxfendazole, target attainment analysis was additionally performed based on $T_{>IC50}$ (i.e., the percent time of a dosing interval (24 h) at steady state during which oxfendazole concentration is above IC_{50}), and the results are summarized in **Table 2**. According to **Table 2**, if maintenance of oxfendazole concentration above IC_{50} ($T_{>IC50}$ = 100%) is required for complete whipworm elimination, 90% of the patients would be completely cured at oxfendazole doses of 30 – 60 mg/kg. If $T_{>IC50} \geq$ 80% is taken as the criteria for complete deworming, 90% target attainment is reached at doses

above 5 mg/kg. At $T_{>IC50} \geq 60\%$, 90% target attainment is achievable at doses above 4 mg/kg. At $T_{>IC50} \geq 40\%$, 90% target attainment is possible at doses as low as 1 mg/kg.

Target attainment analysis for whipworm infection – approach #2

In the second approach to target attainment analysis for whipworm infection, IC_{50} = 5290 ng/mL was estimated as the concentration of oxfendazole in plasma at which 50% of adult whipworms are killed. (Rationales for the proposed IC_{50} is discussed in the **Methods** section.)

Assuming that whipworm elimination depends on oxfendazole $C_{max,ss}$, to achieve 100% cure rate, $C_{max,ss}$ must be higher than IC_{50} of 5290 ng/mL. According to **Figure 7**, the probability of target attainment was less than 90% for all doses other than the 60 mg/kg dose. **Table 3** presents the probability of target attainment based on $T_{>IC50}$. For $T_{>IC50} \geq 40\%$, probability of target attainment is less than 50% at all doses (0.5 – 60 mg/kg).

Target attainment analysis for filariasis

Based on a mouse model of filariasis, the macrofilaricidal effect (i.e., killing of adult worms) of oxfendazole is driven by the maintenance of the minimal efficacious concentration (MEC) of 100 ng/mL in plasma. To account for uncertainty extrapolating exposure-response data from mouse to human, target attainment analysis was performed at different MEC values ranging from 100 to 4000 ng/mL. The mean simulated concentration-time profile of oxfendazole in human plasma following multiple ascending doses (0.5 – 60 mg/kg once daily for 5 days) compared to different MEC levels are presented in **Figure 8A**, and the probability of target attainment at different MEC values and dosing regimens are presented in **Figure 8B**. According to **Figure 8A**, the mean concentrations of oxfendazole at all doses are above MEC throughout the dosing interval given MEC = 100 and 200 ng/mL. Correspondingly, **Figure 8B** demonstrates that the

probability of target attainment at MEC = 100 ng/mL is in the range of 90 – 100% under all dosing regimens. At MEC = 200 ng/mL, probability of target attainment is ≥ 90% at doses of at least 3 mg/kg. At MEC = 500 ng/mL, 90% target attainment is achieved only at doses of 30 mg/kg and above. At MEC = 1000 ng/mL and 1500 ng/mL, even the highest doses (50 and 60 mg/kg) can reach only ~75% and 50% target attainment, respectively. At MEC ≥ 2000 ng/mL, none of the simulated dose can pass 50% target attainment.

3 DISCUSSION

We have successfully developed a popPK/PD model characterizing oxfendazole pharmacokinetics and its effect on hemoglobin concentrations in healthy adults in a multiple ascending dose and food effect evaluation study. Oxfendazole nonlinear pharmacokinetics, attributed to oxfendazole's low solubility, was modeled with dose-dependent bioavailability. The delay in oxfendazole absorption, as well as the increase in oxfendazole exposure in the fed compared to the fasted state were sufficiently captured by the addition of one transit compartment and increase in bioavailability, respectively. According to the model estimated parameters (**Table 1**), oxfendazole bioavailability reduced significantly (22 times) as the dose increased from 0.5 mg/kg to 60 mg/kg. Based on the model developed, oxfendazole's apparent volume of distribution and apparent clearance at the lowest dose were estimated to be 34.5 L and 2.57 L/h (**Table 1**), respectively, indicating that oxfendazole distribution is moderate and oxfendazole hepatic extraction is low. A previous non-compartmental analysis showed that in subjects who have consumed a fatty meal, oxfendazole AUC increased 1.86 times and its T_{max} was delayed by 6.88 h(23) compared to those parameters measured in fasting subjects. In agreement with the published non-compartmental analysis results(23) our present model estimated a 2.08-fold (**Table 1**) increase in oxfendazole bioavailability in the fed state compared to the fasted state. In addition, in the fed state, oxfendazole absorption included one transit compartment with the transit rate constant (0.412 h^{-1}) being much lower than the absorption rate constant of oxfendazole in the fasted state (1.2 h^{-1}) (**Table 1**).

The effect of oxfendazole pharmacokinetics on hemoglobin concentration following multiple doses was sufficiently characterized by a red blood cell lifespan model with k_{in} inhibition. Oxfendazole inhibitory effect on k_{in} followed a linear function with linear coefficient of

0.000458 (95% confidence interval: 0.000377 – 0.000539) (**Table 1**). At baseline, hemoglobin k_{in} in males was estimated to be 0.00509 g/dL/h (equivalent to 0.122 g/dL/day). Hemoglobin k_{in} in females was 91.3% of that in males (**Table 1**), a result expected considering the lower normal range of hemoglobin concentration observed in females than in males (**Figure 5**). Inter-individual variability in oxfendazole pharmacokinetics and pharmacodynamics were low to moderate, ranging from 6% (k_{in} inter-individual variability) to 47% (k_{TR} inter-individual variability) (**Table 1**), which was expected of healthy adult subjects. A limitation of the current pharmacodynamic model is its lack of inclusion of a feedback regulation parameter, thus preventing its use to extrapolate to predict hemoglobin concentration after Day 5.

The developed model was used for exposure-response simulations on safety and efficacy. Regarding oxfendazole safety, simulation of hemoglobin concentration following administration of multiple ascending doses of oxfendazole (3 – 60 mg/kg once daily for 5 days) demonstrated a decrease in hemoglobin concentration with increasing oxfendazole dose (**Figure 5**). However, the median hemoglobin concentrations remained in normal range, with the simulated 5th percentile reading into the Grade 1 or Grade 2 toxicity levels. Note the predicted value of the 5th percentile is below the normal range prior to the administration of oxfendazole.

In terms of oxfendazole efficacy in the treatment of whipworm infection, four scenarios were explored: $C_{max,ss} \geq IC_{50}$ = 480 ng/mL, $T_{>IC50}$ (IC_{50} = 480 ng/mL), $C_{max,ss} \geq IC_{50}$ = 5290 ng/mL, and $T_{>IC50}$ (IC_{50} = 5290 ng/mL). The two different IC_{50} values were estimated based on different *in vitro* models with different sets of assumptions as described in the **Methods** section. $C_{max,ss} \geq IC_{50}$ = 480 ng/mL was the easiest to achieve target with 100% of the population predicted to meet this requirement even at 0.5 mg/kg dose, and 90% of the population predicted to have oxfendazole concentrations in plasma above 480 ng/mL throughout the dosing interval (i.e.,

273 $T_{>IC50}$ = 100%) with 30 – 60 mg/kg dose. In contrast, with IC_{50} = 5290 ng/mL, 90% of the
274 population is predicted to have $C_{max,ss} \geq IC_{50}$ = 5290 ng/mL only at the highest dose of 60 mg/kg,
275 and less than 5% of the population maintains an oxfendazole concentration in plasma above IC_{50}
276 = 5290 ng/mL throughout the dosing interval. Increasing the dose over 60 mg/kg would not
277 improve the probability of target attainment as oxfendazole exposure increased less than dose
278 proportionally with increasing dose. Due to the lack of exposure-response analysis on
279 oxfendazole efficacy in treating whipworm infection, target attainment analysis was performed
280 using two approaches with different sets of assumptions. The two approaches estimated two IC_{50}
281 values which differed by 11 folds and resulted in dramatically differing probabilities of target
282 attainment at clinically relevant doses. In addition, with the increase in dose, the change in
283 efficacy can be very different between IC_{50} = 480 ng/mL and IC_{50} = 5290 ng/mL. At the lower
284 IC_{50}, increasing dose from 15 to 60 mg/kg was predicted to result in only 10% increase in
285 probability of target attainment with the target being $T_{>IC50}$ = 100%. Meanwhile, at the higher
286 IC_{50}, increase in dose from 15 to 60 mg/kg was predicted to increase the probability of target
287 attainment by nearly 30% with the target being $T_{>IC50} \geq 40\%$. Thus, results of target attainment
288 analysis should be considered with caution.

289 For filariasis treatment, assuming that the minimal efficacious concentration of oxfendazole in
290 mouse (100 ng/mL)(26) and human is the same, 90% target attainment is feasible even at low
291 dose (0.5 mg/kg).

292 The *in vitro* study monitoring adult *T. muris* motility in culture as an indication of live worms
293 reported IC_{50} values of 17.7 µM, 24.2 µM, 14.3 µM, and 55.2 µM for albendazole, albendazole
294 sulfoxide, mebendazole and oxfendazole, respectively, indicating that oxfendazole was the least
295 potent among the four benzimidazoles(26). Correspondingly, ED_{50} (dose at 50% worm burden

reduction) values of oxfendazole and albendazole were higher than that of mebendazole in *T. muris* infected mice. The ED_{50} values obtained were: Oxfendazole >300 mg/kg, albendazole = 345 mg/kg and mebendazole = 79 mg/kg(26). The study author speculated that oxfendazole would not be any better than albendazole and mebendazole in treating whipworm infection in humans. However, this conclusion may be premature based on the following two considerations: First, the exposure of oxfendazole in human was 27 times higher than that of albendazole sulfoxide (the major active moiety in human plasma following albendazole oral administration) and 538 times higher than that of mebendazole (23-25). Meanwhile, the oxfendazole IC_{50} determined *in vitro* was higher than that of albendazole sulfoxide and mebendazole by only 2-4 fold(26). Thus, the higher exposure of oxfendazole compared to albendazole sulfoxide and mebendazole could potentially compensate for oxfendazole lower *in vitro* potency. Second, mice might not be a good model for oxfendazole disposition and efficacy in human due to potentially different pharmacokinetic features. Oxfendazole's metabolic profile in mice is not currently available. However, according to oxfendazole pharmacokinetic studies in the rat, following oxfendazole oral administration, oxfendazole sulfone was the most abundant moiety in plasma followed by oxfendazole and fenbendazole(27). Meanwhile, in humans, oxfendazole was the predominant moiety in plasma followed by oxfendazole sulfone and fenbendazole(21). Considering that oxfendazole and fenbendazole are active while oxfendazole sulfone is inactive, the lack of efficacy in mice might be due to oxfendazole pharmacokinetics (i.e., low systemic exposure) rather than pharmacodynamics (i.e., *in vitro* IC_{50}).

It is worth pointing out that the current model was developed based on oxfendazole pharmacokinetics and safety data obtained from healthy adults. When oxfendazole is to be used in patients with neglected tropical infections in Sub-Saharan Africa, South-East Asia and South

America, some differences in subjects' physical and health status might affect oxfendazole's exposure and response. For example, subjects in our study are primarily Caucasian with an average weight of 82.4 kg, which is 32-55% higher than the average weight of adults in Africa and South-East Asia(28). Another difference is with baseline hemoglobin concentrations. Patients in neglected tropical disease pandemic area might have low baseline hemoglobin concentrations due to other conditions, such as malaria(29) or hookworm infection(30). In our study, oxfendazole appeared to have a mild suppressive effect on hemoglobin concentrations, therefore, attention to hemoglobin concentrations in future studies in patients are warranted.

In conclusion, we have developed a robust popPK/PD model which can adequately characterize oxfendazole pharmacokinetics and its effect on hemoglobin concentration in healthy adults following multiple ascending doses (3 – 15 mg/kg), and the effect of food on oxfendazole pharmacokinetics. The model was used to comprehensively evaluate the probability of target attainment of oxfendazole for whipworm infection and filariasis in human following various dose regimens and under different target attainment criteria. Due to the lack of available data on oxfendazole exposure-response in against whipworm infection in human, results of target attainment analysis should be interpreted with caution. Nevertheless, the results of our modeling work, especially the target attainment analysis, are valuable for dose regimen selection in future trials in patient populations with different types of parasitic infections.

4 METHODS

4.1 Clinical data

The data used for popPK/PD model development comes from the multiple ascending dose and food-effect study of oxfendazole in healthy adults that was published recently(23). Key information of the study is summarized below.

The study enrolled 36 subjects, 24 subjects in the multiple ascending dose evaluation and 11 subjects in the food-effect evaluation. Subject demographics are summarized in **Supplementary Table S1**. Overall, demographic and baseline characteristics were similar across dose groups and dosing conditions. In the multiple ascending dose evaluation, subjects were randomized into three groups (8 subjects /group) corresponding to oxfendazole doses of 3, 7.5, and 15 mg/kg. Oxfendazole was administered as an oral suspension in a fasted state once daily for 5 days. Blood samples for pharmacokinetics assessment were collected on Day 1 at pre-dose and at 0.5, 1, 2, 3, 4, 6, 9, and 12 h post-dose. On Day 2, 3 and 4, samples were collected pre-dose and at 2 h post-dose. Samples were collected at 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 72 and 120 h after the last dose. Blood samples for safety assessment including hematology, biochemistry, and coagulation studies were collected at pre-dose on Day 1, 3, and 5, and at 72 h and 120 h after the last dose.

The food-effect evaluation adopted a randomized two-period (separated by 7 days) cross-over study design with subjects taking a single oral dose of oxfendazole at 3 mg/kg following an overnight fast or a high-fat breakfast. Blood samples for oxfendazole quantification were collected at pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 9, 12, and 24 h after each dose and one sample was collected from each subject on Day 14. For safety assessment, blood samples were collected at pre-dose, Day 4, and Day 14.

The oxfendazole concentration in plasma was quantified using a validated liquid chromatography-tandem mass spectrometry assay with linear range of 0.5 – 1000 ng/mL(31). Intra-day and inter-day accuracy of the quantitative method was in the range of 106.9 – 109.5%, and the coefficient of variability was no greater than 13.6%(31). Samples above the upper limit of quantification were diluted appropriately with blank human plasma(31). All samples were analyzed within the previously established stability time frame.

In total, 648 and 252 pharmacokinetics samples were collected and analyzed for the multiple ascending dose evaluation and the food-effect evaluation, respectively. Seven samples were disregarded due to sample misidentification. One hundred and twenty samples from the multiple ascending dose study and 36 samples from the food-effect study were collected and analyzed for safety assessment. There was no drug-related change in any of the safety parameters except for hemoglobin concentrations in subjects in the multiple ascending dose study(23). Therefore, only hemoglobin concentrations from subjects in the multiple ascending dose evaluation were included in the popPK/PD model.

4.2 Population pharmacokinetic-pharmacodynamic development

NONMEM 7.4.0 (Icon Development, Ellicott City, MD, USA) with stochastic approximation expectation-maximization (SAEM) method and ADVAN13 subroutine was employed for nonlinear mixed-effect modeling. Visual predictive checks were performed using Perl-speaks-NONMEM (PsN) 4.8.0 (Uppsala Pharmacometrics Group) interfaced with Pirana 2.9.9 (Certara, Princeton, NJ, USA). R 4.0.2 (R Core Team) and RStudio 1.4.1103 (RStudio, PBC, Boston, MA, USA) were used for data processing and visualization.

4.2.1 Handling of missing and BLQ data

Less than 2% clinical samples (14 out of 893 samples) had a concentration below the limit of quantification (BLQ). Because the number of BLQ samples was low and all BLQ samples were collected after 120 h (i.e., more than 5 half-lives of oxfendazole), BLQ data were omitted from the popPK/PD analysis.

4.2.2 Structural model

Structural pharmacokinetic and pharmacodynamic models were developed sequentially.

Pharmacokinetic model: The structural pharmacokinetic model for oxfendazole and metabolites in healthy adults following the administration of single ascending doses published previously(32) was adopted as the starting model. This model consisted of a depot compartment and a distribution compartment with first-order absorption and elimination. Because oxfendazole is a BCS class II drug with low solubility, the drug's bioavailability decreases with increasing dose, which resulted in a less-than-dose proportional increase in exposure following ascending doses. Additionally, when oxfendazole was administered following a high fat meal, the bioavailability was increased. Consequently, oxfendazole bioavailability (F) was modeling using the following equation,

$$log(F) = \theta_1 log\left(\frac{Dose}{\theta_2}\right) + (fast - 1)log(\theta_3) \quad (1)$$

Where $fast = 0$ if oxfendazole is administered in the fasted state, otherwise $fast = 1$.

According to equation (1), in the fasted state

$$log(F_{fast}) = \theta_1 log\left(\frac{Dose}{\theta_2}\right) - log(\theta_3) \quad (2)$$

And in the fed state

$$log(F_{fed}) = \theta_1 log\left(\frac{Dose}{\theta_2}\right) \quad (3)$$

Therefore

$$log\left(\frac{F_{fed}}{F_{fast}}\right) = log(F_{fed}) - log(F_{fast}) = log(\theta_3) \quad (4)$$

Thus

$$\theta_3 = \frac{F_{fed}}{F_{fast}} \quad (5)$$

To simplify parameter estimation, it was assumed that $F = 1$ at the lowest dose in the fed state. Thus, θ_2 was fixed to 209.25 mg (corresponding to 3 mg/kg dose in a subject weighing 69.8 kg).

To capture the prolonged absorption phase of oxfendazole following consumption of a fatty meal, absorption models with 1 and 2 transit compartments were examined.

Pharmacodynamic model: Exploratory analysis was performed by plotting the observed oxfendazole concentrations versus observed the hemoglobin concentrations (**Supplementary Figure S3**). Based on **Supplementary Figure S3**, no direct relationship was observed between oxfendazole and hemoglobin concentrations. Because hemoglobin concentrations were lower following the administration of multiple higher oxfendazole doses, indirect response models with either inhibition of hemoglobin synthesis or stimulation of hemoglobin elimination are plausible. Since oxfendazole inhibits tubulin assembly(33), oxfendazole has suppressive effect on progenitor cells undergoing extensive cell division in the bone marrow, making more likely indirect response models with oxfendazole reducing hemoglobin synthesis. Two indirect

response models of the inhibition of hemoglobin synthesis were evaluated: the basic indirect response model and the lifespan indirect response model incorporating red blood cell lifespan.

In the basic indirect response model, the change in hemoglobin concentration (C_{Hb}) was modeled using the equation

$$\frac{dC_{Hb}}{dt} = k_{in} f\left(C_{OXF}(t)\right) - k_{out} C_{OXF} \qquad (6)$$

Where k_{in} is the red blood cell synthesis rate constant and k_{out} is the red blood cell elimination rate constant.

The change in hemoglobin concentration (C_{Hb}) in the lifespan model was modeled as follows,

$$\frac{dC_{Hb}}{dt} = k_{in} f\left(C_{OXF}(t)\right) - k_{in} f\left(C_{OXF}(t - T_R)\right) \qquad (7)$$

Where T_R represents red blood cell lifespan, $f\left(C_{OXF}(t)\right)$ is a function reflecting the inhibitory effect of oxfendazole concentration in the body at time t ($C_{OXF}(t)$). Linear and nonlinear correlations between oxfendazole concentration and oxfendazole inhibitory effect was evaluated. For linear correlation,

$$f\left(C_{OXF}(t)\right) = 1 - \theta \cdot C_{OXF}(t) \qquad (8)$$

$$f\left(C_{OXF}(t - T_R)\right) = 1 - \theta \cdot C_{OXF}(t - T_R) \qquad (9)$$

For nonlinear correlation,

$$f\left(C_{OXF}(t)\right) = 1 - \frac{I_{max} C_{OXF}(t)}{IC_{50} + C_{OXF}(t)} \qquad (10)$$

$$f\left(C_{OXF}(t - T_R)\right) = 1 - \frac{I_{max} C_{OXF}(t - T_R)}{IC_{50} + C_{OXF}(t - T_R)} \qquad (11)$$

Where I_{max} is the maximal inhibitory effect, IC_{50} is oxfendazole concentration at 50% inhibition, $C_{OXF}(t)$ is oxfendazole concentration in the body at time t, and $C_{OXF}(t - T_R)$ is oxfendazole concentration in the body at time $t - T_R$.

4.2.3 Stochastic model

Inter-individual variability and inter-occasion variability were evaluated using the exponential model.

$$P_i = TVP \cdot exp\left(\eta_{i,0} + \mathrm{OCC}_1 \cdot \eta_{i,1} + OCC_2 \cdot \eta_{i,2}\right) \quad (5)$$

Where TVP represents the population mean of a pharmacokinetics or pharmacodynamic parameter, P_i represents the individual estimate of the corresponding parameter, $\eta_{i,0}$ represents inter-individual variability, $\eta_{i,1}$ and $\eta_{i,2}$ are inter-occasion variabilities following the first and last doses. $OCC_1 = 1$ for the first dose, otherwise $OCC_1 = 0$. $OCC_2 = 1$ for the last dose, otherwise $OCC_2 = 0$. Inter-individual variability and inter-occasion variability are assumed to have a normal distribution with mean 0 and variance ω^2_{IIV} and ω^2_{IOV}, respectively.

For residual variability, an additive error model, a proportional error model, and a combined additive and proportional error model were examined. The following is an example of the combined additive and proportional error model.

$$C_{ij} = \overline{C_{ij}} \cdot \left(1 + \epsilon_{1ij}\right) + \epsilon_{2ij} \quad (6)$$

C_{ij} is the observed concentrations for individual i at time j, $\overline{C_{ij}}$ is the corresponding model predicted concentration, and ϵ_{1ij} and ϵ_{2ij} are proportional and additive errors, respectively. Additive and proportional residual variabilities were assumed to be normally distributed around 0 with variance of σ_1^2 and σ_2^2, respectively.

4.2.4 Covariate model

Initially, clinically meaningful covariates including sex, age, weight, BMI, and creatinine clearance were plotted against inter-individual variability of each pharmacokinetic and pharmacodynamic parameter. Potential covariates identified through graphical analysis were examined using stepwise forward addition and backward elimination. For stepwise forward addition, a decrease in objective function value (OFV) of more than 6.63 (p-value < 0.01) was considered significant improvement in model performance. For stepwise backward elimination, an increase in OFV of more than 10.83 (p-value < 0.001) was considered significant deterioration in model performance. The effect of continuous variables (i.e., age, weight, BMI, and creatinine clearance) were evaluated using the following general equation,

$$TVP_i = TVP\left(\frac{cov_i}{cov_m}\right)^{\theta_{cov}} \quad (7)$$

Where TVP_i is the individual PK/PD parameter, TVP is the population mean of the corresponding PK/PD parameter, cov_i is the individual covariate, cov_m is the population mean of the covariate, and θ_{cov} is the covariate effect.

The effect of sex, a categorical variable, was assessed using the equation,

$$TVP_i = TVP_{male} \cdot (\theta_{sex})^{Sex} \quad (8)$$

Where TVP_{male} is the value of the PK/PD parameter in males. $Sex = 1$ for females and 0 for males. Thus, θ_{sex} is the ratio of a PK/PD parameter in females over that in males.

4.2.5 Model evaluation

Models were selected based on feasibility and precision of parameter estimates and goodness-of-fit plots including the plots of i) observed concentration versus population predicted

concentration, ii) observed concentration versus individual predicted concentration, iii) conditional weighted residual (CWRES) versus population predicted concentration, and iv) CWRES versus time. For a good model, all data points would scatter evenly around the identity line in the former two plots, and around the zero line in the latter two plots. Nested models were compared based on the difference in OFV (ΔOFV). ΔOFV was assumed to have a χ^2 distribution with the degree of freedom being the difference in the number of parameters between the two nested models. On this basis, the addition of one parameter to the model was considered significantly improved model performance if OFV decreased more than 6.63, corresponding to p-value < 0.01. For non-nested models, AIC was used. The model with a smaller AIC was considered better.

Model predictive performance was evaluated using prediction-corrected visual predictive check of 1000 simulations. Model predictive performance was acceptable if the observed 5th, 50th and 95th percentiles fall within the 95% confidence interval of the corresponding simulated percentiles.

4.3 Simulation

4.3.1 Simulation of exposure-response on safety

The simulation of exposure-response on safety focused on the effect of administration of multiple ascending doses of oxfendazole at 3, 7.5, 15, 30 and 60 mg/kg once daily for 5 days on hemoglobin concentrations in healthy adults. A thousand subjects were simulated for each dose level, assuming 1:1 ratio of female to male subjects. Oxfendazole was administered using weight-normalized dose, but oxfendazole bioavailability depends on the absolute dose present in the gastrointestinal tract; therefore, subject weight needs to be included in the simulation to convert from weight-normalized dose to absolute dose. Subject weight distribution was based on

the observed weight of participants in the oxfendazole multiple ascending dose trial. Female weight was simulated based on a normal distribution of mean 77.4 kg and standard deviation of 14.2 kg. For males, the mean weight was 84.6 kg and standard deviation was 13.0 kg. The simulated hemoglobin concentrations obtained were viewed in light of standard normal sex specific hemoglobin ranges.

4.3.2 Simulation of exposure-response on efficacy

Target attainment analysis for whipworm infection – 1st approach

Factors determining the effect of oxfendazole on *Trichuris trichiura* (human whipworm) is largely unknown. To carry out target attainment analysis, several assumptions were made based on literature information as provided below.

In a drug disposition study by Hansen et al.(22), a single oral dose of oxfendazole at 5 mg/kg was administered to pigs infected with *T. suis*. Blood samples and large intestinal samples were collected from the pigs over 48 h for quantification of oxfendazole in pig plasma, whole cecal tissue, cecal mucosa, cecal content, and in whipworm. This study found that the concentration of oxfendazole in pig plasma ($C_{OXF,plasma}$ (nmol/mL)) was closely associated with the concentration of oxfendazole in whipworm ($C_{OXF,worm}$ (nmol/g)). Since the porcine parasite *T. suis* and the human infecting *T. trichiura* have very similar growth habits, the anterior of the worm penetrating into the intestinal mucosa, with the posterior of the parasite freely moving in the intestinal lumen(22, 34), the findings of the Hansen study(22) are thought to be relevant also for the human *T. trichiura* infection. Further, the relative exposure to oxfendazole and its metabolites (oxfendazole sulfone and fenbendazole) in pigs and in humans is similar(21, 22), suggesting that pig is a good model for oxfendazole disposition in human. Thus, the correlation

between oxfendazole concentration in host plasma and in intestinal dwelling whipworm was assumed to be similar for humans and pigs.

We digitized the data reported by Hansen et al.(22) using Engauge Digitizer(35), and converted oxfendazole concentration in whipworm in nmol/g to concentration in nmol/mL based on body density of nematodes derived using the formulas for nematode body weight and body volume proposed by Andrassy(36).

$$weight(\mu g) = \frac{length(\mu m) \times (diameter(\mu m))^2}{1.6 \times 10^{-6}} \quad (9)$$

$$volume(\mu m^3) = \frac{length(\mu m) \times (diameter(\mu m))^2}{1.7} \quad (10)$$

According to the above equations, all nematodes have the same body density.

$$density = \frac{weight}{volume} = 1.0625 \times 10^{-6} \frac{\mu g}{\mu m^3} \quad (11)$$

Based on the digitized data, the ratio between oxfendazole concentration in worm and oxfendazole concentration in pig plasma was quite consistent over time with the average value of $C_{OXF,worm}(nmol/mL)/C_{OXF,plasma}(nmol/mL) = 3.29$.

The principle pharmacological activity of benzimidazoles, including oxfendazole, stems from the their binding to tubulin resulting in inhibition of tubulin assembly(33). Oxfendazole IC_{50} (drug concentration causing 50% inhibition) to *T. trichiura* tubulin is unknown. However, an *in vitro* experiment evaluating oxfendazole inhibitory effect on tubulin extracted from *Ascaris galli* (roundworm in bird) reported an IC_{50} of 5 µM (~1580 ng/mL)(37). Because *A. galli* and *T. trichiura* are both intestinal nematodes, it was speculated that oxfendazole IC_{50} to *T. trichiura* tubulin and *A. galli* tubulin would be similar.

To summarize, *in vitro* and preclinical efficacy data were applied to target attainment analysis for the treatment of whipworm infection in human based on the following assumptions:

- Assumption 1: $C_{OXF,worm}(nmol/mL)/C_{OXF,plasma}(nmol/mL) = 3.29$.
- Assumption 2: oxfendazole IC_{50} to whipworm tubulin and *A. galli* tubulin is the same and is equivalent to 1580 ng/mL.
- Assumption 3: the worm body is a well-stirred model.

Based on the above assumptions, oxfendazole IC_{50} = 1580 ng/mL at the site of tubulin assembly corresponds to oxfendazole concentration of 480 ng/mL in human plasma.

Oxfendazole concentration time profiles were simulated following the administration of multiple ascending oxfendazole doses of 0.5 – 60 mg/kg once daily for 5 days with 1000 subjects at each dose. Subject sex and weight distribution was the same as in the safety simulation. Because it is unknown whether the antiparasitic efficacy of oxfendazole depends on the achievement of an effective concentration or the maintenance over some time period of the effective concentration, two scenarios were investigated. In the first scenario, oxfendazole antiparasitic efficacy was assumed to be dependent on attainment of a targeted $C_{max,ss}$. The range of targeted $C_{max,ss}$ evaluated was 1 – 40 times IC_{50} (480 ng/mL). In the second scenario. oxfendazole antiparasitic efficacy was assumed to be dependent on maintenance of the oxfendazole concentration above the IC_{50} for a certain amount of time, as reflected by the percent time dosing interval (24 h) at steady state during which oxfendazole concentration is above IC_{50} ($T_{>IC50}$). Ranges of $T_{>IC50}$ explored included <40%, 40-<60%, 60-<80%, 80-<100%, and 100%. The probability of target attainment is the percentage of simulated subjects being able to attain the targeted $C_{max,ss}$ or $T_{>IC50}$.

Target attainment analysis for whipworm infection – 2nd approach

A recent study by Keiser and Haberli evaluated the survival of *Trichuris muris* (whipworm collected from rodents) in culture in the presence of various antiparasitic drugs(26). Whipworm survival was assessed based on whipworm motility. This study reported an IC_{50} of 55.2 µM (~17405 ng/mL) for oxfendazole(26). To utilize this information for target attainment analysis, the following assumptions were made.

- Assumption 1: $C_{OXF,worm}/C_{OXF,plasma} = 3.29$
- Assumption 2: Oxfendazole IC_{50} is the same for *T. muris* and *T. trichiura.*

Based on the above assumptions, oxfendazole IC_{50} of 17405 ng/mL to *T. trichiura* was translated to 5290 ng/mL in human plasma. Two target attainment analysis were carried out, one evaluating $C_{max,ss}$ and the other evaluating $T_{>IC50}$ as described in the 1st approach.

Target attainment analysis for filariasis

To evaluate the macrofilaricidal efficacy of oxfendazole, Hubner et al. utilized *Litomosoides sigmodontis* infected mice, a surrogate model for filariasis in human(13). Oxfendazole was administered to infected mice orally or subcutaneously once daily or twice daily for 1, 5, or 10 days at doses ranging from 1 to 125 mg/kg per day. Macrofilaricidal efficacy was assessed by adult worm count. The study showed that sterile cure was achieved with an oral dose of 12.5 mg/kg twice daily for 5 days, or subcutaneous dose of 25 mg/kg once daily for 5 days(13). Concurrently, oxfendazole pharmacokinetics following the administration of a single subcutaneous dose of 1 and 25 mg/kg or a single oral dose of 5 or 25 mg/kg was evaluated in a parallel untreated group of mice(13). Based on their efficacy and pharmacokinetic results, Hubner et al. suggested that oxfendazole macrofilaricidal efficacy is driven by the maintenance

of its MEC in plasma above 100 ng/mL(13). Thus, for target attainment analysis, the percentage of the simulated population with oxfendazole concentration at steady state being ≥ MEC was computed. To account for the uncertainty extrapolating data from mice to humans, a range of MEC from 100 to 4000 ng/mL was investigated in the present study.

595 **Acknowledgements:** This work was supported by the Division of Microbiology and Infectious
596 Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health
597 through the Vaccine and Treatment Evaluation Unit (contracts no. HHSN272200800008C and
598 HHSN24220130020I) and by the National Center for Advancing Translational Sciences grant to
599 the University of Iowa (grant no. 5U54TR001356) for the work done in the Clinical Research
600 Unit.

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603 **REFERENCES**

604 1. WHO. 2020. Ending the neglect to attain the Sustainable Development Goals - A
605 roadmap for the neglected tropical diseases 2021-2030.
606 https://www.who.int/neglected_diseases/Ending-the-neglect-to-attain-the-SDGs--NTD-
607 Roadmap.pdf. Accessed February 02, 2021.
608 2. Garcia HH, Gilman RH, Horton J, Martinez M, Herrera G, Altamirano J, Cuba JM, Rios-
609 Saavedra N, Verastegui M, Boero J, Gonzalez AE. 1997. Albendazole therapy for
610 neurocysticercosis: a prospective double-blind trial comparing 7 versus 14 days of
611 treatment. Cysticercosis Working Group in Peru. Neurology 48:1421-7.
612 3. Moser W, Schindler C, Keiser J. 2017. Efficacy of recommended drugs against soil
613 transmitted helminths: systematic review and network meta-analysis. BMJ 358:j4307.
614 4. Mihmanli M, Idiz UO, Kaya C, Demir U, Bostanci O, Omeroglu S, Bozkurt E. 2016.
615 Current status of diagnosis and treatment of hepatic echinococcosis. World J Hepatol
616 8:1169-1181.
617 5. Katiyar D, Singh LK. 2011. Filariasis: Current status, treatment and recent advances in
618 drug development. Curr Med Chem 18:2174-85.
619 6. Caravedo MA, Cabada MM. 2020. Human fascioliasis: current epidemiological status
620 and strategies for diagnosis, treatment, and control. Res Rep Trop Med 11:149-158.
621 7. Dawson M, Allan RJ, Watson TR. 1982. The pharmacokinetics and bioavailability of
622 mebendazole in man: a pilot study using [3H]-mebendazole. Br J Clin Pharmacol 14:453-
623 5.
624 8. Dawson M, Braithwaite PA, Roberts MS, Watson TR. 1985. The pharmacokinetics and
625 bioavailability of a tracer dose of [3H]-mebendazole in man. Br J Clin Pharmacol 19:79-
626 86.
627 9. Corti N, Heck A, Rentsch K, Zingg W, Jetter A, Stieger B, Pauli-Magnus C. 2009. Effect
628 of ritonavir on the pharmacokinetics of the benzimidazoles albendazole and
629 mebendazole: an interaction study in healthy volunteers. Eur J Clin Pharmacol 65:999-
630 1006.
631 10. Anonymous. 1990. Freedom of Information Summary, NADA 140-854 Synanthic-
632 original approval.
633 https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/505.
634 11. Alvarez L, Saumell C, Fuse L, Moreno L, Ceballos L, Domingue G, Donadeu M, Dungu
635 B, Lanusse C. 2013. Efficacy of a single high oxfendazole dose against gastrointestinal
636 nematodes in naturally infected pigs. Vet Parasitol 194:70-4.
637 12. Corwin RM, Kennedy JA, Pratt SE. 1979. Dose titration of oxfendazole against common
638 nematodes of swine. Am J Vet Res 40:297-8.
639 13. Hubner MP, Martin C, Specht S, Koschel M, Dubben B, Frohberger SJ, Ehrens A,
640 Fendler M, Struever D, Mitre E, Vallarino-Lhermitte N, Gokool S, Lustigman S,
641 Schneider M, Townson S, Hoerauf A, Scandale I. 2020. Oxfendazole mediates
642 macrofilaricidal efficacy against the filarial nematode Litomosoides sigmodontis in vivo
643 and inhibits Onchocerca spec. motility in vitro. PLoS Negl Trop Dis 14:e0008427.
644 14. Gonzalez AE, Codd EE, Horton J, Garcia HH, Gilman RH. 2019. Oxfendazole: a
645 promising agent for the treatment and control of helminth infections in humans. Expert
646 Rev Anti Infect Ther 17:51-56.

15. Codd EE, Ng HH, McFarlane C, Riccio ES, Doppalapudi R, Mirsalis JC, Horton RJ, Gonzalez AE, Garcia HH, Gilman RH, Cysticercosis Working Group in P. 2015. Preclinical studies on the pharmacokinetics, safety, and toxicology of oxfendazole: toward first in human studies. Int J Toxicol 34:129-37.
16. Gokbulut C, Bilgili A, Hanedan B, McKellar QA. 2007. Comparative plasma disposition of fenbendazole, oxfendazole and albendazole in dogs. Vet Parasitol 148:279-87.
17. Lanusse CE, Gascon LH, Prichard RK. 1995. Comparative plasma disposition kinetics of albendazole, fenbendazole, oxfendazole and their metabolites in adult sheep. J Vet Pharmacol Ther 18:196-203.
18. Moreno L, Lopez-Urbina MT, Farias C, Domingue G, Donadeu M, Dungu B, Garcia HH, Gomez-Puerta LA, Lanusse C, Gonzalez AE. 2012. A high oxfendazole dose to control porcine cysticercosis: pharmacokinetics and tissue residue profiles. Food Chem Toxicol 50:3819-25.
19. Alvarez LI, Saumell CA, Sanchez SF, Lanusse CE. 1996. Plasma disposition kinetics of albendazole metabolites in pigs fed different diets. Res Vet Sci 60:152-6.
20. Allan RJ, Watson TR. 1983. The metabolic and pharmacokinetic disposition of mebendazole in the rat. Eur J Drug Metab Pharmacokinet 8:373-81.
21. An G, Murry DJ, Gajurel K, Bach T, Deye G, Stebounova LV, Codd EE, Horton J, Gonzalez AE, Garcia HH, Ince D, Hodgson-Zingman D, Nomicos EYH, Conrad T, Kennedy J, Jones W, Gilman RH, Winokur P. 2019. Pharmacokinetics, safety, and tolerability of oxfendazole in healthy volunteers: a randomized, placebo-controlled first-in-human single-dose escalation study. Antimicrob Agents Chemother doi:10.1128/AAC.02255-18.
22. Hansen TVA, Williams AR, Denwood M, Nejsum P, Thamsborg SM, Friis C. 2017. Pathway of oxfendazole from the host into the worm: Trichuris suis in pigs. International Journal for Parasitology-Drugs and Drug Resistance 7:416-424.
23. Bach T, Galbiati S, Kennedy JK, Deye G, Nomicos EYH, Codd EE, Garcia HH, Horton J, Gilman RH, Gonzalez AE, Winokur P, An G. 2020. Pharmacokinetics, Safety, and Tolerability of Oxfendazole in Healthy Adults in an Open Label Phase 1 Multiple Ascending Dose and Food Effect Study. Antimicrob Agents Chemother doi:10.1128/AAC.01018-20.
24. Lange H, Eggers R, Bircher J. 1988. Increased systemic availability of albendazole when taken with a fatty meal. Eur J Clin Pharmacol 34:315-7.
25. Janssen. June 2017 2017. Mebendazole label, *on* Janssen. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/208398s000lbl.pdf. Accessed January 26, 219.
26. Keiser J, Haberli C. 2021. Evaluation of Commercially Available Anthelminthics in Laboratory Models of Human Intestinal Nematode Infections. ACS Infect Dis doi:10.1021/acsinfecdis.0c00719.
27. FAO. 1998. Residues of some veterinary drugs in foods and animals: 41-4-oxfendazole. http://www.fao.org/fileadmin/user_upload/vetdrug/docs/41-4-oxfendazole.pdf. Accessed
28. Hayes DJ, Buuren Sv, Ter Kuile FO, Stasinopoulos DM, Rigby RA, Terlouw DJ. 2014. Developing regional weight-for-age growth references for malaria-endemic countries to optimize age-based dosing of antimalarials. Bulletin of the World Health organization 93:74-83.
29. White NJ. 2018. Anaemia and malaria. Malar J 17:371.

30. Layrisse M, Roche M. 1964. The relationship between anemia and hookworm infection. Results of surveys of rural Venezuelan population. Am J Hyg 79:279-301.
31. Bach T, Bae S, D'Cunha R, Winokur P, An G. 2019. Development and validation of a simple, fast, and sensitive LC/MS/MS method for the quantification of oxfendazole in human plasma and its application to clinical pharmacokinetic study. J Pharm Biomed Anal 171:111-117.
32. Bach T, Murry DJ, Stebounova LV, Deye G, Winokur P, An G. 2021. Population Pharmacokinetic Model of Oxfendazole and Metabolites in Healthy Adults following Single Ascending Doses. Antimicrob Agents Chemother doi:10.1128/AAC.02129-20.
33. McKellar QA, Scott EW. 1990. The benzimidazole anthelmintic agents--a review. J Vet Pharmacol Ther 13:223-47.
34. CDC. Trichuriasis. https://www.cdc.gov/parasites/whipworm/biology.html. Accessed 06 April 2021.
35. Mitchell M, Muftakhidinov B, Winchen T, Wilms A, Schaik Bv, Mo-Gul b, Badger TG, Jędrzejewski-Szmek Z, Kensington, Kylesower. Engauge Digitizer Software. https://markummitchell.github.io/engauge-digitizer/. Accessed 06-29-2021.
36. Andrassy I. 1956. Die rauminhalts-und gewichtsbestimmung der fadenwürmer (Nematoden). Acta Zoologica Hungarica 2:1-5.
37. Dawson PJ, Gutteridge WE, Gull K. 1984. A comparison of the interaction of anthelmintic benzimidazoles with tubulin isolated from mammalian tissue and the parasitic nematode Ascaridia galli. Biochem Pharmacol 33:1069-74.

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716 **Figure 1.** A) Structural PK/PD model for oxfendazole and its effect on hemoglobin
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718 B) Structural PK model for oxfendazole following single oral dose in the fed state.

719 **Figure 2.** Time course of population predicted oxfendazole concentration and observed
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735 **Figure 6.** Probability of target attainment for whipworm infection following multiple ascending
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748 subjects (1000 subjects at each dose level) having plasma oxfendazole concentration at steady
749 state above MEC for 100% of the dosing interval (i.e., 24 h).

750

751

752 **Table 1.** Final estimates of oxfendazole population pharmacokinetic-pharmacodynamic model
753 parameters.

Parameter	Definition	Estimate	%RSE[b]	%Shrinkage
θ_1	A parameter in calculation of bioavailability (F)[a]	-0.646	11.2	
θ_2 (mg)	A parameter in calculation of bioavailability (F)[a]	209.25 FIX		
F_{fed}/F_{fast}	Ratio of bioavailability in fed state to bioavailability in fasted state	2.08	12.4	
k_{TR} (h^{-1})	First-order transit rate constant in fed state	0.412	14.7	
k_a (h^{-1})	First-order absorption rate constant in fasted state	1.2	7.1	
V_{OXF} (L)	Oxfendazole apparent volume of distribution	34.5	8.0	
CL_{OXF} (L/h)	Oxfendazole apparent clearance	2.57	8.6	
θ (mL/ng)	Linear coefficient for the inhibitory effect of oxfendazole on hemoglobin synthesis	0.000458	9.5	
k_{in} (g/dL/h)	Zero-order rate constant of hemoglobin synthesis in male	0.00509	1.6	
T_R (h)	Red blood cell lifespan	2880 FIX		
θ_{sex}	The ratio of k_{in} in female to k_{in} in male	0.913	2.7	
k_{TR} IIV (CV%)	Inter-individual variability in k_{TR}	47.4	52.0	44
k_a IIV (CV %)	Inter-individual variability in k_a	28.6	90.6	31
V_{OXF} IIV (CV%)	Inter-individual variability in V_{OXF}	20.1	59.9	17
CL_{OXF} IIV (CV%)	Inter-individual variability in CL_{OXF}	37.3	33.2	7
$\rho_{V\ IIV,CL\ IIV}$	Correlation of V_{OXF} IIV and CL_{OXF} IIV	0.771	10.9	
V_{OXF} IOV (CV%)	Inter-occasion variability in V_{OXF}	24.1	34.6	21
CL_{OXF} IOV (CV%)	Inter-occasion variability in CL_{OXF}	32.1	52.6	26
$\rho_{V\ IOV,CL\ IOV}$	Correlation of V_{OXF} IOV and CL_{OXF} IOV	0.887	15.2	
k_{in} IIV (CV%)	Inter-individual variability in k_{in}	60.8	35.8	20
$\sigma^2_{OXF,prop}$	Variance of oxfendazole proportional residual error	0.0616	10.4	13
$\sigma^2_{OXF,add}$	Variance of oxfendazole additive residual error	0.261	69.0	13
$\sigma^2_{Hb,prop}$	Variance of hemoglobin proportional residual error	0.000600	14.4	10

754 [a]$\log(F) = \theta_1 \log(Dose/\theta_2) + (fast\text{-}1)\log(F_{fed}/F_{fast})$, fast = 0 in fasted state, fast = 1 in fed
755 state.

756 [b]Relative standard error %RSE = standard error × 100%/parameter estimate

757

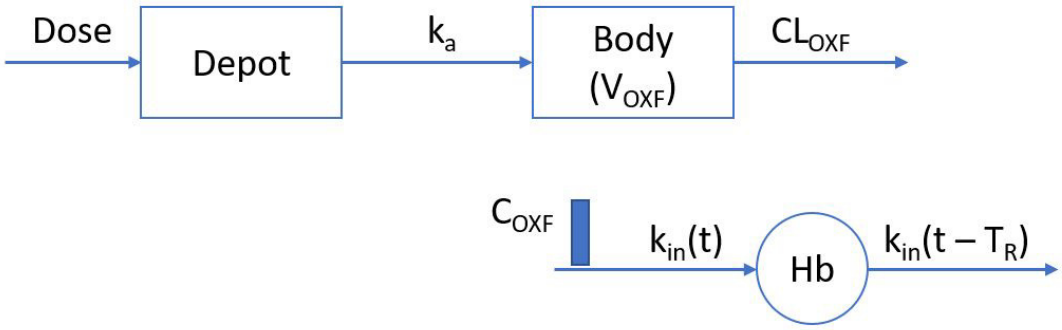
Table 2. Probability of target attainment (%) for whipworm infection following multiple ascending doses of oxfendazole (0.5 – 60 mg/kg once daily for 5 days) – 1st approach. Probability of target attainment is the percentage of simulated subjects (1000 subjects at each dose level) having plasma oxfendazole concentration above IC_{50} for a certain amount of time. $T_{>IC50}$ is the percent time of a dosing interval at steady state during which oxfendazole concentration is above IC_{50} (IC_{50} = 480 ng/mL).

Dose	$T_{>IC50}$ (%)				
(mg/kg)	100	80 – <100	60 – <80	40 – <60	≤40
0.5	27.6	13.0	17.5	26.0	15.9
1	38.0	16.1	17.5	22.0	6.4
1.5	47.0	16.6	15.7	16.7	4.0
3	59.9	14.1	13.0	11.8	1.2
4	64.3	13.7	10.7	10.3	1.0
5	70.9	11.1	9.8	7.3	0.9
7.5	74.9	11.6	7.9	5.2	0.4
10	79.5	10.0	6.2	3.9	0.4
15	82.9	7.6	5.5	3.6	0.4
20	87.9	5.3	4.0	2.6	0.2
30	90.5	5.0	2.8	1.7	0.0
40	90.6	5.3	3.0	0.9	0.2
50	93.5	3.5	2.0	1.0	0.0
60	92.5	3.9	2.7	0.8	0.1

Table 3. Probability of target attainment (%) for whipworm infection following multiple ascending doses of oxfendazole (0.5 – 60 mg/kg once daily for 5 days) – 2nd approach. Probability of target attainment is the percentage of simulated subjects (1000 subjects at each dose level) having plasma oxfendazole concentration above IC_{50} for a certain amount of time. $T_{>IC50}$ is the percent time of a dosing interval at steady state during which oxfendazole concentration is above IC_{50} (IC_{50} = 5290 ng/mL).

Dose	$T_{>IC50}$ (%)				
(mg/kg)	100	80 – <100	60 – <80	40 – <60	≤40
0.5	0.0	0.0	0.0	0.0	100
1	0.0	0.0	0.0	0.1	99.9
1.5	0.0	0.1	0.1	0.3	99.5
3	0.0	0.0	0.0	0.6	99.4
4	0.0	0.0	0.6	1.2	98.2
5	0.0	0.1	0.2	1.4	98.3
7.5	0.1	0.2	0.8	4.1	94.8
10	0.3	0.9	0.7	4.4	93.7
15	0.5	0.3	2.5	7.0	89.7
20	1.0	1.5	2.3	9.9	85.3
30	1.4	1.7	4.5	15.1	77.3
40	1.8	3.5	5.6	18.1	71.0
50	3.7	4.3	6.7	22.1	63.2
60	4.1	5.0	5.9	22.6	62.4

A. Fasted state



B. Fed state