Clinical Pharmacology Review

NDA #:	207695
Submission Date:	January 07, 2016
Brand Name:	Eucrisa®
Generic Name:	Crisaborole ointment, 2%
Dosage Form:	Ointment
Dosage Strength:	2%
Reviewer:	Chinmay Shukla, Ph.D.
Team Leader:	Doanh Tran, Ph.D.
Division Director:	CAPT. Edward Dennis Bashaw, Pharm.D.
OCP Division:	DCP-3
OND Division:	Division of Dermatology and Dental Products
Sponsor:	Anacor Pharmaceuticals, Inc.
Relevant IND(s):	77537
Submission Type:	Original NDA
Indication:	Treatment of mild to moderate atopic dermatitis in subjects 2 years of age and older

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1. Executive Summary

Crisaborole is a phosphodiesterase-4 (PDE-4) inhibitor and is developed for topical treatment of mild to moderate atopic dermatitis (AD). Crisaborole is a new molecular entity (NME) and the applicant has followed a 505(b)(1) regulatory pathway for this NDA application. The specific mechanism(s) by which crisaborole exerts its therapeutic action is not well defined.

In order to support this NDA the applicant has submitted 23 clinical trials and this includes:

- 7 studies in healthy volunteers,
- 7 studies in subjects with AD

- 9 studies in subjects with psoriasis

1.1 Recommendation

From a Clinical Pharmacology standpoint, this application is acceptable provided the labeling comments are adequately addressed by the applicant.

1.2 Post-Marketing Requirement

Conduct a maximal use pharmacokinetic trial in 16 subjects 3 months to 1 year and 11 months with moderate atopic dermatitis with a body surface area involved of ≥ 35%.

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Pharmacokinetics (PK): The applicant has conducted a maximal use PK trial (AN2728-AD-102) and assessed the PK of crisaborole (AN2728), its major metabolite AN7602 and the downstream metabolite of AN7602 (AN8323). The trial enrolled 33 male and female subjects 2 to 17 years of age with mild to moderate AD and Crisaborole Ointment, 2% was applied twice daily to a mean ± SD involved body surface area (BSA) of 49 ± 20%. Systemic concentrations of crisaborole and its metabolites were quantifiable in all subjects and steady state was reached by Day 8. The mean ± SD maximum plasma concentration (C_{max}) and area under the concentration time curve from 0 to 12 hours post dose (AUC_{0-12}) for crisaborole on Day 8 were 127 ± 196 ng/mL and 949 ± 1240 ng*h/mL, respectively. The mean ± SD C_{max} and AUC_{0-12} for AN7602 and AN8323 were 40.8 ± 48.6 ng/mL, 290 ± 313 ng*h/mL and 6150 ± 4790 ng/mL, 63,400 ± 49,000 ng*h/mL, respectively, on Day 8. The mean accumulation factor based on the ratio of AUC_{0-12} between Day 8 and Day 1, were 1.87, 1.71 and 6.28 for crisaborole, AN7602 and AN8323, respectively.

Drug interaction assessment: The applicant conducted in-vitro drug interaction assessment to assess the potential of crisaborole and its metabolites (AN7602 and AN8323) to induce and inhibit cytochrome P450 enzymes. In-vitro studies in human liver microsomes indicated that under the conditions of clinical use, crisaborole and AN7602 are not expected to inhibit CYP 1A2, 2B6, 2C8, 2C9, 2 C19, 2D6 and 3A4.

The downstream metabolite AN8323 did not inhibit the activities of CYP2C19, 2D6 and 3A4. However, it is a weak direct inhibitor of CYP1A2 and 2B6 and a moderate direct inhibitor of CYP2C8 and 2C9. The most sensitive enzyme, CYP2C9, was further investigated for drug interaction potential in a clinical trial (AN2728-PK-101) using 25 mg oral dose of warfarin as a CYP2C9 substrate. The results of this clinical trial showed there was no drug interaction potential. Based on this, further investigation of other enzymes was not warranted. Overall, the data indicates that AN8323 is not expected to inhibit any CYP enzymes under the conditions of clinical use.

In-vitro studies in human hepatocytes showed that under the conditions of clinical use crisaborole, its metabolites AN7602 and the downstream metabolite AN8323 are not expected to induce CYP 1A2, 2B6 and 3A.

TQT assessment: The applicant evaluated the effects of Crisaborole Ointment, 2% on QT/QTc interval compared to vehicle and moxifloxacin positive control in healthy subjects. The therapeutic dose was defined as application to 30% BSA and the supra-therapeutic dose was defined as application to 60% BSA.

Because the trial enrolled healthy adult volunteers, the systemic concentrations of crisaborole following the supra-therapeutic dose were approximately 30% lower than those achieved in the maximal use PK trial in pediatric subjects with AD (AN2728-AD-102). Regression analysis showed no positive relationship in the plot of vehicle-corrected change from time-matched baseline in QTcF ($\Delta\Delta$QTcF) versus concentration of crisaborole. In addition to this, the in-vitro hERG assay did not show any signal and the ECG assessments in the Phase 3 clinical trials also did not show any signal.

Based on the totality of data, there was no evidence that crisaborole has a clinically meaningful effect on the QTc interval.

Pediatric assessment: With this NDA submission, the applicant has assessed efficacy and safety of Crisaborole Ointment, 2% in subjects down to 2 years of age with mild to moderate AD. The applicant has proposed a partial waiver for the requirement to assess pediatric subjects from birth to less than 3 months old due to studies being highly impractical because diagnosis of AD is uncommon and often unreliable in subjects before the age of 3 months.

The applicant has proposed to defer studies in pediatric subjects 3 months to less than 2 years to be conducted post approval with the aim of allowing the Agency to review safety data in subjects ≥ 2 years of age before proceeding with the development in this younger age group.

Reviewer comments: *The applicant has an agreed upon initial pediatric study plan (iPSP) at the time of this NDA submission (see communication dated 10/06/2014 in DARRTS under IND 77537). The pediatric review committee (PeRC) meeting for this NDA was held on August 10, 2016, and at this meeting the PeRC agreed to waive assessment in subjects below 3 months of age and defer studies in subjects 3 months to less than 2 years of age. The PeRC also agreed with conducting maximal use PK trial in subjects 3 months to less than 2 years of age with AD post-approval.*

Clinical Pharmacology Briefing: An Optional Intra-Division Level Clinical Pharmacology briefing was held on August 29, 2016 with the following in attendance: CAPT. E. Dennis Bashaw, Hae-Young Ahn, Doanh Tran, Yanhui Lu and Chinmay Shukla.

2. Question Based Review

2.1 General Attributes of the Drug

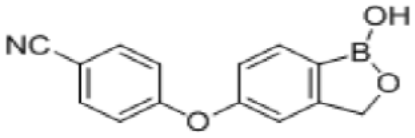
2.1.1 What regulatory pathway has the Applicant followed?

Crisaborole is a new molecular entity (NME) and the applicant has followed a 505(b)(1) regulatory pathway for this NDA application.

2.1.2 What are the highlights of the chemistry and the formulation?

Drug substance: Crisaborole a low molecular weight (mol. Wt. 251.1 daltons) achiral compound and does not possess any stereogenic centers. Its molecular formula is $C_{14}H_{10}BNO_3$ and the structural formula is shown in Figure 1.

Figure 1: Structural formula of crisaborole



Formulation: Crisaborole ointment is a petrolatum-based ointment containing 2% crisaborole (w/w). Table 1 shows the composition of the to-be-marketed formulation.

Table 1: Composition of to-be-marketed crisaborole topical ointment, 2%

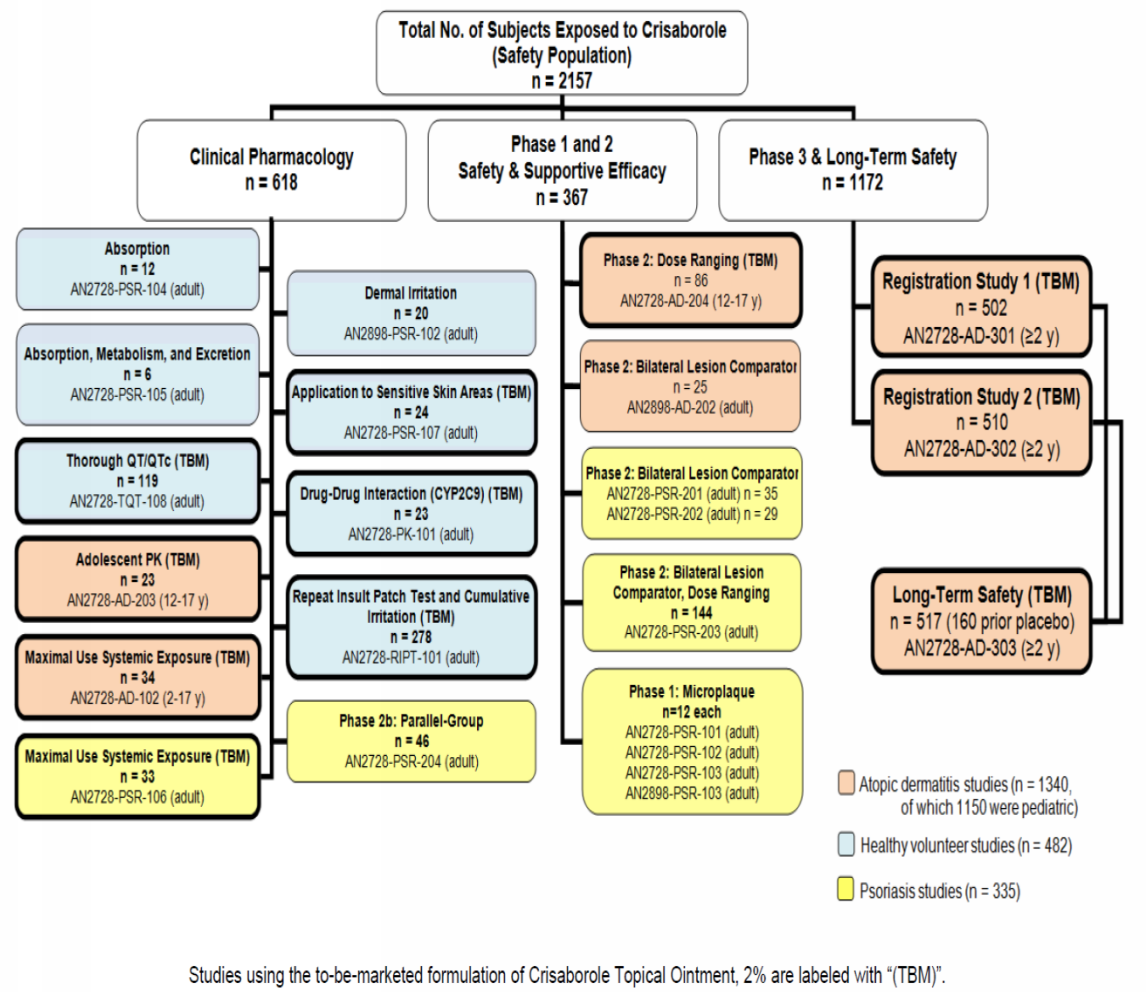
Components	Quality Standard	Function	Concentration (% w/w)
Crisaborole	In-house	Active	2.0000
White Petrolatum	USP	Ointment base	(b) (4)
Propylene Glycol	USP	(b) (4)	
Mono- and Di-glycerides	NF		
Paraffin	NF		
Butylated Hydroxytoluene	NF		
Edetate Calcium Disodium	USP		

Summary of formulation development: Numerous formulations of crisaborole ointments (b) (4) have been developed and evaluated in clinical studies. (b) (4) . These formulations were substantially different from the current to-be-marketed formulation of Crisaborole Ointment, 2%.

In the AD development program, the to-be-marketed formulation was used in the Phase 3 safety and efficacy trials (AN2728-AD-301 and AN2728-AD-302), long term safety trial (AN2728-AD-303), maximal use PK trial (AN2728-AD-102), repeat insult patch test

(AN2728-RIPT-101), thorough QTc trial (AN2728-TQT-108) and other clinical studies, as indicated in Figure 2.

Figure 2: Clinical trials where to-be-marketed (TBM) formulation was used



Studies using the to-be-marketed formulation of Crisaborole Topical Ointment, 2% are labeled with "(TBM)".

2.1.3 What are the proposed mechanism of action and the therapeutic indications?

Mechanism of action: Crisaborole is a phosphodiesterase-4 (PDE-4) inhibitor. PDE-4 inhibition results in increased intracellular cyclic adenosine monophosphate (cAMP) levels. The specific mechanism(s) by which crisaborole exerts its therapeutic action is not well defined.

Therapeutic indication: Topical treatment of mild to moderate AD in subjects 2 years of age and older.

2.1.4 What is the proposed route of administration and dosage?

Proposed route of administration: Topical.

Proposed dosage: Apply twice daily to affected areas.

2.2 General Clinical Pharmacology

2.2.1 What are the clinical trials conducted to support this application?

The applicant has submitted 23 clinical trials (7 studies in healthy volunteers, 7 studies in subjects with AD, and 9 studies in subjects with psoriasis).

(b) (4)

The therapeutic effect of crisaborole to treat AD seen in Phase 2 trials (AN2898-AD-202 and AN2728-AD-204) was confirmed by the results of two identically designed vehicle-controlled, Phase 3 trials (AN2728-AD-301 and AN2728-AD-302). Summary of clinical trials conducted to support this application are shown in Figure 2 under Section 2.1.2.

Reviewer comments: *The 9 studies conducted in subjects with psoriasis will have little regulatory impact and hence these studies will not be covered in this review.*

2.2.2 What is the systemic bioavailability of crisaborole (AN2728), its metabolite (AN7602) and the downstream metabolite of AN7602 (AN8323) under maximal use conditions?

The maximal use PK trial (AN2728-AD-102) was an open label trial to evaluate the PK of crisaborole (AN2728), its major metabolite AN7602 and the downstream metabolite of AN7602 (AN8323) in 33 male and female subjects 2 to 17 years of age with mild to moderate AD. Subjects below 12 years had ≥ 35% body surface area (BSA) involved and subjects above 12 years of age had ≥ 25% BSA involved. The to-be-marketed formulation of Crisaborole Ointment, 2 % was applied twice-a-day at a dose of 3 mg/cm^2 by the clinical staff (approximate dose range was 6 g to 30 g per application) for 9 days (PK phase), thereafter the trial continued for up to 28 days (non-PK safety phase) where subjects applied the drug at home.

PK was evaluated on Day 1 and Day 8 where plasma samples were obtained at baseline (pre-dose) and at 3 h and 12 h post-dose. Trough level samples were obtained on Days 2 (24 hours after the first dose), 7 and 9 (24 hours after the last dose).

Plasma concentrations of crisaborole and its inactive metabolites were at steady state by Day 8. Summary of mean PK parameters is shown in Table 2 and the mean concentration versus time profile of crisaborole (AN2728) and its metabolite AN7602 is shown in Figure 3 and the mean PK profile of the downstream metabolite (AN8323) of AN7602 is shown in Figure 4. See Appendix 1 for detailed report of this trial.

Table 2: Summary of mean PK parameters

PK Parameter	AN2728			AN7602			AN8323		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Day 1									
T_{max}, h[a]	34	3.00	3–12	34	3.00	3–12	34	12.0	3–24
C_{max}, ng/mL	34	111	113	34	37.8	35.0	34	2270	2640
$AUC_{(0-12)}$, ng·h/mL	32	759	730	32	247	224	32	16,800	16,900
$AUC_{(0-T)}$, ng·h/mL	34	863	759	34	288	237	34	30,800	30,500
$AUC_{(0-24)}$, ng·h/mL	29	833	694	29	274	207	29	32,500	32,500
Day 8									
T_{max}, h[a]	33	3.00	3–24	33	3.00	0–12	33	3.00	0–24
C_{max}, ng/mL	33	127	196	33	40.8	48.6	33	6150	4790
$AUC_{(0-12)}$, ng·h/mL	32	949	1240	32	290	313	32	63,400	49,000
$AUC_{(0-T)}$, ng·h/mL	33	1320	1310	33	398	347	33	119,000	87,600
$AUC_{(0-24)}$, ng·h/mL	32	1320	1330	32	391	351	32	116,000	86,700

$AUC_{(0-12)}$, area under the plasma concentration-time curve from time zero to 12 hours post dosing; $AUC_{(0-24)}$, area under the plasma concentration-time curve from time zero to 24 hours post dosing; $AUC_{(0-T)}$, area under the plasma concentration-time curve from time zero to the last measurable concentration; C_{max}, observed maximum plasma concentration after dosing; h, hour(s); PK, pharmacokinetic; SD, standard deviation; T_{max}, time to reach C_{max}.

Note: Cohort 1, Ages 12–17 years, inclusive, with ≥25% Treatable BSA; Cohort 2, Ages 6–11 years, inclusive, with ≥35% Treatable BSA; Cohort 3, Ages 2–5 years, inclusive, with ≥35% Treatable BSA.

[a] For T_{max}, the median and range, rather than the mean and SD, are displayed

Reviewer comments: *$T_{1/2}$ could not be calculated due to insufficient points in the PK profile.*

Figure 3: Mean ± SD plasma concentration-time profiles of AN2728 (Parent) and AN7602 (metabolite)

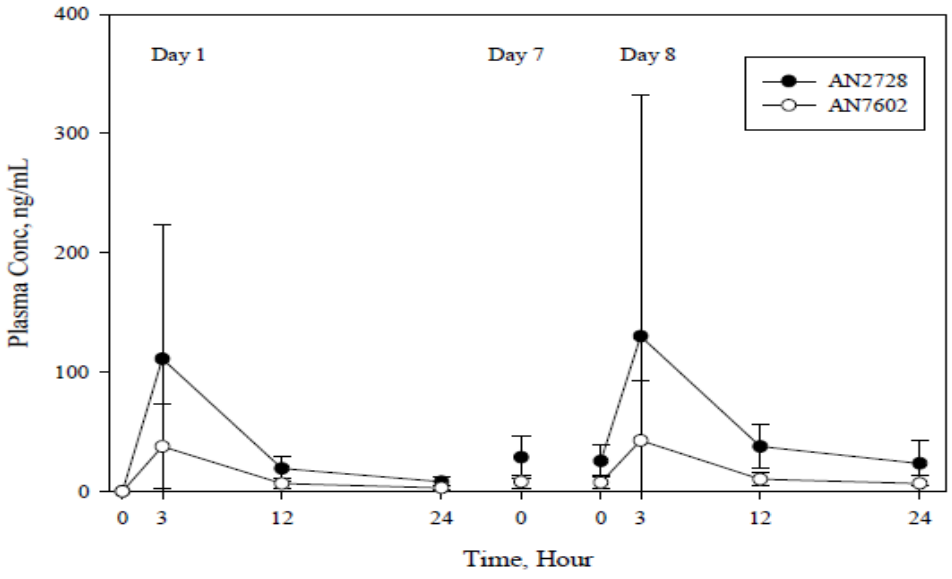
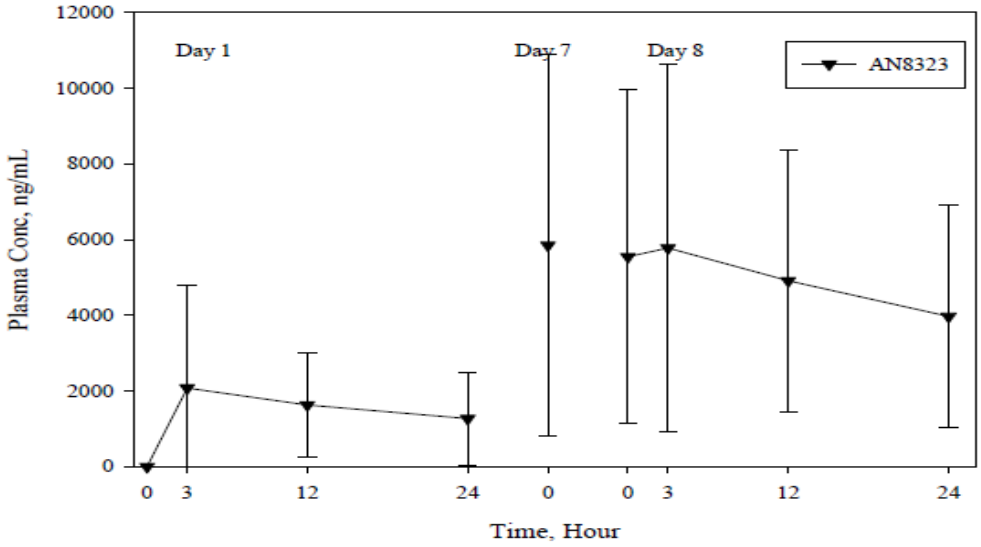


Figure 4: Mean ± SD plasma concentration-time profile of AN8323 (downstream metabolite of AN7602)



Reviewer comments: *The applicant also conducted another PK trial in adolescent subjects (AN2728-AD-203) which was not considered to be under maximal use conditions due to lower % BSA treated. This trial is described in Appendix 2.*

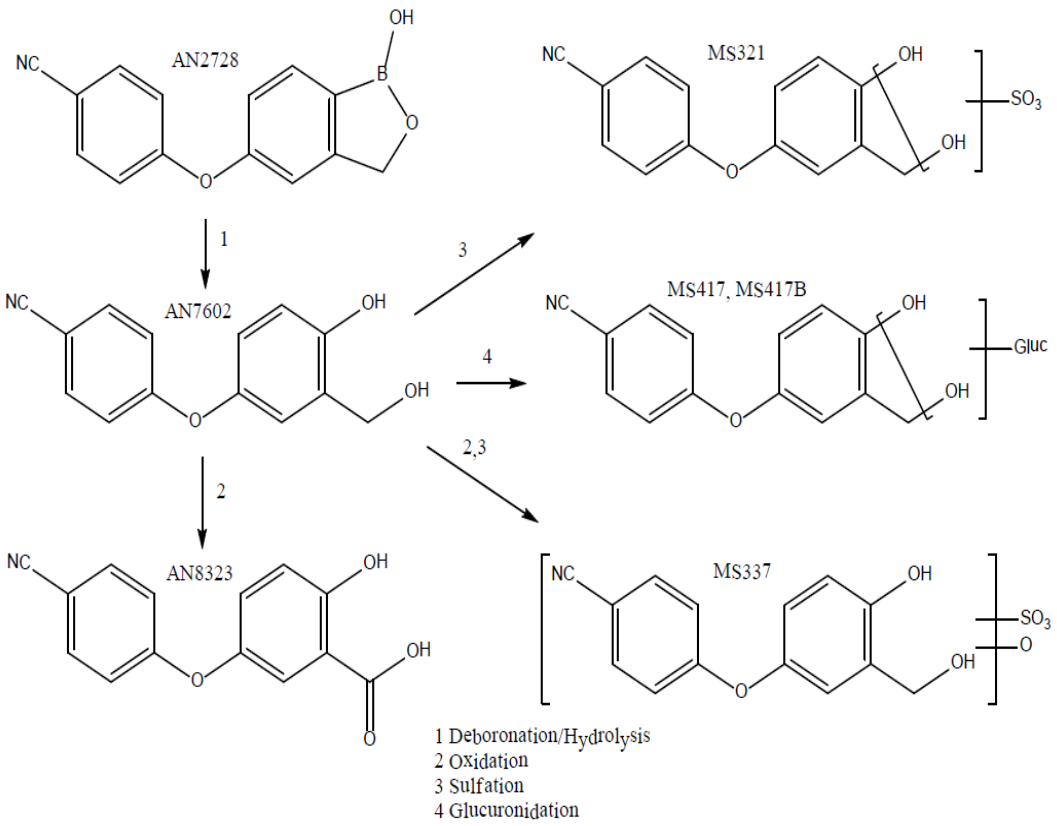
2.2.3 What is the metabolic pathway of crisaborole?

Crisaborole was found to be extensively metabolized. The major metabolite was 5-(4-cyanophenoxy)-2-hydroxyl benzyl alcohol (AN7602), which was formed due to oxidative deboronation and hydrolysis. AN7602 was further metabolized to produce several downstream metabolites, among which 5-(4-cyanophenoxy)-2-hydroxyl benzoic acid (AN8323) (produced by oxidation) and AN7602-sulfate (produced by sulfation) were major circulating components accounting for ~70% and ~30% of the total radioactivity in the plasma, respectively, from 1 to 24 hours post-dose.

CYP3A4 and 1A1/2 seem to play a major role in the formation of AN7602. CYP2B6 and 2E1 also seem to contribute to the formation of AN7602.

Excretion of crisaborole, AN7602 and AN8323 were low in the urine. AN7602-sulfate was mostly excreted in the urine. The proposed metabolic pathway is shown in Figure 5.

Figure 5: Metabolic pathway of crisaborole



AN8323: 5-(4-cyanophenoxy)-2-hydroxybenzoic acid

Nonclinical in-vitro and in-vivo studies were performed to evaluate the potential pharmacologic activity of the AN7602 and AN8323 metabolites. The results indicated lack of PDE-4 inhibitory activity for each of the metabolites.

The PK of AN7602 and AN8323 was assessed in healthy adults (AN2728-PSR-105, AN2728-TQT-108, AN2728-PK-101) and in the maximal use PK trial in subjects with AD (AN2728-AD-102). These trials are described in detail in the Appendix.

2.2.4 What information is known about plasma protein binding?

Based on in-vitro studies, crisaborole was 97% bound to human plasma proteins and the downstream metabolite AN8323 was 99% bound to human plasma proteins.

2.2.5 What is the route of excretion of crisaborole?

Renal excretion is the major route of excretion. This was evident from the Absorption Distribution Metabolism and Excretion (ADME) study (AN2728-PSR-105) conducted in 6 healthy male subjects following a single topical dose of [^{14}C]-AN2728 ointment E, 2%

(not the to-be-marketed formulation). The results indicated that, following a single topical administration, radioactivity readily appeared with median Tmax values of 8 hours and t1/2 values of 20.0 hours in plasma. The overall mean recovery of radioactivity in the study was 97.3% over the 168-hour study. The majority of radioactivity (mean of 72.2%) was recovered from the wrap materials used to cover the dose site.

Approximately 25% of the applied dose was absorbed percutaneously and approximately 81% of the absorbed radioactivity was recovered in the urine within 16 hours post-dose, and approximately 1% of the absorbed radioactivity was recovered in feces. By 168 hours post-dose, the absorbed radioactivity was almost completely recovered. Renal excretion was the major route of elimination for [^{14}C]-AN2728-derived radioactivity in humans after a topical dose.

Reviewer comments: *This trial was conducted with an older formulation. Topical formulation would have direct effect on percutaneous absorption, but the mechanism of disposition of the drug after it is absorbed would not change based on the formulation. Hence, the results support that renal excretion is the major route of elimination following application of Crisaborole Ointment, 2%. See Appendix 3 for further details on this study.*

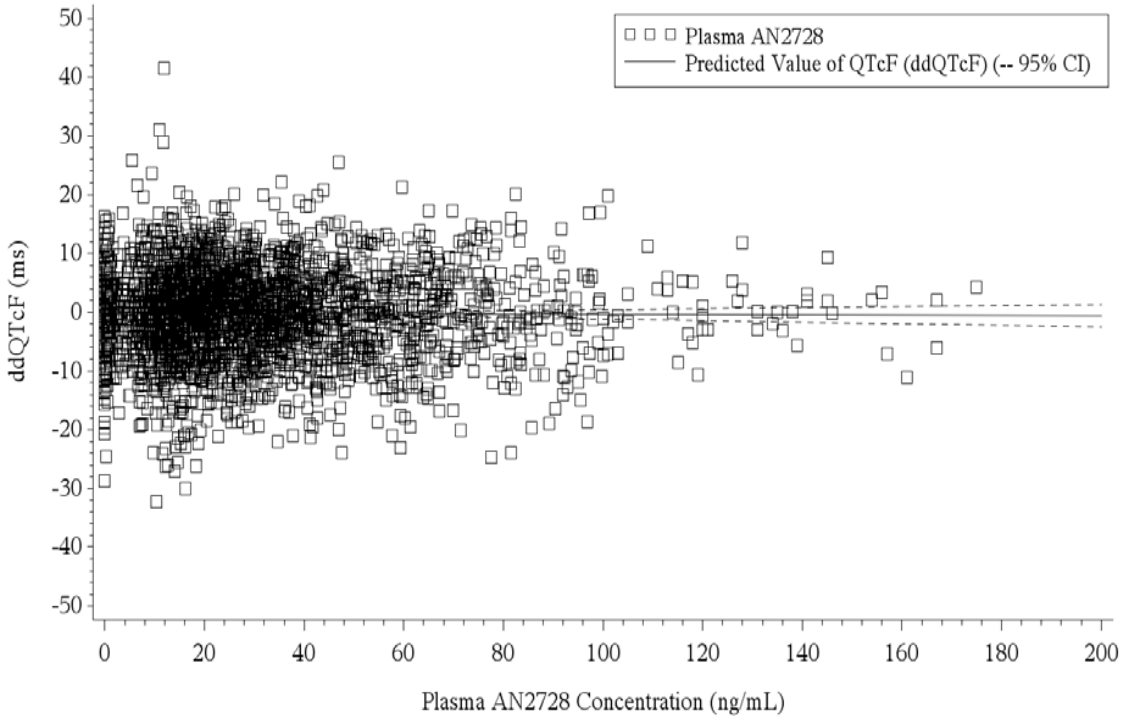
2.2.6 What is the effect of crisaborole on QT interval?

Under the conditions of clinical use, crisaborole is unlikely to cause any QT prolongation. The applicant conducted a TQT trial (AN2728-TQT-108) and the purpose of this trial was to evaluate the effects of Crisaborole Ointment, 2% (to-be-marketed formulation) on QT/QTc interval compared to vehicle and moxifloxacin positive control in healthy subjects. A total of 175 subjects completed this study and this included 78 females and 97 males. Subjects were randomized in a 1:1:1 ratio of one of the 3 cohorts.

- Cohort 1: Vehicle and positive control (moxifloxacin) cohort
- Cohort 2: Therapeutic dose of crisaborole ointment, 2% for 8 days on 30% BSA
- Cohort 3: Supra-therapeutic dose of crisaborole ointment, 2% for 8 days on 60% BSA

The systemic concentrations of crisaborole achieved in this trial were approximately 30% lower than that obtained in the maximal use PK trial (AN2728-AD-102). Based on the review by the QT - Interdisciplinary Review Team (IRT-QT) it was noted that regression analysis showed that there was no positive relationship observed between crisaborole plasma concentration and effect. The plot of vehicle-corrected change from time-matched baseline in QTcF ($\Delta\Delta$QTcF) versus concentration of crisaborole is shown in Figure 6.

Figure 6: Vehicle corrected change from time-matched baseline in QTcF (ΔΔQTcF) versus plasma AN2728 concentrations



Due to the systemic concentration of crisaborole being lower in the TQT trial compared to maximal use PK trial, the applicant was advised to include standard cardiac safety monitoring in the Phase 3 trials (see communication dated 06/16/2014 under IND 77537). The results of the cardiac safety monitoring showed that there were no signals in the Phase 3 trials (see Clinical review for further information). Further, in-vitro hERG assay also showed no signals (see Pharmacology-Toxicology review for further information).

The QT-IRT team concluded that based on the totality of clinical data, there was no evidence that crisaborole has a clinically meaningful effect on the QTc (see IRT-QT review by Dr. Jiang Lu dated 08/02/2016 under NDA 207695).

Reviewer comments: *This reviewer concurs with the assessment of the QT-IRT reviewer and opines that clinically significant effects on QT prolongation are unlikely to occur under the conditions of clinical use of Crisaborole Ointment, 2%. See Appendix 4 for detailed report of this trial.*

2.2.7 How was the dose and the dosing regimen selected for the Phase 3 trials?

The applicant conducted two Phase 2 trials (AN2898-AD-202 and AN2728-AD-204) to obtain preliminary assessments of the safety and efficacy of topical crisaborole ointment for the treatment of AD and to assist with the selection of doses and dose regimens for the Phase 3 trials.

Both Phase 2 studies were randomized, double-blind, bilateral studies in subjects with mild to moderate AD involving ≤35% BSA. Trial AN2898-AD-202 was not conducted using the to-be-marketed formulation. Drug penetration in the skin is formulation dependent. Hence this trial will have little regulatory utility and is not included in this review.

Study AN2728-AD-204: This was a multicenter, randomized, double-blind, 4-week, bilateral study of the safety and efficacy of 2 concentrations of AN2728 ointment administered once or twice a day in subjects with atopic dermatitis. The objective of this study was to determine the safety and efficacy of AN2728 ointment, 2% (to-be-marketed formulation) and 0.5% administered once-a-day (QD) or twice-a-day (BID) in the treatment of AD.

Eighty six adolescent male and female subjects 12-17 years of age with mild-to-moderate AD involving ≤ 35% BSA with 2 target lesions of similar severity located on the trunk or upper or lower extremities were enrolled. The 2% strength was applied to one lesion and the 0.5% strength was applied to another lesion on the same subject. Subjects were randomly assigned in a 1:1 ratio to the QD or BID dosing frequency. The dose was approximately 3 mg/cm^2 to each target lesion and subjects were treated for 29 days.

Each target lesion was evaluated using the Atopic Dermatitis Severity Index (ADSI) at baseline, Days 8, 15, 22 and 28. The primary endpoint was the change from baseline in ADSI score. The results indicated a greater decease in the ADSI score with the 2% BID formulation at day 29 (Table 3).

Table 3: Mean ADSI scores for target lesions over time

	Mean Score (Standard Deviation)			
	QD (N=44)		BID (N=42)	
Study Day	**AN2728, 0.5%**	**AN2728, 2%**	**AN2728, 0.5%**	**AN2728, 2%**
Baseline	8.22 (1.891)	8.02 (1.852)	8.13 (1.811)	8.19 (1.811)
Day 8	5.42 (2.538)	4.40 (1.993)	3.80 (2.156)	3.38 (1.860)
Day 15	4.19 (2.752)	3.68 (2.382)	3.62 (2.804)	2.83 (2.431)
Day 22	4.35 (3.108)	3.48 (2.841)	3.43 (2.617)	2.70 (2.417)
Day 29	3.75 (3.081)	2.95 (2.107)	3.07 (2.834)	2.38 (2.358)

ADSI, Atopic Dermatitis Severity Index; BID, twice daily; ITT, intent-to-treat; QD, once daily

Dose related differences were also observed in the secondary endpoint where the 2% BID formulation showed a greater response (Table 4).

Table 4: Number of lesions with total or partial clearance[a] (n%) based on ADSI score by study day

	QD (N=44)		BID (N=42)	
Study Day	**AN2728, 0.5%**	**AN2728, 2%**	**AN2728, 0.5%**	**AN2728, 2%**
Day 8	4 (9.1%)	8 (18.2%)	12 (28.6%)	12 (28.6%)
Day 15	13 (29.5%)	13 (29.5%)	15 (35.7%)	24 (57.1%)
Day 22	13 (29.5%)	17 (38.6%)	17 (40.5%)	21 (50.0%)
Day 29	19 (43.2%)	18 (40.9%)	21 (50.0%)	26 (61.9%)

ADSI, Atopic Dermatitis Severity Index; BID, twice daily; ITT, intent-to-treat; QD, once daily

[a] Total or partial clearance is defined as ADSI ≤2.

Overall, both the higher dose level (2%) and the more frequent dose (BID) appeared to be more effective. The drug-related treatment emergent adverse events (TEAEs) were all mild application site reactions. One subject discontinued due to a moderate, unrelated AE of "atopic dermatitis flare" and the need for a prohibited concomitant medication (prednisolone). No deaths or severe adverse (SAEs) were reported. Based on this, the 2% strength applied BID was selected for further development.

Reviewer comments: *Strong observations related to safety cannot be made since there was no vehicle arm in this trial.*

2.2.8 What is the summary of efficacy?

The efficacy and safety of Crisaborole ointment, 2% (EUCRISA®) was evaluated in two multicenter, randomized, double-blind, parallel-group, vehicle-controlled trials following application of the drug twice daily for 28 days in subjects with mild to moderate AD. A total of 1522 subjects 2 to 79 years of age with 5% to 95% treatable BSA were randomized in a 2:1 ratio to Treatment:Vehicle arms. Of the enrolled subjects, 38.5% had mild disease and 61.5% had moderate disease.

The primary efficacy endpoint in these trials was the proportion of Crisaborole Ointment, 2% subjects achieving success in ISGA, defined as an ISGA grade of Clear (score=0) or Almost Clear (score=1) with ≥ 2-grade improvement from baseline compared with the vehicle-treated subjects at Day 29. Summary of efficacy is shown in Table 5 below.

Table 5: Summary of efficacy

	Trial 1		Trial 2	
	EUCRISA (N=503)	**Vehicle (N=256)**	**EUCRISA (N=513)**	**Vehicle (N=250)**
Success in ISGA[a]	32.8%	25.4%	31.4%	18.0%
ISGA of Clear or Almost Clear	51.7%	40.6%	48.5 %	29.7%

[a] Defined as an ISGA score of Clear (0) or Almost Clear (1) with ≥2-grade improvement from baseline.

Reviewer comments: *See Clinical and Biostatistics review for further details.*

2.2.9 What is the summary of safety?

The adverse events (AEs) were mostly local and this included application site pain and application site infection. There were no deaths or treatment related severe AEs reported. Safety signals and any abnormalities were not identified in clinical laboratory testing, vital signs and ECG assessment.

Reviewer comments: *See Clinical review for further details.*

2.3 Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

2.3.1.1 Effect of gender and age on PK of crisaborole

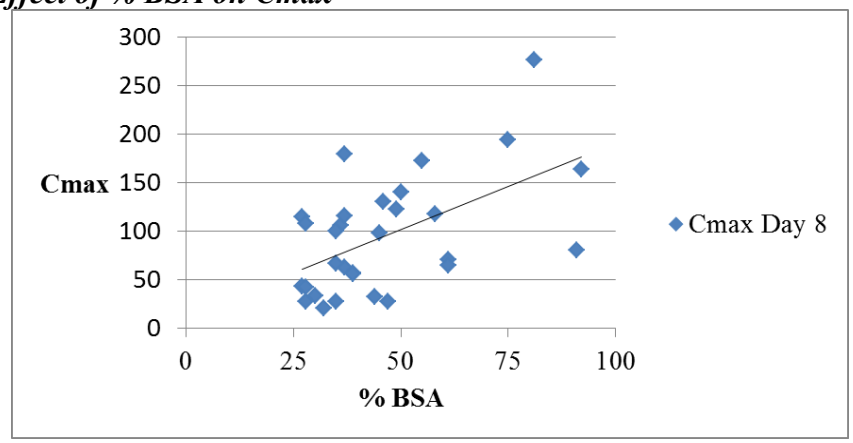
The effect of gender and age on the PK of crisaborole was not explored. Since the maximal use PK trial (AN2728-AD-102) had a wide range of pediatric subjects in terms of age, weight, body surface area, degree of disease severity etc., dosing varied among the subjects. Due to several confounding factors which would directly impact the drug exposure[1], the effect of intrinsic factors was not explored. Furthermore, limited number of subjects would make such analysis non-viable to perform.

[1]***Reference:*** *Maximal Usage Trial: An Overview of the Design of Systemic Bioavailability Trial for Topical Dermatological Products; Bashaw et. al.; Therapeutic Innovation & Regulatory Science; 2015; 49(1); 108-115.*

2.3.1.2 Effect of % body surface area (BSA) on the PK of crisaborole

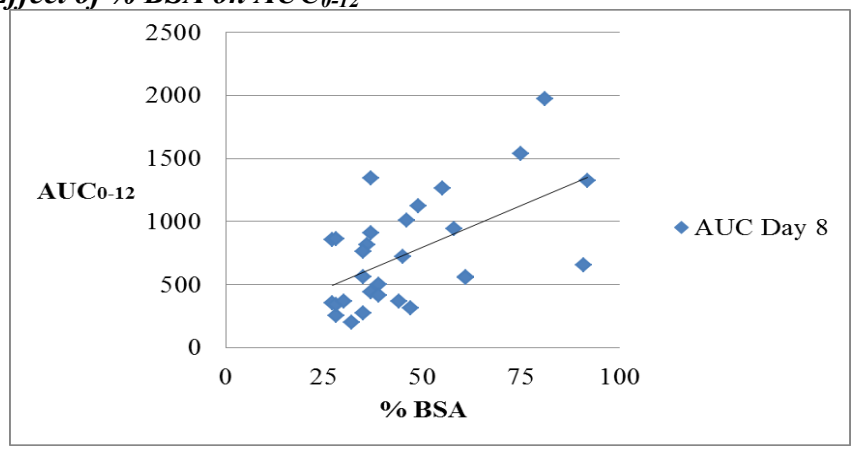
The dose in the maximal use PK trial (AN2728-AD-102) was 3 mg/cm^2. The dosing was directly dependent on the % BSA involved. Hence the effect of % BSA on the exposure of crisaborole was explored (the underlying assumption is that the effect of other intrinsic factors on the exposure of crisaborole is negligible). The values of Cmax and AUC_{0-12} on Day 8 appear to increase with the increase in BSA* (Figure 7 and 8).

Figure 7: Effect of % BSA on Cmax



** Subject 112-09, a 7.3 year old female with moderate disease (BSA 62%), was excluded from this analysis. For reasons unknown, the Cmax on Day 8 was approximately 7.7 fold higher than the mean Cmax.*

Figure 8: Effect of % BSA on AUC_{0-12}



**Subject 112-09, a 7.3 year old female with moderate disease (BSA 62%), was excluded from this analysis. For reasons unknown, the AUC_{0-12} on Day 8 was approximately 6.6 fold higher than the mean AUC_{0-12}.*

Reviewer comments: *The observed increase in Cmax and AUC_{0-12} with increase in %BSA treated is not unusual because of increase in the amount of drug applied with increase in %BSA.*

2.3.2 Pediatric subjects

PK assessment in pediatric subjects: In the maximal use PK trial (AN2728-AD-102) the applicant assessed PK of crisaborole and its metabolites in pediatric subjects aged 2-17 years (Table 6, 7 and 8). Subjects were divided into 3 cohorts as shown below:

- Cohort 1: 12 to 17 years
- Cohort 2: 6 to 11 years
- Cohort 3: 2 to 5 years

Table 6: Mean PK data of crisaborole (AN2728) by age cohort

PK Parameter	Cohort 1			Cohort 2			Cohort 3		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Day 1									
T_{max}, h	12	3.00	3 - 12	12	3.00	3 - 12	10	3.00	3 - 3
C_{max}, ng/mL	12	88.5	128	12	161	126	10	78.4	42.9
$AUC_{(0-12)}$, ng·h/mL	12	599	818	11	1,120	799	9	536	275
$AUC_{(0-T)}$, ng·h/mL	12	738	893	12	1,180	816	10	633	332
$AUC_{(0-24)}$, ng·h/mL	12	738	893	9	1,070	637	8	707	318
Day 8									
T_{max}, h	12	3.00	3 - 3	12	3.00	3 - 12	9	3.00	3 - 24
C_{max}, ng/mL	12	81.4	55.3	12	205	312	9	83.3	36.2
$AUC_{(0-12)}$, ng·h/mL	12	642	392	11	1,490	2,020	9	702	272
$AUC_{(0-T)}$, ng·h/mL	12	916	510	12	1,840	2,010	9	1,150	507
$AUC_{(0-24)}$, ng·h/mL	12	916	510	11	1,890	2,100	9	1,150	507

[a] Expressed as median and range

Table 7: Mean PK data of AN7602 (metabolite) by age cohort

PK Parameter	Cohort 1			Cohort 2			Cohort 3		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Day 1									
T_{max}, h[a]	12	3.00	3 - 12	12	3.00	3 - 12	10	3.00	3 - 3
C_{max}, ng/mL	12	27.4	31.0	12	57.0	44.0	10	27.3	14.0
$AUC_{(0-12)}$, ng·h/mL	12	185	201	11	366	285	9	185	89.0
$AUC_{(0-T)}$, ng·h/mL	12	230	228	12	397	290	10	225	120
$AUC_{(0-24)}$, ng·h/mL	12	230	228	9	351	237	8	253	117
Day 8									
T_{max}, h[a]	12	3.00	0 - 3	12	3.00	3 - 12	9	3.00	3 - 12
C_{max}, ng/mL	12	26.8	18.4	12	62.6	73.9	9	30.3	18.9
$AUC_{(0-12)}$, ng·h/mL	12	203	128	11	426	486	9	241	146
$AUC_{(0-T)}$, ng·h/mL	12	274	159	12	545	494	9	366	236
$AUC_{(0-24)}$, ng·h/mL	12	274	159	11	540	517	9	366	236

[a] Expressed as median and range

Table 8: Mean PK data of AN8323 (downstream metabolite) by age cohort

PK Parameter	Cohort 1			Cohort 2			Cohort 3		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Day 1									
T_{max}, h[a]	12	12.0	3 - 24	12	3.00	3 - 24	10	7.50	3 - 24
C_{max}, ng/mL	12	1,090	938	12	3,410	3,690	10	2,300	2,040
$AUC_{(0-12)}$, ng·h/mL	12	9,640	9,670	11	23,700	20,200	9	18,100	17,900
$AUC_{(0-T)}$, ng·h/mL	12	21,200	19,100	12	40,700	38,200	10.0	30,400	30,300
$AUC_{(0-24)}$, ng·h/mL	12	21,200	19,100	9	45,500	43,400	8.00	34,700	32,600
Day 8									
T_{max}, h[a]	12	3.00	0 - 12	12	3.00	0 - 24	9	0.00	0 - 12
C_{max}, ng/mL	12	4,100	3,550	12	7,750	5,900	9	6,750	3,980
$AUC_{(0-12)}$, ng·h/mL	12	44,400	37,200	11	76,200	62,500	9	73,200	40,900
$AUC_{(0-T)}$, ng·h/mL	12	82,200	63,300	12	146,000	110,000	9	132,000	71,400
$AUC_{(0-24)}$, ng·h/mL	12	82,200	63,300	11	139,000	113,000	9	132,000	71,400

[a] Expressed as median and range

Reviewer comments: *From the data in Tables 6, 7 and 8 the Cmax and AUC_{0-12} on Day 8 in Cohort 2 (ages 6-11 years) appear to be numerically higher than Cohort 1 and Cohort 3. However, definitive conclusions cannot be made regarding the effect of age because of the difference in the dose due to difference in BSA between younger and older subjects.*

PREA requirements: With this NDA submission, the applicant has assessed efficacy (see summary in Section 2.2.8) and safety (see summary in Section 2.2.9) of Crisaborole Ointment, 2% in subjects down to 2 years of age with mild to moderate AD in Phase 3 clinical trials. The applicant has proposed a partial waiver for the requirement to assess pediatric subjects from birth to less than 3 months old due to studies being highly impractical because diagnosis of AD is uncommon and often unreliable in subjects before the age of 3 months.

The applicant has proposed to defer studies in pediatric subjects 3 months to less than 2 years to be conducted post approval with the aim of allowing the Agency to review safety data in subjects ≥ 2 years of age before proceeding with the development in this younger age group.

Reviewer comments: *The applicant has an agreed upon initial pediatric study plan (iPSP) at the time of this NDA submission (see communication dated 10/06/2014 in DARRTS under IND 77537). The pediatric review committee (PeRC) meeting for this NDA was held on August 10, 2016, and at this meeting the PeRC agreed to waive assessment in subjects below 3 months of age and defer studies in subjects 3 months to*

less than 2 years of age. The PeRC also agreed with conducting maximal use PK trial in subjects 3 months to less than 2 years post-approval.

2.3.3 Renal and hepatic impairment

The effect of renal and hepatic impairment on PK was not evaluated by the applicant.

2.3.4 What pregnancy and lactation use information is there in the application?

The applicant has not conducted any trials in pregnant and lactating women.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or response and what is the impact of any differences in exposure or response?

The influence of extrinsic factors on dose-exposure and/or response was not evaluated by the applicant.

2.4.2 Drug-drug interactions

In vitro studies were conducted to determine the inhibition and induction potential of crisaborole and its major metabolites, AN7602 and AN8323, on the activities of cytochrome P450 isoforms.

Drug Interaction Potential for Crisaborole: To assess the potential of crisaborole (molecular weight = 251.1) to interact with other drugs, in-vitro studies were conducted in human liver microsomes to determine whether crisaborole was an inhibitor or inducer of CYP enzymes. Crisaborole did not inhibit (direct or metabolism-dependent) CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, and CYP3A4 at concentrations up to 15 μM.

However, crisaborole was found to competitively inhibit CYP2C19 with a Ki of 8.96 μM and it also showed a metabolism-based inactivation of CYP2C19 with a Ki of 22.3 μM.

To address the potential for crisaborole to inhibit CYP2C19 following clinical application of Crisaborole Ointment, 2%, the exposure of crisaborole, as determined in the maximal use PK trial (AN2728-AD-102) conducted in pediatric subjects with AD, was considered for the calculation of I/Ki. In this study, after topical administration of Crisaborole Ointment, 2% at a dose of 3 mg/cm^2 applied to treatable BSA (mean %BSA = 48.7%), the mean ±SD plasma Cmax on Day 8 was 0.506 ± 0.781 μM (127± 196 ng/mL). Hence the ratios of [I]/ Ki for competitive inhibition (0.506/8.96 or 0.056) and metabolism-based inhibition (0.506/22.3 or 0.023) would be <0.1 and the corresponding R values would be < 1.1 which indicates that the probability of crisaborole to inhibit CYP2C19 under conditions of clinical use is low.

In an in-vitro study using human hepatocytes with mRNA levels as endpoint, crisaborole did not induce CYP1A2, CYP2C9, CYP2C19, and CYP3A4/5. Crisaborole showed weak induction of CYP2B6 in 1 of 3 donors at a concentration of 10 μM. This interaction is not expected to occur under the conditions of clinical use.

Drug Interaction Potential for AN7602: A study was conducted to characterize the in-vitro inhibitory potential of 10 μM AN7602 (molecular weight = 241.1) on the activities of the CYP450 isoforms CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A in human liver microsomes (The mean Cmax in the maximal use PK trial was 0.169 μM). AN7602 did not inhibit of any of the CYP enzymes tested.

A study was conducted to characterize the in-vitro induction potential of 0.1, 1, and 10 μM AN7602 on the activities of the CYP450 isoforms CYP1A2, CYP2B6, and CYP3A4, in human hepatocytes using mRNA levels as the end point. AN7602 did not induce any of the CYP enzymes tested.

Drug Interaction Potential for AN8323: A study was conducted to characterize the in vitro inhibitory potential of AN8323 (molecular weight = 255.23) on the activities of the CYP450 isoforms CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5. AN8323 did not inhibit activities of CYP2C19, CYP2D6, and CYP3A4/5. It was a weak direct inhibitor of CYP1A2 and CYP2B6 and a moderate direct inhibitor of CYP2C8 and CYP2C9. The IC_{50} values were 49.3 μM for CYP1A2, >50 μM for CYP2B6, 7.66 μM for CYP2C8, and 9.52 μM for CYP2C9. The Ki and the inhibition type were further determined for CYP2C8 and CYP2C9. AN8323 inhibited CYP2C8 in a competitive manner with a Ki value of 6.7 μM, whereas AN8323 inhibited CYP2C9 in a mixed (competitive and uncompetitive) manner with a Ki value of 5.2 μM.

To address the potential for inhibition of CYP2C9 and CYP2C8 following clinical application of Crisaborole Topical Ointment, 2%, the [I]/Ki ratio was calculated using plasma AN8323 results from the maximal use PK trial conducted in pediatric subjects with AD (AN2728-AD-102). The mean AN8323 C_{max} value on Day 8 was 24 μM [I]. This led to a [I]/K_i ratio for both CYP2C8 and CYP2C9 of more than 0.1. As a result, clinical DDI studies investigating these two CYP subtypes were warranted.

Because CYP2C9 was the most sensitive of CYP enzymes in-vitro, a clinical DDI study of co-administration of Crisaborole Ointment, 2% and 25 mg oral dose of warfarin (a CYP2C9 substrate) was conducted (AN2728-PK-101). The results of this study showed lack of drug interaction between crisaborole and warfarin as indicated by the 90% confidence interval of the ratio of geometric mean of Cmax, AUC_t and $AUC_{0-\infty}$ for S-and R-warfarin with and without crisaborole, being within the no effect boundary of 80% to 125% (Table 9).

Table 9: Statistical evaluation of S- and R-warfarin plasma PK parameters in healthy adult subjects following a 25 mg warfarin dose with (n = 21) or without (n = 21) AN8323

PK Parameter	With AN8323		Without AN8323		Diff	Diff	%Ratio	CI 90%	CI 90%
	LSM	SE	LSM	SE	LSM	LSM SE		Lower	Upper
S-Warfarin									
ln-C_{max}	7.52409	0.04447	7.58482	0.04447	-0.06073	0.06289	94.11	84.65	104.62
ln-AUC_t	10.90471	0.07227	10.88905	0.07227	0.01566	0.10221	101.58	85.52	120.65
ln-$AUC_{0\text{-}inf}$	10.94359	0.07736	10.92481	0.07736	0.01878	0.10940	101.90	84.75	122.51
R-Warfarin									
ln-C_{max}	7.50020	0.04017	7.55065	0.04017	-0.05045	0.05680	95.08	86.41	104.62
ln-AUC_t	11.36333	0.03975	11.33023	0.03975	0.03310	0.05621	103.36	94.03	113.63
ln-$AUC_{0\text{-}inf}$	11.47451	0.04523	11.42690	0.04523	0.04761	0.06396	104.88	94.17	116.80

Reviewer comments: *Due to the lack of drug interaction between crisaborole and warfarin (CYP2C9 substrate), further investigation of drug interaction potential with CYP2C8 is not needed.*

An in-vitro study in human hepatocytes was conducted to characterize the in-vitro induction potential of AN8323 (at concentrations of 0.1 to 50 μM) on the activities of the CYP450 isoforms CYP1A2, CYP2B6 and CYP3A4/5. AN8323 appears to be a weak inducer of CYP1A2 but not CYP2B6 or CYP3A. However under conditions of clinical use, AN8323 is not expected to induce any of the CYP enzymes.

Reviewer comments: *Details about the in-vitro drug interaction studies are in Appendix 6, 7 and 8; and in-vivo drug interaction study with warfarin (AN2728-PK-101) is in Appendix 5.*

2.5 General Biopharmaceutics

2.5.1 Based on biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

The concept of BCS classification does not apply to topically applied products.

2.5.2 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

The to-be-marketed formulation was used in the maximal use PK trial in AD (AN2728-AD-102), Adolescent subject PK trial (AN2728-AD-203), TQT trial (AN2728-TQT-108), Drug interaction trial for CYP2C9 (AN2728-PK-101), Phase 2 dose ranging trial (AN2728-AD-204), Two Phase 3 trials (AN2728-AD-301 and 302), and long term safety trial (AN2728-AD-303). Hence relative bioavailability assessment is not needed.

2.5.3 What data support or do not support a waiver of in vivo BE data?

The to-be-marketed formulation was used in all the pivotal studies (see Section 2.5.2 for further details). Hence waiver request for in-vivo BE study does not apply.

2.6 Analytical Section

2.6.1 How are the active moieties identified, and measured in the clinical trials?

High performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) was used to quantify crisaborole, its main metabolite AN7602 and the downstream metabolite AN8323 in the plasma.

Reviewer comments: *The bioanalytical method for Maximal use PK trial (AN2728-AD-102), TQT trial (AN2728-TQT-108), Drug interaction trial for CYP2C9 (AN2728-PK-101) was the same. Adolescent subject PK trial (AN2728-AD-203) and the ADME study (AN2728-PSR-105) used different methods.*

2.6.2 Which metabolites have been selected for analysis and why?

AN7602 is the major metabolite of crisaborole and was selected for analysis. AN8323 is a downstream metabolite of AN7602 and this was also selected for analysis because its exposure was very high (~ 66 fold) compared to parent. Both these metabolites are inactive.

2.6.3 For all moieties measured, is free, bound, or total measured?

Total concentration was measured.

2.6.4 What is the range of the standard curve?

The range of standard curve in plasma was:

- For Crisaborole (AN2728): 0.2 ng/mL to 100 ng/mL
- For AN7602: 0.2 ng/mL to 100 ng/mL
- For AN8323: 10 ng/mL to 5000 ng/mL

Reviewer comments: *In the ADME study (AN2728-PSR-105), AN8323 concentrations were not quantified.*

2.6.5 What are the accuracy and precision?

Method validation results (Report # MC13B-022) for assay used in Maximal use PK trial (AN2728-AD-102), TQT trial (AN2728-TQT-108), Drug interaction trial for CYP2C9 (AN2728-PK-101).

Analyte	Inter-Day (N = 18)		Intra-Day (N = 6)	
	Accuracy	*Precision*	*Accuracy*	*Precision*
Crisaborole	-4.33 to 0.50%	1.54 to 4.29%	-8.17 to 1.50%	0.83 to 2.50%
AN7602	-2.63 to 2.00%	1.85 to 6.38%	-3.87 to 3.83%	1.27 to 7.97%
AN8323	-2.67 to -1.75%	1.52 to 2.36%	-3.33 to -1.33%	0.66 to 2.78%

Method validation results (Report # MC13B-0253) for assay used in Adolescent subject PK trial (AN2728-AD-203)

Analyte	Inter-Day (N = 30)		Intra-Day (N = 6)	
	Accuracy	*Precision*	*Accuracy*	*Precision*
Crisaborole	-3.17 to -2.00%	3.64 to 4.86%	-7.50 to 3.00%	2.18 to 5.05%
AN7602	-5.88 to -0.83%	4.13 to 4.57%	- 9.38 to 2.33%	1.37 to 5.32%
AN8323	-5.25 to -0.33%	4.40 to 5.94%	-9.00 to 5.33%	1.68 to 6.71%

Method validation results (Report # MC13B-0154) for assay used in ADME trial (AN2728-AD-105)

Analyte	Inter-Day (N = 24)		Intra-Day (N = 6)	
	Accuracy	*Precision*	*Accuracy*	*Precision*
Crisaborole	-6.67 to -4.38%	4.85 to 5.95%	-8.33 to 2.00%	2.75 to 7.85%
AN7602	-8.83 to -7.13%	4.18 to 7.98%	-10.5 to -5.83%	2.27 to 10.0%

Reviewer comments: *The accuracy and precision for all the quality control samples were within the acceptable limit of ±15%.*

2.6.6 What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler, etc.)?

Stability results for assay used in Maximal use PK trial (AN2728-AD-102), TQT trial (AN2728-TQT-108), Drug interaction trial for CYP2C9 (AN2728-PK-101).

Parameter	*Crisaborole (AN2728) (Parent)*	*AN7602 (Metabolite)*	*AN8323 (Downstream metabolite of AN7602)*
Freeze/Thaw cycle stability	(b) (4)		
Thawed on ice			
Long term stability			

Stability results for assay use in Adolescent subject PK trial (AN2728-AD-203)

Parameter	*Crisaborole (AN2728) (Parent)*	*AN7602 (Metabolite)*	*AN8323 (Downstream metabolite of AN7602)*
Freeze/Thaw cycle stability	(b) (4)		
Thawed on ice			
Long term stability			

Stability results for assay used in ADME trial (AN2728-AD-105)

Parameter	*Crisaborole (AN2728) (Parent)*	*AN7602 (Metabolite)*
Freeze/Thaw cycle stability	(b) (4)	
Thawed on ice		
Long term stability		

Reviewer comments: *The duration of long term PK sample stability was adequate to cover the duration of PK sample storage for all trials.*

2.6.7 What are the results of incurred sample reanalysis (ISR)?

Incurred sample reanalysis (ISR) was evaluated by re-assaying samples and comparing them to their original concentration values. ISR was performed for around 10% of the study samples. The acceptance criteria was individual bias should be within ±20% of the mean values for at least 2/3 (~ 67%) of the repeats. Following are the results of individual studies:

Maximal use PK trial (AN2728-AD-102): ISR was evaluated by re-assaying 27 samples for AN2728 and AN7602 and 26 samples for AN8323 (~ 10 % of the study samples) and comparing them to their original concentration values. All of the samples selected for AN2728 and AN8323 and 23 of the samples selected for AN7602 had a percent difference within ± 20.0% between the original and ISR value. The data indicated that sample reproducibility was within 100%, 85.2% and 100% for AN2728, AN7602 and AN8323 in human plasma, respectively.

Drug interaction trial for CYP2C9 (AN2728-PK-101): ISR was evaluated by re-assaying 28 samples. The results indicated that 28, 27 and 28 samples selected for AN2728, AN7602, and AN8323, respectively had a percent difference within 20.0% between the original and the ISR value. The data indicated that sample reproducibility was within 100%, 96.4% and 100% for AN2728, AN7602 and AN8323 in human plasma, respectively.

TQT trial (AN2728-TQT-108): ISR was evaluated by re-assaying 321 samples. The results showed that 317 samples for AN2728, 283 samples for AN7602 and 319 samples

for AN8323 had a percent difference within 20.0% between the original and the ISR value. The data indicated that sample reproducibility was within 98.8%, 87.9% and 99.4% for AN2728, AN7602 and AN8323 in human plasma.

Adolescent subject PK trial (AN2728-AD-203): ISR was evaluated by re-assaying 46 samples and comparing them to their original concentration values. The results showed that 37 samples for AN2728, 27 of the samples for AN7602 and 35 samples for AN8323 had a percent difference within 20.0% between the original and the ISR value. The data indicated that sample reproducibility was within 80.4%, 58.7% and 76.1% for AN2728, AN7602 and AN8323, respectively.

Reviewer comments: *The adolescent PK trial was not conducted under maximal use conditions and is considered as supportive. Hence the fact that ISR failed for AN7602 does not have an impact on the regulatory decision for this application.*

3. Detailed Labeling Recommendations

The following changes are recommended in the Sponsor's proposed labeling. The **bold and underlined** text indicates insertion recommended by the reviewer and the ~~strikethrough~~ text indicates recommended deletion.

(b) (4)

8.4 Pediatric Use

Safety and effectiveness of EUCRISA have not been established in pediatric patients under 2 years of age.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Crisaborole is a (b) (4) **phosphodiesterase 4 (**PDE-4**)** inhibitor (b) (4) PDE-4 inhibition results in increased intracellular cyclic adenosine monophosphate (cAMP) levels. (b) (4) The specific mechanism(s) by which crisaborole exerts its therapeutic action **for the treatment of atopic dermatitis** is not **known.** (b) (4)

12.2 Pharmacodynamics

(b) (4) **At therapeutic doses, EUCRISA ointment is not expected to prolong QTc to any clinically relevant extent.**

12.3 Pharmacokinetics

(b) (4)

Absorption

The pharmacokinetics (PK) of EUCRISA were investigated in 33 pediatric subjects 2 to 17 years of age with mild to moderate atopic dermatitis and a mean ± SD body surface area involved of 49 ± 20% (range 27% to 92%). In this study, approximately 3 mg/cm^2 of EUCRISA ointment (dose range was approximately 6 g to 30 g per application) was applied twice daily for 8 days.

Plasma concentrations were quantifiable in all the subjects. The mean ± SD maximum plasma concentration (C_{max}) and area under the concentration time curve from 0 to 12 hours post dose (AUC_{0-12}) for crisaborole on Day 8 were 127 ± 196 ng/mL and 949 ± 1240 ng*h/mL, respectively. Systemic concentrations of crisaborole were at steady state by Day 8. Based on the ratios of AUC_{0-12} between Day 8 and Day 1, the mean accumulation factors for crisaborole was 1.9.

Distribution

Based on in vitro study crisaborole is 97% bound to human plasma proteins.

Elimination

Metabolism

Crisaborole is substantially metabolized into inactive metabolites. The major metabolite 5-(4-cyanophenoxy)-2-hydroxyl benzylalcohol (metabolite 1), is formed via hydrolysis; this metabolite is further metabolized into downstream metabolites among which 5-(4-cyanophenoxy)-2-hydroxyl benzoic acid (metabolite 2), formed via oxidation, is also a major metabolite.

PK of metabolites 1 and 2 were assessed in the PK study described above and the systemic concentrations were at or near steady state by Day 8. Based on the ratios of AUC_{0-12} between Day 8 and Day 1, the mean accumulation factors for metabolites 1 and 2 were 1.7 and 6.3, respectively.

Excretion

Renal excretion is the major route of elimination.

Drug Interaction Studies

In vitro studies using human liver microsomes indicated that under the conditions of clinical use crisaborole and metabolite 1 are not expected to inhibit cytochrome P450 (CYP) 1A2, 2B6, 2C8, 2C9, 2 C19, 2D6 and 3A4.

In vitro human liver microsomes studies for metabolite 2 showed that it did not inhibit activities of CYP2C19, 2D6 and 3A4; was a weak inhibitor of CYP1A2 and 2B6; and a moderate inhibitor of CYP2C8 and 2C9. The most sensitive enzyme, CYP2C9, was further investigated in a clinical trial using warfarin as a CYP2C9 substrate. The results of this study showed no drug interaction potential.

In vitro studies in human hepatocytes showed that under the conditions of clinical use crisaborole and metabolites 1 and 2 are not expected to induce CYP enzymes.

4. INDIVIDUAL STUDY REVIEW

Appendix 1: Trial AN2728-AD-102 – Maximal use PK trial

Title: An open-label, maximal use, systemic exposure study to assess the safety and PK profile of AN2728 Topical Ointment, 2% in children and adolescents with atopic dermatitis.

Primary Objective: The objective of the study was to evaluate the systemic exposure, PK, and safety of AN2728 Topical Ointment, 2% when applied under maximal use conditions in pediatric and adolescent subjects with atopic dermatitis (AD).

Study design: This was a multicenter, open-label, maximal use, systemic exposure study with two phases:

- A PK Phase (from Day 1 through the AM dose on Day 9)
- A non-PK Safety Phase (from the PM dose on Day 9 through Day 28)

Eligible subjects were enrolled concurrently in three parallel cohorts. Cohorts were characterized on the basis of age and a specified minimum Treatable Percent Body Surface Area (Treatable %BSA) as follows:

- Cohort 1: Subjects 12–17 years of age, inclusive, with ≥25% Treatable BSA
- Cohort 2: Subjects 6–11 years of age, inclusive, with ≥35% Treatable BSA
- Cohort 3: Subjects 2–5 years of age, inclusive, with ≥35% Treatable BSA

Study treatment was comprised of approximately 3 mg/cm^2 (The dose of the formulation was approximately 6 g to 30 g per application) of AN2728 Topical Ointment, 2%, applied twice daily (BID) to all treatable AD-involved areas identified at Baseline/Day 1 (the Baseline and Day 1 visit were the same) for 28 days except on Days 1 and 8, when study drug was administered each day (QD) in the AM.

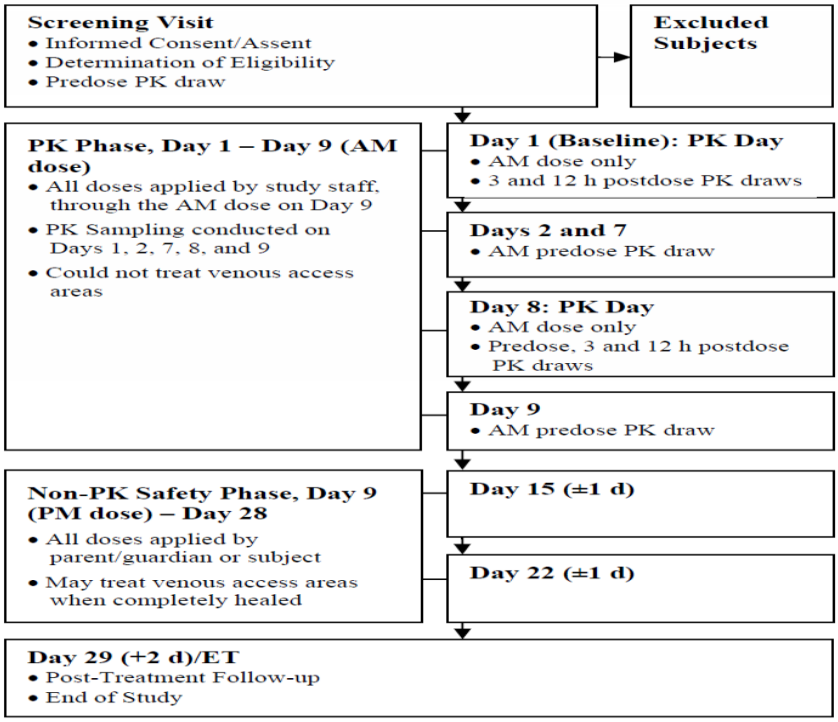
During the PK Phase (Day 1 through the AM dose on Day 9), study staff dispensed and applied all doses of study drug. During the Safety Phase (PM of Day 9 through Day 28), subjects self-administered study drug, or a parent or guardian applied study drug for the subject. Schematic representation of the study design is shown in Figure 9.

PK sampling: During the PK phase of the study, each subject had total 9 blood samples drawn on the following visit days:

- Screening (one draw for predose timepoint)
- Baseline/Day 1 (two draws at 3 h ±20 min and 12 ±1 h after AM dose)
- Day 2 (one draw before AM dose)
- Day 7 (one draw before AM dose)
- Day 8 (three draws, one before AM dose, and at 3 h ±20 min and 12 ±1 h after AM dose)
- Day 9 (one draw before AM dose)

Reviewer comments: *The limited PK sampling scheme was based on adolescent PK trial (TrialAN2728-AD-203) described in Appendix 3. The 3 hour time point would represent T_{max} of crisaborole.*

Figure 9: Schematic representation of study design



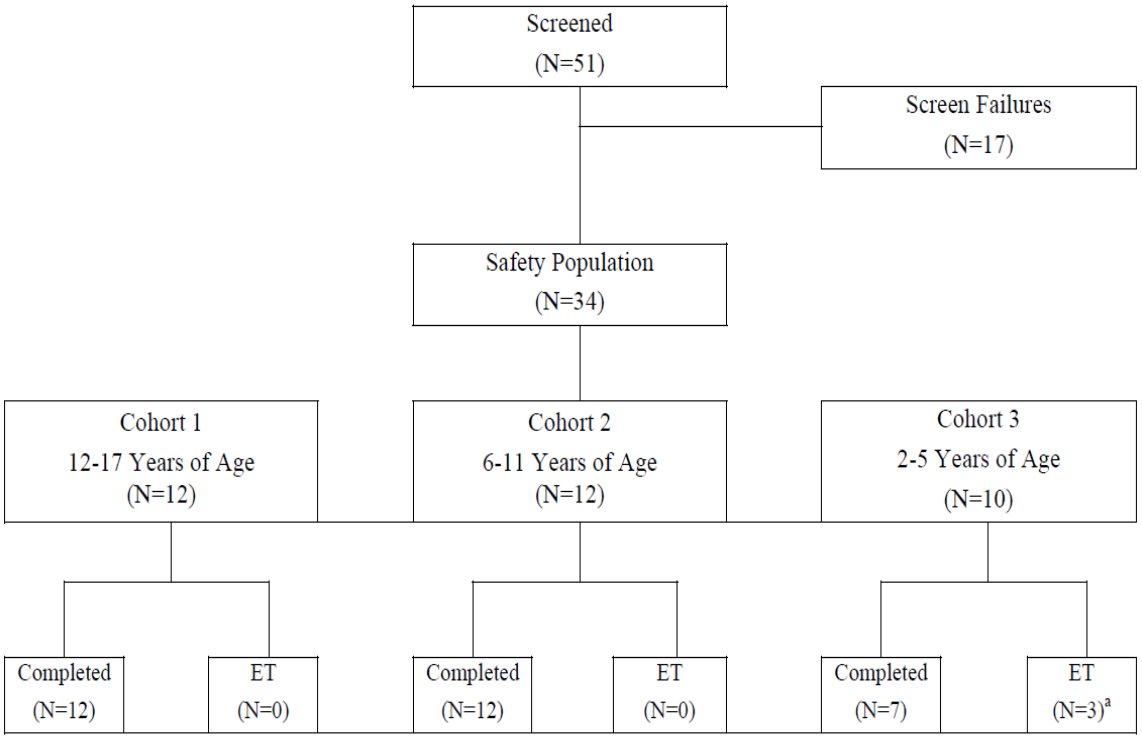
AM, morning; PK, pharmacokinetics; PM, afternoon/evening; ET, early termination from study

Study population: 34 subjects male and female subjects 2 to 17 years old with mild to moderate AD were included based on Investigator's Static Global Assessment (ISGA) score of 2 or 3 (see Table 10) at Baseline/Day 1 were enrolled in this trial and 31 subjects completed. 22 of the 34 enrolled subjects (64.7%) had a baseline ISGA score of 3 (moderate). Of these, 20 (90.9%) completed the study. The two subjects who discontinued early were Subject 11212 who discontinued due to non-compliance of the protocol and Subject 11215 who discontinued due to an adverse event (application site pain). Summary of subject disposition is shown in Figure 10.

Table 10: Investigator's Static Global Assessment (ISGA) scale

Score	Grade	Definition
0	Clear	Minor residual discoloration; no erythema or induration/papulation; no oozing/crusting
1	Almost Clear	Trace faint pink erythema, with barely perceptible induration/papulation and no oozing/crusting
2	Mild	Faint pink erythema with mild induration/papulation and no oozing/crusting
3	Moderate	Pink-red erythema with moderate induration/papulation with or without oozing/crusting
4	Severe	Deep or bright red erythema with severe induration/papulation and with oozing/crusting

Figure 10: Summary of subject disposition



BSA, body surface area; ET, early termination from study

Note: Cohort 1, Ages 12–17 years, inclusive, with ≥25% Treatable BSA; Cohort 2, Ages 6–11 years, inclusive, with ≥35% Treatable BSA; Cohort 3, Ages 2–5 years, inclusive, with ≥35% Treatable BSA.

[a] Primary reasons for early discontinuation in Cohort 3 were: subject withdrew consent and/or did not wish to continue for reasons unrelated to study treatment (Subject 11216), noncompliance with protocol (Subject 11212), and adverse event (Subject 11215, the narrative summary for this subject, a 2.3-year-old White female, is provided in Section 12.3.2).

Reasons for subject discontinuation: 3 subjects discontinued from Cohort 3 (2 - 5 year old) and the reasons for discontinuation are shown below and summarized in Table 11.

- Subject 11216 (mild disease at baseline): Withdrawal of consent/ not wishing to continue for reasons unrelated to study treatment. The last day of dosing was Day 27 (Study duration was for 28 days).
- Subject 11212 (moderate disease at baseline): Noncompliance with the protocol. Last day of dosing was Day 28.
- Subject 11215 (moderate disease at baseline): Adverse event of application site pain. Last day of dosing was Day 12 (PK parameters for this subject were not estimated on Day 8). The subject experienced intermittent application site burning on Days 6, 7, 10, 11, and 12 (nonserious, mild to severe, possibly to definitely related) and worsening of atopic dermatitis on Day 5 (nonserious, moderate, possibly related). She was treated for the worsening dermatitis with sodium hypochlorite (bleach bath), oral cephalexin, topical hydrocortisone and Neosporin. The event resolved following the final application of study drug on Day 12. On Day 13, she was withdrawn from the study at her father's request.

Table 11: Summary of subject discontinuation

	AN2728 Ointment, 2%			
	Cohort 1 12-17 Years (N=12)	**Cohort 2 6-11 Years (N=12)**	**Cohort 3 2-5 Years (N=10)**	**Total (N=34)**
Subjects Enrolled	12	12	10	34
Safety Population [a]	12	12	10	34
Per-Protocol Population [b]	12	12	9	33
Subject Completed Study [c]				
Yes	12 (100%)	12 (100%)	7 (70.0%)	31 (91.2%)
No	0	0	3 (30.0%)	3 (8.8%)
Primary Reason for Early Discontinuation [d]				
Subject Withdrew Consent and/or Did Not Wish to Continue for Reasons Unrelated to Study Treatment	0	0	1 (33.3%)[e]	1 (33.3%)
Subject Withdrew Consent and/or Did Not Wish to Continue for Reasons Related to Study Treatment (Non-AE)	0	0	0	0
Adverse Event	0	0	1 (33.3%)[f]	1 (33.3%)
Pregnancy	0	0	0	0
Lost to Follow-Up	0	0	0	0
Non Compliance with Protocol	0	0	1 (33.3%)[g]	1 (33.3%)
Administrative Reasons (eg, Study discontinued, Study on hold)	0	0	0	0

AE, adverse event; BSA, body surface area

Note: Cohort 1, Ages 12–17 years, inclusive, with ≥25% Treatable BSA; Cohort 2, Ages 6–11 years, inclusive, with ≥35% Treatable BSA; Cohort 3, Ages 2–5 years, inclusive, with ≥35% Treatable BSA.

[a] Safety Population: all subjects who were enrolled, received any amount of study drug, and had at least one post-baseline assessment.

[b] Per-Protocol Population: all subjects from the Safety Population who did not miss more than 1 study visit, did not miss more than 3 consecutive days of treatment, and received 80–120% of the expected doses.

[c] Percentage was calculated based on Safety Population (N=34).

[d] Percentage was calculated based on subjects who did not complete the study.

[e] Subject 11216

[f] Subject 11215 discontinued due to the AE of application site pain. A narrative summary for this subject is provided in Section 12.3.2.

[g] Subject 11212

Reviewer comments: *PK data on Day 8 were available from 33 out of 34 subjects.*

Disease severity at baseline: Summary of disease severity at baseline is shown in Table 12.

Table 12: Summary of disease severity at baseline

	AN2728 Ointment, 2%			
	Cohort 1 12-17 Years (N=12)	**Cohort 2 6-11 Years (N=12)**	**Cohort 3 2-5 Years (N=10)**	**Total (N=34)**
Severity Based on ISGA[a]				
Mean (SD)	2.67 (0.492)	2.58 (0.515)	2.70 (0.483)	2.65 (0.485)
Median	3.00	3.00	3.00	3.00
Min, Max	2.0, 3.0	2.0, 3.0	2.0, 3.0	2.0, 3.0
Signs and Symptoms of AD[b]				
Severity of Erythema				
Mean (SD)	2.00 (0.564)	2.04 (0.916)	2.20 (0.632)	2.07 (0.709)
Median	2.00	2.00	2.00	2.00
Min, Max	1.0, 3.0	0.0, 3.0	1.0, 3.0	0.0, 3.0
Severity of Excoriation				
Mean (SD)	1.08 (0.793)	2.08 (0.793)	2.10 (0.775)	1.74 (0.907)
Median	1.00	2.00	2.00	2.00
Min, Max	0.0, 2.0	1.0, 3.0	1.0, 3.0	0.0, 3.0
Severity of Exudation				
Mean (SD)	0.58 (0.875)	1.42 (1.165)	1.65 (0.944)	1.19 (1.080)
Median	0.50	1.00	1.75	1.00
Min, Max	0.0, 3.0	0.0, 3.0	0.0, 3.0	0.0, 3.0
Severity of Lichenification				
Mean (SD)	1.96 (0.838)	2.21 (0.753)	1.90 (0.810)	2.03 (0.788)
Median	2.00	2.25	1.75	2.00
Min, Max	0.5, 3.0	1.0, 3.0	1.0, 3.0	0.5, 3.0
Severity of Pruritus				
Mean (SD)	1.63 (1.025)	2.63 (0.644)	2.60 (0.459)	2.26 (0.881)
Median	2.00	3.00	2.75	2.50
Min, Max	0.0, 3.0	1.0, 3.0	2.0, 3.0	0.0, 3.0

	AN2728 Ointment, 2%			
	Cohort 1 12-17 Years (N=12)	**Cohort 2 6-11 Years (N=12)**	**Cohort 3 2-5 Years (N=10)**	**Total (N=34)**
Treatable %BSA[c]				
Mean (SD)	35.8 (11.29)	54.9 (19.30)	56.9 (23.03)	48.7 (20.17)
Median	31.0	48.0	47.0	41.5
Min, Max	27, 61	35, 92	35, 91	27, 92

AD, atopic dermatitis; BSA, body surface area; ISGA, Investigator's Global Static Assessment; min, minimum; max, maximum; SD, standard deviation.

Note: Cohort 1, Ages 12–17 years, inclusive, with ≥25% Treatable BSA; Cohort 2, Ages 6–11 years, inclusive, with ≥35% Treatable BSA; Cohort 3, Ages 2–5 years, inclusive, with ≥35% Treatable BSA.

[a] ISGA Score: Disease severity was assessed on a five-point scale: 0 (clear), 1 (almost clear), 2 (mild), 3 (moderate), and 4 (severe).

[b] Signs and symptoms of AD were erythema, excoriation, exudation, lichenification, and pruritus. The severity of each was assessed on a 4-point scale: 0 (none), 1.0 (mild), 2.0 (moderate), and 3.0 (severe).

[c] Treatable %BSA was the percent of the subject's total BSA that was AD-involved, excluding the scalp and venous access areas.

Demographic data: The summary of demographic data is shown in Table 13.

Table 13: Summary of demographic data

	AN2728 Ointment, 2%			
	Cohort 1 12-17 Yrs (N=12)	**Cohort 2 6-11 Yrs (N=12)**	**Cohort 3 2-5 Yrs (N=10)**	**Total (N=34)**
Gender				
Male	7 (58.3%)	4 (33.3%)	4 (40.0%)	15 (44.1%)
Female	5 (41.7%)	8 (66.7%)	6 (60.0%)	19 (55.9%)
Age (Years)				
N	12	12	10	34
Mean (SD)	14.3 (1.85)	8.9 (1.41)	3.7 (1.10)	9.3 (4.54)
Median	13.6	9.4	3.7	9.6
Min, Max	12.1, 17.7	6.3, 10.8	2.1, 5.5	2.1, 17.7
Ethnicity				
Hispanic or Latino	4 (33.3%)	1 (8.3%)	1 (10.0%)	6 (17.6%)
Not Hispanic or Latino	8 (66.7%)	11 (91.7%)	9 (90.0%)	28 (82.4%)
Race				
White	6 (50.0%)	5 (41.7%)	6 (60.0%)	17 (50.0%)
Black or African American	5 (41.7%)	6 (50.0%)	4 (40.0%)	15 (44.1%)
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	1 (8.3%)	1 (8.3%)	0	2 (5.9%)
American Indian or Alaska Native	0	0	0	0
Other	0	0	0	0
Height (cm)				
N	12	12	10	34
Mean (SD)	164.8 (9.67)	131.7 (10.34)	97.5 (8.80)	133.3 (28.96)
Median	162.0	130.2	97.1	132.1
Min, Max	154.9, 182.9	116.8, 149.9	82.6, 111.8	82.6, 182.9
Weight (kg)				
N	12	12	10	34
Mean (SD)	65.0 (18.36)	33.3 (11.69)	16.5 (3.78)	39.6 (23.94)
Median	59.5	31.1	15.8	32.5
Min, Max	48.1, 109.4	21.3, 66.6	11.8, 24.5	11.8, 109.4

BSA, body surface area; max, maximum; min, minimum; SD, standard deviation.

Note: Cohort 1, Ages 12–17 years, inclusive, with ≥25% Treatable BSA; Cohort 2, Ages 6–11 years, inclusive, with ≥35% Treatable BSA; Cohort 3, Ages 2–5 years, inclusive, with ≥35% Treatable BSA.

Identity of investigational product: Details about the investigational product used in this trial is shown in Table 14.

Table 14: Identity of the investigational product used in this trial

Parameter	Investigational Product
Formulations	AN2728 Topical Ointment, 2%
Active Ingredient	AN2728
Chemical Name	5-(4-cyanophenoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole
Chemical Class	Oxaborole
Molecular Formula	$C_{14}H_{10}BNO_3$
CAS[a] Registry Number	906673-24-3

[a] Chemical Abstracts Service

Amount of drug applied: There was no self administration in the PK part of this trial. The dose was 3 mg/cm^2 and doses were individualized based on the body surface area involved. The range of amount of formulation applied by the clinical staff in the PK part of this study was approximately 6 g to 30 g, per application.

Change in treatable body surface area: Summary of % change in treatable body surface area is shown in Table 15.

Table 15: Treatable % BSA at baseline and Day 29/Early termination

	AN2728 Ointment, 2%			
	Cohort 1 12-17 Years (N=12)	**Cohort 2 6-11 Years (N=12)**	**Cohort 3 2-5 Years (N=10)**	**Total (N=34)**
Treatable Body Surface Area (%)[a]				
Baseline				
Mean (SD)	35.8 (11.29)	54.9 (19.30)	56.9 (23.03)	48.7 (20.17)
Median	31.0	48.0	47.0	41.5
Min, Max	27, 61	35, 92	35, 91	27, 92
Day 29/ET				
Mean (SD)	5.7 (8.60)	16.3 (14.54)	18.6 (18.06)	13.2 (14.74)
Median	1.0	15.5	13.5	8.0
Min, Max	0, 29	0, 40	0, 46	0, 46
Change from Baseline				
Mean (SD)	-30.1 (8.64)	-38.6 (14.81)	-38.3 (9.64)	-35.5 (11.83)
Median	-28.5	-39.5	-37.5	-34.5
Min, Max	-53, -15	-68, -10	-55, -23	-68, -10
Percentage Change from Baseline (%)				
Mean (SD)	-86.6 (18.62)	-72.8 (24.39)	-73.0 (21.71)	-77.7 (22.06)
Median	-97.3	-69.6	-73.4	-86.2
Min, Max	-100, -47	-100, -20	-100, -49	-100, -20

AD, atopic dermatitis; ET, early termination from study; max, maximum; min, minimum; N, number of subjects; SD, standard deviation; treatable %BSA, treatable percentage of body surface area.

Note: Baseline is the last non-missing observation prior to study drug application. Change from Baseline is calculated as follow-up evaluation minus baseline evaluation. Subjects with Baseline value=0 are not included in the calculation of percentage change from baseline.

[a] Treatable % BSA was defined as the percent of the subject's total BSA that was AD-involved and not on the scalp or in the venous access areas (see Section 9.4.4 for methods of estimating Treatable %BSA).

Reviewer comments: *Based on the table above, at the end of treatment on Day 29, would not constitute maximal use conditions based on the decrease in the % BSA involved. However, PK assessment was done on Day 8 when systemic concentrations of crisaborole and its metabolites (AN7602 and AN8323) were at steady state.*

PK results: PK parameters were determined by non-compartmental approach based on concentration versus time data of each subject. The mean PK parameters and the PK profile of crisaborole (AN2728), metabolite AN7602 and downstream metabolite AN8323 are described under Section 2.2.3. PK profiles based on cohorts for AN2728, AN7602 and AN8323 are shown in Figure 11, 12 and 13, respectively. Summary of PK parameters by the different age cohorts is shown in Section 2.2.2

Figure 11: Mean (±SD) Plasma Concentration-Time Profiles of AN2728 (parent)

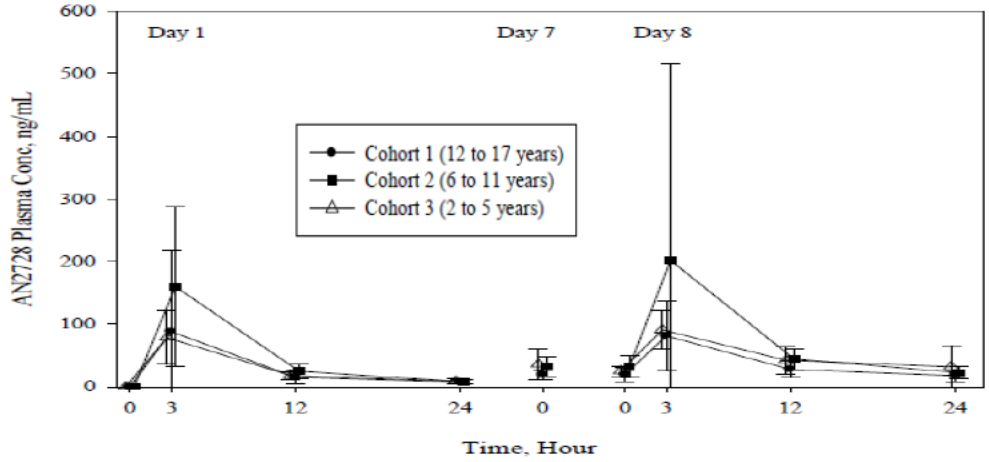


Figure 12: Mean (±SD) Plasma Concentration-Time Profiles of AN7602 (metabolite)

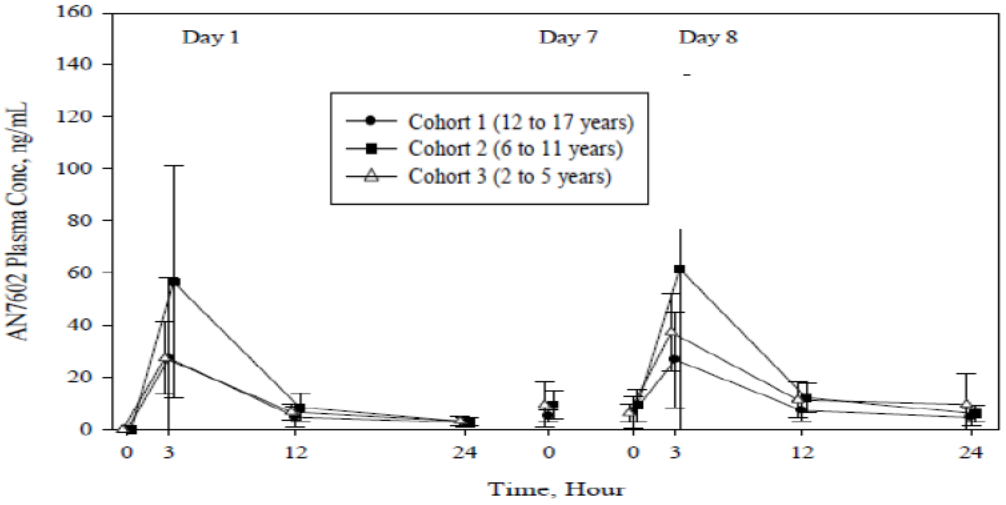
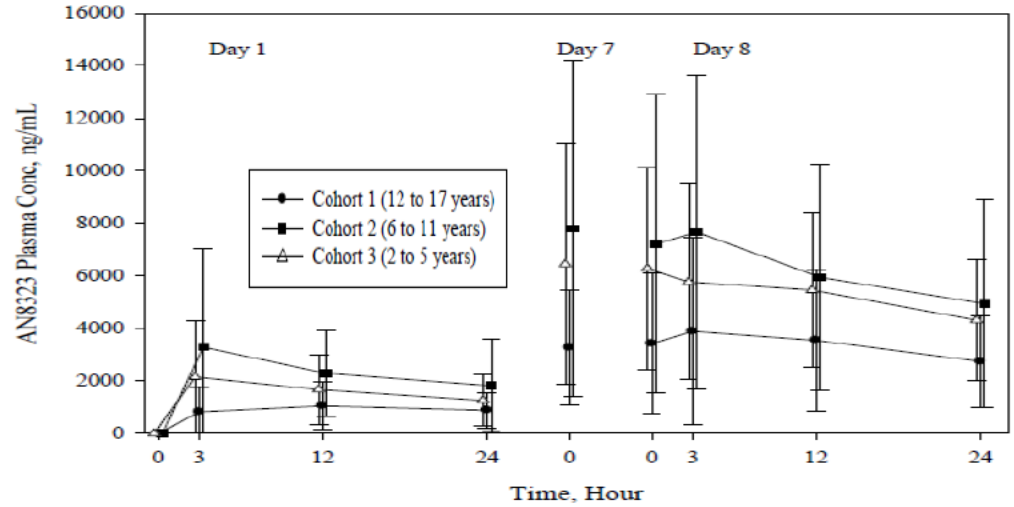


Figure 13: Mean (±SD) Plasma Concentration-Time Profiles of AN8323 (downstream metabolite)



Steady state assessment: By Day 8, systemic concentrations of crisaborole (AN2728), metabolite AN7602 and the downstream metabolite AN8323 were at steady state based on trough plasma concentrations (Figure 14, 15 and 16).

Figure 14: Trough plasma concentrations of crisaborole (AN2728)

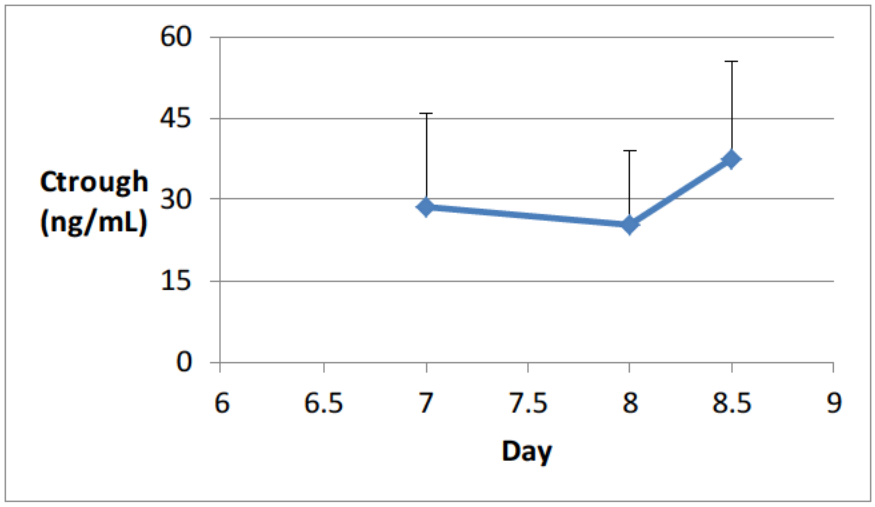


Figure 15: Trough plasma concentrations of metabolite (AN7602)

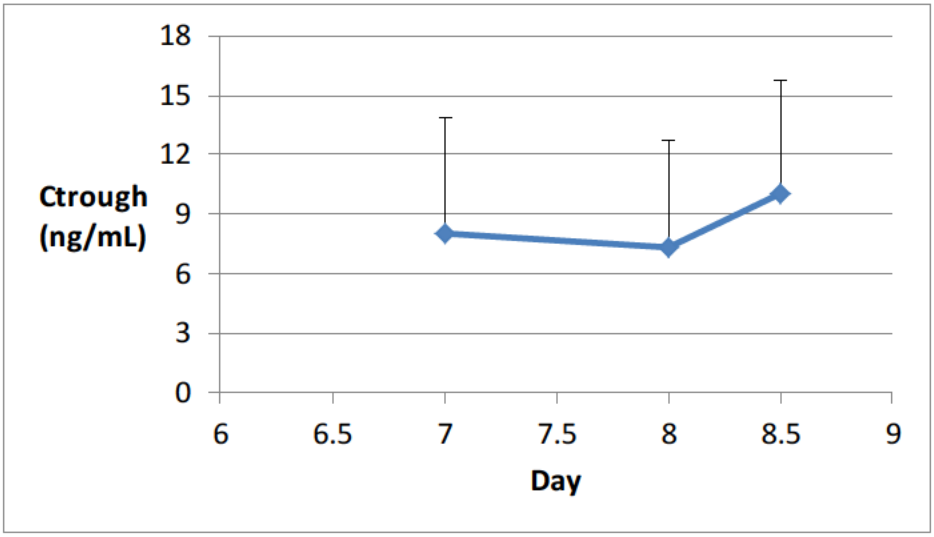
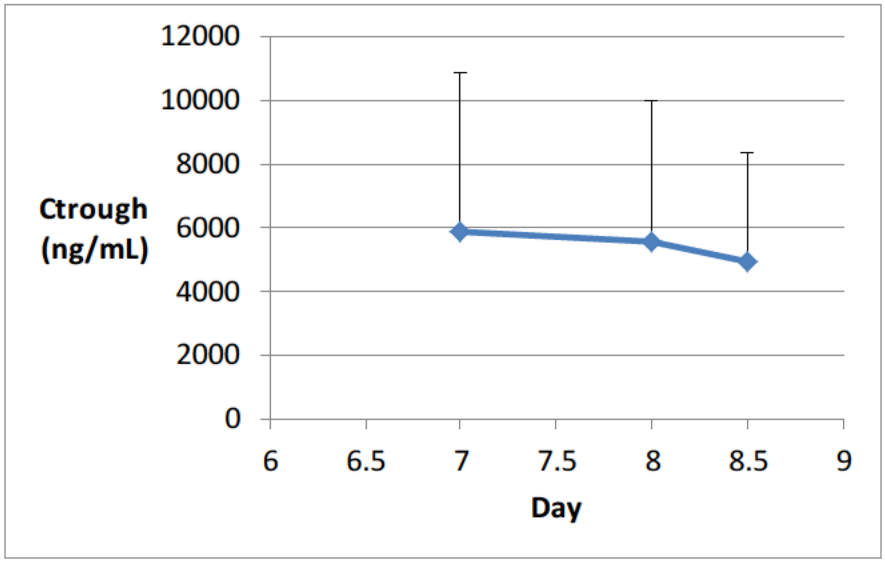


Figure 16: Trough plasma concentrations of downstream metabolite (AN8232)



Reviewer comments: *Additional support for steady state attainment can be obtained from the t1/2 information obtained in the adolescent PK study described in Appendix 3 (see Table 27). The t1/2 values of crisaborole (AN2728), metabolite AN7602 and downstream metabolite AN8323 on Day 8 is reported to be 11.9 h, 10.5 h and 33.5 h. This would suggest that systemic concentrations for all 3 moieties were at steady state by Day 8.*

Accumulation ratio: Summary of mean accumulation ratio between Day 1 and Day 8 is shown in Table 16.

Table 16: Summary of mean ratios of Day8/Day 1 PK parameters

	Day 8/Day 1 Ratio[a]											
	C_{max}			$AUC_{(0-12)}$			$AUC_{(0-T)}$			$AUC_{(0-24)}$		
Analyte	N	Mean (SD)	Median (Range)	N	Mean (SD)	Median (Range)	N	Mean (SD)	Median (Range)	N	Mean (SD)	Median (Range)
AN2728	33	1.74 (1.52)	1.13 (0.283-7.11)	31	1.87 (1.45)	1.23 (0.425-5.88)	33	2.33 (2.15)	1.43 (0.559-10.9)	28	1.93 (1.32)	1.41 (0.559-5.80)
AN7602	33	1.55 (1.27)	1.08 (0.135-5.52)	33	1.71 (1.31)	1.16 (0.370-4.96)	31	1.79 (1.21)	1.24 (0.667-5.33)	28	1.74 (1.09)	1.24 (0.667-4.55)
AN8323	33	3.99 (2.12)	3.51 (1.09-9.23)	31	6.28 (4.91)	4.20 (1.81-21.0)	33	6.07 (6.06)	4.61 (1.57-35.5)	28	4.79 (2.90)	4.07 (1.57-12.4)

$AUC_{(0-12)}$, area under the plasma concentration-time curve from time zero to 12 hours post dosing; $AUC_{(0-24)}$, area under the plasma concentration-time curve from time zero to 24 hours post dosing; $AUC_{(0-T)}$, area under the plasma concentration-time curve from time zero to the last measurable concentration; C_{max}, observed maximum plasma concentration after dosing; PK, pharmacokinetic; SD, standard deviation; T_{max}, time to reach C_{max}.

Note: Cohort 1, Ages 12–17 years, inclusive, with ≥25% Treatable BSA; Cohort 2, Ages 6–11 years, inclusive, with ≥35% Treatable BSA; Cohort 3, Ages 2–5 years, inclusive, with ≥35% Treatable BSA.

[a] Descriptive analysis of the within-subject ratio of Day 8/Day 1 of PK parameters for all subjects.

Summary of safety: AN2728 Topical Ointment, 2%, was generally well tolerated and the summary of safety is as below:

- No subject died, and no subject had a serious TEAE.
- One subject (Subject 11215) discontinued the study due to the TEAE of application site pain.
- Twenty-three of the 34 subjects (67.6%) had ≥1 TEAE, for a total of 63 events and 11subjects (32.4%) experienced no TEAEs. Of the 23 subjects with ≥1 TEAE, 20 reported events of either mild (9 subjects) or moderate (11 subjects) severity. Events that occurred in ≥2 subjects were application site pain (12 subjects), dermatitis atopic (7 subjects), upper respiratory infection (3 subjects), and application site paraesthesia (2 subjects).
- Thirteen subjects had ≥1 related TEAE, for a total of 36 events. Of the 13 subjects with ≥1 related TEAE, 11 reported events of either mild (4 subjects) or moderate (7 subjects) severity. Related TEAEs that occurred in ≥2 subjects were application site pain (12 subjects), dermatitis atopic (4 subjects), and application site paraesthesia (2 subjects). All other TEAEs were reported for one subject each.
- Three subjects (8.8%), one in each cohort, reported a severe TEAE (one unrelated and two related to study drug):
    - Subject 11204 (Cohort 1, 17.7-year-old Black/African American male) had severe, unrelated hand fracture ("right-hand broken thumb") on Day 19 that was ongoing at the end of the study.
    - Subject 11210 (Cohort 2, 6.3-year-old Black/African American female) had severe, definitely related application site pain ("burning sensation back of neck/back at application site") on Day 1 that resolved without treatment on the same day.
    - Subject 11215 (Cohort 3, 2.3-year-old White female) had severe, definitely related application site pain ("intermittent burning sensation at multiple application sites lasting 2–5 minutes") on Day 6 that resolved without treatment on Day 7. This subject discontinued from the study on Day 13.
- No consistent changes in laboratory values, vital signs, or physical examinations were reported after treatment with AN2728 Topical Ointment, 2%.

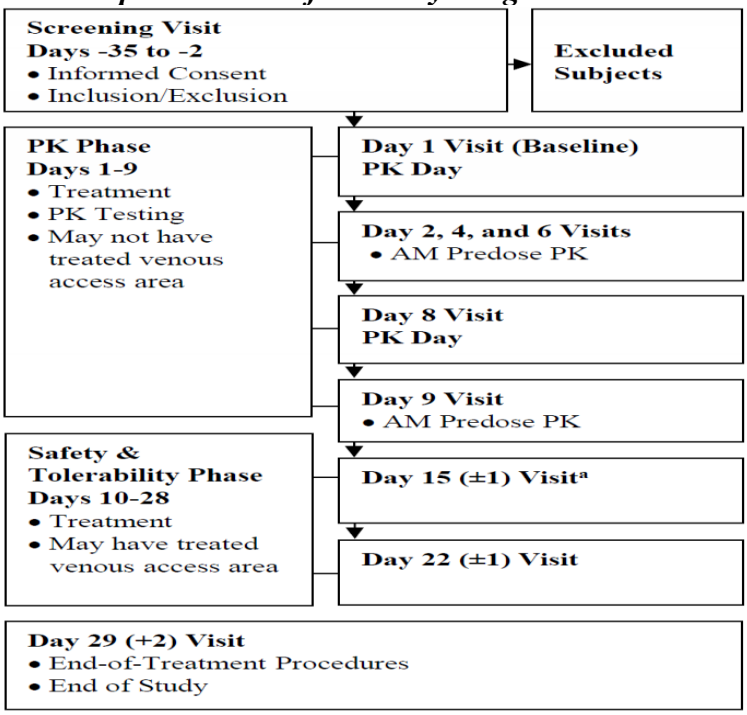
Appendix 2: TrialAN2728-AD-203 – PK study in adolescent subjects with AD

Title: An open-label study to determine the safety, tolerability, and PK profile of AN2728 topical ointment adolescents with atopic dermatitis.

Primary Objective: The objective of the study was to evaluate the safety, tolerability and systemic exposure of AN2728 Topical Ointment, 2% in subjects with AD.

Study design: This study was a multicenter open-label study with a PK Phase (Days 1–9) and a Safety & Tolerability Phase (Days 10–28) in adolescent subjects, 12–17 years of age, with AD involving 10%–35% treatable body surface area (BSA), excluding the scalp and venous access areas. 23 subjects were enrolled and the subjects were treated twice daily with AN2728 Topical Ointment, 2% for 28 days; except on Days 1 and 8 when only a single AM dose was applied. The AM dose on Days 1, 2, 4, 6, 8, and 9 was applied in the clinic. All other doses were applied at home. On Days 1 and 8, only single AM dose was applied, all other days, dosing was BID. Subjects were asked to apply the study drug to all Treatable Areas present at Baseline regardless of whether they became clinically clear, and also could have been applied to any new lesions not in an excluded area. During the Safety & Tolerability Phase (Days 10–28), the Per Application Dosage could have been increased if needed to cover all Treatable Areas. Disease severity, PK, and safety evaluations were scheduled at specific time points during the study (Figure 17). Overall disease severity was assessed using the Investigator's Static Global Assessment (ISGA) scale (shown in Table 10).

Figure 17: Schematic representation of the study design



PK, pharmacokinetic.

[a] For Site 102 (Investigator: Dr. Zoe Draelos), the Day 15 window was ±3 days per a site-specific protocol amendment (version 2, dated 24 July 2012).

PK sampling: A pre-dose blood sample was obtained at base line and full PK profile was obtained on Day 1 and Day 8 with post-dose PK sampling at 1, 2, 4, 6, 8, and 24 hr. Trough samples were collected prior to the AM study drug application on Days 4 and 6.

Treatments Administered: The treatment administered in this study was AN2728 Topical Ointment, 2%. It was to be applied to the Treatable %BSA at a dose of approximately 3 mg/cm^2.

Identity of Investigational Product: Summary of the investigational product is shown in Table 17.

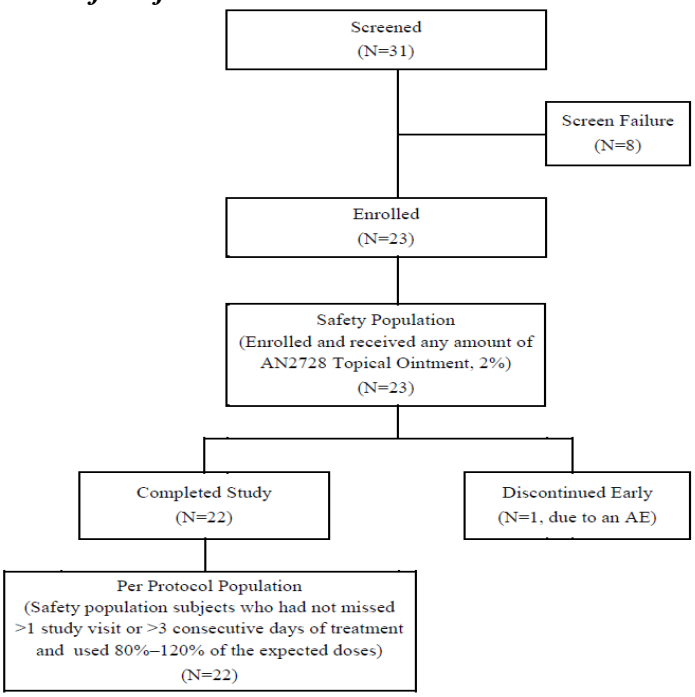
Table 17: Identity of the investigational product

Parameter	Investigational Product
Formulation	AN2728 Topical Ointment, 2%
Active Ingredient	AN2728
Chemical Name	5-(4-cyanophenoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole
Chemical Class	Oxaborole
Molecular Formula	$C_{14}H_{10}BNO_3$
CAS Registry Number	None

No of subjects: 23 Male and female subjects 12 to 17 years of age with mild to moderate AD (ISGA score of 2-3) and BSA involvement of ≥ 10% and ≤ 35% were enrolled.

Disposition of subjects: The disposition of subjects is shown in Figure 18.

Figure 18: Disposition of subjects



Demographic characteristics: The demographic characteristics are shown in Table 18.

Table 18: Demographic characteristics

Characteristic	Results (N=23)
Gender (n [%])	
Male	4 (17.4%)
Female	19 (82.6%)
Age (Years)	
Mean (standard deviation)	15.0 (1.55)
Median	15.1
Minimum, Maximum	12.1, 17.3
Ethnicity (n [%])	
Hispanic/Latino	7 (30.4%)
Not Hispanic/Latino	16 (69.6%)
Race (n [%])	
American Indian or Alaska Native	0
Asian	2 (8.7%)
Black	11 (47.8%)
Pacific Islander	1 (4.3%)
White	9 (39.1%)
Other	0
Height (cm)	
Mean (standard deviation)	162.4 (8.26)
Median	162.6
Minimum, Maximum	151.1, 176.5
Weight (kg)	
Mean (standard deviation)	72.7 (22.93)
Median	66.7
Minimum, Maximum	45.4, 128.9
Treatable Percent Body Surface Area (%BSA) at Baseline (Day 1)[a]	
Mean (standard deviation)	17.6 (5.74)
Median	17.0
Minimum, Maximum	10.0, 31.0

[a] Excludes the scalp and venous access areas.

Measurements of Treatment Compliance: Compliance was assured for the Day 1 and 8 AM doses because the study drug was weighed and applied at the study site by trained study staff. On Days 2, 4, 6, and 9, the AM doses were measured by the subject using Dosing Cards and applied under the supervision of trained study staff. The subject applied all other doses at home and recorded the doses in the dosing diary. Subjects were considered compliant with the treatment regimen if they received 80%–120% of the planned 54 applications (eg, 44–65 applications). 22 out of 23 subjects were treatment compliant. One subject (Subject 10804) discontinued early on Day 23 due to an AE (application site dermatitis).

Change in the treatable % BSA: The change in treatable % BSA is summarized in Table 19.

Table 19: Change is % treatable BSA

Parameter	%BSA by Study Day (N=23)	
	Baseline (Day 1)	Day 29 (End of Treatment)
Number of Subjects	23	23
Treatable Body Surface Area (%)		
Mean (standard deviation)	17.6 (5.74)	8.2 (8.95)
Median	17.0	6.0
Minimum, Maximum	10, 31	0, 40
Change from Baseline		
Mean (standard deviation)	—	-9.4 (8.15)
Median	—	-10.0
Minimum, Maximum	—	-21, 10

BSA, body surface area.

Reviewer comments: *Based on the mean and median % BSA at baseline, this trial is not considered to be conducted under maximal use conditions because % BSA involvement of at least 25% in adolescent subjects with AD is the current recommendation to be under maximal use conditions.*

Investigator's Static Global Assessment and Treatment Success: All subjects had a Baseline ISGA score of 2 (56.5% [13/23]) or 3 (43.5% [10/23]). The mean ISGA score was 2.43 at Baseline and 1.35 on Day 29, which is a mean change from Baseline of -1.08. Treatment success, defined as having an ISGA score of ≤1 (clear or almost clear) with a ≥2-grade improvement from Baseline, was achieved by 34.8% (8/23) of subjects on Day 29. The results are summarized in Table 20.

Table 20: Investigator's Static Global Assessment of Atopic Dermatitis and Treatment Success

	Results by Study Day and Overall Treatment Success (N=23)					
	Baseline	Day 8	Day 15	Day 22	Day 29	Overall[a]
Number of Subjects	23	23	23	23	23	—
Subjects with ISGA Severity Score (n [%])						
0	0	0	1 (4.3%)	4 (17.4%)	3 (13.0%)	—
1	0	9 (39.1%)	12 (52.2%)	12 (52.2%)	14 (60.9%)	—
2	13 (56.5%)	9 (39.1%)	7 (30.4%)	4 (17.4%)	2 (8.7%)	—
3	10 (43.5%)	5 (21.7%)	3 (13.0%)	2 (8.7%)	3 (13.0%)	—
4	0	0	0	1 (4.3%)	1 (4.3%)	—
Mean Score (SD)	2.43 (0.507)	1.83 (0.778)	1.52 (0.790)	1.30 (1.020)	1.35 (1.027)	—
Median	2.00	2.00	1.00	1.00	1.00	—
Minimum, Maximum	2.0, 3.0	1.0, 3.0	0.0, 3.0	0.0, 4.0	0.0, 4.0	—
Change from Baseline						
Mean (SD)	—	-0.61 (0.583)	-0.91 (0.668)	-1.13 (0.757)	-1.08 (0.848)	—
Median	—	-1.00	-1.00	-1.00	-1.00	—
Minimum, Maximum	—	-2.0, 0.0	-2.0, 0.0	-2.0, 1.0	-2.0, 1.0	—
Subjects with ISGA Scores ≤1 (Clear or Almost Clear) (n [%])						
Yes	0	9 (39.1%)	13 (56.5%)	16 (69.6%)	17 (73.9%)	—
No	23 (100.0%)	14 (60.9%)	10 (43.5%)	7 (30.4%)	6 (26.1%)	—
Subjects with ≥2-Grade Improvement from Baseline ISGA Score (n [%])						
Yes	—	1 (4.3%)	4 (17.4%)	7 (30.4%)	8 (34.8%)	—
No	—	22 (95.7%)	19 (82.6%)	16 (69.6%)	15 (65.2%)	—
Subjects with Treatment Success (n [%])[b]						
Yes	—	1 (4.3%)	4 (17.4%)	7 (30.4%)	8 (34.8%)	9 (39.1%)
No	—	22 (95.7%)	19 (82.6%)	16 (69.6%)	15 (65.2%)	14 (60.9%)

ISGA, Investigator's Static Global Assessment; SD, standard deviation.

[a] Each subject who fulfilled the criteria of Treatment Success during the study is included in Overall Treatment Success.

[b] Treatment Success: an ISGA score of 0 (Clear) or 1 (Almost Clear) with a 2-grade improvement from Baseline in ISGA.

PK results: Mean PK parameters are shown in Table 21 and the concentration versus time profiles for crisaborole and metabolite AN7602 are shown in Figure 19 and for the downstream metabolite AN8323 is shown in Figure 20. The accumulation ratios between Day 1 and Day 8 are shown in Table 22.

Figure 19: Mean Plasma profile for AN2728 (Parent) and AN7602 (metabolite)

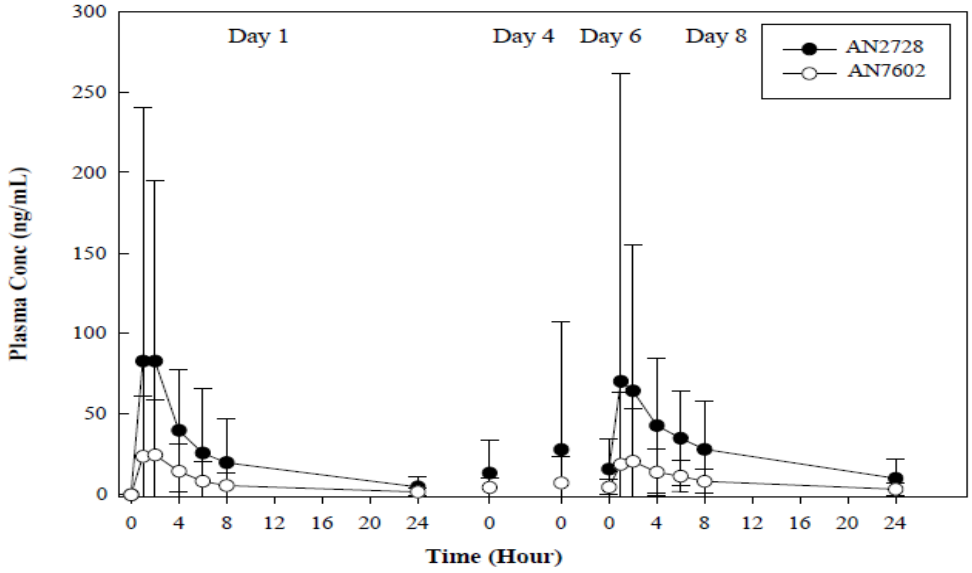


Figure 20: Mean Plasma profile of AN8323 (metabolite)

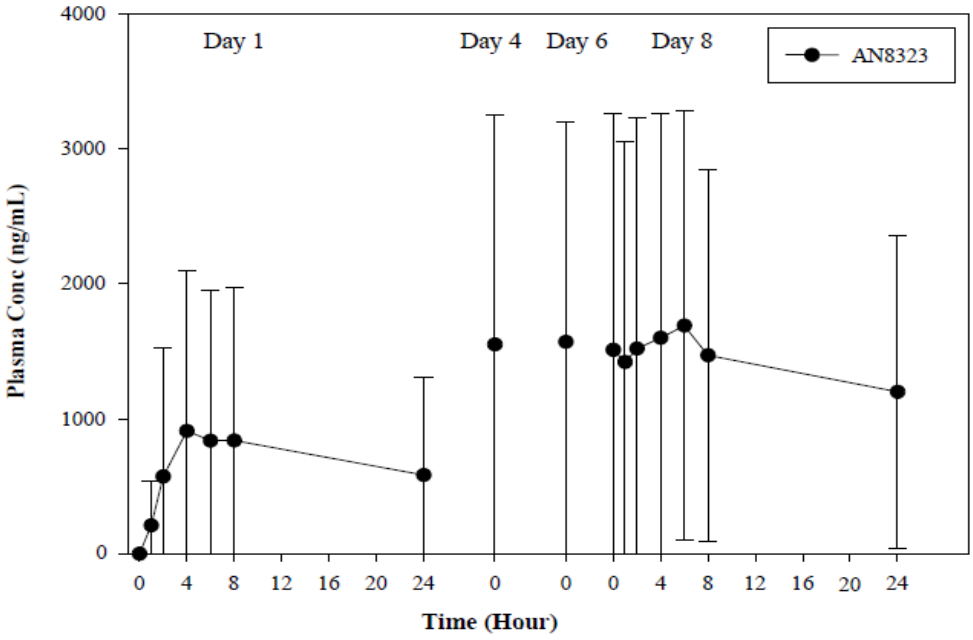


Table 21: Key Plasma Pharmacokinetic Parameters for AN2728 (parent) and its Two Identified Oxidative Metabolites, AN7602 (metabolite) and AN8323

Analyte[a]	Dose Day	Mean (SD) by Analyte (N=23)			
		C_{max} (ng/mL)	T_{max} (median [range]) (h)	$AUC_{(0-12)}$ (ng·h/mL)	$t_{1/2}$ (h)
AN2728	Day 1	105 (160)	2.37 (1.00–24.0)	448 (527)	7.17[b] (2.30)
	Day 8	94.6[c] (189)	2.17[c] (1.00–7.93)	462[c] (506)	11.9[d] (8.28)
AN7602	Day 1	28.2 (37.0)	2.08 (1.00–24.0)	142 (172)	8.19[e] (5.13)
	Day 8	26.3[c] (43.9)	3.94[c] (1.00–6.15)	142[c] (154)	10.5[b] (6.38)
AN8323	Day 1	998 (1220)	6.25 (3.95–25.0)	8900 (11,600)	17.7[f] (1.63)
	Day 8	1850[c] (1830)	6.00[c] (0.00–25.1)	18,200[c] (18,100)	33.5[f] (10.1)

$AUC_{(0-12)}$, area under the plasma concentration-time curve from time zero to 12 hours postdosing; C_{max}, observed maximum plasma concentration after dosing; SD, standard deviation; $t_{1/2}$, apparent half-life; T_{max}, time to reach C_{max}.

[a] AN2728 (3 mg/cm^2) was applied to a mean % body surface area of 17.6% (N=23) at Baseline (Day 1). Subjects received 92-334 mg of AN2728 on Day 1, and 96–334 mg on Day 8.

[b] N=16.

[c] N=22.

[d] N=17.

[e] N=18.

[f] N=6.

Table 22: Summary of Ratios of Day 8/Day 1 Cmax and AUC_{0-12} Values for AN2728, AN7602, and AN8323

Analyte[b]	Day 8/Day 1 Ratio (N=22)[a]			
	C_{max}		$AUC_{(0-12)}$	
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
AN2728	1.99 (2.09)	1.25 (0.0870-7.36)	2.28 (2.40)	1.12 (0.156-9.16)
AN7602	1.96 (1.80)	1.25 (0.0877-6.03)	2.40 (2.69)	1.43 (0.140-10.4)
AN8323	3.65 (4.04)	2.31 (0.139-19.3)	6.64 (11.4)	2.64 (0.176-53.6)

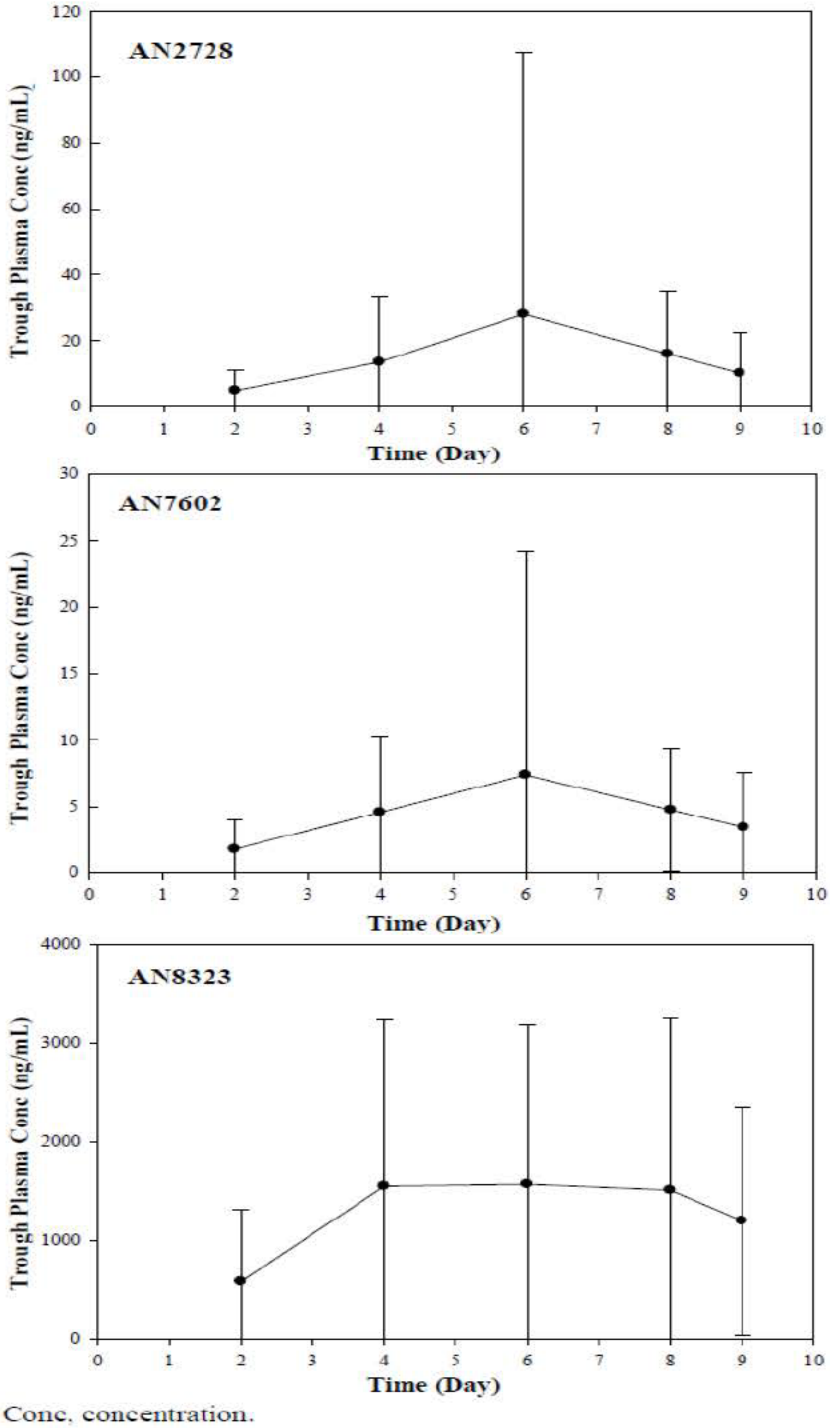
$AUC_{(0-12)}$, area under the plasma concentration-time curve from time zero to 12 hours postdosing; C_{max}, observed maximum plasma concentration after dosing; SD, standard deviation.

[a] Descriptive analysis of the within-subject ratio of Day 8/Day 1 of PK parameters for all subjects.

[b] AN2728 (3 mg/cm^2) was applied to a mean % body surface area of 17.6% (N=23) at Baseline (Day 1). Subjects received 92-334 mg of AN2728 on Day 1, and 96–334 mg on Day 8.

Steady state assessment: Plot of trough plasma concentrations by Day suggests that concentrations of crisaborole (AN2728), metabolite AN7602 and downstream metabolite AN8323 were at steady state by Day 8 (Figure 21).

Figure 21: Trough Plasma Concentrations of AN2728, AN7602, and AN8323 by Day



Conc, concentration.

<u>Reviewer comments:</u> *On Days 1 and 8, only single AM dose was applied; hence, the data points for Day 2 and 9 in Figure 21 represent concentrations at 24 hours post dose instead of 12 hours post dose.*

Summary of safety: No safety signals were identified upon review of AEs and laboratory test, vital sign, and physical examination results. The AE profile was as follows:

- A total of 19 TEAEs were reported by 10/23 subjects (43.5%); 13 subjects (56.5%) reported no TEAEs.
- No TEAE was considered serious, and no deaths occurred.
- All TEAEs were mild (57.9% [11/19]) or moderate (42.1% [8/19]). None was severe.
- The majority of TEAEs (68.4% [13/19]) were unrelated or unlikely to be related to study drug.
- One (5.3%) TEAE was possibly related (application site discomfort), 4 (21.1%) were probably related (application site pain), and 1 (5.3%) was definitely related to study drug (application site dermatitis).
- The most commonly reported TEAEs, application site pain and nasopharyngitis, were each reported by 3 subjects. No other TEAE was reported by more than 1 subject. One subject discontinued due to a TEAE of application site dermatitis ("contact allergic dermatitis to treatment areas").
- No consistent change in laboratory values, vital signs, and physical examination results was reported after dosing.

Appendix 3: Study AN2728-PSR-105 – ADME study

Title: A Phase 1 study to investigate the absorption, metabolism, and excretion of [^{14}C]-AN2728 following a single topical dose of [^{14}C]-AN2728 ointment E, 2% in healthy male subjects.

Objective: The primary objective of this study was to characterize the absorption, metabolism and excretion of [14C]-AN2728 following topical administration of AN2728 Ointment E, 2%.

Study design: This study was an open-label, non-randomized, absorption, metabolism, and excretion study of [^{14}C]-AN2728 (approximately 100 µCi) applied topically in 9.5 g of a 2% ointment to 6 healthy adult (18-55 years old) male subjects.

Subjects were confined at the clinical research unit (CRU) from the time of check-in (Day -1) until study completion for a minimum confinement of 4 days to a maximum confinement of 15 days. Subjects could be discharged from the CRU beginning on Day 4 (72 hours post-dose) provided the total radioactivity had reached the following threshold values:

- blood and plasma radioactivity reached levels below the limit of quantitation (BLQ) and ≥90% of the dose was recovered; or,
- urine total radioactivity reached ≤1% of the administered dose for 2 consecutive collections and fecal total radioactivity reached ≤1% of the administered dose for 2 consecutive collections.

In this study, physical examinations, ECGs, vital signs, How Do You Feel? inquiries, and clinical laboratory evaluations were performed at screening and at specified times during the study. A schematic of the study design is presented in Table 23.

Table 23: Schematic representation of this study

Screening	Check-in	Dose	PK/Radioactivity Sampling	Study Completion / Clinic Discharge
Days -28 to -2	Day -1	Day 1: [^{14}C]-AN2728 (approximately 100 µCi) in 9.5 g of a 2% ointment, topically applied	Days 1 through 15 (0 hour through 336 hours postdose)	Days 4 to 15 [a]
	← Confinement →			

[a] Subjects could be discharged as early as Day 4 (72 hours postdose) or as late as Day 15 (336 hours postdose) based on satisfying Discharge Criteria.

Treatments administered: [^{14}C]-AN2728 Ointment E, 2% was administered topically to healthy male subjects by the clinical staff. Prior to the dosing of each subject, the designated area for treatment was identified and approximate dose of study ointment applied was 5 mg/cm^2. The appropriate quantity of study ointment (9.5 g for 1,900 cm^2) was weighed out and applied as a thin layer and rubbed vigorously into the treatment area by the study staff. Following application, subjects remained seated for a minimum of 4

hours with the dose site uncovered. After approximately 4 hours post-dose, the dose site was wrapped with a gauze wrap and the subjects were allowed to wear loose clothing and to rest in a recumbent position. At approximately 12 hours, the gauze wrap was removed and the dose site was wiped with gauze and washed with soap and water. The gauze wrap and gauze wipes (site-wipes) were collected and submitted for radio-analysis.

Reviewer comments: *The to-be-marketed formulation was not used in this trial. The mechanism of disposition of the drug after it is absorbed should not be altered due to formulation differences. Hence distribution, metabolism, and excretion findings from this trial would be applicable for this application.*

Identity of investigational products: Summary of the identity of the investigational product is shown in Table 24.

Table 24: Identity of investigational product

Drug Name	[^{14}C]-AN2728	AN2728
Description[a]	Off-white Powder	Pale Yellow Powder
Strength	228.4 µCi/mg	Not applicable
Batch/Lot Number	2157-2157-10-001K	02110023R
Storage Condition	Ambient	Ambient
Amount Supplied	0.087 g/20 mCi	40 g
Amount Used	0.027 g/4.3832 mCi	8.11 g
Amount Remaining	0.06 g/15.6168 mCi	31.89 g
Disposition	Incineration	Incineration
Supplier	Anacor Pharmaceuticals, Inc. Palo Alto, California	Anacor Pharmaceuticals, Inc. Palo Alto, California
Manufacturer	(b) (4)	

[a] Specific ingredients/purity was identified on the Certificate of Analysis (or equivalent) that was supplied with the study drug(s).

Treatment Compliance: All doses of study drug were administered at the clinical site.

Sample Collections Times for PK Analysis:
Blood Sample Collection: Blood samples for PK analysis of AN2728 plasma concentrations and [^{14}C]-AN2728 radioactivity (RAD) levels in blood and plasma were collected at pre-dose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and every 24 hours for up to 15 days post-dose until study completion.

Blood samples for metabolite profiling and identification (METAB) were collected at pre- dose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours post-dose.

Urine Sample Collection: Urine samples for PK of AN2728 urine concentrations, radioactivity levels, and metabolite profiling and identification were collected at pre-dose (-12 to 0 hours) and from 0 to 8, 8 to 16, and 16 to 24 hours post-dose and for 24-hour intervals for up to 15 days until study completion.

Fecal Sample Collection: Fecal samples for analysis of [^{14}C]-AN2728 radioactivity levels were collected at pre-dose and from 0 to 12 and 12 to 24 hours post-dose and for 24-hour intervals for up to 15 days until study completion.

PK Variables: For each subject, the following PK parameters were calculated, whenever possible, based on the plasma and whole blood concentrations of total radioactivity and plasma concentrations of AN2728 parent drug and its primary metabolite, AN7602.

- Cmax - maximum observed concentration
- Tmax - time to maximum concentration
- AUC_{0-t} - area under the concentration-time curve from hour 0 to the last measurable concentration
- $AUC_{0-\infty}$ - area under the concentration-time curve extrapolated to infinity
- λz apparent terminal elimination rate constant
- t1/2 apparent terminal elimination half-life

The following PK parameters were calculated, whenever possible, for each subject based on the urine AN7602 concentrations:

- Aeu - amount of drug excreted in urine over sampling interval
- % Excreted - the % excreted

The following PK parameters were calculated, whenever possible, for each subject based on the fecal radioactivity concentration:

- Aef - amount of drug excreted in the feces over sampling interval
- % Excreted – the %excreted in the feces

Drug Concentration Measurements: Concentrations of AN2728 and AN7602 in acidified human plasma and acidified human urine samples were determined with validated liquid chromatography-mass spectrometry (LC-MS/MS) assays.

Radioactivity Measurements: All sample combustions were done in a Model 307 Sample Oxidizer (Packard Instrument Company). Oxidation efficiency was evaluated on each day of sample combustion by analyzing a commercial radiolabeled standard both directly in scintillation cocktail and by oxidation. Acceptance criteria were combustion recoveries of 95 to 105%. All samples were analyzed for radioactivity in Model 2900TR liquid scintillation counters. All samples were analyzed in duplicate if sample size allowed. If results from sample replicates differed by more than 10% from the mean value, the sample was re-homogenized and reanalyzed (if the sample size permitted).

Metabolite Profiling and Identification Measurements: Metabolites were identified by using a LC-MS/MS method.

Disposition of Subject: 6 subjects were enrolled and all the subjects completed the trial.

Demographics and Other Baseline Characteristics: A listing of individual subject demographics is presented in Table 25.

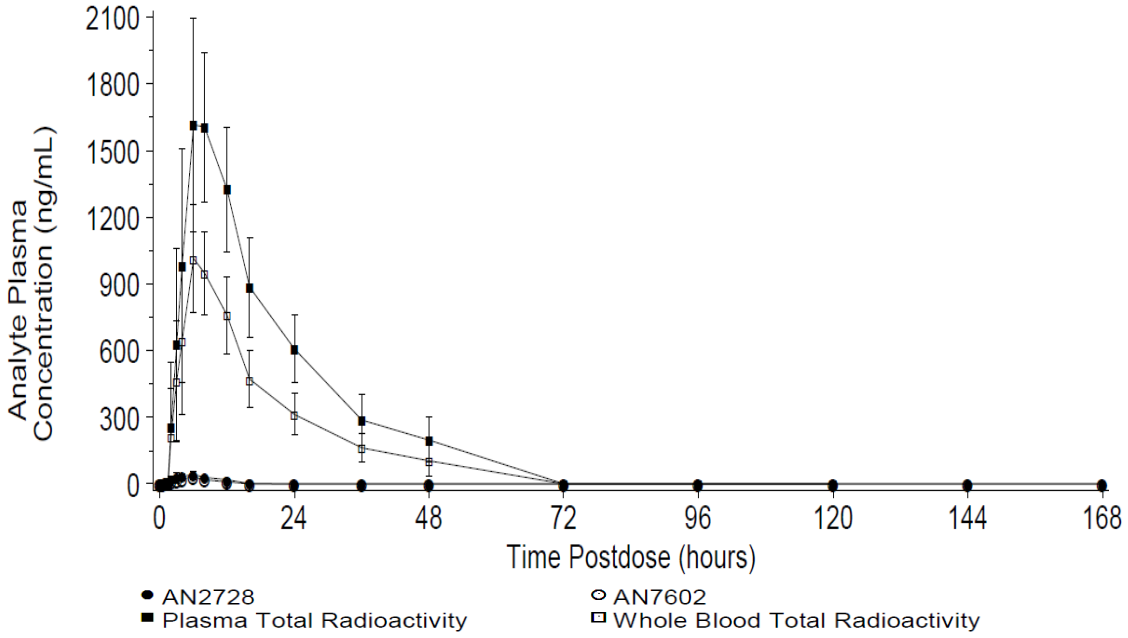
Table 25: Demographic Characteristics

Demographic	**AN2728** **N = 6**
Age (years)	
Mean (SD)	36 (12.2)
Min, Max	22, 55
Sex	
Male	6 (100%)
Race	
White	6 (100%)
Ethnicity	
Not Hispanic or Latino	6 (100%)
Body Weight (kg)	
Mean (SD)	80.3 (9.62)
Min, Max	64.0, 88.8
Height (cm)	
Mean	175.2 (5.35)
Min, Max	168.4, 182.1
BMI (kg/m2)	
Mean (SD)	26.2 (3.07)
Min, Max	21.6, 29.7

N = Number of subjects studied.

PK Results: Mean (SD) plasma concentration-time profiles for total radioactivity in blood and plasma and for AN2728 and AN7602 in plasma following a single dose administration of [^{14}C]-AN2728 is shown in Figure 22.

Figure 22: Mean (SD) plasma concentration-time profiles for AN2728 and AN7602 and plasma and whole blood concentration-time profiles for total radioactivity following a single topical dose of [^{14}C]-AN2728



Summary of the mean (SD) PK parameters for AN2728 and AN7602 in plasma and total radioactivity in plasma and whole blood following a single topical dose administration of [^{14}C]-AN2728 Ointment E, 2% to healthy male subjects is presented in Table 26. A summary of the mean (SD) PK parameter data for total radioactivity in urine and feces is presented in Table 27.

Table 26: Summary of mean (SD) PK parameters for AN2728 and AN7602 in plasma and total radioactivity in plasma and whole blood following a single topical dose of [^{14}C]-AN2728

Matrix	Analyte	N	C_{max} (ng/mL)	t_{max}[a] (h)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	λ_Z (1/h)
Plasma	AN2728	6	47.1 (10.7)	6.00 (3.00, 8.00)	376 (59.9)	380 (67.5)[b]	8.03 (5.83)[b]	0.134 (0.0879)[b]
	AN7602	6	33.4 (10.6)	6.00 (3.00, 6.00)	227 (62.1)	228 (61.9)	2.21 (0.396)	0.324 (0.0659)
	Total Radioactivity	6	1702 (432)	8.00 (6.00, 8.00)	34949 (12491)	37552 (13051)	20.0 (6.89)	0.0383 (0.0135)
Whole Blood	Total Radioactivity	6	1033 (227)	6.00 (6.00, 8.00)	18431 (6397)	21116 (7544)	16.9 (6.35)	0.0443 (0.0105)

Note: Units for total radioactivity in plasma and blood are ng equiv/g for C_{max} and ng equiv·h/g for AUC.
a Median (Min, Max) presented for t_{max}.
b N=5.

Table 27: Summary of mean (SD) PK parameters for AN7602 in urine and total radioactivity in urine and feces following a single topical dose of [^{14}C]-AN2728

	AN7602	Total Radioactivity	
Parameter	Urine	Urine	Feces
A_{eu} (ng)	8022 (2986)	NA	NA
% Excreted	0.00445 (0.00162)	24.9 (4.85)	0.242 (0.0766)

NA Not applicable.
Note: The majority (72.2%) of radioactivity was recovered from the wrap materials used to cover dose site

Total Radioactivity: Following a single topical dose administration of [^{14}C]-AN2728 Ointment E, 2%, total radioactivity readily appeared, with median Tmax values of 8.00 and 6.00 hours observed in plasma and in whole blood, respectively. Total radioactivity was slowly eliminated with mean t1/2 values of 20.0 and 16.9 hours observed in plasma and whole blood, respectively. The mean Cmax for total radioactivity in whole blood (1033 ng equivalents/g) was approximately 60% of the total radioactivity in plasma (1702

ng equivalents/g). The mean AUC_{0-t} and $AUC_{0-\infty}$ ratios of total radioactivity in blood versus plasma were approximately 53% and 56%, respectively.

The overall mean recovery of radioactivity in the study was 97.3% over the 168-hour study, with recovery in individual subjects ranging from 69.8 to 122%. The majority of radioactivity (mean of 72.2%) was recovered from the wrap materials used to cover the dose site. Approximately 25% of the applied dose was absorbed percutaneously. Approximately 81% of the absorbed radioactivity was recovered in the urine within 16 hours post-dose, and approximately 1% of the absorbed radioactivity was recovered in feces. By 168 hours post-dose, the absorbed radioactivity was almost completely recovered. Renal excretion was the major route of elimination for [^{14}C]-AN2728-derived radioactivity in humans after a topical dose.

Radioanalysis/Metabolite Profiling and Identification Results: Biotransformation of AN2728 was extensive and primarily consisted of deboronation/hydrolysis to AN7602, followed by subsequent downstream oxidation, sulfation, and glucuronidation. Overall, AN8323 (carboxyl-AN7602) and AN7602-sulfate were the major circulating components in plasma, accounting for approximately 70% and 30% of the total radioactivity, respectively, from 1 to approximately 24 hours post-dose. AN2728 and AN7602 levels were minimal in plasma and urine. Metabolism is the major clearance mechanism of AN2728 in humans. The schematic representation of the biotransformation pathway in humans is presented in Section 2.2.3.

Appendix 4: Trial AN2728-TQT-108 – TQT trial

Title: A randomized, parallel cohort with nested crossover study of the effects of AN2728 topical ointment, 2% on QT/QTc intervals compared to vehicle and moxifloxacin positive control in healthy subjects.

Primary objective: The primary objective of this trial was to assess the ECG effects of AN2728 relative to vehicle following multiple-dose administration of AN2728 Topical Ointment, 2% BID to designated treatment areas that represent approximately 30% or 60% of BSA in healthy adult male and female subjects.

Primary endpoint: The primary endpoint was the change from time-matched baseline in QTcF (ΔQTcF), based on the effect following multiple-dose administration of AN2728 Topical Ointment, 2%. The vehicle-corrected, change from time-matched baseline in QTcF (ΔΔQTcF) was used in the evaluation of the primary endpoint.

Study design: This was a single-center, randomized, 3-cohort parallel study, with a nested crossover design in healthy adult male and female subjects (schematic representation in Figure 2).

A total of 180 subjects were enrolled and 175 subjects completed the study and this included 78 females and 97 males. Subjects were randomized to 1 of 3 cohorts in a 1:1:1 ratio, according to a randomization schedule. The randomization of subjects was stratified by gender to ensure sufficient representation of males and females within each cohort. Subjects remained in the clinic until completion of all scheduled procedures.

- Cohort 1: This was a vehicle- and positive-control cohort that was further randomized to 1 of 2 blinded sequences (Cohorts 1a and 1b). A total of 60 subjects were enrolled in Cohort 1 with 30 subjects in each sequence. Twenty-eight (28) subjects completed each sequence. Cohort 1 served as the vehicle control cohort for the primary QTc assessment and Cohorts 1a and 1b were used to assess assay sensitivity.
- Cohorts 2: This cohort received therapeutic dose (DT) of AN2728 Topical Ointment, 2%.
- Cohort 3: This cohort received supra-therapeutic dose (DS) of AN2728 Topical Ointment, 2%.

Dosing in cohorts:

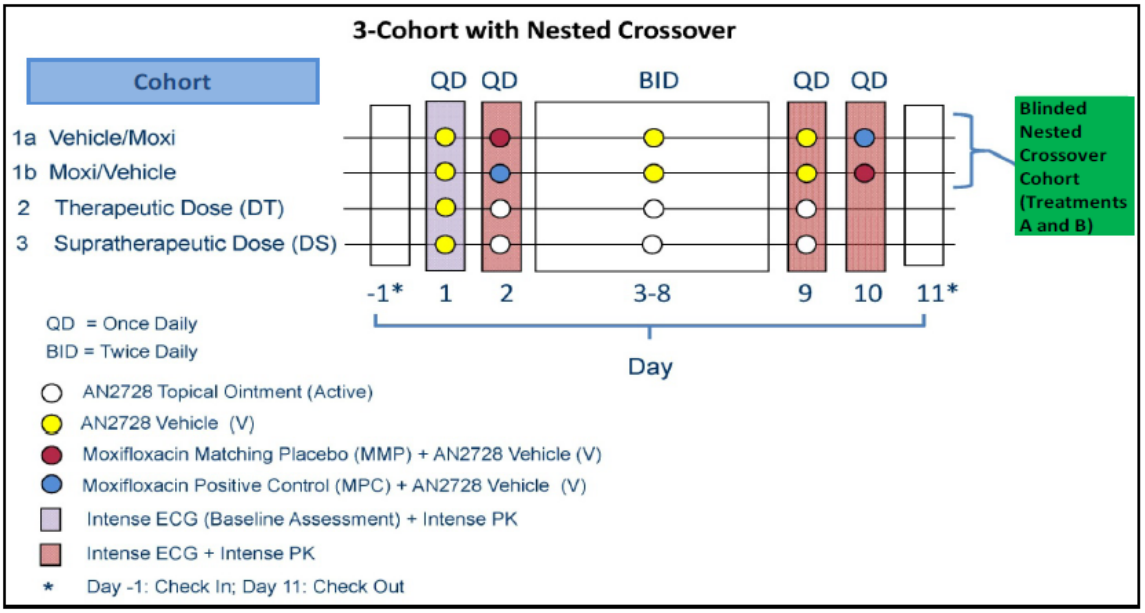
- Cohort 1: All subjects received AN2728 Topical Ointment Vehicle (V) on Days 1 through 10 (QD on Days 1, 2, 9, and 10 and BID on Days 3–8).
  - Cohort 1a: Moxifloxacin matching placebo (MMP) + V was co-administered on Day 2 and Moxifloxacin positive control (MPC) + V was co-administered on Day 10.
  - Cohort 1b: MPC + V was co-administered on Day 2 and MMP + V was co-administered on Day 10.
- Cohort 2: Subjects received V QD on Day 1 followed by 8 days of DT (QD on Days 2 and 9 and BID on Days 3–8).

- Cohort 3: Subjects received V QD on Day 1 followed by 8 days of DS (QD on Days 2 and 9 and BID on Days 3–8).

Subjects, Investigators, and Sponsor were blinded to MMP and MPC treatments. The topical treatments (V, DT, and DS) were open label; however, the central ECG reader was blinded to subject identifiers, treatment, time, and time points.

ECG assessment: Subjects in each cohort underwent cardiodynamic sampling. Twelve (12)-lead Holter monitors captured continuous ECGs from which data were extracted while the subject was lying quietly. The data were analyzed by a core ECG laboratory according to a pre-specified algorithm, starting at approximately 30 minutes prior to the morning dose on Days 1, 2, 9, and 10 until approximately 23.5 hours after that dose.

Figure 23: Schematic representation of the study design



PK sampling: In each cohort, blood samples were taken at the following times to assess the systemic concentrations of the parent and metabolites:
Day 1: Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6.5, 8, 10, 12 and 15 hours post-dose
Day 2: Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6.5, 8, 10, 12 and 15 hours post-dose
Day 3: Pre-dose
Day 8: Pre-dose
Day 9: Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6.5, 8, 10, 12 and 15 hours post-dose
Day 10: Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6.5, 8, 10, 12, 15 and 23.5 hours post-dose

Dosing:

- Cohort 1: In the positive control cohort, 56 subjects received the vehicle (15 g applied to 30% BSA) with wither MPC or MMP as follows.
    - Cohort 1a: 28 subjects received single doses of the vehicle on Days 1, 2, 9, and 10, BID doses administered on Days 3 through 8, with MMP administered on Day 2 and MPC administered on Day 10.

    - Cohort 1b: 28 subjects received single doses of the vehicle on Days 1, 2, 9, and 10, BID doses administered on Days 3 through 8, with MPC administered on Day 2 and MMP administered on Day 10.
- Cohort 2: 60 subjects received the therapeutic dose (DT) of AN2728 (15 g) applied to 30% BSA. Subjects were administered a single dose of the vehicle (15 g, 30% of BSA) on Day1, single doses of DT on Days 2 and 9 with BID administration on Days 3 through 8.
- Cohort 3: 59 subjects received the supra-therapeutic dose (DS) of AN2728 (45 g) applied to 60% of BSA. Subjects were administered a single dose of the vehicle (45 g, 60% of BSA) on Day1, single doses of DS on Days 2 and 9 with BID administration on Days 3 through 8.

QT prolongation assessment: The QT - Interdisciplinary Review Team (QT-IRT) at the Agency reviewed the TQT trial and they have noted that the systemic concentrations in the maximal use PK trial (AN2728-AD-102) were higher than those obtained in the TQT study. However, the reviewer further states that the totality of clinical data suggested that there was no evidence that crisaborole has a clinically meaningful effect on the QTc interval and further studies are not recommended. For further information, see QT-IRT review by Dr. Jiang Lu dated 08/02/2016 under NDA 207695.

Disposition of subjects: A total of 180 subjects entered the study. A total of 175 subjects completed the study as follows (summary shown in Table 28):

- Cohort 1: 56 subjects (28 subjects in Cohort 1a and 28 subjects in Cohort 1b) completed the moxifloxacin control part of the study.
    - Subject 039 (Cohort 1a) withdrew from the study on Day 2 of Period 1 for personal reasons.
    - Subject 126 (Cohort 1a) was discontinued by the PI on Day 7 of Period 1 due to an AE.
    - Subject 101 (Cohort 1b) withdrew from the study on Day 7 of Period 1 for personal reasons.
    - Subject 149 (Cohort 1b) was discontinued by the PI on Day 2 of Period 1 due to an AE.
- Cohort 2: 60 subjects completed the DT part
- Cohort 3: 59 subjects completed the DS part
    - Subject 111 (DS) withdrew consent due to AE occurrences from the study on Day 2 of Period 1

Table 28: Summary of subject disposition

Analysis Group, n (%)	Cohort 1a MMP/MPC N=30 (%)	Cohort 1b MPC/MMP N=30 (%)	Cohort 2 DT N=60 (%)	Cohort 3 DS N=60 (%)	Total N=180 (%)
Randomized	30 (100%)	30 (100%)	60 (100%)	60 (100%)	180 (100%)
Received Study Drug	30 (100%)	30 (100%)	60 (100%)	60 (100%)	180 (100%)
Completed Study	28 (93%)	28 (93%)	60 (100%)	59 (98%)	175 (97%)
Discontinued Study	2 (7%)	2 (7%)	0 (0%)	1 (2%)	5 (3%)
Adverse Event	1 (3%)	1 (3%)	0 (0%)	1 (2%)	3 (2%)
Withdrawal By Subject	1 (3%)	1 (3%)	0 (0%)	0 (0%)	2 (1%)

Demographic information: Summary of demographic information is shown in Table 29.

Table 29: Summary of demographic information

Trait		Cohort 1a MMP/MPC N=30 (%)	Cohort 1b MPC/MMP N=30 (%)	Cohort 2 DT N=60 (%)	Cohort 3 DS N=60 (%)	Total N=180 (%)
Gender	Female	13 (43%)	14 (47%)	28 (47%)	27 (45%)	82 (46%)
	Male	17 (57%)	16 (53%)	32 (53%)	33 (55%)	98 (54%)
Race	American Indian or Alaska Native	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (1%)
	Black or African American	4 (13%)	0 (0%)	2 (3%)	2 (3%)	8 (4%)
	White	26 (87%)	30 (100%)	58 (97%)	57 (95%)	171 (95%)
Ethnicity	Hispanic or Latino	21 (70%)	27 (90%)	52 (87%)	56 (93%)	156 (87%)
	Not Hispanic or Latino	9 (30%)	3 (10%)	8 (13%)	4 (7%)	24 (13%)
Age (yr)	n	30	30	60	60	180
	Mean	33.4	33.4	33.3	33.3	33.3
	SD	7.08	8.28	8.00	7.66	7.73
	CV	21.17	24.77	24.05	23.04	23.19
	Minimum	22	19	18	20	18
	Median	32.5	34.0	34.0	33.5	33.5
	Maximum	45	44	45	45	45
Weight (kg)	n	30	30	60	60	180
	Mean	69.50	69.49	67.44	69.11	68.68
	SD	9.713	11.361	10.301	8.028	9.655
	CV	13.976	16.350	15.276	11.616	14.058
	Minimum	50.7	50.8	50.1	54.4	50.1
	Median	68.45	67.25	67.35	68.85	68.15
	Maximum	87.9	95.7	92.4	88.7	95.7

Trait		Cohort 1a MMP/MPC N=30 (%)	Cohort 1b MPC/MMP N=30 (%)	Cohort 2 DT N=60 (%)	Cohort 3 DS N=60 (%)	Total N=180 (%)
Height (cm)	n	30	30	60	60	180
	Mean	167.1	165.4	165.1	165.7	165.7
	SD	8.63	9.79	9.20	8.69	8.99
	CV	5.16	5.92	5.57	5.24	5.43
	Minimum	151	151	152	151	151
	Median	166.0	165.0	164.0	165.0	165.0
	Maximum	186	187	190	189	190
Body Mass Index (kg/m^2)	n	30	30	60	60	180
	Mean	24.820	25.235	24.636	25.148	24.937
	SD	2.2281	2.0826	2.2759	2.1511	2.1913
	CV	8.9770	8.2530	9.2379	8.5538	8.7873
	Minimum	20.67	21.09	20.61	20.22	20.22
	Median	25.125	25.270	25.105	25.800	25.300
	Maximum	27.94	27.90	28.03	27.91	28.03

PK results: Summary of PK results for crisaborole (parent), metabolite AN7602 and downstream metabolite AN8323 is shown in Table 30, 31 and 32, respectively.

Table 30: Summary Plasma PK Parameters of AN2728 (parent) in Healthy Human Subjects on Days 2 and 9 Following Application of AN2728 Topical Ointment, 2% at Therapeutic Dose (15 g/day) and Supra-therapeutic Dose (45 g/day)

PK Parameters	Therapeutic Dose (DT) (15 g/day AN2728)			Supratherapeutic Dose (DS) (45 g/day AN2728)		
	N	Mean	SD	N	Mean	SD
Day 2						
λz, 1/h	60	0.0532	0.0221	56	0.0619	0.0173
$T_{½}$, h	60	16.0	8.15	56	12.2	4.11
T_{max}, h[a]	60	5.07	2.07 - 10.1	59	6.57	2.07 - 15.1
C_{max}, ng/mL	60	29.0	18.4	59	56.4	26.3
$AUC_{(0-12)}$, ng·h/mL	60	209	107	59	428	187
$AUC_{(0-23.5)}$, ng·h/mL	60	336	134	59	723	246
$AUC_{(0-t)}$, ng·h/mL	60	337	134	59	725	246
$AUC_{(0-inf)}$, ng·h/mL	60	520	160	56	1,040	272
DS/DT Mean C_{max} Ratio	-	1.94	-	-	-	-
DS/DT Mean $AUC_{(0-12)}$ Ratio	-	2.05	-	-	-	-
Day 9						
λz, 1/h	60	0.0518	0.0157	59	0.0656	0.0123
$T_{½}$, h	60	14.9	5.53	59	11.0	2.47
T_{max}, h[a]	60	4.05	1.07 - 8.07	59	3.07	1.07 - 8.07
C_{max}, ng/mL	60	37.2	12.9	59	87.4	29.6
$AUC_{(0-12)}$, ng·h/mL	60	301	88.3	59	697	205
$AUC_{(0-23.5)}$, ng·h/mL	60	455	125	59	1,020	284
$AUC_{(0-t)}$, ng·h/mL	60	456	126	59	1,030	285
DS/DT Mean C_{max} Ratio	-	2.35	-	-	-	-
DS/DT Mean $AUC_{(0-12)}$ Ratio	-	2.32	-	-	-	-

[a] Expressed as median and range

Table 31: Summary Plasma PK Parameters of AN7602 (metabolite) in Healthy Human Subjects on Days 2 and 9 Following Application of AN2728 Topical Ointment, 2% at Therapeutic Dose (15 g/day) and Supra-therapeutic Dose (45 g/day)

PK Parameters	Therapeutic Dose (DT) (15 g/day AN2728)			Supratherapeutic Dose (DS) (45 g/day AN2728)		
	N	Mean	SD	N	Mean	SD
Day 2						
λz, 1/h	60	0.0763	0.0288	57	0.0711	0.0240
$T_{½}$, h	60	10.6	4.56	57	11.1	4.58
T_{max}, h[a]	60	5.07	3.05 - 10.1	59	6.57	2.07 - 15.1
C_{max}, ng/mL	60	13.1	9.57	59	24.3	14.5
$AUC_{(0-12)}$, ng·h/mL	60	91.0	59.0	59	180	96.0
$AUC_{(0-23.5)}$, ng·h/mL	60	138	74.7	59	302	134
$AUC_{(0-t)}$, ng·h/mL	60	138	74.8	59	303	134
$AUC_{(0-inf)}$, ng·h/mL	60	177	84.0	57	421	171
DS/DT Mean C_{max} Ratio	-	1.85	-	-	-	-
DS/DT Mean $AUC_{(0-12)}$ Ratio	-	1.98	-	-	-	-
Day 9						
λz, 1/h	60	0.0752	0.0230	59	0.0843	0.0190
$T_{½}$, h	60	11.3	10.5	59	8.73	2.47
T_{max}, h[a]	60	3.07	2.05 - 8.07	59	3.07	1.07 - 8.07
C_{max}, ng/mL	60	15.1	6.49	59	34.1	15.9
$AUC_{(0-12)}$, ng·h/mL	60	113	44.5	59	270	110
$AUC_{(0-23.5)}$, ng·h/mL	60	159	58.9	59	381	148
$AUC_{(0-t)}$, ng·h/mL	60	159	58.9	59	382	148
DS/DT Mean C_{max} Ratio	-	2.26	-	-	-	-
DS/DT Mean $AUC_{(0-12)}$ Ratio	-	2.39	-	-	-	-

[a] Expressed as median and range

Table 32: Summary Plasma PK Parameters of AN8323 (downstream metabolite of AN7602) in Healthy Human Subjects on Days 2 and 9 Following Application of AN2728 Topical Ointment, 2% at Therapeutic Dose (15 g/day) and Supra-therapeutic Dose (45 g/day)

PK Parameters	Therapeutic Dose (DT) (15 g/day AN2728)			Supratherapeutic Dose (DS) (45 g/day AN2728)		
	N	Mean	SD	N	Mean	SD
Day 2						
λz, 1/h	1	0.0320	NC	0	NC	NC
$T_{½}$, h	1	21.6	NC	0	NC	NC
T_{max}, h[a]	60	23.6	6.57 - 23.6	59	23.6	8.07 - 23.6
C_{max}, ng/mL	60	570	317	59	1,360	569
$AUC_{(0-12)}$, ng·h/mL	60	3,620	2,670	59	7,000	4,130
$AUC_{(0-23.5)}$, ng·h/mL	60	9,380	5,630	59	20,600	9,680
$AUC_{(0-t)}$, ng·h/mL	60	9,430	5,660	59	20,800	9,730
$AUC_{(0-inf)}$, ng·h/mL	1	63,500	NC	0	NC	NC
DS/DT Mean C_{max} Ratio	-	2.39	-	-	-	-
DS/DT Mean $AUC_{(0-12)}$ Ratio	-	1.93	-	-	-	-
Day 9						
λz, 1/h	14	0.0172	8.39E-03	19	0.0147	8.06E-03
$T_{½}$, h	14	50.7	27.4	19	105	217
T_{max}, h[a]	60	1.32	0 - 12.1	59	0.567	0 - 23.6
C_{max}, ng/mL	60	1,530	658	59	3,930	1,740
$AUC_{(0-12)}$, ng·h/mL	60	15,700	6,830	59	39,700	17,000
$AUC_{(0-23.5)}$, ng·h/mL	60	29,500	12,900	59	73,900	31,500
$AUC_{(0-t)}$, ng·h/mL	60	29,600	13,000	59	74,300	31,600
DS/DT Mean C_{max} Ratio	-	2.57	-	-	-	-
DS/DT Mean $AUC_{(0-12)}$ Ratio	-	2.53	-	-	-	-

[a] Expressed as median and range
NC - Not Calculated

Reviewer comments: *This reviewer notes that the T1/2 for the downstream metabolite AN8323 was longer for the supra-therapeutic dose (105 hours) than that observed in the adolescent subject PK trial (33.5 hours) (AN2728-AD-203). This reviewer opines that the T1/2 value of 105 hours observed in this trial does not seem to be reliable due to large variability (i.e. standard deviation (SD) value was of 200 versus SD of 10.1 in the adolescent subject PK trial). Hence the T1/2 value of 50.7 hours under therapeutic dose appears to be more reliable.*

The summary of accumulation ratios for crisaborole (AN2728), its metabolite AN7602 and the downstream metabolite AN8323 is shown in Table 33. The mean concentration versus time profile for crisaborole (AN2728) and its metabolite AN7602 and the

downstream metabolite AN8323following therapeutic dose is shown in Figure 24 and Figure 25 and following supra-therapeutic dose in Figures 26 and 27, respectively.

Table 33: Summary for Ratios of Day 9/Day 2 Cmax and AUC_{0-12} for AN2728, AN7602 and AN8323

Analyte		Day 9/Day 2 C_{max}			Day 9/Day 2 $AUC_{(0-12)}$		
		N	Mean	SD	N	Mean	SD
AN2728							
	DT	60	1.53	0.701	60	1.67	0.671
	DS	59	1.69	0.526	59	1.82	0.650
AN7602							
	DT	60	1.43	0.692	60	1.53	0.703
	DS	59	1.53	0.486	59	1.70	0.659
AN8323							
	DT	60	3.11	1.39	60	5.74	3.36
	DS	59	3.03	1.00	59	6.75	2.91

Figure 24: Mean Plasma Concentration-Time Profiles of AN2728 andAN7602 Following Therapeutic Dose (15 g/day) of AN2728 Topical Ointment, 2%

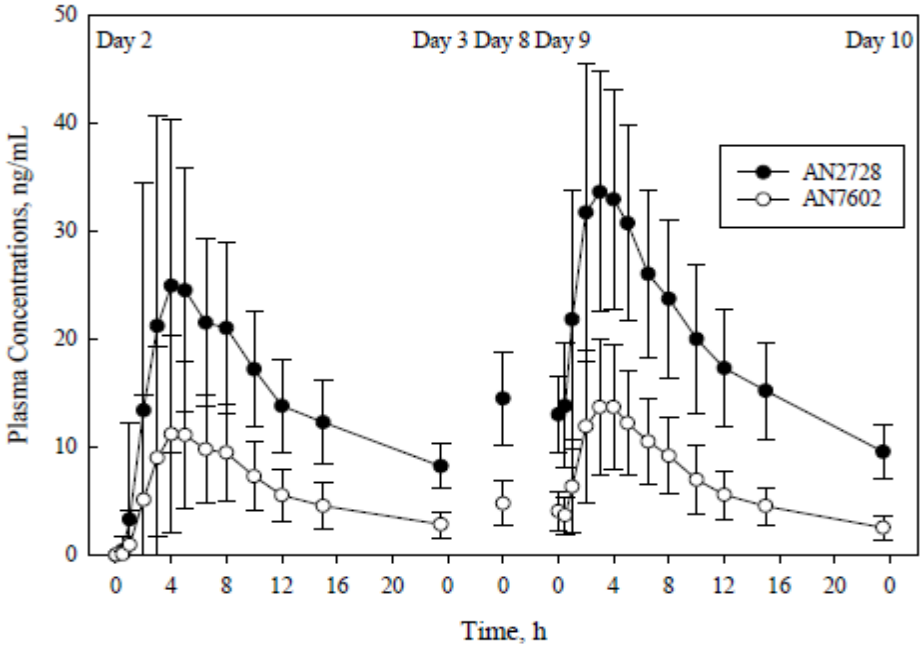


Figure 25: Mean Plasma Concentration-Time Profiles of AN8323 Following Therapeutic Dose (15 g/day) of AN2728 Topical Ointment, 2%

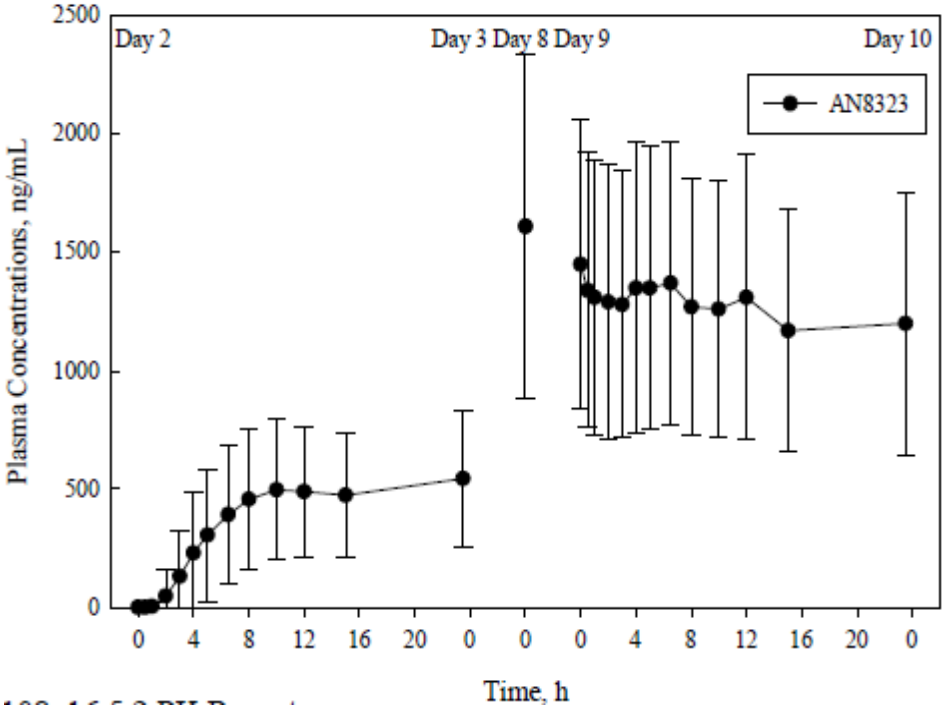


Figure 26: Mean Plasma Concentration-Time Profiles of AN2728 and AN7602 Following Supra-therapeutic Dose (45 g/day) of AN2728 Topical Ointment, 2%

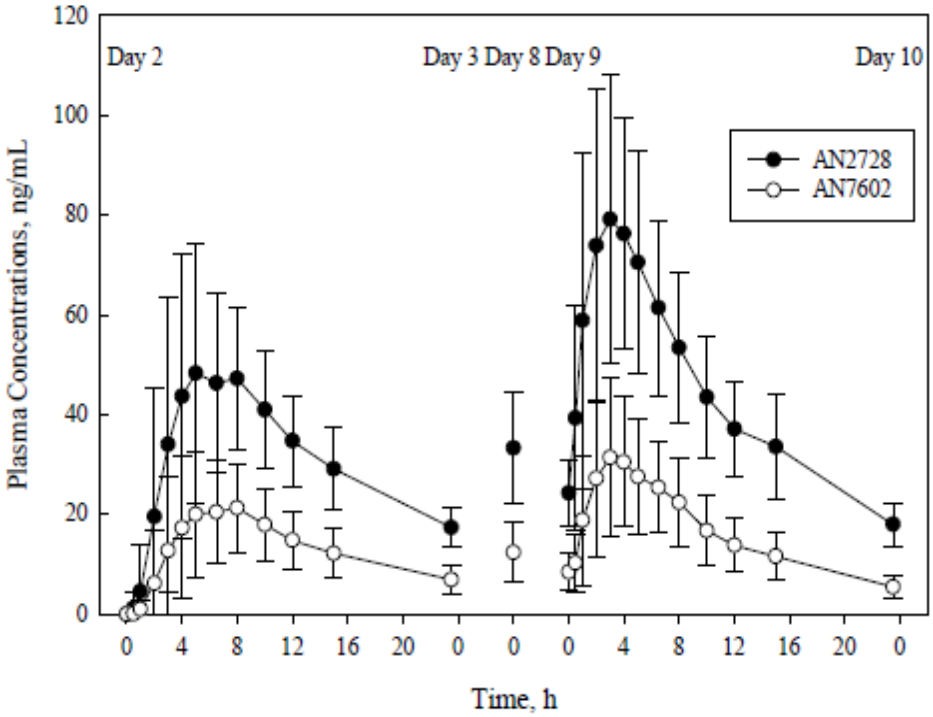
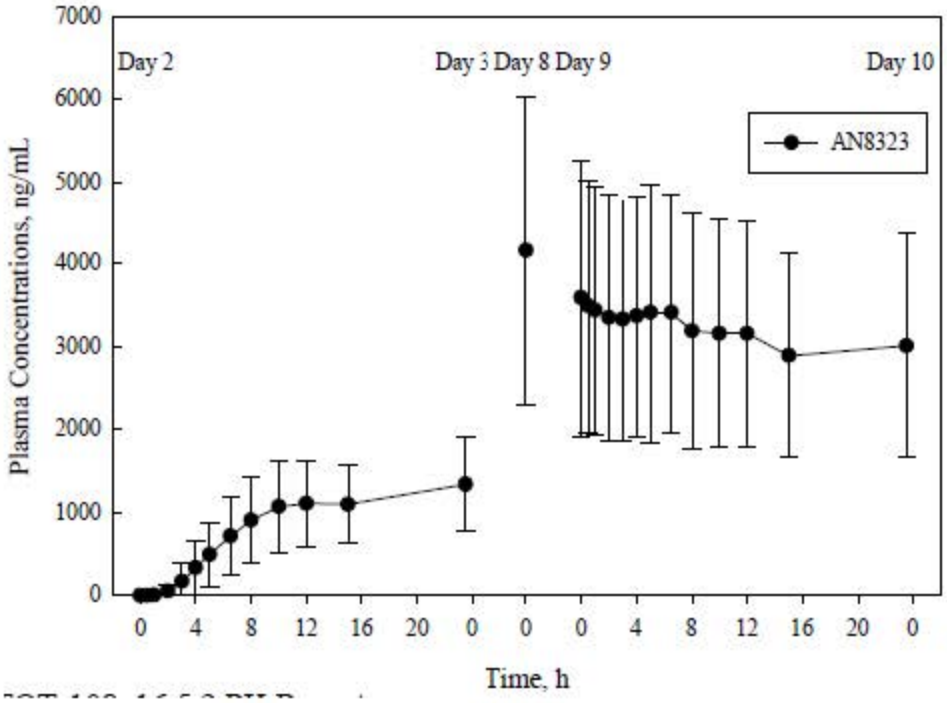


Figure 27: Mean Plasma Concentration-Time Profiles of AN8323 Following Supra-therapeutic Dose (45 g/day) of AN2728 Topical Ointment, 2%



Appendix 5: Trial AN2728-PK-101 – In-vivo DDI study

Title: An open-label, three period, fixed-sequence study to investigate the effect of AN8323, a metabolite of AN2728, on the single dose pharmacokinetics of warfarin, following topical application of AN2728 topical ointment, 2% in healthy adult subjects

Primary objective: The primary objective was to evaluate the effect of AN8323, a metabolite of AN2728, after multiple topical administrations of AN2728 Topical Ointment, 2%, at a supra-therapeutic dose (over 60% body surface area [BSA]), on the PK of a single-dose of warfarin in healthy adult subjects.

Secondary objectives:

- To evaluate the safety and tolerability of multiple topical administrations of AN2728 Topical Ointment, 2%, when administered alone and in combination with orally administered warfarin in healthy adult subjects.
- To determine the effect of AN8323 on the PD of a single-dose of warfarin as assessed by INR for PT (hereafter referred to as INR only), in healthy adult subjects.

Primary PK endpoints: The primary PK endpoints included $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max} for R-warfarin and S-warfarin (Treatments A and C), as appropriate, when administered with and without AN2728 Topical Ointment, 2%.

The following PK parameters were also computed:

- For R-warfarin and S-warfarin (Treatments A and C): $AUC_{\%extrap}$, T_{max}, k_{el}, $t_{½}$, CL/F, and V/F, as appropriate.
- For AN2728 and its metabolites (Treatment B) in plasma after multiple-dose administration of AN2728 Topical Ointment, 2% alone (as appropriate): $AUC_{0\text{-}12}$, $C_{max\text{-}ss}$, $T_{max\text{-}ss}$, C_{min}, C_{trough}, and C_{avg}, as appropriate.

Study design: This was a single-center, open-label, 3-period, fixed-sequence study. 24 healthy, adult, non-tobacco using male and female subjects were enrolled. Women of childbearing potential were not included in the study due to the embryotoxic potential of warfarin.

Period 1 (Treatment A): Subjects received a single oral dose of 25 mg warfarin (a CYP2C9 probe substrate) following an overnight fast of at least 8 hours. There was a washout of at least 14 days between the warfarin dosing in Period 1 and the first AN2728 Topical Ointment, 2%, dose in Period 2.

Period 2 (Treatment B): Subjects were administered AN2728 Topical Ointment, 2%, BID (approximately every 12 hours) for 7 days. There was no washout between the last dose in Period 2 and the first dose in Period 3.

Period 3 (Treatment C): Subjects continued to receive BID application (approximately every 12 hours) of the AN2728 Topical Ointment, 2%, for an additional 7 days. On the

morning of Period 3, Day 1, after an overnight fast of at least 8 hours and 30 minutes following AN2728 Topical Ointment, 2% application, a single oral dose of 25 mg warfarin was administered.

Subjects were confined to the clinical research unit (CRU) at least 10 hours before the first dosing in Period 1 until after the 72-hour blood draw (Day 4) and returned for the subsequent study procedures in Period 1. In Period 2, subjects were housed from at least 10 hours before dosing and remained confined until completion of study procedures on Day 8 of Period 3.

Blood samples were obtained over a period of 168 hours post-dose following warfarin dosing on Day 1 of Period 1 and Day 1 of Period 3 to characterize the PK profile of R- and S-warfarin. Morning pre-dose samples for AN2728 and its metabolites (AN7602 and AN8323) were collected on Days 6 and 7 to confirm steady state. Blood samples were obtained over a period of 12 hours following the morning dose on the seventh day of dosing of Period 2 to characterize the PK of AN2728 and its metabolites (AN7602 and AN8323) when administered alone. In addition, a Pre-dose blood sample prior to the morning AN2728 Topical Ointment, 2% dose on Days 3, 5, and 7 of Period 3, were collected to assess pre-dose levels for AN2728 and its metabolites (AN7602 and AN8323).

Safety was monitored throughout the study. Upon completion of Period 3, subjects returned to the CRU approximately 7 (± 1) days after the last dose of study medication administration in Period 3 for follow-up procedures to determine if any AEs had occurred since the last study visit. Subjects who terminated the study early were contacted if the investigator deemed it clinically necessary. Subjects who were withdrawn or who had participation shortened were not replaced.

Clinical Pharmacology related select inclusion criteria:

1. Healthy adult male or female subjects, 18 – 55 years of age, inclusive, at the time of consent.
2. Medically healthy with no clinically significant laboratory profiles, vital signs, or ECG findings, as deemed by the investigator.
3. Subject had non-clinically significant INR, activated partial thromboplastin time, platelet count values, and hemoglobin levels.

Clinical Pharmacology related select exclusion criteria:

1. History or presence of atopic dermatitis, psoriasis, or other skin diseases that could have altered the absorption of the study ointment. The skin of the areas to be treated with study ointment were to be unbroken and without rash.
2. History or presence of clinically relevant:
   • Bleeding disorders, including relevant familial history
   • Anemia
   • Thromboembolic disease
   • Bleeding in the gastrointestinal tract or central nervous system.
3. Female subjects who were pregnant or lactating

Treatments Administered:

- AN2728 Topical Ointment, 2%
- Warfarin sodium (Coumadin®) tablets

Treatments were as follows:

- Treatment A: Single total oral dose of warfarin sodium (Coumadin) of 25 mg (administered as 2 x 10 mg tablets and 1 x 5 mg tablet) at Hour 0 on Day 1 following an overnight fast of at least 8 hours.
- Treatment B: Multiple topical administrations of AN2728 Topical Ointment, 2%, applied in the amount of 45 g to designated treatment areas which represented approximately 60% of BSA, BID (approximately every 12 hours) on Days 1 to 7.
- Treatment C: Multiple topical administrations of AN2728 Topical Ointment, 2%, applied in the amount of 45 g to designated treatment areas which represented approximately 60% of BSA, BID on Day 1 to Day 7 with a single total oral dose of 25 mg warfarin sodium on Day 1, 30 minutes following application of AN2728 Topical Ointment, 2% and following an overnight fast of at least 8 hours.

AN2728 Topical Ointment, 2%, was applied by clinic study staff and approximately 45 g of ointment was applied BID on to approximately 60% of BSA within ± 1 hour of dosing beginning on Day 1 of Period 2 and continuing through Period 3.

All doses of warfarin were administered orally with approximately 240 mL of water at room temperature. Subjects were instructed not to crush, split, or chew the oral study medication.

PK sampling times:
Warfarin: For all subjects, blood samples for the determination of R- and S-warfarin were collected at pre-dose, and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours following Day 1 dosing of Period 1 (Treatment A) and Period 3 (Treatment C).

AN2728 and Metabolites of AN2728 (AN7602 and AN8323): In Period 2 (Treatment B), for all subjects, blood samples for the determination of AN2728 and its metabolites (AN7602 and AN8323) were collected prior to morning dosing on Days 1, 6, and 7 and at 1, 2, 4, 6, 8, and 12 hours following Day 7 morning dosing.

In Period 3 (Treatment C), for all subjects, blood samples for the determination of AN2728 and its metabolites (AN7602 and AN8323) were collected prior to morning dosing on Days 3, 5, and 7.

Reviewer comments: *The applicant has validated the bioanalytical method for warfarin. The results are not included in this review.*

Disposition of Subjects: A total of 24 subjects were enrolled into the study and 21 subjects completed the study. 3 subjects discontinued prematurely from the study due to the following reasons:

- Subject 5 was discontinued due an AE (increased BP) in Period 2 and completed Treatments A and B.
- Subject 18 was discontinued due to an AE (increased INR) in Period 1 and completed Treatment A only.
- Subject 14 withdrew from the study in Period 3 due to reasons unrelated to the study treatment and completed Treatments A and B.

Demographic and Other Baseline Characteristics: A summary of demographic characteristics is presented in Table 34. Of the 24 healthy subjects participating in the study, 15 were male and 9 were female. With regard to race, 23 subjects were White and one subject was American Indian or Alaska Native. Regarding ethnicity, 22 subjects were Hispanic or Latino and two were not.

Table 34: Demographics

Statistic	Age (years)	Weight (kg)	Height (cm)	BMI* (kg/m^2)
Mean (N= 24)	43.1	76.01	166.7	27.260
Range (N = 24)	21–55	54.1–98.8	148–185	22.43–30.47

*BMI = Body Mass Index

No. of subjects: 24 subjects were enrolled in this study and 21 subjects completed this study. 3 subjects discontinued due to the following reasons:

- Subject 5 was discontinued on Day 1 of Period 3 due to hypertension
- Subject 14 withdrew consent on Day 1 of Period 3 due to personal reasons, and
- Subject 18 was discontinued prior to Period 2 check-in due an AE of elevated INR.

PK results: The PK results of S-warfarin and R-warfarin with and without crisaborole ointment treatment is shown in Table 35 and the relative BA assessment of S- and R-warfarin with and without crisaborole treatment is shown in Table 36. The results in Table 36 indicated lack of any drug interaction potential between crisaborole and warfarin. Summary of plasma PK of crisaborole (AN2728) and its metabolite AN7602 and its downstream metabolite AN8323 is shown in Table 37.

Table 35: PK results of S-warfarin and R-warfarin with and without crisaborole ointment treatment (the interaction was due to downstream metabolite AN8323)

PK Parameter	With AN8323			Without AN8323		
	N	Mean	SD	N	Mean	SD
S-Warfarin						
T_{max}, h*	21	1.00	0.5 - 2.01	21	1.00	0.5 - 3
C_{max}, ng/mL	21	1,880	332	21	2,020	459
AUC_t, ng·h/mL	21	57,300	20,200	21	56,800	21,600
$AUC_{0\text{-}168}$, ng·h/mL	21	57,300	20,200	21	56,800	21,600
$AUC_{0\text{-}inf}$, ng·h/mL	21	60,100	23,200	21	59,400	24,400
$AUC_{\%extrap}$	21	3.79	2.75	21	3.44	2.85
k_{el}, 1/h	21	0.0196	3.65E-03	21	0.0204	3.90E-03
$T_{1/2}$, h	21	36.8	7.54	21	35.3	7.68
CL/F, L/h	21	0.233	0.0718	21	0.238	0.0764
V/F, L	21	11.9	3.19	21	11.6	3.01
R-Warfarin						
T_{max}, h*	21	1.00	0.5 - 4.01	21	1.00	0.5 - 4
C_{max}, ng/mL	21	1,830	296	21	1,940	405
AUC_t, ng·h/mL	21	87,400	14,600	21	84,600	14,200
$AUC_{0\text{-}168}$, ng·h/mL	21	87,400	14,600	21	84,600	14,200
$AUC_{0\text{-}inf}$, ng·h/mL	21	98,000	18,600	21	93,500	17,900
$AUC_{\%extrap}$	21	10.4	3.52	21	9.12	3.89
k_{el}, 1/h	21	0.0135	2.04E-03	21	0.0147	2.76E-03
$T_{1/2}$, h	21	52.3	8.11	21	48.6	9.08
CL/F, L/h	21	0.133	0.0297	21	0.139	0.0324
V/F, L	21	9.82	1.61	21	9.54	1.66

* Expressed in median and range

Table 36: Statistical Evaluation of S- and R-Warfarin Plasma PK Parameters in Healthy Adult Subjects a 25 mg Warfarin Dose With (n = 21) or Without (n = 21) AN8323

PK Parameter	With AN8323		Without AN8323		Diff	Diff	%Ratio	CI 90%	CI 90%
	LSM	SE	LSM	SE	LSM	LSM SE		Lower	Upper
S-Warfarin									
ln-C_{max}	7.52409	0.04447	7.58482	0.04447	-0.06073	0.06289	94.11	84.65	104.62
ln-AUC_t	10.90471	0.07227	10.88905	0.07227	0.01566	0.10221	101.58	85.52	120.65
ln-$AUC_{0\text{-}inf}$	10.94359	0.07736	10.92481	0.07736	0.01878	0.10940	101.90	84.75	122.51
R-Warfarin									
ln-C_{max}	7.50020	0.04017	7.55065	0.04017	-0.05045	0.05680	95.08	86.41	104.62
ln-AUC_t	11.36333	0.03975	11.33023	0.03975	0.03310	0.05621	103.36	94.03	113.63
ln-$AUC_{0\text{-}inf}$	11.47451	0.04523	11.42690	0.04523	0.04761	0.06396	104.88	94.17	116.80

Table 37: Summary of Plasma PK Parameters of AN2728, AN7602 and AN8323 in Healthy Adult Subjects in Period 2 Following the Application of AN2728 Topical Ointment, 2%, Twice a Day for 7 Days

PK Parameter	AN2728			AN7602			AN8323		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Tmax, h*	23	4.00	1 - 4.03	23	4.00	1.09 - 6	23	2.00	0 - 8.01
C_{max}, ng/mL	23	81.1	17.8	23	31.4	8.71	23	5,410	1,920
C_{min}, ng/mL	23	35.7	8.83	23	11.6	3.80	23	5,090	1,720
C_{trough}, ng/mL	23	37.0	8.67	23	12.9	3.79	23	4,380	1,740
C_{avg}, ng/mL	22	56.7	11.7	19	21.3	6.05	7**	4,000	1,610
AUC_t, ng·h/mL	23	685	140	23	251	69.2	23	57,700	22,000
AUC_{0_12}, ng·h/mL	22	681	140	19	256	72.6	7**	48,000	19,400
$AUC_{0\text{-}inf}$, ng·h/mL	22	1,150	280	19	392	113	7**	162,000	92,700
AUC_%Extrap	22	39.2	12.9	19	33.6	11.4	7**	67.4	8.50
K_{el}, 1/h	22	0.0904	0.0292	19	0.106	0.0322	7**	0.0350	0.0120
$T_{1/2}$, h	22	9.27	6.62	19	7.28	2.79	7**	21.7	6.73

* Expressed in median and range

** $AUC_{0\text{-}12}$ (consequently Cavg), $AUC_{0\text{-}inf}$ and AUC_%Extrap were only calculated in 7 subjects since the actual collection times were less than 12 h (11.92 h) and the corresponding $T_{1/2}$ for the remaining subjects were incalculable ($AUC_{0\text{-}12}$ and $AUC_{0\text{-}inf}$ can be calculated using extrapolation if the $T_{1/2}$ is available). $T_{1/2}$ life was only calculable in the 7 subjects as per criteria for calculation $T_{1/2}$ (See Section 3.2)

Reviewer comments: *The mean ± SD Cmax and $AUC_{0\text{-}12}$ of AN8323 in this trial was approximately 12% and 24%, respectively lower than the maximal use PK trial (AN2728-AD-102). Even though the systemic exposure of AN8323 in this trial was slightly lower than the maximal use PK trial, considering the fact that there was no drug interaction potential observed in this trial, the small magnitude of difference in AN8323 exposure is not expected to produce any drug interaction under clinical use conditions.*

The mean plasma concentration versus time profile of S-warfarin and R-warfarin in healthy subjects with and without crisaborole ointment treatment is shown in Figure 28 and 29, respectively.

Figure 28: Mean (SD) Plasma Concentration-Time Profiles of S-Warfarin in Healthy Adult Subjects Following a 25 mg Warfarin Dose With (n = 21) or Without AN8323 (n = 21) of AN8323

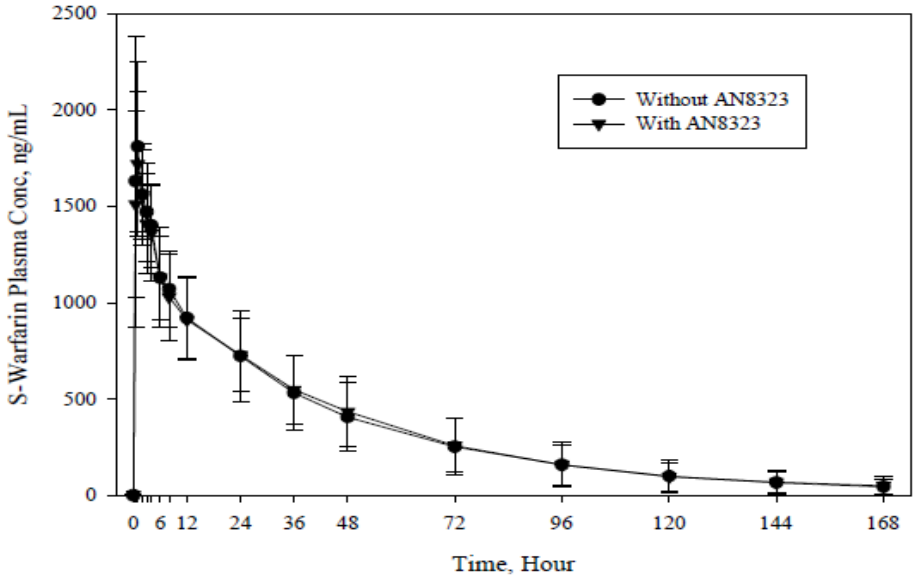
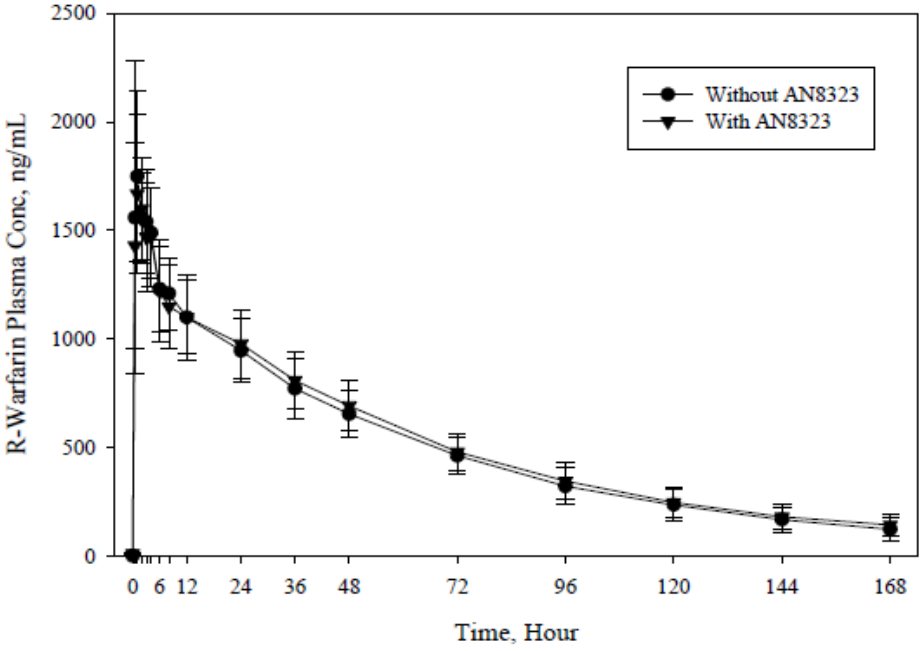


Figure 29: Mean (SD) Plasma Concentration-Time Profiles of R-Warfarin in Healthy Adult Subjects Following a 25 mg Warfarin Dose With (n = 21) or Without AN8323 (n = 21) of AN8323



Appendix 6: In-vitro drug interaction studies for AN2728 (crisaborole)

Study 003-NCL PK-043-01: Inhibitory Potential of AN2728 on Human Hepatic Microsomal Cytochrome P450 Isoenzymes

Objective: The objective of this study was to assess the in-vitro inhibitory potential of AN2728 on the major human hepatic cytochromes P450 including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5.

Method: The direct inhibitory potential of AN2728 on CYP activities was assessed in-vitro using pooled human hepatic microsomes. Hepatic microsomes were incubated with isoenzyme-selective substrates at concentrations approximating the Km in the absence and presence of AN2728 at various concentrations. Also included was a selective inhibitor as a positive control (Table 38). The substrates and positive controls that were used in this study are shown in Table ABC. The extent of inhibition was determined by comparing activities from control and AN2728-treated microsomes.

Table 38: Substrates and positive controls

Activity Assay (Cytochrome P450)	Substrate (μM)	Protein (mg/mL)	Time (minutes)	Analyte	Positive Control and Concentration (μM)
Phenacetin O-deethylase (CYP1A2)	75	0.1	15	Acetaminophen	Fluvoxamine (1)
Bupropion hydroxylase (CYP2B6)	120	0.1	15	Hydroxybupropion	Thiotepa (100)
Amodiaquine *N*-deethylase (CYP2C8)	2	0.025	15	Desethylamodiaquine	Montelukast (0.1)
Diclofenac 4′-hydroxylase (CYP2C9)	11	0.1	15	4′-Hydroxydiclofenac	Sulfaphenazole (3)
S-Mephenytoin 4′-hydroxylase (CYP2C19)	40	0.1	15	4′-Hydroxymephenytoin	Nootkatone (30)
Bufuralol 1′-hydroxylase (CYP2D6)	12	0.1	15	1′-Hydroxybufuralol	Quinidine (0.3)
Testosterone 6β-hydroxylase (CYP3A4/5)	40	0.1	5	6β-Hydroxytestosterone	Ketoconazole (0.1)
Midazolam 1′-hydroxylase (CYP3A4/5)	2.5	0.1	5	1′-Hydroxymidazolam	Ketoconazole (0.1)

Note: Incubation conditions are specific for each characterized lot of human hepatic microsomes.

Results: Marked inhibition was observed with all the known selective CYP inhibitors (positive controls) on the respective CYP isoenzymes. The results obtained in this study showed that AN2728, at concentrations up to approximately 15 μM, did not show notable direct inhibition on the activities of CYP1A2, 2B6, 2C9, 2D6, and 3A4/5, with the remaining activity >90% of the control activity.

AN2728 at 15 μM showed a weak direct inhibition on CYP2C8, with the remaining activity 82.2% of the vehicle control. The IC_{50} for CYP2C8 was not determined, but can be considered to be >15 μM (the highest concentration used in the initial direct inhibition test). AN2728 showed moderate direct inhibition on CYP2C19 in the initial experiment. AN2728 was then tested at higher concentrations, and the IC50 was estimated to be 25.4 μM. Further investigation revealed that AN2728 was a competitive inhibitor of CYP2C19 and its inhibition constant (Ki) was determined to be 8.96 μM. In the maximal use PK

trial the mean ±SD plasma AN2728 Cmax on Day 8 was 0.506 ± 0.781 µM (127 ± 196 ng/mL). Hence the ratios of [I]/ Ki for competitive inhibition (0.506/8.96) would be <0.1 and the corresponding R values will be < 1.1 which indicates that the possibility of crisaborole to inhibit CYP2C19 in the clinic is low.

The metabolism-dependent inhibitory potential of AN2728 on CYP isoenzymes was also assessed in this study. AN2728 (0.0365, 1.35 and 15 µM) was incubated in the absence and presence of NADPH (1 mM) in pooled human hepatic microsomes at 37°C for 30 minutes. The extent of metabolism-dependent inhibitory potential was determined by comparing activities from microsomes pre-incubated with AN2728 in the presence and absence of NADPH.

The results (summarized in Table 39) suggested that AN2728 was a metabolism-dependent inactivator of CYP2C19, but not of other CYP isoenzymes tested and the Ki value was 22.3 µM. Hence the ratio of [I]/Ki (0.506/22.3) would be <0.1 and the corresponding R values will be < 1.1 which indicates that the possibility of crisaborole to inhibit CYP2C19 in the clinic is low.

Table 39: Summary of inhibition on human hepatic CYP isoenzymes by AN2728

	Direct Inhibition			Metabolism-Dependent Inhibition		
CYP Isoenzyme	IC_{50} (µM)	K_i (µM)	Type of Inhibition	Conclusion	K_I (µM)	k_{inact} ($minute^{-1}$)
CYP1A2	>15	ND	NA	NO	NA	NA
CYP2B6	>15	ND	NA	NO	NA	NA
CYP2C8	>15	ND	NA	NO	NA	NA
CYP2C9	>15	ND	NA	NO	NA	NA
CYP2C19	25.4	8.96	Competitive	YES	22.3	0.0568
CYP2D6	>15	ND	NA	NO	NA	NA
CYP3A4/5	>15	ND	NA	NO	NA	NA
CYP3A4/5	>15	ND	NA	NO	NA	NA

IC_{50} The concentration of AN2728 that inhibits 50% of a CYP activity.
K_i Inhibition constant.
K_I Inhibitor concentration that supports half of maximal rate of inactivation.
k_{inact} Maximal rate of inactivation.
NA Not applicable.
ND Not determined.

In conclusion, the in-vitro inhibitory potential of AN2728 on the major human hepatic cytochrome P450 including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 was assessed. AN2728 is not expected to inhibit (direct or metabolism dependent) any of the CYP enzymes under conditions of clinical use.

Study 003-NCL PK-044-01: Evaluation of Cytochrome P450 (CYP) induction by AN2728 following exposure to primary cultures of human hepatocytes

Objective: The objective of this study was to measure the extent of induction of five specific CYP450 marker enzymes, CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4/5, following exposure of human hepatocytes to AN2728, and to compare the effects of the AN2728 with those of prototypical inducers (positive control).

Method: For each CYP enzyme (CYP1A2, 2B6, 2C9, 2C19, and 3A4/5), fresh hepatocytes obtained from three individual human donors were separately incubated for 72 hours with AN2728 (0.5, 2, and 10 µM), a prototype inducer (positive control) for each enzyme, and an appropriate solvent control for AN2728 or each prototypical inducer. The positive control used in this study is shown in Table 40 below.

Table 40: Summary of positive controls

CYP Isoenzyme Induced	Positive Control	Solvent	Dose Concentration (µM)
CYP1A2	Omeprazole	1% ACN in sHMM	25
CYP2B6	Phenobarbital	sHMM	1000
CYP2C9	Rifampicin	1% ACN in sHMM	50
CYP2C19	Rifampicin	1% ACN in sHMM	50
CYP3A4/5	Rifampicin	1% ACN in sHMM	50

Note: A solution of 1% acetonitrile (ACN) in sHMM was prepared for use as a solvent control for omeprazole and rifampicin. ACN percentages are volume/volume.

The induction potential of AN2728 oncytochrome P450 activities of CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP3A4/5 were determined by incubating each culture with a specific probe substrate (Table 41) and measuring the rate of production of relevant metabolites utilizing liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

Table 41: Summary of substrates

CYP Isoenzyme	Substrate	Solvent	Concentration (µM)
CYP1A2	Phenacetin	<1% Methanol in sHMM	100
CYP2B6	Bupropion	<1% Methanol in sHMM	500
CYP2C9	Diclofenac	<1% Methanol in sHMM	100
CYP2C19	*S*-Mephenytoin	<1% Methanol in sHMM	150
CYP3A4/5	Testosterone	<1% Methanol in sHMM	250

Note: Solvent percentages are volume/volume.

Results: All prototype inducers demonstrated appropriate induction of the respective enzyme expression: omeprazole (25 µM) induced CYP1A2 mRNA levels 27.4- to 217-fold, phenobarbital (1,000 µM) induced CYP2B6 mRNA levels 3.17- to 16.5-fold, and rifampicin (50 µM) induced CYP2C9 mRNA levels 2.71- to 5.79-fold, CYP2C19 mRNA levels 1.41- to 9.83-fold, and CYP3A4 mRNA levels 14.5- to 33.5-fold. These results indicated that hepatocytes cultures prepared from the donors were responsive to CYP induction under the experimental conditions.

In this study, AN2728 at 10 μM was found to weakly induce CYP2B6 with ≥ 40% of induction by the positive control phenobarbital in only 1 donor (Donor 2 at 58%). The maximum fold values for induction of CYP2B6 activity by AN2728 over solvent control were 2.53, 1.88, and 1.17, respectively, in hepatocytes from Donors 2, 3, and 4, or 58.1, 34.5 and 3.44% of induction by phenobarbital in hepatocytes from the same individual donors, respectively. AN2728 also showed slight induction of CYP2B6 expression, as represented by CYP2B6 mRNA levels. AN2728 (10 μM) induced CYP2B6 mRNA levels 4.09, 2.70, and 0.843-fold compared to the vehicle control, respectively, in Donors 2, 3, and 4.

AN2728 did not show any notable induction (≤40% of induction by respective prototypical inducers) on CYP1A2, 2C9, 2C19, and CYP3A4 activities under the study conditions.

In conclusion, in this study AN2728 showed weak induction of human CYP2B6 in hepatocytes from one of the three donors at 58.1% of the positive control and did not induce CYP1A2, 2C9, 2C19, and 3A4/5.

Appendix 7: In-vitro drug interaction studies for metabolite AN7602

Study 003-NCL PK-082-01: Evaluation of Cytochrome P450 inhibition in human liver microsomes and induction in human hepatocytes after exposure to AN7602

Objective: The purpose of this study was to determine the potential inhibition and induction of AN7602 on CYP isoforms

Method: For induction study, human plateable cryopreserved hepatocytes were incubated with 0.1, 1 and 10 µM AN7602 for 72 hours at 37°C and for inhibition study, human liver microsomes were incubated with 10 µM AN7602 for 10 minutes at 37°C. Table 42 provides information on the specific reference compounds used in the induction and inhibition studies.

Table 42: List of reference compounds used in the in-vitro CYP induction and inhibition studies

Assay	Source	Reference Compound
CYP1A2 induction (human hepatocytes, mRNA level, 3 donors)	Human plateable cryopreserved hepatocytes (0.70 million viable cells/mL)	Omeprazole
CYP2B6 induction (human hepatocytes, mRNA level, 3 donors)	Human plateable cryopreserved hepatocytes (0.70 million viable cells/mL)	Phenobarbital
CYP3A4 induction (human hepatocytes, mRNA level, 3 donors)	Human plateable cryopreserved hepatocytes (0.70 million viable cells/mL)	Rifampin
CYP1A inhibition (HLM, phenacetin substrate)	Human liver microsomes (0.1 mg/mL)	Furafylline
CYP2B6 inhibition (HLM, bupropion substrate)	Human liver microsomes (0.1 mg/mL)	Clopidogrel
CYP2C8 inhibition (HLM, paclitaxel substrate)	Human liver microsomes (0.1 mg/mL)	Montelukast
CYP2C9 inhibition (HLM, diclofenac substrate)	Human liver microsomes (0.1 mg/mL)	Sulfaphenazole
CYP2C19 inhibition (HLM, omeprazole substrate)	Human liver microsomes (0.1 mg/mL)	Oxybutynin
CYP2D6 inhibition (HLM, dextromethorphan substrate)	Human liver microsomes (0.1 mg/mL)	Quinidine
CYP3A inhibition (HLM, midazolam substrate)	Human liver microsomes (0.1 mg/mL)	Ketoconazole
CYP3A inhibition (HLM, testosterone substrate)	Human liver microsomes (0.1 mg/mL)	Ketoconazole

The extent of inhibition was determined by comparing the activities from control and the AN7602-treated microsomes and the extent of induction was assessed by incubating the hepatocytes with specific probe substrate and them measuring the rate of production of relevant metabolites.

Results:
Summary of CYP inhibition results: The potential of AN7602 to inhibit CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A was tested in vitro at a test concentration of 10 µM in human liver microsomes. These testing concentrations were selected based on the in-vivo Cmax concentration of ~40 ng/mL (0.17 µM) (based on plasma levels from maximal use PK trial AN2728-AD-102).

The results (Table 43) of this study indicate that AN7602 is not considered an inhibitor for CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A at the tested concentration of 10 µM

Table 43: Inhibition of AN7602 on CYP Isoforms

Client Compound I.D.	Test Concentration	% Inhibition of Control Values		
		1st	2nd	Mean
CYP1A inhibition (HLM, phenacetin substrate)				
AN7602	1.0E-05 M	-19.2	-18.1	-18.7
CYP2B6 inhibition (HLM, bupropion substrate)				
AN7602	1.0E-05 M	8.6	0.9	4.8
CYP2C8 inhibition (HLM, paclitaxel substrate)				
AN7602	1.0E-05 M	-12.7	1.6	-5.5
CYP2C9 inhibition (HLM, diclofenac substrate)				
AN7602	1.0E-05 M	6.6	13.3	9.9
CYP2C19 inhibition (HLM, omeprazole substrate)				
AN7602	1.0E-05 M	8.2	-7.8	0.2
CYP2D6 inhibition (HLM, dextromethorphan substrate)				
AN7602	1.0E-05 M	-0.4	-2.2	-1.3
CYP3A inhibition (HLM, midazolam substrate)				
AN7602	1.0E-05 M	-8.1	-8.8	-8.4
CYP3A inhibition (HLM, testosterone substrate)				
AN7602	1.0E-05 M	3.1	3.2	3.1

Reviewer comments: *Based on the data in the table above, it seems that 50% (or more) inhibition was not achieved in any of the enzymes. Hence the IC_{50} values are expected to be > 10 µM. For the sake of argument, suppose if the IC50 value was 10 µM, then the Ki value would be $IC_{50}/2$ = 5 µM. Then the [I]/Ki value is expected to be > 0.1 and the corresponding R value would be > 1.1 ([I] = 0.17 µM).*

Summary of CYP induction results: The potential of AN7602 to induce CYP1A2, CYP2B6 and CYP3A4 was tested in vitro in human hepatocytes at test concentrations of 0.1, 1, and 10 µM using mRNA level as the end point. These test concentrations were selected based on the expected in vivo concentration of 40 ng/mL (based on plasma levels in the maximal use PK trial AN2728-AD-102). Fold induction in mRNA level after incubating AN7602 with hepatocytes was compared to the cutoff value predetermined using a set of known CYP inducers and non-inducers (Table 44).

Table 44: CYP Induction with Reference Compounds

(b) (4) Compound	Test Concentration	Mean of Fold Induction	Hepatocyte Lot #	Cutoff
CYP1A2 induction (human hepatocytes, mRNA level, 3 donors) - Negative Control				
Flumazenil	3.0E-05 M	1.2	CDP	2
Flumazenil	3.0E-05 M	1.2	FOS	2
Flumazenil	3.0E-05 M	1.3	GKV	2
CYP1A2 induction (human hepatocytes, mRNA level, 3 donors) - Positive Control				
Omeprazole	5.0E-05 M	48	CDP	2
Omeprazole	5.0E-05 M	47	FOS	2
Omeprazole	5.0E-05 M	57	GKV	2
CYP2B6 induction (human hepatocytes, mRNA level, 3 donors) - Negative Control				
Flumazenil	3.0E-05 M	1.3	CDP	2
Flumazenil	3.0E-05 M	1.2	FOS	4
Flumazenil	3.0E-05 M	1.1	GKV	2.5
CYP2B6 induction (human hepatocytes, mRNA level, 3 donors) - Positive Control				
Phenobarbital	1.0E-03 M	11.6	CDP	2
Phenobarbital	1.0E-03 M	18.7	FOS	4
Phenobarbital	1.0E-03 M	7.2	GKV	2.5
CYP3A4 induction (human hepatocytes, mRNA level, 3 donors) - Negative Control				
Flumazenil	3.0E-05 M	2.4	CDP	5
Flumazenil	3.0E-05 M	2.3	FOS	15
Flumazenil	3.0E-05 M	1.3	GKV	6
CYP3A4 induction (human hepatocytes, mRNA level, 3 donors) - Positive Control				
Rifampin	1.0E-05 M	61	CDP	5
Rifampin	1.0E-05 M	409	FOS	15
Rifampin	1.0E-05 M	98	GKV	6

Note:
fold induction = (mRNA level in test compound treated cells)/(mRNA level in vehicle control)
Cutoff values were pre-determined using 10 known CYP inducers and 5 known CYP non-inducers.

Based on the cutoff values determined above, if the fold induction at any test concentration was greater than the cutoff value of a CYP isoform in at least one donor, then that compound would be deemed as an in-vitro inducer of that enzyme. The results (Table 45) showed that AN7602 is not considered an inducer for CYP1A2, CYP2B6 and CYP3A4 at the concentrations tested.

Table 45: Induction of AN7602 on CYP Isoforms

(b) (4) Compound I.D.	Client Compound I.D.	Test Concentration	Fold Induction 1st	2nd	3rd	Mean	Hepatocyte Lot#	Cutoff
CYP1A2 induction (human hepatocytes, mRNA level, 3 donors)								
100025032-1	AN7602	1.0E-07 M	0.98	1.10	0.62	0.90	CDP	2
100025032-1	AN7602	1.0E-06 M	0.92	0.89	0.89	0.90	CDP	2
100025032-1	AN7602	1.0E-05 M	0.98	1.24	0.95	1.06	CDP	2
100025032-1	AN7602	1.0E-07 M	0.77	0.93	0.93	0.88	FOS	2
100025032-1	AN7602	1.0E-06 M	1.12	0.96	0.77	0.95	FOS	2
100025032-1	AN7602	1.0E-05 M	0.73	1.01	0.75	0.83	FOS	2
100025032-1	AN7602	1.0E-07 M	0.77	1.27	0.96	1.00	GKV	2
100025032-1	AN7602	1.0E-06 M	0.85	0.63	0.90	0.79	GKV	2
100025032-1	AN7602	1.0E-05 M	1.14	1.10	1.86	1.37	GKV	2
CYP2B6 induction (human hepatocytes, mRNA level, 3 donors)								
100025032-1	AN7602	1.0E-07 M	0.66	0.64	0.57	0.62	CDP	2
100025032-1	AN7602	1.0E-06 M	0.66	0.62	0.65	0.65	CDP	2
100025032-1	AN7602	1.0E-05 M	0.87	0.93	0.79	0.86	CDP	2
100025032-1	AN7602	1.0E-07 M	1.11	1.15	1.03	1.10	FOS	4
100025032-1	AN7602	1.0E-06 M	1.18	0.95	0.77	0.97	FOS	4
100025032-1	AN7602	1.0E-05 M	1.07	1.01	1.19	1.09	FOS	4
100025032-1	AN7602	1.0E-07 M	0.49	0.74	0.51	0.58	GKV	2.5
100025032-1	AN7602	1.0E-06 M	1.08	0.68	0.79	0.85	GKV	2.5
100025032-1	AN7602	1.0E-05 M	0.96	0.98	0.95	0.97	GKV	2.5
CYP3A4 induction (human hepatocytes, mRNA level, 3 donors)								
100025032-1	AN7602	1.0E-07 M	0.63	0.67	0.62	0.64	CDP	5
100025032-1	AN7602	1.0E-06 M	0.98	0.62	0.74	0.78	CDP	5
100025032-1	AN7602	1.0E-05 M	0.74	0.72	0.64	0.70	CDP	5
100025032-1	AN7602	1.0E-07 M	0.92	0.71	0.86	0.83	FOS	15
100025032-1	AN7602	1.0E-06 M	0.82	0.75	0.65	0.74	FOS	15
100025032-1	AN7602	1.0E-05 M	1.44	2.02	1.41	1.62	FOS	15
100025032-1	AN7602	1.0E-07 M	0.66	0.86	0.69	0.74	GKV	6
100025032-1	AN7602	1.0E-06 M	0.82	0.97	0.67	0.82	GKV	6
100025032-1	AN7602	1.0E-05 M	0.96	1.18	0.95	1.03	GKV	6

Note:
fold induction = (mRNA level in test compound treated cells)/(mRNA level in vehicle control)
The value within a bracket is invalid and not included in the calculation of mean.
Cutoff values were pre-determined using 10 known CYP inducers and 5 known CYP non-inducers.

In conclusion, AN7602 at therapeutic concentrations is not expected to inhibit or induce any of the CYP enzymes under conditions of clinical use.

Appendix 8: In-vitro drug interaction studies for downstream metabolite AN8323

Study 003-NCL PK-047-01: Inhibitory potential of AN8323 towards human hepatic microsomal Cytochrome P450 isoenzymes

Objective: The objective of this study was to characterize the *in vitro* inhibitory potential of AN8323 on the activities of the following human hepatic cytochrome P450 (CYP) isoenzymes: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5.

Method: Human hepatic microsomes were pooled from fifty individuals. The inhibitory potential of selected concentrations of test article toward the activities of specific human hepatic CYPs was evaluated. A single CYP-selective substrate concentration was used, approximating the concentration of the substrate that gives half the maximum reaction velocity (Km) for human hepatic microsomes for each cytochrome P450 assay.

Direct inhibition assays were performed in the absence (solvent only) and presence of AN8323 (0.391, 0.781, 1.56, 3.13, 6.25, 12.5, 25, and 50 µM) to determine its inhibition potential on selected cytochrome P450 activities. If >50% inhibition was observed, the concentration of AN8323 that inhibits 50% of the activity (IC50) of each specific isoenzyme of cytochrome P450 was calculated. A further experiment was conducted to determine the inhibition constant (Ki) and the type of inhibition for CYP2C8 and CYP2C9. Details of the incubation conditions for each assay is presented in the Table 46 below.

Table 46: Summary of incubation conditions for each assay

Activity Assay (Cytochrome P450)	Substrate (µM)	Protein (mg/mL)	Time (Minutes)	Analyte	Positive Control (µM)
Phenacetin O-deethylase (CYP1A2)	30	0.1	15	Acetaminophen	Fluvoxamine (1)
Bupropion hydroxylase (CYP2B6)	65	0.1	15	Hydroxybupropion	Thiotepa (100)
Amodiaquine N-deethylase (CYP2C8)	1.0	0.025	10	Desethylamodiaquine	Montelukast (0.1)
Diclofenac 4′-hydroxylase (CYP2C9)	3.5	0.025	10	4′-Hydroxydiclofenac	Sulfaphenazole (3)
S-Mephenytoin 4′-hydroxylase (CYP2C19)	25	0.1	15	4′-Hydroxymephenytoin	Nootkatone (30)
Bufuralol 1′-hydroxylase (CYP2D6)	11	0.1	15	1′-Hydroxybufuralol	Quinidine (0.3)
Testosterone 6β-hydroxylase (CYP3A4/5)	45	0.25	5	6β-Hydroxytestosterone	Ketoconazole (0.2)
Midazolam 1′-hydroxylase (CYP3A4/5)	2.0	0.1	5	1′-Hydroxymidazolam	Ketoconazole (0.2)

Notes: Incubation conditions are specific for each characterized lot of human hepatic microsomes. The stopping solution for the CYP1A2, CYP2C8, CYP2C9, and CYP3A4/5 (midazolam 1′-hydroxylase) assays was 10% acetic acid:acetonitrile (1:1, v/v). The stopping solution for the CYP2B6 and CYP2D6 assays was 5% acetic acid. The stopping solution for the CYP2C19 and CYP3A4/5 (testosterone 6β-hydroxylase) assays was 7% formic acid.

The metabolism-dependent inhibitory potential of AN8323 on CYP isoenzymes was also assessed in this study. Three concentrations of AN8323 (0.781, 6.25 and 50 µM) were incubated in human hepatic microsomes in the absence and presence of NADPH (1 mM). The extent of metabolism-dependent inhibitory potential was determined by comparing activities from microsomes pre-incubated with AN8323 in the presence and absence of NADPH.

Results: Direct inhibition and preliminary metabolism-dependent inhibition of the major human hepatic CYP450 isoenzymes including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 by AN8323 were assessed in this study. IC50 values were determined using sigma plot. The overall summary of results is shown in Table 47.

Table 47: Summary of inhibition on human hepatic CYP isoenzymes by AN8323

P450 Isoenzyme	Activity Assay	Direct Inhibition	Metabolism-Dependent Inhibition
CYP1A2	Phenacetin O-deethylase	IC_{50} = 49.3 µM	Not observed
CYP2B6	Bupropion hydroxylase	IC_{50} >50 µM	Not observed
CYP2C8	Amodiaquine N-deethylase	IC_{50} = 7.66 µM Competitive inhibition K_i = 6.7 µM	Not observed
CYP2C9	Diclofenac 4′-hydroxylase	IC_{50} = 9.52 µM Mixed inhibition K_i =5.2 µM, α = 101	Not observed
CYP2C19	S-Mephenytoin 4′-hydroxylase	Not observed	Not observed
CYP2D6	Bufuralol 1′-hydroxylase	Not observed	Not observed
CYP3A4/5	Testosterone 6β-hydroxylase	Not observed	Not observed
CYP3A4/5	Midazolam 1′-hydroxylase	Not observed	Not observed

α The coefficient to determine the relative contribution of competitive and uncompetitive inhibition in a mixed inhibition.
IC_{50} The concentration of the test article that inhibits 50% of the activity of an enzyme.
K_i Inhibition constant.

Reviewer comments: *The mean Cmax of AN8323 [I] in the maximal use PK trial was 6150 ng/mL = 24.10 µM (The molecular weight of AN8323 was 255.23). Based on the results in the table above, the [I]/Ki for CYP2C9, the most sensitive enzyme will be 5.2/24.10 = 0.22 which is higher than 0.1 and the corresponding R value will be 1.22 which is above the threshold of 1.1. This indicates possibility of observing drug interactions in-vivo.*

The applicant conducted an in-vivo drug interaction study with 25 mg dose of oral warfarin as a CYP2C9 substrate. This study showed lack of drug interaction (see Appendix ABC for results of the in-vivo drug interaction study). Since clinical drug interaction with the most sensitive enzyme showed lack of drug interaction, further assessment of other enzymes for drug interaction in-vivo was considered not warranted.

In conclusion, AN8323 is not expected to inhibit any CYP enzymes under the conditions of clinical use.

Study 003-NCL PK-048-01: Evaluation of Cytochrome P450 induction after exposure of AN8323 to primary cultures of human hepatocytes

Objective: The objective of this study is to assess the induction potential of AN8323 towards CYP1A2, CYP2B6, and CYP3A4/5 in primary cultures of human hepatocytes by measuring changes in mRNA levels and enzyme activities relative to solvent controls and compared with that of prototypical inducers.

Method: Human hepatocytes from three donors were exposed to AN8323 (0.1 to 50 µM) for 72 hours. Hepatocytes were also incubated with solvent control, prototypical inducers omeprazole (CYP1A2), phenobarbital (CYP2B6), and rifampicin (CYPA4/5), and with a non-inducer flumazenil (Table 48).

Table 48: Positive control inducers

Cytochrome P450	Prototypical Inducer/ Non-Inducer	Vehicle	Concentration (µM)
CYP1A2	Omeprazole	1% (v/v) ACN in sHMM	25
CYP2B6	Phenobarbital	1% (v/v) ACN in sHMM	1000
CYP3A4/5	Rifampicin	1% (v/v) ACN in sHMM	50
Non-Inducer	Flumazenil	1% (v/v) ACN in sHMM	20

Note: A solution of 1% (v/v) acetonitrile (ACN) in sHMM was prepared for use as a solvent control.

After exposure, concentrations of mRNA for CYP1A2, CYP2B6, and CYP3A4 were determined by quantitative real time PCR using isoenzyme-specific probes, and cytochrome P450 activities for CYP1A2, CYP2B6, and CYP3A4/5 were determined using isoenzyme-selective cytochrome P450 substrates phenacetin, bupropion, and testosterone, respectively (Table 49).

Table 49: CYP substrates

CYP Isoenzyme	Substrate	Vehicle	Concentration (µM)
CYP1A2	Phenacetin	≤1% Methanol in sHMM (v/v)	100
CYP2B6	Bupropion	≤1% Methanol in sHMM (v/v)	500
CYP3A4/5	Testosterone	≤1% Methanol in sHMM (v/v)	250

Results: The potential for AN8323 to induce CYP1A2, CYP2B6, and CYP3A4/5 was determined in primary cultures of human hepatocytes from three donors. The induction of each cytochrome P450 was evaluated by measuring changes in mRNA levels and enzyme activities relative to solvent controls and compared with that of prototypical inducers.

The results showed that AN8323 produced concentration-dependent increases in mRNA expression and cytochrome P450 activities for CYP1A2. Maximum fold induction of CYP1A2 mRNA expression in AN8323-treated hepatocytes ranged from 0.983 to 2.02 compared with solvent control in the three hepatocyte cultures (Table 50), whereas phenacetin *O*-deethylase activities (CYP1A2) were 2.68 to 5.01 fold compared with solvent control (Table 51).

Table 50: Effects of AN8323 on CYP1A2 mRNA level in human hepatocytes

AN8323 (μM)	CYP1A2 mRNA Average ΔC_T	Mean	$\Delta\Delta C_T$	Fold[a]	Percent of Positive Control
		Donor 2			
0[b]	10.9	10.2	0.00	1.00	14.7
	10.3				
	9.46				
0.1	10.7	10.4	0.200	0.871	12.8
	9.55				
	11.0				
1	10.5	10.3	0.0815	0.945	13.9
	9.61				
	10.8				
10	10.0	9.48	-0.730	1.66	24.4
	10.2				
	8.24				
50	8.36	9.20	-1.01	2.02	29.7
	9.09				
	10.1				
		Donor 3			
0[b]	8.84	8.65	0.00	1.00	34.1
	8.18				
	8.93				
0.1	8.86	8.84	0.195	0.874	29.8
	9.00				
	8.67				
1	8.98	8.49	-0.157	1.11	38.0
	7.69				
	8.82				
10	8.78	8.82	0.175	0.886	30.2
	9.19				
	8.51				
50	7.91	7.77	-0.880	1.84	62.8
	8.16				
	7.24				
		Donor 4			
0[b]	10.6	9.09	0.00	1.00	11.5
	8.43				
	8.23				
0.1	11.8	11.7	2.61	0.164	1.89
	11.5				
	11.8				
1	11.6	11.6	2.56	0.170	1.96
	11.7				
	11.6				
10	10.5	10.5	1.37	0.387	4.46
	10.8				
	10.0				
50	9.16	9.12	0.0250	0.983	11.3
	8.61				
	9.57				

a Fold induction is calculated relative to the vehicle control.
b Vehicle control is supplemented hepatocyte maintenance medium (sHMM).

Table 51: Effects of AN8323 on phenacetin O-deethylase activity (CYP1A2 substrate) in human hepatocytes

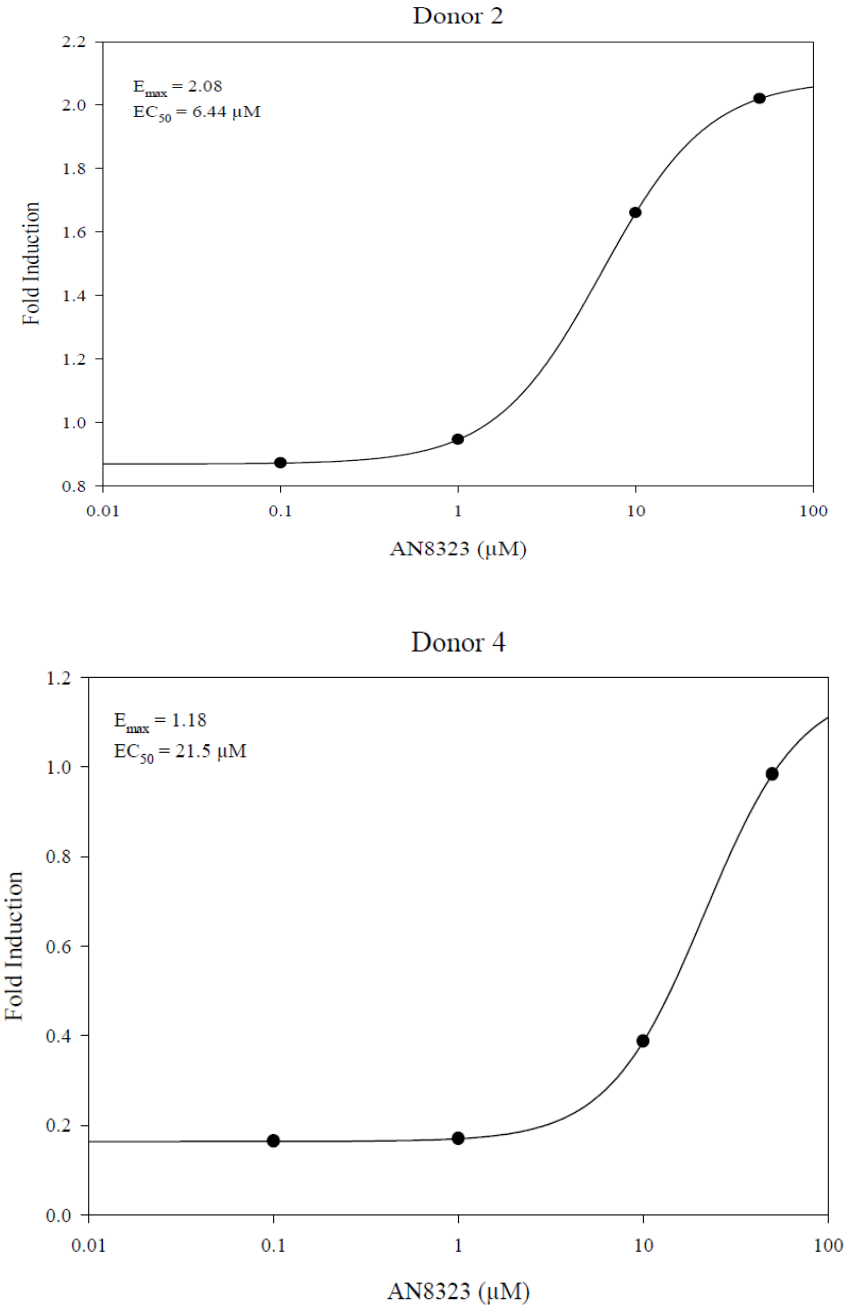
AN8323 (μM)	Acetaminophen (μM)	Activity (pmol/min/million cells) Replicate	Mean	SD	Fold[a]	Percent of Positive Control
		Donor 2				
0[b]	0.145	4.03	4.12	0.137	NA	NA
	0.146	4.06				
	0.154	4.28				
0.1	0.137	3.81	3.80	0.181	0.921	-0.373
	0.143	3.97				
	0.130	3.61				
1	0.138	3.83	4.02	0.212	0.975	-0.117
	0.153	4.25				
	0.143	3.97				
10	0.211	5.86	5.87	0.0976	1.42	2.01
	0.215	5.97				
	0.208	5.78				
50	0.480	13.3	13.4	0.598	3.24	10.6
	0.503	14.0				
	0.460	12.8				
		Donor 3				
0[b]	0.245	6.81	6.07	0.766	NA	NA
	0.221	6.14				
	0.190	5.28				
0.1	0.185	5.14	5.48	0.500	0.902	-0.362
	0.189	5.25				
	0.218	6.06				
1	0.270	7.50	6.53	0.882	1.07	0.277
	0.208	5.78				
	0.227	6.31				
10	0.323	8.97	8.46	1.87	1.39	1.46
	0.230	6.39				
	0.361	10.0				
50	0.584	16.2	16.3	0.946	2.68	6.22
	0.553	15.4				
	0.621	17.3				
		Donor 4				
0[b]	0.0521	1.45	1.45	0.163	NA	NA
	0.0462	1.28				
	0.0579	1.61				
0.1	0.0308	0.856	1.18	0.282	0.813	-0.794
	0.0462	1.28				
	0.0500	1.39				
1	0.0661	1.84	1.81	0.0417	1.25	1.07
	0.0661	1.84				
	0.0635	1.76				
10	0.0754	2.09	2.15	0.0510	1.49	2.08
	0.0783	2.18				
	0.0788	2.19				
50	0.274	7.61	7.25	0.749	5.01	17.1
	0.230	6.39				
	0.279	7.75				

NA Not applicable.
SD Standard deviation.
a Fold induction is calculated relative to the vehicle control.
b Vehicle control is supplemented hepatocyte maintenance medium (sHMM).

Reviewer's comments: *From the fold change data in Table 50, Donor 3 was bracketed between Donor 2 and Donor 4. The same effect was observed with a known substrate of CYP1A2 (as shown in Table 51).*

The applicant assessed the maximum induction (Emax) of CYP1A2 was 2.08 and 1.18 fold in hepatocytes from Donors 2 and 4, respectively, with corresponding EC_{50} values of 6.44 and 21.5 µM, respectively (Figure 30).

Figure 30: Concentration-response curves for CYP1A2 mRNA expression in AN8323-treated Hepatocytes



Reviewer comments: *The applicant did not assess the Emax and EC_{50} values for Donor 3. However this reviewer notes that the fold change values for Donor 3 were bracketed in between Donor 2 and Donor 4. Hence further assessment using data from Donor 2 and Donor 4 would be applicable to Donor 3.*

AN8323 produced no substantive increases in mRNA expression or in cytochrome P450 activities for CYP2B6 and CYP3A4/5 compared with solvent and positive control inducers for each cytochrome P450. The fold increases in CYP2B6 and CYP3A4/5 activities were less than 1% of that observed for the positive control inducers.

Flumazenil (non-inducer) produced no increases in mRNA expression or cytochrome P450 activity for CYP1A2, CYP2B6, and CYP3A4/5 in hepatocytes from any donor.

Reviewer comments: *For CYP1A2, this reviewer calculated the R_3 values using the following equation and the results are shown in Table 50.*

$$R_3 = 1/(1 + d \times E_{max} \times [I]/(EC_{50} + [I]))$$

Table 50: R3 values estimation

Donor #	Emax	EC50	R3
2	2.08	6.44 µM	0.616
4	1.18	21.5 µM	0.379

[I] = 24.10 µM [This is the mean Cmax of AN8323 observed in the maximal use PK trial (AN2728-AD-102)]
d = 1 [d is a scailing factor and is assumed to be 1 for the basic model, as per the Draft Drug Interaction Guidance – Feb 2012]

Since R_3 values are below the threshold of 0.9, the results indicate that AN8323 is likely to be an inducer of CYP1A2.

Mechanistic model for induction and calculation of AUCR: *This reviewer used the equation provided in the Draft Drug Interaction Guidance and modified it as described below.*

Following equation is provided in the Draft Guidance for calculating AUCR.

Equation 1:

$$\mathbf{AUCR} = \left(\frac{1}{[A_g \times B_g \times C_g] \times (1 - F_g) + F_g} \right) \times \left(\frac{1}{[A_h \times B_h \times C_h] \times f_m + (1 - f_m)} \right)$$

In Equation 1 above, the subscript 'g' is for gut and 'h' is for hepatic.

This model takes into account detailed drug disposition and drug interaction mechanisms such as bioavailability in gut and liver, fractional metabolism, and inhibition and induction parameters.

Because crisaborole is administered by topical dermal route, this reviewer has assumed that the drug does not reach the gut. Thus, for the purpose of estimating the AUCR for crisaborole (downstream metabolite AN8323), this reviewer has adapted the above model and excluded the gut effect. Since in-vitro studies have not indicated AN8323 as a time-dependent inhibitor, the parameter 'B_h' is assumed to be 1. Hence the modified AUCR equation will be considering only hepatic reversible inhibition 'A_h' and induction 'C_h' as shown in Equation 2 below.

Equation 2:

$$AUCR = \frac{1}{([A_h \times C_h]) \times f_m + (1 - f_m)}$$

Where:

$$A_h = \frac{1}{1 + \frac{[I]_h}{K_i}}$$

$$C_h = 1 + \frac{d \times E_{max} \times [I]_h}{[I]_h + EC_{50}}$$

In the equation above 'f_m' is the fraction of systemic clearance of the substrate medicated by CYP enzyme that is subject to inhibition/induction and this value is 1 for all CYP enzymes except for CYP3A4 where this value is 0.93. The parameter 'd' is the induction scaling factor of in-vitro to in-vivo for individual hepatocyte lots. This parameter is unknown and this reviewer has assumed this to be 1.

Hepatic exposure to orally-administered drugs is through the systemic circulation and via the portal vein following gut absorption. Because crisaborole is administered by dermal route, hepatic exposure is only through systemic drug levels in blood, and the inhibitor concentration in the liver [I]h is equal to the unbound crisaboorole (in this case the unbound metabolite AN8323)in plasma (blood). AN8323 plasma protein binding was 99% when measured in vitro in human plasma. Hence the value of fraction unbound ($f_{u,b}$)in Equation 3 below will be (100-99)/100 = 0.01.

Equation 3:

$[I]_h = f_{u,b} \times [I]_{max,b} = 0.01 \times 24.1\ \mu M = 0.241\ \mu M$

The Ki values used in the model was calculated using the IC_{50} value for CYP1A2 shown in Table 47. The Ki value was calculated as $IC_{50}/2 = 49.3\ \mu M/2 = 24.65\ \mu M$. The E_{max} and EC_{50} values used to calculate 'C_h' value are shown in Table 50 value.

Based on the mechanistic model the AUCR values were calculated by using Equation 2 and the results showed that the AUCR values were 0.971 and 0.997 for Donor 2 and 4, respectively. These values are more than the threshold of 0.8 (i.e. between 0.8 and 1.25) which would indicate that AN8323 is not expected to induce CYP1A2.

In conclusion, the downstream metabolite AN8323 is not expected to induce CYP1A2, 2B6 and 3A4 under the conditions of clinical use.

Study 003-NCL PK-050-01: Evaluation of AN8323 as a substrate and inhibitor of a panel of human drug transporters

Objective: The substrate and inhibition potential of AN8323, the major downstream metabolite of AN2728, for human uptake transporters OAT1, OAT3, OCT2, OATP1B1, and OATP1B3 and human efflux transporters P-gp and BCRP was assessed using cell models specific to each transporter.

Method: For assessment of substrate, AN8323 (1 and 10 µM) was incubated with Chinese hamster ovary (CHO) cells individually expressing OAT1, OAT3, OCT2, and OATP1B3 as well as vector control, with Human embryonic kidney (HEK293) cells expressing OATP1B1, and in Caco-2 cells for assessing P-gp and BCRP-mediated transport.

To assess transporter-mediated uptake or efflux, incubations were also conducted in the absence or presence of transporter selective inhibitors. For inhibition assessment, AN8323 (5 and 50 µM) was incubated with CHO, HEK293, and Caco-2 cells mediating the transport of positive control substrates as shown in the Table 52.

Table 52: Positive control substrates

Transporter	Known Substrate (µM)	Known Inhibitor (µM)
OAT1	^{14}C-para-Aminohippurate (1)	Probenecid (200)
OAT3	^{3}H-Estrone-3-sulfate (1)	Probenecid (200)
OCT2	^{14}C-Tetraethylammonium (1)	Quinidine (256)
OATP1B1	^{3}H-Estradiol-17β-D-glucuronide (0.5)	Cyclosporine A (10)
OATP1B3	^{3}H-Cholecystokinin octapeptide (1)	Cyclosporine A (10)
P-gp	^{3}H-Digoxin (1)	Zosuquidar (2)
BCRP	^{3}H-Estrone-3-sulfate (0.1)	Ko143 (1)

Note: The vector control was incubated with each substrate at the same concentrations as the transporters.

Results: AN8323 was not a substrate for OCT2 or OATP1B3. AN8323 was a weak substrate for uptake transporters OAT1 and OAT3 and showed maximum 2.77- and 2.96-fold uptake normalized to vector control, respectively, which was inhibited approximately 81% and 88% by probenecid, respectively, suggesting that the AN8323 uptake was transporter mediated. AN8323 showed a maximum 2.41-fold uptake in OATP1B1-expressing HEK293 cells normalized to vector control, which cyclosporine A decreased by approximately 26%, suggesting that AN8323 may be a weak substrate for OATP1B1.

In Caco-2 cells, AN8323 efflux ratios ranged from 18.3 to 35.9 for the low AN8323 concentration (1 µM) and from 14.1 to 30.8 for the high concentration (10 µM). In the presence of the P-gp inhibitor zosuquidar, efflux ratios for AN8323 decreased to 1.66 to 5.51 at 1 µM AN8323 and to 4.12 to 12.3 at 10 µM AN8323, consistent with P-gp-mediated transport of AN8323. Efflux ratios for AN8323 in the presence of the BCRP

inhibitor, Ko143, ranged from 3.97 to 5.47 at 1 µM AN8323 and from 2.91 to 5.47 at 10 µM AN8323, consistent with BCRP-mediated transport of AN8323.

AN8323 did not inhibit OCT2 or OATP1B1. AN8323 inhibited OAT1, OAT3, and OATP1B3 uptake activities, with maximum inhibition of 73.1%, 75.5%, and 66.0%, respectively, at the highest AN8323 concentration (50 µM). Kinetic analyses showed that AN8323 was a competitive inhibitor of OAT3 and OATP1B3, with apparent Ki values of 9.79 µM and 70.7 µM, respectively. In addition, AN8323 inhibited P-gp- and BCRP-mediated transport of 3H-digoxin and 3H-estrone 3-sulfate, respectively, in a concentration-dependent manner. AN8323 decreased 3H-digoxin efflux ratios from 5.32 to 1.18 and 3H-estrone 3-sulfate efflux ratios from 4.30 to 1.48. The summary of AN8323 as a substrate and inhibitor of selected human uptake and efflux transporters is shown in Table 53.

Table 53: Summary of results

			DDI Potential/Clinical Relevance	
Transporter	Substrate	Inhibitor	Substrate	Inhibitor
OAT1	Weak	Yes	None	NCR C_{max}[a]/K_i <0.1[b]
OAT3	Weak	Yes (K_i = 9.79 µM)	NCR	NCR C_{max}[a]/K_i = 0.024
OCT2	No	No	None	None
OATP1B3	No	Yes (K_i = 70.7 µM)	None	NCR R[c] = 1.003
OATP1B1	Weak	No	None	None
P-gp	Yes	Yes	NCR Topical dose for AN2728; AN8323 is an inactive metabolite	NCR
BCRP	Yes	Yes	NCR Topical dose for AN2728; AN8323 is an inactive metabolite	NCR

NCR Not clinically relevant.
a Unbound AN8323 C_{max} from study AN2728-AD-102 = 0.24 µM.
b Since the inhibition of OAT1 (K_i not determined) is less potent compared to OAT3, the unbound C_{max}/K_i is also expected to be <0.1.
c R-value = $1 + (fu \times I_{in,max}/IC_{50})$ where $I_{in,max} = C_{max} + (K_a \times Dose \times F_aF_g/Qh)$.

Observation: The substrate and inhibition potential of AN8323, the major metabolite of AN2728, for human uptake transporters OAT1, OAT3, OCT2, OATP1B1, and OATP1B3 and human efflux transporters P-gp and BCRP was assessed using cell models specific to each transporter. The data indicate that AN8323 is not a substrate of OCT2 and OATP1B3.

AN8323 was found to be a weak substrate for uptake transporters OAT1, OAT3, OATP1B1, and a strong substrate for efflux transporters P-gp and BCRP. AN8323 is not an inhibitor of OCT2 and OATP1B1 but was found to be an inhibitor of OAT1, OAT3, OATP1B3, P-gp, and BCRP.

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