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本項で使用する用語・略語

用語・略号	省略していない表現または定義
ABC	ATP-binding cassette
ABT	1-aminobenzotriazole：1-アミノベンゾトリアゾール
AI	accumulation index：累積係数
ATP	adenosine triphosphate：アデノシン三リン酸
AUC	area under the plasma concentration-time curve：血漿中濃度 - 時間曲線下面積
AUC_{inf}	area under the plasma concentration-time curve from zero to infinity： 0 時間から無限大時間までの血漿中濃度 - 時間曲線下面積
AUC_{last}	area under the plasma concentration-time curve from zero to time of last measurable concentration：0 時間から最終定量可能時間までの血漿中濃度 - 時間曲線下面積
AUCR	ratio of AUC：AUC 変化率
AUC_t	area under the plasma concentration-time curve from zero to time t： 0 時間から t 時間までの血漿中濃度 - 時間曲線下面積
BCRP	breast cancer resistance protein
BSA	bovine serum albumin：ウシ血清アルブミン
BSEP	bile salt export pump
C_b/C_p	ratio of drug concentration in blood/plasma：血液/血漿中濃度比
C_{max}	maximum plasma concentration：最高血漿中濃度
CYP	cytochrome P450
EDTA	ethylenediaminetetraacetic acid：エチレンジアミン四酢酸
EMA	European Medicines Agency：欧州医薬品庁
F	bioavailability：バイオアベイラビリティ
F_a	fraction of orally administered drug absorbed：吸収率
FDA	Food and Drug Administration：米国食品医薬品局
F_g	fraction of orally administered drug that escapes gut metabolism：消化管利用率
f_m	fraction metabolized
f_u	fraction unbound：非結合型分率
GLP	Good Laboratory Practice：医薬品の安全性に関する非臨床試験の実施の基準
HEK	human Embryonic Kidney：ヒト胎児腎臓
HPBCD	hydroxypropyl-β-cyclodextrin：ヒドロキシプロピル-β-シクロデキストリン
HPLC	high performance liquid chromatography：高速液体クロマトグラフィ
IC_{50}	50% inhibitory concentration：50%阻害濃度
IV	intravenous：静脈内
k_a	1^{st} order absorption rate constant：吸収速度定数
LC	liquid chromatography：液体クロマトグラフィー
LSC	liquid scintillation counter：液体シンチレーションカウンター
MATE	multidrug and toxin extrusion
MDCK	Madin-Darby canine kidney
MHLW	Ministry of Health, Labour and Welfare：厚生労働省
mRNA	messenger ribonucleic acid：メッセンジャーRNA
MS/MS	tandem mass spectrometry：タンデム質量分析法
NADPH	nicotinamide adenine dinucleotide phosphate： ニコチンアミドアデニンジヌクレオチドリン酸
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
PBS	phosphate buffered saline：リン酸緩衝生理食塩水

PD	pharmacodynamics；薬力学
PEG400	polyethylene glycol 400：ポリエチレングリコール 400
P-gp	P-glycoprotein
PK	pharmacokinetics：薬物動態
PO	per os：経口
Q_h	hepatic blood flow：肝血流量
RAF	Relative activity factor
SLC	solute carrier
$t_{1/2}$	apparent terminal elimination half-life：終末相の見かけの消失半減期
TK	toxicokinetics：トキシコキネティクス
T_{max}	time of occurrence of C_{max}：最高血漿中濃度到達時間
UGT	uridine diphosphate glucuronosyl transferase：ウリジン二リン酸グルクロン酸転移酵素

パラメータの略号および算出法

略号・用語	定義および算出法
T_{max}	最高血漿中濃度到達時間：(hr)
C_{max}	最高血漿中濃度：(ng/mL)
AUC_t	ゼロから最終検出可能時点 (t) までの血漿中濃度－時間曲線下面積：(ng･hr/mL) 台形法を用いて算出した。 AUC_{24} はゼロから投与後 24 時間までの血漿中濃度－時間曲線下面積
AUC_{inf}	ゼロから無限時間まで外挿した血漿中濃度－時間曲線下面積：(ng･hr/mL) $AUC_{inf} = AUC_t + C_t / k_{el}$ C_t は最終検出可能時点 (t) の血漿中濃度，k_{el} は消失速度定数：(hr^{-1})
$t_{1/2}$	消失半減期：(hr)　以下の式を用いて算出した。 $t_{1/2} = \ln 2 / k_{el}$
CL	全身クリアランス：(mL/h/kg)　以下の式を用いて算出した。 CL= 投与量／AUC_{inf}
V_{ss}	定常状態における分布容積：(L/kg)　以下の式を用いて算出した。 $V_{ss} = CL / k_{el}$
F	バイオアベイラビリティ：(%)　以下の式を用いて算出した。 $\text{ﾊﾞｲｵｱﾍﾞｲﾗﾋﾞﾘﾃｨ}\ (\%) = \left(\frac{\text{経口投与したときの } AUC_{inf} \times \text{静脈内投与量}}{\text{静脈内投与したときの } AUC_{inf} \times \text{経口投与量}} \right) \times 100$
排泄率	尿中排泄率は以下の式により算出した。 $\text{尿中排泄率（%）} = \Sigma \left(\frac{\text{尿中薬物（放射能）濃度} \times \text{尿量}}{\text{投与量（放射能）}} \right) \times 100$ 糞中排泄率および胆汁中排泄率の算出も上式を用いて同様に算出した。
IC_{50}	IC_{50} 値は酵素反応を 50%阻害するときの被験薬物濃度：(μmol/L)
P_{app}	In vitro 細胞膜透過試験における被験薬物の見かけの透過係数：($\times 10^{-6}$ cm/sec) 頂側膜側に被験薬物を添加したときの頂側膜側から側底膜側への見かけの透過係数は $P_{appA \rightarrow B}$ と表し，側底膜側に被験薬物を添加したときの側底膜側から頂側膜側への見かけの透過係数は $P_{appB \rightarrow A}$ と表した。

1. まとめ

エトラシモドの薬物動態について，種々の in vivo および in vitro 試験を実施して評価した。エトラシモドは APD334（遊離酸）の一般名であり，AR401959 とも記載され，非臨床試験では L-アルギニン塩（エトラシモド L-アルギニン）または遊離酸として投与した。In vitro 代謝試験は，肝細胞画分，肝細胞ならびに遺伝子組み換えの P450 分子種および UGT 分子種を用いて実施した。In vivo 薬物動態試験は，BALB/c マウス，CD-1 マウス，Sprague Dawley ラット，Long-Evans ラット，ビーグル犬およびカニクイザルを用いて実施した。In vivo 薬物動態試験で用いた動物種は，Long-Evans 有色ラットおよびカニクイザルを除き，非臨床薬理試験および毒性試験でも用いた。エトラシモドを用いた in vitro および in vivo 薬物動態試験の一覧を概要表（M2.6.5 Table 1）に示した。In vivo 試験で用いられた用量は遊離酸相当量に換算して表記した。

Caco-2 細胞単層膜を用いた in vitro 試験では，エトラシモドは高い膜透過性を示し，排出トランスポーターによる輸送はほとんど認められなかった。この結果と一致して，評価した動物種（BALB/c マウス，Sprague Dawley ラット，ビーグル犬およびカニクイザル）への単回経口および静脈内投与試験に基づくエトラシモドの経口バイオアベイラビリティは，43.8%～約 100%であった。検討したいずれの動物種においても，薬物動態に明らかな性差は認められなかった。全身クリアランスは概して低く，評価したすべての動物種で肝血流量の 4%以下であり，雄ラットで最も高く，雄イヌで最も低かった。単回静脈内投与後の血漿中 $t_{1/2}$ は，8 時間（ラットおよびサル）～31.1 時間（イヌ）であった。エトラシモドの V_{ss} はすべての動物種で総体液量と同等かそれ以上であり，全身への分布が示唆された。反復投与毒性試験でエトラシモドを 1 日 1 回投与したところ，累積係数（AI）は 0.453（報告書番号 APD334.TOX.008）～6.23（報告書番号 TX[redacted]008）であった。経口投与時の曝露量は，ラット（1～250 mg/kg/日）では用量に比例して増加したが，イヌ（20～300 mg/kg/日）では用量比を下回って増加した。

In vitro 血漿蛋白結合試験では，エトラシモドは非臨床動物種の血漿中で 95%超，ヒト血漿中では 97.9%の蛋白結合率を示した。ラット試料を用いた in vitro 血球移行性試験において，エトラシモドは主として血漿成分に分布した。10 mg/kg の［^{14}C］エトラシモドを雄雌の Sprague Dawley（アルビノ）ラットおよび Long-Evans（有色）ラットに単回経口投与した後の組織分布は速やかであり，投与後 0.5 時間の初回試料採取時に放射能が検出され，投与後 4～8 時間に C_{max} に到達した。雌雄の Sprague Dawley ラットおよび Long-Evans ラットで，放射能は消化管，消失器官（肝臓・腎臓），心筋，分泌腺，褐色脂肪および脳室周囲以外の中枢神経系組織（脊髄，小脳，大脳，延髄および嗅脳）を含む複数の臓器で認められた。Long-Evans（有色）ラットでは，メラニン含有組織への顕著な放射能の蓄積は認められなかった。Sprague Dawley ラットおよび Long-Evans ラットのいずれにおいても，［^{14}C］エトラシモド由来放射能は血液脳関門，血液精巣関門および血液卵胞関門を通過した。

マウス，ラット，イヌ，サルおよびヒトの肝ミクロソームを用いた in vitro 代謝試験では，エトラシモドの代謝速度は遅いことが示された。ラット，イヌ，サルおよびヒトの肝細胞と［^{14}C］エトラシモドをインキュベートしたときの代謝物プロファイルは類似していたが，代謝物の相対比率は異なっていた。ラットおよびイヌに［^{14}C］エトラシモドを経口投与したときの代謝物プロファイリングにより，P450 酵素による酸化および UGT 酵素と硫酸転移酵素による抱合反応の代謝経路が認められた。エトラシモド 2～4 mg を経口服用した thorough QT 試験の被験者（APD334-008 試験）および［^{14}C］エトラシモド（2 mg）を経口服用した被検者（APD334-107

試験）の血中代謝物は，ラットおよびイヌで認められた代謝物と類似していた。これらのことから，臨床試験を支持するためのエトラシモドおよびその代謝物の毒性を評価するために用いるげっ歯類および非げっ歯類として，ラットおよびイヌを選択することは適切であると考えた。

［^{14}C］エトラシモドを雌雄 Sprague Dawley ラットおよび雌雄ビーグル犬に経口投与したときの放射能の回収率は，それぞれ 97%超および 83%超であった。エトラシモドおよびその代謝物の主な排泄経路は胆汁中排泄であり，尿中排泄はわずかであった。

様々な動物種およびヒトの肝ミクロソームならびに組み換え P450 分子種を用いて実施した in vitro 試験の結果から，エトラシモドは CYP2C8，CYP2C9 および CYP3A4 によって主に代謝され，それらより程度は低いものの CYP2C19 および CYP2J2 によっても代謝され，また複数の UGT 分子種（UGT1A7，UGT1A1 および UGT1A4 等）により直接的または間接的に抱合されることが示された。エトラシモドは CYP1A2，CYP2B6，CYP2C9，CYP2C19，CYP2D6 または CYP3A4/5 を阻害しなかった（10 µmol/L のエトラシモドで阻害率 17%未満）。エトラシモドは CYP2C8 を可逆的に阻害したが（プローブ基質としてパクリタキセルおよびアモジアキンを用いたときの IC_{50} 値はそれぞれ 35 µmol/L および 4.0 µmol/L），時間依存的阻害作用は示さなかった。メカニズムに基づく静的薬物速度論モデルを用いた評価では，エトラシモドが臨床的に意味のある CYP2C8 阻害を介した薬物相互作用を引き起こす可能性は低いと考えられた（AUCR < 1.25，2.7.2.3 項）。ヒト肝細胞を用いた mRNA の発現レベルに基づく評価では，エトラシモドは CYP1A2，CYP2B6，CYP3A4，CYP2C8，CYP2C9 および CYP2C19 を誘導しなかった。

エトラシモドは P-gp，BCRP，OATP1B1/OATP1B3，OAT1/OAT3 および OCT1/OCT2 の基質とはならず，P-gp，BCRP，OATP1B1/OATP1B3，OAT1/OAT3，OCT1/OCT2，MATE1，MATE2-K および BSEP を臨床的に意味のある濃度で阻害しなかった。

雄 BALB/c マウス，Sprague Dawley ラット，ビーグル犬およびカニクイザルを用いた PK/PD モデリング試験において，血中リンパ球の用量依存的な減少が認められた。

2. 分析法

2.1. 放射性同位元素標識化合物

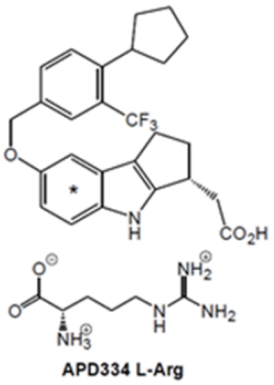
報告書番号 APD334.DMPK.002，APD334.DMPK.001

［^{14}C］エトラシモドを用いたラットおよびイヌの試験（報告書番号 APD334.DMPK.002, APD334.DMPK.001）について，組織，体液および装置からすべての残留放射能を回収するための方法を開発した（報告書番号 APD334.DMPK.002）。すなわち，シンチレーションカクテルと混合して LSC で分析を行う前に，血液サンプルを可溶化し，EDTA とインキュベートした後，過酸化水素で脱色した。血漿は追加の処理を行わずにシンチレーションカクテルと混合した。胆汁および尿をアセトニトリルで 1:1 に希釈した。糞便をアセトニトリル（50:50 = v:v）でホモジネートし，次に 1N 水酸化ナトリウムと混合し，溶解するまで加熱した後，シンチレーションカクテルと混合した。ケージリンス，ジャケットリンスおよびケージ洗浄液は，追加の処理を行わずにシンチレーションカクテルと混合した。胆管カニューレおよびケージワイパーは十分なアセトニトリルに浸して放射性試料を抽出してから直接分析した。少なくとも 5 分間または 100,000 カウントで LSC 測定を実施し，サンプルが十分な場合には 2 回分析した。同一検体を 2 回分析したときに，その平均値からの差が 10%を超える場合，検体を再度ホモジネートし，再分析を行った。

全身オートラジオグラフィーに供する組織は凍結し，40 µm の切片を作製した後，［^{14}C］エトラシモドを添加した血液標準品とともに放射線画像解析装置で放射能を測定した。

[^{14}C]エトラシモド L-アルギニンの構造および標識位置を Figure 1 に示す。

Figure 1.　[^{14}C]エトラシモド L-アルギニンの構造



APD334 L-Arg

*[^{14}C]標識位置

2.2. HPLC/MS/MS による定量法

報告書番号 APD334.MV.001，CA16857-01，AA95243-01，CA16858-01
CA15809-01，AA96474-01，AA95242-01，概要表 2.6.5.2

FDA ガイダンス（FDA 2001[1], FDA 2018[2]）および EMA ガイドライン（EMA 2012a[3]）に基づき，マウス，ラット，ウサギおよびイヌ血漿中のエトラシモドを定量するために，LC-MS/MS を用いた高感度かつ特異的な分析法を開発し，バリデートした。これらの方法を用いて，マウス，ラット，ウサギおよびイヌのトキシコキネティクス（TK）解析（GLP 適用）で用いる血漿中エトラシモド濃度を測定した（Table 1）。内標準物質としてエトラシモドの構造類似化合物（AR436434）または d_9-エトラシモドを使用した。

GLP 非適用試験では，バリデートされた分析法と同様の適切な LC-MS/MS 法を，バリデーションを行わずに試験ごとに用いた。GLP 非適用試験では，各試験報告書に方法および試験実施施設を記載した。

Table 1.　非臨床検体中のエトラシモドの定量に用いた HPLC/MS/MS 法

動物種 検体	抗凝固剤	サンプル量(µL)	分析範囲 [a]	内標準物質	サンプル処理	凍結融解安定性	バリデーション報告書番号
マウス 血漿	K_2EDTA	25	0.50-250 µg/mL	d_9-エトラシモド	蛋白質沈殿	-70°C，121 日間	APD334.MV.001 (900-170)[b]
ラット 血漿	K_2EDTA	25	0.005-2.5 µg/mL	d_9-エトラシモド	蛋白質沈殿	-80°C，20 日間	CA16857-01
	K_2EDTA	20	0.100-25.0 µg/mL	AR436434	蛋白質沈殿	-20°C，21 日間 -80°C，1390 日間	AA95243-01
ウサギ 血漿	K_2EDTA	25	0.005-2.5 µg/mL	d_9-エトラシモド	蛋白質沈殿	-80°C，91 日間	CA16858-01
	K_2EDTA	25	0.250-100 µg/mL	d_9-エトラシモド	蛋白質沈殿	-80°C，304 日間	CA15809-01
イヌ 血漿	K_2EDTA	50	0.001-0.5 µg/mL	AR436434	蛋白質沈殿	-80°C，307 日間	AA96474-01
	K_2EDTA	20	1.00-250 µg/mL	AR436434	蛋白質沈殿	-20°C，17 日間 -80°C，307 日間	AA95242-01

[a] 分析範囲の下限はアッセイの定量下限を示す。

[b] 報告書番号 APD334.TOX.001

3. 吸収

標準的な in vitro および in vivo 試験において，エトラシモドの吸収に関する薬物動態を検討した。エトラシモドの透過性および吸収を評価するために実施した試験一覧を M2.6.5 Table 1 に示す。

3.1. 膜透過性

報告書番号 PDM-[redacted]-286，APD334.DMPK.007，概要表 2.6.5.3 Table 3，Table 4

Caco-2 細胞単層膜を用いて，エトラシモドの双方向の膜透過性を評価した。

0.5～20 µmol/L の濃度範囲で，P_{appAB} は 20.6 × 10^{-6} cm/s 以上の高値を示した。0.5，5.0 および 20 µmol/L の濃度範囲での Efflux ratio（P_{appBA}/P_{appAB}）は，それぞれ 0.81，1.61 および 2.01 であった。Efflux ratio（2 以下）に基づくと，エトラシモドは排出トランスポーターである P-gp または BCRP の基質とはならないと考えられた。

以上のことから，エトラシモドは高い膜透過性を示し，排出トランスポーターである P-gp および BCRP による輸送をほとんど受けないと考えられた。

3.2. 単回投与時の血漿中濃度

雄の BALB/c マウス，Sprague Dawley ラット，ビーグル犬およびカニクイザルを用いて，単回静脈内投与および経口投与後の血漿中エトラシモド濃度推移を検討した。

3.2.1. 静脈内投与

報告書番号 MPR-[redacted]-014，MPR-[redacted]-013，MPR-[redacted]-015，MPR-[redacted]-016，概要表 2.6.5.3 Table 5

雄の BALB/c マウス，Sprague Dawley ラット，ビーグル犬およびカニクイザルに 1.0 mg/kg のエトラシモドを静脈内投与したときの PK パラメータを Table 2 に示す。マウスの PK パラメータは，時間ごとに異なる動物から採取したサンプルの濃度を組み合わせて求めたため（スパースサンプリング），標準偏差は算出できなかった。$t_{1/2}$ に動物種間でばらつきがみられ，8 時間（ラット，サル）～31.1 時間（イヌ）であった（Table 2）。いずれの動物種においても CL は低度であり，肝血流量の 4%以下であった [4]。総体液量と比較したときの V_{ss} は，げっ歯類では 2～3 倍であり，イヌでは同等であり，サルでは約 60%であった [4]。

Table 2.　マウス，ラット，イヌおよびサルにおける単回静脈内投与後の薬物動態パラメータ

動物種	投与量 (mg/kg)	$t_{1/2}$ (h)	CL (L/h/kg)	V_{ss} (L/kg)	AUC_{inf} (µg•h/mL)
マウス [a]	1.0	12.1	0.110	1.93	9.13
ラット [b]	1.0	8.02 ± 1.10	0.145 ± 0.024	1.61 ± 0.38	7.03 ± 1.24
イヌ [c]	1.0	31.1 ± 3.8	0.014 ± 0.003	0.609 ± 0.103	74.5 ± 15.2
サル [d]	1.0	8.00 ± 1.23	0.059 ± 0.012	0.433 ± 0.022	17.6 ± 4.0

[a] 雄 BALB/c マウス，絶食，溶媒：40% HPBCD，スパースサンプリング，平均値，n=3/時点（報告書番号 MPR-[redacted]-014，Table 4）

[b] 雄 Sprague Dawley ラット，絶食，溶媒：100% PEG400，各個体から経時的に採血，平均値±標準偏差，n=3（報告書番号 MPR-[redacted]-013，Table 4）

[c] 雄ビーグル犬，絶食，溶媒：100% PEG400，各個体から経時的に採血，平均値±標準偏差，n=3（報告書番号 MPR-[redacted]-015，Table 4）

[d] 雄カニクイザル，絶食，溶媒：100% PEG400，各個体から経時的に採血，平均値±標準偏差，n=3（報告書番号 MPR-[redacted]-016，Table 4）

3.2.2. 経口投与

報告書番号 MPR-[redacted]-014，MPR-[redacted]-013，MPR-[redacted]-015，MPR-[redacted]-016，TX[redacted]010
APD334.TOX.001，TX[redacted]009，TX[redacted]019，TX[redacted]008
概要表 2.6.5.3，2.6.5.4，2.6.5.7

雄の BALB/c マウスおよび Sprague Dawley ラットに 1.0 mg/kg のエトラシモドを，雄のビーグル犬およびカニクイザルに 3.0 mg/kg のエトラシモドを単回経口投与したときの PK パラメータを Table 3 に示す。マウスの PK パラメータは，スパースサンプリングのため，標準偏差は算出できなかった。エトラシモドの経口投与後の吸収は中等度であった。T_{max} は，マウス，ラットおよびイヌでは同程度で約 7～8 時間，サルではより短く約 3 時間であった。経口バイオアベイラビリティは 43.8%～約 100%であった（Table 3）。

Table 3.　マウス，ラット，イヌ，サルおよびヒトにおける単回経口投与後の薬物動態パラメータ

動物種	投与量 (mg/kg)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (µg/mL)	AUC_{inf} (µg•h/mL)	F (%)
マウス [a]	1.0	12.3	8.00	0.780	10.3	~100
ラット [b]	1.0	9.64 ± 3.33	7.33 ± 2.31	0.218 ± 0.130	3.79 ± 0.90	54.0 ± 12.8
イヌ [c]	3.0	28.8 ± 3.1	7.33 ± 1.15	3.71 ± 0.12	164 ± 25	73.2 ± 11.0
サル [d]	3.0	6.37 ± 0.68	3.33 ± 1.15	2.87 ± 0.77	23.1 ± 8.1	43.8 ± 15.3
ヒト [e]	2.0 mg（固定用量）	26.2 ± 7.3	8.0 (2.0 – 12.0)	0.0438 ± 0.0129	1.85 ± 0.472	ND

F (%) = ([AUC_{inf} (PO) × 投与量 (IV)] / [AUC_{inf} (IV) × 投与量 (PO)]) × 100

[a] 雄 BALB/c マウス，絶食，溶媒：0.5%メチルセルロース，スパースサンプリング，平均値，n=3/時点（報告書番号 MPR-[redacted]-014，Table 4）

[b] 雄 Sprague Dawley ラット，絶食，溶媒：0.5%メチルセルロース，各個体から経時的に採血，平均値±標準偏差，n=3（報告書番号 MPR-[redacted]-013，Table 5）

[c] 雄ビーグル犬，絶食，溶媒：0.5%メチルセルロース，各個体から経時的に採血，平均値±標準偏差，n=3（報告書番号 MPR-[redacted]-015，Table 5）

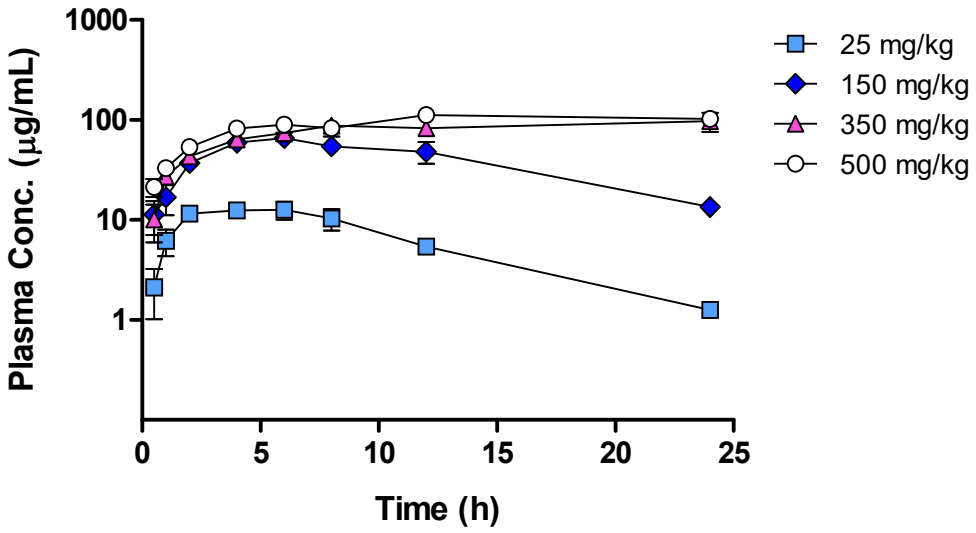
[d] 雄カニクイザル，絶食，溶媒：0.5%メチルセルロース，各個体から経時的に採血，平均値±標準偏差，n=3（報告書番号 MPR-[redacted]-016，Table 5）

[e] 男女健康被検者，絶食，エトラシモドカプセル，各個体から経時的に採血，試験 1 日，平均値±標準偏差［T_{max} のみ中央値（最小値-最大値）で示す］，n=9～10 (APD334-002 試験，Table 7)

雌雄のマウス，ラットおよびイヌを用いた GLP 適用反復経口投与毒性試験の初回投与後に，血漿中エトラシモド濃度推移を検討した（Table 4，全ての TK 結果については概要表 2.6.5 を参照）。雌雄マウスに 2～300 mg/kg/日，雌雄ラットに 2～500 mg/kg/日およびイヌに 0.02～15 mg/kg/日の範囲で経口投与したところ，初回投与時の C_{max} および $AUC_{last(24)}$は概ね用量比例的に増加した。高用量（20～300 mg/kg/日）を投与したイヌでは，曝露量の増加は用量比を下回っていた。300 mg/kg/日超を投与したイヌでは，用量比例的な曝露量の増加は認められなかったが，これらの用量では顕著な用量制限毒性が認められた。

試験，動物種および用量を通して，単回経口投与後の血漿中エトラシモド濃度に一貫した性差は認められなかった。ラットでは，用量増加に伴い T_{max} が増加し，見かけの吸収相の延長と一致していた（Figure 2，Table 4）。

Figure 2.　雄Sprague Dawleyラットにおける単回経口投与後のエトラシモドの血漿中濃度



平均値±標準偏差，n=3，Conc：濃度

出典：報告書番号TX[redacted]010, Appendix J (TK Report, MPR-[redacted]-008), Figure 1

Table 4.　動物にエトラシモドを単回経口投与したときの TK パラメータ

動物種	用量 (mg/kg)	$t_{1/2}$ (h)		T_{max} (h)		C_{max} (µg/mL)		AUC_{last} (µg·h/mL)			AUC_{last} 用量比例性	
		雄	雌	雄	雌	雄	雌	雄	雌	雌:雄比	雄	雌
マウス[a]	20	NR	NR	4.00	2.00	16.5	10.6	237	167	0.71	あり	あり
	60	NR	NR	4.00	4.00	56.0	40.4	778	669	0.86		
	200	NR	NR	12.00	4.00	152	127	2800	1120	0.40		
ラット[b]	25	6.88	4.98	4.00	4.00	10.6	13.1	114	155	1.36	あり	あり
	75	8.19	7.93	6.00	6.00	32.4	50.1	385	556	1.44		
	150	5.96	12.8	8.00	6.00	54.8	78.0	803	1150	1.43		
	250	13.6	31.0	12.00	8.00	81.2	91.1	1310	18500	1.41		
ウサギ[c]	2	NA	NR	NA	6.00 ± 2.31	NA	0.258 ± 0.0792	NA	2.21 ± 0.493	NA	NA	あり
	10	NA	NR	NA	4.00 ± 0.00	NA	1.80 ± 0.29	NA	14.4 ± 3.8	NA		
	20	NA	NR	NA	4.00 ± 0.00	NA	4.89 ± 1.30	NA	35.0 ± 11.5	NA		
イヌ[d]	2	32.2 ± 11.1	31.6 ± 13.8	10.0 ± 4.0	8.00 ± 2.83	2.03 ± 0.55	1.88 ± 0.19	38.0 ± 7.2	36.9 ± 3.4	0.97	あり	あり
	5	25.7 ± 4.5	33.7 ± 6.4	5.50 ± 1.91	6.50 ± 3.79	12.2 ± 4.3	5.23 ± 1.78	173 ± 52	98.8 ± 28.3	0.57		
	10	24.8 ± 8.0	22.4 ± 1.3	11.3 ± 1.6	8.33 ± 2.94	11.5 ± 2.4	10.2 ± 2.3	213 ± 37	191 ± 35	0.90		
	15	32.0 ± 7.1	29.2 ± 6.5	9.33 ± 3.01	8.00 ± 8.10	22.5 ± 6.6	19.8 ± 3.0	418 ± 107	379 ± 47	0.91		

[a] CD-1 マウス，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 APD334.TOX.001，Appendix 11，Table 1）

[b] Sprague Dawley ラット，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 TX[redacted]009，Appendix K，Table 12）

[c] New Zealand White ウサギ，非絶食，各個体から経時的に採血，平均値±標準偏差，交配後雌 n=4/群，妊娠 7 日に初回投与（報告書番号 TX[redacted]019，Appendix G，Table 7-9）

[d] ビーグル犬，非絶食，各個体から経時的に採血，平均値±標準偏差，n=4/性/群（2 および 5 mg/kg），n=6/性/群（10 および 15 mg/kg）(報告書番号 TX[redacted]008，Appendix L, Table 13-20）

3.3. 反復投与時の血漿中濃度

報告書番号 TX[redacted]008，APD334.TOX.001，APD334.TOX.003，TX[redacted]009
APD334.TOX.011，APD334.TOX.002，TX[redacted]019，TX[redacted]008
概要表 2.6.5.4，2.6.5.7

CD-1 マウス，Sprague Dawley ラット，妊娠 New Zealand White ウサギおよびビーグル犬を用いた反復経口投与毒性試験の TK から反復投与時の血漿中エトラシモド濃度について検討した。結果の要約を Table 5 に，詳細を概要表 2.6.5 に示す。

定常状態における曝露量（AUC_{last}，最終投与日）を単回投与後の曝露量の推定値（AUC_{last}，投与初日）と比較し，AI を算出した。いずれの動物種においても，Table 6 に示した用量範囲における AI は雌雄ともに 0.67～3.76 の範囲であった。雌イヌに 80 mg/kg/日を 28 日間投与したときの AI は 6.23 で，最も高値を示した（報告書番号 TX[redacted]008）。

雌雄マウスに 28 日間反復経口投与したところ，評価した用量範囲における AI は 0.858～1.31 であった（報告書番号 TX[redacted]003）。3 ヵ月間反復経口投与後，評価した用量範囲における雌雄の AI は 0.67～1.40 であった（Table 6）。一方，反復投与時の AI の閾値を 2 倍とした場合，動物種間で明らかな違いが認められた。雌雄マウスおよび雄ラットの AI はいずれも 1～2 であり，反復投与による明らかな蓄積性は認められず，定常状態に達していることが示唆された。雌ウサギに 1 日 1 回 14 日間経口投与した後の AI は 1 をわずかに下回り，反復投与により曝露量は低下する傾向が示唆された。しかしながら，雌ラットおよび雌雄イヌの AI は概して 2 を超えており（雌イヌでは 3 を超える），定常状態における蓄積性が示唆された。ラットと比較したとき，イヌではわずかに高い AI を示した（Table 6）。動物種間および性別間の AI の傾向によらず，いずれの用量においても，動物種内または性別内で用量に関連した明らかな AI の差は認められなかった。

マウス，ラット，妊娠ウサギおよびイヌに 1 日 1 回単回経口投与したときの TK 結果を踏まえると，いずれの動物種においても定常状態では T_{max} が短くなる傾向が認められ，イヌおよびラットでは定常状態で $t_{1/2}$ が長くなる可能性が示唆された（Table 4，Table 5）。ラットでは，定常状態において AUC_{last} の性差がより顕著に認められた。性差が認められた 2 試験の用量は異なるものの，すべての用量において，ラットに 182 日間および 364 日間投与したときの AUC_{last} の雌:雄比は 2 を超えていたのに対し（Table 5），初回投与後は 1.5 未満であった（Table 4）。雌ラットでは AI が雄ラットよりも高値であったことも踏まえると（Table 6），わずかな差ではあるものの，反復投与後の雌ラットにおいては，雄ラットよりも高い吸収率または低い CL を示す可能性が示唆される。

幼若ラットおよびラットを用いた胚・胎児発生に関する試験の TK 結果を概要表 2.6.5.4 および 2.6.5.7 にそれぞれ示した。妊娠ラットでは，妊娠 6，17 および授乳 20 日におけるエトラシモドの曝露量（C_{max} および AUC_{last}）は用量増加に伴い増加した（Table 6）。ラット出生児の血漿中エトラシモド濃度は母動物への投与用量増加に伴い増加する傾向が認められ，母動物の血漿中濃度に対する胎児の血漿中濃度の割合は 31.3%～73.1%であった。幼若ラットにおける TK 結果は，Table 5 に示したげっ歯類の TK 結果と類似していた。

Table 5.　動物にエトラシモドを反復経口投与したときの TK パラメータ

動物種 試験日	用量 (mg/kg)	$t_{1/2}$ (h)		T_{max} (h)		C_{max} (µg/mL)		AUC_{last} (µg·h/mL)			AUC_{last} 用量比例性	
		雄	雌	雄	雌	雄	雌	雄	雌	雌:雄比	雄	雌
マウス 90 日 [a]	20	NR	NR	2.00	2.00	22.4	14.9	331	220	0.67	なし	なし
	60	NR	NR	4.00	4.00	51.4	37.0	894	569	0.64		
	200	NR	NR	2.00	4.00	106	90.7	1870	1150	0.62		
マウス 364 日 [b]	2	NR	NR	4.00	12.0	2.40	2.86	39.5	44.6	1.13	あり	あり
	6	NR	NR	4.00	2.00	6.59	4.85	109	73.4	0.672		
	20	NR	NR	4.00	4.00	19.8	12.4	305	210	0.690		
ラット 182 日 [c]	25	5.09	6.32	2.00	2.00	21.5	45.7	210	442	2.10	あり	あり
	75	6.11	8.93	6.00	6.00	45.4	106	554	1650	2.98		
	150	5.84	10.9	8.00	6.00	113	201	1440	3180	2.21		
	250	11.6	9.94	6.00	8.00	159	275	2060	4470	2.17		
ラット 364 日 [d]	2	NR	NR	6.00	2.00	1.99	4.55	27.6	65.2	2.36	なし	なし
	6	NR	NR	4.00	4.00	5.19	8.44	68.2	147	2.16		
	20	NR	NR	6.00	6.00	12.1	24.2	172	387	2.25		
ウサギ 14 日 [e]	2	NA	NR	NA	4.00 ± 0.00	NA	0.234 ± 0.102	NA	1.70 ± 0.76	NA	NA	あり
	10	NA	NR	NA	5.00 ± 2.00	NA	1.40 ± 0.49	NA	12.6 ± 2.9	NA		
	20	NA	NR	NA	5.00 ± 2.00	NA	2.88 ± 0.72	NA	24.2 ± 2.9	NA		
イヌ 273 日 [f]	2	45.1 ± ID	51.6 ± 25.1	3.00 ± 2.00	2.75 ± 1.50	6.24 ± 1.81	6.80 ± 2.47	114 ± 36.5	128 ± 46	1.12	あり	あり
	5	39.7 ± ID	80.1 ± 5.2	4.00 ± 5.42	2.00 ± 1.41	14.4 ± 6.8	17.9 ± 3.5	269 ± 93	335 ± 60	1.25		
	10	42.8 ± ID	53.4 ± ID	2.25 ± 1.47	7.50 ± 8.48	27.3 ± 3.0	28.7 ± 4.8	542 ± 106	586 ± 112	1.08		
	15	31.4 ± 7.5	31.1 ± ID	1.25 ± 1.54	7.83 ± 8.82	46.9 ± 11.5	45.7 ± 11.0	879 ± 177	861 ± 170	0.98		

Table 5.　動物にエトラシモドを反復経口投与したときの TK パラメータ

動物種 試験日	用量 (mg/kg)	$t_{1/2}$ (h)		T_{max} (h)		C_{max} (µg/mL)		AUC_{last} (µg·h/mL)			AUC_{last} 用量比例性	
		雄	雌	雄	雌	雄	雌	雄	雌	雌:雄比	雄	雌

ID： n < 3 のため算出されなかった。

[a] CD-1 マウス，試験 90 日，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 APD334.TOX.001，Appendix 11，Table 1）

[b] CD-1 マウス，試験 364 日，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 APD334.TOX.003，Appendix 12，Table 3）

[c] Sprague Dawley ラット，試験 182 日，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 TX[redacted]009，Appendix K，Table 12）

[d] Sprague Dawley ラット，試験 364 日，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 APD334.TOX.002，Appendix 12，Table 1）

[e] 妊娠 New Zealand White ウサギ，試験 14 日（妊娠 20 日），非絶食，各個体から経時的に採血，平均値±標準偏差，交配後雌 n=4/時点，妊娠 7 日に初回投与（報告書番号 TX[redacted]019，Appendix G，Table 7-9）

[f] ビーグル犬，試験273日，非絶食，各個体から経時的に採血，平均値±標準偏差，n=4（2および5 mg/kg），n=6（10および15 mg/kg）（報告書番号 TX[redacted]008，Appendix L，Table 13-20）

Table 6.　動物にエトラシモドを反復経口投与したときの血漿中濃度および AI

動物種	試験日	用量 (mg/kg)	C_{max} (μg/mL)		AUC_{last} (μg·h/mL)		AI	
			雄	雌	雄	雌	雄	雌
マウス[a]	1 日	20	16.5	10.6	237	167	NA	NA
		60	56.0	40.4	778	669	NA	NA
		200	152	127	2800	1120	NA	NA
	90 日	20	22.4	14.9	331	220	1.40	1.32
		60	51.4	37.0	894	569	1.15	0.85
		200	106	90.7	1870	1150	0.67	1.03
ラット[b]	1 日	25	10.6	13.1	114	155	NA	NA
		75	32.4	50.1	385	556	NA	NA
		150	54.8	78.0	803	1150	NA	NA
		250	81.2	91.1	1310	1850	NA	NA
	182 日	25	21.5	45.7	210	442	1.84	2.85
		75	45.4	106	554	1650	1.40	2.97
		150	113	201	1440	3180	1.79	2.77
		250	159	275	2060	4470	1.57	2.42
ラット[c]	1 日（妊娠 6 日）	0.4	NA	0.339	NA	3.73	NA	NA
		2	NA	1.40	NA	16.6	NA	NA
		4	NA	4.00	NA	43.1	NA	NA
	12 日（妊娠 17 日）	0.4	NA	0.290	NA	4.32	NA	1.16
		2	NA	1.64	NA	21.6	NA	1.30
		4	NA	3.52	NA	51.2	NA	1.19
	授乳 20 日	0.4	NA	0.269	NA	2.39	NA	0.639
		2	NA	1.34	NA	11.4	NA	0.687
		4	NA	2.96	NA	36.7	NA	0.851
ウサギ[c]	1 日（妊娠 7 日）	2	NA	0.258	NA	2.21	NA	NA
		10	NA	1.80	NA	14.4	NA	NA
		20	NA	4.89	NA	35.0	NA	NA
	14 日（妊娠 20 日）	2	NA	0.234	NA	1.70	NA	0.786
		10	NA	1.40	NA	12.6	NA	0.962
		20	NA	2.88	NA	24.2	NA	0.742
イヌ[c]	1 日	2	2.03	1.88	38.0	36.9	NA	NA
		5	12.2	5.23	173	98.8	NA	NA
		10	11.5	10.2	213	191	NA	NA
		15	22.5	19.8	418	379	NA	NA
	273 日	2	6.24	6.80	114	128	2.94	3.43
		5	14.4	17.9	269	335	1.57	3.76
		10	27.3	28.7	542	586	2.57	3.16
		15	46.9	45.7	879	861	2.28	2.28

Table 6.　動物にエトラシモドを反復経口投与したときの血漿中濃度および AI

動物種	試験日	用量 (mg/kg)	C_{max} (µg/mL)		AUC_{last} (µg·h/mL)		AI	
			雄	雌	雄	雌	雄	雌

AI：$AUC_{last,\ 最終投与日}/AUC_{last,\ 投与初日}$

[a] CD-1 マウス，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 APD334.TOX.001，Appendix 11，Table 1）

[b] Sprague Dawley ラット，非絶食，スパースサンプリング，平均値，n=3/性/時点（報告書番号 TX[redacted]009，Appendix K，Table 12）

[c] 妊娠 Sprague Dawley ラット，非絶食，スパースサンプリング，平均値，交配後雌 n=4/時点，妊娠 6 日に初回投与，妊娠 6 日のデータには異常動物は含まない（報告書番号 APD334.TOX.011，Appendix 37，Table 2.2，Table 2.3，Table 2.4）

[d] 妊娠 New Zealand White ウサギ，非絶食，各個体から経時的に採血，平均値，交配後雌 n=4/時点，妊娠 7 日に初回投与（報告書番号 TX[redacted]019，Appendix G，Table 7-9）

[e] ビーグル犬，非絶食，各個体から経時的に採血，平均値，n=4（2および5 mg/kg），n=6（10および15 mg/kg）（報告書番号TX[redacted]008，Appendix L，Table 13-20）

4. 分布

5 つの in vitro 試験および 2 つの in vivo 試験で，エトラシモドの分布を検討した（Table 7）。In vitro 試験では，CD-1 マウス，Sprague Dawley ラット，New Zealand White ウサギ，ビーグル犬，カニクイザルおよびヒトの血漿を用いて血漿蛋白結合率を評価した。また，ラット，イヌ，サルおよびヒトの血液および血漿を用いて血液/血漿中濃度比（C_b/C_p）を評価した。In vivo 試験では，[^{14}C]エトラシモドを用い，ラットおよびイヌの組織分布を評価した。

Table 7.　エトラシモドの分布に関する試験一覧

試験名	試験系/動物種	報告書番号
In Vitro		
Determination of APD334 Plasma Protein Binding in Mouse, Rat, Rabbit, Dog, Monkey, and Human Plasma Using an Ultracentrifugation Method	Ex vivo 血漿： マウス（CD-1），ラット（Sprague Dawley），ウサギ（New Zealand White），イヌ（ビーグル），サル（カニクイ），ヒト	MPR-[redacted]-001
In Vitro Blood-to-Plasma Partitioning of [^{14}C]APD334 in Rat, Dog, Monkey and Human	Ex vivo 全血： ラット（Sprague Dawley），イヌ（ビーグル），サル（カニクイ），ヒト	APD334.DMPK.003
APD334 Microsomal Protein Binding	肝ミクロソーム： ヒト	APD334.DMPK.004
Determination of the Binding of APD334 to Human Serum Albumin, α_1-Acid Glycoprotein and Lipoproteins	血漿蛋白（ヒトの血清アルブミン，α_1-酸性糖蛋白質およびリポ蛋白： ヒト	ARE-R10656
Non-Specific Binding for APD334	HEK293 細胞： ヒト	[redacted]8101
In Vivo		
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats	ラット： Sprague Dawley ラット（胆管カニューレ処置および非処置），Long-Evans ラット	APD334.DMPK.002
Pharmacokinetics, Absorption, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs	イヌ（ビーグル）	APD334.DMPK.001

4.1. In vitro

報告書番号 MPR-[redacted]-001，APD334.DMPK.003，APD334.DMPK.004，ARE-R10656，[redacted]8101
概要表 2.6.5.6，2.6.5.8

雄の CD-1 マウス，Sprague Dawley ラット，New Zealand White ウサギ，ビーグル犬，カニクイザルおよびヒトにおけるエトラシモドの血漿蛋白結合率を超遠心分離法により測定した（報告

書番号 MPR-[redacted]-001）。エトラシモド（10 µmol/L）を各動物種の血漿試料とインキュベートしたときの血漿蛋白結合率（%）を Table 8 に示す。

Table 8.　エトラシモドの血漿蛋白結合率

動物種	エトラシモド濃度（µmol/L）	蛋白結合率（%）
マウス（CD-1）	10	99.1 ± 0.3
ラット（Sprague Dawley）	10	99.1 ± 0.2
ウサギ（New Zealand White）	10	98.2 ± 0.4
イヌ（ビーグル）	10	99.2 ± 0.1
サル（カニクイ）	10	95.4 ± 1.4
ヒト	10	97.9 ± 0.4

平均±標準偏差（n=3）
報告書番号 MPR-[redacted]-001，Table 1

雄の Sprague Dawley ラット，ビーグル犬およびカニクイザルのプール血液（いずれの動物種も n=3）を用いて，血球移行性に関する in vitro 評価を実施した。また男性被検者から採血したヒト血液（n=3，プールせず）も評価した（報告書番号 APD334.DMPK.003）。検討した濃度範囲 100～2000 ng/mL で，C_b/C_p はラット血液で 0.859～0.956，イヌ血液で 0.647～0.755，サル血液で 0.828～0.999 およびヒト血液で 0.643～0.821 であり，[^{14}C]エトラシモドは血液中の細胞外成分に分布する傾向が示された。

[^{14}C]エトラシモドの血漿中と細胞内との分配については，概して濃度依存性はみられなかったが，濃度が高くなるにつれほとんどの動物種で血球移行性の若干の飽和が認められた。このように，[^{14}C]エトラシモドをラット，イヌ，サルおよびヒト血中でインキュベーションした結果，エトラシモドの細胞内への分布はわずかであることが示唆され，100～2000 ng/mL の濃度範囲で濃度依存性は認められなかった。

さらに，ヒト肝ミクロソームへの結合（報告書番号 APD334.DMPK.004），種々の血漿蛋白質への結合（報告書番号 ARE-R10656），HEK293 細胞に対するエトラシモドの非特異的結合（報告書番号 [redacted]8101）を評価するための in vitro 試験を実施した。

エトラシモドとヒト肝ミクロソーム蛋白質との結合の評価（報告書番号 APD334.DMPK.004）では，エトラシモド（最終濃度：10 µmol/L）をヒト肝ミクロソーム蛋白質（0.0125 mg protein/mL）に添加し，平衡透析法によりミクロソーム蛋白結合を測定した。同時に陽性対照としてパロキセチン（1 µmol/L）のミクロソーム蛋白結合を測定した。エトラシモド（10 µmol/L）と 4，16，20 および 24 時間インキュベートした後の，エトラシモドの非特異的結合率はそれぞれ 79.5%，95.4%，96.6%および 96.1%，平衡の程度はそれぞれ 2.0%，57.8%，50.7%および 113.2%であった。このことから，主試験における透析時間を 24 時間に設定した。0.0125 mg protein/mL においてエトラシモド（10 µmol/L）を 24 時間インキュベートしたときの非結合型分率（f_u）は 3.9%，蛋白結合率は 96.1%であった。一方，1 mg protein/mL における陽性対照であるパロキセチンの f_u は，4.4%であった。

ヒトの血清アルブミン，α_1-酸性糖蛋白質およびリポ蛋白に対するエトラシモドの結合を検討する in vitro 試験（報告書番号 ARE-R10656）では，エトラシモド（20 および 200 ng/mL）の結合について，超遠心分離法を用いて総エトラシモド濃度および上清中濃度（遊離型）を測定して

評価した。ヒト血清アルブミンに対してはワルファリン（100 µmol/L），α_1-酸性糖蛋白質およびリポ蛋白に対してはプロプラノロール（100 µmol/L）を陽性対照として同様に評価した。エトラシモドはヒト血清アルブミンとの結合性が極めて高く，20 ng/mL においては遊離型濃度を測定できなかったが，200 ng/mL における f_u は 0.0792%±0.0050 であった。ヒト血清アルブミンへの結合率と比較し，エトラシモドのα_1-酸性糖蛋白質への結合率は低く，エトラシモドのリポ蛋白への結合率はさらに低値を示した。In vitro において，エトラシモドの血漿蛋白への結合は 20～200 ng/mL の範囲で濃度依存性は認められず，ヒト血清アルブミン，α_1-酸性糖蛋白質およびリポ蛋白の f_u は，それぞれ約 0.1%，約 1.1%～1.5%および約 14%～17%であった。

さらに，トランスポーターに関する試験に用いられる HEK293 細胞に対するエトラシモドの非特異的結合を評価した（報告書番号 [redacted]8101）。高濃度および低濃度のエトラシモド（1，10 および 30 µmol/L）を培地に添加した後，HEK293 細胞の存在下および非存在下でインキュベートした（培地中に，0%，1%，2%および 4%の BSA を含む）。インキュベート後に，LC-MS/MS により分析し，各濃度のエトラシモド溶液（100%溶液）と比較した。エトラシモドは，BSA 非存在下において，評価した濃度範囲（1～30 µmol/L）で非特異的結合を示し，非特異的結合率は約 60%であった。なお，HEK293 細胞に対する非特異的結合率は概ね BSA 濃度増加（1～4%）に伴い低下した。

4.2. In vivo

報告書番号 APD334.DMPK.002，APD334.DMPK.001，概要表 2.6.5.5

雌雄 Sprague Dawley ラットに[^{14}C]エトラシモドを単回経口投与し，組織分布を評価した。また，雌雄 Long-Evans 有色ラットに[^{14}C]エトラシモドを単回および 7 日間反復経口投与した後の組織分布を評価した（報告書番号 APD334.DMPK.002）。

雌雄の Sprague Dawley ラットおよび Long-Evans ラットに[^{14}C]エトラシモドを 10 mg/kg の用量で単回経口投与したときの放射能は，血液および血漿を含む検討したすべての臓器（眼の水晶体を除く）で定量可能であった。性別および系統種によらず，大部分の組織の C_{max} は投与後 4～8 時間に認められた。本項では C_{max} の概要を示し，詳細は概要表 2.6.5.5 項に示した。高い放射能濃度（C_{max}）が認められたのは，消化管，消失器官（腎臓・肝臓），心筋，分泌腺［副腎（S1P 感受性組織[5,6]），甲状腺］，および脳室周囲以外の中枢神経系組織（脊髄，小脳，大脳，延髄および嗅脳）であった（雄 Sprague Dawley ラット：8000 ng eq./g 超，雌 Sprague Dawley ラット：10000 ng eq./g 超，雄 Long-Evans ラット：9000 ng eq./g 超，雌 Long-Evans ラット：9000 ng eq./g 超）。雌雄 Sprague Dawley ラットでは褐色脂肪に高い放射能濃度が認められ，概して曝露量（AUC_t）も高かった。いずれの系統種においても，[^{14}C]エトラシモド由来の放射能は血液脳関門，血液精巣関門および血液卵胞関門を通過した。投与後 72 時間までの組織中：血中濃度の比は，消化管，尿管，消失器官（肝臓・腎臓），皮膚，分泌腺，生殖器，脂肪および中枢神経系を除いて概ね 2 倍未満となり，雌雄の Sprague Dawley ラットおよび Long-Evans ラットでは，血液中と同程度の割合でほとんどの組織から放射能が消失することが示唆された。

雌雄 Long-Evans 有色ラットに[^{14}C]エトラシモド 10 mg/kg を 1 日 1 回 7 日間経口投与後の放射能は，血液，血漿および検討したすべての臓器（眼の水晶体を含む）で定量可能であった。雌雄 Long-Evans ラットにおける投与後 7 日の組織分布は，単回投与後の組織分布と類似していた。7 日間投与後の C_{max} は，雌の骨（投与後 0.5 時間）を除くすべての臓器において投与後 4～8 時間

に認められた。反復投与後に高い C_{max}（12000 ng eq./g 超）が認められた臓器は，単回経口投与時と類似しており，加えて包皮腺および卵巣においても高い C_{max} が認められた。これらの組織の曝露量（AUC_t）は概して高値を示した。さらに，皮膚（有色および非有色），精巣，脾臓および胸腺の AUC_t も同様に高値を示したが，これらの組織における C_{max} は 12000 ng eq./g 未満であった。

雌雄 Long-Evans ラットへの[^{14}C]エトラシモドの反復投与により，単回投与後に比べて曝露量は増加し，反復投与後の終末相における長い $t_{1/2}$ と一致していた。単回投与後と同様に，[^{14}C]エトラシモド由来放射能は血液脳関門，血液精巣関門および血液卵胞関門を通過し，AUC 比も同程度に推移し消失した。このことから，放射能は曝露量が高い組織ほど血液より遅く消失し，曝露量が低い組織（AUC 比：2 未満）ほど血液と同等またはそれより速く消失することが示唆された。

また，雌雄 Long-Evans ラットでは，非有色皮膚と比較して有色皮膚における放射能濃度はやや高く（2 倍未満），メラニン含有組織に[^{14}C]エトラシモド由来放射能が中等度に結合および蓄積した。

本試験の C_b/C_p（0.565～0.800）は in vitro 試験結果と同程度（0.859～0.956，4.1 項）であり，系統種および雌雄の間に差は認められなかった。すべての動物における C_b/C_p が 1 未満であったことから，放射能は主に血漿中に存在し，血液中の細胞成分への分布はわずかであることが示された。[^{14}C]エトラシモドを用いたビーグル犬のマスバランスおよび排泄試験（報告書番号 APD334.DMPK.001）においても同様の傾向（C_b/C_p：0.56～0.72）が認められた。

Table 9. Sprague Dawley ラットおよび Long-Evans ラットに[^{14}C]エトラシモドを経口投与したときの組織分布

Tissue	Sprague Dawley 単回投与				Long-Evans 単回投与				Long-Evans 反復投与（7 日間）			
	雄		雌		雄		雌		雄		雌	
	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比
Adrenal glands	577275	6.59	1117360	12.25	1300485	10.81	1393640	12.29	3178485	17.88	6218825	36.03
Blood (LSC)	115742	1.32	128657	1.41	128119	1.06	136250	1.20	187799	1.06	192958	1.12
Blood (QWBA)	87533	1.00	91200	1.00	120337	1.00	113387	1.00	177737	1.00	172600	1.00
Bone	4444	0.05	3626	0.04	4040	0.03	3605	0.03	3977	0.02	5443	0.03
Bone marrow	130024	1.49	150613	1.65	110187	0.92	125669	1.11	162700	0.92	210289	1.22
Brain	130373	1.49	320890	3.52	194852	1.62	258224	2.28	221134	1.24	334584	1.94
Brain cerebellum	137435	1.57	308529	3.38	214250	1.78	276953	2.44	235423	1.32	347595	2.01
Brain cerebrum	127746	1.46	284540	3.12	170896	1.42	253687	2.24	181543	1.02	288286	1.67
Brain medulla	164724	1.88	429841	4.71	246904	2.05	331298	2.92	293358	1.65	444344	2.57
Brain olfactory lobe	102890	1.18	311474	3.42	124253	1.03	165350	1.46	151213	0.85	247295	1.43
Bulbo urethral gland	195659	2.24	-	-	175522	1.46	-	-	268563	1.51	-	-
Cecum	101375	1.16	173040	1.90	173800	1.44	174650	1.54	221714	1.25	233710	1.35
Diaphragm	101859	1.16	117265	1.29	116608	0.97	124992	1.10	139939	0.79	162736	0.94
Epididymis	54136	0.62	-	-	166711	1.39	-	-	130257	0.73	-	-
Esophagus	123924	1.42	210018	2.30	185252	1.54	168701	1.49	200531	1.13	234210	1.36
Exorbital lacrimal gland	118503	1.35	205181	2.25	165818	1.38	190622	1.68	224260	1.26	285995	1.66
Eye lens	NC	NC	NC	NC	NC	NC	NC	NC	39299	0.22	37237	0.22
Eye uveal tract	62989	0.72	78037	0.86	98366	0.82	104195	0.92	143417	0.81	154929	0.90
Eye(s)	13084	0.15	13431	0.15	18879	0.16	17833	0.16	49627	0.28	47673	0.28
Fascia	80353	0.92	107466	1.18	143002	1.19	81305	0.72	201964	1.14	152274	0.88
Fat (abdominal)	34855	0.40	30977	0.34	23392	0.19	6254	0.06	108128	0.61	78352	0.45
Fat (brown)	251942	2.88	321448	3.52	257525	2.14	276207	2.44	422808	2.38	532970	3.09
Harderian gland	244307	2.79	358460	3.93	227257	1.89	367458	3.24	333903	1.88	495379	2.87
Intra-orbital lacrimal gland	113267	1.29	183042	2.01	165883	1.38	194698	1.72	221349	1.25	271398	1.57

Table 9. Sprague Dawley ラットおよび Long-Evans ラットに[^{14}C]エトラシモドを経口投与したときの組織分布

Tissue	Sprague Dawley 単回投与				Long-Evans 単回投与				Long-Evans 反復投与（7 日間）			
	雄		雌		雄		雌		雄		雌	
	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比
Kidney cortex	177935	2.03	234195	2.57	198260	1.65	233929	2.06	246038	1.38	376345	2.18
Kidney medulla	148575	1.70	185505	2.03	168225	1.40	193853	1.71	209872	1.18	318990	1.85
Kidney(s)	168430	1.92	271935	2.98	214888	1.79	240652	2.12	269137	1.51	353290	2.05
Large intestine	121624	1.39	139372	1.53	148652	1.24	149400	1.32	164099	0.92	224570	1.30
Liver	543072	6.20	608078	6.67	654627	5.44	684075	6.03	989000	5.56	1147703	6.65
Lung(s)	100148	1.14	123758	1.36	143859	1.20	153363	1.35	191234	1.08	214200	1.24
Lymph node(s)	103807	1.19	160346	1.76	126422	1.05	178276	1.57	319675	1.80	427315	2.48
Muscle	53893	0.62	59504	0.65	60462	0.50	64468	0.57	65433	0.37	81253	0.47
Myocardium	159505	1.82	208935	2.29	188569	1.57	229928	2.03	212190	1.19	283820	1.64
Nasal turbinates	92044	1.05	152115	1.67	118256	0.98	139257	1.23	208956	1.18	293628	1.70
Ovary(ies)	-	-	365523	4.01	-	-	263646	2.33	-	-	801940	4.65
Pancreas	150760	1.72	286567	3.14	196981	1.64	227086	2.00	231937	1.30	336279	1.95
Pituitary gland	135910	1.55	222774	2.44	152710	1.27	201037	1.77	156899	0.88	261621	1.52
Plasma (LSC)	191171	2.18	200812	2.20	213022	1.77	208203	1.84	324086	1.82	315114	1.83
Preputial gland	170143	1.94	50918	0.56	189727	1.58	218561	1.93	557130	3.13	629375	3.65
Prostate gland	66934	0.76	-	-	93243	0.77	-	-	148479	0.84	-	-
Salivary gland(s)	202647	2.32	291992	3.20	211501	1.76	275014	2.43	293252	1.65	353849	2.05
Seminal vesicle(s)	38822	0.44	-	-	33787	0.28	-	-	100672	0.57	-	-
Skin (non-pigmented)	115827	1.32	134997	1.48	149372	1.24	182643	1.61	256887	1.45	512823	2.97
Skin (pigmented)	-	-	-	-	212949	1.77	237125	2.09	444790	2.50	688345	3.99
Small intestine	138415	1.58	145550	1.60	119234	0.99	174970	1.54	224766	1.26	274674	1.59
Spinal cord	279172	3.19	421485	4.62	330930	2.75	429635	3.79	330439	1.86	587928	3.41
Spleen	99892	1.14	159974	1.75	117140	0.97	138671	1.22	212486	1.20	293953	1.70
Stomach	128744	1.47	183402	2.01	141099	1.17	153334	1.35	145063	0.82	226660	1.31
Stomach mucosa	113215	1.29	167150	1.83	73905	0.61	176655	1.56	166859	0.94	238028	1.38

Table 9. Sprague Dawley ラットおよび Long-Evans ラットに[^{14}C]エトラシモドを経口投与したときの組織分布

Tissue	Sprague Dawley 単回投与				Long-Evans 単回投与				Long-Evans 反復投与（7 日間）			
	雄		雌		雄		雌		雄		雌	
	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比	AUC_t (ng eq.·h/g)	AUC 比
Stomach wall	64351	0.74	91303	1.00	44928	0.37	94118	0.83	104943	0.59	146252	0.85
Testis(es)	114832	1.31	-	-	157422	1.31	-	-	251664	1.42	-	-
Thymus	130545	1.49	222477	2.44	131628	1.09	178926	1.58	206405	1.16	323910	1.88
Thyroid	286020	3.27	444963	4.88	617710	5.13	512663	4.52	6554100	36.88	4510850	26.13
Urinary bladder	136153	1.56	146384	1.61	183843	1.53	96300	0.85	179575	1.01	193957	1.12
Uterus	-	-	134707	1.48	-	-	117242	1.03	-	-	272169	1.58

報告書番号 APD334.DMPK.002，Appendix 4，Tables 4-1，4-4，4-7，4-10，4-13，4-16
AUC 比：組織中 AUC/血中 AUC
-：性別または系統種により存在しない組織

5. 代謝（動物種間の比較）

エトラシモドの代謝を in vitro および in vivo で評価した（Table 10）。In vitro において，種々の動物種およびヒトの肝ミクロソームおよび肝細胞を用いた評価により代謝物を同定した。In vivo において，非標識エトラシモドを投与したマウスの血漿中および排泄物中における代謝物を同定した。また，[^{14}C]エトラシモドを単回経口投与したラットおよびイヌの血漿中および排泄物中においても代謝物を同定し特性を明らかにした。さらに，エトラシモドの代謝に関与するヒトの P450 分子種および UGT 分子種も同定した。

Table 10.　エトラシモドの代謝に関する試験一覧

試験名	試験系/動物種	報告書番号
In Vitro		
Species Comparison of the Metabolic Stability of APD334 in Mouse, Rat, Rabbit, Dog, Monkey, and Human Liver Microsomes in the Presence of NADPH	肝ミクロソーム：マウス（CD-1），ラット（Sprague-Dawley），ウサギ（New Zealand White），イヌ（ビーグル），サル（カニクイ），ヒト	MPR-[redacted]-003
Identification of the In Vitro Metabolites of APD334 in Liver Microsomes from Mouse, Rat, Rabbit, Dog, Monkey, and Human	肝ミクロソーム：マウス（CD-1），ラット（Sprague-Dawley），ウサギ（New Zealand White），イヌ（ビーグル），サル（カニクイ），ヒト	MPR-[redacted]-005
In Vitro Identification of ^{14}C-APD334 Metabolites in Hepatocytes of Various Species	肝細胞：ラット（Sprague-Dawley），イヌ（ビーグル），サル（カニクイ），ヒト	MPR-[redacted]-003
Identification of Human CYP Enzymes Involved in the Metabolism of APD334 by Using Recombinant CYPs	組み換えヒト P450	MPR-[redacted]-002
In Vitro Cytochrome P450 (CYP) Phenotyping of APD334 in Human Liver Microsomes and Recombinant Human CYP Enzymes	組み換えヒト P450 肝ミクロソーム：ヒト	[redacted]4054
APD334: Identification of Etrasimod Oxidative Metabolites in Human Liver Microsomes and Recombinant CYPs	組み換えヒト P450 肝ミクロソーム：ヒト	APD334.DMPK.005
Recombinant CYP Phenotyping (RCP) for Etrasimod (APD334) to Determine Fraction Metabolized	組み換えヒト P450 肝ミクロソーム：ヒト	APD334.DMPK.010
Identification of the Enzyme(s) that Catalyze the Conversion of Etrasimod (APD334) Metabolites M2 to M6, M3 to M6, M6 to M2 and M6 to M3	肝ミクロソーム：ヒト	APD334.DMPK.009

Table 10.　エトラシモドの代謝に関する試験一覧

試験名	試験系/動物種	報告書番号
Species Comparison APD334-glucuronide Formation and Identification of Human UGT Enzymes Involved in the Glucuronidation of APD334	肝ミクロソーム：マウス（CD-1），ラット（Sprague-Dawley），イヌ（ビーグル），サル（カニクイ），ヒト	MPR-[redacted]-004
In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters	Caco-2，MDCKII-BCRP，MDCKII-P-gp，HEK293 細胞および小胞膜	[redacted]8106
Inhibition Potential of APD334 on Human Liver Microsomal CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 Catalytic Activities	肝ミクロソーム：ヒト	MPR-[redacted]-004
APD334: Cytochrome P450 (CYP2C8) Inhibition in Human Liver Microsomes	肝ミクロソーム：ヒト	APD334.DMPK.011
In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes	肝ミクロソーム：ヒト	[redacted]5088
In Vitro Evaluation of APD334 as an Inducer of Cytochrome P450 Expression in Cultured Human and Rat Hepatocytes	肝細胞：ラット，ヒト	[redacted]3079
In Vivo		
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats	ラット	APD334.DMPK.002
Pharmacokinetics, Absorption, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs	イヌ	APD334.DMPK.001

5.1. キラル変換

エトラシモドは 1 つの不斉炭素を有し，生体内でエナンチオマーである AR401967 に変換する可能性がある。エトラシモドから AR401967 への変換を検出するため，キラル LC-MS/MS 法を開発し，バリデートした。キラル LC-MS/MS 法については，M2.7.1.1 に記載し，プール血漿検体を用いたエナンチオマー変換の評価結果については，M2.7.2.2 に記載した。

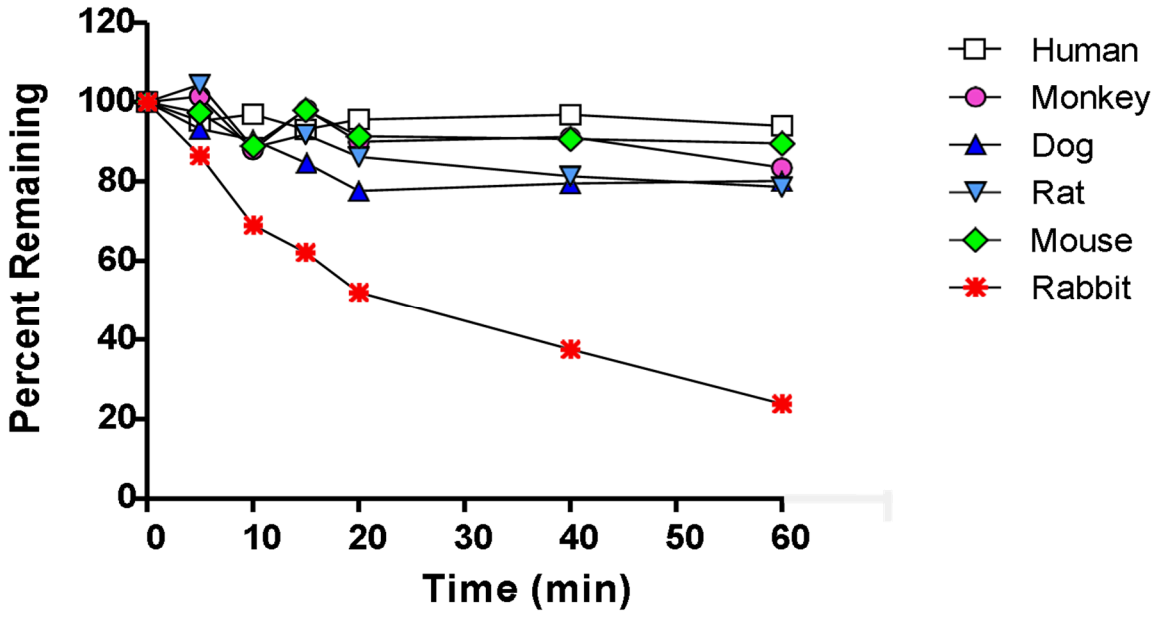
5.2. In vitro 代謝安定性

報告書番号 MPR-[redacted]-003，MPR-[redacted]-003，概要表 2.6.5.10

雄の CD-1 マウス，Sprague Dawley ラット，ビーグル犬，カニクイザルおよび男女のヒト（プール）の肝ミクロソームを用いて NADPH 存在下でインキュベートしたとき，エトラシモドはあまり代謝を受けず，未変化体の消失に基づく $t_{1/2}$ は 60 分超であった（報告書番号 MPR-[redacted]-003）。一方，雄の New Zealand White ウサギの肝ミクロソームにおいては，未変化体が大きく減少し（$t_{1/2}$：約 30 分），他の動物種と比較してウサギでは代謝活性が高いことが示唆された（Figure 3）。NADPH 非存在下では，エトラシモドの代謝は認められなかった。ウサギの肝ミクロソームで認められた未変化体の速やかな消失は，in vivo で曝露量が低いことと一致していた（Table 5）。

凍結保存したラット，イヌ，サルおよびヒトの肝細胞で 24 時間インキュベートしたとき，未変化体（[^{14}C]エトラシモド）はそれぞれ 42.82%，25.89%，62.31%および 36.02%消失した（報告書番号 MPR-[redacted]-003）。In vitro で認められた低い代謝活性は，動物およびヒトにおける低い全身クリアランスと一致していた（Table 2，Table 3）。

Figure 3.　各動物種の肝ミクロソームにおけるエトラシモドの消失



報告書番号 MPR-[redacted]-003，Figure 1
平均値，n = 2

5.3. In vitro における代謝物の同定

報告書番号 MPR-[redacted]-005，MPR-[redacted]-003，概要表 2.6.5.10

エトラシモドの代謝物を同定するため，d_9-エトラシモドを用いて雄の CD-1 マウス，Sprague Dawley ラット，ビーグル犬，カニクイザル，雌 New Zealand White ウサギおよび男女ヒトの肝ミクロソームとインキュベートする in vitro 代謝試験を行った。さらに，[^{14}C]エトラシモドを用いてラット，イヌ，サルおよびヒトの凍結肝細胞とインキュベートする in vitro 代謝試験を行った。

肝ミクロソームとインキュベート（報告書番号 MPR-[redacted]-005）したとき，5 種類の代謝物（M1，M2，M3，M4，M5）が認められた（Table 11）。代謝物 M1 はエトラシモドのアシルグルクロン酸抱合体であったが，M2 および M3 はモノヒドロキシル体であった。

ヒドロキシル代謝物（M2，M3）およびそれらのグルクロン酸抱合体（M4，M5，O グルクロニドかアシルグルクロニドかは不明）は，それぞれレジオアイソマーまたはジアステレオマーである可能性がある。ラットまたはイヌの肝ミクロソームにおいては，ヒト肝ミクロソームで認められたすべての代謝物が認められ，毒性試験で用いる動物種としてラットおよびイヌを用いることの適切性が裏付けられた（Table 11）。なお，本試験では代謝物の定量は実施していない。

[^{14}C]エトラシモド（20 µmol/L）を肝細胞とインキュベート（報告書番号 MPR-[redacted]-003）したとき，29 種類の代謝物が認められた（Table 12，Figure 4）。[^{14}C]エトラシモドの代謝は，サルの肝細胞において最も高く，次いでラット，ヒトおよびイヌであった。ヒト肝細胞では，ヒドロキシル体（M3）およびケトン体（M6）が最も多く認められ，それぞれ総放射能の 4.3%および 9.0%を占めた。ヒト肝細胞で認められたその他すべての代謝物は，グルクロン酸抱合体（M1）および酸化とグルクロン酸抱合が組み合わされた他の代謝物を含めて，総放射能の 4%未満であった。ヒト肝細胞で認められたすべての代謝物は，評価した動物種においても認められた。

Table 11. エトラシモドをマウス，ラット，ウサギ，イヌ，サルおよびヒトの肝ミクロソームとインキュベートしたときの in vitro 代謝物

動物種	代謝物				
	M1 (AR440843)	M2 (AR503640)	M3 (AR503641)	M4	M5
マウス（CD-1）	X	X	X	–	X
ラット（Sprague Dawley）	X	X	X	–	X
ウサギ（New Zealand White）	X	X	X	X	X
イヌ（ビーグル）	X	X	X	–	–
サル（カニクイ）	X	X	X	X	X
ヒト	X	X	X	–	X

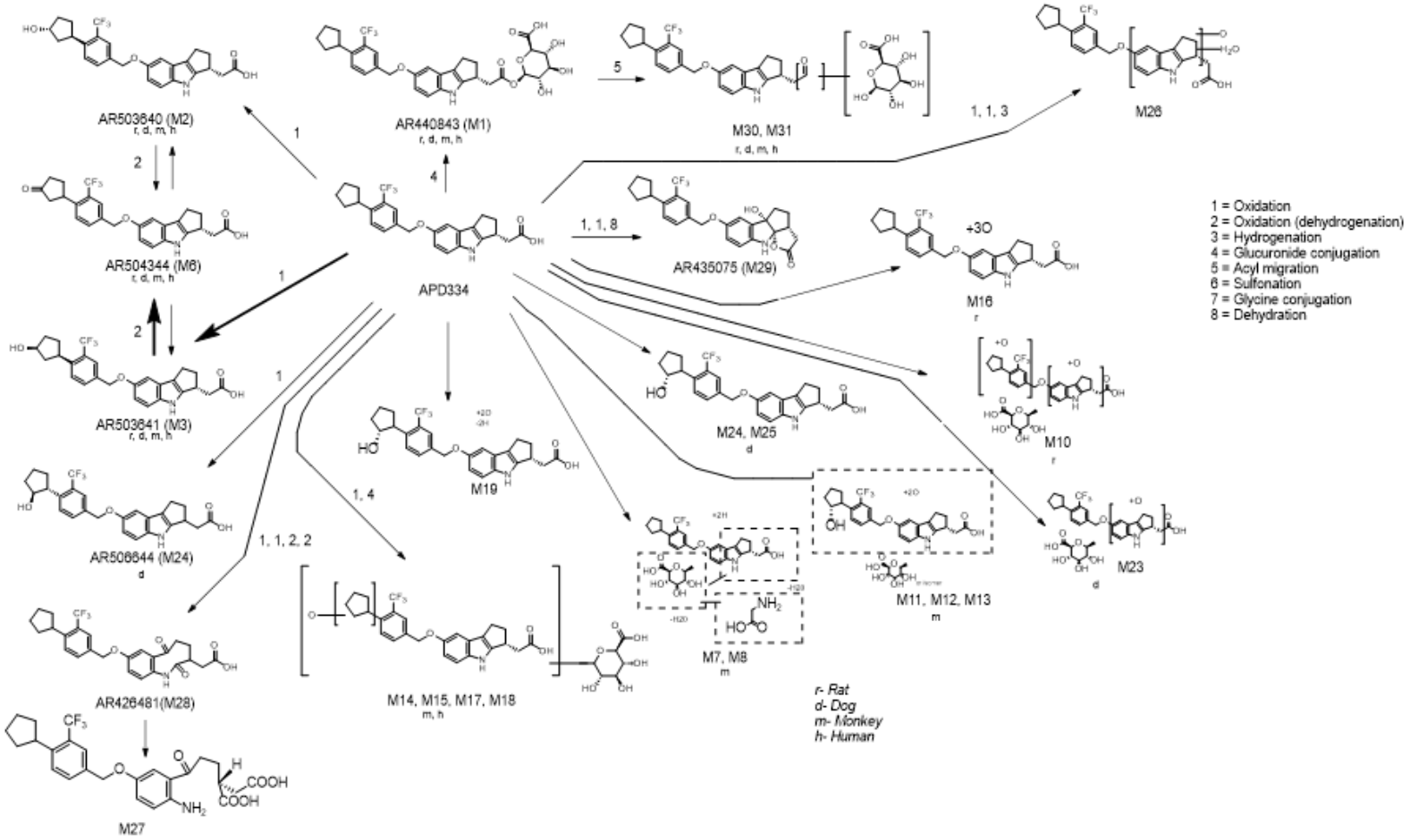
報告書番号 MPR-[redacted]-005，Table 2
X：代謝物が検出されたことを示す，–：代謝物が検出されなかったことを示す
AR の名称を有する代謝物には標準物質が存在する。
CD-1 マウス（雄），Sprague Dawley ラット（雄），New Zealand White ウサギ（雌），ビーグル犬（雄），カニクイザル（雄）またはヒト（男女）の肝ミクロソーム（プール）を用いた。

Table 12. [^{14}C]エトラシモドをラット，イヌ，サルおよびヒト肝細胞とインキュベートしたときの in vitro 代謝物

代謝物	ラット	イヌ	サル	ヒト
M1（AR440843）	X	X	X	X
M2（AR503640）	X	X	X	X
M3（AR503641）	X	X	X	X
M6（AR504344）	X	X	X	X
M7	–	–	X	–
M8	–	–	X	–
M9	–	–	X	–
M10	X	–	–	–
M11	–	–	X	–
M12	–	–	X	–
M13	–	–	X	–
M14	–	–	X	X
M15	–	–	X	X
M16	X	–	–	–
M17	–	–	X	X
M18	–	–	X	X
M19	X	–	–	X
M20	X	–	X	X
M21	X	–	X	X
M22	X	–	X	X
M23	–	X	–	–
M24	–	X	–	–
M25	–	X	–	–
M26	X	X	X	X
M27	X	X	X	X
M28	X	X	X	X
M29	X	X	X	X
M30（AR440843 の異性体 B）	X	X	X	X
M31（AR440843 の異性体 C）	X	X	X	X

報告書番号 MPR-[redacted]-003，Table 3
X：代謝物が検出されたことを示す，–：代謝物が検出されなかったことを示す
AR の名称を有する代謝物には標準物質が存在する。

Figure 4.　ラット，イヌ，サルおよびヒトの肝細胞におけるエトラシモドの in vitro 推定代謝経路



報告書番号MPR-[redacted]-003，Table 2，Table 3，Figure 1より作図

5.4. In vivo における代謝物の同定

報告書番号 APD334.TOX.001，APD334.DMPK.002，APD334.DMPK.001，概要表 2.6.5.9

CD-1 マウスを用いた 90 日間経口反復投与毒性試験ならびに[^{14}C]エトラシモドを投与した Sprague Dawley ラットおよびビーグル犬を用いたマスバランス試験において，エトラシモドの in vivo 代謝を評価した。

20 mg/kg/日を雌雄マウスに反復経口投与したとき，酸化開裂エトラシモド（M28）および酸化エトラシモド（M42）が血漿中の主要代謝物として認められた。200 mg/kg/日を投与した雌雄マウスでは，M6，M28 および M29 が血漿中の主な代謝物として認められた。マウスにおける推定代謝経路を Figure 5 に示す（報告書番号 APD334.TOX.001）。

無処置 Sprague Dawley ラットおよび胆管カニューレ挿入ラット（10 mg/kg 単回経口投与）ならびに無処置 Long-Evans ラット（10 mg/kg/日を 1 日 1 回 7 日間経口投与）を用いたマスバランス試験において，[^{14}C]エトラシモドは広範に代謝され，29 種類の放射性物質が生成された。このうち，25 種類の代謝物が LC/MS によって同定された（報告書番号 APD334.DMPK.002）。

ラットの血漿，糞および胆汁中に認められた代謝物は，主に酸化代謝物（水酸化体およびケトン体）および抱合体であった（Figure 6）。血中で主に認められた放射能は未変化体であり，雄および雌でそれぞれ投与後 48 時間までの放射能の 50.0%および 65.9%を占めた。単回投与後に最も多く認められた血中代謝物は M6 であり，[^{14}C]エトラシモドのシクロペンチル環が酸化および脱水素化された構造である。M6 は雄および雌で放射能 $AUC_{0.25\text{-}48}$ のそれぞれ 26.1%および 17.8%を占めた。[^{14}C]エトラシモドのシクロペンチル環が酸化された代謝物である M3 は，雄および雌で放射能 $AUC_{0.25\text{-}48}$ のそれぞれ 7.27%および 9.40%を占めた。血漿中には硫酸抱合体やグルクロン酸抱合体（未変化体の 2.5%未満）などの微量代謝物も認められた。糞中で認められた主な代謝物は M6 および M3 であり，雄および雌でそれぞれ 19.6%および 13.7%を占めた。胆管カニューレ挿入 Sprague Dawley ラットを用いた別の試験で，10 mg/kg[^{14}C]エトラシモドを経口投与したところ，胆汁中に代謝物が認められた。胆汁中で最も多く認められた代謝物は，雄では M21（オキシジデヒドロエトラシモドのアシルグルクロニド異性体 2），雌では M1（エトラシモドアシルグルクロニド 1）であった。ラット胆汁中に回収された代謝物 M20/M21/M22（in vivo または合成中にアシル転移により生成される）は，雄および雌でそれぞれ投与放射能の 22.7%および 11.9%を占めていた。同様に，ラット胆汁中に回収された代謝物 M1/M30/M31（上記と同様に，in vivo または合成中にアシル転移により生成される）は，雄および雌でそれぞれ投与放射能の 20.2%および 22.9%を占めていた。その他の代謝物として，エトラシモドのより広範な酸化により生成された M39，M42，M44 および M28，ならびにこれらの酸化体のグルクロン酸抱合体および硫酸抱合体（M40，M45，M46，M47，M35，M48，M49）が認められ，投与放射能の 5%未満であった。M42（トリオキシジデヒドロエトラシモド 2）は雄の胆汁中でのみ定量された代謝物であり，雌の胆汁中では認められなかった。

また，無処置 Long-Evans ラットに[^{14}C]エトラシモド 10 mg/kg を 7 日間反復経口投与したときの代謝物プロファイルも評価した。Long-Evans ラットの代謝プロファイルは Sprague Dawley ラットと同様であり，代謝物の生成量のみが異なっていた。反復投与後の雄のプール血漿中における主な成分は M6 であり，放射能の 48.0%を占めた。一方，血漿中の[^{14}C]エトラシモドは 24.6%であった。Long-Evans ラットにおける M6 の相対存在量は，雌雄ともに反復投与後の方が単回

投与後よりも多かった。3 番目に多く認められたのは M3 であり，雄および雌でそれぞれ放射能 $AUC_{0.25\text{-}48}$ の 7.77%および 6.68%であり，単回投与時の量と同程度であった。血漿中で定量されたその他の代謝物は，放射能 $AUC_{0.25\text{-}48}$ の 2.5%未満であった。

雌雄ビーグル犬に[^{14}C]エトラシモド 2 mg/kg を単回経口投与したマスバランス試験では（報告書番号 APD334.DMPK.001），エトラシモドは中等度に代謝され，親化合物の大部分が直接抱合により消失した。アシルグルクロン酸抱合体は胆汁を介して速やかに消失し，その後消化管内で加水分解されて，アグリコンに変換され，再吸収された。また，血中および排泄物中の主な成分は未変化体であった。尿中に回収された放射能は，投与放射能の 1.5%未満であった。単回経口投与後の雌雄イヌにおけるエトラシモドの代謝により，18 の放射性成分が認められ，そのうち LC/MS によって 11 成分が同定された（Table 14，Figure 7）。雌雄イヌにおける血中および糞中の代謝物は，シクロペンチル環の酸化体である M2，M3，M24 および M6 と考えられた。その他の中等度に認められた代謝物として，二次酸化体（M26，M43），硫酸抱合体（M36）および主要なアシルグルクロン酸抱合体のグルクロン酸転移体（M30，M31）が認められた。このように，エトラシモドの代謝には，酸化，脱水素ならびに硫酸またはグルクロン酸による抱合が関与しており，雌雄イヌの代謝に顕著な性差は認められなかった。イヌにおける推定代謝経路を Figure 7 に示す。

以上，[^{14}C]エトラシモドを用いた in vivo の代謝試験の結果から，in vitro の肝細胞および肝ミクロソームを用いた代謝試験で同定された代謝物プロファイルと in vivo における代謝物プロファイルの間に良好な相関性が認められた。ヒトで in vitro および in vivo において同定された代謝物は（M2.7.2.3），ラットおよびイヌでも認められたことから，毒性試験の動物種としてラットおよびイヌを選択したことの適切性を裏付けるものであった。一方，in vitro 試験で認められたグルクロン酸抱合体（M4，M5）は in vivo 試験では認められなかった。これらのグルクロン酸抱合体は，in vivo でみられた競合する排泄経路のない静的な in vitro の系において過大評価されたものと考えられる。

Table 13.　ラットに[^{14}C]エトラシモド 10 mg/kg を投与後に血漿，胆汁および糞中で検出または同定された代謝物

代謝物	保持時間（分）	代謝物の同定（推定）	試料		
			血漿	胆汁	糞
APD334	67.50	Parent compound	X	X	X
M1 (AR440843)	62.25	APD334 acyl glucuronide-1	–	X	–
M2 (AR503640)	35.75	Oxy-APD334-1	–	–	X
M3 (AR503641)	40.25-40.50	Oxy-APD334-2	X	–	X
M6 (AR504344)	43.00	Oxy-didehydro-APD334	X	X	X
M8	38.86	Dioxy-APD334-1	–	X	–
M20	30.50	Oxy-didehydro-APD334 acyl glucuronide-1	–	X	–
M21/M22	31.75	Isomers of M20, oxy-didehydro-APD334 acyl glucuronide-2/3	–	X	–
M28	49.00-49.25	Dioxy-APD334-2	–	X	X
M29	62.00	Oxy-APD334 lactone	–	–	X
M30 (Isomer B of AR440843)	62.75	APD334 acyl glucuronide-2	–	X	–
M31 (Isomer C of AR440843)	63.25	APD334 acyl glucuronide-3	–	X	–
M35	38.75	Dioxy-APD334 glucuronide-1	–	X	–
M36	53.00-53.25	Oxy-APD334 sulfate	X	X	X
M38	9.00	Trioxy-APD334-1	–	–	X
M39	12.25	Trioxy-didehydro-APD334-1	–	X	–
M40	13.00-13.25	Dioxy-APD334 sulfate glucuronide	–	X	–
M41	19.25	Trioxy-APD334-2	X	–	–
M42	20.00	Trioxy-didehydro-APD334-2	–	X	–
M43	27.25	Dioxy-APD334-3	–	–	X
M44	28.25	Dioxy-dehydro-APD334	X	X	X
M45	29.00-29.25	Oxy-APD334 glucuronide	–	X	–
M46	34.50-34.75	Oxy-APD334 sulfate glucuronide-1	–	X	–
M47	35.75-36.00	Oxy-APD334 sulfate glucuronide-2	–	X	–
M48	40.00	Dioxy-APD334 glucuronide-2	–	X	–
M51	36.00-36.50	Unknown	–	X	–
M52	74.00-74.50	Unknown	X	–	X
M53	75.00	Unknown	X	–	X
M54	75.50-76.00	Unknown	–	–	X

報告書番号 APD334.DMPK.002，Appendix 5

X：代謝物が検出されたことを示す，–：代謝物が検出されなかったことを示す

保持時間の範囲は，全試料の解析に基づき記載した。

Table 14.　イヌに[^{14}C]エトラシモド 2 mg/kg を投与後に血漿および糞中で検出または同定された代謝物

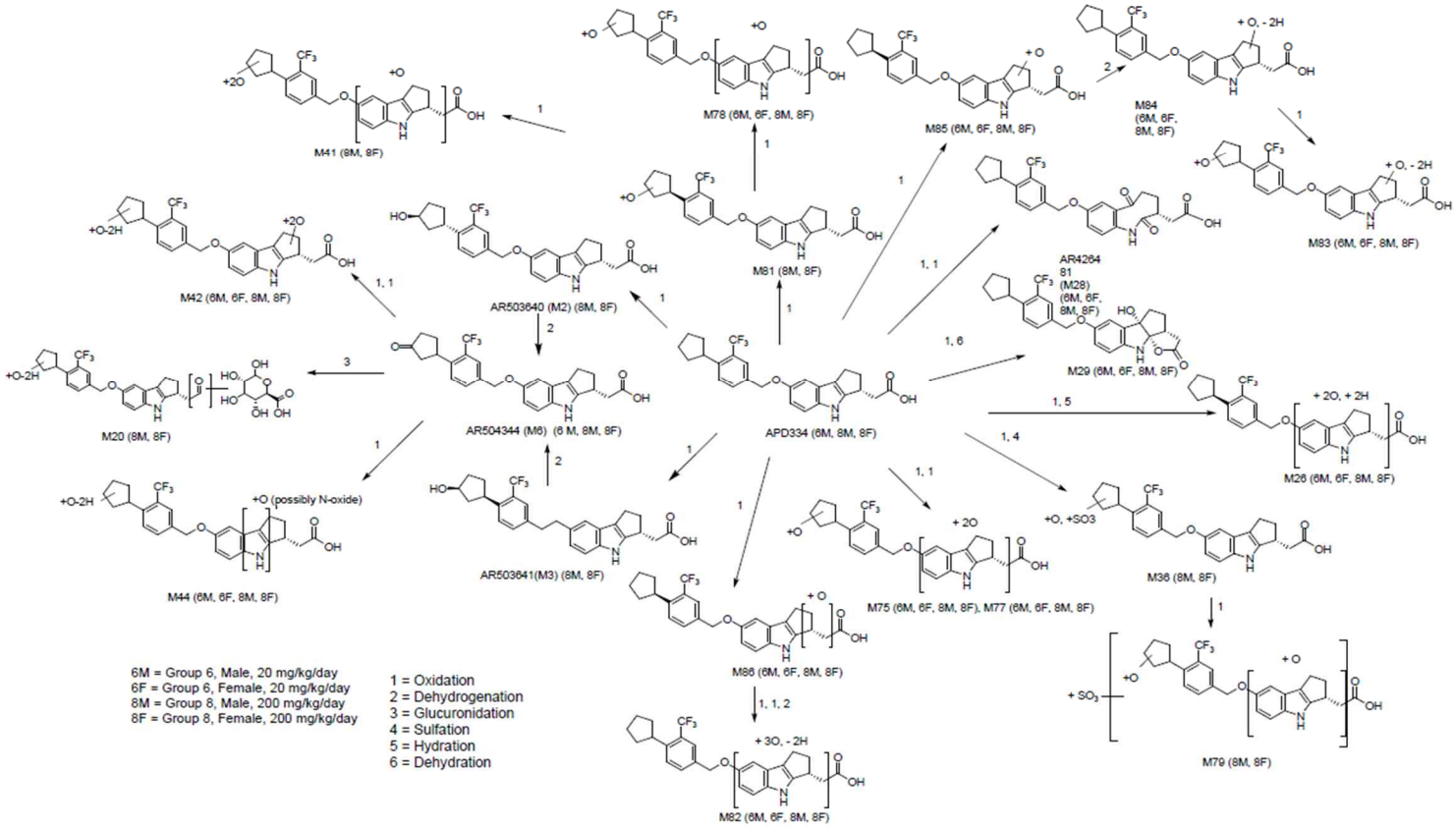
代謝物	保持時間（分）	代謝物の同定	試料	
			血漿	糞
APD334	67.75	Parent compound	X	X
M2 (AR503640)	35.75	Oxy-APD334-1	–	X
M3 (AR503641)	40.50	Oxy-APD334-2	–	X
M6 (AR504344)	43.00	Oxy-didehydro-APD334	–	X
M24 (AR503644)	41.75	Oxy-APD334-3	–	X
M26	49.00	Dioxy-dihydro-APD334	X	X
M29	62.00	Oxy-APD334 lactone	X	X
M30 (Isomer B of AR440843)	63.00	APD334 acyl glucuronide-2	–	X
M31 (Isomer C of AR440843)	63.50	APD334 acyl glucuronide-3	–	X
M36	54.75-55.00	Oxy-APD334 sulfate	–	X
M43	27.25	Dioxy-APD334-3	–	X
M52	74.25-74.75	Unknown	X	X
M53	75.00	Unknown	–	X
M55	2.00	Unknown	X	–
M56	2.50	Unknown	X	–
M57	64.50-64.75	Unknown	–	X
M58	66.25	Unknown	–	X
M59	73.75-74.00	Unknown	–	X

報告書番号 APD334.DMPK.001，Appendix 4

X：代謝物が検出されたことを示す，–：代謝物が検出されなかったことを示す

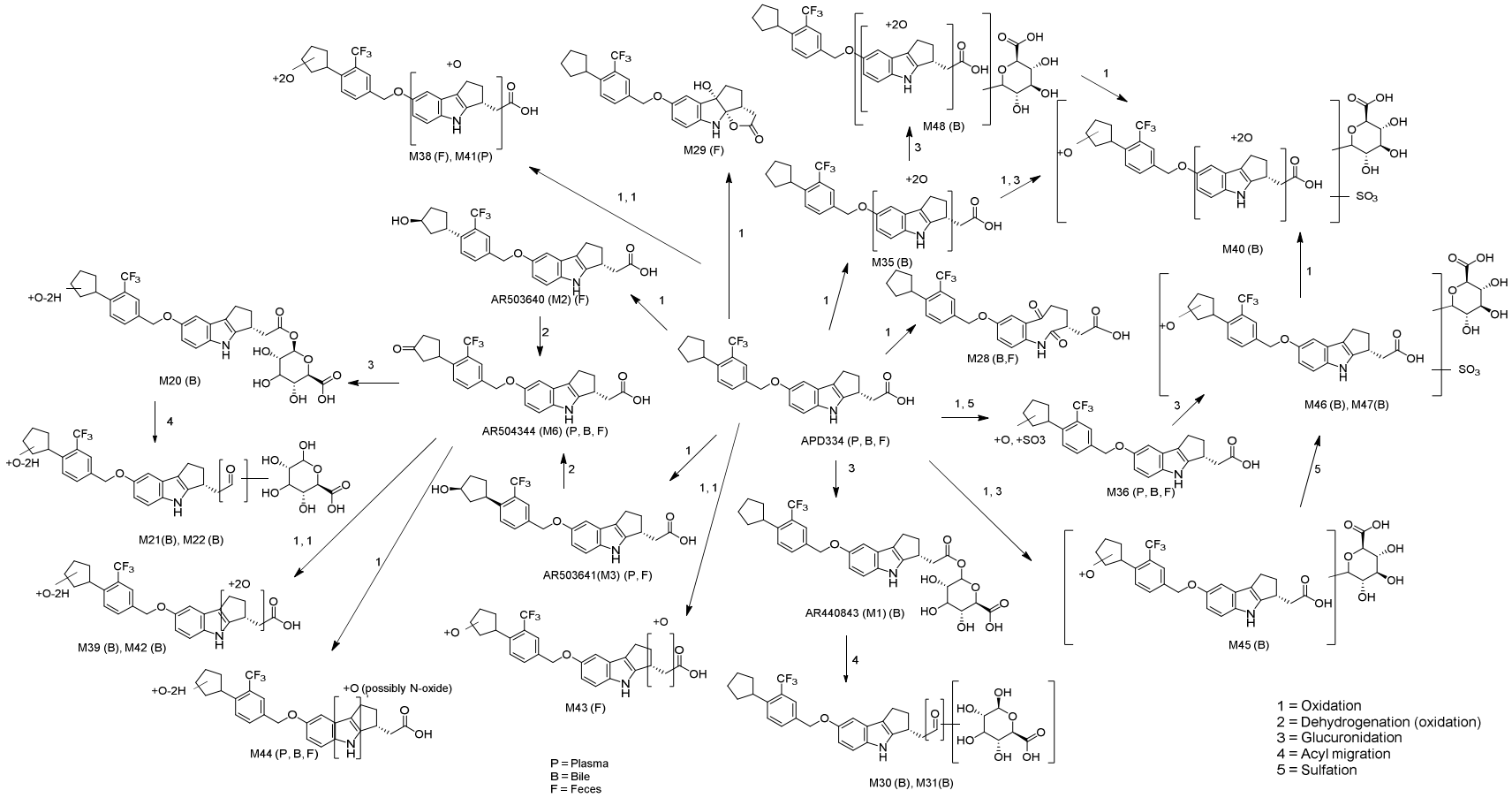
保持時間の範囲は，全試料の解析に基づき記載した。

Figure 5.　マウスにおけるエトラシモドの推定代謝経路



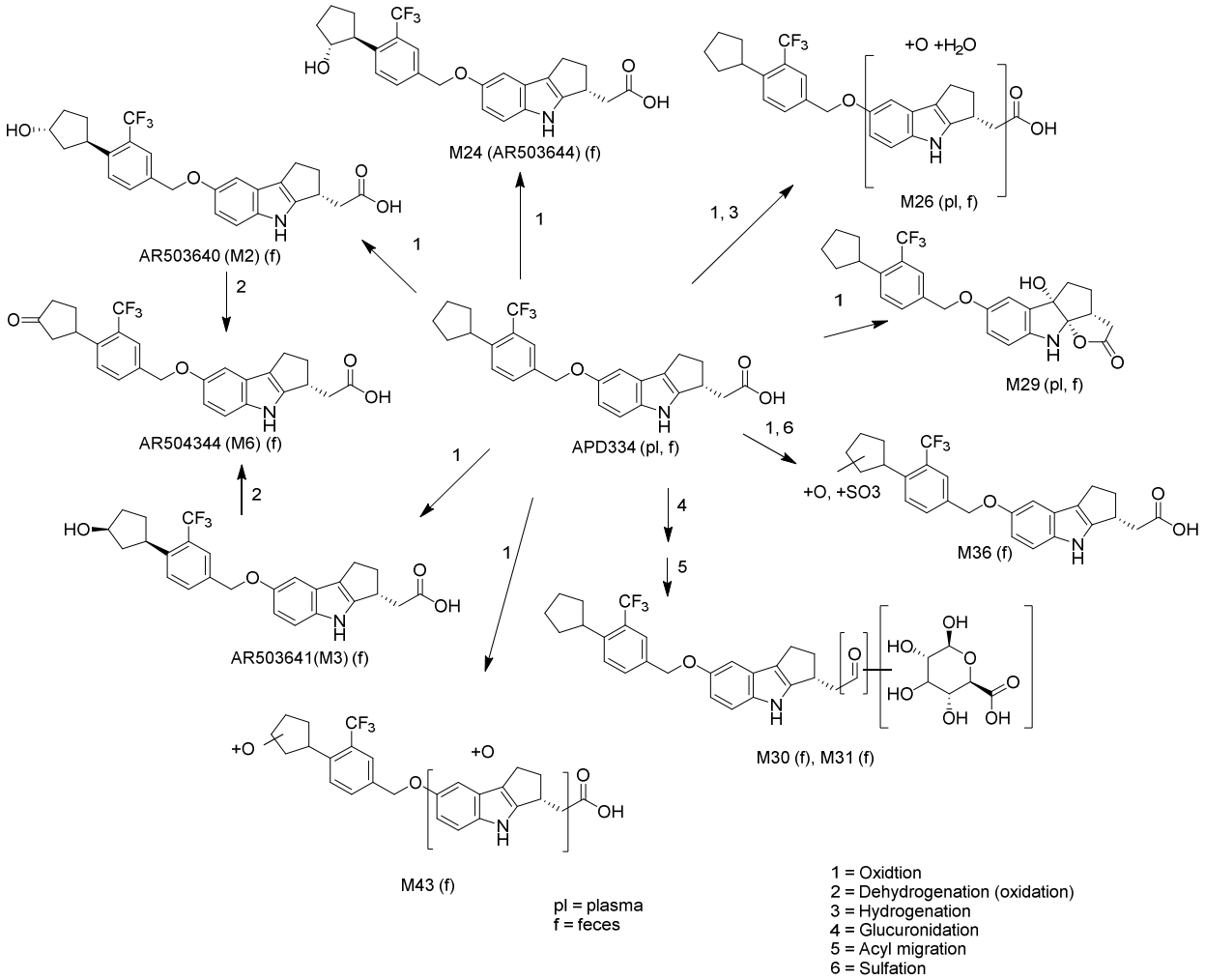
報告書番号APD334.TOX.001，Appendix 12，Figure 42
いずれの群および性別においても，総ピーク面積の1%超の代謝物のみを記載する。

Figure 6.　ラットにおけるエトラシモドの推定代謝経路



報告書番号APD334.DMPK.002，Appendix 5，Figure 5-46

Figure 7.　イヌにおけるエトラシモドの推定代謝経路



報告書番号APD334.DMPK.001，Appendix 4，Figure 4-30

5.5. 代謝酵素の同定

報告書番号 MPR-[redacted]-002，[redacted]4054，APD334.DMPK.005，APD334.DMPK.010
APD334.DMPK.009，MPR-[redacted]-004，概要表 2.6.5.10

P450 分子種および主要代謝物（M2，M3，M6）への代謝に着目した一連の試験において，エトラシモドの代謝に関与する酵素を検討した。ヒト肝ミクロソームにおけるエトラシモドの代謝が遅いことから，バキュロウイルス/昆虫細胞に発現させたヒト組み換え P450 を用いて，代謝に関与する P450 分子種の同定を行った。未変化体の消失に基づき，評価した組み換え P450 分子種のうち CYP2C8，CYP2C9 および CYP2C19 が同定された（報告書番号 MPR-[redacted]-002）。同定された P450 分子種をバリデートするため，ヒト肝ミクロソームおよび組み換え P450 分子種を用いて，P450 リアクションフェノタイピングを実施したところ，マススペクトル解析により当初推定した代謝物が同定された（報告書番号 [redacted]4054）。また，主要代謝物の標準物質を用いた追加試験により，代謝物を同定した（報告書番号 APD334.DMPK.005）。先に実施した試験（報告書番号 [redacted]4054）では，CYP2C8 によって生成される酸化代謝物 1（後の M2），CYP2C8 および CYP2C9 によって生成される酸化代謝物 2（後の M3）および CYP2C8 によって生成されるケトン代謝物 1（後の M6）の主要な 3 つの代謝物が同定された。

P450 により代謝される割合を明らかにするため，組み換え P450 を用いて基質の減少を測定する方法およびヒト肝ミクロソーム活性に対する組み換え P450 活性の比を求める Relative activity factor（RAF）法を組み合わせた試験により，エトラシモドの代謝に関与する種々の P450 分子種の相対的寄与を明らかにした。組み換え P450 を添加しインキュベートした後に M2，M3 および M6 が生成され，CYP2C8，CYP2C9，CYP3A4，CYP2C19 および CYP2J2 がエトラシモドの代謝に関与していた。RAF で補正したエトラシモドのヒト肝ミクロソームにおける推定固有クリアランス値から，組み換え CYP2C8，CYP2C9，CYP3A4，CYP2C19 および CYP2J2 の推定寄与率（f_m）は，それぞれ 0.38，0.37，0.22，0.01 および 0.01 と推定された（報告書番号 APD334.DMPK.010）。複数の P450 リアクションフェノタイピング試験および RAF 法を用いた試験結果から，エトラシモドの代謝に関与する P450 は，CYP2C8，CYP2C9 および CYP3A4 で，また，これらより程度は低いものの CYP2C19 および CYP2J2 も関与すると考えられた。

このように，主要な水酸化代謝物である M2 および M3 と，ケトン代謝物である M6 の生成には，主に CYP2C8 および CYP2C9 が関与しており，それより程度は低いものの CYP3A4，CYP2C19，CYP2D6 および CYP2J2 も関与していた（報告書番号 [redacted]4054，APD334.DMPK.010）。M6 の生成に関与する主要な P450 分子種は，ヒト肝ミクロソームおよび選択的阻害剤を用いた検討から，当初 CYP2C8 と考えられていた。しかしながら，ヒト肝ミクロソーム，ヒト肝サイトゾル，組換えヒトカルボニル還元酵素および非特異的 P450 阻害剤である ABT を用いた更なる検討（報告書番号 APD334.DMPK.009）では，M2 から M6，次いで M3 への一方向の変換が認められ，M3 は安定な代謝物であることが示された。NADPH 存在下でサイトゾルでインキュベートしたとき，M3 から M6 または M2 への変換は認められなかった。また，ミクロソーム中の 11β-ヒドロキシステロイドデヒドロゲナーゼは，M3 ではなく M2 から M6 への変換に関与していると考えられた。一方，ミクロソーム中においてはサイトゾル酵素と異なり，M6 は M2 および M3 へ同程度に変換されたことから，ミクロソーム酵素ではなくサイトゾル酵素によるエナンチオマー選択性が示唆された。P450 阻害薬の存在下における M2 から M6 の変換の程度は阻害薬非存在下と比べてより少なかったことから M6 の生成に P450 が関与

していることが示唆されたものの，P450 阻害薬の存在下および非存在下における M2，M3 および M6 の動態はほぼ類似していた。以上より，M2 のエナンチオマー選択的酸化により M6 が生成された後，サイトゾル酵素によって M3 に還元されるが，M3 はサイトゾル酵素（NADPH 存在下）またはミクロソーム酵素により M6 または M2 に相互変換されないと考えられる。

5.3 項および 5.4 項で考察したように，in vitro における代謝物同定試験ではグルクロン酸抱合体と推定される代謝物（M4 および M5）が同定されたが，これらは in vivo で検出されなかったことから，M4 および M5 は生成されたとしてもごく微量であるか，in vivo では速やかに消失することが示唆された。

In vivo 試験に先立ち，雄 CD-1 マウス，Sprague Dawley ラット，ビーグル犬，カニクイザルおよび男女ヒト（プール）の肝ミクロソームを用いた in vitro 試験において，グルクロン酸抱合体の生成を比較し，さらに，組み換え UGT 分子種を用いた試験を実施した（報告書番号 MPR-[redacted]004）。エトラシモドからグルクロン酸抱合体への生成における UGT 分子種の寄与の程度は，UGT1A7 >> UGT1A1 > UGT1A4 > UGT1A9 > UGT1A6 ～ UGT1A3 = UGT1A8 の順であった。UGT1A10，UGT2B4，UGT2B10，UGT2B15 および UGT2B17 によるグルクロン酸抱合体の生成量は対照群と同等であり，生成への寄与は認められなかった。各 UGT 分子種の相対的存在量は不明であることから，エトラシモドの抱合には様々な UGT 分子種が関与する可能性がある。

5.5.1. 代謝酵素の阻害および誘導

報告書番号 MPR-[redacted]-004，[redacted]5088，APD334.DMPK.011，[redacted]3079，概要表 2.6.5.12

代謝酵素に対するエトラシモドの阻害または誘導能を，ヒト肝ミクロソーム（阻害），組み換え UGT 分子種（阻害），ラットおよびヒトの肝細胞（誘導のみ）を用い in vitro で検討した。

P450 阻害活性については，選択的プローブ基質を用いて，最高 50 µmol/L の濃度まで評価した。

パクリタキセルをプローブ基質とする試験で（報告書番号 MPR-[redacted]-004），エトラシモドは CYP2C8 を可逆的に阻害し（IC_{50} 値：34.7 µmol/L），アモジアキンを用いた試験（報告書番号 [redacted]5088）では，エトラシモド（10 µmol/L）は CYP2C8 活性を 100%阻害した。アモジアキンを用いた追加試験において，エトラシモドは CYP2C8 を阻害し，IC_{50} 値は 4.0 µmol/L であった（報告書番号 APD334.DMPK.011）。薬物相互作用に関するガイダンス（MHLW 2018[7]，EMA 2012b[8]，FDA 2020[9]）に基づき，高感度なプローブ基質であるレパグリニドを用いた評価では，エトラシモドの存在下および非存在下の AUCR は 1.25 未満であり，エトラシモドが臨床的に意味のある CYP2C8 阻害剤となる可能性は低いと考えられた。エトラシモド（最高 50 µmol/L）による CYP1A2，CYP2C9，CYP2C19，CYP2D6 および CYP3A4 に対する阻害率は 50%未満であった（報告書番号 MPR-[redacted]-004）。また，P450 分子種（CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP3A4/5）に対して時間依存的阻害作用を示さなかった（報告書番号 [redacted]5088）。エトラシモドは UGT1A1 および UGT1A6 の活性を可逆的に阻害し，検討した最高濃度（10 µmol/L）での阻害率はそれぞれ 28%および 48%であった。一方，エトラシモドは UGT1A3，UGT1A4，UGT1A9，UGT2B7 および UGT2B17 を阻害せず，10 µmol/L での阻害率は 8%未満であった（報告書番号 [redacted]5088）。

雌ラット肝細胞（3 ロット）を用いた P450 誘導能の評価では，エトラシモド（3 および 30 µmol/L）による，CYP1A1，CYP1A2 および CYP3A1 の mRNA 発現レベルに対する影響はほとんど認められなかった。しかしながら，エトラシモドは CYP2B1（30 µmol/L）および CYP2C11（3 および 30 µmol/L）の mRNA 発現を 2 倍以上増加させた。また，雄ラット肝細胞（3 ロット）においては，エトラシモド（3 および 30 µmol/L）による CYP1A1，CYP3A1 および CYP3A2 の mRNA 発現レベルに対する影響はほとんど認められなかったが，CYP1A2（30 µmol/L），CYP2B1（30 µmol/L）および CYP2C11（3 および 30 µmol/L）の mRNA 発現が 2 倍超増加した（報告書番号 [redacted]3079）。

ヒト肝細胞では，用いた 3 ロットのいずれにおいてもエトラシモド（10 µmol/L）は CYP1A2 の mRNA を誘導しなかった。3 ロット中 1 ロットで，エトラシモド（10 µmol/L）は CYP2B6 の mRNA を最大 3.73 倍誘導したが，誘導の程度は陽性対照であるフェノバルビタールによる CYP2B6 の mRNA 誘導能の 11%未満であった。また，3 ロット中 1 ロットで，エトラシモド（10 µmol/L）は CYP3A4 の mRNA を最大 8.94 倍誘導したが，誘導の程度は陽性対照であるリファンピシンによる CYP3A4 の mRNA 誘導能の 10%未満であった（報告書番号 [redacted]3079）。薬物相互作用に関するガイダンス（MHLW 2018[7]，EMA 2012b[8]，FDA 2020[9]）によると，3 ロット中 1 ロットで mRNA の増加が濃度依存的であり，mRNA 増加が 2 倍超である場合，さらなる誘導の評価が必要となる。誘導の可能性を評価するもう 1 つの方法（MHLW 2018[7]，EMA 2012b[8]，FDA 2020[9]）は，肝薬物濃度を推定することである。肝薬物濃度の推定には以下の 2 つの方法がある。

- 方法 1：$I_h = f_{u,p} \times [C_{max} + (F_a \times F_g \times k_a \times Dose)/Q_h/R_B]$[7,8]
  I_h：肝薬物濃度，$f_{u,p}$：血漿中非結合型分率，C_{max}：定常状態における血漿中総最高濃度，F_a：吸収率，F_g：消化管バイオアベイラビリティ，k_a：吸収速度定数，Q_h：肝血流量，R_B：血液/血漿中濃度比（C_b/C_p）

- 方法 2：$I_{max,u} \times 30$[9]
  $I_{max,u}$：定常状態における最高血漿中非結合型濃度

エトラシモド 2 mg を 1 日 1 回投与後の定常状態における肝薬物濃度は 6.8 nmol/L（方法 1）または 156 nmol/L（方法 2）と推定され，いずれもヒト肝細胞を用いた誘導評価に用いられたエトラシモド濃度（1 µmol/L，3 ロット中 1 ロットで mRNA が 3.44 倍増加，3 ロット中 2 ロットでの増加は 2 倍未満）よりも低濃度であった。このことから，エトラシモド 2 mg の 1 日 1 回投与時における P450 誘導の可能性は低いと考えられた。

さらに，ヒト肝細胞を 0.001～125 µmol/L のエトラシモドで処理したところ，CYP3A4，CYP2B6，CYP1A2，CYP2C8，CYP2C9 または CYP2C19 の誘導は認められなかった（報告書番号 PF-07915503_[redacted]_074315）。この追加試験の結果から，臨床においてエトラシモドが CYP3A4，CYP2B6，CYP1A2 及び CYP2C を誘導するリスクは低いと考えられた。

6. 排泄

報告書番号 APD334.DMPK.002，APD334.DMPK.001，概要表 2.6.5.13

無処置または胆管カニューレ挿入ラットあるいはイヌに[^{14}C]エトラシモドを経口投与したときの薬物動態を検討した試験において，エトラシモドの排泄を評価した。

10 mg/kg の[^{14}C]エトラシモドを雌雄の無処置 Sprague Dawley ラットに単回経口投与したところ（報告書番号 APD334.DMPK.002），主な排泄経路は糞中排泄であり，投与後 48 時間以内に投与放射能の 87%～89%，投与後 168 時間までに投与放射能の 95%超が回収された。雌雄いずれにおいても尿中排泄の寄与は小さく，投与放射能の排泄は 1%未満であった。雌雄の無処置 Sprague Dawley ラットにおける放射能の総回収率は 97%超であった。また，胆管カニューレ挿入ラットでは（報告書番号 APD334.DMPK.002），投与後 24 時間以内に 80%超の放射能が回収され，速やかに排泄された。投与後 48 時間以内に雄および雌で投与放射能のそれぞれ 80.6%および 64.8%が胆汁中に回収され，投与後 120 時間までに投与放射能のそれぞれ 83.0%および 66.7%が胆汁中に排泄された。雌雄いずれにおいても尿中排泄の寄与は小さく，投与放射能の排泄は 1%未満であった。雌雄の胆汁中排泄および尿中排泄の結果から，[^{14}C]エトラシモドは経口投与後に良好に吸収された（67%～84%）ことが示唆される。胆管カニューレ挿入ラットにおける投与後 168 時間までの放射能の回収率は，投与放射能の約 100%であった。放射能の排泄に性差は認められなかった。

雌雄のビーグル犬に[^{14}C]エトラシモドを 2 mg/kg の用量で単回経口投与したとき（報告書番号 APD334.DMPK.001），主な排泄経路は糞中排泄であり，投与後 96 時間までに 68%超が回収され，投与後 168 時間までに投与放射能の 81%超が回収された。雌雄いずれにおいても，投与後 96 時間超までに糞中排泄された放射能の 1 日当たりの回収率は 2%～7%であり，[^{14}C]エトラシモドの排泄は投与後 168 時間までには完了しないと推察された。尿中排泄の寄与は小さく，投与放射能の排泄は 2%未満であった。雌雄の放射能の総回収率は，投与放射能の 83%を上回った。放射能の排泄に性差は認められなかった。

以上の結果より，[^{14}C]エトラシモドは無処置ラットおよびイヌにおいて主に糞中に排泄され，また胆管カニューレ挿入ラットにおいては経口投与後に良好に吸収され，主に胆汁中に排泄されることが示された。

健康被験者のマスバランス試験（APD334-107 試験，M2.7.2.2）では，ヒトにおいても放射能は主に糞中に排泄され，尿中排泄はわずかであることが示されている。このように，動物およびヒトにおける主な排泄経路はいずれも胆汁中であることが示唆された。

7. 薬物動態学的薬物相互作用

エトラシモドのヒトのトランスポーターに対する阻害能または基質性を評価するための in vitro 試験を実施した（7.1 項）。なお，動物を用いた薬物相互作用試験は実施していない。

7.1. ヒト ABC および SLC トランスポーターに対する阻害能または基質性に関する in vitro 評価

報告書番号 [redacted]8106，概要表 2.6.5.14

ヒトトランスポーター（P-gp，BCRP，BSEP，OATP1B1，OATP1B3，OAT1，OAT3，OCT1，OCT2，MATE1 および MATE2-K）に対するエトラシモドの阻害能および基質性を評価した（報告書番号 [redacted]8106）。

各種トランスポーターを発現する細胞または膜小胞を特異的基質およびエトラシモドとインキュベートして，エトラシモドによるトランスポーター阻害活性を測定した。10 µmol/L および 100 µmol/L あるいは 1 および 10 µmol/L の濃度での阻害作用を評価し，その後必要に応じて，0.03，0.1，0.3，1，3，10 および 30 µmol/L での試験を行い IC_{50} 値を求めた。基質性に関する評価では，1 および 10 µmol/L の濃度（当該試験用に開発した LC-MS/MS 法で測定）で試験した（報告書番号 [redacted]8106）。

トランスポーター阻害に関する IC_{50} 値およびトランスポーターの基質となる可能性を Table 15 および Table 16 に示す。

Table 15.　エトラシモドのトランスポーターに対する in vitro 阻害能

トランスポーター	試験系	基質	エトラシモド濃度	IC_{50} 値 (µmol/L)
P-gp	Caco-2	ジゴキシン(10 µmol/L)	10 および 100 µmol/L	約 100
BCRP	MDCKII-BCRP	プラゾシン(1 µmol/L)	0.03～30 µmol/L	35.7
BSEP	膜小胞	[^{3}H]タウロコール酸(0.4 µmol/L)	1 および 10 µmol/L	>10
OATP1B1	HEK293	[^{3}H]エストラジオール-17β-グルクロニド(50 nmol/L)		約 10
OATP1B3				>10
OAT1		[^{3}H]*p*-アミノ馬尿酸(1 µmol/L)		>10
OAT3		[^{3}H]エストロン-3-硫酸塩(50 nmol/L)		>10
OCT1		[^{14}C]テトラエチルアンモニウムブロミド(5 µmol/L)		>10
OCT2		[^{14}C]メトホルミン(10 µmol/L)		>10
MATE1				>10
MATE2-K				>10

報告書番号 [redacted]8106，Table 1～10.

Table 16.　エトラシモドのトランスポーターに対する基質性

トランスポーター	試験系	エトラシモド濃度	基質性
P-gp	MDCKII-P-gp	1 および 10 µmol/L	なし
BCRP	MDCKII-BCRP		なし
OATP1B1	HEK293		なし
OATP1B3			なし
OAT1			なし
OAT3			なし
OCT1			なし
OCT2			なし

P-gp および BCRP については Efflux ratio，OATP1B1/3，OAT1/3 および OCT1/2 については取込みに関連する
報告書番号 [redacted]8106，Table 11～18

エトラシモドは，P-gp，BCRP および OATP1B1 を阻害し，IC_{50} 値はそれぞれ約 100，35.7 および約 10 µmol/L であった。その他のトランスポーター（BSEP，OATP1B3，OAT1，OAT3，

OCT1，OCT2，MATE1 および MATE2-K）に対する阻害能は，最大 10 µmol/L において 50%未満であった。

薬物相互作用に関するガイダンス（MHLW 2018[7]，EMA 2012b[8]，FDA 2020[9]）に基づき，エトラシモドは P-gp，BCRP および OATP1B1 に対して臨床的に意味のある薬物相互作用を引き起こす可能性は低いと考えられる。さらに，検討した条件において，エトラシモドは P-gp，BCRP，OATP1B1，OATP1B3，OAT1，OAT3，OCT1 および OCT2 の基質とはならなかった。概して，エトラシモドは臨床においてトランスポーターを介した薬物相互作用を引き起こす可能性は低いと考えられる。

8. その他の薬物動態試験

8.1. 薬物動態と薬力学との関係

雄の BALB/c マウス，Sprague Dawley ラット，ビーグル犬およびカニクイザルを用いて，エトラシモドの血漿中濃度（PK）とエトラシモドによる血中リンパ球減少（PD）との関連性を検討した（報告書番号 MPR-[redacted]-011，MPR-[redacted]-012，MPR-[redacted]-013，MPR-[redacted]-014）。

PK/PD モデルから，雄のマウス，ラット，イヌおよびサルへのエトラシモド経口投与により血中リンパ球数が用量依存的に減少することが明らかとなった。ヒトの PK/PD 解析結果は，M2.7.2.3 に記載した。

9. 考察及び結論

複数の動物種を用いてエトラシモドの薬物動態を検討した。エトラシモドはすべての動物種で速やかに吸収された。単回静脈内投与後の血漿 $t_{1/2}$ は，マウスで 12.1 時間，ラットで 8.02 時間，イヌで 31.1 時間，サルで 8.00 時間であり，イヌにおける $t_{1/2}$ は他の動物種と比べて長かった。げっ歯類の CL（マウスで 0.110 L/h/kg，ラットで 0.145 L/h/kg）と比較してイヌの CL（0.014 L/h/kg）は低値であり，長い $t_{1/2}$ は低い CL と相関していた。一方，イヌの $t_{1/2}$ はヒトの $t_{1/2}$（30 時間，M2.7.2.3）に最も類似していた。サルの $t_{1/2}$ はラットと同程度であったが，単回静脈内投与後のサルの CL（0.059 L/h/kg）はイヌと同程度であった。サルの V_{ss}（0.433 L/kg）は，げっ歯類（マウスで 1.93 L/kg，ラットで 1.61 L/kg）よりも小さく，イヌ（0.609 L/kg）よりわずかに小さかった。エトラシモドを単回経口投与したとき，T_{max} はマウス，ラットおよびイヌで同程度（マウスで 8.00 時間，ラットおよびイヌで 7.33 時間）であったが，サル（3.33 時間）ではやや短かった。一方，経口バイオアベイラビリティはマウスおよびイヌで高く（それぞれ約 100%および 73%），ラットおよびサルで中等度（それぞれ 54%および 44%）であった。ヒトマスバランス試験（APD334-107 試験）におけるエトラシモドの吸収は良好で，ヒトにおいても高いバイオアベイラビリティを示す可能性が示された（M2.7.2.2，M2.7.2.3）。

マウス，ラットおよびイヌにエトラシモドを反復投与したときの TK は，全動物種で 4 週間および 3 ヵ月間，さらに，マウスで 2 年間，ラットで 6 ヵ月間および 2 年間，イヌで 9 ヵ月間の試験で評価した。妊娠ラットおよび妊娠ウサギを用いた反復投与 TK も実施した。マウスにエトラシモドを 4 週間および 3 ヵ月間，1 日 1 回経口投与したところ，エトラシモド曝露量（AUC）

は用量比例的に増加したが，反復投与後の曝露量（AUC）は高用量群（200 mg/kg/日以上）では雌雄ともに用量比例性を下回った。

ラットでは，初回経口投与後に 25 から 200 mg/kg/日の範囲で曝露量は用量比例的に増加した一方，182 日間の反復投与後の最大耐量（250 mg/kg/日）ならびに 364 日間反復投与後の 20 mg/kg/日の用量で，曝露量（AUC）は用量比例性を下回った。ラットでは曝露量に性差が認められ，特に 182 および 364 日間の反復投与後の雌で最も高い蓄積性が認められた。イヌでは 0.02～2 mg/kg/日で用量比例性が認められたが，20～1000 mg/kg/日では用量比例性は認められなかった。イヌにおけるエトラシモドの蓄積性は，ラットおよびマウスと比較してわずかに大きかったが，ヒトと同程度であり，これはラットおよびマウスの $t_{1/2}$（約 8～12 時間）と比べてイヌの $t_{1/2}$（約 31 時間）が長いことと一致していた。

アルビノラットおよび有色ラットに[^{14}C]エトラシモドを投与したとき，放射能は消化管，消失器官（腎臓および肝臓），心筋，分泌腺（副腎および甲状腺）および脳室周囲以外の中枢神経系組織（脊髄，小脳，大脳，延髄および嗅脳）に分布した。また，アルビノラットおよび有色ラットのいずれにおいても，エトラシモドは血液脳関門，血液精巣関門および血液卵胞関門を通過した。雌雄 Long-Evans ラットに[^{14}C]エトラシモドを反復投与したところ，単回投与した場合に比べて曝露量が増加した。有色皮膚における放射能濃度および曝露量は，非有色皮膚と比べてやや高く（2 倍未満），雌雄 Long-Evans ラットにおいて[^{14}C]エトラシモド由来放射能がメラニン含有組織に中等度に結合および蓄積することと一致していた。[^{14}C]エトラシモドの C_b/C_p は，in vitro 試験の値と同程度であり，Sprague Dawley ラットおよび Long-Evans ラットの系統間あるいは雌雄間で差は認められなかった。ヒトで認められた結果（M2.7.2.2）と同様に，評価したすべての動物種における C_b/C_p が 1 未満であったことから，放射能は主に血漿コンパートメントに存在し，血液中の細胞成分への分布はわずかであることが示された。すべての動物種およびヒトの血漿中におけるエトラシモドの蛋白結合率は高く（95%超），主に血漿コンパートメントに分布し，血球成分への結合率は低いことが示された。

種々の動物種およびヒトにおけるエトラシモドの in vitro での代謝率は低く，肝ミクロソーム（補因子の存在下）および肝細胞では，さまざまな酸化代謝物，抱合体および二次酸化代謝物/抱合代謝物が認められた。エトラシモドの代謝に関与する主なヒト酵素は，CYP2C8，CYP2C9 および CYP3A4 であり，これらより程度は低いものの CYP2C19 および CYP2J2 も関与している。また，複数のヒト UGT もエトラシモドの代謝に関与している。

エトラシモドはヒト P450 または UGT を阻害しなかった。mRNA の発現倍率に基づく評価では，ヒト CYP1A2，CYP2B6，CYP3A4，CYP2C8，CYP2C9 および CYP2C19 を誘導しなかった。また，ヒト ABC トランスポーター（P-gp，BCRP および BSEP）および SLC トランスポーター（OATP1B1，OATP1B3，OAT1/3，OCT1，OCT2 および MATE1/2-K）を阻害せず，また基質にもならなかった。したがって，エトラシモドが他の併用薬に対して臨床的に意味のある薬物相互作用を引き起こす可能性は低いと考えられる。同様に，CYP2C8，CYP2C9 または CYP3A4 に対する中等度から強力な阻害剤あるいは誘導剤を併用する場合を除き，エトラシモドの代謝に関与する複数の P450 を介してエトラシモドが被相互作用薬となる可能性は低いと考えられる。

動物およびヒトの肝細胞を[^{14}C]エトラシモドとインキュベーションしたときの代謝物プロファイルは同様であり，ヒト特異的な代謝物は認められなかった。

無処置の雌雄ラットおよびイヌならびに雌雄胆管カニューレ挿入ラットに[^{14}C]エトラシモドを投与したマスバランス試験では，ヒトと同様の結果が得られた（M2.7.2.2）。ラットおよびイヌにおける薬物由来放射能は主に糞中に排泄され，投与放射能のそれぞれ 95%超および 81%を占めた一方，尿中排泄はわずかであった。In vivo において，エトラシモドはラットで広範に代謝され，in vitro 試験で認められた酸化，抱合および酸化/抱合の組み合わせと一致していたが，イヌにおける代謝の程度はラットより低かった。ヒトと同様に，ラットおよびイヌの血中に認められた主な放射能由来物質は未変化体であった。このように，マスバランス試験の結果および代謝の類似性から，毒性試験に用いる非臨床動物種としてラットおよびイヌを選択したことの適切性とヒトの安全性評価への外挿性が支持された。

マウス，ラット，イヌおよびサルにおける PK/PD 評価から，選択的 S1P 受容体調節薬であるエトラシモドの経口投与により，すべての動物種で用量依存的に血中リンパ球数が低下し，ヒトで認められた結果と一致していた（M2.7.2.3）。間接反応 PK/PD モデルを用いて濃度-反応関係を検討したところ，全動物種において血漿中濃度とリンパ球減少との間に一過性の逆相関関係が認められた。

以上，in vivo および in vitro の非臨床試験（動物およびヒト試料を含む）においてエトラシモドの薬物動態特性は十分に評価され，良好な薬物動態特性が示された。また臨床開発を進める上で，ラットおよびイヌが毒性試験に用いる適切な非臨床動物種となることが示された。

10. 図表

図表は，本文中の該当箇所または薬物動態試験概要表（M2.6.5）に記載し，概要表を参照する場合は，参照箇所を本文中に示した。

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2.6.5.1. PHARMACOKINETICS: OVERVIEW

Table 1: Overview of Nonclinical Pharmacokinetics Studies

Type of Study	Tox Study Number Report Number	Test System	Method of Administration	Dose Level or Concentration	GLP[a]	Test Facility
Absorption After a Single Dose						
Permeability in Caco-2 cells	NA PDM-[redacted]-286	Caco-2 cells	In vitro	20 µM	No	Arena Pharmaceuticals, Inc.
Permeability and P-gp and BCRP substrate potential in Caco-2 cells	NA APD334.DMPK.007	Caco-2 cells	In vitro	0.5, 5.0 µM	No	[redacted]
Single-dose PK	NA MPR-[redacted]-014	Mouse/BALB/c	Single intravenous or oral gavage	1 mg/kg[b]	No	Arena Pharmaceuticals, Inc.
Single-dose PK	NA MPR-[redacted]-013	Rat/ Sprague Dawley	Single intravenous or oral gavage	1 mg/kg[b]	No	Arena Pharmaceuticals, Inc.
Single- and repeat-dose ADME	NA APD334.DMPK.002	Rat/ Sprague Dawley and Long-Evans	Single and daily oral gavage for 7 days	10 mg/kg/day[b]	No	[redacted]
Single-dose PK	NA MPR-[redacted]-015	Dog/ Beagle	Single intravenous or oral gavage	1 mg/kg[b] intravenous; 3 mg/kg[b] oral	No	[redacted] Arena Pharmaceuticals, Inc. (BA/PK)
Single- and repeat-dose TK	TX[redacted]007 AVA00130	Dog/ Beagle	Single and daily oral gavage for 3 days	100, 300[c], 1000 mg/kg	No	[redacted]
Single-dose ADME	NA APD334.DMPK.001	Dog/ Beagle	Single oral gavage	2 mg/kg[b]	No	[redacted]
Single-dose PK	NA MPR-[redacted]-016	Monkey/ Cynomolgus	Single intravenous or oral gavage	1 mg/kg[b] intravenous; 3 mg/kg[b] oral	No	[redacted]; Arena Pharmaceuticals, Inc. (BA/PK)

Type of Study	Tox Study Number Report Number	Test System	Method of Administration	Dose Level or Concentration	GLP[a]	Test Facility
Absorption After Repeat Doses						
Single- and repeat-dose ADME	NA APD334.DMPK.002	Rat/Sprague Dawley and Long-Evans	Single and daily oral gavage for 7 days	10 mg/kg/day[b]	No	[redacted]
Repeat-dose TK	TX[redacted]003 MPR-[redacted]-013	Mouse/CD-1	Daily oral gavage for 28 days	30, 100, and 300/200 mg/kg/day[b]	No	[redacted].
Repeat-dose TK	APD334.TOX.001 900-169	Mouse/CD-1	Daily oral gavage for 91 days	20, 60, 200 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	APD334.TOX.003 900-174	Mouse/CD-1	Daily oral gavage for 104 weeks	2, 6, 20 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	TX[redacted]010 MPR-[redacted]-008	Rat/Sprague Dawley	Daily oral gavage for 28 days	25, 150, 350/250, 500 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	TX[redacted]004 MPR-[redacted]-009	Rat/Sprague Dawley	Daily oral gavage for 3 months	25, 100, 200 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	TX[redacted]009 MPR-[redacted]-007	Rat/Sprague Dawley	Daily oral gavage for 6 months	25, 75, 150, 250 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	APD334.TOX.002 900-173	Rat/Sprague Dawley	Daily oral gavage for 104 weeks	2, 6, 20 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	TX[redacted]007 AVA00130	Dog/Beagle	Single and daily oral gavage for 3 days	100, 300[c],1000 mg/kg /day	No	[redacted]
Repeat-dose TK	TX[redacted]008 AVA00131	Dog/Beagle	Daily oral gavage for 7 days	30, 300 mg/kg/day	No	[redacted]
Repeat-dose TK	TX[redacted]007 MPR-[redacted]-012	Dog/Beagle	Daily oral gavage for 28 days	0.02, 0.2, 2.0 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	TX[redacted]008 MPR-[redacted]-011	Dog/Beagle	Daily oral gavage for 28 days	20, 40, 80 100, 500/200, 1000/ 300 mg/kg/day[b]	Yes	[redacted]

Type of Study	Tox Study Number Report Number	Test System	Method of Administration	Dose Level or Concentration	GLP[a]	Test Facility
Repeat-dose TK	TX[redacted]005 MPR-[redacted]-008	Dog/Beagle	Daily oral gavage for 3 months	1, 5, 15 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK	TX[redacted]008 MPR-[redacted]-011	Dog/Beagle	Daily oral gavage for 9 months	2, 5, 10, 15 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK in juvenile animals	APD334.TOX.008 900-177	Rat/Sprague Dawley	Daily oral gavage from Postnatal Day 21 through Postnatal Day 34	25, 100, 200 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK in juvenile animals	APD334.TOX.009 900-180	Rat/Sprague Dawley	Daily oral gavage for 7 weeks starting on Postnatal Day 21	10, 30, 100 mg/kg/day[b]	Yes	[redacted]
Organ Distribution						
Single- and repeat-dose ADME	NA APD334.DMPK.002	Rat/Sprague Dawley and Long-Evans	Single or repeat 7-day oral gavage	10 mg/kg/day[b]	No	[redacted]
Single-dose ADME	NA APD334.DMPK.001	Dog/Beagle	Single oral gavage	2 mg/kg[b]	No	[redacted]
Plasma Protein Binding						
Plasma protein binding	NA MPR-[redacted]-001	Mouse/CD-1, Rat/Sprague Dawley, Rabbit/New Zealand White, Dog/Beagle, Monkey/Cynomolgus, Human	In vitro	10 µM	No	Arena Pharmaceuticals, Inc.
Blood-to-plasma partitioning	NA APD334.DMPK.003	Rat/Sprague Dawley, Dog/Beagle, Monkey/ Cynomolgus, Human	In vitro	0.10, 0.25, 0.50, 1.0, 2.0 µg/mL	No	[redacted]
Study in Pregnant or Nursing Animals						

Type of Study	Tox Study Number Report Number	Test System	Method of Administration	Dose Level or Concentration	GLP[a]	Test Facility
Repeat-dose TK in pregnant animals	TX[redacted]017 MPR-[redacted]-008	Rat/Sprague Dawley	Daily oral gavage from Gestation Day 6 through Gestation Day 17	1, 2, 4 mg/kg/day[b]	Yes	[redacted]
Repeat-dose TK in pregnant and nursing animals	APD334.TOX.012 20271969	Rat/Sprague Dawley	Daily oral gavage for Gestation Day 6 through Lactation Day 20	0.05, 0.2, 1.0, 2.0 mg/kg/day[b]	No	[redacted]
Repeat-dose TK in pregnant and nursing animals	APD334.TOX.011	Rat/Sprague Dawley	Daily oral gavage for Gestation Day 6 through Lactation Day 20	0.4, 2.0, 4.0 mg/kg/day [b]	Yes	[redacted]
Repeat-dose TK in pregnant animals	TX[redacted]019 MPR-[redacted]-009	Rabbit/New Zealand White	Daily oral gavage from Gestation Day 7 through Gestation Day 20	2, 10, 20 mg/kg/day[b]	Yes	[redacted]
Other Distribution						
Microsomal protein binding	NA APD334.DMPK.004	HLM	In vitro	10 µM	No	[redacted]
Human serum albumin, α_1-acid glycoprotein, and lipoprotein binding	NA ARE-R10656	NA	In vitro	0.020, 0.20 µg/mL	No	[redacted]
Non-specific binding	NA [redacted]8101	HEK293	In vitro	1, 10, 30 µM	No	[redacted]
Metabolism In Vivo						
Repeat-dose TK	APD334.TOX.001 900-169	Mouse/CD-1	Daily oral gavage for 91 days	20, 60, 200 mg/kg/day[b]	Yes	[redacted]
Single- and repeat-dose ADME	NA APD334.DMPK.002	Rat/Sprague Dawley and Long Evans	Single or repeat 7-day oral gavage	10 mg/kg/day[b]	No	[redacted]

Type of Study	Tox Study Number Report Number	Test System	Method of Administration	Dose Level or Concentration	GLP[a]	Test Facility
Single-dose ADME	NA APD334.DMPK.001	Dog/Beagle	Single oral gavage	2 mg/kg[b]	No	[redacted]
Metabolism In Vitro						
Metabolic stability	NA MPR-[redacted]-003	Mouse/CD-1, Rat/Sprague Dawley, Rabbit/New Zealand White, Dog/Beagle, Monkey/Cynomolgus, and Human liver microsomes	In vitro	1.0 µM	No	Arena Pharmaceuticals, Inc.
Metabolite identification	NA MPR-[redacted]-005	Mouse/CD-1, Rat/Sprague Dawley, Rabbit/New Zealand White, Dog/Beagle, Monkey/Cynomolgus, and Human liver microsomes	In vitro	100 µM	No	Arena Pharmaceuticals, Inc.
Metabolite identification	NA MPR-[redacted]-003	Rat/Sprague Dawley, Dog/Beagle, Monkey/ Cynomolgus, and Human hepatocytes	In vitro	20 µM	No	Arena Pharmaceuticals, Inc.
CYP reaction phenotyping	NA MPR-[redacted]-002	HLM and recombinant CYP enzymes	In vitro	1.0, 10 µM	No	Arena Pharmaceuticals Inc.
CYP reaction phenotyping	NA [redacted]4054	HLM and recombinant CYP enzymes	In vitro	1, 10 µM	No	[redacted]
Metabolite Clarification	NA APD334.DMPK.005	HLM and recombinant CYP enzymes	In vitro	10 µM	No	[redacted]
CYP reaction phenotyping	NA APD334.DMPK.010	HLM and recombinant CYP enzymes	In vitro	0.25, 1.0 µM	No	[redacted]

Type of Study	Tox Study Number Report Number	Test System	Method of Administration	Dose Level or Concentration	GLP[a]	Test Facility
Non-CYP enzyme identification	NA APD334.DMPK.009	HLM, human cytosol, recombinant CBR	In vitro	0.250, 1.0 µM (metabolites)	No	[redacted]
UGT reaction phenotyping	NA MPR-[redacted]-004	Mouse/CD-1, Rat/Sprague Dawley, Dog/Beagle, Monkey/Cynomolgus, and Human liver microsomes	In vitro	1.0 µM	No	Arena Pharmaceuticals, Inc.
Induction/Inhibition of Drug-Metabolizing Enzymes						
CYP inhibition	NA MPR-[redacted]-004	HLM	In vitro	0 to 50 µM	No	Arena Pharmaceuticals, Inc.
CYP and UGT inhibition	NA [redacted]5088	HLM	In vitro	1, 10 µM	No	[redacted]
CYP inhibition	NA APD334.DMPK.011	HLM	In vitro	0.03 to 30 µM	No	[redacted]
CYP induction	NA [redacted]3079	Human and rat hepatocytes	In vitro	1, 10 µM (Human), 3 to 150 µM (Rat)	No	[redacted]
Excretion						
Single- and repeat-dose ADME	NA APD334.DMPK.002	Rat/Sprague Dawley and Long-Evans	Single and daily oral gavage for 7 days	10 mg/kg/day[b]	No	[redacted]
Single-dose ADME	NA APD334.DMPK.001	Dog/Beagle	Single oral gavage	2 mg/kg[b]	No	[redacted]
Excretion into Bile						
Single- and repeat-dose ADME	NA APD334.DMPK.002	Rat/Sprague Dawley	Single and daily oral gavage for 7 days	10 mg/kg/day[b]	No	[redacted]
Drug-Drug Interactions						

Type of Study	Tox Study Number Report Number	Test System	Method of Administration	Dose Level or Concentration	GLP[a]	Test Facility
ABC and SLC transporters substrate and inhibition	NA [redacted]8106	Caco-2 cells, MDCKII cells, HEK293 cells, and Sf9 vesicles	In vitro	0.03 to 100 µM	No	[redacted]
Other						
PK/PD	NA MPR-[redacted]-011	Mouse/BALB/c	Single dose, oral gavage	0.03, 0.10, 0.30, 1.0 mg/kg[b]	No	Arena Pharmaceuticals, Inc.
PK/PD	NA MPR-[redacted]-012	Rat/Sprague Dawley	Single dose, oral gavage	0.10, 0.30, 1.0 mg/kg[b]	No	Arena Pharmaceuticals, Inc.
PK/PD	NA MPR-[redacted]-013	Dog/Beagle	Single dose, oral gavage	0.30, 1.0, 3.0 mg/kg[b]	No	Arena Pharmaceuticals, Inc.
PK/PD	NA MPR-[redacted]-014	Monkey/Cynomolgus	Single dose, oral gavage	0.10, 1.0 mg/kg[b]	No	Arena Pharmaceuticals, Inc.

[a] Yes indicates that the study was conducted in compliance with US FDA GLP Regulations (21 CFR Part 58).

[b] Expressed as free acid equivalents.

[c] Only 300 mg/kg/day was administered as repeat dose.

ABC, ATP-binding cassette; ADME, absorption, distribution, metabolism, excretion; ATP, adenosine triphosphate; BA/PK, bioanalytical and pharmacokinetic analysis; BCRP, breast cancer resistance protein; Caco-2, human colorectal adenocarcinoma cell line; CBR, carbonyl reductases; CD-1, Crl: CD1 (ICR) mouse strain; CFR, Code of Federal Regulations; CYP, cytochrome P450; FDA, Food and Drug Administration; GLP, Good Laboratory Practice; HEK293, human embryonic kidney cells 293; HLM, human liver microsomes; MDCKII, Madin-Darby canine kidney strain II cells; NA, not applicable; P-gp, P-glycoprotein; PK, pharmacokinetic; PK/PD, pharmacokinetics/pharmacodynamics; SLC, solute carrier; TK, toxicokinetic; UGT, uridine 5′-diphospho-glucuronosyltransferase; USA, United States of America.

2.6.5.2. ANALYTICAL METHODS AND VALIDATION REPORTS

Table 2: Bioanalytical Methods for Quantitation of Etrasimod in Nonclinical Samples

Species Matrix	Anti-coagulant	Sample Volume (µL)	Method	Analytical Range[a]	Internal Standard	Sample Processing	Established Frozen Matrix Stabilities	GLP Compliant (Y/N)	Validation Report	Method ID Number	Test Facility[b]	Study Number(s)
Mouse/ BALB/c Plasma	K-EDTA	NR	LC-MS/MS	0.005 – 10 µg/mL	AR116082	PPT	NR	N	NA	NA	Arena	MPR-[redacted]-014
Mouse/CD-1 Plasma	K_2EDTA	25	LC-MS/MS	0.50 – 250 µg/mL	d_9-etrasimod	PPT	121 days at –70°C	Y	APD334.MV.001 (900-170)	AM-900-170[c]	[redacted]	APD334.TOX.001 APD334.TOX.003
Rat/Sprague Dawley Plasma	K_2EDTA	25	LC-MS/MS	0.0005 – 1 µg/mL	d_9-etrasimod	LLE	NR	N	NA	NA	[redacted]	APD334.TOX.012
Rat/Sprague Dawley Plasma	K-EDTA	NR	LC-MS/MS	0.0025 – 5.0 µg/mL	AR116082	PPT	NR	N	NA	NA	Arena	MPR-[redacted]-013
Rat/Sprague Dawley, Long-Evans Plasma, Blood	K_2EDTA	NR	LSC	NR	NR	PPT	NR	N	NA	NA	[redacted]	APD334.DMPK.002
Rat/Sprague Dawley Plasma	K_2EDTA	25	LC-MS/MS	0.0050 – 2.5 µg/mL	d_9-etrasimod	PPT	20 days at –80°C	Y	CA16857-01	CA16857-01	[redacted]	TX[redacted]017 APD334.TOX.002 APD334.TOX.008 APD334.TOX.009 APD334.TOX.011
Rat/Sprague Dawley Plasma	K_2EDTA	20	LC-MS/MS	0.100 – 25.0 µg/mL	AR436434	PPT	21 days at –20°C, 1390 days at –80°C	Y	AA95243-01	AA95243-01	[redacted]	TX[redacted]010 TX[redacted]004 TX[redacted]009

Species Matrix	Anti-coagulant	Sample Volume (µL)	Method	Analytical Range[a]	Internal Standard	Sample Processing	Established Frozen Matrix Stabilities	GLP Compliant (Y/N)	Validation Report	Method ID Number	Test Facility[b]	Study Number(s)
Rabbit/New Zealand White Plasma	K_2EDTA	25	LC-MS/MS	0.005 – 2.5 µg/mL	*d*9-etrasimod	PPT	91 days at –80°C	Y	CA16858-01	CA16858-01	[redacted]	TX[redacted]019
Rabbit/New Zealand White Plasma	K_2EDTA	25	LC-MS/MS	0.250 – 100 µg/mL	*d*9-etrasimod	PPT	304 days at –80°C	Y	CA15809-01	CA15809-01	[redacted]	TX[redacted]019
Dog/Beagle Plasma	K_3EDTA	NR	LC-MS/MS	0.01 – 10 µg/mL	AR116082	PPT	NR	N	NA	NA	Arena	MPR-[redacted]-015
Dog/Beagle Plasma, Blood	K_2EDTA	NR	LSC	NR	NR	NR	NR	N	NA	NA	[redacted]	APD334.DMPK.001
Dog/Beagle Plasma	K-EDTA	10	NR	0.05 – 500 µg/mL	NR	NR	NR	N	NA	NA	Arena	TX[redacted]007 TX[redacted]008
Dog/Beagle Plasma	K_2EDTA	50	LC-MS/MS	0.001 – 0.5 µg/mL	AR436434	PPT	307 days at –80°C	Y	AA96474-01	AA96474-01	[redacted]	TX[redacted]007 TX[redacted]005 TX[redacted]008
Dog/Beagle Plasma	K_2EDTA	20	LC-MS/MS	1.00 – 250 µg/mL	AR436434	PPT	17 days at –20°C, 307 days at –80°C	Y	AA95242-01	AA95242-01	[redacted]	TX[redacted]008 TX[redacted]005 TX[redacted]008
Monkey/ Cynomolgus Plasma	K_2EDTA	NR	LC-MS/MS	0.100 – 25 µg/mL, 0.005 – 5 µg/mL, and 0.01 – 2.5 µg/mL	AR116082	PPT	NR	N	NA	NA	Arena	MPR-[redacted]-016

Species Matrix	Anti-coagulant	Sample Volume (µL)	Method	Analytical Range[a]	Internal Standard	Sample Processing	Established Frozen Matrix Stabilities	GLP Compliant (Y/N)	Validation Report	Method ID Number	Test Facility[b]	Study Number(s)

[a] Low end of range indicates assay lower limit of quantitation.

[b] Test facility names are abbreviated as follows: Arena, Arena Pharmaceuticals, Inc.; [redacted], [redacted]; [redacted], [redacted]; [redacted], [redacted].

[c] Presented in Study Report APD334.TOX.001.

C, Celsius; LC-MS/MS; liquid chromatography with tandem mass spectrometry; LLE, liquid-liquid extraction; GLP, Good Laboratory Practice; ID, identification; K_2EDTA, dipotassium ethylenediaminetetraacetic acid; K_3EDTA, tripotassium ethylenediaminetetraacetic acid; LSC, liquid scintillation counter; PPT, protein precipitation; N, no; NA, not applicable; NR, not reported; Y, yes

2.6.5.3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

Table 3: Permeability of Etrasimod Across Caco-2 Cell Monolayers

Test Article	Etrasimod (APD334, AR401959)
Study Number	PDM-[redacted]-286 (non-GLP)
Study Type	In vitro
Study System	Caco-2 cells
Method	The bidirectional transport of etrasimod (20 µM) and positive control ^{3}H-digoxin (0.03 µM) was determined in the absence and presence of P-gp inhibitor cyclosporin A (5 µM) in Caco-2 cell monolayers.
Report Title: Preliminary study on bidirectional permeation of AR401959 across Caco-2 cell monolayers	

Compound	**Dose (µM)**	**P_{appAB} ($\times10^{-6}$ cm/s)**	**P_{appBA} ($\times10^{-6}$ cm/s)**	**Efflux Ratio[a]**	**P-gp Substrate Classification**
Etrasimod[b]	20	21.8 ± 3.03	43.8 ± 4.20	2.01	Negative
Etrasimod (+ cyclosporin)[b]	20 (5)	19.7 ± 1.23	26.2 ± 4.25	1.33	
^{3}H-digoxin[c]	0.03	1.18	12.8	10.8	Positive
^{3}H-digoxin (+ cyclosporin)[c]	0.03 (5)	5.05	6.01	1.19	

[a] Efflux ratio = P_{appBA}/P_{appAB}.
[b] Three inserts were used for each condition. Result is presented as mean ± standard deviation (n = 3).
[c] One insert was used for each condition.
GLP, Good Laboratory Practice; n, number; P_{appAB}, apparent permeability in the apical to basolateral direction; P_{appBA}, apparent permeability in the basolateral to apical direction; P-gp, P-glycoprotein-1

Table 4: Permeability of Etrasimod Across Caco-2 Cell Monolayers and P-gp and BCRP Substrate Potential

Test Article	Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.007 (non-GLP)
Study Type	In vitro
Study System	Caco-2 cells
Method	The bidirectional transport of etrasimod (0.5 and 5.0 µM) was determined in the absence and presence of valspodar (P-gp inhibitor) and Ko143 (BCRP inhibitor) in Caco-2 cell monolayers. Digoxin and cladribine are known substrates of P-gp and BCRP, respectively, and were used as positive controls.

Report Title: Assessment of Permeability and Substrate of P-Glycoprotein and Breast Cancer Resistance Protein Using Caco-2 Monolayers

Compound	**Concentration (µM)**	**P_{appAB} ($\times 10^{-6}$ cm/s)**	**P_{appBA} ($\times 10^{-6}$ cm/s)**	**Efflux Ratio**	**P-gp Substrate Classification**	**BCRP Substrate Classification**
Etrasimod	0.5	20.6	16.7	0.809	Negative	Negative
Etrasimod + valspodar (1 µM)	0.5	29.0	12.7	0.440		
Etrasimod + Ko143 (0.5 µM)	0.5	27.4	9.42	0.343		
Etrasimod	5.0	23.5	37.9	1.61	Negative	Negative
Etrasimod + valspodar (1 µM)	5.0	33.3	30.7	0.922		
Etrasimod + Ko143 (0.5 µM)	5.0	36.9	22.5	0.610		
Digoxin	10	0.513	7.71	15.0	Positive	NA
Digoxin + valspodar (1 µM)	10	2.65	2.32	0.876		
Cladribine	10	1.38	12.2	8.82	NA	Positive
Cladribine + Ko143 (0.5 µM)	10	1.67	1.94	1.16		

Note: Determinations were made in duplicate, and the mean is presented.
BCRP, breast cancer resistance protein; GLP, Good Laboratory Practice; NA, not applicable; P_{appAB}, apparent permeability in the apical to basolateral direction; P_{appBA}, apparent permeability in the basolateral to apical direction; P-gp, P-glycoprotein 1

Table 5: Pharmacokinetic Parameters of Etrasimod in Plasma After a Single Intravenous or Oral Administration – Mouse, Rat, Dog, and Monkey

Test Article		Etrasimod (APD334, AR401959)			
Study Number		MPR-[redacted]-014 (non-GLP)	MPR-[redacted]-013 (non-GLP)	MPR-[redacted]-015 (non-GLP)	MPR-[redacted]-016 (non-GLP)
Species		Mouse/BALB/c	Rat/Sprague Dawley	Dog/Beagle	Monkey/Cynomolgus
Sex/Number of Animals		Male/3 per timepoint per route of administration (11 timepoints for IV, 12 timepoints for oral)	Male/3 per route of administration	Male/3 per route of administration	Male/3 per route of administration
Vehicle/Formulation		40% HPBCD (IV), 0.5% methylcellulose (oral)	100% PEG400 (IV), 0.5% methylcellulose (oral)	100% PEG400 (IV), 0.5% methylcellulose (oral)	100% PEG400 (IV), 0.5% methylcellulose (oral)
Feeding Condition		Fasted	Fasted	Fasted	Fasted
Method of Administration		Oral, IV	Oral, IV	Oral, IV	Oral, IV
Dose (mg/kg)		1.0 (IV), 1.0 (oral)	1.0 (IV), 1.0 (oral)	1.0 (IV), 3.0 (oral)	1.0 (IV), 3.0 (oral)
Sample		Plasma	Plasma	Plasma	Plasma
Analyte		Etrasimod	Etrasimod	Etrasimod	Etrasimod
Assay		LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK Parameter	**Route**				
$t_{½}$ (h)	IV	12.1	8.02 ± 1.10	31.1 ± 3.8	8.00 ± 1.23
	Oral	12.3	9.64 ± 3.33	28.8 ± 3.1	6.37 ± 0.68
t_{max} (h)	IV	NA	NA	NA	NA
	Oral	8.00	7.33 ± 2.31	7.33 ± 1.15	3.33 ± 1.15
C_{max} (µg/mL)	IV	NA	NA	NA	NA
	Oral	0.780	0.218 ± 0.130	3.71 ± 0.12	2.87 ± 0.77
AUC_{last} (µg·h/mL)	IV	8.57	5.90 ± 1.25	48.3 ± 8.3	17.5 ± 3.8
	Oral	9.90	2.86 ± 1.19	112 ± 10	23.0 ± 8.0

Test Article		Etrasimod (APD334, AR401959)			
Study Number		MPR-[redacted]-014 (non-GLP)	MPR-[redacted]-013 (non-GLP)	MPR-[redacted]-015 (non-GLP)	MPR-[redacted]-016 (non-GLP)
Species		Mouse/BALB/c	Rat/Sprague Dawley	Dog/Beagle	Monkey/Cynomolgus
AUC_{∞} (µg·h/mL)	IV	9.13	7.03 ± 1.24	74.5 ± 15.2	17.6 ± 4.0
	Oral	10.3	3.79 ± 0.90	164 ± 25	23.1 ± 8.1
$\%AUC_{extrap}$	IV	6.10	16.4 ± 3.1	34.9 ± 2.3	0.884 ± 0.500
	Oral	3.65	26.5 ± 13.0	31.5 ± 4.1	0.512 ± 0.296
CL (L/h/kg)	IV	0.110	0.145 ± 0.024	0.0138 ± 0.0030	0.0585 ± 0.0116
	Oral	NA	NA	NA	NA
MRT_{last} (h)	IV	14.5	6.74 ± 0.20	18.2 ± 0.4	7.15 ± 1.48
	Oral	11.0	9.71 ± 1.38	19.5 ± 1.0	8.29 ± 1.33
V_{ss} (L/kg)	IV	1.93	1.61 ± 0.38	0.609 ± 0.103	0.433 ± 0.022
	Oral	NA	NA	NA	NA
%F	IV	NA	NA	NA	NA
	Oral	~100%	54.0 ± 12.8	73.2 ± 11.0	43.8 ± 15.3

Note: Composite sampling was used for mouse, so variance was not calculated. Serial sampling was used for all other species, and data are presented as mean ± SD.
% F: ratio of the dose-normalized exposures after oral and intravenous administration, ie, $(AUC_{\infty}/Dose)_{oral}/(Mean\ AUC_{\infty}/Dose)_{IV} \times 100$.
$MRT_{last} = AUMC_{last}/AUC_{last}$
AUC, area under the concentration-time curve; $\%AUC_{extrap}$, percent of AUC_{∞} corresponding to the area under the extrapolated portion of the log plasma concentration-time curve; %F, absolute bioavailability; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 21 h (rat) or 48 h (mouse, dog, monkey); $AUMC_{last}$, area under the moment curve; C_{max}, maximum plasma drug concentration; CL, clearance; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; IV, intravenous; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MRT_{last}, mean residence time; NA, not applicable; PEG, polyethylene glycol; PK, pharmacokinetic; SD, standard deviation; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration; V_{ss}, volume of distribution at steady state

Table 6: Toxicokinetic Parameters of Etrasimod in Plasma After a Single Oral Administration – Dog

Test Article	Etrasimod (APD334, AR401959)
Study Number	TX[redacted]007 (Appendix 3) (non-GLP)
Species	Dog/Beagle
Sex/Number of Animals	Males, Females/1 per sex
Vehicle/Formulation	0.5% methylcellulose
Feeding Condition	Fed
Method of Administration	Oral
Dose (mg/kg)	100, 300, 1000
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Report Title: AR401959: Oral Rising-Dose Toxicity Study in Dogs			**Study Number:** TX[redacted]007 (Appendix 3)
PK Parameter	**Dose (mg/kg)**	**Males**	**Females**
$t_{½}$ (h)	100	NR	NR
	300	NR	NR
	1000	NR	NR
t_{max} (h)	100	6.00	24.0
	300	6.00	6.00
	1000	24.0	8.00
C_{max} (µg/mL)	100	29.5	50.5
	300	48.5	50.8
	1000	77.8	123

Report Title: AR401959: Oral Rising-Dose Toxicity Study in Dogs			**Study Number:** TX[redacted]007 (Appendix 3)
PK Parameter	**Dose (mg/kg)**	**Males**	**Females**
AUC_{last} (µg·h/mL)	100	973	1750
	300	1500	1540
	1000	2790	4160
AUC_{∞} (µg·h/mL)	100	1560	NA[a]
	300	2510	2680
	1000	NA[a]	5720
$\%AUC_{extrap}$	100	37.6	NA
	300	40.4	42.6
	1000	NA	27.3

[a] Not applicable since the terminal phase was undefined.

AUC, area under the concentration-time curve; $\%AUC_{extrap}$, percent of AUC_{∞} corresponding to the area under the extrapolated portion of the log plasma concentration-time curve; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 48 h; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 7: Pharmacokinetic Parameters of [^{14}C]Etrasimod in Blood and Plasma After a Single Oral Administration – Rat and Dog

Test Article		[^{14}C]Etrasimod (APD334, AR401959)					
Study Number		APD334.DMPK.002 (non-GLP)		APD334.DMPK.002 (non-GLP)		APD334.DMPK.001 (non-GLP)	
Species		Rat/Sprague Dawley[a]		Rat/Long-Evans[b]		Dog/Beagle	
Sex/Number of Animals		Male, Female/12 per sex		Male, Female/7 per sex		Male, Female/3 per sex	
Vehicle/Formulation		5% HPBCD		5% HPBCD		5% HPBCD	
Feeding Condition		Fasted		Fasted		Fasted	
Method of Administration		Oral		Oral		Oral	
Dose (mg/kg)		10		10		2.0	
Sample		Plasma, blood		Plasma, blood		Plasma, blood	
Analyte		Radioactivity[c]		Radioactivity[c]		Radioactivity[c]	
Assay		Radioactivity		Radioactivity		Radioactivity	
PK Parameter	**Matrix**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female[d]**
$t_{½}$ (h)	blood	25.8	27.3	12.9	33.6	43.9 ± 5.36	45.3
	plasma	20.0	22.2	13.0	11.9	41.2 ± 3.29	42.3
t_{max} (h)	blood	2.00	2.00	4.00	4.00	6.00 ± 2.00	4.00
	plasma	2.00	2.00	4.00	4.00	4.33 ± 1.53	3.50
C_{max} (µg eq./g)	blood	5.54	8.34	9.34	8.39	1.93 ± 0.027	2.40
	plasma	9.30	13.6	15.4	14.1	2.94 ± 0.036	3.92
AUC_{last} (µg eq.·h/g)	blood	107	121	128	136	92.4 ± 5.71	106
	plasma	167	179	213	208	145 ± 11.4	174
AUC_{0-24} (µg eq.·h/g)	blood	84.2	97.6	NC	NC	37.7 ± 1.63	44
	plasma	132	146	NC	NC	57.3 ± 3.03	71.6
AUC_{∞} (µg eq.·h/g)	blood	108	123	129	137	98.2 ± 7.43	114
	plasma	168	180	215	210	153 ± 13.9	185

[a] Rat/Sprague Dawley (Group 1)
[b] Rat/Long-Evans (Group 5)
[c] Total Radioactivity, [^{14}C]
[d] One animal was excluded from PK evaluation and descriptive statistics due to vomitus following dosing; n = 2.
Note: Composite sampling was used for rat, so variance was not calculated. Serial sampling was used for dog, and data are presented as mean ± SD.
AUC, area under the concentration-time curve; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 120 h (Rat/Sprague Dawley) or 168 h (Rat/Long-Evans, Dog/Beagle); AUC_{0-24}, AUC from time zero to 24 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; NC, not calculated; PK, pharmacokinetic; SD, standard deviation; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

2.6.5.4. PHARMACOKINETICS: ABSORPTION AFTER REPEAT-DOSE ADMINISTRATION

Table 8: Pharmacokinetic Parameters of [^{14}C]Etrasimod in Blood and Plasma After Multiple Dose, 7-Day Repeat Administration – Rat

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male, Female/1 per sex per timepoint (7 timepoints) (Group 6)
Vehicle/Formulation	5% HPBCD
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Dose (mg/kg)	10
Sample	Plasma, blood
Analyte	Radioactivity[a]
Assay	Radioactivity

Study Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats			**Study Number:** APD334.DMPK.002
PK Parameter	**Matrix**	**Males**	**Females**
$t_{½}$ (h)	blood	47.6	70.8
	plasma	42.6	59.8
t_{max} (h)	blood	4.00	4.00
	plasma	4.00	4.00
C_{max} (µg eq./g)	blood	13.2	12.6
	plasma	23.7	21.6
AUC_{last} (µg eq.·h/g)	blood	187.8	193.0
	plasma	324.1	315.1

Study Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats			**Study Number:** APD334.DMPK.002
PK Parameter	**Matrix**	**Males**	**Females**
AUC_{∞} (µg eq.·h/g)	blood	192.0	201.0
	plasma	329.2	323.6

[a] Total Radioactivity, [^{14}C]
Note: Composite sampling was used, so variance was not calculated.
AUC, area under the concentration-time curve; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 168 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 9: Toxicokinetic Parameters of Etrasimod in Plasma After 28-Day Oral Administration – Mouse

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]003 (Appendix G) (non-GLP)
Species	Mouse/CD-1
Sex/Number of Animals	Male, Female/3 per sex timepoint (13 timepoints total)
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral
Dose (mg/kg/day)	30, 100, 300/200 [a] (once daily for 28 days)
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: Oral Dose Range-Finding Toxicity Study in Mice				**Study Number:** TX[redacted]003 (Appendix G)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	30	ID	9.23	9.24	9.49
	100	ID	ID	9.31	7.88
	300/200	ID	22.7	8.04	ID
t_{max} (h)	30	8.00	4.00	2.00	2.00
	100	8.00	8.00	2.00	4.00
	300/200	12.0	8.00	4.00	8.00
C_{max} (µg/mL)	30	30.2	27.4	34.0	31.8
	100	91.8	67.7	109	95.0
	300/200	222	181	168	96.7

Study Title: APD334 L-Arginine: Oral Dose Range-Finding Toxicity Study in Mice				**Study Number:** TX[redacted]003 (Appendix G)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
AUC_{last} (µg·h/mL)	30	439	409	577	456
	100	1720	1200	1780	1030
	300/200	4530	3360	2180	1400
AI AUC_{last}	30	NA	NA	1.31	1.11
	100	NA	NA	1.19	0.858
	300/200	NA	NA	NA[a]	NA[a]
AUC_{last} F:M Ratio	30	NA	0.93	NA	0.79
	100	NA	0.70	NA	0.58
	300/200	NA	0.74	NA	0.64

[a] The dose was reduced from 300 mg/kg/day to 200 mg/kg/day on Day 7 due to mortality and clinical signs in both sexes. AI was not calculated due to dose reduction.
Note: PK parameters were determined from composite concentration-time profiles, so variance was not calculated.
AI, accumulation index (AUC_{last} Day 28/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; ID, insufficient data to calculate parameter due to undefined terminal phase; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 10: Toxicokinetic Parameters of Etrasimod in Plasma After 91-Day Oral Administration – Mouse

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.001 (Appendix 11) (GLP)
Species	Mouse/CD-1
Sex/Number of Animals	Male, Female/3 per sex per timepoint (13 timepoints total)
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 91 days)
Dose (mg/kg/day)	20, 60, 200
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: A 91-Day Oral Toxicity Study in Mice				**Study Number:** APD334.TOX.001 (Appendix 11)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 90**	
		Male	**Female**	**Male**	**Female**
t_{max} (h)	20	4.00	2.00	2.00	2.00
	60	4.00	4.00	4.00	4.00
	200	12.0	4.00	2.00	4.00
C_{max} (µg/mL)	20	16.5	10.6	22.4	14.9
	60	56.0	40.4	51.4	37.0
	200	152	127	106	90.7
AUC_{last} (µg·h/mL)	20	237	167	331	220
	60	778	669	894	569
	200	2800	1120	1870	1150

Study Title: APD334 L-Arginine: A 91-Day Oral Toxicity Study in Mice				**Study Number:** APD334.TOX.001 (Appendix 11)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 90**	
		Male	**Female**	**Male**	**Female**
AI AUC_{last}	20	NA	NA	1.40	1.32
	60	NA	NA	1.15	0.85
	200	NA	NA	0.67	1.03
AUC_{last} F:M Ratio	20	NA	0.71	NA	0.67
	60	NA	0.86	NA	0.64
	200	NA	0.40	NA	0.62

Note: PK parameters were determined from composite concentration-time profiles, so variance was not calculated.
AI, accumulation index (AUC_{last} Day 90/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; t_{max}, time to maximum plasma drug concentration

Table 11: Toxicokinetic Parameters of Etrasimod in Plasma After 104-Week Oral Administration – Mouse

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.003 (Appendix 12) (GLP)
Species	Mouse/CD-1
Sex/Number of Animals	Male, Female/ 3 cohorts of 3 animals/sex/group
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once-daily for up to 104 weeks)
Dose (mg/kg/day)	2, 6, 20
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: A 104-Week Oral Oncogenicity Study in Mice			**Study Number:** APD334.TOX.003 (Appendix 12)
PK Parameter	**Dose (mg/kg/day)**	**Day 364**	
		Male	**Female**
t_{max} (h)	2	4.00	12.0
	6	4.00	2.00
	20	4.00	4.00
C_{max} (µg/mL)	2	2.40	2.86
	6	6.59	4.85
	20	19.8	12.4
AUC_{last} (µg·h/mL)	2	39.5	44.6
	6	109	73.4
	20	305	210

Study Title: APD334 L-Arginine: A 104-Week Oral Oncogenicity Study in Mice			**Study Number:** APD334.TOX.003 (Appendix 12)
PK Parameter	**Dose (mg/kg/day)**	**Day 364**	
		Male	**Female**
AUC_{last} F:M Ratio	2	NA	1.13
	6	NA	0.672
	20	NA	0.690

AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; t_{max}, time to maximum plasma drug concentration

Table 12: Toxicokinetic Parameters of Etrasimod in Plasma After 28-Day Oral Administration – Rat

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]010 (Appendix J) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Male, Female/3 cohorts of 3 animals per sex per dose group sampled at alternating timepoints
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 28 days)
Dose (mg/kg/day)	25, 150, 350/250[a], 500[b]
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: 4-Week Oral Toxicity Study in Rats with a 4-Week Recovery Period				**Study Number:** TX[redacted]010 (Appendix J)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	25	5.36	5.11	4.65	5.80
	150	7.82	13.4	3.54	6.49
	350/250	UD	23.0	5.21	11.3
	500	UD	47.7	NS[a]	NS[a]
t_{max} (h)	25	6.00	2.00	2.00	4.00
	150	6.00	6.00	4.00	8.00
	350/250	24.0	12.0	4.00	8.00
	500	12.0	6.00	NS[a]	NS[a]

Study Title: APD334 L-Arginine: 4-Week Oral Toxicity Study in Rats with a 4-Week Recovery Period				**Study Number:** TX[redacted]010 (Appendix J)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
C_{max} (µg/mL)	25	12.6	18.8	15.6	28.6
	150	65.8	70.4	56.2	83.4
	350/250	97.0	129	88.6	152
	500	112	101	NS[a]	NS[a]
AUC_{last} (µg·h/mL)	25	147	198	165	271
	150	908	1130	700	1290
	350/250	1870	2290	1300	2370
	500	2210	1860	NS[a]	NS[a]
AI AUC_{last}	25	NA	NA	1.12	1.37
	150	NA	NA	0.77	1.14
	350/250	NA	NA	NA[b]	NA[b]
	500	NA	NA	NS[a]	NS[a]
AUC_{last} F:M Ratio	25	NA	1.35	NA	1.64
	150	NA	1.24	NA	1.84
	350/250	NA	1.22	NA	1.82
	500	NA	0.84	NS[a]	NS[a]

a Due to animal morbundity and/or mortality in the 500 mg/kg group, no samples were collected for Day 28. AI AUC_{last} was not calculated for this group since no samples were collected for Day 28.

b Due to toxicity and mortality in the 350 mg/kg/day group, animals in this cohort were not dosed on Days 5, 6, or 7. Dosing for the surviving animals was restarted on Day 8, but at a lower dose level of 250 mg/kg/day for the remainder of the 28-day study. AI AUC_{last} was not calculated for this group due to dose reduction.

Note: PK parameters were determined from composite concentration-time profiles, so variance was not calculated.

AI, accumulation index (AUC_{last} Day 28/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NS, no sample collected due to animal mortality/death prior to Day 28; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration; UD, underdefined terminal phase

Table 13: Toxicokinetic Parameters of Etrasimod in Plasma After 90-Day Oral Administration – Rat

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]004 (Appendix J) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Male, Female/3 cohorts of 3 animals per sex per dose group sampled at alternating timepoints
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 90 days)
Dose (mg/kg/day)	25, 100, 200
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: A 3-Month Toxicity Study in Rats with a 4-Week Recovery Period				**Study Number:** TX[redacted]004 (Appendix J)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 90**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	25	5.35	8.36	5.28	13.1
	100	5.59	8.38	4.77	9.10
	200	ID	ID	5.44	ID
t_{max} (h)	25	4.00	4.00	1.00	1.00
	100	6.00	4.00	6.00	4.00
	200	12.0	12.0	4.00	8.00
C_{max} (µg/mL)	25	11.0	13.6	19.3	29.6
	100	35.7	37.6	53.1	76.8
	200	71.2	95.3	79.7	150

Study Title: APD334 L-Arginine: A 3-Month Toxicity Study in Rats with a 4-Week Recovery Period				**Study Number:** TX[redacted]004 (Appendix J)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 90**	
		Male	**Female**	**Male**	**Female**
AUC_{last} (µg·h/mL)	25	131	157	218	314
	100	428	490	586	670
	200	1470	1970	1010	1730
AI AUC_{last}	25	NA	NA	1.66	1.99
	100	NA	NA	1.37	1.37
	200	NA	NA	0.687	0.878
AUC_{last} F:M Ratio	25	NA	1.20	NA	1.44
	100	NA	1.14	NA	1.14
	200	NA	1.34	NA	1.71

Note: PK parameters were determined from composite concentration-time profiles, so variance was not calculated.
AI, accumulation index (AUC_{last} Day 90/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 14: Toxicokinetic Parameters of Etrasimod in Plasma After 182-Day Oral Administration – Rat

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]009 (Appendix K) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Male, Female/3 cohorts of 3 animals per sex per dose group sampled at alternating timepoints
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 182 days)
Dose (mg/kg/day)	25, 75, 150, 250
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: A 6-Month Oral Toxicity Study in Rats with a 4-Week Recovery Period				**Study Number:** TX[redacted]009 (Appendix K)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 182**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	25	6.88	4.98	5.09	6.32
	75	8.19	7.93	6.11	8.93
	150	5.96	12.8	5.84	10.9
	250	13.6	31.0	11.6	9.94
t_{max} (h)	25	4.00	4.00	2.00	2.00
	75	6.00	6.00	6.00	6.00
	150	8.00	6.00	8.00	6.00
	250	12.0	8.00	6.00	8.00

Study Title: APD334 L-Arginine: A 6-Month Oral Toxicity Study in Rats with a 4-Week Recovery Period				**Study Number:** TX[redacted]009 (Appendix K)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 182**	
		Male	**Female**	**Male**	**Female**
C_{max} (µg/mL)	25	10.6	13.1	21.5	45.7
	75	32.4	50.1	45.4	106
	150	54.8	78.0	113	201
	250	81.2	91.1	159	275
AUC_{last} (µg·h/mL)	25	114	155	210	442
	75	385	556	554	1650
	150	803	1150	1440	3180
	250	1310	1850	2060	4470
AI AUC_{last}	25	NA	NA	1.84	2.85
	75	NA	NA	1.40	2.97
	150	NA	NA	1.79	2.77
	250	NA	NA	1.57	2.42
AUC_{last} F:M Ratio	25	NA	1.36	NA	2.10
	75	NA	1.44	NA	2.98
	150	NA	1.43	NA	2.21
	250	NA	1.41	NA	2.17

Note: PK parameters were determined from composite concentration-time profiles, so variance was not calculated.
AI, accumulation index (AUC_{last} Day 182/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 15: Toxicokinetic Parameters of Etrasimod in Plasma After 104-Week Oral Administration – Rat

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.002 (Appendix 12) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Male, Female/2 cohorts of 3 animals/sex/group at alternating timepoints
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once-daily for up to 91 weeks; 104 weeks intended)
Dose (mg/kg/day)	2, 6, 20
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-arginine: A 104-Week Oral Oncogenicity Study in Rats			**Study Number:** APD334.TOX.002 (Appendix 12)
PK Parameter	**Dose (mg/kg/day)**	**Day 364**	
		Male	**Female**
t_{max} (h)	2	6.00	2.00
	6	4.00	4.00
	20	6.00	6.00
C_{max} (µg/mL)	2	1.99	4.55
	6	5.19	8.44
	20	12.1	24.2
AUC_{last} (µg·h/mL)	2	27.6	65.2
	6	68.2	147

Study Title: APD334 L-arginine: A 104-Week Oral Oncogenicity Study in Rats			**Study Number:** APD334.TOX.002 (Appendix 12)
PK Parameter	**Dose (mg/kg/day)**	**Day 364**	
		Male	**Female**
	20	172	387
AUC_{last} F:M Ratio	2	NA	2.36
	6	NA	2.16
	20	NA	2.25

AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; t_{max}, time to maximum plasma drug concentration

Table 16: Toxicokinetic Parameters of Etrasimod in Plasma After 3-Day Oral Administration – Dog

Test Article	Etrasimod (APD334, AR401959)
Study Number	TX[redacted]007 (Appendix 3) (non-GLP)
Species	Dog/Beagle
Sex/Number of Animals	Male, Female/1 animal per sex
Vehicle/Formulation	0.5% methylcellulose
Feeding Condition	Fed
Method of Administration	Oral (once daily for 3 days)
Dose (mg/kg/day)	300
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS
Study Title	AR401959: Oral Rising-Dose Toxicity Study in Dogs

PK Parameter	**Dose (mg/kg/day)**	**Day 22**		**Day 23**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	300	NR	NR	NR	NR
t_{max} (h)	300	8.00	3.00	8.00	3.00
C_{max} (µg/mL)	300	73.6	141	94.7	126
AUC_{last} (µg·h/mL)	300	1530	2840	3280	4350
AUC_{last} F:M Ratio	300	NA	1.86	NA	1.33

Note: Single escalating doses of 100, 300, and 1000 mg/kg etrasimod were administered on Days 3, 9, and 15, respectively (single-dose PK parameters presented in Table 6). Daily doses of 300 mg/kg/day etrasimod were administered for 3 days beginning on Day 21. Days 22 and 23 are the second and third day of repeat dosing, respectively.
AUC, area under the concentration-time curve; AUC_{last}, AUC time zero to time of last measurable concentration through 48 h after Day 23 dose; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 17: Toxicokinetic Parameters of Etrasimod in Plasma After 7-Day Oral Administration – Dog

Test Article	Etrasimod (APD334, AR401959)
Study Number	TX[redacted]008 (Appendix 4) (non-GLP)
Species	Dog/Beagle
Sex/Number of Animals	Male, Female/2 per sex per dose level
Vehicle/Formulation	0.5% methylcellulose
Feeding Condition	Fed
Method of Administration	Oral (once daily for 7 days)
Dose (mg/kg/day)	30, 300
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: AR401959: 7-Day Oral Toxicity Study in Dogs				**Study Number:** TX[redacted]008 (Appendix 4)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 7**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	30	NR	NR	NR	NR
	300	NR	NR	NR	NR
t_{max} (h)	30	6.00	4.50	2.00	5.50
	300	10.0	24.0	6.00	5.50
C_{max} (µg/mL)	30	19.2	25.7	29.9	57.5
	300	73.4	86.0	229	181
AUC_{last} (µg·h/mL)	30	372	514	542	1130
	300	1341	1489	4405	3770

Study Title: AR401959: 7-Day Oral Toxicity Study in Dogs				**Study Number:** TX[redacted]008 (Appendix 4)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 7**	
		Male	**Female**	**Male**	**Female**
AI AUC_{last}	30	NA	NA	1.46	2.20
	300	NA	NA	3.28	2.53
AUC_{last} F:M Ratio	30	NA	1.38	NA	2.08
	300	NA	1.11	NA	0.86

AI AUC_{last}, accumulation index for AUC_{last} (AUC_{last} Day 7/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 48 h for Day 1 dose and 24 h for Day 7 dose; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 18: Toxicokinetic Parameters of Etrasimod in Plasma After 28-Day Oral Administration – Dog

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]007 (Appendix L) (GLP)
Species	Dog/Beagle
Sex/Number of Animals	Male, Female/3 per sex per dose level
Vehicle/Formulation	20% ethanol
Feeding Condition	Fed
Method of Administration	Oral (once daily for 28 days)
Dose (mg/kg/day)	0.02, 0.2, 2.0
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: 4-Week Oral Toxicity Study in Dogs				**Study Number:** TX[redacted]007 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	0.02	21.2 ± 1.7	43.1 ± 28.8	40.2 ± 8.2	41.3 ± 10.6
	0.2	31.6 ± 9.8	27.6 ± 1.8	110 ± 85	52.6 ± 20.0
	2.0	29.7 ± 8.2	20.9 ± 4.5	33.5 ± 9.1	52.6 ± 17.3
t_{max} (h)	0.02	5.33 ± 3.06	6.00 ± 5.29	4.67 ± 1.15	3.33 ± 1.15
	0.2	4.00 ± 2.00	4.67 ± 1.15	3.33 ± 1.15	4.67 ± 1.15
	2.0	4.67 ± 1.15	4.00 ± 0.00	4.67 ± 1.15	4.67 ± 1.15
C_{max} (µg/mL)	0.02	0.0288 ± 0.0034	0.0216 ± 0.0031	0.0620 ± 0.0021	0.0640 ± 0.0062
	0.2	0.216 ± 0.079	0.334 ± 0.040	0.726 ± 0.017	0.574 ± 0.073
	2.0	3.04 ± 0.33	2.80 ± 0.46	5.84 ± 0.79	5.31 ± 0.44

Study Title: APD334 L-Arginine: 4-Week Oral Toxicity Study in Dogs				**Study Number:** TX[redacted]007 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
AUC_{last} (µg·h/mL)	0.02	0.531 ± 0.050	0.410 ± 0.035	1.27 ± 0.05	1.22 ± 0.14
	0.2	4.05 ± 1.25	5.79 ± 0.89	14.3 ± 0.7	11.2 ± 0.9
	2.0	56.3 ± 5.1	46.8 ± 7.0	112 ± 21	104 ± 11
AI AUC_{last}	0.02	NA	NA	2.41	3.01
	0.2	NA	NA	3.71	1.96
	2.0	NA	NA	1.99	2.25
AUC_{last} F:M Ratio	0.02	NA	0.77	NA	0.96
	0.2	NA	1.43	NA	0.78
	2.0	NA	0.83	NA	0.93

Note: Data presented as mean ± standard deviation except for AI AUC_{last} and AUC_{last} F:M Ratio which are presented as mean.
AI AUC_{last}, accumulation index for AUC_{last} (AUC_{last} Day 28/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 19: Toxicokinetic Parameters of Etrasimod in Plasma After 28-Day Oral Administration – Dog

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]008 (Appendix L) (GLP)
Species	Dog/Beagle
Sex/Number of Animals	Male, Female/4 per sex per dose level in the 20 and 100 mg/kg/day groups and 6 per sex per dose level in the 40, 80, 500/200, and 1000/300 mg/kg/day groups
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 28 days)
Dose (mg/kg/day)	20, 40, 80, 100[a], 500/200[b], 1000/300[b]
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: 4-Week Oral Toxicity Study in Dogs With A 4-Week Recovery Period				**Study Number:** TX[redacted]008 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	20	24.0 ± 4.1	30.4 ± 9.2	46.6 ± 7.2	49.1 ± 18.8
	40	44.7 ± 39.3	43.6 ± 18.0	34.8 ± 6.8	58.2 ± 56.8
	80	61.7 ± 25.7	60.2 ± 45.2	30.1 ± 3.9	27.3 ± 7.8
	100	28.2[c]	184 ± 271	ID	ID
	500/ 200	55.0 ± 17.7	19.6 ± 0.8	ID	ID
	1000/ 300	37.5 ± ID	38.7 ± 15.0	ID	ID
t_{max} (h)	20	13.0 ± 7.6	7.00 ± 3.46	4.50 ± 3.0	5.50 ± 1.00
	40	9.00 ± 3.52	9.00 ± 3.52	5.08 ± 2.87	6.00 ± 1.79

Study Title: APD334 L-Arginine: 4-Week Oral Toxicity Study in Dogs With A 4-Week Recovery Period				**Study Number:** TX[redacted]008 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
	80	15.0 ± 7.4	6.67 ± 3.01	2.00 ± 2.83	4.08 ± 3.64
	100	16.5 ± 9.0	13.0 ± 8.3	ID	ID
	500/ 200	10.0 ± 3.4	18.7 ± 8.3	ID	ID
	1000/ 300	10.7 ± 7.0	15.7 ± 9.2	ID	ID
C_{max} (µg/mL)	20	21.3 ± 12.0	20.2 ± 9.1	48.8 ± 5.8	47.1 ± 20.6
	40	43.1 ± 19.5	41.8 ± 16.4	100 ± 11	90.8 ± 22.6
	80	49.0 ± 26.1	34.8 ± 23.8	139 ± 25	150 ± 42.9
	100	94.6 ± 27.0	51.6 ± 32.4	ID	ID
	500/ 200	57.8 ± 32.8	100 ± 34	ID	ID
	1000/ 300	80.1 ± 37.7	75.5 ± 44.2	ID	ID
AUC_{last} (µg·h/mL)	20	344 ± 191	355 ± 129	925 ± 165	910 ± 445
	40	740 ± 343	748 ± 267	1940 ± 298	1860 ± 518
	80	875 ± 501	630 ± 409	2590 ± 555	2750 ± 1000
	100	1560 ± 235	862 ± 486	ID	ID
	500/ 200	1070 ± 542	1480 ± 314	ID	ID
	1000/ 300	1520 ± 571	1160 ± 523	ID	ID
AI AUC_{last}	20	NA	NA	4.46	2.97
	40	NA	NA	3.19	3.21
	80	NA	NA	4.19	6.23
	100	NA	NA	NA	NA
	500/ 200	NA	NA	NA	NA

Study Title: APD334 L-Arginine: 4-Week Oral Toxicity Study in Dogs With A 4-Week Recovery Period				**Study Number:** TX[redacted]008 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 28**	
		Male	**Female**	**Male**	**Female**
	1000/ 300	NA	NA	NA	NA
AUC_{last} F:M Ratio	20	NA	1.03	NA	0.98
	40	NA	1.01	NA	0.96
	80	NA	0.72	NA	1.06
	100	NA	0.55	NA	NA
	500/ 200	NA	1.38	NA	NA
	1000/ 300	NA	0.76	NA	NA

[a] Dosing in the 100 mg/kg/day cohort was discontinued on Day 14 due to toxicity.
[b] Due to toxicity in the 500 mg/kg/day and 1000 mg/kg/day cohorts, dosing was halted on Day 7 and 8 and continued on Day 9 at reduced doses of 200 mg/kg/day and 300 mg/kg/day, respectively. Dosing was discontinued on Day 14 due to toxicity.
[c] n = 1; therefore, no associated statistical error term.
Note: Data presented as mean ± standard deviation.
AI AUC_{last}, accumulation index for AUC_{last} (AUC_{last} Day 28/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; ID, insufficient data to calculate parameters when n < 3; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; ; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 20: Toxicokinetic Parameters of Etrasimod in Plasma After 90-Day Oral Administration – Dog

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]005 (Appendix L) (GLP)
Species	Dog/Beagle
Sex/Number of Animals	Male, Female/6 per sex per dose level
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 90 days)
Dose (mg/kg/day)	1, 5, 15
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: 3-Month Oral Toxicity Study in Dogs With A 4-Week Recovery Period				**Study Number:** TX[redacted]005 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 90**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	1	48.7 ± 23.0	32.9 ± 13.4	37.8 ± 14.8	32.7 ± 6.8
	5	31.9 ± 8.1	23.0 ± 3.8	38.9 ± 6.7	40.3 ± 13.2
	15	26.3 ± 6.6	38.5 ± 16.3	98.2 ± 68.4	51.3 ± 8.5
t_{max} (h)	1	5.00 ± 1.10	6.33 ± 2.94	2.67 ± 1.03	4.00 ± 1.26
	5	5.33 ± 3.50	5.00 ± 1.10	5.00 ± 1.10	3.67 ± 1.97
	15	7.33 ± 2.73	6.33 ± 3.20	8.67 ± 7.76	7.00 ± 2.76
C_{max} (µg/mL)	1	0.982 ± 0.186	0.957 ± 0.095	2.81 ± 0.43	2.62 ± 0.52
	5	5.50 ± 2.04	5.55 ± 2.18	13.4 ± 1.4	14.0 ± 2.4
	15	17.3 ± 6.8	13.2 ± 5.0	52.3 ± 17.4	49.6 ± 8.6

Study Title: APD334 L-Arginine: 3-Month Oral Toxicity Study in Dogs With A 4-Week Recovery Period				**Study Number:** TX[redacted]005 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 90**	
		Male	**Female**	**Male**	**Female**
AUC_{last} (µg·h/mL)	1	18.3 ± 3.1	17.4 ± 1.2	52.6 ± 7.5	50.1 ± 11.9
	5	101 ± 34	96.4 ± 43.1	259 ± 34	265 ± 40
	15	313 ± 110	253 ± 103	1030 ± 330	1000 ± 130
AI AUC_{last}	1	NA	NA	2.90	2.88
	5	NA	NA	2.90	4.72
	15	NA	NA	3.66	4.55
AUC_{last} F:M Ratio	1	NA	0.95	NA	0.95
	5	NA	0.95	NA	1.02
	15	NA	0.81	NA	0.97

Note: Data presented as mean ± standard deviation.
AI AUC_{last}, accumulation index for AUC_{last} (AUC_{last} Day 90/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 21: Toxicokinetic Parameters of Etrasimod in Plasma After 273-Day Oral Administration – Dog

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]008 (Appendix L) (GLP)
Species	Dog/Beagle
Sex	Male, Female/4 per sex per dose level for the 2 and 5 mg/kg/day groups and 6 per sex per dose level for the 10 and 15 mg/kg/day groups
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 273 days)
Dose (mg/kg/day)	2, 5, 10, 15
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: 9-Month Oral Toxicity Study in Dogs With A 4-Week Recovery Period				**Study Number:** TX[redacted]008 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 273**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	2	32.2 ± 11.1	31.6 ± 13.8	45.1 ± ID	51.6 ± 25.1
	5	25.7 ± 4.5	33.7 ± 6.4	39.7 ± ID	80.1 ± 5.2
	10	24.8 ± 8.0	22.4 ± 1.3	42.8 ± ID	53.4 ± ID
	15	32.0 ± 7.1	29.2 ± 6.5	31.4 ± 7.5	31.1 ± ID
t_{max} (h)	2	10.0 ± 4.0	8.00 ± 2.83	3.00 ± 2.00	2.75 ± 1.50
	5	5.50 ± 1.91	6.50 ± 3.79	4.00 ± 5.42	2.00 ± 1.41
	10	11.3 ± 1.6	8.33 ± 2.94	2.25 ± 1.47	7.50 ± 8.48
	15	9.33 ± 3.01	8.00 ± 8.10	1.25 ± 1.54	7.83 ± 8.82

Study Title: APD334 L-Arginine: 9-Month Oral Toxicity Study in Dogs With A 4-Week Recovery Period				**Study Number:** TX[redacted]008 (Appendix L)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1**		**Day 273**	
		Male	**Female**	**Male**	**Female**
C_{max} (µg/mL)	2	2.03 ± 0.55	1.88 ± 0.19	6.24 ± 1.81	6.80 ± 2.47
	5	12.2 ± 4.3	5.23 ± 1.78	14.4 ± 6.8	17.9 ± 3.5
	10	11.5 ± 2.4	10.2 ± 2.3	27.3 ± 3.0	28.7 ± 4.8
	15	22.5 ± 6.6	19.8 ± 3.0	46.9 ± 11.5	45.7 ± 11.0
AUC_{last} (µg·h/mL)	2	38.0 ± 7.2	36.9 ± 3.4	114 ± 36.5	128 ± 46
	5	173 ± 52	98.8 ± 28.3	269 ± 93	335 ± 60
	10	213 ± 37	191 ± 35	542 ± 106	586 ± 112
	15	418 ± 107	379 ± 47	879 ± 177	861 ± 170
AI AUC_{last}	2	NA	NA	2.94	3.43
	5	NA	NA	1.57	3.76
	10	NA	NA	2.57	3.16
	15	NA	NA	2.28	2.28
AUC_{last} F:M Ratio	2	NA	0.97	NA	1.12
	5	NA	0.57	NA	1.25
	10	NA	0.90	NA	1.08
	15	NA	0.91	NA	0.98

Note: Data presented as mean ± standard deviation.
AI AUC_{last}, accumulation index for AUC_{last} (AUC_{last} Day 273/AUC_{last} Day 1); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; AUC_{last} F:M ratio, female/male ratio for AUC_{last}; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; ID, insufficient data to calculate parameters when n < 3; LC-MS/MS, liquid chromatography tandem mass spectrometry; NA, not applicable; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 22: Toxicokinetic Parameters of Etrasimod in Plasma After 14-Day Oral Administration in Juvenile Rats

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.008 (Appendix 10) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Male, Female/3 cohorts of 3 animals per sex per dose group sampled at alternating timepoints
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 14 days starting on postnatal day 21)
Dose (mg/kg/day)	25, 100
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: An Oral (Gavage) Dose Range-Finding Toxicity Study in Juvenile Sprague Dawley Rats with a Toxicokinetic Phase				**Study Number:** APD334.TOX.008 (Appendix 10)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1 (Postnatal Day 21)**		**Day 14 (Postnatal Day 34)**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	25	NR	NR	NR	NR
	100	NR	NR	NR	NR
t_{max} (h)	25	4	4	1	4
	100	4	4	2	4
C_{max} (µg/mL)	25	9.84	13.6	9.45	11.7
	100	50.5	43.4	27.8	34.1
AUC_{0-24} (µg·h/mL)	25	120	142	88.9	132
	100	732	696	332	408

Study Title: APD334 L-Arginine: An Oral (Gavage) Dose Range-Finding Toxicity Study in Juvenile Sprague Dawley Rats with a Toxicokinetic Phase				**Study Number:** APD334.TOX.008 (Appendix 10)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1 (Postnatal Day 21)**		**Day 14 (Postnatal Day 34)**	
		Male	**Female**	**Male**	**Female**
AI AUC_{0-24}	25	NA	NA	0.740	0.934
	100	NA	NA	0.453	0.587
AUC_{0-24} F:M Ratio	25	NA	1.18	NA	1.49
	100	NA	0.951	NA	1.23

Note: Etrasimod was administered once daily from Postnatal Day 21 to 34; GLP toxicity study.
Composite sampling was used, so variance was not calculated.
AI AUC_{0-24}, accumulation index for AUC_{0-24} (AUC_{0-24} Day 14/AUC_{0-24} Day 1); AUC, area under the concentration-time curve; AUC_{0-24}, AUC from time zero to 24 h; AUC_{0-24} F:M Ratio, AUC_{0-24} female/AUC_{0-24} male; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 23: Toxicokinetic Parameters of Etrasimod in Plasma After 49-Day Oral Administration in Juvenile Rats

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.009 (Appendix 19) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Male, Female/3 animals per sex per timepoint per dose group on Day 1 and 3 cohorts of 3 animals per sex per dose group sampled at alternating timepoints on Day 49
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 49 days starting on Postnatal Day 21)
Dose (mg/kg/day)	10, 30, 100
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: An Oral (Gavage) GLP 7-Week Toxicity and Toxicokinetic Study in Juvenile Sprague Dawley Rats with a 4-week Recovery Period				**Study Number:** APD334.TOX.009 (Appendix 19)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1 (Postnatal Day 21)**		**Day 49 (Postnatal Day 69)**	
		Male	**Female**	**Male**	**Female**
$t_{½}$ (h)	10	NR	NR	NR	NR
	30	NR	NR	NR	NR
	100	NR	NR	NR	NR
t_{max} (h)	10	4	2	2	2
	30	4	2	2	2
	100	8	6	6	6
C_{max} (µg/mL)	10	7.40	5.71	6.85	10.5

Study Title: APD334 L-Arginine: An Oral (Gavage) GLP 7-Week Toxicity and Toxicokinetic Study in Juvenile Sprague Dawley Rats with a 4-week Recovery Period				**Study Number:** APD334.TOX.009 (Appendix 19)	
PK Parameter	**Dose (mg/kg/day)**	**Day 1 (Postnatal Day 21)**		**Day 49 (Postnatal Day 69)**	
		Male	**Female**	**Male**	**Female**
	30	22.2	23.7	16.2	32.9
	100	70.7	71.8	60.2	72.3
AUC_{0-24} (µg·h/mL)	10	62.7	69.6	74.5	128
	30	236	269	181	363
	100	956	1010	631	957
AI AUC_{0-24}	10	NA	NA	1.19	1.84
	30	NA	NA	0.765	1.35
	100	NA	NA	0.660	0.943
AUC_{0-24} F:M Ratio	10	NA	1.11	NA	1.72
	30	NA	1.14	NA	2.01
	100	NA	1.06	NA	1.52

Note: Etrasimod was administered once daily from Postnatal Day 21 to 69.
Composite sampling was used, so variance was not calculated.
AI AUC_{0-24}, accumulation index for AUC_{0-24} (AUC_{0-24} Day 49/AUC_{0-24} Day 1); AUC, area under the concentration-time curve; AUC_{0-24}, AUC from time zero to 24 h; AUC_{0-24} F:M ratio, AUC_{0-24} female/AUC_{0-24} male; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

2.6.5.5. PHARMACOKINETICS: ORGAN DISTRIBUTION

Table 24: Blood and Plasma Distribution Following a Single Oral Administration of [^{14}C]Etrasimod to Male and Female Sprague Dawley Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Male, Female/5 per sex (Group 4)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	Radioactivity

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Sampling Time				
	(0.50 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Animal ID (Male)	**R0301**	**R0302**	**R0303**	**R0304**	**R0305**
Blood	4160	7780	5320	730	70.3
Plasma	6840	12900	8790	1210	104
Blood:Plasma Ratio	0.608	0.601	0.606	0.604	0.679

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Sampling Time				
	(0.50 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Animal ID (Female)	**R0901**	**R0902**	**R0903**	**R0904**	**R0905**
Blood	5960	9260	4860	977	112
Plasma	9030	15200	7460	1480	158
Blood:Plasma Ratio	0.630	0.609	0.651	0.661	0.706

eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; ID, identification number

Table 25: Blood and Plasma Distribution Following a Single Oral Administration of [^{14}C]Etrasimod to Male and Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male, Female/7 per sex (Group 5)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	Radioactivity

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats						**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**					
	Sampling Time					
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Animal ID (Male)	**R0401**	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
Blood	6230	9340	5600	938	209	71.5	BLQ
Plasma	10100	15400	9410	1550	356	119	BLQ
Blood:Plasma Ratio	0.616	0.606	0.595	0.606	0.587	0.603	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Sampling Time						
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Animal ID (Female)	**R1001**	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
Blood	3930	8390	5730	1330	275	92.0	18.9
Plasma	6190	14100	8930	2110	413	128	BLQ
Blood:Plasma Ratio	0.634	0.597	0.642	0.628	0.667	0.721	NA

BLQ, below limit of quantitation; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; ID, identification number; NA, not applicable

Table 26: Blood and Plasma Distribution Following 7 Days of Oral Administration of [^{14}C]Etrasimod to Male and Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male, Female/7 per sex (Group 6)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg/day)	10
Assay	Radioactivity

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats						**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**					
	Sampling Time					
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Animal ID (Male)	**R0501**	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
Blood	8460	13200	7250	1240	412	187	61.9
Plasma	15600	23700	12300	2180	693	280	82.9
Blood:Plasma Ratio	0.544	0.558	0.590	0.567	0.594	0.667	0.747

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Sampling Time						
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Animal ID (Female)	**R1101**	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
Blood	9490	12600	8540	1020	270	178	78.5
Plasma	16800	21600	13700	1680	435	246	98.2
Blood:Plasma Ratio	0.565	0.584	0.624	0.608	0.622	0.723	0.800

Note: 7 days of oral administration of [^{14}C]etrasimod 10 mg/kg/day.
eq., equivalents; GLP, Good Laboratory Practice; HPBCD, 2-hydroxypropyl-β-cyclodextrin; ID, identification number

Table 27: Blood and Plasma Distribution Following a Single Oral Administration of [^{14}C]Etrasimod in Dog

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.001 (non-GLP)
Species	Dog/Beagle
Sex/Number of Animals (Group)	Male, Female/3 per sex
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg/day)	2
Assay	Radioactivity

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs					**Study Number:** APD334.DMPK.001
Species	**Time (h)**	**Blood:Plasma Concentration Ratio**			
		Male		**Female**	
		Mean	**SD**	**Mean**	**SD**
Dog	0.25	0.715	0.0129	0.607	0.0299
	0.50	0.707	0.00704	0.616	0.0302
	1	0.670	0.0649	0.585	0.0421
	2	0.623	0.0753	0.596	0.0915
	3	0.621	0.0174	0.613	0.0282
	4	0.662	0.00734	0.625	0.00744
	6	0.657	0.0226	0.636	0.0244

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs				**Study Number:** APD334.DMPK.001	
Species	**Time (h)**	**Blood:Plasma Concentration Ratio**			
		Male		**Female**	
		Mean	**SD**	**Mean**	**SD**
Dog	8	0.666	0.00861	0.614	0.0186
	24	0.650	0.0108	0.598	0.0336
	48	0.584	0.0699	0.559	0.0444
	72	0.639	0.0486	0.604	0.0607
	96	0.608	0.0514	0.608	0.0516
	120	0.660	0.0159	0.632	0.0290
	168	0.659	0.0118	0.619	0.0530

GLP, Good Laboratory Practice; HPBCD, 2-hydroxypropyl-β-cyclodextrin; SD, standard deviation

Table 28: Tissue Distribution Pattern Following a Single Oral Administration of [^{14}C]Etrasimod to Male Sprague Dawley Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Male/5 (Group 4)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Adrenal gland(s)	5250[a]	15300[a]	21300[a]	6570[a]	3590[a]
Bile	25200	53300	48400	ND	ND
Blood	3580	7230	4750	720	ND
Bone	332	865	268	ND	ND
Bone marrow	2740	5600	5160	1390	311
Brain (whole)	455	5750	8190	3250	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Brain cerebellum	531	5950	9070	2920	ND
Brain cerebrum	499	5970	8300	2670	ND
Brain medulla	443	5770	10300	4900	ND
Brain olfactory lobe	406	3930	6750	2480	ND
Bulbo-urethral gland	1850	10500	8460	1880	326
Cecum	1500	5940	5970	2050	BLQ
Diaphragm	2650	6500	6480	923	ND
Epididymis	314	3490	NR	1250	BLQ
Esophagus	4090	6160	4910	1150	281
Exorbital lacrimal gland	1330	8390	7430	1260	ND
Eye lens	ND	ND	ND	ND	ND
Eye uveal tract	990	3950	4210	512	ND
Eye(s)	178	722	858	180	ND
Fascia	ND	3630	3390	784	323
Fat (abdominal)	138	1950	625	467	253
Fat (brown)	4360	7210	10300	3000	716
Harderian gland	767	8590	11300	2790	345
Intra-orbital lacrimal gland	1210	7700	3810	927	592
Kidney cortex	4440	12100	11200	1460	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Kidney medulla	4500	9220	9620	1100	ND
Kidney(s)	4470	11000	10800	1280	BLQ
Large intestine	1100	5410	5500	1290	119
Liver	18400	26400	28400	3400	603
Lung(s)	3510	7520	5860	791	ND
Lymph node(s)	1180	5110	5150	752	280
Muscle	787	3990	3410	407	ND
Myocardium	5000	10300	10200	1110	ND
Nasal turbinates	562	2560	3540	1270	220
Pancreas	2660	10400	9740	1130	BLQ
Pituitary gland	3060	8520	8960	1030	ND
Preputial gland	789	9100[a]	5180	1750[a]	1110[a]
Prostate gland	723	5040	4070	736	ND
Salivary gland(s)	2810	10900	10400	1350	373
Seminal vesicle(s)	318	2810	2240[a]	656[a]	ND
Skin (nonpigmented)	561	3340	3010	1900	470
Small intestine	1620	8340	9310	1350	ND
Spinal cord	424	6080	9310	4730	461
Spleen	2870	5840	6590	794	BLQ

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Stomach	1520	7970	6340	926	116
Stomach mucosa	ND	9350	7020	994	ND
Stomach wall	ND	5140	3950	697	ND
Testis(es)	157	2250	3380	1850	545
Thymus	685	5460	6570	1160	245
Thyroid	3420	9120	6650	3530	2730
Urinary bladder	1760[a]	6670	6630[a]	974	423
Urine	636	3950	1040	166	ND

[a] Tissue appeared to be fat soaked.
BLQ, below the limit of quantitation (< 116 ng eq. [^{14}C]etrasimod/g); eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; ND, not detectable (sample shape not discernible from background or surrounding tissue); NR, not represented (tissue not present in section); QWBA, quantitative whole body autoradiography

Table 29: Tissue:Plasma Concentration Ratios Following a Single Oral Administration of [^{14}C]Etrasimod to Male Sprague Dawley Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Male/5 (Group 4)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Adrenal gland(s)	0.768	1.19	2.42	5.43	34.5
Blood	0.523	0.560	0.540	0.595	NA
Bone	0.0485	0.0671	0.0305	NA	NA
Bone marrow	0.401	0.434	0.587	1.15	2.99
Brain (whole)	0.0665	0.446	0.932	2.69	NA
Brain cerebellum	0.0776	0.461	1.03	2.41	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Brain cerebrum	0.0730	0.463	0.944	2.21	NA
Brain medulla	0.0648	0.447	1.17	4.05	NA
Brain olfactory lobe	0.0594	0.305	0.768	2.05	NA
Bulbo-urethral gland	0.270	0.814	0.962	1.55	3.13
Cecum	0.219	0.460	0.679	1.69	NA
Diaphragm	0.387	0.504	0.737	0.763	NA
Epididymis	0.0459	0.271	NA	1.03	NA
Esophagus	0.598	0.478	0.559	0.950	2.70
Exorbital lacrimal gland	0.194	0.650	0.845	1.04	NA
Eye lens	NA	NA	NA	NA	NA
Eye uveal tract	0.145	0.306	0.479	0.423	NA
Eye(s)	0.0260	0.0560	0.0976	0.149	NA
Fascia	NA	0.281	0.386	0.648	3.11
Fat (abdominal)	0.0202	0.151	0.0711	0.386	2.43
Fat (brown)	0.637	0.559	1.17	2.48	6.88
Harderian gland	0.112	0.666	1.29	2.31	3.32
Intra-orbital lacrimal gland	0.177	0.597	0.433	0.766	5.69
Kidney cortex	0.649	0.938	1.27	1.21	NA
Kidney medulla	0.658	0.715	1.09	0.909	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Kidney(s)	0.654	0.853	1.23	1.06	NA
Large intestine	0.161	0.419	0.626	1.07	1.14
Liver	2.69	2.05	3.23	2.81	5.80
Lung(s)	0.513	0.583	0.667	0.654	NA
Lymph node(s)	0.173	0.396	0.586	0.621	2.69
Muscle	0.115	0.309	0.388	0.336	NA
Myocardium	0.731	0.798	1.16	0.917	NA
Nasal turbinates	0.0822	0.198	0.403	1.05	2.12
Pancreas	0.389	0.806	1.11	0.934	NA
Pituitary gland	0.447	0.660	1.02	0.851	NA
Preputial gland	0.115	0.705	0.589	1.45	10.7
Prostate gland	0.106	0.391	0.463	0.608	NA
Salivary gland(s)	0.411	0.845	1.18	1.12	3.59
Seminal vesicle(s)	0.0465	0.218	0.255	0.542	NA
Skin (nonpigmented)	0.0820	0.259	0.342	1.57	4.52
Small intestine	0.237	0.647	1.06	1.12	NA
Spinal cord	0.0620	0.471	1.06	3.91	4.43
Spleen	0.420	0.453	0.750	0.656	NA
Stomach	0.222	0.618	0.721	0.765	1.12

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0301	**R0302**	**R0303**	**R0304**	**R0305**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Stomach mucosa	NA	0.725	0.799	0.821	NA
Stomach wall	NA	0.398	0.449	0.576	NA
Testis(es)	0.0230	0.174	0.385	1.53	5.24
Thymus	0.100	0.423	0.747	0.959	2.36
Thyroid	0.500	0.707	0.757	2.92	26.3
Urinary bladder	0.257	0.517	0.754	0.805	4.07

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; NA, not applicable; QWBA, quantitative whole body autoradiography

Table 30: Estimated Pharmacokinetic Parameters Following a Single Oral Administration of [^{14}C]Etrasimod to Male Sprague Dawley Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 4) (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Male/5 (Group 4)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Adrenal gland(s)	577275	862402	21300	8.00	55.1
Blood (QWBA)	87533	NC	7230	4.00	NC
Brain	130373	186634	8190	8.00	12.0
Brain cerebellum	137435	178656	9070	8.00	9.79
Brain cerebrum	127746	165412	8300	8.00	9.78
Brain medulla	164724	270255	10300	8.00	14.9
Brain olfactory lobe	102890	142519	6750	8.00	11.1
Bulbo urethral gland	195659	204590	10500	4.00	19.0

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Eye lens	NC	NC	NC	NC	NC
Eye uveal tract	62989	66877	4210	8.00	5.26
Eye(s)	13084	14928	858	8.00	7.10
Fat (brown)	251942	275930	10300	8.00	23.2
Harderian gland	244307	252229	11300	8.00	15.9
Kidney cortex	177935	190981	12100	4.00	6.19
Kidney medulla	148575	156691	9620	8.00	5.11
Kidney(s)	168430	179570	11000	4.00	6.03
Liver	543072	559806	28400	8.00	19.2
Lymph node(s)	103807	117410	5150	8.00	33.7
Myocardium	159505	168819	10300	4.00	5.82
Pancreas	150760	160358	10400	4.00	5.89
Pituitary gland	135910	143528	8960	8.00	5.13
Plasma (LSC)	191171	192768	12900	4.00	10.6
Preputial gland	170143	287176	9100	4.00	73.1
Salivary gland(s)	202647	216566	10900	4.00	25.9
Skin (nonpigmented)	115827	132014	3340	4.00	23.9
Small intestine	138415	149601	9310	8.00	5.74
Spinal cord	279172	288915	9310	8.00	14.6
Testis(es)	114832	136236	3380	8.00	27.2

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Thyroid	286020	795911	9120	4.00	129
Urinary bladder	136153	160497	6670	4.00	39.9

Note: Tissues with $AUC_{last} > 1.5 \times AUC_{last}$ of blood (QWBA) (i.e., >131,300) are displayed, in addition to other select tissues. Refer to Table 4-1 in APD334.DMPK.002 (Appendix 4) for full presentation of data.
AUC_{∞}, area under the concentration-time curve from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 72 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counter; NC, not calculated; QWBA, quantitative whole body autoradiography; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 31: Tissue Distribution Pattern Following a Single Oral Administration of [^{14}C]Etrasimod to Female Sprague Dawley Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Female/5 (Group 4)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Adrenal gland(s)	12000[a]	16000[a]	33800[a]	15400[a]	8440[a]
Bile	42500	49000	44900	ND	ND
Blood	5690	7680	4410	865	ND
Bone	190	703	305	ND	ND
Bone marrow	4330	7340	5730	1540	327
Brain (whole)	929	8640	11400	5160	313

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Brain cerebellum	1020	8350	12200	4540	329
Brain cerebrum	998	9410	11400	4000	219
Brain medulla	801	9060	13700	7660	506
Brain olfactory lobe	706	7530	11800	5010	146
Cecum	2520	8690	8810	1330	198
Diaphragm	4520	8700	6640	1150	ND
Esophagus	6190	8040	5380	3270	377
Exorbital lacrimal gland	2450	11500	9790	1650	269
Eye lens	ND	ND	ND	ND	ND
Eye uveal tract	993	6870	4240	986	ND
Eye(s)	178	1060	746	205	ND
Fascia	ND	6010	4140	1040	427
Fat (abdominal)	1070	1870	976	233	192
Fat (brown)	8860	15200	14200	2540	977
Harderian gland	2770	16000	16400	3560	625
Intra-orbital lacrimal gland	1970	NR	8570	2060	356
Kidney cortex	8360	17300	13300	2450	ND
Kidney medulla	7730	13900	10200	1990	ND
Kidney(s)	8050	15700	11800	2310	210

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Large intestine	1820	7840	5670	1350	268
Liver	25900	31400	26800	4690	852
Lung(s)	7590	8970	6590	1130	ND
Lymph node(s)	1900	7690	6610	1650	367
Muscle	1550	4610	3370	677	ND
Myocardium	9780	13700	12400	1750	ND
Nasal turbinates	1100	5990	4970	2150	373
Ovary(ies)	5630	8550	10300	5590[a]	1680[a]
Pancreas	6480	16100	14600	1990	148
Pituitary gland	7120	11400	9280	1920	481
Preputial gland	2410[a]	7450[a]	9080[a]	NR	NR
Salivary gland(s)	5070	16400	13100	2490	403
Skin (nonpigmented)	1470	3470	3030	2030	991
Small intestine	3090	11400	7550	2640	ND
Spinal cord	1030	8700	11600	7240	1630
Spleen	5000	8680	7400	1240	156
Stomach	3890	10200	7570	1760	223
Stomach mucosa	4090	13000	9270	2190	ND
Stomach wall	2540	6790	5020	1320	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Thymus	1720	8220	8190	2750	763
Thyroid	7080	8710	8430	4640	6890
Urinary bladder	2200	7170	5140	1740	334[a]
Urine	2380	1470	184	BLQ	BLQ
Uterus	1940	7270	4670	1390	516[a]

[a] Tissue appeared to be fat soaked.

BLQ, below the limit of quantitation (< 116 ng eq. [^{14}C]etrasimod/g); eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; ND, not detectable (sample shape not discernible from background or surrounding tissue); NR, not represented (tissue not present in section); QWBA, quantitative whole body autoradiography

Table 32: Tissue:Plasma Concentration Ratios Following a Single Oral Administration of [^{14}C]Etrasimod to Female Sprague Dawley Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Female/5 (Group 4)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Adrenal gland(s)	1.33	1.05	4.53	10.4	53.4
Blood	0.630	0.505	0.591	0.584	NA
Bone	0.0210	0.0463	0.0409	NA	NA
Bone marrow	0.480	0.483	0.768	1.04	2.07
Brain (whole)	0.103	0.568	1.53	3.49	1.98
Brain cerebellum	0.113	0.549	1.64	3.07	2.08

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Brain cerebrum	0.111	0.619	1.53	2.70	1.39
Brain medulla	0.0887	0.596	1.84	5.18	3.20
Brain olfactory lobe	0.0782	0.495	1.58	3.39	0.924
Cecum	0.279	0.572	1.18	0.899	1.25
Diaphragm	0.501	0.572	0.890	0.777	NA
Esophagus	0.685	0.529	0.721	2.21	2.39
Exorbital lacrimal gland	0.271	0.757	1.31	1.11	1.70
Eye lens	NA	NA	NA	NA	NA
Eye uveal tract	0.110	0.452	0.568	0.666	NA
Eye(s)	0.0197	0.0697	0.100	0.139	NA
Fascia	NA	0.395	0.555	0.703	2.70
Fat (abdominal)	0.118	0.123	0.131	0.157	1.22
Fat (brown)	0.981	1.00	1.90	1.72	6.18
Harderian gland	0.307	1.05	2.20	2.41	3.96
Intra-orbital lacrimal gland	0.218	NA	1.15	1.39	2.25
Kidney cortex	0.926	1.14	1.78	1.66	NA
Kidney medulla	0.856	0.914	1.37	1.34	NA
Kidney(s)	0.891	1.03	1.58	1.56	1.33
Large intestine	0.202	0.516	0.760	0.912	1.70

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Liver	2.87	2.07	3.59	3.17	5.39
Lung(s)	0.841	0.590	0.883	0.764	NA
Lymph node(s)	0.210	0.506	0.886	1.11	2.32
Muscle	0.172	0.303	0.452	0.457	NA
Myocardium	1.08	0.901	1.66	1.18	NA
Nasal turbinates	0.122	0.394	0.666	1.45	2.36
Ovary(ies)	0.623	0.563	1.38	3.78	10.6
Pancreas	0.718	1.06	1.96	1.34	0.937
Pituitary gland	0.788	0.750	1.24	1.30	3.04
Preputial gland	0.267	0.490	1.22	NA	NA
Salivary gland(s)	0.561	1.08	1.76	1.68	2.55
Skin (nonpigmented)	0.163	0.228	0.406	1.37	6.27
Small intestine	0.342	0.750	1.01	1.78	NA
Spinal cord	0.114	0.572	1.55	4.89	10.3
Spleen	0.554	0.571	0.992	0.838	0.987
Stomach	0.431	0.671	1.01	1.19	1.41
Stomach mucosa	0.453	0.855	1.24	1.48	NA
Stomach wall	0.281	0.447	0.673	0.892	NA
Thymus	0.190	0.541	1.10	1.86	4.83

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**				
	Animal (Sampling Time)				
	R0901	**R0902**	**R0903**	**R0904**	**R0905**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(72 h)**
Thyroid	0.784	0.573	1.13	3.14	43.6
Urinary bladder	0.244	0.472	0.689	1.18	2.11
Uterus	0.215	0.478	0.626	0.939	3.27

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; NA, not applicable; QWBA, quantitative whole body autoradiography

Table 33: Estimated Pharmacokinetic Parameters Following a Single Oral Administration of [^{14}C]Etrasimod to Female Sprague Dawley Rats

Test Article:	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 4) (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Female/5 (Group 4)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Adrenal gland(s)	1117360	1791005	33800	8.00	55.3
Blood (QWBA)	91200	NC	7680	4.00	NC
Bone marrow	150613	158423	7340	4.00	16.6
Brain	320890	326412	11400	8.00	12.2
Brain cerebellum	308529	314399	12200	8.00	12.4
Brain cerebrum	284540	288102	11400	8.00	11.3
Brain medulla	429841	439441	13700	8.00	13.2
Brain olfactory lobe	311474	313566	11800	8.00	9.93

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Cecum	173040	178029	8810	8.00	17.5
Esophagus	210018	218923	8040	4.00	16.4
Exorbital lacrimal gland	205181	210362	11500	4.00	13.3
Eye lens	NC	NC	NC	NC	NC
Eye uveal tract	78037	88374	6870	4.00	7.27
Eye(s)	13431	15939	1060	4.00	8.48
Fat (brown)	321448	348024	15200	4.00	18.9
Harderian gland	358460	375704	16400	8.00	19.1
Intra-orbital lacrimal gland	183042	192775	8570	8.00	19.0
Kidney cortex	234195	258691	17300	4.00	6.93
Kidney medulla	185505	205687	13900	4.00	7.03
Kidney(s)	271935	275438	15700	4.00	11.6
Large intestine	139372	145400	7840	4.00	15.6
Liver	608078	625238	31400	4.00	14.0
Lymph node(s)	160346	169089	7690	4.00	16.5
Myocardium	208935	225070	13700	4.00	6.39
Nasal turbinates	152115	161546	5990	4.00	17.5
Ovary(ies)	365523	426448	10300	8.00	25.1
Pancreas	286567	288754	16100	4.00	10.2
Pituitary gland	222774	234164	11400	4.00	16.4

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Plasma (LSC)	200812	203580	15200	4.00	12.1
Salivary gland(s)	291992	299957	16400	4.00	13.7
Skin (nonpigmented)	134997	193704	3470	4.00	41.1
Small intestine	145550	182729	11400	4.00	9.76
Spinal cord	421485	474484	11600	8.00	22.5
Spleen	159974	162742	8680	4.00	12.3
Stomach	183402	187666	10200	4.00	13.3
Stomach mucosa	167150	191654	13000	4.00	7.76
Thymus	222477	244471	8220	4.00	20.0
Thyroid	444963	NC	8710	4.00	NC
Urinary bladder	146384	154571	7170	4.00	17.0
Uterus	134707	151224	7270	4.00	22.2

Note: Tissues with AUC_{last} > 1.5 × AUC_{last} of blood (QWBA) (i.e., >136,800) are displayed, in addition to other select tissues. Refer to Table 4-4 in APD334.DMPK.002 (Appendix 4) for full presentation of data.
AUC_{∞}, area under the concentration-time curve from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 72 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counter; NC, not calculated; QWBA, quantitative whole body autoradiography; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 34: Tissue Distribution Pattern Following a Single Oral Administration of [^{14}C]Etrasimod to Male Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male/7 (Group 5)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	12300[a]	20300[a]	24100[a]	16000[a]	7720[a]	6470[a]	1360[a]
Bile	ND	65000	20900	8020	ND	ND	ND
Blood	6180	8750	5430	922	202	ND	ND
Bone	162	735	480	ND	ND	ND	ND
Bone marrow	4010	7030	4770	840	327	288	ND
Brain (whole)	650	7810	9470	2910	406	135	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebellum	713	9120	10200	3050	530	242	ND
Brain cerebrum	626	8000	9910	1900	212	BLQ	ND
Brain medulla	676	9040	11900	3750	638	195	ND
Brain olfactory lobe	686	5740	6810	1580	138	BLQ	ND
Bulbo-urethral gland	3450	12200	8070	1340	535	211	ND
Cecum	2670	10800	7180	2520	162	156	BLQ
Diaphragm	3270	8370	6060	739	275	BLQ	ND
Epididymis	583[a]	6780	6920	1320	380	426	205
Esophagus	17700	10400	4740	2350	456	459	ND
Exorbital lacrimal gland	1810	12600	8680	1300	179	BLQ	ND
Eye lens	BLQ	BLQ	ND	ND	ND	ND	ND
Eye uveal tract	1230	7080	5370	643	233	ND	ND
Eye(s)	223	1980	948	191	BLQ	ND	ND
Fascia	ND	5160	3770	1000	501	674	281
Fat (abdominal)	399	1610	590	186	BLQ	196	ND
Fat (brown)	5090	7630	7920	2080	917	951	393
Harderian gland	1250	16700	9710	2540	497	192	ND
Intra-orbital lacrimal gland	1540	12900	8860	1160	219	BLQ	ND
Kidney cortex	7470	16800	11000	1290	ND	ND	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Kidney medulla	6880	14300	9140	1180	ND	ND	ND
Kidney(s)	7120	16000	10500	1390	265	124	ND
Large intestine	1750	7840	9400	847	401	BLQ	BLQ
Liver	30000	37700	30700	3820	1110	609	139
Lung(s)	6550	11300	6580	1020	182	ND	ND
Lymph node(s)	1270	7770	5990	880	588	261	ND
Muscle	1490	5400	3380	429	BLQ	ND	ND
Myocardium	8110	13500	9660	1150	177	ND	ND
Nasal turbinates	853	5160	4190	1070	388	237	216
Pancreas	4540	15900	10300	1160	173	BLQ	ND
Pituitary gland	4520	12200	7510	820	169	197	ND
Preputial gland	1490[a]	7300[a]	2990[a]	1460[a]	649[a]	906[a]	632[a]
Prostate gland	1080	7620	4560	747	164	BLQ	ND
Salivary gland(s)	4470	15100	10700	1390	353	222	ND
Seminal vesicle(s)	287[a]	2350[a]	936	1880	BLQ	ND	ND
Skin (nonpigmented)	725	3530	4070	2370	827	264	227
Skin (pigmented)	1470	3870	4030	2190	1510	855	497
Small intestine	2670	5240	6430	1340	262	BLQ	ND
Spinal cord	646	8330	10700	6480	1970	1210	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Spleen	5070	8370	5850	735	201	BLQ	ND
Stomach	4220	10900	6740	831	260	127	ND
Stomach mucosa	4510	13100	7880	ND	ND	ND	ND
Stomach wall	2630	7730	5340	ND	ND	ND	ND
Testis(es)	266	6280	4270	2300	793	330	121
Thymus	1540	8610	6460	983	387	226	ND
Thyroid	4980	12600	7790	2650	4090	2740	3480
Urinary bladder	1720	11300	11000	1240	269	BLQ	ND
Urine	565	4050	1110	233	BLQ	BLQ	ND

[a] Tissue appeared to be fat soaked.

BLQ, below the limit of quantitation (< 116 ng eq. [^{14}C]etrasimod/g); eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; ND, not detectable (sample shape not discernible from background or surrounding tissue); QWBA, quantitative whole body autoradiography

Table 35: Tissue:Plasma Concentration Ratios Following a Single Oral Administration of [^{14}C]Etrasimod to Male Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male/7 (Group 5)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	1.22	1.32	2.56	10.3	21.7	54.4	NA
Blood	0.612	0.568	0.577	0.595	0.567	NA	NA
Bone	0.0160	0.0477	0.0510	NA	NA	NA	NA
Bone marrow	0.397	0.456	0.507	0.542	0.919	2.42	NA
Brain (whole)	0.0644	0.507	1.01	1.88	1.14	1.13	NA
Brain cerebellum	0.0706	0.592	1.08	1.97	1.49	2.03	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebrum	0.0620	0.519	1.05	1.23	0.596	NA	NA
Brain medulla	0.0669	0.587	1.26	2.42	1.79	1.64	NA
Brain olfactory lobe	0.0679	0.373	0.724	1.02	0.388	NA	NA
Bulbo-urethral gland	0.342	0.792	0.858	0.865	1.50	1.77	NA
Cecum	0.264	0.701	0.763	1.63	0.455	1.31	NA
Diaphragm	0.324	0.544	0.644	0.477	0.772	NA	NA
Epididymis	0.0577	0.440	0.735	0.852	1.07	3.58	NA
Esophagus	1.75	0.675	0.504	1.52	1.28	3.86	NA
Exorbital lacrimal gland	0.179	0.818	0.922	0.839	0.503	NA	NA
Eye lens	NA	NA	NA	NA	NA	NA	NA
Eye uveal tract	0.122	0.460	0.571	0.415	0.654	NA	NA
Eye(s)	0.0221	0.129	0.101	0.123	NA	NA	NA
Fascia	NA	0.335	0.401	0.645	1.41	5.66	NA
Fat (abdominal)	0.0395	0.105	0.0627	0.120	NA	1.65	NA
Fat (brown)	0.504	0.495	0.842	1.34	2.58	7.99	NA
Harderian gland	0.124	1.08	1.03	1.64	1.40	1.61	NA
Intra-orbital lacrimal gland	0.152	0.838	0.942	0.748	0.615	NA	NA
Kidney cortex	0.740	1.09	1.17	0.832	NA	NA	NA
Kidney medulla	0.681	0.929	0.971	0.761	NA	NA	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002		
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Kidney(s)	0.705	1.04	1.12	0.897	0.744	1.04	NA
Large intestine	0.173	0.509	0.999	0.546	1.13	NA	NA
Liver	2.97	2.45	3.26	2.46	3.12	5.12	NA
Lung(s)	0.649	0.734	0.699	0.658	0.511	NA	NA
Lymph node(s)	0.126	0.505	0.637	0.568	1.65	2.19	NA
Muscle	0.148	0.351	0.359	0.277	NA	NA	NA
Myocardium	0.803	0.877	1.03	0.742	0.497	NA	NA
Nasal turbinates	0.0845	0.335	0.445	0.690	1.09	1.99	NA
Pancreas	0.450	1.03	1.09	0.748	0.486	NA	NA
Pituitary gland	0.448	0.792	0.798	0.529	0.475	1.66	NA
Preputial gland	0.148	0.474	0.318	0.942	1.82	7.61	NA
Prostate gland	0.107	0.495	0.485	0.482	0.461	NA	NA
Salivary gland(s)	0.443	0.981	1.14	0.897	0.992	1.87	NA
Seminal vesicle(s)	0.0284	0.153	0.0995	1.21	NA	NA	NA
Skin (nonpigmented)	0.0718	0.229	0.433	1.53	2.32	2.22	NA
Skin (pigmented)	0.146	0.251	0.428	1.41	4.24	7.18	NA
Small intestine	0.264	0.340	0.683	0.865	0.736	NA	NA
Spinal cord	0.0640	0.541	1.14	4.18	5.53	10.2	NA
Spleen	0.502	0.544	0.622	0.474	0.565	NA	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0401	**R0402**	**R0403**	**R0404**	**R0405**	**R0406**	**R0407**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Stomach	0.418	0.708	0.716	0.536	0.730	1.07	NA
Stomach mucosa	0.447	0.851	0.837	NA	NA	NA	NA
Stomach wall	0.260	0.502	0.567	NA	NA	NA	NA
Testis(es)	0.0263	0.408	0.454	1.48	2.23	2.77	NA
Thymus	0.152	0.559	0.687	0.634	1.09	1.90	NA
Thyroid	0.493	0.818	0.828	1.71	11.5	23.0	NA
Urinary bladder	0.170	0.734	1.17	0.800	0.756	NA	NA

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; NA, not applicable; QWBA, quantitative whole body autoradiography

Table 36: Estimated Pharmacokinetic Parameters Following a Single Oral Administration of [^{14}C]Etrasimod to Male Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 4) (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male/7 (Group 5)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Adrenal gland(s)	1300485	1391289	24100	8.00	46.3
Blood (QWBA)	120337	NC	8750	4.00	NC
Brain	194852	196962	9470	8.00	10.8
Brain cerebellum	214250	218834	10200	8.00	13.1
Brain cerebrum	170896	173110	9910	8.00	7.24
Brain medulla	246904	250070	11900	8.00	11.3
Brain olfactory lobe	124253	125664	6810	8.00	7.09
Bulbo urethral gland	175522	181001	12200	4.00	18.0

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Esophagus	185252	196864	17700	0.500	17.5
Eye lens	NC	NC	NC	NC	NC
Eye uveal tract	98366	103875	7080	4.00	16.4
Eye(s)	18879	20787	1980	4.00	6.92
Fat (brown)	257525	308740	7920	8.00	90.3
Harderian gland	227257	230385	16700	4.00	11.3
Kidney cortex	198260	208188	16800	4.00	5.33
Kidney(s)	214888	217351	16000	4.00	13.8
Liver	654627	662919	37700	4.00	41.3
Lymph node(s)	126422	136729	7770	4.00	27.4
Myocardium	188569	190370	13500	4.00	7.05
Pancreas	196981	198705	15900	4.00	6.91
Plasma (LSC)	213022	215247	15400	4.00	13.0
Preputial gland	189727	358190	7300	4.00	185
Salivary gland(s)	211501	217310	15100	4.00	18.1
Skin (nonpigmented)	149372	165680	4070	8.00	49.8
Skin (pigmented)	212949	263859	4030	8.00	71.0
Spinal cord	330930	365540	10700	8.00	19.8

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Testis(es)	157422	169000	6280	4.00	66.3
Thyroid	617710	NC	12600	4.00	NC
Urinary bladder	183843	188068	11300	4.00	10.9

Note: Tissues with AUC_{last} > 1.5 × AUC_{last} of blood (QWBA) (i.e., >180,506) are displayed, in addition to other select tissues. Refer to Table 4-7 in APD334.DMPK.002 (Appendix 4) for full presentation of data.
AUC, area under the concentration-time curve; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 168 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counting; NC, not calculated; QWBA, quantitative whole body autoradiography; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 37: Tissue Distribution Pattern Following a Single Oral Administration of [^{14}C]Etrasimod to Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Female/7 (Group 5)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats						**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**					
	Animal (Sampling Time)					
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	4600[a]	12800[a]	21100[a]	18100[a]	13100[a]	5180[a]	2880[a]
Bile	ND	ND	ND	ND	ND	ND	ND
Blood	3530	7540	5050	1230	246	BLQ	ND
Bone	149	757	234	ND	ND	ND	ND
Bone marrow	2200	5950	5570	1360	557	224	ND
Brain (whole)	410	6480	10000	5310	988	266	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002		
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebellum	432	7310	10900	5260	1240	393	ND
Brain cerebrum	401	7420	10600	4570	998	309	ND
Brain medulla	395	7010	12300	7720	946	343	ND
Brain olfactory lobe	413	5410	7320	3040	362	129	ND
Cecum	1550	7370	5570	3480	566	419	ND
Diaphragm	2570	8130	6630	996	262	BLQ	ND
Esophagus	3340	11500	5250	1530	1210	563	ND
Exorbital lacrimal gland	1110	9930	9090	2160	566	290	ND
Eye lens	ND	BLQ	ND	ND	ND	ND	ND
Eye uveal tract	915	5550	6420	654	356	ND	ND
Eye(s)	201	1140	1150	207	BLQ	ND	ND
Fascia	ND	2030	4160	803	493	350	ND
Fat (abdominal)	193	1010	1040	BLQ	ND	ND	ND
Fat (brown)	2820	5700	9890	2660	1390	751	389
Harderian gland	904	12600	18900	5360	729	392	ND
Intra-orbital lacrimal gland	972	9130	9760	2150	546	401	ND
Kidney cortex	4310	14300	11400	2580	507	ND	ND
Kidney medulla	4880	12300	8910	2210	389	ND	ND
Kidney(s)	4630	13600	11700	2470	481	204	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Large intestine	1540	6480	8270	1420	347	216	ND
Liver	19100	36500	27300	6820	1370	920	240
Lung(s)	3840	9030	7390	1470	281	148	ND
Lymph node(s)	970	6480	6290	1500	587	542	261
Muscle	910	4560	4030	656	BLQ	ND	ND
Myocardium	5080	12000	12300	2070	352	160	ND
Nasal turbinates	502	4510	6180	2140	506	383	ND
Ovary(ies)	1160[a]	6200[a]	5250[a]	3070[a]	1530[a]	1060[a]	497[a]
Pancreas	3190	14200	11400	2200	329	130	ND
Pituitary gland	3330	12700	9360	2090	388	170	ND
Preputial gland	620[a]	4110[a]	4780[a]	3260[a]	1330[a]	653[a]	371[a]
Salivary gland(s)	2160	13000	12600	2360	650	446	133
Skin (nonpigmented)	595	3020	3200	1870	1550	726	416
Skin (pigmented)	640	3420	2930	2060	2020	1270	580
Small intestine	1850	7130	8780	1750	819	173	ND
Spinal cord	476	7380	11400	6650	1890	1670	176
Spleen	3070	8630	6600	1250	299	166	ND
Stomach	1080	9560	7770	1400	314	174	ND
Stomach mucosa	ND	12500	9090	1680	440	ND	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Stomach wall	ND	5800	5010	958	259	ND	ND
Thymus	920	7240	7460	2430	844	540	BLQ
Thyroid	2940	9990	8300	8840	1780	1860	1150
Urinary bladder	726	3970	4760	1120	349	132	ND
Urine	768	4830	7200	681	BLQ	240	ND
Uterus	1170[a]	4170[a]	5720[a]	1580[a]	334[a]	204[a]	ND

[a] Tissue appeared to be fat soaked.
BLQ, below the limit of quantitation (< 116 ng eq. [^{14}C]etrasimod/g); eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; ND, not detectable (sample shape not discernible from background or surrounding tissue); QWBA, quantitative whole body autoradiography

Table 38: Tissue:Plasma Concentration Ratios Following a Single Oral Administration of [^{14}C]Etrasimod to Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Female/7 (Group 5)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	0.743	0.908	2.36	8.58	31.7	40.5	NA
Blood	0.570	0.535	0.566	0.583	0.596	NA	NA
Bone	0.0241	0.0537	0.0262	NA	NA	NA	NA
Bone marrow	0.355	0.422	0.624	0.645	1.35	1.75	NA
Brain (whole)	0.0662	0.460	1.12	2.52	2.39	2.08	NA
Brain cerebellum	0.0698	0.518	1.22	2.49	3.00	3.07	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebrum	0.0648	0.526	1.19	2.17	2.42	2.41	NA
Brain medulla	0.0638	0.497	1.38	3.66	2.29	2.68	NA
Brain olfactory lobe	0.0667	0.384	0.820	1.44	0.877	1.01	NA
Cecum	0.250	0.523	0.624	1.65	1.37	3.27	NA
Diaphragm	0.415	0.577	0.742	0.472	0.634	NA	NA
Esophagus	0.540	0.816	0.588	0.725	2.93	4.40	NA
Exorbital lacrimal gland	0.179	0.704	1.02	1.02	1.37	2.27	NA
Eye lens	NA	NA	NA	NA	NA	NA	NA
Eye uveal tract	0.148	0.394	0.719	0.310	0.862	NA	NA
Eye(s)	0.0325	0.0809	0.129	0.0981	NA	NA	NA
Fascia	NA	0.144	0.466	0.381	1.19	2.73	NA
Fat (abdominal)	0.0312	0.0716	0.116	NA	NA	NA	NA
Fat (brown)	0.456	0.404	1.11	1.26	3.37	5.87	NA
Harderian gland	0.146	0.894	2.12	2.54	1.77	3.06	NA
Intra-orbital lacrimal gland	0.157	0.648	1.09	1.02	1.32	3.13	NA
Kidney cortex	0.696	1.01	1.28	1.22	1.23	NA	NA
Kidney medulla	0.788	0.872	0.998	1.05	0.942	NA	NA
Kidney(s)	0.748	0.965	1.31	1.17	1.16	1.59	NA
Large intestine	0.249	0.460	0.926	0.673	0.840	1.69	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002		
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Liver	3.09	2.59	3.06	3.23	3.32	7.19	NA
Lung(s)	0.620	0.640	0.828	0.697	0.680	1.16	NA
Lymph node(s)	0.157	0.460	0.704	0.711	1.42	4.23	NA
Muscle	0.147	0.323	0.451	0.311	NA	NA	NA
Myocardium	0.821	0.851	1.38	0.981	0.852	1.25	NA
Nasal turbinates	0.0811	0.320	0.692	1.01	1.23	2.99	NA
Ovary(ies)	0.187	0.440	0.588	1.45	3.70	8.28	NA
Pancreas	0.515	1.01	1.28	1.04	0.797	1.02	NA
Pituitary gland	0.538	0.901	1.05	0.991	0.939	1.33	NA
Preputial gland	0.100	0.291	0.535	1.55	3.22	5.10	NA
Salivary gland(s)	0.349	0.922	1.41	1.12	1.57	3.48	NA
Skin (nonpigmented)	0.0961	0.214	0.358	0.886	3.75	5.67	NA
Skin (pigmented)	0.103	0.243	0.328	0.976	4.89	9.92	NA
Small intestine	0.299	0.506	0.983	0.829	1.98	1.35	NA
Spinal cord	0.0769	0.523	1.28	3.15	4.58	13.0	NA
Spleen	0.496	0.612	0.739	0.592	0.724	1.30	NA
Stomach	0.174	0.678	0.870	0.664	0.760	1.36	NA
Stomach mucosa	NA	0.887	1.02	0.796	1.07	NA	NA
Stomach wall	NA	0.411	0.561	0.454	0.627	NA	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002		
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R1001	**R1002**	**R1003**	**R1004**	**R1005**	**R1006**	**R1007**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Thymus	0.149	0.513	0.835	1.15	2.04	4.22	NA
Thyroid	0.475	0.709	0.929	4.19	4.31	14.5	NA
Urinary bladder	0.117	0.282	0.533	0.531	0.845	1.03	NA
Uterus	0.189	0.296	0.641	0.749	0.809	1.59	NA

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; NA, not applicable; QWBA, quantitative whole body autoradiography

Table 39: Estimated Pharmacokinetic Parameters Following a Single Oral Administration of [^{14}C]Etrasimod to Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 4) (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Female/7 (Group 5)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Adrenal gland(s)	1393640	1625866	21100	8.00	55.9
Blood (QWBA)	113387	NC	7540	4.00	NC
Brain	258224	262489	10000	8.00	11.1
Brain cerebellum	276953	284224	10900	8.00	12.8
Brain cerebrum	253687	259193	10600	8.00	12.4
Brain medulla	331298	336585	12300	8.00	10.7
Brain olfactory lobe	165350	167309	7320	8.00	10.5
Cecum	174650	184103	7370	4.00	15.6

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Exorbital lacrimal gland	190622	197554	9930	4.00	16.6
Eye lens	NC	NC	NC	NC	NC
Eye uveal tract	104195	118243	6420	8.00	27.4
Eye(s)	17833	19764	1150	8.00	6.47
Fat (brown)	276207	308059	9890	8.00	56.8
Harderian gland	367458	374652	18900	8.00	12.7
Intra-orbital lacrimal gland	194698	206160	9760	8.00	19.8
Kidney cortex	233929	240511	14300	4.00	9.00
Kidney medulla	193853	198852	12300	4.00	8.91
Kidney(s)	240652	243898	13600	4.00	11.0
Liver	684075	700779	36500	4.00	48.2
Lymph node(s)	178276	215566	6480	4.00	99.0
Myocardium	229928	232928	12300	8.00	13.0
Ovary(ies)	263646	319193	6200	4.00	77.5
Pancreas	227086	228944	14200	4.00	9.91
Pituitary gland	201037	203755	12700	4.00	11.1
Plasma (LSC)	208203	210395	14100	4.00	11.9
Preputial gland	218561	246439	4780	8.00	52.1
Salivary gland(s)	275014	285209	13000	4.00	53.1
Skin (nonpigmented)	182643	222752	3200	8.00	66.8

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002 (Appendix 4)
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Skin (pigmented)	237125	296480	3420	4.00	70.9
Small intestine	174970	178558	8780	8.00	14.4
Spinal cord	429635	438086	11400	8.00	33.3
Stomach mucosa	176655	182590	12500	4.00	9.35
Thymus	178926	196159	7460	8.00	22.1
Thyroid	512663	797930	9990	4.00	172
Uterus	117242	122025	5720	8.00	16.3

Note: Tissues with AUC_{last} > 1.5 × AUC_{last} of blood (QWBA) (i.e., >170,081) are displayed, in addition to other select tissues. Refer to Table 4-10 in APD334.DMPK.002 (Appendix 4) for full presentation of data.
AUC, area under the concentration-time curve; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 168 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counting; NC, not calculated; QWBA, quantitative whole body autoradiography; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 40: Tissue Distribution Pattern Following 7 Days of Oral Administration of [^{14}C]Etrasimod to Male Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male/7 (Group 6)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	42000[a]	67500[a]	52200	29700	29800	11500	6670
Bile	ND	73900	92700	11500	ND	ND	ND
Blood	6990	11900	8100	1270	452	157	ND
Bone	539	582	358	ND	ND	ND	ND
Bone marrow	4010	7600	5230	1090	684	480	143
Brain (whole)	4200	8360	10900	2790	591	200	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebellum	4320	9030	11500	2930	691	228	ND
Brain cerebrum	3690	7300	10500	1670	255	189	ND
Brain medulla	5600	10200	13900	4070	854	251	ND
Brain olfactory lobe	2670	6140	8840	1330	230	194	ND
Bulbo-urethral gland	6370	14100	11200	1590	684	NR	165
Cecum	6080	9850	11400	2110	574	220	ND
Diaphragm	3860	10900	6970	862	367	ND	ND
Epididymis	3680	6270	5490	1440	501	305	BLQ
Esophagus	5040	7750	5870	1910	971	592	118
Exorbital lacrimal gland	4590	10800	11100	1520	600	217	120
Eye lens	376	654	295	438	194	216	141
Eye uveal tract	2710	5500	4520	833	408	382	475
Eye(s)	802	1620	1070	384	201	186	158
Fascia	3770	7410	5960	1480	888	629	383
Fat (abdominal)	1660	3920	1050	543	498	661	357
Fat (brown)	6840	12000	11100	4020	2630	1350	596
Harderian gland	5880	10500	17000	2420	880	496	281
Intra-orbital lacrimal gland	4150	7580	11900	1600	555	240	123
Kidney cortex	7060	15800	11100	1800	792	555	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Kidney medulla	6190	13600	9230	1620	649	518	ND
Kidney(s)	6810	14700	10400	1720	772	538	233
Large intestine	4010	8470	5290	1280	668	370	158
Liver	27000	52800	39100	6290	3160	1890	645
Lung(s)	7280	12600	9010	1230	502	223	ND
Lymph node(s)	3670	10100	6430	1760	1870	1440	910
Muscle	1690	4540	3540	410	119	ND	ND
Myocardium	6390	15000	10400	1160	536	258	ND
Nasal turbinates	2900	6240	6300	1660	842	635	526
Pancreas	4390	11500	12000	1270	523	240	160
Pituitary gland	4560	11700	6490	1070	514	439	ND
Preputial gland	8400[a]	13800	9590[a]	5090[a]	4120[a]	2000[a]	1500[a]
Prostate gland	2910	8390	7690	957	456	351[a]	BLQ
Salivary gland(s)	5760	14000	11900	1740	980	670	244
Seminal vesicle(s)	1590	2850	1960	1070	268	329	409[a]
Skin (nonpigmented)	3410	5860	5050	2630	1150	1040	729
Skin (pigmented)	6980	8440	6590	4460	2410	2160	1180
Small intestine	5760	7610	7790	2260	817	528	215
Spinal cord	5480	10100	12400	4540	1120	487	223

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Spleen	4070	8650	7120	1340	904	575	369
Stomach	2820	9750	7100	768	548	279	BLQ
Stomach mucosa	2910	12100	8020	794	645	342	ND
Stomach wall	861	6300	5870	608	290	148	ND
Testis(es)	4550	6530	6110	3260	1260	800	282
Thymus	3400	7820	6960	1410	784	672	278
Thyroid	58400	46000	65000	46800	36200	47900	19500
Urinary bladder	5190	8580	6250	1230	504	532	123
Urine	3030	6020	995	376	BLQ	119	ND

[a] Tissue appeared to be fat soaked.

BLQ, below the limit of quantitation (< 109 ng eq. [^{14}C]etrasimod/g); eq., equivalents; GLP, Good Laboratory Practice; HPBCD, 2-hydroxypropyl-β-cyclodextrin; ND, not detectable (sample shape not discernible from background or surrounding tissue); NR, not represented (tissue not present in section); QWBA, quantitative whole body autoradiography

Table 41: Tissue:Plasma Concentration Ratios Following 7 Days of Oral Administration of [^{14}C]Etrasimod to Male Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male/7 (Group 6)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	2.69	2.85	4.24	13.6	43.0	41.1	80.5
Blood	0.448	0.502	0.659	0.583	0.652	0.561	NA
Bone	0.0346	0.0246	0.0291	NA	NA	NA	NA
Bone marrow	0.257	0.321	0.425	0.500	0.987	1.71	1.72
Brain (whole)	0.269	0.353	0.886	1.28	0.853	0.714	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebellum	0.277	0.381	0.935	1.34	0.997	0.814	NA
Brain cerebrum	0.237	0.308	0.854	0.766	0.368	0.675	NA
Brain medulla	0.359	0.430	1.13	1.87	1.23	0.896	NA
Brain olfactory lobe	0.171	0.259	0.719	0.610	0.332	0.693	NA
Bulbo-urethral gland	0.408	0.595	0.911	0.729	0.987	NA	1.99
Cecum	0.390	0.416	0.927	0.968	0.828	0.786	NA
Diaphragm	0.247	0.460	0.567	0.395	0.530	NA	NA
Epididymis	0.236	0.265	0.446	0.661	0.723	1.09	NA
Esophagus	0.323	0.327	0.477	0.876	1.40	2.11	1.42
Exorbital lacrimal gland	0.294	0.456	0.902	0.697	0.866	0.775	1.45
Eye lens	0.0241	0.0276	0.0240	0.201	0.280	0.771	1.70
Eye uveal tract	0.174	0.232	0.367	0.382	0.589	1.36	5.73
Eye(s)	0.0514	0.0684	0.0870	0.176	0.290	0.664	1.91
Fascia	0.242	0.313	0.485	0.679	1.28	2.25	4.62
Fat (abdominal)	0.106	0.165	0.0854	0.249	0.719	2.36	4.31
Fat (brown)	0.438	0.506	0.902	1.84	3.80	4.82	7.19
Harderian gland	0.377	0.443	1.38	1.11	1.27	1.77	3.39
Intra-orbital lacrimal gland	0.266	0.320	0.967	0.734	0.801	0.857	1.48
Kidney cortex	0.453	0.667	0.902	0.826	1.14	1.98	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Kidney medulla	0.397	0.574	0.750	0.743	0.937	1.85	NA
Kidney(s)	0.437	0.620	0.846	0.789	1.11	1.92	2.81
Large intestine	0.257	0.357	0.430	0.587	0.964	1.32	1.91
Liver	1.73	2.23	3.18	2.89	4.56	6.75	7.78
Lung(s)	0.467	0.532	0.733	0.564	0.724	0.796	NA
Lymph node(s)	0.235	0.426	0.523	0.807	2.70	5.14	11.0
Muscle	0.108	0.192	0.288	0.188	0.172	NA	NA
Myocardium	0.410	0.633	0.846	0.532	0.773	0.921	NA
Nasal turbinates	0.186	0.263	0.512	0.761	1.22	2.27	6.34
Pancreas	0.281	0.485	0.976	0.583	0.755	0.857	1.93
Pituitary gland	0.292	0.494	0.528	0.491	0.742	1.57	NA
Preputial gland	0.538	0.582	0.780	2.33	5.95	7.14	18.1
Prostate gland	0.187	0.354	0.625	0.439	0.658	1.25	NA
Salivary gland(s)	0.369	0.591	0.967	0.798	1.41	2.39	2.94
Seminal vesicle(s)	0.102	0.120	0.159	0.491	0.387	1.17	4.93
Skin (nonpigmented)	0.219	0.247	0.411	1.21	1.66	3.71	8.79
Skin (pigmented)	0.447	0.356	0.536	2.05	3.48	7.71	14.2
Small intestine	0.369	0.321	0.633	1.04	1.18	1.89	2.59
Spinal cord	0.351	0.426	1.01	2.08	1.62	1.74	2.69

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R0501	**R0502**	**R0503**	**R0504**	**R0505**	**R0506**	**R0507**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Spleen	0.261	0.365	0.579	0.615	1.30	2.05	4.45
Stomach	0.181	0.411	0.577	0.352	0.791	0.996	NA
Stomach mucosa	0.187	0.511	0.652	0.364	0.931	1.22	NA
Stomach wall	0.0552	0.266	0.477	0.279	0.418	0.529	NA
Testis(es)	0.292	0.276	0.497	1.50	1.82	2.86	3.40
Thymus	0.218	0.330	0.566	0.647	1.13	2.40	3.35
Thyroid	3.74	1.94	5.28	21.5	52.2	171	235
Urinary bladder	0.333	0.362	0.508	0.564	0.727	1.90	1.48

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; NA, not applicable; QWBA, quantitative whole body autoradiography

Table 42: Estimated Pharmacokinetic Parameters Following 7 Days of Oral Administration of [^{14}C]Etrasimod to Male Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 4) (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Male/7 (Group 6)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats				**Study Number:** APD334.DMPK.002 (Appendix 4)	
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Adrenal gland(s)	3178485	4353973	67500	4.00	122
Blood (QWBA)	177737	NC	11900	4.00	NC
Brain	221134	224777	10900	8.00	12.6
Brain cerebellum	235423	239709	11500	8.00	13.0
Brain cerebrum	181543	185707	10500	8.00	15.3
Brain medulla	293358	297683	13900	8.00	11.9
Brain olfactory lobe	151213	156050	8840	8.00	17.3
Bulbo urethral gland	268563	279958	14100	4.00	47.9

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats				**Study Number:** APD334.DMPK.002 (Appendix 4)	
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Eye lens	39299	83795	654	4.00	219
Eye uveal tract	143417	NC	5500	4.00	NC
Eye(s)	49627	131989	1620	4.00	361
Fat (brown)	422808	469514	12000	4.00	54.3
Harderian gland	333903	367016	17000	8.00	81.7
Kidney(s)	269137	293354	14700	4.00	72.0
Liver	989000	1039954	52800	4.00	54.8
Lymph node(s)	319675	480648	10100	4.00	123
Plasma (LSC)	324086	329184	23700	4.00	42.6
Preputial gland	557130	740040	13800	4.00	84.5
Prostate gland	148479	165276	8390	4.00	33.2
Salivary gland(s)	293252	314879	14000	4.00	61.4
Seminal vesicle(s)	100672	NC	2850	4.00	NC
Skin (nonpigmented)	256887	450215	5860	4.00	184
Skin (pigmented)	444790	639830	8440	4.00	115
Spinal cord	330439	349129	12400	8.00	58.1
Testis(es)	251664	275137	6530	4.00	57.7
Thyroid	6554100	9826236	65000	8.00	116

Note: Tissues with AUC_{last} > 1.5 × AUC_{last} of blood (QWBA) (i.e., >266,606) are displayed, in addition to other select tissues. Refer to Table 4-13 in APD334.DMPK.002 (Appendix 4) for full presentation of data.
AUC, area under the concentration-time curve; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 168 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counter; NC, not calculated; QWBA, quantitative whole body autoradiography; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 43: Tissue Distribution Pattern Following 7 Days of Oral Administration of [^{14}C]Etrasimod to Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Female/7 (Group 6)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	68900[a]	41500[a]	73300	69600	27700[a]	43300	11200
Bile	ND	50700	54200	11100	ND	ND	ND
Blood	8670	12800	8360	1030	255	ND	ND
Bone	832	636	697	ND	ND	ND	ND
Bone marrow	6330	7820	7980	1520	698	522	209
Brain (whole)	6490	11600	17800	4020	647	348	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebellum	7340	12500	17800	4250	779	362	ND
Brain cerebrum	5570	11000	17100	2790	291	176	ND
Brain medulla	11000	13600	22900	5850	871	370	ND
Brain olfactory lobe	4550	8900	15200	2260	214	207	ND
Cecum	5680	12200	8840	2700	1250	350	ND
Diaphragm	6750	9730	9260	840	279	ND	ND
Esophagus	8260	10600	8720	1960	849	381	173
Exorbital lacrimal gland	5930	14500	13700	1930	517	426	129
Eye lens	266	492	384	288	249	191	163
Eye uveal tract	2510	6990	6730	781	535	316	187
Eye(s)	751	1380	1270	348	196	182	119
Fascia	3200	6970	7270	1780	315	323	ND
Fat (abdominal)	1080	2000	1790	328	183	504	200
Fat (brown)	8970	15400	14900	4890	2200	2000	785
Harderian gland	9460	20500	24700	3530	1050	664	353
Intra-orbital lacrimal gland	5380	13800	12200	1540	511	556	218
Kidney cortex	11600	18300	17400	2160	758	590	286
Kidney medulla	10800	15000	15200	1880	723	381	236
Kidney(s)	11000	17000	16400	2030	743	535	271

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Large intestine	5730	7960	9630	1540	571	502	257
Liver	50100	53700	46600	7580	2880	1920	921
Lung(s)	8650	12800	11600	1090	320	285	ND
Lymph node(s)	5460	9940	11000	3190	1150	2010	1190
Muscle	2370	5390	5210	525	BLQ	ND	ND
Myocardium	10800	16000	16000	1460	382	321	ND
Nasal turbinates	3130	7760	7610	2440	1120	1310	581
Ovary(ies)	10500[a]	17200[a]	15200[a]	8200[a]	2940[a]	4050[a]	1810[a]
Pancreas	8510	16900	18300	1750	389	323	191
Pituitary gland	9150	15900	13200	1740	516	376	ND
Preputial gland	7250[a]	10900[a]	13900[a]	3270[a]	4520[a]	2640[a]	2140[a]
Salivary gland(s)	8190	15500	15300	2260	762	786	327
Skin (nonpigmented)	5320	6710	7570	3590	2000	3220	1840
Skin (pigmented)	8120	7500	8790	5100	3910	4020	2480
Small intestine	ND	14200	12800	1790	544	514	286
Spinal cord	9510	14800	23300	5510	2250	1800	171
Spleen	7370	11900	10800	1890	769	717	569
Stomach	4220	11600	10800	1440	445	318	170
Stomach mucosa	5480	14000	12500	1600	533	398	ND

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Concentration (ng eq. [^{14}C]etrasimod/g)**						
	Animal (Sampling Time)						
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Stomach wall	4570	8560	7560	969	327	182	ND
Thymus	5290	10600	11900	2440	1140	895	515
Thyroid	32400	40600	70300	30800	18000	19500	28600
Urinary bladder	5560	8190	6880	1570	480[a]	407	333
Urine	1360	1140	1420	284	NR	ND	ND
Uterus	4660[a]	10700	11900	1390	567[a]	821[a]	272

[a] Tissue appeared to be fat soaked.

eq., equivalents; GLP, Good Laboratory Practice; HPBCD, 2-hydroxypropyl-β-cyclodextrin; ND, not detectable (sample shape not discernible from background or surrounding tissue); NR, not represented (tissue not present in section); QWBA, quantitative whole body autoradiography

Table 44: Tissue:Plasma Concentration Ratios Following 7 Days of Oral Administration of [^{14}C]Etrasimod to Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Female/7 (Group 6)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats						**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**					
	Animal (Sampling Time)					
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Adrenal gland(s)	4.10	1.92	5.35	41.4	63.7	176	114
Blood	0.516	0.593	0.610	0.613	0.586	NA	NA
Bone	0.0495	0.0294	0.0509	NA	NA	NA	NA
Bone marrow	0.377	0.362	0.582	0.905	1.60	2.12	2.13
Brain (whole)	0.386	0.537	1.30	2.39	1.49	1.41	NA
Brain cerebellum	0.437	0.579	1.30	2.53	1.79	1.47	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Brain cerebrum	0.332	0.509	1.25	1.66	0.669	0.715	NA
Brain medulla	0.655	0.630	1.67	3.48	2.00	1.50	NA
Brain olfactory lobe	0.271	0.412	1.11	1.35	0.492	0.841	NA
Cecum	0.338	0.565	0.645	1.61	2.87	1.42	NA
Diaphragm	0.402	0.450	0.676	0.500	0.641	NA	NA
Esophagus	0.492	0.491	0.636	1.17	1.95	1.55	1.76
Exorbital lacrimal gland	0.353	0.671	1.00	1.15	1.19	1.73	1.31
Eye lens	0.0158	0.0228	0.0280	0.171	0.572	0.776	1.66
Eye uveal tract	0.149	0.324	0.491	0.465	1.23	1.28	1.90
Eye(s)	0.0447	0.0639	0.0927	0.207	0.451	0.740	1.21
Fascia	0.190	0.323	0.531	1.06	0.724	1.31	NA
Fat (abdominal)	0.0643	0.0926	0.131	0.195	0.421	2.05	2.04
Fat (brown)	0.534	0.713	1.09	2.91	5.06	8.13	7.99
Harderian gland	0.563	0.949	1.80	2.10	2.41	2.70	3.59
Intra-orbital lacrimal gland	0.320	0.639	0.891	0.917	1.17	2.26	2.22
Kidney cortex	0.690	0.847	1.27	1.29	1.74	2.40	2.91
Kidney medulla	0.643	0.694	1.11	1.12	1.66	1.55	2.40
Kidney(s)	0.655	0.787	1.20	1.21	1.71	2.17	2.76
Large intestine	0.341	0.369	0.703	0.917	1.31	2.04	2.62

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Liver	2.98	2.49	3.40	4.51	6.62	7.80	9.38
Lung(s)	0.515	0.593	0.847	0.649	0.736	1.16	NA
Lymph node(s)	0.325	0.460	0.803	1.90	2.64	8.17	12.1
Muscle	0.141	0.250	0.380	0.313	NA	NA	NA
Myocardium	0.643	0.741	1.17	0.869	0.878	1.30	NA
Nasal turbinates	0.186	0.359	0.555	1.45	2.57	5.33	5.92
Ovary(ies)	0.625	0.796	1.11	4.88	6.76	16.5	18.4
Pancreas	0.507	0.782	1.34	1.04	0.894	1.31	1.95
Pituitary gland	0.545	0.736	0.964	1.04	1.19	1.53	NA
Preputial gland	0.432	0.505	1.01	1.95	10.4	10.7	21.8
Salivary gland(s)	0.488	0.718	1.12	1.35	1.75	3.20	3.33
Skin (nonpigmented)	0.317	0.311	0.553	2.14	4.60	13.1	18.7
Skin (pigmented)	0.483	0.347	0.642	3.04	8.99	16.3	25.3
Small intestine	NA	0.657	0.934	1.07	1.25	2.09	2.91
Spinal cord	0.566	0.685	1.70	3.28	5.17	7.32	1.74
Spleen	0.439	0.551	0.788	1.13	1.77	2.91	5.79
Stomach	0.251	0.537	0.788	0.857	1.02	1.29	1.73
Stomach mucosa	0.326	0.648	0.912	0.952	1.23	1.62	NA
Stomach wall	0.272	0.396	0.552	0.577	0.752	0.740	NA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats							**Study Number:** APD334.DMPK.002
Tissue	**Tissue:Plasma Concentration Ratio**						
	Animal (Sampling Time)						
	R1101	**R1102**	**R1103**	**R1104**	**R1105**	**R1106**	**R1107**
	(0.5 h)	**(4 h)**	**(8 h)**	**(24 h)**	**(48 h)**	**(72 h)**	**(168 h)**
Thymus	0.315	0.491	0.869	1.45	2.62	3.64	5.24
Thyroid	1.93	1.88	5.13	18.3	41.4	79.3	291
Urinary bladder	0.331	0.379	0.502	0.935	1.10	1.65	3.39
Uterus	0.277	0.495	0.869	0.827	1.30	3.34	2.77

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; NA, not applicable; QWBA, quantitative whole body autoradiography

Table 45: Estimated Pharmacokinetic Parameters Following 7 Days of Oral Administration of [^{14}C]Etrasimod to Female Long-Evans Rats

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 4) (non-GLP)
Species	Rat/Long-Evans
Sex/Number of Animals (Group)	Female/7 (Group 6)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral (once daily for 7 days)
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Assay	QWBA

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats				**Study Number:** APD334.DMPK.002 (Appendix 4)	
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Adrenal gland(s)	6218825	7218328	73300	8.00	61.9
Blood (QWBA)	172600	NC	12800	4.00	NC
Brain	334584	341411	17800	8.00	13.6
Brain cerebellum	347595	354650	17800	8.00	13.5
Brain cerebrum	288286	291343	17100	8.00	12.0
Brain medulla	444344	450777	22900	8.00	12.1
Brain olfactory lobe	247295	251452	15200	8.00	13.9
Exorbital lacrimal gland	285995	296910	14500	4.00	58.6

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats				**Study Number:** APD334.DMPK.002 (Appendix 4)	
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Eye lens	37237	91682	492	4.00	232
Eye uveal tract	154929	178847	6990	4.00	88.7
Eye(s)	47673	75774	1380	4.00	164
Fat (brown)	532970	620999	15400	4.00	77.7
Harderian gland	495379	537559	24700	8.00	82.8
Intra-orbital lacrimal gland	271398	299143	13800	4.00	88.2
Kidney cortex	376345	412289	18300	4.00	87.1
Kidney medulla	318990	348163	15200	8.00	85.7
Kidney(s)	353290	387048	17000	4.00	86.3
Liver	1147703	1250349	53700	4.00	77.3
Lymph node(s)	427315	645255	11000	8.00	127
Myocardium	283820	293992	16000	4.00	22.0
Nasal turbinates	293628	361669	7760	4.00	81.2
Ovary(ies)	801940	1016196	17200	4.00	82.1
Pancreas	336279	369224	18300	8.00	120
Pituitary gland	261621	273401	15900	4.00	21.7
Plasma (LSC)	315114	323585	21600	4.00	59.8
Preputial gland	629375	1051004	13900	8.00	137
Salivary gland(s)	353849	396618	15500	4.00	90.7
Skin (nonpigmented)	512823	828468	7570	8.00	119
Skin (pigmented)	688345	1222517	8790	8.00	149

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats				**Study Number:** APD334.DMPK.002 (Appendix 4)	
Matrix	**AUC_{last} (ng eq.·h/g)**	**AUC_{∞} (ng eq.·h/g)**	**C_{max} (ng eq./g)**	**t_{max} (h)**	**$t_{½}$ (h)**
Small intestine	274674	326003	14200	4.00	124
Spinal cord	587928	595255	23300	8.00	29.7
Spleen	293953	523296	11900	4.00	279
Thymus	323910	404698	11900	8.00	109
Thyroid	4510850	NC	70300	8.00	NC
Uterus	272169	300014	11900	8.00	71.0

Note: Tissues with AUC_{last} > 1.5 × AUC_{last} of blood (QWBA) (i.e., >258,900) are displayed, in addition to other select tissues. Refer to Table 4-16 in APD334.DMPK.002 (Appendix 4) for full presentation of data.
AUC, area under the concentration-time curve; AUC_{∞}, AUC from zero to infinity; AUC_{last}, AUC from time zero to last measurable concentration through 168 h; C_{max}, maximum plasma drug concentration; eq., equivalents; GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counter; NC, not calculated; QWBA, quantitative whole body autoradiography; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

2.6.5.6. PHARMACOKINETICS: PLASMA PROTEIN BINDING

Table 46: Plasma Protein Binding of Etrasimod in Mouse, Rat, Rabbit, Dog, Monkey, and Human

Test Article	Etrasimod (APD334, AR401959)
Study Number	MPR-[redacted]-001 (non-GLP)
Study Type	In vitro
Study System	Plasma
Species	Mouse, rat, rabbit, dog, monkey, and human
Method	Plasma from mouse, rat, rabbit, dog, monkey, and human was combined with 10 µM etrasimod and ultracentrifuged to determine plasma protein binding.
Report Title	Determination of APD334 Plasma Protein Binding in Mouse, Rat, Rabbit, Dog, Monkey, and Human Plasma Using an Ultracentrifugation Method

Species	**Etrasimod Concentration (µM)**	**Percent Bound**
Mouse[a]	10	99.1 ± 0.3
Rat[b]	10	99.1 ± 0.2
Rabbit[c]	10	98.2 ± 0.4
Dog[d]	10	99.2 ± 0.1
Monkey[e]	10	95.4 ± 1.4
Human[f]	10	97.9 ± 0.4

[a] Mouse/CD-1; male; mean ± SD, n = 3.
[b] Rat/Sprague Dawley; male; mean ± SD, n = 3.
[c] Rabbit/New Zealand White; male; mean ± SD, n = 3.
[d] Dog/Beagle; male; mean ± SD, n = 3.
[e] Monkey/Cynomolgus; male; mean ± SD, n = 3.
[f] Human; male; mean ± SD, n = 3.
GLP, Good Laboratory Practice; n, number; SD, standard deviation

Table 47: Blood-to-Plasma Partitioning of Etrasimod in Rat, Dog, Monkey, and Human

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.003 (non-GLP)
Study Type	In vitro
Study System	Plasma and whole blood
Species	Rat, dog, monkey, human
Method	[^{14}C]Etrasimod was spiked into whole blood at 5 concentrations for each species and incubated for 60 minutes at 37°C. Blood was processed to plasma, and radioactivity was measured by liquid scintillation counting in whole blood and plasma samples.

Report Title: In Vitro Blood-to-Plasma Partitioning of [^{14}C]APD334 in Rat, Dog, Monkey and Human		**Study Number:** APD334.DMPK.003
Species	**Etrasimod Concentration (µg/mL)**	**Mean Blood Cell Ratio (± SD)**
Rat[a]	0.10	0.956 ± 0.036
	0.25	0.890 ± 0.077
	0.50	0.859 ± 0.052
	1.0	0.925 ± 0.095
	2.0	0.883 ± 0.009
Dog[b]	0.10	0.734 ± 0.034
	0.25	0.755 ± 0.087
	0.50	0.686 ± 0.082
	1.0	0.647 ± 0.008
	2.0	0.659 ± 0.070

Report Title: In Vitro Blood-to-Plasma Partitioning of [^{14}C]APD334 in Rat, Dog, Monkey and Human		**Study Number:** APD334.DMPK.003
Species	**Etrasimod Concentration (µg/mL)**	**Mean Blood Cell Ratio (± SD)**
Monkey[c]	0.10	0.921 ± 0.085
	0.25	0.999 ± 0.082
	0.50	0.943 ± 0.060
	1.0	0.893 ± 0.084
	2.0	0.828 ± 0.047
Human 1[d]	0.10	0.643 ± 0.035
	0.25	0.649 ± 0.069
	0.50	0.681 ± 0.077
	1.0	0.667 ± 0.059
	2.0	0.769 ± 0.106
Human 2[d]	0.10	0.730 ± 0.066
	0.25	0.674 ± 0.058
	0.50	0.678 ± 0.042
	1.0	0.747 ± 0.048
	2.0	0.702 ± 0.082
Human 3[d]	0.10	0.821 ± 0.078
	0.25	0.774 ± 0.011
	0.50	0.761 ± 0.061
	1.0	0.741 ± 0.029
	2.0	0.750 ± 0.025

Report Title: In Vitro Blood-to-Plasma Partitioning of [^{14}C]APD334 in Rat, Dog, Monkey and Human		**Study Number:** APD334.DMPK.003
Species	**Etrasimod Concentration (µg/mL)**	**Mean Blood Cell Ratio (± SD)**

[a] Rat/Sprague Dawley; male; mean ± SD, n = 3; pooled.
[b] Dog/Beagle; male; mean ± SD, n = 3; pooled.
[c] Monkey/Cynomolgus; male; mean ± SD, n = 3; pooled.
[d] Human (male) 1, 2, or 3; mean ± SD, n = 3; not pooled; 1, 2, and 3 represent individual volunteers.
GLP, Good Laboratory Practice; n, number; SD, standard deviation

2.6.5.7. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS

Table 48: Toxicokinetic Parameters of Etrasimod in Plasma After Repeated Doses in Pregnant Rats

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]017 (Appendix I) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Female/2 cohorts of 4 animals per dose level sampled at alternating timepoints
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 12 days starting on Gestation Day 6)
Dose (mg/kg/day)	1, 2, 4
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rats			**Study Number:** TX[redacted]017 (Appendix I)
PK Parameter	**Dose (mg/kg/day)**	**Day 1 (Gestation Day 6)**	**Day 12 (Gestation Day 17)**
$t_{½}$ (h)	1	NR	NR
	2	NR	NR
	4	NR	NR
t_{max} (h)	1	4.00	4.00
	2	4.00	4.00
	4	4.00	4.00
C_{max} (µg/mL)	1	0.45	0.80
	2	1.17	2.12
	4	2.74	3.62

Study Title: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rats			**Study Number:** TX[redacted]017 (Appendix I)
AUC_{last} (µg·h/mL)	1	7.00	11.0
	2	14.7	22.8
	4	34.2	44.5
AI AUC_{last}	1	NA	1.57
	2	NA	1.55
	4	NA	1.30

Note: Etrasimod was administered once daily from Gestation Day 6 through 17.
AI AUC_{last}, accumulation index for AUC_{last} (AUC_{last} Day 17/AUC_{last} Day 6); AUC, area under the concentration-time curve; AUC_{last}, AUC from time zero to time of last measurable concentration through 24 h; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

Table 49: Toxicokinetic Parameters of Etrasimod in Plasma After Repeated Doses in Pregnant and Nursing Rats (Dose Range-Finding)

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.012 (Appendix 23) (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Female/4 cohorts of 8 animals per dose level sampled at alternating timepoints
Vehicle/Formulation	0.5% (v/v) TWEEN® 20 in 10 mM Tris, pH 8.5 to 9.0
Feeding Condition	Fed
Method of Administration	Oral (once daily from Gestation Day 6 to Lactation Day 20)
Dose (mg/kg/day)	0.05, 0.2, 1.0, 2.0
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: A Dose Range-Finding Prenatal/Postnatal Development Study of APD334 Administered by Oral Gavage to Sprague Dawley Rats		**Study Number:** APD334.TOX.012 (Appendix 23)
PK Parameter	**Dose (mg/kg/day)**	**Lactation Day 20**
t_{max} (h)	0.05	1
	0.2	1
	1.0	1
	2.0	1
C_{max} (µg/mL)	0.05	0.0378 ± 0.00244
	0.2	0.154 ± 0.0269
	1.0	0.633 ± 0.0747
	2.0	1.43 ± 0.123

Study Title: A Dose Range-Finding Prenatal/Postnatal Development Study of APD334 Administered by Oral Gavage to Sprague Dawley Rats		**Study Number:** APD334.TOX.012 (Appendix 23)
PK Parameter	**Dose (mg/kg/day)**	**Lactation Day 20**
C_{max}/Dose ((µg/mL)/(mg/kg/day))	0.05	0.755
	0.2	0.772
	1.0	0.633
	2.0	0.714
AUC_{last} (µg·h/mL)	0.05	0.303 ± 0.0157
	0.2	1.11 ± 0.0347
	1.0	5.21 ± 0.384
	2.0	9.71 ± 0.599
AUC_{last}/Dose ((µg·h/mL)/(mg/kg/day))	0.05	6.06
	0.2	5.54
	1.0	5.21
	2.0	4.86

Note: Data displayed as mean ± standard error for C_{max} and AUC_{last}.
AUC_{last}, area under the plasma concentration-time curve from time zero to time of last measurable concentration through 24 h; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; PK, pharmacokinetic; t_{max}, time to maximum plasma drug concentration

Table 50: Toxicokinetic Parameters of Etrasimod in Plasma After Repeated Doses in Pregnant and Nursing Rats

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.011 (Appendix 37) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Female/3 cohorts of 8 animals per dose level sampled at alternating timepoints
Vehicle/Formulation	0.5% (v/v) TWEEN® 20 in 10 mM Tris, pH 8.5 to 9.0
Feeding Condition	Fed
Method of Administration	Oral (once daily from Gestation Day 6 to Lactation Day 20)
Dose (mg/kg/day)	0.4, 2, 4
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: An Oral Pre- and Postnatal Development Study of APD334 in Rats, Including Toxicokinetics and a Postnatal Neurobehavioral and Reproductive Functional Evaluation				**Study Number:** APD334.TOX.011 (Appendix 37)
PK Parameter	**Dose (mg/kg/day)**	**Gestation Day 6**	**Gestation Day 17**	**Lactation Day 20**
t_{max} (h)	0.4	1	4	2
	2	2	4	1
	4	4	4	1
C_{max} (µg/mL)	0.4	0.339	0.290	0.296
	2	1.40	1.64	1.34
	4	4.00	3.52	2.96
C_{max}/Dose ([µg/mL]/[mg/kg/day])	0.4	0.847	0.726	0.739
	2	0.700	0.820	0.668

Study Title: An Oral Pre- and Postnatal Development Study of APD334 in Rats, Including Toxicokinetics and a Postnatal Neurobehavioral and Reproductive Functional Evaluation				**Study Number:** APD334.TOX.011 (Appendix 37)
PK Parameter	**Dose (mg/kg/day)**	**Gestation Day 6**	**Gestation Day 17**	**Lactation Day 20**
	4	1.00	0.881	0.739
AUC_{last} (µg·h/mL)	0.4	3.73	4.32	2.39
	2	16.6	21.6	11.4
	4	43.1	51.2	36.7
AUC_{last}/Dose ([µg·h/mL]/[mg/kg/day])	0.4	9.34	10.8	5.96
	2	8.31	10.8	5.71
	4	10.8	12.8	9.17

Note: Data displayed as mean for C_{max} and AUC_{last}; GD6 parameters excluded anomalous animals.
AUC_{last}, area under the plasma concentration-time curve from time zero to time of last measurable concentration through 24 h; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; PK, pharmacokinetic; t_{max}, time to maximum plasma drug concentration

Table 51: Mean Litter-to-Maternal Etrasimod Concentration Ratios in Rats

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.011 (Appendix 37) (GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals	Female/3 cohorts of 8 animals per dose level sampled at alternating timepoints
Vehicle/Formulation	0.5% (v/v) TWEEN® 20 in 10 mM Tris, pH 8.5 to 9.0
Feeding Condition	Fed
Method of Administration	Oral (once daily from Gestation Day 6 to Lactation Day 20)
Dose (mg/kg/day)	0.4, 2.0, 4.0
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: An Oral Pre- and Postnatal Development Study of APD334 in Rats, Including Toxicokinetics and a Postnatal Neurobehavioral and Reproductive Functional Evaluation					**Study Number:** APD334.TOX.011 (Appendix 37)	
Dose (mg/kg/day)	**Time postdose (hr)**	**Mean Maternal Concentration (ng/mL)**	**Male Litter Concentration (ng/mL)**	**Male Litter/Maternal Ratio (%)**	**Female Litter Concentration (ng/mL)**	**Female Litter/Maternal Ratio (%)**
0.4	2	296	176	59.5	129	43.6
	4	179	117	65.4	118	65.9
2.0	2	1330	416	31.3	580	43.6
	4	1130	363	32.1	451	39.9
4.0	2	2790	1710	61.3	1740	62.4
	4	2380	1370	57.6	1740	73.1

Note: Mean concentrations are following oral administration of etrasimod on Postnatal Day 20/Lactation Day 20; the mean concentrations are per time point, and not matched with dams as pup samples were not necessarily collected from the associated dams.
GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry

Table 52: Toxicokinetic Parameters of Etrasimod in Plasma After Repeated Doses in Pregnant Rabbits

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	TX[redacted]019 (Appendix G) (GLP)
Species	Rabbit/New Zealand White
Sex/Number of Animals	Female/4 per dose group
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral (once daily for 14 days starting on Gestation Day 7)
Dose (mg/kg/day)	2, 10, 20
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS

Study Title: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rabbits			**Study Number:** TX[redacted]019 (Appendix G)
PK Parameter	**Dose (mg/kg/day)**	**Day 1 (Gestation Day 7)**	**Day 14 (Gestation Day 20)**
$t_{½}$ (h)	2	NR	NR
	10	NR	NR
	20	NR	NR
t_{max} (h)	2	6.00 ± 2.31	4.00 ± 0.00
	10	4.00 ± 0.00	5.00 ± 2.00
	20	4.00 ± 0.00	5.00 ± 2.00
C_{max} (µg/mL)	2	0.258 ± 0.0792	0.234 ± 0.102
	10	1.80 ± 0.29	1.40 ± 0.49
	20	4.89 ± 1.30	2.88 ± 0.72

Study Title: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rabbits			**Study Number:** TX[redacted]019 (Appendix G)
AUC_{last} (µg·h/mL)	2	2.21 ± 0.493	1.70 ± 0.76
	10	14.4 ± 3.8	12.6 ± 2.9
	20	35.0 ± 11.5	24.2 ± 2.9
AI AUC_{last}	2	NA	0.786 ± 0.416
	10	NA	0.962 ± 0.474
	20	NA	0.742 ± 0.209

Note: Etrasimod was administered once daily from Gestation Day 7 through 20.
Data are presented as mean ± standard deviation.
AI AUC_{last}, accumulation index for AUC_{last} (AUC_{last} Day 20/AUC_{last} Day 7); AUC, area under the concentration-time curve; AUC_{last}, AUC time zero to time of last measurable concentration through 24 h; C_{max}, maximum plasma drug concentration; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; PK, pharmacokinetic; $t_{½}$, half-life; t_{max}, time to maximum plasma drug concentration

2.6.5.8. PHARMACOKINETICS: OTHER DISTRIBUTION STUDY

Table 53: Microsomal Binding of Etrasimod

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.DMPK.004 (non-GLP)
Study Type	In vitro
Study System	Human liver microsomes (HLM)
Species	Human
Method	Etrasimod (final concentration: 10 µM) was added to human liver microsomes (0.0125 mg protein/mL) to determine microsomal protein binding by equilibrium dialysis. Etrasimod concentrations were analyzed by LC-MS/MS.

Report Title: APD334 Microsomal Protein Binding			**Study Number:** APD334.DMPK.004
Matrix	**Etrasimod Concentration (µM)**	**Percent Free**	**Percent Bound**
HLM (0.0125 mg/mL)	10.0	3.9 ± 1.3	96.1 ± 1.3

Note: values are mean ± standard deviation
GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry

Table 54: Binding of Etrasimod to Human Serum Albumin, α1-Acid Glycoprotein, and Lipoproteins

Test Article	Etrasimod (APD334)
Study Number	ARE-R10656 (non-GLP)
Study Type	In vitro
Study System	Human serum albumin (HSA), α1-acid glycoprotein (AGP), and lipoproteins (LP)
Species	Human
Method	Binding of etrasimod at 20 and 200 ng/mL was assessed by an ultracentrifugation method. Total etrasimod concentrations and supernatant samples (protein free) were analyzed by LC-MS/MS.

Report Title: Determination of the Binding of APD334 to Human Serum Albumin, α1-Acid Glycoprotein, and Lipoproteins		**Study Number:** ARE-R10656
Matrix	**Etrasimod Concentration (ng/mL)**	**Percent Unbound (mean ± SD)**
AGP[a]	20	1.49 ± 0.247
	200	1.11 ± 0.101
HSA[b]	20	NA
	200	0.0792 ± 0.00501
LP[c]	20	17.4 ± 4.43
	200	14.0 ± 0.690

[a] 1.8 mg/mL
[b] 41.8 mg/mL
[c] mixture containing high density LP (2.73 mg/mL), low density LP (2.73 mg/mL), and very low density LP (1.36 mg/mL)
AGP α1-acid glycoprotein, GLP, Good Laboratory Practice; HSA, human serum albumin; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LP, lipoproteins

Table 55: Etrasimod Non-Specific Binding

Test Article	Etrasimod (APD334)
Study Number	[redacted]8101 (non-GLP)
Study Type	In vitro
Study System	Human embryonic kidney 293 (HEK293) cells
Species	Human
Method	Etrasimod was mixed with applicable incubation media at 1, 10, and 30 µM concentrations in the presence and absence of HEK293 control cells. The incubation media included bovine serum albumin (BSA) at 0, 1, 2, and 4%. At the end of the incubation period, aliquots of the mixture were collected and analyzed by LC-MS/MS and compared to the dose solution (100% solution).

Report Title: Non-Specific Binding for APD334					**Study Number:** [redacted]8101	
BSA Content (% v/v)	**Etrasimod Concentration (µM)**	**Sample Type**	**Time (min)**	**Average Area Ratio ± SD**	**Percent Unbound**	**Non-Specific Binding (%)**
0	1	Dose solution	0	9.98 ± 0.31	100	NA
	10			226 ± 236	100	NA
	30			136 ± 140	100	NA
	1	Media, no cells	30	2.81 ± 0.17	28.2	71.8
	10			37.5 ± 30.0	16.6	83.4
	30			5.49 (n = 2)	4.0	96.0
	1	Media exposed to HEK293 control cells	30	7.81 ± 0.71	78.3	21.7
	10			11.0 (n = 2)	4.8	95.2
	30			50.4 ± 54.0	37.1	62.9

Report Title: Non-Specific Binding for APD334					**Study Number:** [redacted]8101	
BSA Content (% v/v)	**Etrasimod Concentration (µM)**	**Sample Type**	**Time (min)**	**Average Area Ratio ± SD**	**Percent Unbound**	**Non-Specific Binding (%)**
1	1	Dose solution	0	37.3 ± 1.6	100	NA
	10			345 ± 23	100	NA
	30			1370 ± 359	100	NA
	1	Media, no cells	30	37.7 ± 1.4	101	0
	10			331 ± 32	95.9	4.06
	30			1580 ± 285	115	0
	1	Media exposed to HEK293 control cells	30	31.8 ± 2.8	85.3	14.7
	10			291 (n = 2)	84.4	15.6
	30			1070 ± 106	77.8	22.2
2	1	Dose solution	0	38.2 ± 0.2	100	NA
	10			461 ± 162	100	NA
	30			1820 ± 203	100	NA
	1	Media, no cells	30	38.2 ± 2.3	100	0
	10			349 ± 39.6	75.7	24.3
	30			1760 ± 297	96.3	3.75
	1	Media exposed to HEK293 control cells	30	34.7 ± 3.7	90.8	9.25
	10			345 ± 75	74.8	25.2
	30			1540 ± 223	84.2	15.8

Report Title: Non-Specific Binding for APD334					**Study Number:** [redacted]8101	
BSA Content (% v/v)	**Etrasimod Concentration (µM)**	**Sample Type**	**Time (min)**	**Average Area Ratio ± SD**	**Percent Unbound**	**Non-Specific Binding (%)**
4	1	Dose solution	0	39.9 ± 0.8	100	NA
	10			359 ± 15	100	NA
	30			1990 ± 118	100	NA
	1	Media, no cells	30	37.5 ± 1.8	94.1	5.93
	10			342 ± 37	95.2	4.82
	30			1390 ± 115	69.7	30.3
	1	Media exposed to HEK293 control cells	30	37.3 ± 2.7	93.5	6.52
	10			326 ± 24	90.7	9.28
	30			1540 ± 72	77.3	22.7

Note: Values are the average of triplicate determinations rounded to 3 significant figures. Standard deviation is rounded to the same degree of accuracy. Percentages are rounded to one decimal place except percentages ≥ 100, which are rounded to the nearest whole number.
BSA, bovine serum albumin; GLP, Good Laboratory Practice; HEK293, human embryonic kidney 293; LC-MS/MS, liquid chromatography with tandem mass spectrometry; n, number of replicates; SD, standard deviation

2.6.5.9. PHARMACOKINETICS: METABOLISM IN VIVO

Table 56: Metabolite Profiling in Plasma Following Repeat Administration of Etrasimod in Mouse

Test Article	Etrasimod L-arginine (APD334 L-arginine, AR401959)
Study Number	APD334.TOX.001 (Appendix 12) (GLP)
Species	Mouse/CD-1
Sex/Number of Animals	Male, Female/3 per sex per timepoint (7 timepoints total)
Vehicle/Formulation	Deionized water
Feeding Condition	Fed
Method of Administration	Oral
Dose (mg/kg/day)	20, 200
Sample	Plasma
Analyte	Etrasimod
Assay	LC-MS/MS
Method	Pooled plasma samples Day 90 following daily oral dosing of etrasimod were used for semi-quantification of etrasimod and its metabolites with LC-MS.

Report Title: APD334 L-Arginine: A 91-Day Oral Toxicity Study in Mice			**Study Number:** APD334.TOX.001 (Appendix 12)	
Component Designation	**Relative Metabolite Abundance (%)**			
	20 mg/kg/day		**200 mg/kg/day**	
	Male	**Female**	**Male**	**Female**
Etrasimod	0.0364	ND	6.63	5.62
AR440843 (M1)	ND	ND	0.163	0.150
AR503640 (M2)	ND	ND	8.02	4.06
AR503641 (M3)	ND	ND	2.78	2.75

Report Title: APD334 L-Arginine: A 91-Day Oral Toxicity Study in Mice			**Study Number:** APD334.TOX.001 (Appendix 12)	
Component Designation	**Relative Metabolite Abundance (%)**			
	20 mg/kg/day		**200 mg/kg/day**	
	Male	**Female**	**Male**	**Female**
AR504344 (M6)	0.0160	ND	22.0	18.9
M15	ND	ND	0.274	0.0804
M17	ND	ND	0.118	0.0666
M20	ND	ND	1.05	0.551
M26	0.0910	0.0448	3.84	1.86
M27	0.409	0.446	0.299	0.464
AR426481 (M28)	62.3	63.8	12.4	15.4
M29	1.34	0.981	17.9	15.8
M36	ND	ND	1.82	1.08
M41	ND	ND	2.12	3.74
M42	20.2	17.2	4.03	10.1
M43	0.0704	0.00323	0.794	0.936
M44	0.511	0.171	6.85	8.85
M45	ND	ND	0.0863	0.0928
M68	ND	ND	0.0389	0.0220
M74	ND	ND	0.406	0.271
M75	3.23	2.65	0.683	0.852
M76	ND	ND	0.421	0.509
M77	1.43	1.60	0.312	0.738
M78	0.234	0.0287	2.56	1.67

Report Title: APD334 L-Arginine: A 91-Day Oral Toxicity Study in Mice				**Study Number:** APD334.TOX.001 (Appendix 12)
Component Designation	**Relative Metabolite Abundance (%)**			
	20 mg/kg/day		**200 mg/kg/day**	
	Male	**Female**	**Male**	**Female**
M79	ND	ND	0.591	1.25
M80	ND	ND	0.354	0.640
M81	ND	ND	1.01	0.303
M82	0.868	1.84	0.0358	0.0779
M83	1.67	1.23	0.301	0.446
M84	4.69	5.36	0.299	0.578
M85	0.245	0.539	1.28	1.22
M86	2.70	4.16	0.499	0.821
Total	100	99.8	100	100

Note: Relative metabolite abundance expressed as percent of total peak area.
Samples pooled by sex and timepoint; samples further pooled by group and sex to generate AUC_{0-24} representative pooled samples.
GLP, Good Laboratory Practice; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; ND, not detected

Table 57: Summary of Metabolite Profiling in Plasma, Bile, and Feces Following a Single Administration of [^{14}C]Etrasimod in Rat

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 5) (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Male, Female/3 per sex
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Analyte	Etrasimod and metabolites
Assay	LC-MS/MS
Method	Rats were administered a single dose of [^{14}C]etrasimod. Plasma samples from male and female rats were collected from 0.25 to 48 hours postdose and pooled by sex and timepoint. Bile samples were collected from 0 to 48 hours postdose for male and female rats in Group 3 and were pooled by sex. Feces samples were collected from male and female rats from 0 to 72 hours in Group 2 and from 0 to 48 hours in Group 3 and were pooled by sex and group. All samples were analyzed with LC-MS to detect metabolites.

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats			**Study Number:** APD334.DMPK.002 (Appendix 5)		
Metabolite Designation	**Retention Time (Minutes)**	**Proposed Identification**	**Matrix**		
			Plasma	**Bile**	**Feces**
M38	9.00	Trioxy-APD334-1	-	-	X
M39	12.25	Trioxy-didehydro-APD334-1	-	X	-
M40	13.00-13.25	Dioxy-APD334 sulfate glucuronide	-	X	-
M41	19.25	Trioxy-APD334-2	X	-	-

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats			**Study Number:** APD334.DMPK.002 (Appendix 5)		
Metabolite Designation	**Retention Time (Minutes)**	**Proposed Identification**	**Matrix**		
			Plasma	**Bile**	**Feces**
M42	20.00	Trioxy-didehydro-APD334-2	-	X	-
M43	27.25	Dioxy-APD334-3	-	-	X
M44	28.25	Dioxy-dehydro-APD334	X	X	X
M45	29.00-29.25	Oxy- APD334 glucuronide	-	X	-
M20	30.50	Oxy-didehydro-APD334 acyl glucuronide-1	-	X	-
M21/ M22	31.75	Isomers of M20, Oxy-didehydro-APD334 acyl glucuronide-2/3	-	X	-
M46	34.50-34.75	Oxy-APD334 sulfate glucuronide-1	-	X	-
M2 (AR503640)	35.75	Oxy-APD334-1	-	-	X
M47	35.75-36.00	Oxy-APD334 sulfate glucuronide-2	-	X	-
M51	36.00-36.50	Unknown	-	X	-
M35	38.75	Dioxy-APD334 glucuronide-1	-	X	-
M8	38.86	Dioxy-APD334-1	-	X	-
M48	40.00	Dioxy-APD334 glucuronide-2	-	X	-
M3 (AR503641)	40.25-40.50	Oxy-APD334-2	X	-	X
M6 (AR504344)	43.00	Oxy-didehydro-APD334	X	X	X
M28	49.00-49.25	Dioxy-APD334-2	-	X	X
M36	53.00-53.25	Oxy-APD334 sulfate	X	X	X
M29	62.00	Oxy-APD334 lactone	-	-	X
M1 (AR440843)	62.25	APD334 acyl glucuronide-1	-	X	-
M30 (Isomer B of AR440843)	62.75	APD334 acyl glucuronide-2	-	X	-
M31 (Isomer C of AR440843)	63.25	APD334 acyl glucuronide-3	-	X	-

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats			**Study Number:** APD334.DMPK.002 (Appendix 5)		
Metabolite Designation	**Retention Time (Minutes)**	**Proposed Identification**	**Matrix**		
			Plasma	**Bile**	**Feces**
APD334	67.50	parent compound	X	X	X
M52	74.00-74.50	Unknown	X	-	X
M53	75.00	Unknown	X	-	X
M54	75.50-76.00	Unknown	-	-	X

Note: Retention time ranges are from profiling analyses of all matrices.
'X' indicates that metabolite is found in matrix. '–' indicates that metabolite is not found in matrix.
GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry

Table 58: Metabolite Profiling in Plasma Following a Single Dose of [^{14}C]Etrasimod in Dog

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.001 (Appendix 4) (non-GLP)
Species	Dog/Beagle
Sex/Number of Animals (Group)	Male, Female/3 per sex
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	2
Analyte	Etrasimod and metabolites
Assay	LC-MS/MS
Method	Dogs were administered a single dose of [^{14}C]etrasimod. Plasma samples obtained from male and female dogs from 0.25 to 120 hours postdose were pooled by sex to generate 0.25- to 24-hour and 24- to 120-hour pooled samples. Feces samples obtained from male and female dogs from 0 to 120 hours postdose were pooled by sex to generate 0- to 120-hour pooled samples. Samples were analyzed with LC-MS to detect metabolites.

Report Title: Pharmacokinetics, Absorption, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs			**Study Number:** APD334.DMPK.001 (Appendix 4)	
Metabolite Designation	**Retention Time (Minutes)**	**Metabolite Identification**	**Matrix**	
			Plasma	**Feces**
M55	2.00	Unknown	X	-
M56	2.50	Unknown	X	-
M43	27.25	Dioxy-APD334-3	-	X
M2 (AR503640)	35.75	Oxy-APD334-1	-	X

Report Title: Pharmacokinetics, Absorption, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs			**Study Number:** APD334.DMPK.001 (Appendix 4)	
Metabolite Designation	**Retention Time (Minutes)**	**Metabolite Identification**	**Matrix**	
			Plasma	**Feces**
M3 (AR503641)	40.50	Oxy-APD334-2	-	X
M24 (AR502644)	41.75	Oxy-APD334-3	-	X
M6 (AR504344)	43.00	Oxy-didehydro-APD334	-	X
M26	49.00	Dioxy-dihydro-APD334	X	X
M36	54.75-55.00	Oxy-APD334 sulfate	-	X
M29	62.00	Oxy-APD334 lactone	X	X
M30 (Isomer B of AR440843)	63.00	APD334 acyl glucuronide-2	-	X
M31 (Isomer C of AR440843)	63.50	APD334 acyl glucuronide-3	-	X
M57	64.50-64.75	Unknown	-	X
M58	66.25	Unknown	-	X
APD334	67.75	Parent compound	X	X
M59	73.75-74.00	Unknown	-	X
M52	74.25-74.75	Unknown	X	X
M53	75.00	Unknown	-	X

Note: 'X' indicates that metabolite is found in matrix. '–' indicates that metabolite is not found in matrix.
GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry

2.6.5.10. PHARMACOKINETICS: METABOLISM IN VITRO

Table 59: Etrasimod Liver Microsomal Metabolic Stability Species Comparison

Test Article	Etrasimod (APD334, AR401959)	
Study Number	MPR-[redacted]-003 (non-GLP)	
Study Type	In vitro	
Study System	Mouse, rat, rabbit, dog, monkey, and human liver microsomes	
Method	Pooled liver microsomes were incubated with 1.0 µM etrasimod in the presence of NADPH for up to 60 minutes at 37°C, and etrasimod was detected with LC-MS/MS.	
Report Title	Species Comparison of the Metabolic Stability of APD334 in Mouse, Rat, Rabbit, Dog, Monkey, and Human Liver Microsomes in the Presence of NADPH	
Species	**Sex**	**$t_{1/2}$ (min)**
Mouse	Male	> 60
Rat	Male	> 60
Rabbit	Male	30
Dog	Male	> 60
Monkey	Male	> 60
Human	Mixed	> 60

Note: Pooled liver microsomes were from mouse/CD-1 (male), rat/Sprague Dawley (male), rabbit/New Zealand White (male), dog/beagle (male), monkey/cynomolgus (male), and human (male and female)
GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; $t_{½}$, half-life

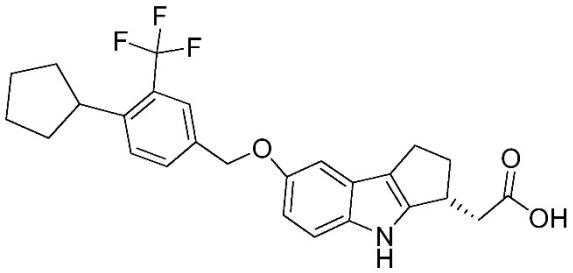
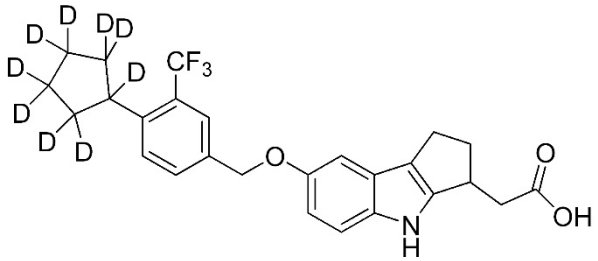
Table 60: Species Comparison of Etrasimod and Metabolites Identified in Liver Microsomes

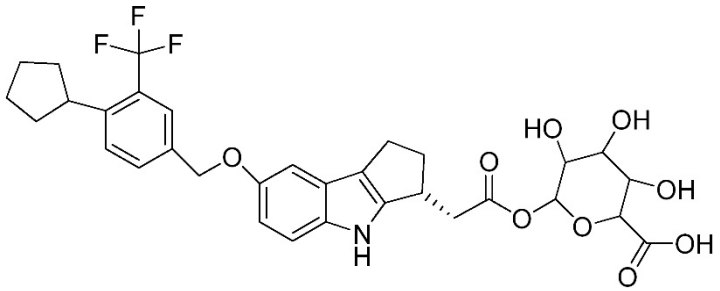
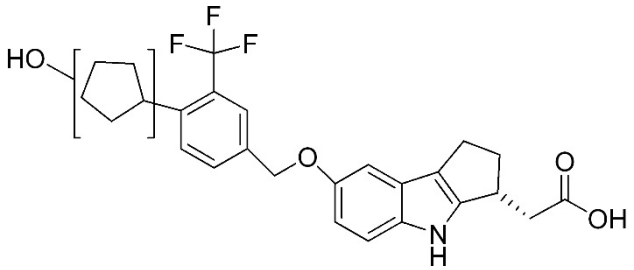
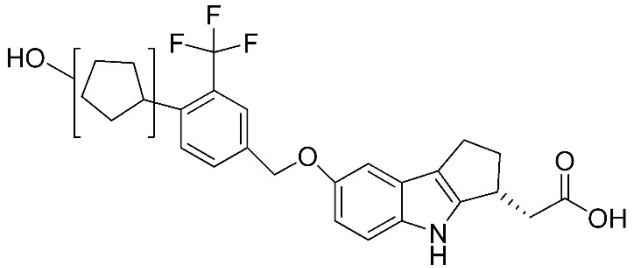
Test Article	Etrasimod (APD334, AR401959)				
Study Number	MPR-[redacted]-005 (non-GLP)				
Study Type	In vitro				
Study System	Mouse, rat, rabbit, dog, monkey, and human liver microsomes				
Method	Pooled liver microsomes were incubated with 100 µM etrasimod or etrasimod deuterium analog and NADPH, UDPGA, or NADPH plus UDPGA, and samples were analyzed by LC-MS.				
Report Title	Identification of the In Vitro Metabolites of APD334 in Liver Microsomes from Mouse, Rat, Rabbit, Dog, Monkey, and Human				
Species	**Metabolite**				
Mouse	M1	M2	M3	ND	M5
Rat	M1	M2	M3	ND	M5
Rabbit	M1	M2	M3	M4	M5
Dog	M1	M2	M3	ND	ND
Monkey	M1	M2	M3	M4	M5
Human	M1	M2	M3	ND	M5

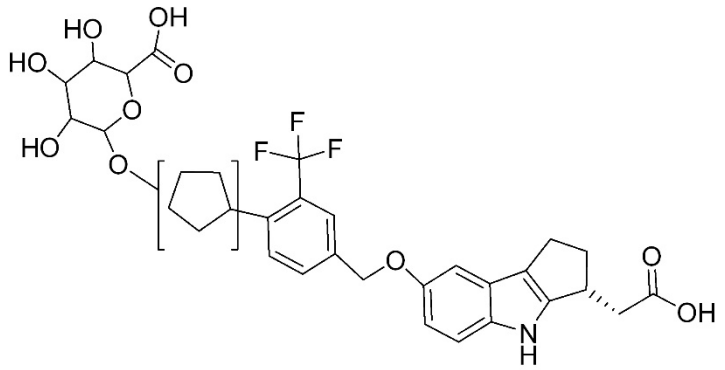
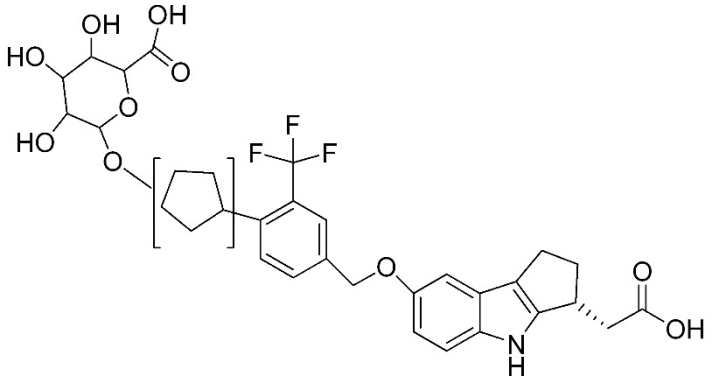
Note: Pooled liver microsomes were from mouse/CD-1 (male), rat/Sprague Dawley (male), rabbit/New Zealand White (female), dog/Beagle (male), monkey/Cynomolgus (male), and human (male and female).
GLP, Good Laboratory Practice; LC-MS, liquid chromatography-mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; ND, not detected; UDPGA, uridine 5'-diphosphoglucuronic acid

Table 61: Molecular Structures of Etrasimod and Metabolites Identified in Liver Microsomes of Various Species

Test Article	Etrasimod (APD334, AR401959)
Study Number	MPR-[redacted]-005 (non-GLP)
Study Type	In vitro
Study System	Mouse, rat, rabbit, dog, monkey, and human liver microsomes
Method	Pooled liver microsomes were incubated with 100 µM etrasimod or etrasimod-deuterium analog and NADPH, UDPGA, or NADPH plus UDPGA, and samples were analyzed by LC-MS.

Report Title: Identification of the In Vitro Metabolites of APD334 in Liver Microsomes from Mouse, Rat, Rabbit, Dog, Monkey, and Human		**Study Number:** MPR-[redacted]-005
Analyte	**Nomenclature**	**Molecular Structure**
Parent	Etrasimod	
Parent-d_9	Etrasimod-deuterium isotope (APD334-d_9)	

Report Title: Identification of the In Vitro Metabolites of APD334 in Liver Microsomes from Mouse, Rat, Rabbit, Dog, Monkey, and Human		**Study Number:** MPR-[redacted]-005
Analyte	**Nomenclature**	**Molecular Structure**
M1	Etrasimod-glucuronide	
M2	Etrasimod–oxidative metabolite, regioisomer or diastereomer	
M3	Etrasimod-oxidative metabolite, regioisomer or diastereomer	

Report Title: Identification of the In Vitro Metabolites of APD334 in Liver Microsomes from Mouse, Rat, Rabbit, Dog, Monkey, and Human		**Study Number:** MPR-[redacted]-005
Analyte	**Nomenclature**	**Molecular Structure**
M4	Etrasimod-oxidative metabolite → glucuronide, regioisomer or diastereomer	
M5	Etrasimod–oxidative metabolite → glucuronide, regioisomer or diastereomer	

GLP, Good Laboratory Practice; LC-MS, liquid chromatography-mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; UDPGA, uridine 5'-diphosphoglucuronic acid

Table 62: Relative Abundance of [^{14}C]Etrasimod and Metabolites Identified in Hepatocytes from Different Species

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	MPR-[redacted]-003 (non-GLP)
Study Type	In vitro
Study System	Rat, dog, monkey, and human hepatocytes
Method	Pooled hepatocytes were incubated with 20 µM etrasimod (a mixture of [^{14}C]-labeled and cold) for 24 hours and analyzed with radiometric detection and LC-MS.

Report Title: In Vitro Identification of ^{14}C-APD334 Metabolites in Hepatocytes of Various Species				**Study Number:** MPR-[redacted]-003
Analyte	**Percentage of Total Radioactivity (%)**			
	Rat	**Dog**	**Monkey**	**Human**
M7	BDL	BDL	2.84	BDL
M8, M9	BDL	BDL	3.43	BDL
M10	1.06	BDL	BDL	BDL
M11	BDL	BDL	2.20	BDL
M12	BDL	BDL	2.28	BDL
M13	BDL	BDL	1.31	BDL
M14	BDL	BDL	6.86	0.85
M15	BDL	BDL	8.53	0.84
M16	1.17	BDL	BDL	BDL
M17	BDL	BDL	5.39	1.76
M18	BDL	BDL	3.67	2.40
M19	1.32	BDL	BDL	0.94
M20	2.42	BDL	1.84	0.31
M21	1.03	BDL	0.97	0.48

Report Title: In Vitro Identification of ^{14}C-APD334 Metabolites in Hepatocytes of Various Species				**Study Number:** MPR-[redacted]-003
Analyte	**Percentage of Total Radioactivity (%)**			
	Rat	**Dog**	**Monkey**	**Human**
M22	0.77	BDL	1.56	0.51
M23	BDL	1.45	BDL	BDL
M2	0.42	0.89	0.45	1.01
M3	0.97	0.82	2.59	4.30
M24	BDL	0.93	BDL	BDL
M6	22.00	1.50	8.93	9.00
M25	BDL	0.93	BDL	BDL
M26, M27	0.87	0.81	2.62	1.66
M28	2.46	1.52	1.41	2.26
M29	0.90	2.63	1.23	3.25
M1	5.38	9.05	2.32	1.91
M30	1.31	2.57	0.95	1.53
M31	0.74	2.79	0.93	3.01
APD334	47.26	66.70	27.81	55.23
Total (%)	90.08	92.59	90.12	91.25

Note: Cryopreserved plateable hepatocytes (pooled) from rat/Sprague Dawley, dog/beagle, monkey/cynomolgus, and human.
BDL, below the detection limit; GLP, Good Laboratory Practice; LC-MS, liquid chromatography-mass spectrometry

Table 63: Summary of [^{14}C]Etrasimod Metabolites Identified in Hepatocytes of Various Species

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	MPR-[redacted]-003 (non-GLP)
Study Type	In vitro
Study System	Rat, dog, monkey, and human hepatocytes
Method	Pooled hepatocytes were incubated with 20 µM etrasimod (a mixture of [^{14}C]-labeled and cold) for 24 hours and analyzed with radiometric detection and LC-MS.
Report Title:	In Vitro Identification of ^{14}C-APD334 Metabolites in Hepatocytes of Various Species
Species	**Metabolite**
Rat	M1, M2, M3, M6, M10, M16, M19, M20, M21, M22, M26, M27, M28, M29, M30, M31
Dog	M1, M2, M3, M6, M23, M24, M25, M26, M27, M28, M29, M30, M31
Monkey	M1, M2, M3, M6, M7, M8, M9, M11, M12, M13, M14, M15, M17, M18, M20, M21, M22, M26, M27, M28, M29, M30, M31
Human	M1, M2, M3, M6, M14, M15, M17, M18, M19, M20, M21, M22, M26, M27, M28, M29, M30, M31

Note: Table was compiled using data reported in Table 62.
GLP, Good Laboratory Practice; LC-MS, liquid chromatography-mass spectrometry

Table 64: Identification of Human CYP Enzymes Involved in the Metabolism of Etrasimod

Test Article	Etrasimod (APD334, AR401959)
Study Number	MPR-[redacted]-002 (non-GLP)
Study Type	In vitro
Study System	HLM and recombinant human CYP enzymes
Method	Recombinant CYP enzymes (50 pmol/mL), human reductase, Sf9 insect control cell and insect cell control were incubated separately with 1.0 µM of etrasimod in 100 mM potassium phosphate buffer. For the incubation of metabolite identification catalyzed by recombinant CYP, the final concentration of etrasimod was 10 µM. Incubations were also performed with HLM (0.5 mg/mL) and 10.0 µM of etrasimod in 100 mM potassium phosphate buffer. Reactions were terminated after 30 minutes. Samples were centrifuged and the supernatant was analyzed with LC-MS/MS.

Report Title: Identification of Human CYP Enzymes Involved in the Metabolism of APD334 by Using Recombinant CYPs	**Study Number:** MPR-[redacted]-002
CYP Enzymes	**Etrasimod (µM) ± SD[a]**
1A1	0.751 ± 0.023
1A2	0.876 ± 0.022
2A6	0.887 ± 0.040
1B1	0.939 ±0.033
2B6	0.914 ± 0.012
2C8	0.760 ± 0.008
2C9*1	0.387 ± 0.016
2C18	0.821 ± 0.100
2C19	0.699 ± 0.062
2D6*1	0.869 ± 0.042
2E1	0.873 ± 0.020
3A4	0.837 ± 0.076
3A5	0.896 ± 0.11

Report Title: Identification of Human CYP Enzymes Involved in the Metabolism of APD334 by Using Recombinant CYPs	**Study Number:** MPR-[redacted]-002
CYP Enzymes	**Etrasimod (µM) ± SD**[a]
3A7	0.836 ± 0.009
4A11	0.915 ±0.047
2J2	0.856 ± 0.116
4F2	0.915 ± 0.109
4F12	0.988 ± 0.020
4F3A	0.911 ± 0.078
4F3B	0.840 ± 0.001
Sf9	0.913 ± 0.054
Insect CCS	0.882 ± 0.072
Reductase+b5	0.974 ± 0.004

[a] Amount of etrasimod that remains in the incubation mixture after 30 minutes of incubation with various CYP enzymes. Incubation was conducted with 1.0 µM of APD334 and quantification was achieved with LC-MS/MS.

Note: Incubation with rCYP2C-family showed the disappearance of etrasimod with maximum disappearance observed with CYP2C9*1 (~60%); CYP2C19 and CYP2C8 also contributed in the metabolism of etrasimod but to a lesser extent compared to CYP2C9*1. Additionally, this study showed that M2 was catalyzed by HLM, CYP2C8, and CYP2C19; M3 was catalyzed by HLM, CYP2C8, CYP2C9*1, and CYP2C19; and M6 was catalyzed by HLM, CYP2C8, and CYP2C9*1.

CCS, controlled cells with CYP expression; CYP, cytochrome P450; GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry; rCYP, recombinant CYP; SD, standard deviation

Table 65: Metabolism of Etrasimod by Recombinant Human CYP Enzymes

Test Article	Etrasimod (APD334, AR401959)
Study Number	[redacted]4054 (non-GLP)
Study Type	In vitro
Study System	HLM and recombinant human CYP enzymes
Method	Pooled HLM were incubated with etrasimod (1 and 10 µM) and recombinant CYP enzymes (50 pmol), and samples were analyzed with LC-MS/MS.

Report Title: In Vitro Cytochrome P450 (CYP) Reaction Phenotyping of APD334 in Human Liver Microsomes and Recombinant Human CYP Enzymes			**Study Number:** [redacted]4054
Test System	**Incubation Time (min)**	**Etrasimod Substrate Remaining (%)**	
		1 µM	**10 µM**
Control bactosomes	0	100	100
	30	104	99.7
	60	102	102
	120	102	102
Reductase bactosomes	0	100	100
	30	105	103
	60	99.3	101
	120	98.9	99.8
CYP1A2	0	100	100
	30	101	102
	60	100	98.0
	120	95.2	97.6

Report Title: In Vitro Cytochrome P450 (CYP) Reaction Phenotyping of APD334 in Human Liver Microsomes and Recombinant Human CYP Enzymes			**Study Number:** [redacted]4054
Test System	**Incubation Time (min)**	**Etrasimod Substrate Remaining (%)**	
		1 µM	**10 µM**
CYP2B6 + b_5	0	100	100
	30	97.3	102
	60	98.7	104
	120	99.5	101
CYP2C8 + b_5	0	100	100
	30	54.7	65.4
	60	35.8	50.7
	120	25.1	38.6
CYP2C9 + b_5	0	100	100
	30	94.9	89.4
	60	91.2	85.1
	120	77.0	76.1
CYP2C19 + b_5	0	100	100
	30	84.3	89.9
	60	79.0	85.0
	120	64.4	76.8
CYP2D6	0	100	100
	30	103	102
	60	100	93.0
	120	98.6	96.6

Report Title: In Vitro Cytochrome P450 (CYP) Reaction Phenotyping of APD334 in Human Liver Microsomes and Recombinant Human CYP Enzymes			**Study Number:** [redacted]4054
Test System	**Incubation Time (min)**	**Etrasimod Substrate Remaining (%)**	
		1 µM	**10 µM**
CYP3A4 + b_5	0	100	100
	30	92.7	101
	60	91.0	91.0
	120	88.5	89.3

Note: Values are the mean of duplicates. No loss indicates that the value was zero or negative.
b_5, expressed with cytochrome b_5; CYP, cytochrome P450; GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry

Table 66: Conversion of Etrasimod to Metabolites by a Panel of Recombinant Human CYP Enzymes

Test Article	Etrasimod (APD334, AR401959)
Study Number	[redacted]4054 (non-GLP)
Study Type	In vitro
Study System	HLM and recombinant human CYP enzymes
Method	Pooled HLM were incubated with etrasimod (1 and 10 µM) and recombinant CYP enzymes (50 pmol), and samples were analyzed with LC-MS/MS.

Report Title: In Vitro Cytochrome P450 (CYP) Reaction Phenotyping of APD334 in Human Liver Microsomes and Recombinant Human CYP Enzymes						**Study Number:** [redacted]4054
Test System	**Incubation Time (min)**	**Metabolite Detected (Area Ratio)**				
		Ketone Metabolite 1		**Oxidative Metabolite 1**		**Oxidative Metabolite 2**	
		1 µM	**10 µM**	**1 µM**	**10 µM**	**1 µM**	**10 µM**
Control bactosomes	0	ND	ND	ND	ND	ND	ND
	30	ND	ND	ND	ND	ND	ND
	60	ND	ND	ND	ND	ND	ND
	120	ND	ND	ND	ND	ND	ND
Reductase bactosomes	0	ND	ND	ND	ND	ND	ND
	30	ND	ND	ND	ND	ND	ND
	60	ND	ND	ND	ND	ND	ND
	120	ND	ND	ND	ND	ND	ND
CYP1A2	0	ND	ND	ND	ND	ND	ND
	30	ND	ND	ND	ND	ND	ND
	60	ND	ND	ND	0.00290	ND	0.00146
	120	ND	ND	ND	0.00348	ND	0.00444

Report Title: In Vitro Cytochrome P450 (CYP) Reaction Phenotyping of APD334 in Human Liver Microsomes and Recombinant Human CYP Enzymes							**Study Number:** [redacted]4054
Test System	**Incubation Time (min)**	**Metabolite Detected (Area Ratio)**					
		Ketone Metabolite 1		**Oxidative Metabolite 1**		**Oxidative Metabolite 2**	
		1 µM	**10 µM**	**1 µM**	**10 µM**	**1 µM**	**10 µM**
CYP2B6 + b_5	0	ND	ND	ND	0.00375	ND	ND
	30	ND	ND	ND	0.00853	ND	0.0201
	60	ND	ND	ND	0.0127	ND	0.0312
	120	ND	ND	ND	0.0160	0.00382	0.0408
CYP2C8 + b_5	0	ND	ND	ND	0.00333	ND	ND
	30	0.0605	0.426	0.140	1.53	0.0860	0.830
	60	0.0822	0.673	0.114	1.40	0.0850	0.933
	120	0.0982	0.816	0.0886	1.25	0.0808	0.986
CYP2C9 + b_5	0	ND	ND	ND	ND	ND	ND
	30	ND	0.00468	0.00685	0.0593	0.0874	0.862
	60	ND	0.0110	0.0118	0.115	0.157	1.62
	120	ND	0.0244	0.0163	0.186	0.252	2.48
CYP2C19 + b_5	0	ND	ND	ND	ND	ND	ND
	30	ND	0.00599	0.123	1.42	0.00948	0.106
	60	ND	0.0121	0.190	2.22	0.0142	0.184
	120	ND	0.0185	0.256	2.81	0.0190	0.230
CYP2D6	0	ND	ND	ND	0.00924	ND	ND
	30	ND	ND	ND	0.0169	ND	0.00822
	60	ND	ND	ND	0.0291	ND	0.0144
	120	ND	ND	0.00390	0.0479	ND	0.0221

Report Title: In Vitro Cytochrome P450 (CYP) Reaction Phenotyping of APD334 in Human Liver Microsomes and Recombinant Human CYP Enzymes							**Study Number:** [redacted]4054
Test System	**Incubation Time (min)**	**Metabolite Detected (Area Ratio)**					
		Ketone Metabolite 1		**Oxidative Metabolite 1**		**Oxidative Metabolite 2**	
		1 µM	**10 µM**	**1 µM**	**10 µM**	**1 µM**	**10 µM**
CYP3A4 + b_5	0	ND	ND	ND	ND	ND	ND
	30	ND	ND	ND	0.0187	ND	0.0114
	60	ND	ND	0.00286	0.0259	0.00114	0.0172
	120	ND	ND	0.00334	0.0321	0.00200	0.0214

Note: Values are the mean of duplicates.
b_5, expressed with cytochrome b_5; CYP, cytochrome P450; GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry; ND, not detected

Table 67: Metabolism of Etrasimod by Human Liver Microsomes and Recombinant CYP Enzymes

Test Article	Etrasimod (APD334, AR401959)						
Study Number	APD334.DMPK.005 (non-GLP)						
Study Type	In vitro						
Study System	HLM and recombinant human CYP enzymes						
Method	Etrasimod (10 µM) was incubated with pooled HLM (0.5 mg protein/mL) and recombinant CYPs (50 pmol/incubation) for 0 or 120 minutes. Etrasimod and oxidative metabolites were identified by LC-MS/MS.						
Report Title	APD334: Identification of Etrasimod Oxidative Metabolites in Human Liver Microsomes and Recombinant CYPs						
Test System	**Incubation Time (min)**	**Protein or CYP Concentration**	**Etrasimod Detected (Area Ratio)**	**Etrasimod Loss (%)**	**Metabolite Formation (Area Ratio)**		
					M2	**M3**	**M6**
Human liver microsomes	0	0.1 mg	21.5	NA	ND	ND	ND
Control bactosomes		0 pmol	25.7				
Reductase bactosomes			21.9				
CYP2C8 + b_5		50 pmol	26.6				
CYP2C9 + b_5			34.4				
CYP2C19+ b_5			24.0				
Human liver microsomes	120	0.1 mg	19.4	9.8	0.0495	0.147	0.0973
Control bactosomes		0 pmol	18.8	27.0	ND	ND	ND
Reductase bactosomes			18.8	14.2			
CYP2C8 + b_5		50 pmol	10.4	60.7	0.0557	0.204	0.894
CYP2C9 + b_5			16.2	53.0	0.0115	0.376	0.0571
CYP2C19+ b_5			11.8	51.0	0.205	0.0535	0.0444

Note: Values are the mean of duplicate determinations.
b_5, expressed with cytochrome b_5; CYP, cytochrome P450; GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; ND, not detected

Table 68: Recombinant CYP Depletion Rate Constants for Etrasimod

Test Article	Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.010 (non-GLP)
Study Type	In vitro
Study System	Recombinant human CYP enzymes
Method	Etrasimod (250 and 1000 nM) was incubated with recombinant CYPs (1 mg/mL) for 0 to 120 minutes. Substrate depletion was measured by LC-MS/MS, and depletion rate constants were determined.

Report Title: Recombinant CYP Phenotyping (RCP) for Etrasimod (APD334) to Determine Fraction Metabolized						**Study Number:** APD334.DMPK.010	
Matrix	**Time (min)**	**Test Article Concentration**	**Average % of Test Article Remaining[a]**	**y-intercept (ln% of Test Article Remaining)[a]**	**$r^{2,a}$**	**-k (min^{-1})[a]**	**Quantified k (min^{-1})[a]**
rCYP2C8	120	250 nM	90.4	NQS	NQS	NQS	0.00133[b]
	60		99.3				
	30		99.4				
	15		99.6				
	3		101				
	1		105				
	0		100				
	120	1000 nM	87.0	4.63	0.557[c]	0.00133	
	60		97.6				
	30		98.6				
	15		96.2				
	3		98.1				
	1		110				
	0		100				

Report Title: Recombinant CYP Phenotyping (RCP) for Etrasimod (APD334) to Determine Fraction Metabolized						**Study Number:** APD334.DMPK.010	
Matrix	**Time (min)**	**Test Article Concentration**	**Average % of Test Article Remaining**[a]	**y-intercept (ln% of Test Article Remaining)**[a]	**r^2**[a]	**-k (min^{-1})**[a]	**Quantified k (min^{-1})**[a]
rCYP2C9	60	250 nM	25.7	4.44	0.958	0.0211	0.0208[d]
	30		38.8				
	15		62.6				
	3		81.1				
	1		87.9				
	0		100				
	60	1000 nM	29.7	4.55	0.959	0.0205	
	30		46.4				
	15		65.0				
	3		91.5				
	1		101				
	0		100				
rCYP2C19	120	250 nM	93.5	NQS	NQS	NQS	0.00121[b]
	60		90.5				
	30		92.1				
	15		97.9				
	3		95.0				
	1		99.3				
	0		100				
	120	1000 nM	84.1	4.56	0.541[c]	0.00121	
	60		87.0				
	30		90.5				
	15		96.0				
	3		93.2				
	1		98.8				
	0		100				

Report Title: Recombinant CYP Phenotyping (RCP) for Etrasimod (APD334) to Determine Fraction Metabolized						**Study Number:** APD334.DMPK.010	
Matrix	**Time (min)**	**Test Article Concentration**	**Average % of Test Article Remaining**[a]	**y-intercept (ln% of Test Article Remaining)**[a]	**r^2**[a]	**-k (min^{-1})**[a]	**Quantified k (min^{-1})**[a]
rCYP2J2	120	250 nM	66.1	4.55	0.863[c]	0.00321	0.00335[d]
	60		75.2				
	30		85.1				
	15		87.4				
	3		94.0				
	1		99.3				
	0		100				
	120	1000 nM	65.8	4.58	0.954	0.00349	
	60		77.4				
	30		85.7				
	15		90.2				
	3		100				
	1		100				
	0		100				

Report Title: Recombinant CYP Phenotyping (RCP) for Etrasimod (APD334) to Determine Fraction Metabolized						**Study Number:** APD334.DMPK.010	
Matrix	**Time (min)**	**Test Article Concentration**	**Average % of Test Article Remaining**[a]	**y-intercept (ln% of Test Article Remaining)**[a]	**r^2**[a]	**-k (min^{-1})**[a]	**Quantified k (min^{-1})**[a]
rCYP3A4	120	250 nM	83.6	4.61	0.758[c]	0.00173	0.00228[e]
	60		89.2				
	30		91.7				
	15		98.7				
	3		104				
	1		102				
	0		100				
	120	1000 nM	76.4	4.57	0.644[c]	0.00228	
	60		81.4				
	30		85.0				
	15		90.7				
	3		102				
	1		101				
	0		100				

[a] The numbers in this table have been rounded, which may have led to slight differences between experiment documentation and report.
[b] 1000 nM slope chosen
[c] $r^2 < 0.95$; signal considered quantified after evaluation
[d] Average of 250 nM and 1000 nM k
[e] 1000 nM/ 250 nM K > 120%; 1000 nM k used for calculations of CL_{int}
CL_{int}, intrinsic clearance; CYP, cytochrome P450; GLP, Good Laboratory Practice; k, depletion rate constant; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NQS, no quantified signal; r^2, coefficients of determination; RCP, recombinant CYP phenotyping

Table 69: Prediction of Etrasimod Intrinsic Clearance and Fraction Metabolized by Recombinant CYPs

Test Article	Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.010 (non-GLP)
Study Type	In vitro
Study System	HLM and recombinant human CYP enzymes
Method	Etrasimod (0.25 and 1.0 µM) was incubated with pooled HLM (0.25 mg/mL) and recombinant CYPs (1 mg/mL) for 0 to 120 minutes, and substrate depletion was measured by LC-MS/MS. Substrate depletion and relative activity factor were used to scale heterologously expressed rCYP activity to HLM activity that predicted the relative contributions of different CYPs to etrasimod metabolism.
Report Title	Recombinant CYP Phenotyping (RCP) for Etrasimod (APD334) to Determine Fraction Metabolized

rCYP	-k (min^{-1})[a]	Inc. Protein Concentration ($mg \cdot mL^{-1}$)	CL_{int}[b] ($\mu L \cdot min^{-1} \cdot mg^{-1}$)[a]	rCYP P450 Content ($pmol \cdot mg^{-1}$)	CL_{int} ($\mu L \cdot min^{-1} \cdot pmol\ CYP^{-1}$)[a]	RAF[c] ($pmol\ CYP \cdot mg^{-1}$)	Scaled[d] $CL_{int,\ HLM}$ ($\mu L \cdot min^{-1} \cdot mg^{-1}$)[a]	$f_{m,CYP}$[a,e]
HLM	0.00146	1.0	1.46	NA	NA	NA	NA	NA
rCYP2C8	0.00133	1.0	1.33	115	0.0116	78.5	0.908	0.3832
rCYP2C9	0.0208	1.0	20.8	244	0.0853	10.4	0.887	0.3744
rCYP2C19	0.00121	1.0	1.21	90.9	0.0133	2.34	0.0311	0.0132
rCYP2J2	0.00335	1.0	3.35	179	0.0187	1.23	0.0230	0.00972
rCYP3A4	0.00228	1.0	2.28	139	0.0164	31.7	0.520	0.2195

[a] The numbers in this table have been rounded, which may have led to slight differences between experimental documentation and report.

[b] CL_{int} determined using $CLint = k(min^{-1}) \cdot 1000\ \mu L/mL \cdot (mg\ microsomal\ protein/mL)^{-1} = \mu L \cdot min^{-1} \cdot mg^{-1}$

[c] RAF which accounts for differences in probe CYP substrate CL_{int} between rCYP and HLM matrices was determined using Equation 2 ($CL_{int(HLM\ CYPx\ probe)(\mu L*min\text{-}1*mg\text{-}1)}/CL_{int(rCYPx\ probe)(\mu L*min\text{-}1*pmol\text{-}1)}=RAF_{(pmol\ rCYPx*mg\text{-}1)}$)

[d] Scaled $CL_{int,\ HLM}$ determined using $Scaled\ HLM\ CLint = CLint_{(\mu L \cdot min^{-1} \cdot mg^{-1})} = rCYPx\ CLint_{(\mu L*min^{-1} \cdot pmol^{-1})} \cdot RAF_{(pmol\ rCYPx \cdot mg^{-1})}$

[e] $f_{m,\ CYP}$ determined using $f_{m,\ CYPx} = Scaled\ HLM\ CL_{int\ CYPx} / \sum HLM\ CL_{int\ All\ CYPs}$

CL_{int}, intrinsic clearance; CYP, cytochrome P450; $f_{m,\ CYP}$, fraction metabolized by a particular CYP; GLP, Good Laboratory Practice; HLM, human liver microsomes; k, depletion rate constant; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; RAF, relative activity factor, which accounts for differences in probe CYP substrate CL_{int}; RCP, recombinant CYP phenotyping; rCYP, recombinant CYP

Table 70: Identification of Enzymes that Catalyze the Interconversion of Etrasimod Metabolites M2, M3, and M6

Test Article	Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.009 (non-GLP)
Study Type	In vitro
Study System	HLM, cytosol, and recombinant CBR1 protein
Method	Metabolites were each incubated separately at 250 nM with HLM (± NADPH for 60 minutes, ± ABT) or with CRB1 (± NADPH for 30 minutes). Metabolites were incubated separately at 1000 nM with HLM (± NADPH for 60 minutes, ± CYP inhibitor ABT with TA) or with cytosol (± NADPH for 60 minutes). Samples were analyzed with UPLC with MS detection.

Report Title: Identification of the Enzyme(s) that Catalyze the Conversion of Etrasimod (APD334) Metabolites M2 to M6, M3 to M6, M6 to M2 and M6 to M3		**Study Number:** APD334.DMPK.009	
M2 Conversion to M3 and M6			
Reaction Conditions	**M2 Concentration (nM)**	**% Conversion**	
		M3	**M6**
Recombinant CBR1 + NADPH	250	NC	NC
Recombinant CBR1 – NADPH	250	NC	NC
HLM + NADPH	250	1.0	16
HLM – NADPH	250	NC	9.4
HLM + NADPH + ABT	250	NC	15
HLM – NADPH + ABT	250	NC	7
Cytosol + NADPH	1000	25.8	20.7
Cytosol – NAPDH	1000	0.2	73.7
HLM + NADPH	1000	3.8	53.6
HLM – NADPH	1000	0.1	24.2
HLM + NADPH + ABT + TA	1000	2.7	38.0
HLM – NADPH + ABT + TA	1000	NC	22.0

Report Title: Identification of the Enzyme(s) that Catalyze the Conversion of Etrasimod (APD334) Metabolites M2 to M6, M3 to M6, M6 to M2 and M6 to M3		**Study Number:** APD334.DMPK.009	
M3 Conversion to M2 and M6			
Reaction Conditions	**M3 Concentration (nM)**	**% Conversion**	
		M2	**M6**
Recombinant CBR1 + NADPH	250	NC	NC
Recombinant CBR1 – NADPH	250	2.4	NC
HLM + NADPH	250	NC	12
HLM – NADPH	250	NC	8.8
HLM + NADPH + ABT	250	NC	6.1
HLM – NADPH + ABT	250	NC	8.0
Cytosol + NADPH	1000	NC	1.3
Cytosol – NAPDH	1000	NC	25.3
HLM + NADPH	1000	NC	8.0
HLM – NADPH	1000	NC	5.1
HLM + NADPH + ABT +TA	1000	NC	4.2
HLM – NADPH + ABT + TA	1000	NC	4.0

Report Title: Identification of the Enzyme(s) that Catalyze the Conversion of Etrasimod (APD334) Metabolites M2 to M6, M3 to M6, M6 to M2 and M6 to M3		**Study Number:** APD334.DMPK.009	
M6 Conversion to M2 and M3			
Reaction Conditions	**M6 Concentration (nM)**	**% Conversion**	
		M2	**M3**
Recombinant CBR1 + NADPH	250	3.4	9.6
Recombinant CBR1 – NADPH	250	NC	NC
HLM + NADPH	250	13	19
HLM – NADPH	250	1.3	4.9
HLM + NADPH + ABT	250	27	29
HLM – NADPH + ABT	250	1.4	5.3
Cytosol + NADPH	1000	26.7	70.0
Cytosol – NAPDH	1000	1.6	1.2
HLM + NADPH	1000	19.7	16.6
HLM – NADPH	1000	1.4	3.7
HLM + NADPH + ABT + TA	1000	36.0	31.3
HLM – NADPH + ABT + TA	1000	1.6	3.6

ABT, 1-aminobenzotriazole; CRB1, carbonyl reductase 1; CYP, cytochrome P450; GLP, Good Laboratory Practice; HLM, human liver microsomes; MS, mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; NC, no conversion observed; TA, tienilic acid; UPLC, ultra performance liquid chromatography

Table 71: Rate of Etrasimod-glucuronide Formation in Liver Microsomes of Various Species

Test Article	Etrasimod (APD334, AR401959)
Study Number	MPR-[redacted]-004 (non-GLP)
Study Type	In vitro
Study System	Mouse, rat, dog, monkey, and human liver microsomes
Method	Pooled liver microsomes were incubated with 1 µM etrasimod in the presence of 3 mM UDPGA. Samples were analyzed with LC-MS/MS.
Report Title	Species Comparison APD334-Glucuronide Formation and Identification of Human UGT Enzymes Involved in the Glucuronidation of APD334

Species	**Rate of Etrasimod-Glucuronide Formation (pmol/mg protein/min)**
Mouse	3.633
Rat	5.367
Dog	4.033
Monkey	5.700
Human	10.00

Note: Liver microsomes were from mouse/CD-1 (male), rat/Sprague Dawley (male), dog/beagle (male), monkey/cynomolgus (male), and human (male and female).
GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; UDPGA, uridine 5'-diphosphoglucuronic acid; UGT, uridine 5'-diphospho-glucuronosyltransferase

Table 72: Formation of Etrasimod-glucuronide Metabolite (M1) by Various Human Recombinant UGT Enzymes

Test Article	Etrasimod (APD334, AR401959)		
Study Number	MPR-[redacted]-004 (non-GLP)		
Study Type	In vitro		
Study System	Recombinant human UGT enzymes		
Method	Recombinant human UGTs were incubated with 1.0 µM etrasimod and 3 mM UDPGA. Parallel control reactions were performed with insect control cells not expressing UGT enzyme. Samples were analyzed with LC-MS/MS.		
Report Title	Species Comparison APD443-glucuronide formation and Identification of Human UGT Enzymes Involved in the Glucuronidation of APD334		
Enzyme	**APD334-Glucuronide (M1)**		
	Concentration (ng/mL)	**Product Formation (pmol product/0.25 mg protein/30 min)**	**Formation Rate (pmol/mg protein/30 min)**
UGT1A1	0.016	25.25	101
UGT1A3	0.010	15.75	63
UGT1A4	0.015	23.67	94.68
UGT1A6	0.011	17.36	69.44
UGT1A7	0.041	64.7	258.8
UGT1A8	0.010	15.75	63
UGT1A9	0.013	20.51	82.04
UGT1A10	0.002	3.16	12.64
UGT2B4	0.002	3.16	12.64
UGT2B7	0.008	12.6	50.4
UGT2B10	0.002	3.16	12.64
UGT2B15	0.002	3.16	12.64
UGT2B17	0.002	3.16	12.64
Insect cells	0.002	3.16	12.64
Buffer	0.002	3.16	12.64

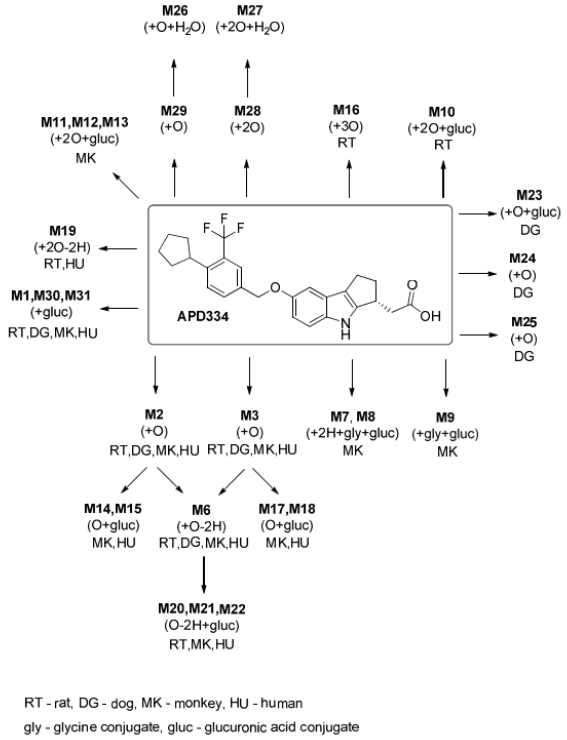
Report Title	Species Comparison APD443-glucuronide formation and Identification of Human UGT Enzymes Involved in the Glucuronidation of APD334		
Enzyme	**APD334-Glucuronide (M1)**		
	Concentration (ng/mL)	**Product Formation (pmol product/0.25 mg protein/30 min)**	**Formation Rate (pmol/mg protein/30 min)**

GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; UDPGA, uridine 5'-diphosphoglucuronic acid; UGT, uridine 5'-diphospho-glucuronosyltransferases

2.6.5.11. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

Table 73: Proposed Etrasimod Metabolic Pathway In Vitro – Hepatocytes of Various Species

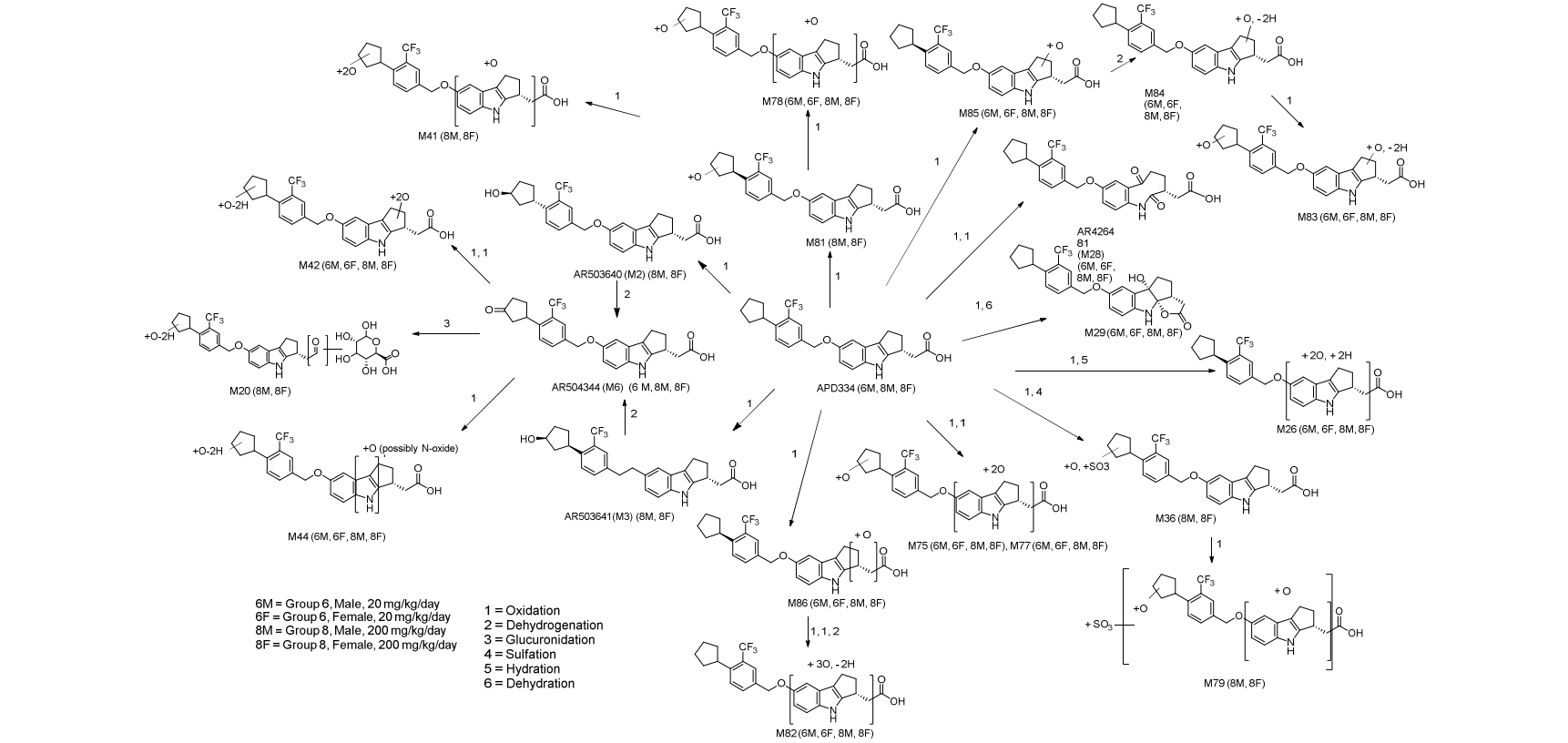
Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	MPR-[redacted]-003 (non-GLP)
Study Type	In vitro
Study System	Rat, dog, monkey, and human hepatocytes
Report Title	In Vitro Identification of ^{14}C-APD334 Metabolites in Hepatocytes of Various Species



GLP, Good Laboratory Practice

Table 74: Proposed Etrasimod Metabolic Pathway In Vivo – Mouse

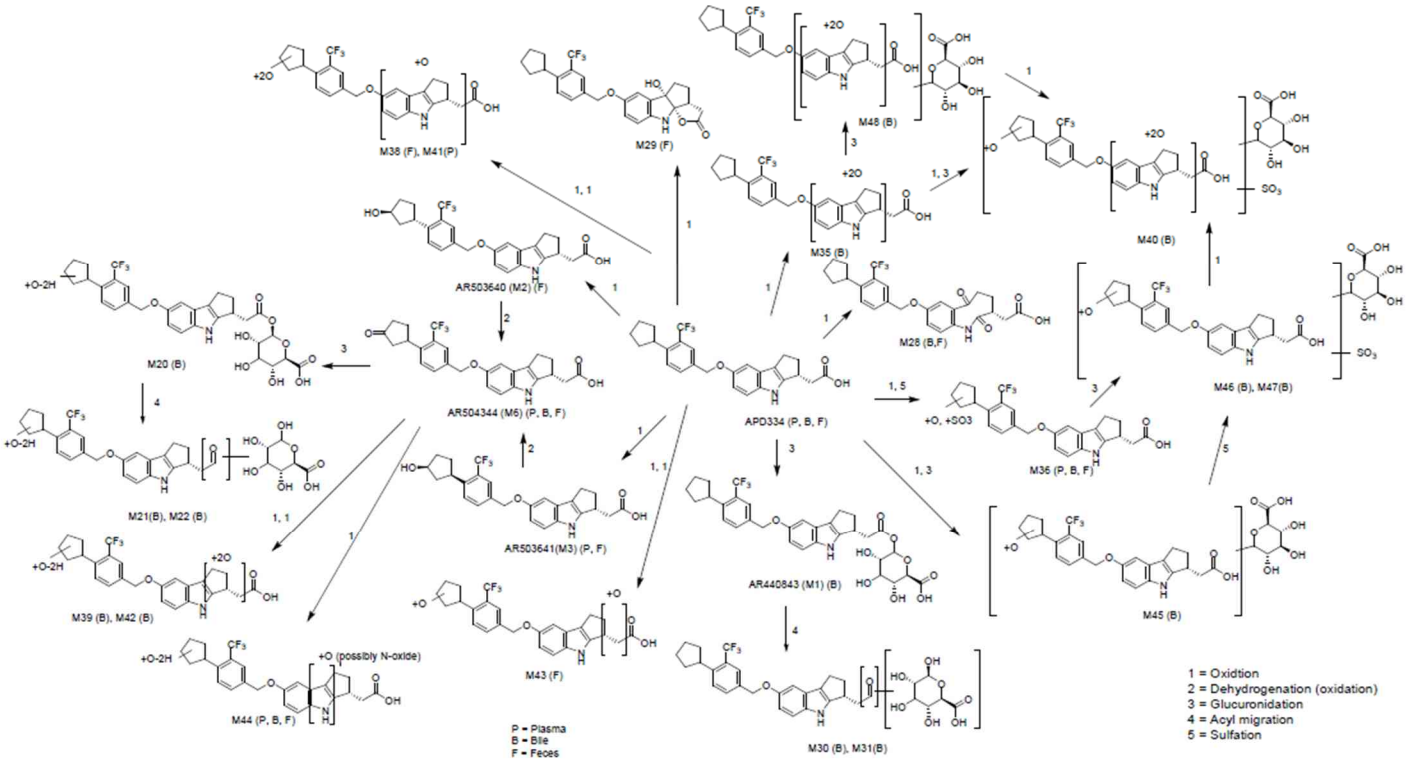
Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.TOX.001 (Appendix 12) (GLP)
Study Type	In vivo
Study System	Mouse plasma
Report Title	APD334 L-arginine: A 91-Day Oral Toxicity Study in Mice



GLP, Good Laboratory Practice

Table 75: Proposed Etrasimod Metabolic Pathway In Vivo – Rat

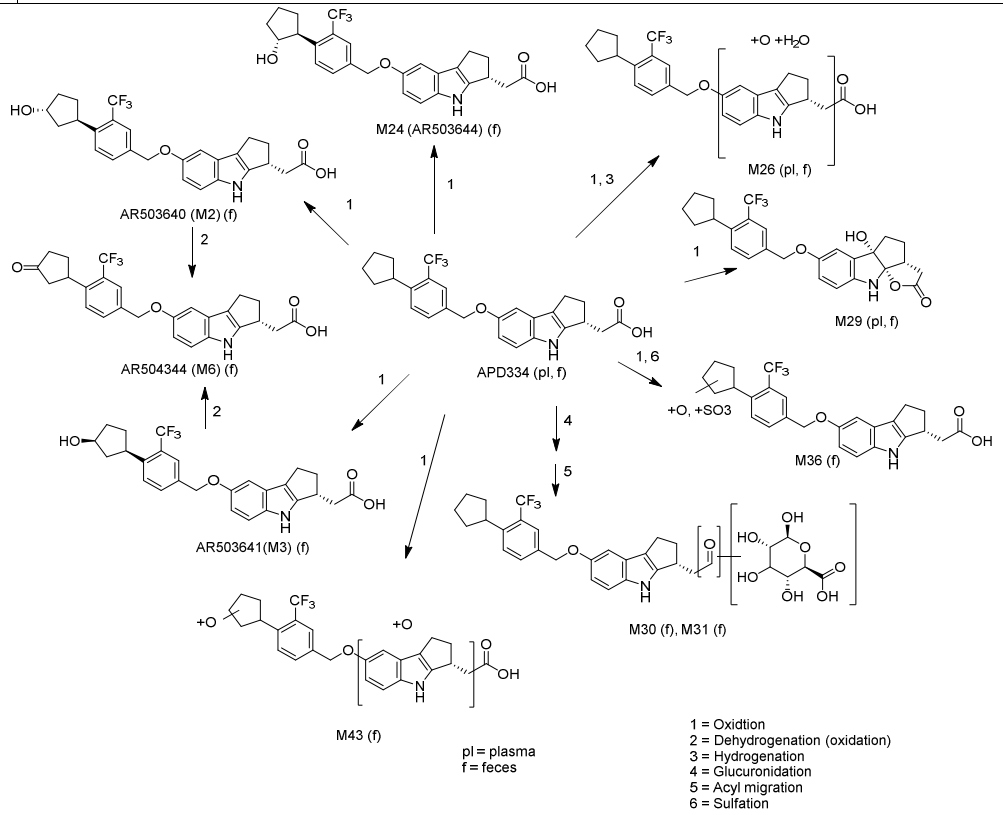
Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (Appendix 5) (non-GLP)
Study Type	In vivo
Study System	Rat plasma, bile, and feces
Report Title	Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats



Note: Pathways are proposed based on general knowledge of metabolism and do not imply definitive pathways. Direct experimentation was not performed.
GLP, Good Laboratory Practice

Table 76: Proposed Etrasimod Metabolic Pathway In Vivo - Dog

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.001 (Appendix 4) (non-GLP)
Study Type	In vivo
Study System	Dog plasma and feces
Report Title	Pharmacokinetics, Absorption, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs



Note: Pathways are proposed based on general knowledge of metabolism and do not imply definitive pathways. Direct experimentation was not performed.
GLP, Good Laboratory Practice

2.6.5.12. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG-METABOLIZING ENZYMES

Table 77: Inhibition of CYP Enzymes in Human Liver Microsomes

Test Article	Etrasimod (APD334, AR401959)	
Study Number	MPR-[redacted]-004 (non-GLP)	
Study Type	In vitro	
Study System	HLM	
Method	Pooled HLM were incubated with reaction buffer, etrasimod (0 to 50 µM), NADPH (1 mM), and CYP substrates, and samples were analyzed by LC-MS/MS.	
Report Title	Inhibition Potential of APD334 on Human Liver Microsomal CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 Catalytic Activities	
CYP Isoform	**Enzyme Activity Inhibited**	**IC_{50}[a] (µM)**
CYP1A2	Phenacetin *O*-deethylase	> 50
CYP2C8	Paclitaxel 6α-hydroxylase	34.7
CYP2C9	Tolbutamide 4′-hydroxylase	> 50
CYP2C19	(*S*)-Mephenytoin 4′-hydroxylase	> 50
CYP2D6	Dextromethorphan *O*-demethylase	> 50
CYP3A4	Midazolam 1′-hydroxylase	> 50

[a] For IC_{50} values greater than 50 µM: Addition of etrasimod up to 50 µM in the microsomal incubation did not produce 50% inhibition of the initial enzyme activity.
CYP, cytochrome P450; GLP, Good Laboratory Practice; HLM, human liver microsomes; IC_{50}, half maximal inhibitory concentration; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate

Table 78: Inhibition of CYP Enzymes in Human Liver Microsomes

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	[redacted]5088 (non-GLP)
Study Type	In vitro
Study System	HLM
Method	Pooled HLM were incubated with etrasimod, reaction buffer, an NADPH-regenerating system, and marker substrate. To evaluate metabolism-dependent inhibition, etrasimod was pre-incubated with the other components before adding the marker substrate. Samples were analyzed with LC-MS/MS.

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes | **Study Number:** [redacted]5088

Enzyme	Zero-min Preincubation				30-min Preincubation				
	Sample Name	Mean Velocity (pmol/min/mg protein)	Mean Percent of Solvent Control (%)	Mean % Inhibition	Sample Name	Mean Velocity (pmol/min/mg protein)	Mean Percent of Solvent Control (%)	Mean % Inhibition	Increase in Inhibition after Preincubation (%)
CYP1A2	No solvent control	348	NA	NA	No solvent control	234	NA	NA	NA
	Solvent control	344	100	NA	Solvent control	283	100	NA	NA
	1 µM etrasimod	343	99.7	0.3	1 µM etrasimod	275	97.3	2.7	2.4
	10 µM etrasimod	350	102	0	10 µM etrasimod	277	97.9	2.1	2.1
	0.1% MeOH control	349	100	NA	0.1% DMSO control	266	100	NA	NA
	0.5 µM α-naphthoflavone	58.1	16.6	83.4	2 µM furafylline	79.9	30.0	70.0	57.5
	0.1% DMSO control	328	100	NA					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes					**Study Number:** [redacted]5088				
Enzyme	**Zero-min Preincubation**				**30-min Preincubation**				
	Sample Name	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Increase in Inhibition after Preincubation (%)**
	2 µM furafylline	287	87.5	12.5					
CYP2B6	No solvent control	63.1	NA	NA	No solvent control	46.5	NA	NA	NA
	Solvent control	56.6	100	NA	Solvent control	40.8	100	NA	NA
	1 µM etrasimod	55.0	97.2	2.8	1 µM etrasimod	39.0	95.6	4.4	1.6
	10 µM etrasimod	47.7	84.2	15.8	10 µM etrasimod	29.2	71.5	28.5	12.7
	0.2% DMSO control	52.2	100	NA	Solvent control	48.0	100	NA	NA
	750 µM orphenadrine	18.5	35.4	64.6	30 µM phencyclidine	23.8	49.6	50.4	43.3
	Solvent control	62.8	100	NA					
	30 µM phencyclidine	58.3	92.8	7.2					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes									**Study Number:** [redacted]5088
Enzyme	**Zero-min Preincubation**				**30-min Preincubation**				
	Sample Name	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Increase in Inhibition after Preincubation (%)**
CYP2C8	No solvent control	837	NA	NA	No solvent control	627	NA	NA	NA
	Solvent control	951	100	NA	Solvent control	795	100	NA	NA
	1 µM etrasimod	740	77.8	22.2	1 µM etrasimod	570	71.7	28.3	6.1
	10 µM etrasimod	0	0	100	10 µM etrasimod	0	0	100	0
	0.1% MeOH control	862	100	NA	0.5% acetonitrile with 0.1% (v/v) formic acid	587	100	NA	NA
	0.05 µM montelukast	155	18.0	82.0	5 µM gemfibrozil glucuronide	242	41.2	58.8	45.5
	0.5% acetonitrile with 0.1% (v/v) formic acid	845	100	NA					
	5 µM gemfibrozil glucuronide	733	86.7	13.3					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes									**Study Number:** [redacted]5088
Enzyme	**Zero-min Preincubation**				**30-min Preincubation**				
	Sample Name	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Increase in Inhibition after Preincubation (%)**
CYP2C9	No solvent control	734	NA	NA	No solvent control	793	NA	NA	NA
	Solvent control	769	100	NA	Solvent control	829	100	NA	NA
	1 µM etrasmiod	766	99.6	0.4	1 µM etrasimod	784	94.6	5.4	5.0
	10 µM etrasimod	657	85.4	14.6	10 µM etrasimod	686	82.8	17.2	2.6
	0.1% MeOH control	713	100	NA	0.002 mg/mL tris base	753	100	NA	NA
	2 µM sulfaphenazole	213	29.9	70.1	0.25 µM tienilic acid	184	24.4	75.6	63.1
	0.002 mg/mL tris base	715	100	NA					
	0.25 µM tienilic acid	626	87.6	12.5					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes									**Study Number:** [redacted]5088
Enzyme	**Zero-min Preincubation**				**30-min Preincubation**				
	Sample Name	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Increase in Inhibition after Preincubation (%)**
CYP2C19	No solvent control	26.1	NA	NA	No solvent control	27.5	NA	NA	NA
	Solvent control	21.9	100	NA	Solvent control	22.7	100	NA	NA
	1 µM etrasimod	22.1	101	0	1 µM etrasimod	23.4	103	0	0
	10 µM etrasimod	19.0	86.9	13.1	10 µM etrasimod	19.3	85.0	15.0	1.9
	0.1% DMSO control	23.0	100	NA	0.5% 40:60 (v/v) MeOH/0.1 M tris buffer pH 9.0	27.9	100	NA	NA
	400 µM modafinil	5.96	25.9	74.1	10 µM esomeprazole	5.45	19.5	80.5	42.6
	0.5% 40:60 (v/v) MeOH/0.1 M tris buffer pH 9.0	26.9	100	NA					
	10 µM esomeprazole	16.7	62.1	37.9					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes									**Study Number:** [redacted]5088
Enzyme	**Zero-min Preincubation**				**30-min Preincubation**				
	Sample Name	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Increase in Inhibition after Preincubation (%)**
CYP2D6	No solvent control	98.4	NA	NA	No solvent control	51.8	NA	NA	NA
	Solvent control	105	100	NA	Solvent control	108	100	NA	NA
	1 µM etrasimod	107	102	0	1 µM etrasimod	109	101	0	0
	10 µM etrasimod	102	97.6	2.4	10 µM etrasimod	99.9	92.6	7.4	5.0
	Solvent control	93.1	100	NA	Solvent control	50.7	100	NA	NA
	5 µM quinidine	7.20	7.7	92.3	1 µM paroxetine	7.89	15.6	84.4	45.0
	Solvent control	94.9	100	NA					
	1 µM paroxetine	57.5	60.6	39.4					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes									**Study Number:** [redacted]5088
Enzyme	**Zero-min Preincubation**				**30-min Preincubation**				
	Sample Name	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Increase in Inhibition after Preincubation (%)**
CYP3A4/5 (midazolam)	No solvent control	716	NA	NA	No solvent control	772	NA	NA	NA
	Solvent control	600	100	NA	Solvent control	650	100	NA	NA
	1 µM etrasimod	586	97.6	2.4	1 µM etrasimod	654	101	0	0
	10 µM etrasimod	537	89.4	10.6	10 µM etrasimod	495	76.2	23.8	13.2
	0.1% MeOH control	699	100	NA	0.1% acetonitrile	722	100	NA	NA
	0.075 µM ketaconazole	99.9	14.3	85.7	7.5 µM troleandomycin	144	19.9	80.1	25.4
	0.1% acetonitrile	657	100	NA					
	7.5 µM troleandomycin	298	45.4	54.6					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes									**Study Number:** [redacted]5088
Enzyme	**Zero-min Preincubation**				**30-min Preincubation**				
	Sample Name	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**	**Increase in Inhibition after Preincubation (%)**
CYP3A4/5 (testosterone)	No solvent control	1440	NA	NA	No solvent control	1380	NA	NA	NA
	Solvent control	1250	100	NA	Solvent control	1020	100	NA	NA
	1 µM etrasimod	1050	84.1	15.9	1 µM etrasimod	1070	105	0	0
	10 µM etrasimod	1030	83.0	17.0	10 µM etrasimod	842	82.4	17.6	0.6
	0.1% MeOH control	1440	100	NA	0.1% acetonitrile	1340	100	NA	NA
	0.075 µM ketaconazole	193	13.4	86.6	7.5 µM troleandomycin	235	17.5	82.5	66.1
	0.1% acetonitrile	1470	100	NA					
	7.5 µM troleandomycin	1230	83.7	16.3					

Note: Percentages < 100% are rounded to 1 decimal point. Percentages ≥ 100% are rounded to the nearest whole number. Negative values are reported as zero.
CYP, cytochrome P450; DMSO, dimethyl sulfoxide; GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MeOH, methanol; NA, not applicable; NADPH, nicotinamide adenine dinucleotide phosphate; UDP, uridine diphosphate; UGT, UDP-glucuronosyltransferase

Table 79: Inhibition of CYP2C8 in Human Liver Microsomes

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	APD334.DMPK.011 (non-GLP)
Study Type	In vitro
Study System	HLM
Method	Etrasimod was incubated with probe substrate (amodiaquine) and HLM in buffer mixture. Reactions were initiated by cofactor (NADPH-regenerating system) and terminated by the addition of the appropriate stop reagent. Samples were centrifuged and the supernatant fractions were analyzed by LC-MS/MS.

Report Title: APD334: Cytochrome P450 (CYP2C8) Inhibition in Human Liver Microsomes					**Study Number:** APD334.DMPK.011		
Enzyme	**Direct inhibition (0-min preincubation)**		**Time-dependent inhibition (30-min preincubation without NADPH)**		**Metabolism-dependent inhibition (30-min preincubation with NADPH)**		**Inhibition potential conclusion**
	IC_{50} (µM) ± SE	**Maximum Inhibition (%)**	**IC_{50} (µM) ± SE**	**Maximum Inhibition (%)**	**IC_{50} (µM) ± SE**	**Maximum Inhibition (%)**	
CYP2C8	4.0 ± 0.3	100	4.4 ± 0.3	100	4.6 ± 0.4	100	Direct

CYP, cytochrome P450; GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry; IC_{50}, half maximal inhibitory concentration; NADPH, nicotinamide adenine dinucleotide phosphate; SE, standard error

Table 80: Inhibition of UGT Enzymes in Human Liver Microsomes

Test Article	Etrasimod L-arginine (APD334 L-arginine)
Study Number	[redacted]5088 (non-GLP)
Study Type	In Vitro
Study System	HLM
Method	Pooled HLM were incubated with etrasimod, reaction buffer, and marker substrate. Samples were analyzed with LC-MS/MS.

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes				**Study Number:** [redacted]5088
Enzyme	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**
UGT1A1	No solvent control	580	NA	NA
	Solvent control	683	100	NA
	1 µM etrasimod	604	88.5	11.5
	10 µM etrasimod	492	72.0	28.0
	0.5% DMSO control	692	100	NA
	12 µM troglitazone	116	16.8	83.2
UGT1A3	No solvent control	48.0	NA	NA
	Solvent control	52.4	100	NA
	1 µM etrasimod	50.7	96.8	3.2
	10 µM etrasimod	53.5	102	0
	0.5% DMSO control	53.7	100	NA
	100 µM troglitazone	10.4	19.4	80.6

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes				**Study Number:** [redacted]5088
Enzyme	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**
UGT1A4	No solvent control	NR	NA	NA
	Solvent control	514	100	NA
	1 µM etrasimod	515	100	0
	10 µM etrasimod	548	107	0
	0.5% DMSO control	471	100	NA
	3 µM troglitazone	111	23.6	76.4
UGT1A6	No solvent control	11200	NA	NA
	Solvent control	9470	100	NA
	1 µM etrasimod	8990	94.9	5.1
	10 µM etrasimod	4900	51.7	48.3
	0.5% DMSO control	8450	100	NA
	20 µM troglitazone	3600	42.6	57.4
UGT1A9	No solvent control	462	NA	NA
	Solvent control	604	100	NA
	1 µM etrasimod	560	92.7	7.3
	10 µM etrasimod	558	92.4	7.6
	0.5% DMSO control	520	100	NA
	20 µM troglitazone	62.6	12.0	88.0

Report Title: In Vitro Evaluation of APD334 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes				**Study Number:** [redacted]5088
Enzyme	**Sample Name**	**Mean Velocity (pmol/min/mg protein)**	**Mean Percent of Solvent Control (%)**	**Mean % Inhibition**
UGT2B7	No solvent control	875	NA	NA
	Solvent control	906	100	NA
	1 µM etrasimod	971	107	0
	10 µM etrasimod	933	103	0
	0.5% DMSO control	832	100	NA
	100 µM troglitazone	152	18.3	81.7
UGT2B17	No solvent control	277	NA	NA
	Solvent control	279	100	NA
	1 µM etrasimod	293	105	0
	10 µM etrasimod	266	95.4	4.6
	0.5% DMSO control	272	100	NA
	50 µM troglitazone	98.9	36.4	63.6

Note: Percentages < 100% are rounded to 1 decimal point. Percentages ≥ 100% are rounded to the nearest whole number.
CYP, cytochrome P450; DMSO, dimethylsulfoxide; GLP, Good Laboratory Practice; HLM, human liver microsomes; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NA, not applicable; NR, not reported; UDP, uridine diphosphate; UGT, UDP-glucuronosyltransferase

Table 81: CYP mRNA Induction in Rat Hepatocytes

Test Article	Etrasimod (APD334, AR401959)
Study Number	[redacted]3079 (non-GLP)
Study Type	In vitro
Study System	Rat hepatocytes
Method	Hepatocytes were incubated with etrasimod (3, 30, or 150 µM) or known CYP inducers (positive controls) once daily for 3 consecutive days. approximately 24 hours following the final treatment, RNA was isolated and used in quantitative RT-PCR to measure CYP induction.

Report Title: In Vitro Evaluation of APD334 as an Inducer of Cytochrome P450 Expression in Cultured Human and Rat Hepatocytes						**Study Number:** [redacted]3079
Enzyme	**mRNA Fold Change**					
CYP1A1	**Sex**	**Male**			**Female**		
	Sample ID	**1610291**	**1610409**	**1710038**	**1510028**	**1510131**	**1710044**
	1% MeOH	1.00	1.00	1.00	1.00	1.00	1.00
	3 µM etrasimod	1.23	0.918	0.698	1.48	1.53	1.03
	30 µM etrasimod	1.07	1.71	1.10	2.18	2.78	1.52
	150 µM etrasimod	ND	ND	ND	ND	ND	ND
	25 µM flumazenil	0.0370	0.594	0.0920	4.36	4.01	0.677
	33 µM beta-naphthoflavone	32.8	70.2	41.4	65.1	66.2	63.3
CYP1A2	**Sex**	**Male**			**Female**		
	Sample ID	**1610291**	**1610409**	**1710038**	**1510028**	**1510131**	**1710044**
	1% MeOH	1.00	1.00	1.00	1.00	1.00	1.00
	3 µM etrasimod	1.19	1.62	1.40	1.63	1.23	1.41
	30 µM etrasimod	2.36	3.23	2.18	1.92	1.79	2.82
	150 µM etrasimod	ND	ND	ND	ND	ND	ND
	25 µM flumazenil	0.911	0.839	0.406	1.54	1.94	0.862
	33 µM beta-naphthoflavone	6.11	40.9	49.3	131	151	63.7

Report Title: In Vitro Evaluation of APD334 as an Inducer of Cytochrome P450 Expression in Cultured Human and Rat Hepatocytes						**Study Number:** [redacted]3079
Enzyme	**mRNA Fold Change**					
CYP2B1	**Sex**	**Male**			**Female**	
	Sample ID	**1610291**	**1610409**	**1710038**	**1510028**	**1510131**	**1710044**
	1% MeOH	1.00	1.00	1.00	1.00	1.00	1.00
	3 µM etrasimod	7.28	35.1	62.1	7.48	12.0	2.22
	30 µM etrasimod	14.4	244	243	11.7	18.6	21.2
	150 µM etrasimod	ND	ND	ND	ND	ND	ND
	25 µM flumazenil	0.991	1.91	0.588	1.67	3.49	0.416
	750 µM phenobarbital	1.92	259	1250	70.2	130	48.9
	10 µM dexamethasone	ND	3.01	19.2	2.47	3.47	12.8
CYP2C11	**Sex**	**Male**			**Female**	
	Sample ID	**1610291**	**1610409**	**1710038**	**1510028**	**1510131**	**1710044**
	1% MeOH	1.00	1.00	1.00	1.00	1.00	1.00
	3 µM etrasimod	1.22	2.47	2.56	1.32	2.26	1.08
	30 µM etrasimod	3.87	4.26	3.28	0.804	1.25	2.41
	150 µM etrasimod	ND	ND	ND	ND	ND	ND
	25 µM flumazenil	1.16	0.977	0.627	1.03	1.70	0.732
	750 µM phenobarbital	1.80	3.08	6.83	1.94	3.41	2.24
	10 µM dexamethasone	4.77	3.79	6.66	2.30	3.53	6.90
CYP3A1	**Sex**	**Male**			**Female**	
	Sample ID	**1610291**	**1610409**	**1710038**	**1510028**	**1510131**	**1710044**
	1% MeOH	1.00	1.00	1.00	1.00	1.00	1.00
	3 µM etrasimod	6.76	4.69	4.50	2.07	3.49	1.42
	30 µM etrasimod	11.4	9.28	5.42	1.82	2.63	3.21
	150 µM etrasimod	ND	ND	ND	ND	ND	ND
	25 µM flumazenil	1.31	1.19	0.494	1.16	2.14	1.23
	750 µM phenobarbital	22.1	54.5	92.3	17.6	46.1	44.6

Report Title: In Vitro Evaluation of APD334 as an Inducer of Cytochrome P450 Expression in Cultured Human and Rat Hepatocytes						**Study Number:** [redacted]3079	
Enzyme	**mRNA Fold Change**						
	10 µM dexamethasone	1240	942	1170	92.8	156	1040
CYP3A2[a]	**Sex**	**Male**			**Female**		
	Sample ID	**1610291**	**1610409**	**1710038**	**1510028**	**1510131**	**1710044**
	1% MeOH	1.00	1.00	1.00	-	-	-
	3 µM etrasimod	1.04	1.25	1.09	-	-	-
	30 µM etrasimod	1.27	1.57	1.17	-	-	-
	150 µM etrasimod	ND	ND	ND	-	-	-
	25 µM flumazenil	0.909	0.986	0.735	-	-	-
	750 µM phenobarbital	1.13	2.22	3.71	-	-	-
	10 µM dexamethasone	1.16	3.44	4.09	-	-	-

[a] CYP3A2 mRNA induction was not measured in female rat hepatocytes.

Note: Fold change values are relative to vehicle control and normalized to β-actin. Six separate preparations of cryopreserved rat hepatocytes (n = 3/sex) were used. The mean of quadruplicate determinations is presented. β-naphthoflavone, phenobarbital, and dexamethasone are known CYP inducers and were used as positive controls.

'-', not measured; CYP, cytochrome P450; GLP, Good Laboratory Practice; ID, identification; MeOH, methanol; mRNA, messenger ribonucleic acid; ND, not determined; RT-PCR, reverse transcription-polymerase chain reaction

Table 82: CYP mRNA Induction in Human Hepatocytes

Test Article	Etrasimod (APD334, AR401959)			
Study Number	[redacted]3079 (non-GLP)			
Study Type	In vitro			
Study System	Human hepatocytes			
Method	Hepatocytes were incubated with etrasimod (1, or 10 µM) or known CYP inducers (positive controls) once daily for 3 consecutive days. approximately 24 hours following the final treatment, RNA was isolated and used in quantitative RT-PCR to measure CYP induction.			
Report Title	In Vitro Evaluation of APD334 as an Inducer of Cytochrome P450 Expression in Cultured Human and Rat Hepatocytes			
Enzyme	**mRNA Fold Change**			
CYP1A2	**Sample ID**	**HC10-23**	**HC5-24**	**HC7-12**
	1% MeOH	1.00	1.00	1.00
	1 µM etrasimod	1.03	0.916	1.68
	10 µM etrasimod	1.18	1.85	1.19
	25 µM flumazenil	1.41	0.821	1.08
	50 µM omeprazole	115	41.4	91.1
CYP2B6	**Sample ID**	**HC10-23**	**HC5-24**	**HC7-12**
	1% MeOH	1.00	1.00	1.00
	1 µM etrasimod	1.38	2.23	1.62
	10 µM etrasimod	1.44	3.73	1.24
	25 µM flumazenil	1.01	0.941	1.94
	750 µM phenobarbital	14.2	26.0	15.1
CYP3A4	**Sample ID**	**HC10-23**	**HC5-24**	**HC7-12**
	1% MeOH	1.00	1.00	1.00
	1 µM etrasmiod	1.10	3.44	1.36
	10 µM etrasimod	1.91	8.94	1.46
	25 µM flumazenil	1.25	1.05	1.34
	20 µM rifampin	115	81.8	56.8

Note: Fold change values are relative to vehicle control and normalized to GAPDH. Three separate preparations of cryopreserved human hepatocytes (HC10-23, HC5-24, and HC7-12) were used. The mean of quadruplicate determinations is presented. Omeprazole, phenobarbital, and rifampin are known CYP inducers and were used as positive controls.
CYP, cytochrome P450; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLP, Good Laboratory Practice; ID, identification; MeOH, methanol; mRNA, messenger ribonucleic acid; RT-PCR, reverse transcription-polymerase chain reaction

2.6.5.13. PHARMACOKINETICS: EXCRETION

Table 83: Percent of Radioactive Dose in Urine and Feces Following a Single Oral Administration of [^{14}C]Etrasimod in Rat

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Male, Female/3 per sex (Group 2)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Analyte	Radioactivity
Assay	Radioactivity
Method	Rats were administered a single dose of [^{14}C]etrasimod. Feces and urine samples were collected from male and female rats from 0 to 168 hours postdose in Group 2. All samples were analyzed with LSC to measure radioactivity.

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002				
Time (h)	**Percent of Radioactive Dose**				**Time (h)**	**Percent of Radioactive Dose**			
	Feces					**Urine**			
	Male		**Female**			**Male**		**Female**	
	Mean	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**
0-24	67.2	0.683	63.2	6.89	0-8	0.301	0.0442	0.205	0.0694
24-48	20.1	1.85	25.2	4.40	8-24	0.326	0.0638	0.177	0.0241

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats					**Study Number:** APD334.DMPK.002				
Time (h)	**Percent of Radioactive Dose**				**Time (h)**	**Percent of Radioactive Dose**			
	Feces					**Urine**			
	Male		**Female**			**Male**		**Female**	
	Mean	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**
48-72	5.23	1.71	4.81	1.57	24-48	0.0997	0.0157	0.0291	0.00486
72-96	1.92	0.403	1.11	0.228	48-72	0.0315	0.00667	0.00617	0.00558
96-120	0.825	0.0829	0.624	0.149	72-96	0.0151	0.00257	0.00332	0.00288
120-144	0.489	0.0180	0.393	0.117	96-120	0.00775	0.000851	0.00118	0.00204
144-168	0.312	0.0211	0.286	0.0855	120-144	0.00496	0.000787	0.00	0.00
Subtotal	96.1	1.05	95.6	1.25	144-168	0.00121	0.00210	0.00	0.00
					Subtotal	0.787	0.0809	0.421	0.0802

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counting; SD, standard deviation

Table 84: Percent of Radioactive Dose in Urine and Feces Following a Single Oral Administration of [^{14}C]Etrasimod in Dog

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.001 (non-GLP)
Species	Dog/Beagle
Sex/Number of Animals (Group)	Male, Female/3 per sex
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	2
Analyte	Radioactivity
Assay	Radioactivity
Method	Dogs were administered a single dose of [^{14}C]etrasimod. Feces and urine samples were obtained from male and female dogs from 0 to 168 hours postdose. Samples were analyzed with LSC to detect radioactivity.

Report Title: Pharmacokinetics, Absorption, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs						**Study Number:** APD334.DMPK.001			
Time (h)	**Percent of Radioactive Dose**				**Time (h)**	**Percent of Radioactive Dose**			
	Feces					**Urine**			
	Male		**Female**			**Male**		**Female**	
	Mean	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**
0-24	9.11	9.36	0.00	0.00	0-4	0.000496	0.000859	0.0729	0.0889
24-48	36.7	1.88	40.8	2.50	4-8	0.312	0.442	0.0640	0.111
48-72	15.4	3.56	18.3	2.06	8-24	0.327	0.193	0.288	0.185

Report Title: Pharmacokinetics, Absorption, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Dogs					**Study Number:** APD334.DMPK.001				
Time (h)	**Percent of Radioactive Dose**				**Time (h)**	**Percent of Radioactive Dose**			
	Feces					**Urine**			
	Male		**Female**			**Male**		**Female**	
	Mean	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**
72-96	9.28	3.54	9.69	1.31	24-48	0.233	0.0451	0.172	0.0146
96-120	5.78	1.77	6.34	0.887	48-72	0.134	0.0275	0.105	0.00952
120-144	3.53	2.60	3.53	0.190	72-96	0.131	0.0452	0.0680	0.00687
144-168	2.55	0.612	2.33	0.261	96-120	0.0814	0.0262	0.0519	0.00608
Subtotal	82.3	2.85	81.0	1.51	120-144	0.0486	0.0197	0.0284	0.00396
					144-168	0.0336	0.00811	0.0224	0.00238
					Subtotal	1.30	0.298	0.872	0.0861

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counting; SD, standard deviation

Table 85: Cumulative Percent of Radioactive Dose in Bile Following a Single Oral Administration of [^{14}C]Etrasimod in Rat

Test Article	[^{14}C]Etrasimod (APD334, AR401959)
Study Number	APD334.DMPK.002 (non-GLP)
Species	Rat/Sprague Dawley
Sex/Number of Animals (Group)	Male, Female/3 per sex (Group 3)
Vehicle/Formulation	5% HPBCD in deionized water
Feeding Condition	Fasted
Method of Administration	Oral
Radionuclide	^{14}C
Specific Activity	26.0 mCi/mmol
Dose (mg/kg)	10
Analyte	Radioactivity
Assay	Radioactivity
Method	Rats were administered a single dose of [^{14}C]etrasimod. Bile samples were collected from 0 to 120 hours postdose. Samples were analyzed with LSC to measure radioactivity.

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats			**Study Number:** APD334.DMPK.002	
Time (h)	**Cumulative Percent of Radioactive Dose in Bile**			
	Male		**Female**	
	Mean	**SD**	**Mean**	**SD**
0-2	1.44	1.38	1.43	0.481
0-4	9.48	4.94	7.36	1.30
0-8	35.2	1.47	26.7	0.659
0-24	71.5	2.53	58.5	3.20

Report Title: Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]APD334 Following Oral Administration to Rats			**Study Number:** APD334.DMPK.002	
Time (h)	**Cumulative Percent of Radioactive Dose in Bile**			
	Male		**Female**	
	Mean	**SD**	**Mean**	**SD**
0-48	80.6	3.08	64.8	3.86
0-72	82.0	3.20	65.9	4.00
0-96	82.6	3.26	66.4	4.13
0-120	83.0	3.31	66.7	4.16

GLP, Good Laboratory Practice; HPBCD, hydroxypropyl-β-cyclodextrin; LSC, liquid scintillation counter; SD, standard deviation

2.6.5.14. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS

Table 86: Inhibition of P-gp and BCRP in Caco-2 and MDCKII Cells

Test Article	Etrasimod (APD334, AR401959)
Study Number	[redacted]8106 (non-GLP)
Study Type	In vitro
Study System	Caco-2 and MDCKII cells
Method	Caco-2 or MDCKII cells were incubated with probe substrates in the absence or presence of etrasimod or a known inhibitor. Probe substrate was measured with LC-MS/MS.

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters								**Study Number:** [redacted]8106
Transporter	**Test System**	**Substrate**	**Inhibitor (µM)**	**P_{app} ($\times 10^{-6}$ cm/s) (Mean ± SD)**		**Efflux Ratio**	**Percent of Control**	**IC_{50}**
				Apical to Basal	**Basal to Apical**			
P-gp	Caco-2	Digoxin (10 µM)	Solvent Control (0)	0.670 ± 0.283	30.7 ± 0.9	45.8	100	~100 µM
			Etrasimod (10)	0.602 ± 0.221	29.5 ± 0.9	49.0	107	
			Etrasimod (100)	1.01 ± 0.76	24.8 ± 0.6	24.6	52.7	
			Valspodar (1)	9.33 ± 1.68	12.6 ± 0.4	1.35	0.8	
BCRP[a]	MDCKII	Prazosin (1 µM)	Solvent Control (0)	14.4 ± 2.0	91.7 ± 13.8	6.38	100	ND
			Etrasimod (10)	26.9 ± 3.0	71.9 ± 4.9	2.67	31.1	
			Etrasimod (100)	24.4 ± 11.5	4.31 (n = 2)	0.177	0.0	
			Ko143 (1)	46.4 ± 2.8	40.9 ± 3.4	0.882	0.0	

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters								**Study Number:** [redacted]8106
Transporter	**Test System**	**Substrate**	**Inhibitor (µM)**	**P_{app} ($\times 10^{-6}$ cm/s) (Mean ± SD)**		**Efflux Ratio**	**Percent of Control**	**IC_{50}**
				Apical to Basal	**Basal to Apical**			
BCRP[b]	MDCKII	Prazosin (1 µM)	Solvent Control (0)	7.38 ± 0.84	73.8 ± 7.0	10.0	100	IC_{50} 35.7 µM Slope 0.669 Min 0% Max 100%
			Etrasimod (0.03)	5.56 ± 2.65	69.0 ± 9.6	12.4	126	
			Etrasimod (0.1)	5.05 (n = 2)	53.6 ± 4.9	10.6	107	
			Etrasimod (0.3)	6.44 (n = 2)	56.2 ± 2.1	8.73	85.8	
			Etrasimod (1)	9.66 ± 1.84	77.7 ± 12.7	8.04	78.2	
			Etrasimod (3)	6.67 ± 1.73	59.2 ± 9.6	8.87	87.4	
			Etrasimod (10)	5.85 ± 3.00	46.6 ± 12.9	7.97	77.4	
			Etrasimod (30)	7.02 ± 5.69	38.0 ± 13.2	5.41	49.0	
			Ko143 (1)	27.6 ± 4.5	30.4 ± 3.1	1.10	1.1	NA

[a] BCRP inhibition potential could not be determined since post-assay TEER values were below acceptance criteria, and lucifer yellow P_{app} values were above acceptable ranges at both 10 and 100 µM.

[b] A second BCRP experiment was performed for BCRP IC_{50} determination

Note: Values are triplicate determinations rounded to 3 significant figures with standard deviations rounded to the same degree of accuracy. Percentages are rounded to 1 decimal place except percentages ≥ 100, which are rounded to the nearest whole number.

ABC, ATP-binding cassette; ATP, adenosine triphosphate; BCRP, breast cancer resistance protein; Caco-2, human epithelial colorectal adenocarcinoma cells; GLP Good Laboratory Practice; IC_{50}, half maximal inhibitory concentration; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MDCKII, Madin-Darby canine kidney strain II cells; Min, minimum; Max, maximum; ND; could not be determined; P_{app}, apparent permeability; P-gp, P-glycoprotein; SD, standard deviation; SLC, solute carrier; TEER, trans-epithelial electrical resistance

Table 87: BSEP Inhibition in BSEP Vesicles

Test Article		Etrasimod (APD334, AR401959)							
Study Number		[redacted]8106 (non-GLP)							
Study Type		In vitro							
Study System		BSEP Vesicles							
Method		BSEP vesicles were incubated with probe substrates in the absence or presence of etrasimod or a known inhibitor. Probe substrate was measured with LC-MS/MS.							
Report Title		In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters							
Transporter	**Test System**	**Substrate**	**Inhibitor**	**[Inhibitor] (µM)**	**Uptake (pmol/mg protein)**			**Percent of Control**	**+ATP / +AMP Ratio**
					+ATP	**+AMP**	**ATP-dependent**		
BSEP	Vesicles	[^{3}H]-Taurocholic acid (0.4 µM)	Solvent control	0	26.9	1.43	25.4	100	18.8
			APD334	1	27.0	1.62	25.4	99.8	16.7
				10	27.2	1.65	25.5	100	16.4
			Cyclosporine	20	3.45	1.48	1.96	7.7	2.32

Note: Values are duplicate determinations rounded to 3 significant figures. Percentages are rounded to 1 decimal place except percentages ≥ 100, which are rounded to the nearest whole number.
ABC, ATP-binding cassette; AMP, adenosine monophosphate; ATP, adenosine triphosphate; BSEP, bile salt export pump; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; SLC, solute carrier

Table 88: SLC Transporter Inhibition in HEK Cells

Test Article	Etrasimod (APD334, AR401959)
Study Number	[redacted]8106 (non-GLP)
Study Type	In vitro
Study System	HEK293 Cells
Methods	Transporter-expressing HEK293 cells were incubated with radiolabeled probe substrates in the absence or presence of etrasimod or a known inhibitor. Probe substrate was measured with a scintillation counter.

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters							**Study Number:** [redacted]8106
Transporter	**Probe Substrate**	**Inhibitor (µM)**	**Uptake (pmol/mg) (Mean ± SD)**		**Background Corrected Uptake Rate (pmol/mg/min)**	**Percent of Control**	**IC_{50}**
			Control	**Transporter**			
OATP1B1	[^{3}H]-Estradiol-17β-glucuronide (50 nM)	Solvent control (0)	0.0117 ± 0.0106	2.47 ± 0.19	1.23	100	~10 µM
		APD334 (1)	0.00605 ± 0.00479	2.45 ± 0.08	1.22	99.2	
		APD334 (10)	0.00513 ± 0.00168	1.34 ± 0.15	0.669	54.4	
		Rifampin (10)	0.00330 ± 0.00110	0.157 ± 0.015	0.0770	6.3	
OATP1B3	[^{3}H]-Estradiol-17β-glucuronide (50 nM)	Solvent control (0)	0.0117 ± 0.0106	0.362 ± 0.008	0.175	100	> 10 µM
		APD334 (1)	0.00605 ± 0.00479	0.374 ± 0.018	0.184	105	
		APD334 (10)	0.00513 ± 0.00168	0.233 ± 0.022	0.114	65.1	
		Rifampin (10)	0.00330 ± 0.00110	0.0116 ± 0.0045	0.00415	2.4	
OAT1	[^{3}H]-p-Aminohippurate (1 µM)	Solvent control (0)	0.164 ± 0.080	11.3 ± 1.9	11.1	100	> 10 µM
		APD334 (1)	0.138 (n = 2)	9.26 ± 1.44	9.13	82.2	
		APD334 (10)	0.316 ± 0.194	12.3 ± 1.6	12.0	108	
		Probenecid (100)	0.187 ± 0.207	1.29 ± 0.08	1.10	9.9	

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters					**Study Number:** [redacted]8106		
Transporter	**Probe Substrate**	**Inhibitor (µM)**	**Uptake (pmol/mg) (Mean ± SD)**		**Background Corrected Uptake Rate (pmol/mg/min)**	**Percent of Control**	**IC_{50}**
			Control	**Transporter**			
OAT3	[^{3}H]-Estrone-3-sulfate (50 nM)	Solvent control (0)	0.0206 ± 0.0082	1.56 ± 0.09	0.768	100	> 10 µM
		APD334 (1)	0.0206 ± 0.0101	1.50 ± 0.08	0.741	96.4	
		APD334 (10)	0.0180 ± 0.0094	1.05 ± 0.14	0.516	67.1	
		Probenecid (100)	0.0265 ± 0.0021	0.184 ± 0.025	0.0788	10.3	
OCT1	[^{14}C]-Tetraethylammonium bromide (5 µM)	Solvent control (0)	5.48 ± 0.83	302 ± 26	19.7	100	> 10 µM
		APD334 (1)	5.04 ± 0.99	295 ± 23	19.3	97.9	
		APD334 (10)	6.21 ± 1.71	282 ± 27	18.4	93.2	
		Quinidine (100)	3.87 ± 2.61	35.7 ± 4.8	2.12	10.8	
OCT2	[^{14}C]-Metformin (10 µM)	Solvent control (0)	7.67 ± 1.70	202 ± 24	97.3	100	> 10 µM
		APD334 (1)	5.66 ± 1.19	207 ± 14	101	104	
		APD334 (10)	6.87 ± 0.25	206 ± 16	99.7	102	
		Quinidine (300)	1.97 ± 0.88	6.76 ± 0.99	2.39	2.5	
MATE1	[^{14}C]-Metformin (10 µM)	Solvent control	0.755 ± 0.146	22.8 ± 4.3	NR	100	> 10 µM
		APD334 (1)	0.772 ± 0.068	27.5 ± 3.1	NR	121	
		APD334 (10)	0.697 ± 0.126	25.8 ± 5.2	NR	114	
		Cimetidine (10)	0.532 ± 0.062	2.18 ± 0.32	NR	7.5	
MATE2-K	[^{14}C]-Metformin (10 µM)	Solvent control	0.483 ± 0.033	14.8 ± 2.3	NR	100	> 10 µM
		APD334 (1)	0.895 ± 0.029	14.9 ± 1.9	NR	97.8	
		APD334 (10)	0.853 ± 0.050	14.6 ± 2.0	NR	96.0	
		Cimetidine (100)	0.416 ± 0.014	2.72 ± 0.36	NR	16.1	

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters					**Study Number:** [redacted]8106		
Transporter	**Probe Substrate**	**Inhibitor (µM)**	**Uptake (pmol/mg) (Mean ± SD)**		**Background Corrected Uptake Rate (pmol/mg/min)**	**Percent of Control**	**IC_{50}**
			Control	**Transporter**			

Note: Values are triplicate determinations rounded to 3 significant figures with standard deviations rounded to the same degree of accuracy. Percentages are rounded to 1 decimal place except percentages ≥ 100, which are rounded to the nearest whole number.
Note: Uptake expressed in units of µL/mg protein for MATE1 and MATE2-K
ABC, ATP-binding cassette; ATP, adenosine triphosphate; GLP, Good Laboratory Practice; HEK, human embryonic kidney; IC_{50}, half maximal inhibitory concentration; MATE, multidrug and toxin extrusion protein; NR, not reported; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; SD, standard deviation; SLC, solute carrier

Table 89: Substrate Determination for P-gp and BCRP in MDCKII Cells

Test Article	Etrasimod (APD334, AR401959)
Study Number	[redacted]8106 (non-GLP)
Study Type	In vitro
Study System	MDCKII Cells
Methods	MDCKII cells in transwell plates were incubated with etrasimod in the absence or presence of a known transporter inhibitor. Samples from the donor and receiver wells were analyzed with LC-MS/MS.

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters								**Study Number:** [redacted]8106	
Transporter	**Substrate**	**Time (h)**	**Etrasimod Transported (pmol) in Receiver Wells (Mean ± SD)**		**P_{app} ($\times 10^{-6}$ cm/s) (Mean ± SD)**		**Efflux Ratio**	**Recovery (%)**	
			Apical to Basal	**Basal to Apical**	**Apical to Basal**	**Basal to Apical**		**Apical to Basal**	**Basal to Apical**
P-gp	Etrasimod (1 µM)	0.5	0.496 ± 0.258	0.0921 ± 0.0181	0.314 ± 0.110	0.351 ± 0.081	1.12	18.1	24.2
		1	0.0506 ± 0.0264	0.205 ± 0.022					
		2	0.989 (n = 2)	1.15 ± 0.09					
	Etrasimod (1 µM) (+) Valspodar (10 µM)	0.5	0.322 (n = 1)	0.0517 (n = 1)	0.0646 ± 0.0567	0.199 ± 0.033	3.08	27.1	42.0
		1	0.0329 (n = 1)	0.201 ± 0.032					
		2	0.164 (n = 2)	0.688 ± 0.044					
	Etrasimod (10 µM)	0.5	0.257 (n = 1)	0.272 ± 0.093	0.129 ± 0.038	0.410 ± 0.078	3.17	24.8	59.6
		1	0.224 (n = 2)	2.18 ± 0.17					
		2	2.62 ± 1.42	7.63 ± 1.29					
	Etrasimod (10 µM) (+) Valspodar (10 µM)	0.5	0.270 (n = 1)	0.198 ± 0.074	0.127 ± 0.027	0.408 ± 0.102	3.20	28.5	52.3
		1	0.789 ± 0.212	2.20 ± 0.49					
		2	3.63 ± 0.15	11.6 ± 5.4					

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters							**Study Number:** [redacted]8106	
Transporter	**Substrate**	**Time (h)**	**Etrasimod Transported (pmol) in Receiver Wells (Mean ± SD)**		**P_{app} ($\times 10^{-6}$ cm/s) (Mean ± SD)**		**Efflux Ratio**	**Recovery (%)**	
			Apical to Basal	**Basal to Apical**	**Apical to Basal**	**Basal to Apical**		**Apical to Basal**	**Basal to Apical**
BCRP	Etrasimod (1 µM)	0.5	NC	0.289 (n = 1)	0.685	1.15 ± 0.32	1.67	31.0	32.4
		1	NC	0.723 ± 0.075					
		2	2.15 (n = 2)	5.95 (n = 2)					
	Etrasimod (1 µM) (+) Ko143 (1 µM)	0.5	NC	0.203 (n = 1)	0.518 ± 0.030	0.735 ± 0.155	1.42	27.4	45.2
		1	1.08 (n = 1)	0.890 ± 0.259					
		2	1.89 (n = 2)	3.89 ± 1.18					
	Etrasimod (10 µM)	0.5	NC	2.20 ± 2.21	0.398 ± 0.097	2.93 ± 0.78	7.36	29.1	47.7
		1	2.66 (n = 2)	15.5 ± 8.3					
		2	11.6 ± 5.7	94.2 ± 91.2					
	Etrasimod (10 µM) (+) Ko143 (1 µM)	0.5	1.50 (n = 1)	1.14 ± 0.11	0.925 ± 0.199	2.93 ± 0.92	3.17	32.9	34.5
		1	5.61 ± 2.20	9.86 ± 2.10					
		2	25.7 ± 7.5	98.9 ± 71.6					

Note: Values are triplicate determinations rounded to 3 significant figures with standard deviations rounded to the same degree of accuracy. Percentages are rounded to 1 decimal place except percentages ≥ 100, which are rounded to the nearest whole number.
ABC, ATP-binding cassette; ATP, adenosine triphosphate; BCRP, breast cancer resistance protein; GLP, Good Laboratory Practice; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MDCKII, Madin-Darby canine kidney strain II cells; n, number of replicates; NC, not calculated; P_{app}, apparent permeability; P-gp, P-glycoprotein; SD, standard deviation; SLC, solute carrier

Table 90: Substrate Determination for SLC Transporters in HEK Cells

Test Article	Etrasimod (APD334, AR401959)
Study Number	[redacted]8106 (non-GLP)
Study Type	In vitro
Study System	HEK293 cells
Methods	HEK293 cells were incubated with etrasimod in the absence or presence of a known transporter inhibitor. Samples were analyzed with LC-MS/MS.

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters					**Study Number:** [redacted]8106	
Transporter	**Substrate**	**Substrate Concentration (µM)**	**Incubation Time (min)**	**Uptake (pmol/mg protein) (Mean ± SD)**		**Uptake Ratio**
				Control	**Transporter**	
OATP1B1	Etrasimod	1	1	45.8 ± 11.7	65.0 ± 2.5	1.42
			10	140 ± 12	151 ± 19	1.08
		10	1	413 ± 59	604 ± 26	1.46
			10	1610 ± 160	1740 ± 210	1.08
	Etrasimod (+) Rifampin (10 µM)	1	1	41.6 ± 2.1	64.0 ± 2.2	1.54
			10	145 ± 13	171 ± 21	1.18
		10	1	400 ± 24	619 ± 26	1.55
			10	1710 ± 80	1920 ± 220	1.13

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters					**Study Number:** [redacted]8106
Transporter	**Substrate**	**Substrate Concentration (µM)**	**Incubation Time (min)**	**Uptake (pmol/mg protein) (Mean ± SD)**	**Uptake Ratio**
				Control / **Transporter**	
OATP1B3	Etrasimod	1	1	45.8 ± 11.7 / 62.7 ± 10.1	1.37
			10	140 ± 12 / 170 ± 9	1.22
		10	1	413 ± 59 / 559 ± 61	1.35
			10	1610 ± 160 / 1820 ± 20	1.13
	Etrasimod (+) Rifampin (10 µM)	1	1	41.6 ± 2.1 / 56.9 ± 7.6	1.37
			10	145 ± 13 / 163 ± 14	1.13
		10	1	400 ± 24 / 593 ± 81	1.48
			10	1710 ± 80 / 1870 ± 230	1.09
OAT1	Etrasimod	1	1	46.4 ± 3.0 / 56.8 ± 13.3	1.23
			10	244 ± 10 / 245 ± 21	1.00
		10	1	534 ± 30 / 640 ± 116	1.20
			10	2760 ± 120 / 2700 ± 160	0.977
	Etrasimod (+) Probenecid (100 µM)	1	1	46.9 ± 1.1 / 60.4 ± 13.2	1.29
			10	255 ± 14 / 260 ± 24	1.02
		10	1	524 ± 55 / 611 ± 99	1.17
			10	2660 ± 80 / 2610 ± 80	0.979

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters					**Study Number:** [redacted]8106
Transporter	**Substrate**	**Substrate Concentration (µM)**	**Incubation Time (min)**	**Uptake (pmol/mg protein) (Mean ± SD)**	**Uptake Ratio**
				Control / **Transporter**	
OAT3	Etrasimod	1	1	177 ± 6 / 126 ± 11	0.711
			10	580 ± 121 / 392 ± 46	0.675
		10	1	1900 ± 100 / 1200 ± 90	0.634
			10	5730 ± 840 / 3980 ± 450	0.694
	Etrasimod (+) Probenecid (100 µM)	1	1	195 ± 13 / 131 ± 5	0.673
			10	569 ± 90 / 410 ± 44	0.721
		10	1	1850 ± 30 / 1160 ± 60	0.630
			10	6200 ± 700 / 3870 ± 200	0.624
OCT1	Etrasimod	1	1	9.81 ± 1.27 / 7.66 ± 0.10	0.780
			10	48.2 ± 3.4 / 37.9 ± 2.6	0.786
		10	1	218 ± 14 / 173 ± 19	0.793
			10	1220 ± 70 / 1000 ± 90	0.823
	Etrasimod (+) Quinidine (100 µM)	1	1	9.94 ± 0.97 / 7.22 ± 1.73	0.726
			10	50.4 ± 3.9 / 42.3 (n = 2)	0.840
		10	1	130 ± 17 / 106 ± 23	0.816
			10	1110 ± 40 / 865 ± 47	0.776

Report Title: In Vitro Evaluation of APD334 as an Inhibitor and a Substrate of Human ABC and SLC Transporters						**Study Number:** [redacted]8106
Transporter	**Substrate**	**Substrate Concentration (µM)**	**Incubation Time (min)**	**Uptake (pmol/mg protein) (Mean ± SD)**		**Uptake Ratio**
				Control	**Transporter**	
OCT2	Etrasimod	1	1	124 ± 31	79.3 ± 11.2	0.641
			10	502 ± 35	290 ± 86	0.577
		10	1	1250 ± 80	880 ± 96	0.705
			10	4810 ± 420	2720 ± 130	0.566
	Etrasimod (+) Quinidine (300 µM)	1	1	109 ± 7	90.8 ± 12.9	0.831
			10	502 ± 83	311 ± 76	0.620
		10	1	553 ± 52	440 ± 44	0.795
			10	3690 ± 150	2050 ± 30	0.556

Note: Values are triplicate determinations rounded to 3 significant figures with standard deviations rounded to the same degree of accuracy. Percentages are rounded to 1 decimal place except percentages ≥ 100, which are rounded to the nearest whole number.
ABC, ATP-binding cassette; ATP, adenosine triphosphate; GLP, Good Laboratory Practice; HEK, human embryonic kidney; LC-MS/MS, liquid chromatography with tandem mass spectrometry; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; SD, standard deviation; SLC, solute carrier

2.6.5.15. PHARMACOKINETICS: OTHER

The relationship between etrasimod plasma concentration (pharmacokinetic [PK]) and etrasimod-induced blood lymphocyte reduction (pharmacodynamic [PD]) was examined in male BALB/c mice, Sprague Dawley rats, beagle dogs, and cynomolgus monkeys. PK/PD modeling revealed that oral administration of etrasimod to male mice, rats, dogs, and monkeys decreased blood lymphocyte counts in a dose-dependent manner. The results of this modeling exercise were superseded by results from human studies, which are found in Module 2.7.2 Section 3.2.