CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207988Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date: December 18, 2015
From: Timothy J. McGovern, PhD, ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 207988
Agency receipt date: December 29, 2014
Drug: ZURAMPIC (lesinurad, RDEA594)
Sponsor: Ardea Biosciences, Inc.

Indication: Treatment of hyperuricemia associated with gout in combination with xanthine oxidase inhibitor

Reviewing Division: Division of Pulmonary, Allergy, and Rheumatology Products

The primary pharmacology/toxicology reviewer and team leader concluded that the nonclinical data for ZURAMPIC (lesinurad) support approval for the indication listed above.

ZURAMPIC is an oral therapy for the (b) (4) treatment of hyperuricemia associated with gout in combination with a xanthine oxidase inhibitor. The recommended dose of ZURAMPIC is 200 mg administered once daily in combination with allopurinol or febuxostat. Lesinurad reduces serum uric acid levels by inhibiting the function of two apical transporters responsible for uric acid reabsorption in the kidney. The Established Pharmacologic Class (EPC) for lesinurad was discussed internally. After an initial recommendation to classify lesinurad in a manner similar to probenecid, uricosuric, it was eventually concluded that the sponsor's proposed EPC of URAT1 inhibitor was acceptable.

Pivotal nonclinical toxicology studies were conducted in rats and cynomolgus monkeys; cynomolgus monkeys were selected as the non-rodent species since a major human metabolite (M4) is not produced in dogs. Studies up to 6 months duration in rats and 12 months duration in monkeys identified common target organs of toxicity, kidney and gastrointestinal tract. Deaths associated with these toxicities occurred in both species at the high dose of 600 mg/kg. Metabolic enzyme induction was observed in both species and was especially apparent in monkeys at doses of 300 mg/kg or greater after 3 months treatment. The NOAELs were identified as 100 mg/kg/day in both species. Systemic exposures at these doses provided safety margins of approximately 15-fold (rats) and 3-fold (monkeys) compared to the maximum recommended clinical dose of 200 mg per day based on systemic exposure comparisons.

A standard battery of genetic toxicology studies was conducted with lesinurad and the results were negative. Carcinogenicity studies conducted in rats and TgrasH2 mice produced no evidence of tumorigenicity.

Development and reproductive endpoints were evaluated as part of a battery of studies in rats and rabbits. No significant effects on fertility parameters were identified in rats and

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no developmental effects occurred in the absence of maternal toxicity. Observations at maternally toxic doses included prolonged estrous cycles, an increase in mean number of days to mate, decreased mean numbers of corpora lutea and implantation sites, reduced pup survival and body weights, and mean time to vaginal patency in F1 offspring.

Two disproportionate human metabolites, M3 and M4, were identified. M3 was qualified based on its exposure at the NOAEL in the 12 month monkey study and at the high dose in the 2 year rat carcinogenicity study. M4 is formed from an epoxide intermediate, M3c, that presents an alert for mutagenic potential. M3c was qualified for general toxicity concerns in the 12 month monkey study based on calculated exposures at the NOAEL dose but not with respect to carcinogenicity due to the low or absent levels of exposure in rodents. However, direct testing of M3c is not technically feasible given its reactive nature. Further, concern for carcinogenic potential is low given the transient nature of exposure and since M3c is converted to a dihydrodiol (M4) in humans.

The sponsor submitted labeling in general conformance with the PLR and PLLR and the Division provided further revisions. The proposed revisions to the nonclinical sections of the label appear appropriate. The Division could consider deletion of text in Section 8.1 "Animal Data" that references (b) (4) It is generally not recommended to include findings associated with (b) (4) in the label.

Conclusion: I agree with the Division pharmacology/toxicology conclusion that this NDA can be approved from the pharmacology/toxicology perspective. The two disproportionate human metabolites have been adequately addressed. The recommended EPC for lesinurad, URAT1 inhibitor, is appropriate.

I have discussed and am in general agreement with labeling revisions proposed by the Division. Consideration can be given to deleting text in Section 8.1 under "Animal Data" that references (b) (4) .

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/s/

TIMOTHY J MCGOVERN
12/18/2015

Pharmacology and Toxicology Secondary Review for NDA 207988

TO: NDA 207988 (ZURAMPIC; Lesinurad)

FROM: Timothy W. Robison, Ph.D., D.A.B.T.
Pharmacology and Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: November 24, 2015

After discussion with the Clinical Team, the Sponsor's proposed Established Pharmacological Classification (EPC) for ZURAMPIC (Lesinurad) of URAT1 inhibitor was judged to be acceptable.

Lesinurad reduces serum uric acid levels by inhibiting the function of transporter proteins involved in uric acid reabsorption in the kidney. Lesinurad inhibited the function of two apical transporters responsible for uric acid reabsorption, uric acid transporter 1 (URAT1) and organic anion transporter 4 (OAT4), with IC50 values of 7.3 and 3.7 μM, respectively. URAT1 is responsible for the majority of the reabsorption of filtered uric acid from the renal tubular lumen.

Recommendation: From the nonclinical perspective, approval of the application is recommended.

There are no outstanding nonclinical issues.

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/s/

TIMOTHY W ROBISON
11/24/2015

Pharmacology and Toxicology Review for NDA 207988

TO: NDA 207988 (ZURAMPIC; Lesinurad (RDEA594))

FROM: Matthew Whittaker, Ph.D.
Pharmacology and Toxicology reviewer
Division of Pulmonary, Allergy, and Rheumatology Products

SUBJECT: Addendum to NDA 209788 Nonclinical Review dated September 3, 2015

RDEA594-mediated pathologies to kidney and GI tract observed in chronic rat and monkey toxicology studies are summarized below.

Rat (6 month study, Doses tested: 30, 100, 300, 600 mg/kg/day)

- 13/60 rats treated with 600 mg/kg/d RDEA594 died within 21 days after the start of dosing.
  - GI Tract: Single cell necrosis (minimal – moderate), congestion, and hemorrhage (all GI tract components)
  - Kidney: Single cell necrosis (minimal – mild) and tubular degeneration (minimal – moderate)
- The remaining 600 mg/kg/day rats were euthanized on study day 23.
  - GI Tract findings: Minimal – mild erosion of the glandular stomach (sporadic)
  - Kidney findings: minimal-moderate tubular degeneration and single cell tubular necrosis.

- The following findings were observed after 3 to 6 months of 300 mg/kg/day RDEA594 treatment
  - GI Tract: erosions (minimal – mild) of the glandular stomach and minimal hemorrhage/congestion of the stomach mucosa (incidence: 2/10 – 3/10 animals)
  - Evidence for potential treatment-related kidney toxicity at 300 mg/kg/d included increases of water consumption, urine volume (decreased ability to concentrate urine), and serum creatinine. A decreased BUN:creatinine ratio was seen. However, there was limited microscopic evidence for kidney pathology at either the 3 or 6 month time points (increased incidences of tubular dilation [possibly due to tubular obstruction with crystalline deposits]).

- The NOAEL with respect to GI tract/kidney pathology in rats is 100 mg/kg/day.

Monkey (12 month study, Doses tested: 0, 30, 100, 300, 600 mg/kg/day)

- There were 4 premature deaths in animals treated with 600 mg/kg/day RDEA594.
  - Deaths were secondary to severe diarrhea, emesis and decreased food consumption.
  - GI tract: discoloration of duodenum, jejunum, ileum, cecum and colon; erosions and hemorrhage in colon and rectum; and GI components being filled with gas or yellowish material.

- Clinical observations of diarrhea, emesis and soft stool were observed in monkeys treated with ≥30 mg/kg/day, increased with dose, and persisted throughout the study.
- There were no treatment related microscopic lesions of GI tract or kidney at up to 300 mg/kg/day.

Species	RDEA594 (mg/kg/day)	Notable pathology	AUC_{0-24h} (µg*h/mL)	Exposure margin[1]
Rat	100	NOAEL	415	15
	300	▪ Stomach: Erosions/hemorrhage (minimal – mild) ▪ Kidney: Cysts, tubular dilatation (minimal-mild)	1017	36
	600	▪ Death ▪ GI Tract Single cell necrosis (min – moderate) Congestion Hemorrhage ▪ Kidney: Single cell necrosis (minimal – mild) Tubular degeneration (minimal – moderate)	3340	119
Monkey	100	NOAEL	83	3
	300	▪ Liver Bile duct hyperplasia	138	5
	600	▪ Death ▪ GI Tract Diarrhea, emesis Discoloration of GI tract tissue Colon, rectum: Hemorrhage	321	11

[1] Mean AUC_{0-24} (M & F combined, n = 12) for 200 mg RDEA594 administered 1x/d for 14 days in healthy adults = **28.0 µg*hr/ml** (Study RDEA594-105)

Condensed description: Chronic toxicology studies showed evidence of kidney toxicity in rats and GI tract toxicity in both rats and monkeys. In rats, the dose of 600 mg/kg/day (119 x clinical exposure) was lethal due to kidney toxicity (tubular degeneration and single cell necrosis) and gastrointestinal toxicity (erosion, hemorrhage, congestion, single necrosis). At the dose of 300 mg/kg/day (36 x clinical exposure), kidney findings were limited to tubular dilatation and changes of clinical chemistry parameters. Low incidences of GI tract erosion were observed. For monkeys, the dose of 600 mg/kg/day (11 x clinical exposure) was lethal due to GI tract toxicity (erosions and hemorrhage in colon and rectum and severe diarrhea and emesis). There was no GI tract toxicity at lower doses; however, bile duct hyperplasia was observed at 200 mg/kg/day. NOAELs of 100 mg/kg/day in both rats and monkeys provide exposure margins of 15- and 3-fold relative to the clinical exposure.

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/s/

MATTHEW T WHITTAKER
09/25/2015

TIMOTHY W ROBISON
09/25/2015
I concur

Pharmacology and Toxicology Secondary Review for NDA 207988

TO: NDA 207988 (ZURAMPIC; Lesinurad)

FROM: Timothy W. Robison, Ph.D., D.A.B.T.
Pharmacology and Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: September 10, 2015

ZURAMPIC (Lesinurad) is an oral therapy for the (b) (4) treatment of hyperuricemia (elevated serum uric acid levels) associated with gout, to be used in combination with a xanthine oxidase (XO) inhibitor. Lesinurad is to be administered at 200 mg once daily in combination with allopurinol or febuxostat.

Dr. Whittaker's reviews dated July 29, 2015 and September 3, 2015 evaluated the Sponsor's nonclinical safety assessment of lesinurad.

I concur with the recommendations of Dr. Whittaker's reviews dated July 29, 2015 and September 3, 2015 that the nonclinical pharmacology and toxicology of lesinurad have been adequately studied and ZURAMPIC should be approved from the nonclinical perspective.

The Sponsor has a complete nonclinical development program for lesinurad.

Pharmacology: Lesinurad inhibits the function of multiple carrier proteins that transport uric acid including uric acid transporter 1 (URAT1) and Organic Anion Transporter 4 (OAT4), located on the apical (or lumenal) surface of kidney proximal tubule epithelial cells, and OAT1 and OAT3, located on the basolateral surface. Lesinurad inhibits the transport of [^{14}C]uric acid into human embryonic kidney-293 cells expressing URAT1 or OAT4 with IC_{50} values of 7.3 and 3.7 μM, respectively. Lesinurad inhibits OAT1 and OAT3 transport of specific substrates in vitro with IC_{50} values of 4.3 and 3.5 μM, respectively. URAT1 is responsible for the majority of the reabsorption of filtered uric acid from the renal tubular lumen.

ADME: A mass balance study in humans (study RDEA594-112) identified a disproportionate human metabolite (20.7% of initial dose) of lesinurad, a dihydrodiol metabolite termed M4. The M4 metabolite is formed in humans by an epoxide intermediate termed M3c. Epoxide functional groups are known structural alerts for mutagenicity and could be potential carcinogens. M3c formation may represent a potential clinical concern given that lesinurad is to be administered chronically. M4 was not detected in rats suggesting that the M3c epoxide was not present at significant levels. However, cysteine conjugates (M9a and M9b) of the M3c epoxide were detected in monkeys. Based upon the 12-month monkey study, the human M3c metabolite, an epoxide intermediate, was considered qualified for safety with respect to general toxicity. Although the metabolite was not qualified for safety with respect to reproductive

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toxicity or carcinogenicity, no additional nonclinical studies were required. This conclusion was based on the consideration that it was not technically feasible to directly administer M3c in a stand-alone reproductive toxicity or carcinogenicity study due to its chemical instability. There is an undefined risk to human subjects from exposure to the epoxide intermediate. Some concern with regard to the potential reproductive toxicity and carcinogenic effects of M3c might be mitigated by its transient nature and the fact that, in humans, this molecule can be detoxified to a dihydrodiol (M4).

Toxicology: Chronic toxicology studies with lesinurad were conducted in rats (26 weeks) and monkeys (12 months). In both studies, animals received oral doses up to 600 mg/kg/day. Common target organs of toxicity in the rat and monkey included the kidneys and gastrointestinal tract. Premature deaths related to toxicities in these organs were observed at the high dose (600 mg/kg/day) in both rats and monkeys. Evidence for auto-induction of metabolism was also observed in both species and systemic exposure was markedly reduced in monkeys receiving doses ≥300 mg/kg/day after 12 weeks of treatment. The monkey was the more sensitive species. The systemic exposure in monkeys at the NOAEL supports the maximum clinical lesinurad exposure at the proposed dose of 200 mg/day.

Genetic Toxicity: Lesinurad was negative in a standard battery of genotoxicity assays.

Carcinogenicity: There was no evidence of tumorigenic potential in a 2-year carcinogenicity study conducted in rats or in a 26 week carcinogenicity study in TgRasH2 mice. The study with TgrasH2 mice was for hazard identification purposes only; so exposure multiples relative to the clinical dose would not be calculated.

Reproductive Toxicity: Mating and reproductive performance were unaffected in male and female rats that received lesinurad at oral doses up to 300 mg/kg/day. Female rats treated with lesinurad at 300 mg/kg/day exhibited prolonged estrous cycles and a slight increase in the mean number of days to mate compared to controls. In addition, slightly decreased mean numbers of corpora lutea and implantation sites were observed in this dose group. All of these findings were observed in the presence of overt toxicity. There was no evidence of teratogenicity or any other embryo-fetal developmental toxicity in rats and rabbits that received lesinurad at oral doses up to 300 and 75 mg/kg/day, respectively, during the period of organogenesis. Lesinurad was found to distribute to the milk of lactating rats at exposure levels comparable to plasma. Mean time to vaginal patency was significantly delayed in F1 female offspring of rats treated with doses ≥200 mg/kg/day in a pre- and postnatal development study.

Safety Evaluation of Impurities: See Dr. Whittaker's review dated September 1, 2015 for a safety evaluation of observed impurities in the drug substance and a degradant in the drug product.

Labeling: Dr. Whittaker's reviews dated July 29, 2015 and September 3, 2015 recommend changes to product labeling in Indications and Usage under Highlights of Prescribing Information, Section 8.1 (Pregnancy), (b) (4)

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Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility). I concur with Dr. Whittaker's recommendations for changes to the product label. See Dr. Whittaker's reviews for additional details of changes to the product labeling.

The pharmacological actions and binding affinities of lesinurad were relatively similar to probenicid. The most appropriate Established Pharmacological Classification (EPC) for Lesinurad was judged to be uricosuric, which was identical to the commonly used EPC for probenicid. See Dr. Whittaker's review dated September 3, 2015 for the details of this decision.

Recommendation: From the nonclinical perspective, approval of the application is recommended.

There are no outstanding nonclinical issues.

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/s/

TIMOTHY W ROBISON
09/10/2015

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 207988
Supporting document/s:	Supporting document 1
Applicant's letter date:	December 29, 2014
CDER stamp date:	December 29, 2014
Product:	Lesinurad (RDEA594)
Indication:	Treatment of hyperuricemia associated with gout (in combination with a xanthine oxidase inhibitor)
Applicant:	Ardea Biosciences, Inc.
Review Division:	Division of Pulmonary, Allergy & Rheumatology Products
Reviewer:	Matthew Whittaker, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Michelle Jordan Garner

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 207988 are owned by Ardea Biosciences, Inc. or are data for which Ardea Biosciences, Inc. has obtained a written right of reference.

Any information or data necessary for approval of NDA 207988 that Ardea Biosciences, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 207988.

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1 Executive Summary

1.1 Introduction

Ardea Biosciences, Inc. is developing lesinurad (RDEA594) as an oral therapy for the (b) (4) treatment of hyperuricemia (elevated serum uric acid levels) associated with gout, to be used in combination with a xanthine oxidase (XO) inhibitor. Lesinurad is to be administered at 200 mg once daily in combination with allopurinol or febuxostat.

Lesinurad is an inhibitor of multiple carrier proteins expressed in renal proximal tubule cells that transport uric acid. These include uric acid transporter 1 (URAT1) and organic anion transporters 1, 3, and 4 (OAT1, OAT3, OAT4). URAT1 is responsible for the transport of the majority of reabsorbed uric acid. XO inhibition is intended to reduce the production of uric acid, while lesinurad is designed to reduce the reabsorption of uric acid from the glomerular filtrate.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical data supports the approval of 200 mg lesinurad as an oral therapy for the treatment of hyperuricemia associated with gout.

The current review includes a detailed evaluation of the relevant nonclinical Pharmacology and Pharmacokinetics/ADME studies. A complete review of the pre- and postnatal reproductive toxicology study conducted in rats is also included. The results of relevant general toxicology, genetic toxicology, carcinogenicity and reproductive toxicology studies reviewed previously are summarized here with reference to the original nonclinical reviews.

Lesinurad decreases the reabsorption of uric acid from the renal proximal tubule by inhibiting the function of carrier proteins that transport uric acid. Inhibition of URAT1 and OAT4, located on the apical surface of renal tubular epithelial cells, is likely to be principal mechanism of action. Inhibition of the function of OAT1 and OAT3, located on the basolateral membrane of tubular epithelial cells, is not expected to play a major role in the pharmacodynamic effect of lesinurad. Lesinurad inhibits transporter function with potency in the 3 – 7 μM range. The pharmacological actions and binding affinities of lesinurad are relatively similar to probenicid. The most appropriate Established Pharmacological Classification (EPC) for lesinurad was judged to be uricosuric, which is identical to the commonly used EPC for probenicid.

Rats and monkeys were chosen by the sponsor as the species to be used in the relevant studies in their *in vivo* nonclinical development program. Lesinurad is absorbed rapidly after oral administration in rats and monkeys. Oral bioavailability is greater in rats (73%) than in monkeys (41%). Lesinurad is primarily eliminated via feces in nonclinical species while humans eliminate lesinurad primarily in urine. All metabolites observed in humans are also synthesized in at least one nonclinical species. A mass balance study in humans (study RDEA594-112) identified a disproportionate human metabolite (20.7% of initial dose) of RDEA594, a didhydrodiol metabolite termed M4. The M4 metabolite is formed in humans via an epoxide intermediate termed M3c. Epoxide functional groups are known structural alerts for mutagenicity. M3c formation may represent a potential clinical concern given that RDEA594 is

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to be administered chronically. M4 was not detected in rats suggesting that the M3c epoxide was not present at significant levels. However, cysteine conjugates (M9a and M9b) of the M3c epoxide were detected in monkeys. Based upon the 12-month monkey study, the human M3c metabolite, an epoxide intermediate, is qualified for safety with respect to general toxicity. Although the metabolite is not qualified for safety with respect to carcinogenicity, no additional nonclinical studies are required. This conclusion is based on the consideration that it is not technically feasible to directly administer M3c in a stand-alone carcinogenicity study due its chemical instability. Some concern with regard to the potential carcinogenic effects of M3c may be mitigated by its transient nature and the fact that, in humans, this molecule is detoxified to a didhydrodiol (M4).

Chronic toxicology studies with RDEA594 were conducted in rats (26 weeks) and monkeys (12 months). In both studies, animals were dosed by oral gavage at up to 600 mg/kg/day. Common target organs of toxicity in the rat and monkey include the kidney and GI tract. Premature deaths related to toxicities in these organs were observed in HD animals (600 mg/kg/d) in both rats and monkeys. Evidence for autoinduction of metabolism was also observed in both species, and systemic exposure was markedly reduced in monkeys receiving ≥ 300 mg/kg/d after 12 weeks of treatment. The monkey is the more sensitive species, with an $AUC_{0\text{-}24h}$ of 82.6 µg*hr/ml. This NOAEL supports the maximum clinical RDEA594 exposure at the proposed dose of 200 mg/day.

Lesinurad was negative in a standard battery of genotoxicity assays. There was no evidence of tumorigenic potential in a 2 year carcinogenicity study conducted in rats or in a 26 week carcinogenicity study in TgRasH2 mice.

Female rats that were treated with lesinurad (oral) at 300 mg/kg/day exhibited prolonged estrous cycles and a slight increase in mean number of days to mate compared to controls. In addition, slightly decreased mean numbers of corpora lutea and implantation sites were observed in this dose group. All of these findings were observed in the presence of overt toxicity. There was no evidence of teratogenicity or any other embryo-fetal developmental toxicity in rats and rabbits that received oral lesinurad doses up to 300 and 75 mg/kg/day, respectively, during the period of organogenesis. Lesinurad was found to distribute to the milk of lactating rats at exposure levels comparable to plasma. Mean time to vaginal patency was significantly delayed in F1 females offspring of rats treated with ≥ 200 mg/kg/day in a pre- and postnatal development study.

1.3 Recommendations

1.3.1 Approvability

Ardea has submitted a complete nonclinical pharmacology and toxicology program for lesinurad. From the nonclinical perspective, the application is recommended for approval. There are no outstanding nonclinical issues.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are required.

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1.3.3 Labeling

Labeling recommendations are provided for Indications and Usage (under Highlights of Prescribing Information), Sections 8.1 - 8.3, Section 12.1, and Section 13. The Pediatric and Maternal Health Staff was consulted for Sections 8.1 - 8.3 to achieve compliance with the Pregnancy and Lactation Labeling Rule. Additions are shown in underlined text and deletions are shown in strikethrough text.

---------- **INDICATIONS AND USAGE** ----------

ZURAMPIC is a uricosuric agent ~~URAT1 inhibitor~~ indicated for the treatment of hyperuricemia associated with gout in combination with a xanthine oxidase inhibitor. (1)

8.1 Pregnancy

Risk Summary

There are no available data on use of Zurampic in pregnant women to inform a drug-associated risk. No teratogenicity was observed in embryo-fetal development studies with oral administration of lesinurad to pregnant rats and rabbits during organogenesis at doses that produced maternal exposures up to approximately 45 and 10 times, respectively, the exposure at the maximum recommended human dose (MRHD). No adverse developmental effects were observed in a pre- and postnatal development study with administration of lesinurad to pregnant rats from organogenesis through lactation at a dose approximately 5 times the MRHD.

[*see* (b) (4) *Data*]

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data

Animal Data

In an embryo-fetal development study in pregnant rats dosed during the period of organogenesis from gestation days 6-17, lesinurad was not teratogenic at exposures up to approximately 45 times the MRHD (on an AUC basis at maternal oral doses up to 300 mg/kg/day). In an embryo-fetal development study in pregnant rabbits dosed during the period of organogenesis from gestation days 7-20, lesinurad was not teratogenic at exposures up to approximately 10 times the MRHD (on an AUC basis at maternal oral doses up to 75 mg/kg/day). Severe maternal toxicity, including mortality, was observed in rats and rabbits at exposures equal to or greater than approximately 45 and 4 times the MRHD (on an AUC basis at maternal oral doses of 300 mg/kg/day in rats and 25 mg/kg/day and higher in rabbits) respectively.

In a pre- and post-natal development study in pregnant female rats dosed from gestation day 7 through lactation day 20, lesinurad had no effects on delivery or growth and development of offspring at a dose approximately 5 times the MRHD (on a mg/m^2 basis at a maternal dose oral dose of 100 mg/kg/day). (b) (4)

(b) (4)

(b) (4)

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(b) (4)

8.2 Lactation

Risk Summary

There is no information regarding the presence of Zurampic in human milk, the effects on the breastfed infant, or the effects on milk production. Lesinurad is present in the milk of rats *[see Use in Specific Populations (8.1)]*. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Zurampic and any potential adverse effects on the breastfed infant from Zurampic or from the underlying maternal condition. (b) (4)

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

The carcinogenic potential of lesinurad was evaluated in Sprague-Dawley rats and TgRasH2 mice. No evidence of tumorigenicity was observed in male or female rats that received lesinurad for 91 to 100 weeks at oral doses up to 200 mg/kg/day (approximately 35 times the MRHD on an AUC basis). No evidence of tumorigenicity was observed in TgRasH2 mice that received at lesinurad for 26 weeks at oral doses up to 125 and 250 mg/kg/day in male and female mice, respectively. (b) (4)

(b) (4)

Lesinurad tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* chromosomal aberration assay in Chinese hamster ovary ~~(CHO)~~ cells, and *in vivo* rat bone marrow micronucleus assay ~~in rat bone marrow~~.

(b) (4)

Fertility and reproductive performance were unaffected in male or female rats that received lesinurad at oral doses up to 300 mg/kg/day (approximately 15 times the MRHD on a mg/m^2 basis).

(b) (4)

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2 Drug Information

2.1 Drug

CAS Registry Number: 878672-00-5

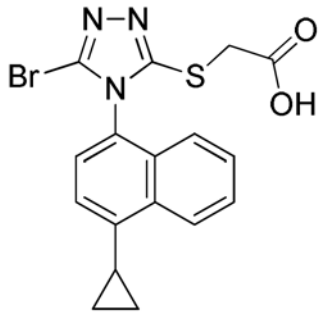
Generic Name: Lesinurad

Code Name: RDEA594

Chemical Name: 2-((5-bromo-4-(4-cyclopropylnaphthalen-1-yl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid

Molecular Formula/Molecular Weight: $C_{17}H_{14}BrN_3O_2S$; 404.28 g/mol

Structure:



Pharmacologic Class: Lesinurad is a uric acid reabsorption inhibitor. It inhibits the function of URAT1 and OAT4 with comparable potency (3 – 7 µM). There is currently no established pharmacologic class (EPC) text phrase specific for uric acid reabsorption inhibitors. Probenecid is the only approved product that acts via inhibition of uric acid reabsorption. This product is described as a Uricosuric. Given that the pharmacologic profile of lesinurad is comparable to that of probenecid, the proposed pharmacologic class for lesinurad is recommended as uricosuric.

2.2 Relevant INDs, NDAs, BLAs and DMFs

Application	Relevance
IND 102128	Original IND for RDEA594

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2.3 Clinical Formulation

2.3.1 Drug Substance

Ardea has chosen to proceed with a crystalline free acid form of the lesinurad drug substance (DS) for commercial development. Nonclinical studies were conducted with free acid and (b) (4) sodium salt forms of lesinurad. Lesinurad free acid and sodium salt had comparable systemic exposures in rats and monkeys (Table 65).

2.3.2 Drug Product

Lesinurad drug product (DP) is to be administered as an oral, immediate release tablet. The maximum recommended human dose of lesinurad drug product is 200 mg/day. Table 1 shows the drug product formulation for a single 200 mg immediate release tablet.

Table 1. Clinical formulation of a single 200 mg immediate release lesinurad tablet.

Component Name	Quality Standard	Function	Quantity per Unit (mg)	% w/w
Lesinurad	In-house	Active ingredient	200.00	(b) (4)
Hypromellose 2910	USP, Ph Eur	(b) (4)		
Microcrystalline Cellulose	NF, Ph Eur			
Lactose Monohydrate	NF, Ph Eur			
Crospovidone	NF, Ph Eur			
Magnesium Stearate	NF, Ph Eur			
Opadry Blue (b) (4)	Supplier			
(b) (4)	(b) (4)			
		Total Target Weight:	(b) (4) mg/tablet	100.0%

2.4 Comments on Novel Excipients

All excipients in the drug product are within the range of excipients in FDA-approved products.

2.5 Comments on Impurities/Degradants of Concern

Organic impurities (b) (4) in the drug substance have specification limits that exceed the 0.15% qualification established in the ICHQ3A(R2) Guidance.

The specification limit for the drug product degradant (b) (4) exceeds the 0.2% qualification threshold established in the ICH Q3B(R2) Guidance.

The specification limits for the DS impurities and DP degradant are all considered to be acceptable. See chemistry consult review dated September 1, 2015.

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2.6 Proposed Clinical Population and Dosing Regimen

Lesinurad is to be administered as an immediate release tablet to adults with gout at a dose of 200 mg once daily in combination with a xanthine oxidase inhibitor. This is not a fixed dose combination drug product. Administration of lesinurad is to be followed by a xanthine oxidase inhibitor, such as allopurinol or febuxostat. The therapeutic goal is to lower serum uric acid (sUA) levels to a target of <6 mg/dL.

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2.7 Regulatory Background

- The initial IND for lesinurad (IND 102128) was submitted on September 30, 2009.

- An End of Phase 2 meeting was held on July 21, 2011. The Sponsor's nonclinical questions and FDA responses are reproduced below:

Question 1:
Does the FDA agree that the completed nonclinical safety pharmacology studies with lesinurad are sufficient to support the NDA?
Response:
We agree that the completed nonclinical safety pharmacology studies with lesinurad are sufficient to support the NDA.
Discussion:
None

Question 2:
Does the FDA agree that the single-agent, repeat-dose chronic toxicity studies of lesinurad in rats and monkeys are sufficient to support the proposed Phase 3 studies of lesinurad and the NDA?
Response:
We agree that your completed nonclinical rat and monkey chronic oral toxicology studies support your proposed Phase 3 clinical trials and an NDA for lesinurad administered orally once daily up to 400 mg per day.
Discussion:
None

Question 3:
Does the FDA agree that the ongoing 13-week lesinurad and allopurinol combination repeat-dose toxicity study in rats is sufficient to support the proposed Phase 3 studies of lesinurad in combination with allopurinol and the NDA?
Response:
As your Phase 3 clinical studies propose the co-administration of lesinurad and allopurinol products, not a fixed-dose combination product, and the toxicology profile of allopurinol is well characterized, the 13-week lesinurad and allopurinol combination repeat-dose toxicology study is not essential to support your Phase 3 clinical studies or the NDA. However, submit the completed 13-week combination repeat-dose toxicity study as supportive data to your IND.

Discussion:
None

Question 4:
Does the FDA agree that the ongoing 13-week combination repeat-dose toxicity study in rats with lesinurad and allopurinol, the most widely used drug in the xanthine oxidase inhibitor class, is also sufficient to support the proposed Phase 3 study of lesinurad in combination with febuxostat, a member of the same class?
Response:
No nonclinical combination toxicology study is required to support the clinical coadministration of lesinurad and febuxostat. (Refer to our response to question 3)

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Discussion:
None

Question 5:
Does the FDA agree that the completed genotoxicity studies with lesinurad, its metabolites and drug substance starting materials/intermediates are sufficient to support the proposed Phase 3 studies and the NDA?
Response:
We agree that the completed genotoxicity studies with lesinurad and its metabolites support the proposed Phase 3 studies and the NDA. As was discussed in your July 6, 2011 EOP2 CMC meeting, you proposed to complete bacterial reverse mutation assays (Ames assays) for starting materials or impurities that contain structural alerts.

For those impurities that are positive in the Ames assay or are known carcinogens, you proposed to control these impurities to < (b) (4) *mcg/day. We agree with your proposed approach to qualify or control impurities containing structural alerts. We also remind you for impurities that do not contain structural alerts, Monitor impurities and degradation products of all active ingredients and refer to ICH Guidance [ICH Q3A(R) and ICHQ3B(R)] for possible qualification requirements.*

Discussion:
None

Question 6:
Does the FDA agree that the ongoing transgenic mouse and rat carcinogenicity studies are sufficient to support the NDA?
Response:
We acknowledge that completion of the transgenic mouse and rat carcinogenicity studies may fulfill the carcinogenicity assessment requirement to support an NDA filing for lesinurad. However, review and discussions with the Executive Carcinogenicity Assessment Committee of the completed carcinogenicity study reports are necessary prior to concurring with the validity of your carcinogenicity studies.

Discussion:
None

Question 7:
Does the FDA agree that the completed fertility and early embryonic development study and the embryo-fetal development studies with lesinurad along with the planned prenatal and postnatal development study are sufficient to allow the inclusion of women of child bearing potential (WOCBP) in the proposed Phase 3 studies and to support the NDA?
Response:
We agree that your currently completed and planned (Pre- and Post-natal development toxicity) reproductive toxicology studies are sufficient to allow the inclusion of WOCBP in the proposed Phase 3 studies and to support an NDA. The adequacy of the pre- and post-natal development study will be a review issue.

Discussion:
None

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- A pre-NDA meeting was held on September 26, 2014. Sponsor questions and FDA responses that included content related to nonclinical studies are reproduced below.

Question 2:
Does the Agency agree that the proposed lesinurad label statements for Section 13 Nonclinical Toxicology of the proposed Prescribing Information for lesinurad are acceptable?
Response:
We cannot comment on your proposed labeling for sections 8.1, 13.1, and 13.2 at this time. The acceptability of these sections of the label will be review issues. It is unlikely that Section 13.2 would be needed.

Discussion:
None.

Question 4:
Does the Agency agree that, based on new information available since the CMC End of Phase 2 meeting, the compound known as (b) (4) can be designated as a starting material in the manufacturing process for lesinurad drug substance?
Response:
Yes, we agree. For all structure alert impurities (including those related to the starting materials and process generated) provide in the NDA, in a tabular form the structure, in-silico test results, point of occurrence, summary with link to details of toxicological assessment, and chemical assessment (control, purge and fate). Impurities that are identified as structural alerts should be at or below acceptable qualification thresholds to support an NDA as described in the ICH M7 Guideline. See the response to Question 8.

We also note that any non-genotoxic impurities or degradants of the active ingredient exceeding qualification limits per the ICH Q3A (R2) and Q3B (R2) Guidances, respectively, should be appropriately qualified in a toxicology study with minimum duration of 13 weeks. Batch analysis of impurities in drug substance lots used in toxicology studies with lesinurad can be considered in the qualification process as needed. Levels of impurities and degradants in the drug product exceeding qualification limits should be supported by No Observed Adverse Effect Levels (NOAELs) identified in toxicology studies with an appropriate safety margin (e.g., (b) (4) fold on mg/m2 basis).

Discussion:
None.

Question 8:
Does the Agency agree with the process used for assessment of the potential genotoxic impurities in the lesinurad drug substance?
Response:
The process used for assessment of potential genotoxic impurities generally appears to be acceptable per the ICH M7 Guideline. A final decision regarding the assessment and control of genotoxic impurities will be made upon review of the NDA submission.

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Discussion:
None.

Question 9:
Does the Agency agree that the completed nonclinical package is sufficient to support an NDA for lesinurad?
Response:
The sufficiency of the nonclinical package will be a review issue. We note the presence of the disproportionate human metabolite M4, formed via the epoxide intermediate M3c. Provide justification in your NDA that the M3c/M4 metabolites have been adequately assessed and are qualified with respect to general toxicity, genetic toxicity, carcinogenicity, and reproductive toxicity. This should include information that the M3c epoxide intermediate was formed in a nonclinical species at sufficient levels to permit assessments of potential toxicity. If this is not possible, the M3c metabolite should be qualified for safety according to the ICH M3(R2) Guidance and FDA Guidance for Industry: Safety testing of drug metabolites (February, 2008).

Discussion:
None.

- NDA 207988 for lesinurad was submitted to the FDA on December 29, 2014.

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3 Studies Submitted

3.1 Studies Reviewed

Study #	Title
PHARMACOLOGY	
Primary pharmacology	
SR08-018*	Assessment of SLC transporters (OAT1 and OAT3) interaction with ardea compounds in oocytes [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-047*	Drug-drug interaction study of RDEA806, RDEA594, and Tenofovir in rats [see nonclinical review dated 6/18/10 (IND 102128)]
SR11-023	Evaluation of lesinurad (RDEA594) on URAT1 orthologs in rat and mouse (rRST and mRST), version 2
SR08-012	Inhibitory Effects of RDEA 806 and its Metabolites on Uric Acid Transporter URAT1
SR09-019	Evaluation of RDEA594 on Uric Acid Transporters Expressed in HEK293 Cells and Xenopus Laevis Oocytes
SR10-006	Interaction of RDEA594 with Renal Uric Acid Transporters URAT1 , OAT1, and OAT3
SR11-020	Testing of RDEA594 on BCRP, NPT1, MATE1, and MATE2K transporters
SR08-008	Effect of RDEA806 and metabolites on xanthine oxidase conversion of xanthine and hypoxanthine to uric acid
SR08-007	Effect of RDEA806 and metabolites on enzymatic and cellular activity of purine nucleoside phosphorylase
SR11-039	Inhibition of URAT1 and OAT4 by Lesinurad Metabolites
SR11-015	In vitro and in vivo anti-inflammatory activities of RDEA594, Version 2
AIR-ARD-3, (b)(4)10-0145, (b)(4)10-0167, CPE-ARD-1, AIA-ARD-4	In vivo pharmacology studies conducted with RDEA594 in rats
Secondary pharmacology	
SR08-086	(b)(4) Spectrum screen of RDEA594, Version 2
SR08-072*	Evaluation of RDEA806, RDEA594, (b)(4) for functional interaction with metabolic disease-related nuclear receptors [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-025*	In vitro anti-HIV activity of RDEA594 [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-061*	Exploratory non-GLP cardiovascular assessment of intravenously administered M6 metabolite of RDEA806 (RDEA594) in tethered conscious male Sprague-Dawley rats [see nonclinical review dated 6/18/10 (IND 102128)]
SR09-060*	Mitochondrial toxicity comparison of benzbromarone, RDEA594 and URAT1 second generation molecules [see nonclinical review dated 6/18/10 (IND 102128)]
SR11-013	In vitro pharmacology: Functional assays – study of compounds 0495594 (b)(4)
SR11-015	In vitro and in vivo anti-inflammatory activities of RDEA594, Version 2
SAFETY PHARMACOLOGY [See nonclinical review dated 6/18/10 (IND 102128)]	
Cardiovascular/Respiratory	
SR08-021	Effects of RDEA594 on cloned hERG potassium channels expressed in human embryonic kidney cells
SR08-054, SR08-085	Effect of RDEA594 on action potentials in isolated rabbit cardiac Purkinje fibers in the presence of human serum albumin
SR08-020	RDEA594: Cardiovascular and respiratory assessment when administered orally to

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Study #	Title
	conscious monkeys
Neurologic	
SR08-027	RDEA594: Central nervous system assessment with functional observational battery when administered orally to conscious rats
Renal/ urinary	
SR08-088	RDEA594: Renal function-diuresis and electrolyte excretion in conscious rats via oral administration
Gastrointestinal	
SR08-033	RDEA594: Effects on gastrointestinal motility after a single oral administration in male Sprague Dawley rats
PK/ADME	
Absorption	
SR09-066	Bidirectional transport (Papp) studies of RDEA594 on Caco-2 monolayers
SR08-060	Pharmacokinetic evaluation of RDEA594 and [^{14}C]RDEA594-derived radioactivity in rat plasma or whole blood following a single oral or intravenous administration of [^{14}C]RDEA594
SR08-059	Pharmacokinetic evaluation of RDEA594 and [^{14}C]RDEA594-derived radioactivity in cynomolgus monkey plasma or whole blood following a single oral and intravenous administration of [^{14}C]RDEA594.
SR08-071*	RDEA594: Single oral dose pharmacokinetic study in male Sprague-Dawley rats [see nonclinical review dated 6/18/10 (IND 102128)]
SR09-012*	Drug-drug interaction study of RDEA594 and febuxostat in rats [see nonclinical review dated 6/18/10 (IND 102128)]
SR09-065*	Evaluation of potential drug-drug interaction between RDEA594 and allopurinol/oxypurionl in plasma following oral gavage in male cynomolgus monkeys
SR09-018*	RDEA594: Single oral dose pharmacokinetic study in male and female ICR (CD-1) mice [see nonclinical review dated 6/18/10 (IND 102128)]
SR10-040*	A single dose oral gavage pharmacokinetic study in male TgRasH2 (wild type) mice [see nonclinical review dated 4/29/11 (IND 102128)]
Distribution	
SR08-045	Evaluation of the plasma protein binding potential of RDEA594 in mouse, rat, dog, monkey and human plasma by equilibrium dialysis
SR12-039	Evaluation of the plasma protein binding of RDEA594-M4 in human plasma by equilibrium dialysis
SR08-046	The quantitative tissue distribution of total radioactivity in the male rat following single oral and single intravenous administration of [^{14}C]RDEA594
SR10-028	Metabolite profile of radioactivity in plasma, excreta and bile of male cynomolgus monkeys following an oral gavage dose of [^{14}C]RDEA594
SR10-029	Metabolite profile of radioactivity in plasma, excreta and bile of male cynomolgus monkeys following 28 day repeated oral gavage doses of [^{14}C]RDEA594
Metabolism	
SR08-026*	In vitro evaluation of (b) (4), RDEA594 and (b) (4) as inducers of cytochrome P450 expression in cultured human hepatocytes [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-038*	Qualitative reaction phenotyping of P450 isozymes responsible for the metabolism of RDEA594 [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-056	Evaluation of in vitro metabolism of [^{14}C]RDEA594 in liver microsomes and cryopreserved hepatocytes from animals and humans
SR11-031	Identification of liver microsomal enzymes responsible for oxidative metabolism of RDEA594
SR12-026	Evaluation of RDEA594-M4 formation in induced rat liver microsomes and liver S9

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Study #	Title
	fractions
SR08-048	In vitro evaluation of RDEA594 as an inhibitor of major human liver cytochrome P450 Isozymes
SR12-043	In vitro evaluation of RDEA594 as an inhibitor of human cytochrome P450 2B6 isozyme
SR10-002	Qualitative reaction phenotyping of UGT isozymes responsible for glucuronidation of RDEA594
SR10-001	In vitro evaluation of RDEA594 as an inhibitor of human UDP-glucuronosyltransferase isozymes 1A1 and 2B7
SR10-063	In vitro evaluation of RDEA594 as an inducer of cytochrome P450 2B and 2C expression in cultured human hepatocytes
RDEA594-112	An AME (Absorption, Metabolism and Excretion) study of [^{14}C]RDEA594 orally administered to healthy adult male volunteers.
SR11-037	Evaluation of the metabolic profile of RDEA594 in urine and feces in wild type CBYB6F1-Tg(Hras)2JIC mice following a single oral administration of [^{14}C]RDEA594 at 100 mg/kg (60 µCi/kg)
SR08-120	Evaluation of the metabolic profile of [^{14}C]RDEA594 in rat plasma, urine, and feces following oral administration to male and female Sprague-Dawley rats
SR10-021	Plasma, urine and fecal metabolite profile, urinary and fecal excretion evaluation in male and female Sprague Dawley rats following a single oral dose of [^{14}C]RDEA594 at 300 mg/kg after repeated daily doses of RDEA594
SR09-070-MET	Exploratory non-GLP plasma metabolic profiling of TK samples in 24 month rat carcinogenicity study
SR08-119	In vivo metabolism study of RDEA594 in monkey plasma, urine and feces following single intravenous or oral administration to male cynomolgus monkeys.
SR10-029-MET	[^{14}C]RDEA594 metabolite profile of radioactivity in plasma, excreta and bile of male cynomolgus monkeys following 28-day repeated oral gavage doses of 300 mg/kg RDEA594
SR08-094-MET	RDEA594: A 12-month repeat dose toxicity and toxicokinetic study with a 3-month recovery period and a 6-month pull in cynomolgus monkey
Elimination	
SR08-028	Pharmacokinetic, blood to plasma ratio, and mass balance of [^{14}C]RDEA594 administered to Sprague-Dawley rats following single intravenous and oral doses
SR11-068	RDEA594 excretion to rat milk
SR09-056	Evaluation of biliary excretion, enterohepatic circulation, and metabolism of [^{14}C]RDEA594 after a single oral dose followed by intraduodenal readministration in rats
SR12-032	Evaluation of the metabolic profile of RDEA594 in bile and urine in male bile-duct cannulated Sprague-Dawley rats following a single oral administration of [^{14}C]RDEA594 at a dose of 200 mg/kg (100 µCi/kg)
SR08-017	[^{14}C]RDEA594: Mass balance and blood to plasma ratio of radioactivity in male cynomolgus monkeys following single oral and intravenous doses
GENERAL TOXICOLOGY [see nonclinical review dated 12/24/12 (IND 102128)]	
SR08-015*	RDEA594: A 14-day oral dose range finding toxicity and toxicokinetic study in Sprague-Dawley rats [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-016*	RDEA594: A 14-day oral dose range finding and toxicokinetic study in cynomolgus monkeys [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-034*	RDEA594: A 28-day repeated oral dose toxicity and toxicokinetic study followed by a 28-day recovery period in Sprague-Dawley rats with a a14-day pull [see nonclinical review dated 6/18/10 (IND 102128)]
SR08-035*	RDEA594: A 28-day repeated oral dose toxicity and toxicokinetic study followed by a 28-day recovery period in cynomolgus monkeys

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Study #	Title
SR08-094	RDEA594: A 12-month repeat dose toxicity and toxicokinetic study with a 3-month recovery period and a 6-month pull in cynomolgus monkeys
SR08-095	RDEA594: A 6 month repeat oral dose toxicity and toxicokinetic study with a 2-month recovery period and a 3-month pull with a 28-day recovery period in Sprague Dawley rats
SR10-018*	RDEA594: 4-week dose range finding oral gavage toxicity and toxicokinetic study in TgHras2 (wild type) mice [see nonclinical review dated 4/29/11 (IND 102128)]
SR10-060*	Bioanalysis and toxicokinetics of RDEA594: a 28 day oral gavage toxicokinetic study in TgHras2 (wild type) mice [see nonclinical review dated 4/29/11 (IND 102128)]
SR13-019	(b) (4) A 13-week oral gavage toxicity study in Sprague-Dawley rats
GENETIC TOXICOLOGY [see nonclinical review dated 6/23/11 (IND 102128)]	
SR08-022	Bacterial reverse mutation assay, test article: RDEA594
SR06-086	In vitro mammalian chromosome aberration screening test
SR08-023	RDEA594: In vitro mammalian chromosome aberration test
SR08-024	Rat bone marrow erythrocyte micronucleus test following oral administration of RDEA594
SR09-015	Bacterial reverse mutation assay, test article: RDEA594
CARCINOGENICITY [see nonclinical review dated 7/29/15 (NDA 207988)]	
SR09-070	RDEA594: An oral gavage 24-month carcinogenicity study in Sprague Dawley rats
SR10-019	RDEA594: 26-week oral gavage carcinogenicity and toxicokinetic study in CBYB6F1-Tg(Hras)2Jic mice
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	
SR10-007	RDEA594: Oral gavage study of fertility and early embryonic development to implantation in rats [see nonclinical review dated 6/22/11 (IND 102128)]
SR10-008	RDEA594: Rat oral gavage embryo-fetal developmental toxicity and toxicokinetic study [see nonclinical review dated 6/22/11 (IND 102128)]
SR10-009	RDEA594: Rabbit oral gavage embryo-fetal developmental toxicity and toxicokinetic study [see nonclinical review dated 6/22/11 (IND 102128)]
SR11-068	A Developmental and Perinatal/Postnatal Reproduction Study by Oral Gavage in Rats, Including a Postnatal Behavioral/Functional Evaluation
* Indicates that study was reviewed previously but is not discussed in the current NDA review	

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3.2 Studies Not Reviewed

Study #	Title
PHARMACOLOGY	
Secondary pharmacology	
SR09-085	Evaluation of RDEA806, RDEA594, (b) (4) for functional interaction with neuropeptide Y receptors NPY5 and NPY4
SR11-014	Evaluation of muscle cell toxicity of RDEA594, colchicine and simvastatin
SR14-008	Evaluation of effects of RDEA594-M4 metabolite on selected inflammation targets
PK/ADME	
Absorption	
SR08-030	RDEA594: A pharmacokinetic cross-over study following oral and intravenous administration in male beagle dogs
SR08-058	Pharmacokinetics of RDEA594 following a single oral and intravenous administration to male beagle dogs
SR08-132	Evaluation of protein binding potential of REA594 in Purkinje assay medium by equilibrium dialysis
SR09-062	Drug-drug interaction study of RDEA594 and AZT in rats
SR11-006	Bioanalysis and pharmacokinetics of "An allopurinol/RDEA594 combination of single oral dose administration pharmacokinetic study in male and female Sprague-Dawley rats (non-GLP)"
SR11-022	An allopurinol alone and allopurinol/RDEA594 combination single oral dose administration with different formulations pharmacokinetic study in male and female Sprague Dawley rats
SR11-051	Bioanalysis and pharmacokinetics of RDEA594 administered orally to cynomolgus monkeys as a split bolus dose
SR11-056	A pharmacokinetic study in male and female Sprague-Dawley rats after single oral administration with two different doses of febuxostat plus RDEA594
SR12-025	Comparative plasma pharmacokinetic profiles of RDEA594 following multiple oral formulations of RDEA594 in beagle dogs
SR12-042	Pharmacokinetic evaluation of RDEA594 in plasma and urine of nephrectomy rats
SR12-045	Pharmacokinetics of RDEA594-M6 in plasma following a single intravenous or oral administration of RDEA594-M6 to male Sprague-Dawley rats
SR12-047	Pharmacokinetics of RDEA594-M6 in plasma and/or urine following a single intravenous or oral administration of RDEA594-M6 to female cynomolgus monkeys
SR12-048	Evaluation of RDEA594 covalent binding in rat and human liver microsomes and human hepatocytes
SR13-026	Photosafety assessment of lesinurad
Metabolism	
SR10-035	Urinary and fecal excretion of RDEA594 in male/female Sprague-Dawley and Wistar Han rats following a single oral administration of [^{14}C]RDEA594
SR10-056	In vitro debromination of RDEA594 in rat blood and intestinal microflora
SR11-082	Qualitative reaction phenotyping of rat cytochrome P450 enzymes responsible for oxidation of RDEA594
SR12-007	Comparative plasma pharmacokinetic profiles of RDEA594-M6 following multiple formulations of RDEA594-M6 in Sprague-Dawley rats
SR12-027	Glutathione and cysteine conjugation metabolites of RDEA594 in human and liver microsomes and S9 fractions
SR12-028	Qualitative reaction phenotyping of monkey CYP450 isozymes responsible for oxidation of RDEA594
SR12-019	Pharmacokinetics of RDEA594-M6 in plasma and urine following a single oral administration of RDEA594-M6 to male cynomolgus monkeys

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Study #	Title
Elimination	
SR10-041	Excretion, distribution, and metabolism of [^{14}C]RDEA594-M6 following a single oral administration to bile-duct cannulated monkeys
SR12-050	Investigation into source of the unidentified urinary objects observed in Sprague Dawley rats administered RDEA594
GENERAL TOXICOLOGY	
SR09-037	RDEA594: Toxicity study by oral gavage administration to Han Wistar rats for 4 weeks
SR10-016	Allopurinol: A 4-week repeat dose oral toxicity and toxicokinetic study in Sprague Dawley rats
SR10-032	Allopurinol: A 13-week oral tolerance and toxicokinetic study in male Sprague Dawley rats
SR11-002	RDEA594 and Allopurinol: A 13-week repeat combination oral toxicity and toxicokineic study in Sprague-Dawley rats
SR13-051	RDEA594 and Febuxostat: A 13-week repeat combination oral toxicity and toxicokinetic study in Sprague-Dawley rats
GENETIC TOXICOLOGY	
SR09-088	Bacterial reverse mutation assay: (b) (4)
SR11-052	Bacterial reverse mutation assay- Test article (b) (4)
SR11-069	Bacterial reverse mutation assay- Test article (b) (4)
SR11-073	Bacterial reverse mutation assay- Test article: (b) (4)
SR11-074	Bacterial reverse mutation assay- Test article: (b) (4)
SR11-080	Bacterial reverse mutation assay- Test article: (b) (4)
SR12-005	Bacterial reverse mutation assay- Test article: (b) (4)
SR12-006	Bacterial reverse mutation assay- Test article: (b) (4)
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	
SR09-068	RDEA594: Rabbit oral gavage dose range-finding developmental toxicity study
SR09-069	RDEA594: Rat oral gavage dose range-finding developmental toxicity study

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3.3 Previous Reviews Referenced

Date	Review	Author
6/18/10	Initial 30 d safety review of IND 102128	Kathleen Young, Ph.D.
4/29/11	Nonclinical review of dose pharmacokinetic and dose-range finding studies in TgRasH2 mice	Kathleen Young, Ph.D.
6/22/11	Nonclinical review of reproductive and developmental toxicology studies with RDEA594	Grace Lee, Ph.D.
6/23/11	Nonclinical review of genetic toxicology studies with RDEA594	Marcie Wood, Ph.D.
12/24/12	Nonclinical review of 6 month rat toxicology study (SR08-095) & 12 month monkey toxicology (SR08-094)	Matthew Whittaker, Ph.D.
7/29/15	Nonclinical review of 2 year rat carcinogenicity study and 26 week carcinogenicity study in TgRasH2 mice	Matthew Whittaker, Ph.D.
9/1/15	Chemistry consultation review. Safety qualification of specification limits for impurities in lesinurad DS and degradant in lesinurad DP	Matthew Whittaker, Ph.D.

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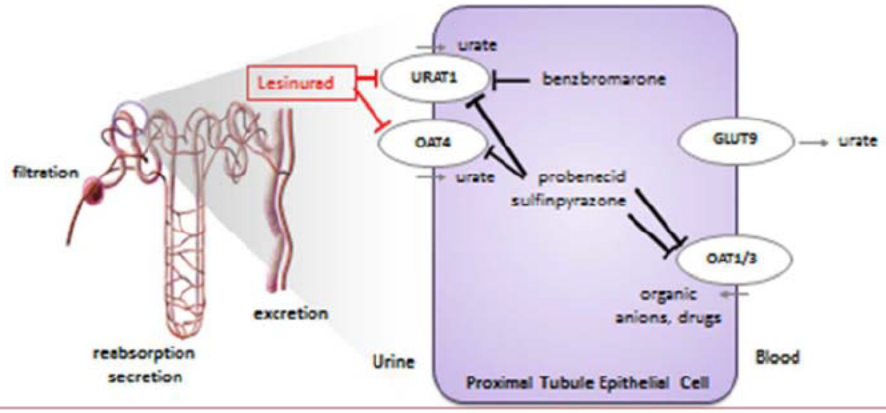
4 Pharmacology

Ardea proposes to administer lesinurad in combination with a xanthine oxidase (XO) inhibitor to treat hyperuricemia (elevated serum uric acid levels). XO inhibition reduces the production of uric acid, while lesinurad is designed to reduce the reabsorption of uric acid from the glomerular filtrate. The rationale behind co-administering lesinurad with an XO inhibitor is to limit the potential for urate microcrystal formation in renal proximal tubules in treated patients.

Uric acid transport protein 1 (URAT1) and organic anion transport protein 4 (OAT4) are transport proteins expressed on the lumenal membrane of renal proximal tubule epithelial cells (Figure 1). These carrier proteins are responsible for reabsorption of uric acid from the glomerular filtrate.

Ardea conducted several in vitro studies examining the inhibitory effects of lesinurad on the function of carrier proteins that transport uric acid. The results are summarized in Table 2.



Abbreviations: GLUT9, glucose transporter 9; OAT, organic anion transporter; URAT1, uric acid transporter 1

Figure 1. Sponsor's diagrammatic representation of the location of relevant transport proteins expressed in kidney proximal tubule epithelial cells.

Lesinurad inhibits URAT1 and organic anion transport protein 4 (OAT4) with potencies of 7.3 and 3.7 μM respectively. While the IC_{50} values indicate a low potency effect, the concentration of lesinurad in the urine in the lumen of the proximal tubule (i.e. the site of action) in patients receiving the 200 mg/day dose would be expected to exceed these IC_{50} values.

Clinical study RDEA594-112 determined that 31.3% of the initial lesinurad dose is detected in urine as unchanged lesinurad. A rough estimate of the steady state concentration of lesinurad in urine can be calculated as follows:

- 200 mg * 0.313 = 62 mg
- Lesinurad molecular weight: 404.28 g/mol

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- 0.062 g * (1 mol/404.28 g) = 1.5×10^{-4} mol
- Urine volume: ~2 L
- Lesinurad concentration: 1.5×10^{-4} mol/ 2 L = ~75 μM

OAT1 and OAT3 are located on the basolateral surface of renal proximal tubule cells and are capable of transporting uric acid from the blood into the proximal tubule cell[1]. Lesinurad inhibited the OAT1 and OAT3 transporters with similar potencies to URAT1 and OAT4.

Given that OAT1 and OAT3 contribute to removal of uric acid from the blood, inhibition of their function is not a desired effect of lesinurad. However, lesinurad is also a substrate for OAT1 and OAT3 (Study SR10-006). An additional potential mechanism of action for lesinurad exists whereby it is transported from the blood to the tubular epithelial cell cytoplasm by OAT1 and OAT3 and inhibits URAT1 function from the cytoplasmic side (termed "trans inhibition").

[1] Kim et al. (2007) Effects of increased uric acid intake on the abundance of urate-anion exchanger and organic anion transporter proteins in the rat kidney. *Electrolyte & Blood Pressure*. 5, 62-67.

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Table 2. Summary of in vitro effects of RDEA594 and other related molecules on the function of selected uric acid transport proteins.

Transporter	Location	Substrate	Expression system	Compound	IC_{50} (µM)	Study#
URAT1	Apical	[^{14}C]uric acid	Xenopus oocyte	RDEA594	52.5	SR08-012
		[^{14}C]uric acid	Xenopus oocyte	RDEA594	52.5	SR10-006
		[^{14}C]uric acid	HEK-293	RDEA594	7.3	SR09-019
		[^{14}C]uric acid	HEK-293	RDEA594	3.4	SR14-018[c]
		[^{14}C]uric acid	HEK-293	Probenecid	10.0	SR14-018[c]
		[^{14}C]uric acid	HEK-293	RDEA594 – M3[a]	>30	SR11-039
		[^{14}C]uric acid	HEK-293	RDEA594 – M4[b]	>30	SR11-039
		[^{14}C]uric acid	HEK-293	Benzbromarone	0.16	SR09-019
OAT4	Apical	[^{14}C]uric acid	HEK-293	RDEA594	3.7	SR09-019
		[^{14}C]uric acid	HEK-293	Probenecid	11.1	SR09-019
		6-carboxyfluorescein	HEK-293	RDEA594 – M3[a]	>30	SR11-039
		6-carboxyfluorescein	HEK-293	RDEA594 – M4[b]	>30	SR11-039
		[^{14}C]uric acid	HEK-293	Benzbromarone	1.8	SR09-019
OAT1	Basolateral	[^{3}H]para-aminohippuric acid (PAH)	Flp-In 293 cells	RDEA594	4.3	SR10-006
		6-carboxyfluorescein	HEK-293	RDEA594	3.6	SR14-018[c]
		[3H]cidofovir	CHO-K1 cells	Probenecid	3.9	Chu et al. (2007)[d]
OAT3	Basolateral	[^{3}H]estrone sulfate	Xenopus oocyte	RDEA594	3.5	SR10-006
		[^{3}H]cimetidine	CHO-K1 cells	Probenecid	3.1	Chu et al. (2007)[d]

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SLC2A9v1	Apical	[^{14}C]uric acid	Xenopus oocyte	RDEA594	No effect	SR09-019
SLC2A9v2	Apical	[^{14}C]uric acid	Xenopus oocyte	RDEA594	No effect	SR09-019

[a] RDEA594-M3 is a disproportionate human metabolite. Total amount in urine & feces is 12.3% of the total dose.
[b] RDEA594-M4 is a disproportionate human metabolite. Total amount in urine & feces is 20.7% of the total dose.
[c] Data are from study SR14-018. This study was submitted to IND 102128 but not NDA207988.
[d] Chu et al. (2007) Transport of the dipeptidyl peptidase-4 inhibitor sitagliptin by human organic anion trasporter 3, organic anion transporting polypeptide 4C1, and multidrug resistance P-glycoprotein. *Journal of Pharmacology and Experimental Therapeuctics*. 321, 673 – 683.

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4.1 Primary Pharmacology

In vitro pharmacology

Study SR11-023: Evaluation of lesinurad (RDEA594) on URAT1 orthologs in rat and mouse (rRST and mRST), version 2

rRST and mRST represent orthologs of URAT1 in rat and mouse respectively. hURAT1, rRST, and mRST were each expressed in HEK293 cells in order to evaluate the effect of RDEA594 on uric acid transport. rRST and mRST share 75% and 73% protein sequence homology with URAT1 and are expressed in the kidney proximal tubule.

Cells were incubated with RDEA594 (3.7 – 100 µM) plus [^{14}C]uric acid for 10 minutes at RT. Uric acid uptake into cells was measured using a scintillation counter. RDEA594 had no effect on uric acid uptake in cells expressing rRST or mRST at any of the doses tested. This evidence suggests that the rat is not a pharmacologically relevant species for RDEA594 with respect to the effects of inhibition of substrate transport by rRST.

RDEA594 inhibited URAT1 uptake of uric acid with an IC_{50} of approximately 33 µM.

Study SR08-012: Inhibitory Effects of RDEA 806 and its Metabolites on Uric Acid Transporter URAT1

Lesinurad (RDEA 594) was identified by the sponsor as a metabolite (M6) of RDEA806, a non-nucleoside reverse transcriptase inhibitor (NNRTI). (b) (4)

In this experiment, human URAT1 was expressed in *Xenopus* oocytes. Uptake of [^{14}C]uric acid (17 µM) was measured in the presence of RDEA806 or its metabolites to examine their inhibitory effects on URAT1 function. Radioactivity associated with oocytes was measured via liquid scintillation counting. The M6 metabolite (lesinurad) inhibited [^{14}C]uric acid uptake with an IC_{50} of **52.5 µM**.

Study SR09-019: Evaluation of RDEA594 on Uric Acid Transporters Expressed in HEK293 Cells and Xenopus Laevis Oocytes

This study investigated the effects of RDEA594 on [^{14}C]uric acid uptake by URAT1, OAT4, and the transporter SLC2A9 (variants 1 and 2). All of these transporters are capable of transporting uric acid and are expressed on the apical membrane of renal proximal tubule cells.

Experiments in HEK293 cells

Human URAT1 (**hURAT1**), **hOAT4**, and **hSLC2A9** variant 2 (hSLC2A9v2) were stably expressed in human embryonic kidney (HEK293) cells. [^{14}C]uric acid uptake was measured over the course of 15 minutes of incubation at 37°C (hOAT4 and hSLC2A9v2) or 10 minutes at room temperature (URAT1) in the presence of test compounds (RDEA594, benzbromarone, probenecid).

The IC_{50} value for RDEA594 inhibition of [^{14}C]uric acid uptake by URAT1 was determined to be **7.3 µM** while that for benzbromarone was less than **0.01 µM.**

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The IC_{50} value for RDEA594 inhibition of [^{14}C]uric acid uptake by OAT4 was determined to be **3.7 µM** while the IC_{50} values for benzbromarone and probenecid were 1.84 and 11.1 µM respectively.

Experiments in Xenopus oocytes
The effect of RDEA594 on **SLC2A9v1** or **SLC2A9v2** transport of [^{14}C]uric acid was also tested on these transporters expressed in Xenopus oocytes. Transfected cells were incubated with RDEA594 or benzbromarone plus [^{14}C]uric acid for 10 minutes. There was no effect of RDEA594 on uric acid uptake by either of these transporters.

Study SR10-006: Interaction of RDEA594 with Renal Uric Acid Transporters URAT1 , OAT1, and OAT3
As shown in Figure 1, URAT1 is expressed on the lumenal surface of proximal tubule epithelial cells while OAT1 and OAT3 are expressed on the basolateral surface. The current study examined the extent to which RDEA594 may be a substrate for any of these transporters. Further, this study measured the influence of RDEA594 on the specific transport function of each protein.

Uptake of RDEA594 into cells expressing OAT1
OAT1 was expressed in Flp-In 293 cells. [^{14}C]RDEA594 was incubated with cells for 5 minutes at 37°C. The reaction was stopped with ice-cold transport medium and cells were lysed with 0.2 ml of 1 N NAOH and radioactivity was measured using liquid scintillation counting.

- There was saturable uptake of [^{14}C]RDEA594 into oocytes expressing **OAT1**. The K_m was **0.85 µM** and V_{max} was 13.0 pmol/min/mg protein.

Uptake of RDEA594 into cells expressing URAT1 or OAT3
Experiments exploring the transport of [^{14}C]RDEA594 by URAT1 and OAT3 were conducted in *Xenopus* oocytes expressing each of these respective transporters.
- There was no significant uptake of [^{14}C]RDEA594 into **URAT1** expressing oocytes. RDEA 594 is **NOT** transported by URAT1.
- There was saturable uptake of [^{14}C]RDEA594 into oocytes expressing **OAT3**. The K_m was **1.96 µM** and V_{max} was 0.2 pmol/min/oocyte

Inhibition of transporter function
OAT1 transporter:
RDEA594 was added simultaneously with 50 nM [^{3}H]para-aminohippuric acid (PAH) to Flp-In 293 cells expressing OAT1. Uptake was conducted for 5 min at 37°C.
- The IC_{50} for RDEA594 inhibition of **OAT1** mediated [^{3}H]PAH uptake was **4.3 µM**.

URAT1 and OAT3 transporters
The effects of RDEA594 on URAT1 and OAT3 function were investigated using *Xenopus* oocyte expression systems by measuring uptake of [^{14}C]uric acid (URAT1 expressing cells) and

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[^{3}H]estrone-3-sulfate (OAT3 expressing cells) respectively in the presence of varying concentrations of RDEA594. Uptake was conducted for 60 min at 25°C.

In all experiments, uptake of radiolabeled substrate was quantified via liquid scintillation counting.

- The IC_{50} for RDEA594 inhibition of **URAT1** mediated [^{14}C]uric acid uptake was **52.5 µM**, consistent with the reported results in Study SR08-012.
- The IC_{50} for RDEA594 inhibition of **OAT3** mediated [^{3}H]$E_1$3S uptake was **3.5 µM**.

"Trans"-effect on transporter function
The current experiment also investigated whether *intracellular* RDEA594 could inhibit URAT1 function. This is termed trans-inhibition. This is an important consideration given that RDEA594 is a substrate for the basolateral transporters OAT1 and OAT3 and could accumulate inside of tubular epithelial cells.

RDEA594 was microinjected into URAT1 expressing oocytes and cells were subsequently incubated with [^{14}C]uric acid for 15 minutes at 25°C.

- RDEA594 showed a dose-dependent increase in trans-inhibition of [^{14}C]uric acid uptake at estimated intracellular concentrations of ≥ **44 µM**.

This experiment suggests that, in vivo, RDEA594 may act both intracellularly (trans-inhibition) and extracellularly (cis-inhibition) to inhibit URAT1 function.

<u>Study SR11-020</u>: Testing of RDEA594 on BCRP, NPT1, MATE1, and MATE2K transporters

The effects of RDEA594 on the function of these selected transport proteins were examined to evaluate potential risks associated with drug-drug interactions. RDEA594 was tested at concentrations of 1, 10, and 100 µM.

BCRP (breast cancer resistance protein) is an efflux transporter whose functions include regulation of intestinal absorption as well as biliary and renal secretion of substrates. Inhibitors of BCRP function can present risks associated with drug-drug interactions by altering the PK of co-administered drugs.

NPT1 is also known as SLC17A1 (Solute carrier family 17 (Organic Anion Transporter), Member 1). This transporter has been shown to be localized to the apical membrane of the renal proximal tubule. It transports multiple substrates, including uric acid. It has recently been reported to mediate urate **excretion**[2]**.** This is notable given that URAT1, the target for

[2] Chiba T et al. (2015) NPT1/SLC17A1 is a renal urate exporter in humans and its common gain-of-function variant decreases the risk of renal underexcretion in gout. *Arthritis Rheumatol*. 61, 281-287.

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RDEA594, mediates renal urate **reabsorption**. NPT1 function was evaluated by measuring the uptake of [^{3}H]estradiol 17**β**-D-glucuronide.

MATE1 (multidrug and toxin extrusion protein 1) is also known as SLC47A1. It is primarily expressed in the liver and kidney and is involved in the **excretion** of endogenous and exogenous organic cations. In the kidney, MATE1 is expressed on the luminal membrane of proximal tubule cells. Evaluation of the effect of RDEA594 on the function of this transporter is relevant to understanding potential drug-drug interactions. MATE1 function was evaluated by measuring the uptake of [^{14}C]tetraethylammonium (TEA).

MATE2K (multidrug and toxin extrusion protein 2) is also known as SLC47A2. It is a renal transporter of organic cations. Evaluation of the effect of RDEA594 on the function of this transporter is relevant to understanding potential drug-drug interactions. MATE2 function was evaluated by measuring the uptake of [^{14}C]tetraethylammonium (TEA).

BCRP function was evaluated by measuring the transport of [^{3}H]methotrexate in a BCRP-expressing membrane vessel preparation purchased from (b) (4). Incubation was for 2 minutes at 37°C. 100 μM RDEA594 inhibited **BCRP** function (37.3% of control, Table 3)

The effect of RDEA594 on the function of NPT1, MATE1 and MATE2K was studied in HEK293 cells expressing these transporters. Experiments were conducted at 37°C and incubation times were either 5 minutes (NPT1) or 10 minutes (MATE1 and MATE2K).

100 μM RDEA594 had an apparent inhibitory effect on **MATE1** function (80.5% of control) but not **MATE2K** function.

RDEA594 inhibited **NPT1** function at all doses tested. Given the potential role of NPT1 in excretion of uric acid from proximal tubule cells, the inhibition of the function of this transporter by RDEA594 could negatively impact its in vivo efficacy.

Table 3. RDEA594 inhibition of the transport proteins BCRP, MATE1, MATE2K, and NPT1.

RDEA594 dose	Substrate transport (% control)			
	BCRP	MATE1	MATE2K	NPT1
0	100	100	100	100
1	81.4	91.2	121.1	81.7
10	81.7	97.9	116.3	45.0
100	37.3	80.5	109.3	23.9

Reference ID: 3815621

SR08-008: Effect of RDEA806 and metabolites on xanthine oxidase conversion of xanthine and hypoxanthine to uric acid.
RDEA806 is a non-nucleoside reverse transcriptase inhibitor of HIV-1. RDEA594 is the M6 metabolite of RDEA806.

This study measured the effects of test compounds on xanthine oxidase function using the Amplex Red Xanthine/Xanthine Oxidase Assay kit from Invitrogen. In this assay, xanthine or hypoxanthine is converted to uric acid + superoxide by the xanthine oxidase enzyme. The superoxide then degrades to H_2O_2 which reacts with the Amplex red reagent in the presence of HRP to produce a fluorescent product which can be monitored fluorimetrically.

Xanthine and hypoxanthine were used as substrates in separate experiments. There was no clear effect of RDEA594 (up to 100 µM) on xanthine oxidase activity with xanthine as the substrate. Similarly, there was no clear effect of RDEA594 (up to 100 µM) on xanthine oxidase activity with hypoxanthine as the substrate. Allopurinol, the positive control XO inhibitor, inhibited conversion of xanthine and hypoxanthine to uric acid with IC_{50} values of 0.71 and 1.8 µM respectively.

SR08-007: Effect of RDEA806 and metabolites on enzymatic and cellular activity of purine nucleoside phosphorylase.
Purine nucleoside phosphorylase (PNP) catalyzes the conversion of purine nucleosides to the purine base plus ribose phosphate. Hypoxanthine is one of the purine bases formed in this reaction and is a substrate for further metabolism by xanthine oxidase to uric acid.

As with study SR08-008, this study was conducted when the primary molecule of interest was RDEA806. RDEA594 represents the M6 metabolite of RDEA806. The study's objective was to establish whether inhibition of PNP by RDEA806 or its metabolites might be a possible mechanism of the observed decrease in serum uric acid levels in healthy volunteers treated with RDEA806.

In the PNP assay, 2-amino-6-mercapto-7-methylpurine riboside (MESG; (b) (4) is converted to ribose 1-phosphate and 2-amino-6-mercapto-7-methylpurine in the presence of inorganic phosphate. Production of 2-amino-6-mercapto-7-methylpurine can be monitored spectrophotometrically at 360 nm. PNP activity was measured in a 30 minute reaction carried out at room temperature. RDEA594 was tested at concentrations up to 150 µM. Tenofovir was used as the positive control.

RDEA594 exhibited no inhibition of PNP at any dose tested.

RDEA594 inhibition of PNP was also tested in cytotoxicity assays in CCRF-CEM and MOLT-4 T-cell leukemia cell lines. PNP inhibitors are cytotoxic to these cells in the presence of deoxyguanosine (dG). RDEA594 was tested at concentrations from 1 – 100 µM. RDEA594 cytotoxicity was not enhanced in the presence of dG, suggesting a lack of PNP inhibition at the doses tested.

Taken together, the data suggests that RDEA594 is not an inhibitor of PNP.

Reference ID: 3815621

Study SR11-039: Inhibition of URAT1 and OAT4 by Lesinurad Metabolites

As described in Section 5.1: Metabolism, lesinurad metabolites M2, M3, and M4 are detected in human urine and feces after a single dose of lesinurad. Metabolite M2 is a debrominated metabolite of the parent compound. M3 is a hydroxylated metabolite and M4 is a didhydrodiol metabolite of lesinurad (Figure 2).

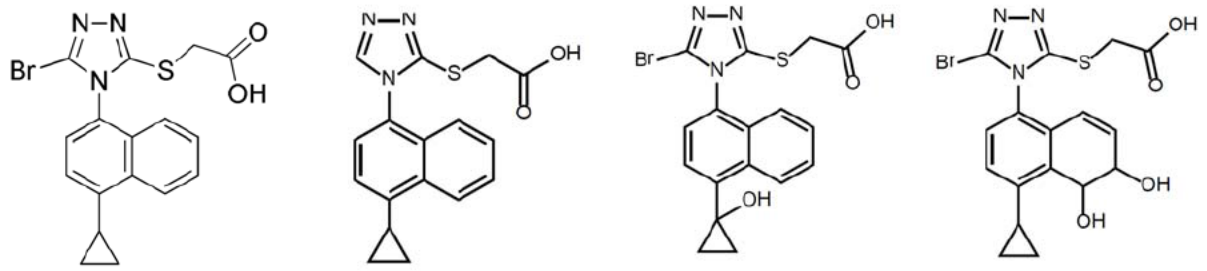


Figure 2. Chemical structures of RDEA594 (lesinurad) and metabolites M2, M3, and M4.

Metabolites M3 and M4 are disproportionate human metabolites, as they are present in urine & feces at 12.3 and 20.7% of the initial dose respectively. Metabolite M6 is formed at high levels in monkeys (Study SR08-094) but not in humans.

The current study examined the effects of these metabolites on the function of human URAT1 and OAT4. These transporters were stably expressed in HEK-293 cells and metabolites were tested for their effect on the uptake of [^{14}C]uric acid (URAT1) or 6-carboxyfluorescein (OAT4).

The URAT1 assay was conducted by incubating URAT1 expressing cells with [^{14}C]uric acid plus varying concentrations of metabolites (up to 30 µM) for 10 minutes at RT and detecting radioactivity in the cells via liquid scintillation counting. The OAT4 assay was conducted in a 96 well plate format using 6-carboxyfluorescein as the substrate. 6-carboxyfluorescein plus or minus varying concentrations of metabolites were incubated with OAT4 expressing cells for 10 minutes at RT before the reaction was stopped and free 6-carboxyfluorescein removed. Plates were read using a Molecular Devices Analyst HT plate reader.

	IC_{50} value (µM)	
Metabolite	**hURAT1**	**hOAT4**
M2	>30	12.2
M3	>30	>30
M4	>30	>30
M6	8.85	13.0

Reference ID: 3815621

The M4 metabolite, the metabolite present at the highest concentration in humans, does not appear to significantly affect the function of URAT1 or OAT4 at levels that might be present in humans.

The inhibitory effects of M6 on URAT1 and OAT4 are not physiologically relevant as the metabolite is not formed in humans.

Reference ID: 3815621

In vivo pharmacology

SR11-015: In vitro and in vivo anti-inflammatory activities of RDEA594, Version 2

This study report includes reference to 5 separate in vivo pharmacology studies. The results of these studies are summarized in Table 4. Briefly, oral treatment with RDEA594 was shown to have efficacy in the following rat models: (1) Rat air pouch model of monosodium urate crystal induced arthropathy, (2) rat model of monosodium urate-induced gouty arthritis, and (3) rat carrageenan paw edema model.

RDEA594 treatment was not efficacious in the OVA induced airway inflammation model in mice or the rat adjuvant induced arthritis model.

Reference ID: 3815621

Table 4. Summary of *in vivo* pharmacology studies conducted with RDEA594 in rats.

Study #	Study Title	Conducting laboratory	Brief summary
AIR-ARD-3	Evaluation of anti-inflammatory potency of compound RDEA594, alone or in combination with colchicine in a rat air pouch model of **crystal-induced arthropathy**	(b) (4)	▪ Model: injection with monosodium urate (MSU) into an "air pouch" created by injecting air subcutaneously into the nape of the neck of the rat . ▪ Rats were dosed with RDEA 594 (oral, 0.1, 1, 10 mg/kg/d) for 4 – 6 days prior to injection of MSU into the air pouch ▪ MSU injected into air pouch for 4 hours ▪ Air pouch exudate volume & cell content were measured ▪ RDEA594 at all doses significantly reduced the number of WBCs in the air pouch ▪ Exudate volume was significantly reduced at 10 mg/kg/day only
(b) (4)10-0145	Effect of testing compounds [RDEA594] on **monosodium urate-induced gouty arthritis** in SD rats		▪ Rats were injected in the right knee with monosodium urate crystals ▪ Once daily dosing of RDEA594 (60 mg/kg, oral) for 7 days significantly reduced right knee diameter vs. vehicle control ▪ No additive effect of RDEA594 (60 mg/kg) + colchicine (0.1 mg/kg).
(b) (4)10-0167	Efficacy of test article [RDEA594] in **OVA induced asthma** in BALB/c mice		▪ RDEA594 at 60 mg/kg (oral) had no significant effect on OVA induced airway inflammation in mice.
CPE-ARD-1	Evaluation of the anti-inflammatory activity of Ardea Biosciences, Inc. compound [RDEA594] in a rat model of **carrageenan-induced paw edema**		▪ Pre-treatment of oral RDEA594 at 60 mg/kg for 1 week prior to carrageenan challenge resulted in significant paw edema inhibition
AIA-ARD-4	Evaluation of the anti-inflammatory activity of Ardea Biosciences, Inc. compound [RDEA594] in a rat model of **antigen-induced paw edema**		▪ Rat model of adjuvant-induced arthritis (mycobacterium tuberculosis, heat-killed, intra-dermal inoculation at base of tail) ▪ RDEA594 (60 mg/kg, oral) starting 2 days prior to disease induction had no effect on arthritic index

Reference ID: 3815621

4.2 Secondary Pharmacology

<u>Study SR08-086</u>: (b) (4) Spectrum screen of RDEA594, Version 2

This study evaluated the ability of RDEA594 to inhibit specific radioligand binding to 169 different receptors. RDEA594 was tested at 100 µM. There were 10 receptors where ligand binding was inhibited by greater than 50% by 100 µM RDEA594. These receptors were further assayed to establish an IC_{50} value.

RDEA594 inhibited binding to the thromboxane A2 (Prostanoid TP) receptor with an IC_{50} of **4.9 µM.** RDEA594 inhibits ligand binding to the thromboxane A2 receptor with similar potency to its inhibition of URAT1 function (7.3 µM). This receptor is involved in thrombosis/hemostasis as well as modulation of the immune response.

Ardea tested the ability of RDEA594 to act as an agonist or antagonist at TP receptors in a separate in vitro functional assay with TP receptors expressed in HEK-293 cells (see discussion of *Study SR11-013* below). At a concentration of 100 µM, RDEA594 has no agonist effects at this receptor. RDEA594 (100 µM) showed minimal antagonism of this receptor as it inhibited the reference agonist response by approximately 15%.

Ardea further argues that the IC_{50} of 4.9 µM at TP receptors is not physiologically relevant given that RDEA594 is 98% protein bound in human plasma. However, the Division generally considers total drug concentrations when assessing safety rather than free drug concentrations. This approach is taken based on the consideration that free and bound drug are in equilibrium *in vivo* and any number of factors can alter this equilibrium.

Lesinurad also inhibits ligand binding to the angiotensin AT2 and prostanoid D2 (DP1) receptors with potencies that are approximately 4 and 6-fold lower than its potency at URAT1 (Table 5).

Reference ID: 3815621

Table 5. Sponsor's table of RDEA594 IC_{50} values for inhibition of ligand binding at selected off-target receptors.

Radiolabeled Ligand Binding Inhibition by RDEA594			
Receptor Assay	Species	% INH at 100 µM	IC_{50} µM
Prostanoid, Thromboxane A2 (TP)	Human	94	4.94
Angiotensin AT2	Human	84	31.2
Prostanoid DP	Human	74	41.8
Colchicine	Rat	65	71.4
Adrenergic α1D	Human	54	84.2
Retinoid X Receptor RXRα	Human	53	86.9
Androgen (Testosterone) AR	Rat	50	104
Purinergic P2γ	Rat	50	105
Somatostatin sst4	Human	42	>100

SR11-013: In vitro pharmacology: Functional assays – study of compounds 0495594 and (b) (4)

This study was conducted at (b) (4) to examine the ability of RDEA594 to act as a functional **agonist** or an **antagonist** at the **thromboxane A2 receptor (TP)**. Recombinant human TP receptors were expressed in HEK-293 cells. The reference agonist used was U44069 and the reference antagonist used was L 670596. The functional measurement was intracellular Ca^{2+} concentration (fluorimetry) after exposure of cells to reference ligand and/or varying concentrations of RDEA594 (30 nM – 100 µM).

RDEA594 had no agonist effect at TP receptors at up to 100 µM. 100 µM RDEA594 showed slight inhibition of the agonist induced response (~-15% vs. control) at expressed TP receptors. There was no evidence of antagonism at any other dose tested.

Reference ID: 3815621

SR11-015: In vitro and in vivo anti-inflammatory activities of RDEA594, Version 2

The in vivo portions of this study report are discussed in Section 4.1: In vivo pharmacology above. The study report also includes **in vitro** studies which examined the effects of RDEA594 on the functional activity of **TP receptors** and prostanoid **DP receptors.**

Consistent with Study SR08-086, RDEA594 (Lot 0904728) inhibited radioligand binding to **DP1** and **TP** with IC_{50}s of 6.5 and 3.2 µM respectively (Table 6).

The effects of RDEA594 on **TP receptor function** were tested in a rat aortic ring contraction assay. RDEA594 inhibited TP receptor mediated aortic contraction with a reported IC_{50} of 39.7 µM.

RDEA594 had no effect on TP receptor mediated **platelet aggregation** in rabbit platelet rich plasma at up to 100 µM.

Table 6. Sponsor's table summarizing results of additional *in vitro* studies conducted with RDEA594 at DP1 and TP receptors presented in Study SR11-015.

Target - Assay	Species	RDEA594 IC50 (µM)	SD	N
DP1 - Binding	Human	6.5	1.3	3
TP - Binding	Human	3.2	1.5	3
DP1 - Functional Cell-Based	Human	11.5	-	1
TP - Functional Cell-Based	Human	11.3	-	1
TP - Aortic Contraction	Rat	39.7	-	4[a]
TP - Platelet Aggregation	Rabbit	>100	-	2[b]

[a] Three independent experiments using a single drug concentration data supporting IC50: 99% inhibition at 100uM, 11%, and 14% inhibition at 10µM

[b] Two independent experiments single concentration data supporting estimated IC50 value: 2% and 7% inhibition at 100µM

Reference ID: 3815621

4.3 Safety Pharmacology

A complete battery of safety pharmacology studies has been conducted (see nonclinical review dated 6/18/10). The effects of RDEA594 on the cardiovascular, respiratory, CNS, renal, and gastrointestinal systems were examined. Specific studies included:

- in vitro hERG assay
- rabbit Purkinje fiber assay
- CNS function (rats)
- Respiratory system function (conscious monkeys)
- Cardiovascular system (conscious telemetered monkeys)
- Gastrointestinal motility (SD rats)
- Renal/urinary function (SD rats)

Adequate margins of safety were achieved in each study. Additional discussion of the results of safety pharmacology studies is found in Section 11.

Reference ID: 3815621

5 Pharmacokinetics/ADME/Toxicokinetics

Absorption, distribution, metabolism, and elimination studies for lesinurad have been conducted in rats, dogs, and monkeys primarily via the oral route of administration.

5.1 PK/ADME

Absorption

RDEA594 absorption characteristics were examined using an in vitro transport assay as well as in *in vivo* studies in rats and monkeys in which animals received a single oral or intravenous dose of [^{14}C]RDEA594.

Table 7. RDEA594 absorption parameters following a single oral dose of [14C]RDEA594 in rats and monkeys.

Species	Study	Sex	Dose (mg/kg)	C_{max} (µg/ml)	T_{max} (h)	$T_{1/2}$ (h)	F (%)
Rat	SR08-060	Male	20	33.3	0.25	3.39	75.3
		Female	20	28.7	0.25	3.98	71.1
Monkey	SR-059	Male	20	16.2	1.33	21.8	41.1

In vitro

Study SR09-066: Bidirectional transport (Papp) studies of RDEA594 on Caco-2 monolayers

The objective of this study was to investigate whether RDEA594 is a substrate for active transport in the Caco-2 monolayer efflux assay. RDEA594 was tested at 1, 10, and 100 µM.

The results showed higher basolateral – apical permeability than apical - basal permeability at all doses. The efflux ratios ranged from 2.92 – 3.50, indicating active transport of RDEA594.

Table 8. Sponsor's summary table of permeability and efflux ratio values for RDEA594 in Caco-2 cell monolayer.

Date:	October 29, 2009			
Compound		P_{app} A-B	P_{app} B-A	ER
Controls	Antipyrine	104.17	NA	NA
	mannitol	1.30	NA	NA
	digoxin	2.85	18.11	6.35
RDEA594	1 µM	4.40	12.82	2.92
	10 µM	4.80	13.19	2.75
	100 µM	4.44	15.54	3.50
digoxin	10 µM	3.32	21.22	6.40
	+ 60 µM verapamil	8.28	7.27	0.88
	+ 50 µM RDEA594	4.29	16.15	3.77

Reference ID: 3815621

Follow-up experiments examined whether the permeability glycoprotein (P-glycoprotein, MDR-1) is the transporter responsible for the efflux of RDEA594. RDEA594 was incubated with Caco-2 cells in the presence of MDR-1 inhibitors PSC833 and verapamil. The results of these experiments were somewhat equivocal but suggested that MDR-1 was a minor contributor to the active transport of REA594 through Caco-2 monolayers.

Reference ID: 3815621

Rats

Study SR08-060: Pharmacokinetic evaluation of RDEA594 and [^{14}C]RDEA594-derived radioactivity in rat plasma or whole blood following a single oral or intravenous administration of [^{14}C]RDEA594

A single 20 mg/kg oral or IV dose of [^{14}C]RDEA594 was administered to male and female Sprague-Dawley rats. Blood was collected from 3 animals per sex per time point (total *n* = 9/sex/route) at the following time points after dosing: 5 min (IV only), 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 hours post-dose. Blood and plasma samples were analyzed for radioactivity content by liquid scintillation counting. Plasma was analyzed for RDEA594 via LC/MS/MS.

RDEA594 was absorbed quickly after oral administration, with T_{max} = 0.25 hr in males and females (Table 9). The volume of distribution after IV dosing was 0.931 L/kg in males and 0.546 L/kg in females. Given an estimated body weight of 0.25 kg for rats, the volume of distribution was ~230 ml and 137 ml in males and females respectively. These volumes are close to the total body water volume of 167 ml in rats[3]. Systemic RDEA594 clearance (~0.2 L/hr/kg) was markedly lower than hepatic blood flow in rats (~3.3 L/hr/kg) [3].

Oral bioavailability was 75.3% in males and 71.1% in females. There was no evidence for differences in systemic exposure between males and females after oral or IV dosing.

Table 9. Summary of pharmacokinetic parameters after a single oral or IV lesinurad dose (20 mg/kg) in rats.

Route	Dose (mg/kg)	Gender	T_{max} (hr)	T_{last} (hr)	C_{max} (µg/mL)	C_{last} (µg/mL)	AUC_{0-t} (µg·hr/mL)	$AUC_{0-\infty}$ (µg·hr/mL)	CL (L/hr/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (hr)	F (%)
PO	20	Male	0.250	36.0	33.3	0.00370	73.6	73.6	NE	NE	3.39	75.3
		Female	0.250	36.0	28.7	0.00600	81.7	81.8	NE	NE	3.98	71.1
IV	20	Male	0.0833	72.0	60.9	0.0061	97.7	97.8	0.205	0.931	7.40	NA
		Female	0.0833	72.0	62.3	0.0033	115	115	0.174	0.546	5.06	NA

NA: Not applicable
NE: Not estimated for oral dosing

Pharmacokinetic parameters of RDEA594 in rats after multiple doses

[3] Davies & Morris (1993) Physiological parameters in laboratory animals and humans. *Pharmaceutical Research*. 10, 1093 – 1095.

Reference ID: 3815621

Toxicokinetic data from repeat dose oral studies in rats of durations of 14 days (SR08-015), 28 days (SR08-034), and 6 months (SR08-095) has been provided and reviewed previously (See IND 102128 nonclinical reviews dated 6/18/10, 4/29/11 and 12/24/12).

In the chronic rat toxicology study (SR08-095), RDEA594 was administered at 10, 30, 100, 300, and 600 mg/kg/d doses. Systemic RDEA594 exposure increased with increasing dose on day 1 and at the 3 month and 6 month time points. T_{max} ranged from 1 – 6 hours.

At the two lowest doses (10 mg/kg/d and 30 mg/kg/d), males had higher systemic exposure than females at all time points. There were no differences in exposure between males and females at doses ≥ 100 mg/kg/d. There was evidence of slight accumulation (1.31 – 1.78) at the 10 mg/kg and 30 mg/kg doses in males and females after 3 and 6 months of treatment.

However, systemic RDEA594 exposures were lower in 100 mg/kg or 300 mg/kg animals at both 3 months and 6 months compared to day 1 (accumulation ratio: 0.62 – 0.92). This evidence suggests potential liver enzyme induction at doses ≥ 100 mg/kg. Systemic exposure in rats in the 100 mg/kg and 300 mg/kg dose groups remained consistent between the 3 and 6 month time points, suggesting that steady state exposure was achieved by 3 months of dosing.

Reference ID: 3815621

Monkeys

Study SR08-059: Pharmacokinetic evaluation of RDEA594 and [^{14}C]RDEA594-derived radioactivity in cynomolgus monkey plasma or whole blood following a single oral and intravenous administration of [^{14}C]RDEA594.

A single dose of [^{14}C]RDEA594 was administered to male cynomolgus monkeys (*n* = 3/route) via the oral (20 mg/kg) or IV (10 mg/kg) route of administration. Blood was collected from all monkeys at the following time points: Predose, 5 min (IV only), 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 hours post-dose. Blood and plasma samples were analyzed for radioactivity content by liquid scintillation counting. Plasma was analyzed for RDEA594 via LC/MS/MS.

The RDEA594 T_{max} after oral dosing was 1.33 hours (Table 10). Mean clearance after IV administration (0.173 L/hr/kg) was lower than monkey hepatic blood flow[3] (~2.6 L/hr/kg). The mean volume of distribution was established at 0.289 L/kg. This is equivalent to 1.45 L (based on average weight of 5 kg), below the total body water volume of ~3.5 L in monkeys. The oral bioavailability of RDEA594 was calculated to be 41.1% in monkeys.

Table 10. Summary of relevant pharmacokinetic data after a single oral or IV dose of [^{14}C]RDEA594 to male cynomolgus monkeys (Study SR08-059).

Route	Dose (mg/kg)	T_{max} (hr)	C_{max} (µg/ml)	AUC_{0-t} (µg*hr/ml)	$t_{1/2}$ (hr)	V_D (L/kg)	CL (L/hr/kg)	F (%)
Oral	20	1.33	16.2	50.0	21.8	NA	NA	41.1
IV	10	0.083	97.8	59.4	43.8	0.289	0.173	NA

Pharmacokinetic parameters of RDEA594 in monkeys after multiple doses

Toxicokinetic data from repeat dose oral studies in rats of durations of 14 days (SR08-016), 28 days (SR08-035), and 12 months (SR08-094) has been provided and reviewed previously (See IND 102128 nonclinical reviews dated 6/18/10, 4/29/11 and 12/24/12).

In the chronic monkey study (SR08-094), T_{max} was observed to range from 1.0 h to 4.0 hrs. Systemic exposure and C_{max} were similar in males and females, and increased with increasing dose. Values for RDEA594 AUC decreased markedly over time (accumulation ratios ranged from 0.14 – 0.37) starting at week 3 and persisted to the end of the study. This evidence suggests treatment-induced liver enzyme induction (autoinduction). An exploratory study on plasma samples from animals dosed with 600 mg/kg/d was carried out to identify potential metabolites of RDEA594. It was determined that metabolite **M6**, an S-dealkylated metabolite of RDEA594 was the major RDEA594 metabolite formed in monkeys.

Reference ID: 3815621

Distribution

In vitro

Study SR08-045: Evaluation of the plasma protein binding potential of RDEA594 in mouse, rat, dog, monkey and human plasma by equilibrium dialysis

This study examined the extent of in vitro protein binding of RDEA594 to C57BL/6 mouse, Sprague-Dawley rat, cynomolgus monkey, beagle dog, and human plasma by equilibrium dialysis. Dialysis experiments were conducted in a Pierce Equilibrium Dialysis Device.

Plasma from each species was spiked with [^{14}C]RDEA594 and unlabeled RDEA594 to achieve concentrations of 1, 10, and 50 µM. Concentrations up to 1,000 µM were tested in rat and monkey plasma. 500 µl PBS was loaded into the buffer chamber while 300 µl of plasma + [^{14}C]RDEA594 solution was loaded into the sample chamber. Assays were conducted in triplicate and proceeded over the course of 20 h at 37°C. 100 µl aliquots were removed from each chamber and analyzed by liquid scintillation counting. The % free RDEA594 concentration was calculated as [RDEA594 in buffer chamber]/[RDEA594 in plasma sample chamber], while % bound is equivalent to: 100% - percent free.

RDEA594 is highly protein bound (~98% bound) across all species tested (Table 11).

Table 11. Sponsor's table summarizing % plasma protein binding data for [^{14}C}RDEA594 across species.

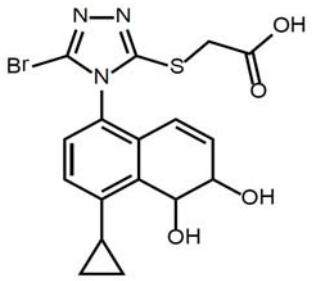
Species	% Protein Binding (Mean ± SD, N=3)					
	1 µM	10 µM	50 µM	200 µM	500 µM	1000 µM
Mouse	95.2 ± 0.45	94.9 ± 0.35	94.0 ± 0.15	NM	NM	NM
Rat	97.7 ± 0.34	98.1 ± 0.09	97.7 ± 0.01	97.6 ± 0.11	96.4 ± 0.06	95.1 ± 0.18
Dog	98.3 ± 0.06	98.2 ± 0.11	98.1 ± 0.13	NM	NM	NM
Monkey	98.2 ± 0.15	98.3 ± 0.03	98.2 ± 0.10	97.8 ± 0.06	97.3 ± 0.02	NM
Human	98.5 ± 0.06	98.4 ± 0.02	97.9 ± 0.17	NM	NM	NM

NM: not measured

Reference ID: 3815621

<u>Study SR12-039</u>: Evaluation of the plasma protein binding of <u>RDEA594-M4</u> in human plasma by equilibrium dialysis

This study used equilibrium dialysis methods generally similar to those described in Study SR08-045 in order to establish the extent of protein binding of the M4 (didhydrodiol) metabolite (Figure 3) of RDEA594 to human plasma protein. M4 is a disproportionate human metabolite, representing approximately 20.7% of the initial dose (Table 22).



M4

Figure 3. Chemical structure of M4 metabolite

Human plasma samples were spiked with M4 to achieve final concentrations of 0.5, 2, and 5 µM. Dialysis was conducted with plasma + M4 in the sample chamber and PBS in the buffer chamber for 4 hours at 37°C. [^{3}H]propranolol protein binding was measured as a positive control. M4 levels in plasma and PBS were determined by LC-MS/MS.

The M4 metabolite was approximately **24% protein bound** across all concentrations tested.

<u>Study SR08-046</u>: The quantitative tissue distribution of total radioactivity in the male rat following single oral and single intravenous administration of [^{14}C]RDEA594

<u>Methods</u>

The study was conducted in 3 phases:

- Phase 1: Albino male Sprague-Dawley rats received a single **Oral** dose of 20 mg/kg [^{14}C]RDEA594. Animals were sacrificed at 2, 4, and 24 h post dose (3 rats/time point). Quantitative whole body phosphor imaging (QWBPI) was conducted on whole body sections
- Phase 2: Albino male Sprague-Dawley rats received a single **IV** dose of 10 mg/kg [^{14}C]RDEA594. Rats were sacrificed at 0.5 and 4 h post dose (3 rats/time point).
- Phase 3: Pigmented (Lister Hooded) male rats received a single **Oral** dose of 20 mg/kg [^{14}C]RDEA594. Animals were sacrificed at 2, 4, 24, 72, 168, and 336 h post dose (3 rats/time point).

Blood samples were taken from each animal immediately prior to sacrifice in order to measure [^{14}C]RDEA594 concentration. Animals were sacrificed by CO_2 narcosis at the specified time points after dose administration. They were then frozen rapidly by immersion in a mixture of hexane / solid CO_2 and set in a block of sodium carboxymethylcellulose. Sagittal whole body sections were cut at 40 µm.

Reference ID: 3815621

A Fuji model FLA5000 phosphor imager system was used to quantify radioactivity in whole body sections. Aida software (v3.27) was used to calibrate individual scans and to quantify radioactivity. Freeze dried sections were placed against a phosphor screen with calibration and QC standards. The screens were then enclosed in lead shielding and exposed for 18 h. Screens were scanned by laser and radioactivity in individual tissues was quantified using Aida software.

Results

Distribution after oral administration to pigmented rats

In the first 24 hours after oral dosing, radioactivity was seen to the greatest extent in the cecum, stomach, small intestine, large intestine, liver and kidney. These are expected findings for an orally administered drug. The highest tissue concentrations were observed at 4 h after dosing. It is also notable that radioactivity levels in urine were also high (maximum level recorded 2 h post dose). A representative image of the distribution of radioactivity in a pigmented rat at 4 h after dosing is seen in Figure 4.

Uptake into other tissues was limited. The kidney and liver were the only tissues which achieved tissue:blood ratios greater than 2. The thyroid gland showed an increase in tissue:blood ratio over the 4-24 h post dose period, providing evidence of slower distribution to, and elimination from, this tissue. All tissues had markedly decreased radioactivity by 24-h post dose and elimination was complete by 168 h. There was no evidence of binding to the pigmented tissues of pigmented rats

Reference ID: 3815621

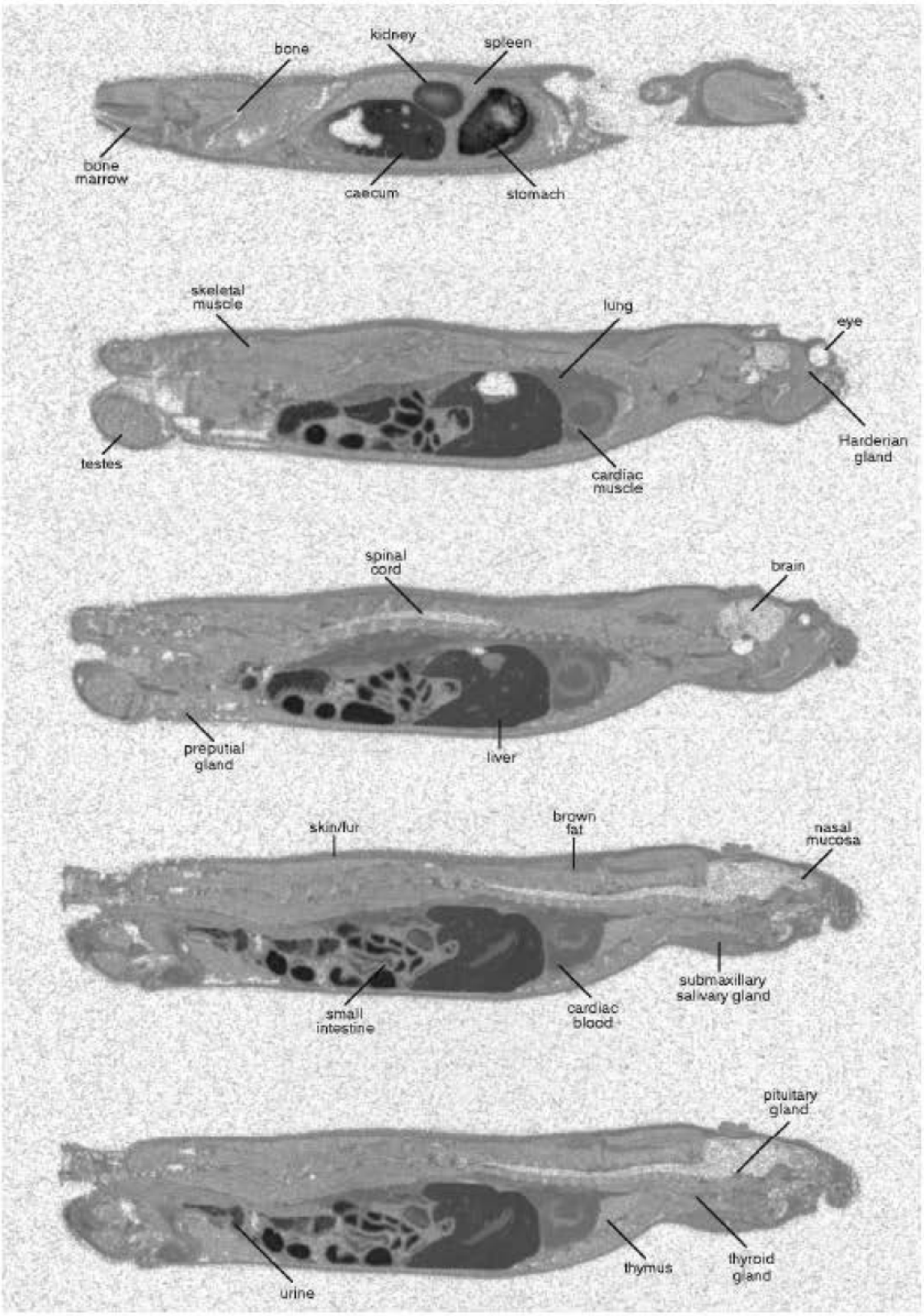


Figure 4. Distribution of radioactivity at t = 4 h after oral administration of 20 mg/kg [^{14}C]RDEA594 to pigmented rats. Representative images of sagittal whole body sections scanned using quantitative whole body phosphor-imaging.

<u>Study SR10-028</u>: Metabolite profile of radioactivity in plasma, excreta and bile of male cynomolgus monkeys following an oral gavage dose of [^{14}C]RDEA594

<u>Methods:</u> Two daily oral doses of 300 mg/kg [^{14}C]RDEA594 (80 µCi/kg) were administered to male cynomolgus monkeys (*n* = 8).

Blood, urine and feces were collected at established intervals.

	Time after dosing on day 1(h)	Time after dosing on day 2
Blood	predose and 0.25, 0.5, 1, 2, 4, 6, 8, 12 & 24 h post-dose	Immediately prior to termination
Urine	predose and 0 - 6, 6 – 12, 12 – 24 h post-dose	**Animals 229M & 231M only**: predose and 0 - 6, 6 – 12, 12 – 24 h post-dose
Feces	predose and 0 - 6, 6 – 12, 12 – 24 h post-dose	**Animals 229M & 231M only**: predose and 0 - 6, 6 – 12, 12 – 24 h post-dose

After sacrifice, any bile in the gall bladder was aspirated with a syringe and needle and acidified with formic acid. The liver and kidneys were removed intact from each carcass and weighed.

Plasma, urine, and cage wash solutions were mixed directly with scintillation cocktail for measurement of radioactivity. Samples from kidneys and liver were weighed, then homogenized with acetonitrile:water. Feces were weighed and homogenized with acetonitrile:water

Replicate samples of whole blood, feces, cage debris, kidney & liver tissue, and bile were placed on Combustocones and burned in oxygen using an automatic sample oxidizer. Combustion products were absorbed in Carbosorb E and mixed with Permafluor E+ for measurement of radioactivity concentrations.

<u>Results</u>: The whole blood: plasma ratio ranged from 0.52 – 0.57, providing evidence that there was little to no radioactivity distributed into blood cells.

Radioactivity was greatest in plasma at between 2 – 6 h after dose 1, and was nearly completely removed at 24 h.

Mean concentrations of radioactivity in bile, kidneys, and liver were greatest at 2 hours after the 2nd dose (Table 13). Bile contained by far the highest levels of radioactivity. Only a small fraction of the initial dose was detected in kidney and liver tissue (0.2% and 1.6% of initial dose respectively) at this time point (Table 12).

Radioactivity levels in bile were still high at 24 hours following the second dose of [^{14}C]RDEA594 suggesting that enterohepatic circulation may be occurring. This is consistent with the results of study SR09-056, which showed evidence for this phenomenon in rats.

Reference ID: 3815621

Table 12. Mean percentage of total [14C]RDEA594 dose detected in kidneys and liver at time points following the 2nd of 2 daily oral doses of [14C]RDEA594 to male cynomolgus monkeys. *n* = 2 animals per time point.

Tissue/organ	µg equivalents RDEA594 free acid/g			
	Day 2: 2 h	Day 2: 6 h	Day 2: 12 h	Day 2: 24 h
Whole blood	176	73.5	8.71	16.0
Bile	17700	13900	5560	6560
Kidneys	388	198	33.8	21.9
Liver	428	187	21.1	50.3

Table 13. Mean concentrations of radioactivity in tissues at time points following the 2nd of 2 daily oral doses of [14C]RDEA594 to male cynomolgus monkeys. *n* = 2 animals per time point.

Tissue/organ	Total % dose per organ			
	Day 2: 2 h	Day 2: 6 h	Day 2: 12 h	Day 2: 24 h
Kidneys	0.2304	0.1074	0.0278	0.0184
Liver	1.546	0.8669	0.1086	0.3037

<u>Study SR10-029</u>: Metabolite profile of radioactivity in plasma, excreta and bile of male cynomolgus monkeys following 28 day repeated oral gavage doses of [^{14}C]RDEA594

<u>Methods:</u> The study was conducted in a similar manner to study SR10-028 above. The main exception is that monkeys (*n* =12) were dosed once daily with 300 mg/kg/day RDEA594 (oral) for 25 days. 300 mg/kg/day [^{14}C]RDEA594 was administered for an additional 3 days to allow for a total dosing period of 28 days. Sample collection and analysis methods were generally the same as those in SR10-028.

<u>Results</u>:
All animals showed slight to moderate levels of salivation and retching.

The overall results after 28 days of treatment were consistent with the results after 2 days of dosing. Maximal radioactivity in plasma was observed between 2 – 6 hours post dose on day 28.

Mean concentrations of radioactivity in bile, kidneys, and liver were greatest at 2 hours after the final dose, with highest concentrations by far in bile (Table 15). Only a small fraction of the initial dose was detected in kidney and liver tissue (0.2% and 1.1% of initial dose respectively) at this time point (Table 14).

Reference ID: 3815621

Table 14. Mean percentage of total [^{14}C]RDEA594 dose detected in kidneys and liver at time points following 28 daily oral doses of [^{14}C]RDEA594 to male cynomolgus monkeys. *n* = 2 animals per time point.

Tissue/organ	µg equivalents RDEA594/tissue g			
	Day 28: 2 h	Day 28: 6 h	Day 28: 12 h	Day 28: 24 h
Whole Blood	60.9	43.1	18.5	12.7
Bile	12800	14500	5500	3940
Kidney	329	157	74.4	23.4
Liver	265	131	49.2	39.4

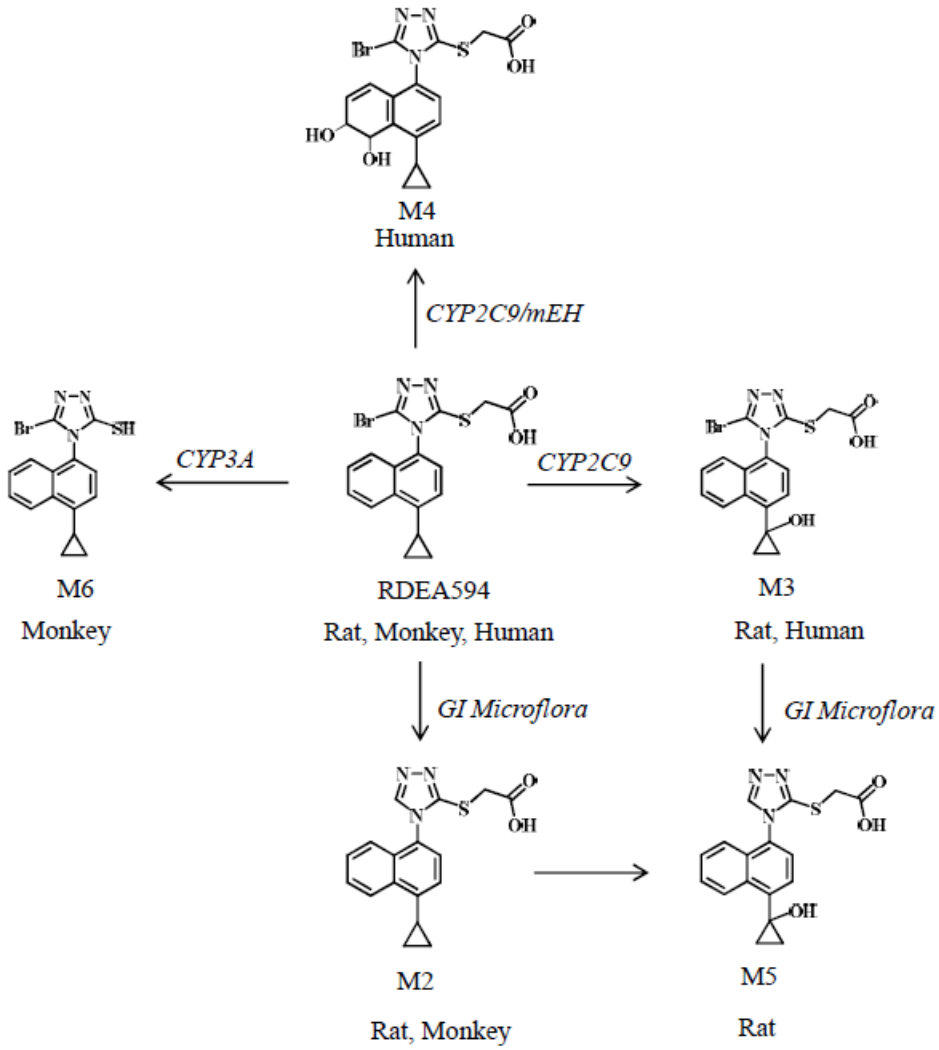
Table 15. Mean concentrations of radioactivity in tissues at time points following the 28 daily oral doses of [^{14}C]RDEA594 to male cynomolgus monkeys. *n* = 2 animals per time point.

Organ	Total % dose per organ			
	Day 28: 2 h	Day 28: 6 h	Day 28: 12 h	Day 28: 24 h
Kidney	0.1808	0.1058	0.0505	0.0150
Liver	1.142	0.5713	0.2720	0.1844

Reference ID: 3815621

Metabolism

The structures of the major metabolites observed in humans, rats and monkeys are seen in Figure 5. All metabolites observed in humans were also observed in at least one nonclinical species.



Abbreviations: CYP, cytochrome P450; GI, gastrointestinal; mEH, microsomal epoxide hydrolase

Figure 5. Structures of relevant RDEA594 metabolites in rats, monkeys and humans.

Reference ID: 3815621

In vitro metabolism

Study SR08-056: Evaluation of in vitro metabolism of [^{14}C]RDEA594 in liver microsomes and cryopreserved hepatocytes from animals and humans

The in vitro metabolism of RDEA594 was tested using cryopreserved hepatocytes and liver microsomes derived from humans, dogs, monkeys, and rats.

- Microsomes

[^{14}C]RDEA594 (1 µM or 30 µM) was incubated with microsomes for 60 mins at 37°C and the reaction terminated by addition of acetonitrile. Samples were centrifuged and the supernatant analyzed by HPLC with β-Ram detection or MS.

- Hepatocytes

[^{14}C]RDEA594 (5 µM) was incubated with hepatocytes (0.5 ml; 2 million cells/ml) in a 24-well tissue culture plate at 37°C under an atmosphere of 95% O_2 / 5% CO_2 for 4 hours. The reaction was stopped by adding the contents of each well to a centrifuge tube containing acetonitrile. The mixture was centrifuged and samples of the supernatant were analyzed by HPLC-MS/MS.

Results:

RDEA594 was very stable upon incubation with liver microsomes from all species tested. The only evidence for RDEA594 metabolism in this setting was the formation of the M3 metabolite in monkeys at 7.9% of the total dose.

Both the M3 and M4 metabolites (Figure 5, Table 16) were detected at low levels after incubation with monkey and human hepatocytes. M3 and M4 were not detected after incubation with rat hepatocytes.

Table 16. RDEA594 metabolite formation in liver microsomes and hepatocytes from multiple species.

Matrix	Species	% Radioactivity			
		M3	M4	RDEA594	Total
Microsome	Rat	-	-	100	100
	Dog	-	-	100	100
	Monkey	7.9	-	92.1	100
	Human	-	-	100	100
Hepatocytes	Rat	-	-	100	100
	Dog	-	-	100	100
	Monkey	1.45	0.47	98.1	100
	Human	2.24	5.69	92.1	100

Reference ID: 3815621

<u>Study SR11-031</u>: Identification of liver microsomal enzymes responsible for oxidative metabolism of RDEA594

In vitro experiments were conducted to identify the enzymes involved in the formation of oxidative metabolites of RDEA594.

<u>Methods</u>:

RDEA594 was incubated with 14 different recombinant **human** CYPs (1A1, 1A2, 2A6, 1B1, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 3A4, 3A5, 2E1, and 2J2) as well as human liver microsome preparations in the presence or absence of specific enzyme inhibitors. Human CYP Supersomes™ [redacted] (b) (4)) or human liver microsomes were incubated with RDEA594 (50 µM) for 60 mins at 37°C. Reactions were initiated by the addition of NADPH and terminated by the addition of chilled acetonitrile containing 0.5 µM [redacted] (b) (4) as an internal standard. The precipitated proteins were removed by centrifuge and the supernatant was analyzed by LC/MS/MS.

<u>Results:</u>

- *Metabolite M3*

Examination of RDEA594 metabolism across the panel of recombinant human CYPs revealed that **CYP2C9** is the major enzyme responsible for the formation of the M3, M3a, M3b, and M3c metabolites (Figure 6). This finding was confirmed in subsequent experiments in which human liver microsomes were incubated with RDEA594 + 50 µM sulfaphenazole (CYP2C9 inhibitor). In the presence sulfaphenazole, formation of M3, M3a, and M3b was inhibited by approximately 96%, 92%, and 99% respectively.

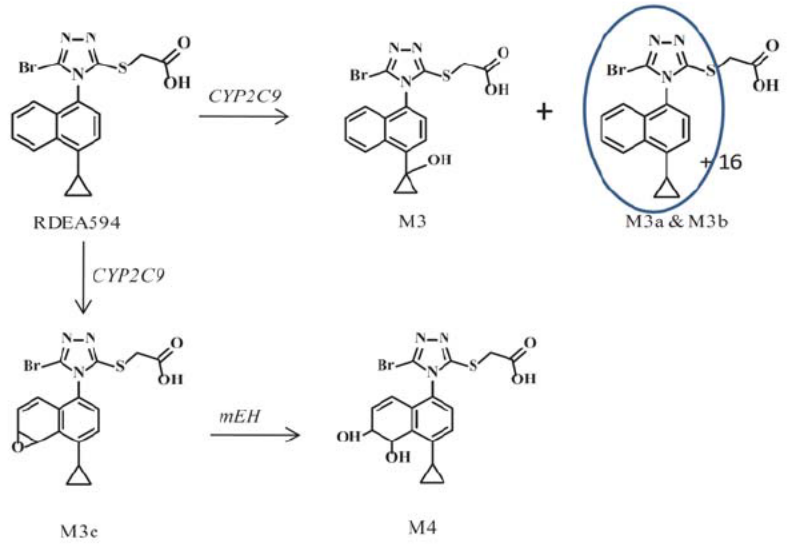


Figure 6. Sponsor's proposed metabolic pathway for formation of the M4 metabolite in humans. The M3c epoxide intermediate is formed via oxidation of RDEA594 by CYP2C9. M3c is then converted to M4 via the activity of microsomal epoxide hydrolase (mEH).

Reference ID: 3815621

- *Metabolite M4*

Metabolite M4, a dihydrodiol metabolite of RDEA594, is a major metabolite (20.7% of total dose) found in human urine and feces (Table 22). This metabolite was detected after incubation of RDEA594 in human liver microsomes but not after incubation with recombinant CYP2C9 enzyme alone.
Metabolite M3c (Figure 6), an epoxide intermediate between RDEA594 and M4, was detected following incubation with recombinant CYP2C9 enzyme but not after incubation with human liver microsomes. This led the sponsor to conclude that there may be more than one enzyme involved in conversion of RDE594 to M4.

RDEA594 was incubated with CYP2C9 plus 1 of 5 hydrolases including: Microsomal epoxide hydrolase (mEH), butyrylcholinsterase, esterase, carboxypeptidase, and leukotriene hydrolase for 60 mins at 37°C. The reactions were carried out as described above. M4 was formed in the presence of CYP2C9 + mEH only.

The results of these experiments led to the conclusion that the pathway for M4 formation involves the conversion of RDEA594 to the epoxide intermediate M3c by CYP2C9, followed by hydrolysis of M3c to M4 by mEH.

Computational toxicology assessment of metabolites M3c and M4 (September 26, 2014)
Quantitative structure-activity relationship (QSAR) model analysis conducted by CDER/OTS/OCP/DARS (*Derek Nexus* 4.0.5 (*DX*), *Leadscope Model Applier* 1.8.3-1 (*LMA*), and *CASE Ultra* 1.4.6.6 (*CU*) predicted that M3c would be positive for bacterial mutagenicity based on the presence of the epoxide functional group. Metabolite M4 was predicted to be negative for *Salmonella* mutagenicity. No call could be made for *E. coli* mutagenicity due to inadequate coverage of the structural features of M4 in the model training sets.

See Section 7.4 (Other Genetic Toxicity Studies) below.

Study SR12-026: Evaluation of RDEA594-M4 formation in induced rat liver microsomes and liver S9 fractions
Study SR08-056 identified M4 as a metabolite of RDEA594 in human and monkey hepatocytes, but not in rat microsomes or hepatocytes. Furthermore, Study SR11-031 identified that human CYP2C9 metabolizes RDEA594 to the M3c epoxide intermediate prior to further metabolism to M4 by epoxide hydrolase.

The current study examined whether M3c or M4 is produced in the presence of chemically induced rat liver subcellular fractions. The cell preparations included aroclor 1254 treated rat liver microsomes and β-naphthoflavone + phenobarbital treated rat liver S9 fractions.

Methods: 10 µM RDEA594 was incubated for 1 h at 37°C with 1 mg/ml rat liver microsomes (from + or – aroclor 1254 treated rats) or 6 mg/ml rat liver S9 fractions (from + or - β-naphthoflavone and phenobarbital treated rats). Incubations were terminated with the addition of acetonitrile. Samples were vortexed and the resulting supernatant was analyzed using LC/MS/MS analysis.

Reference ID: 3815621

Comparable experiments with human liver S9 fractions and microsomes were conducted as positive controls.

Results: M4 was not detectable upon incubation of RDEA594 with induced rat S9 or induced rat microsomes. M4 was detectable following incubation of RDEA594 with human liver S9 fraction or microsomes.

The M3c metabolite was not identified after incubation with either human or rat liver preparations. The inability to detect M3c in human liver preparations was likely due to its rapid metabolism to M4 by epoxide hydrolase.

Study SR08-048: In vitro evaluation of RDEA594 as an inhibitor of major human liver cytochrome P450 Isozymes

Pooled human liver microsomes were used to evaluate the inhibitory effects of RDEA594 at CYPs 1A2, 2C8, 2C9, 2C19, 2D6, and 3A4. The effects of RDEA594 were compared against the effects of known inhibitors of each isozyme.

Reversible Inhibition of CYP enzymes

RDEA594 was tested at concentrations ranging from 0.0152 – 100 µM. RDEA594 was mixed with substrate specific for each isozyme + microsomes and incubated at 37°C for 5 – 30 minutes. The reaction was stopped by the addition of 400 µl acetonitrile containing 10 µM labetalol. Reaction mixtures were centrifuged and the supernatant was analyzed by LC/MS/MS.

RDEA594 was found to inhibit **CYP2C8** and **CYP2C9** with IC_{50} values of 16.2 and 40.7 µM respectively (Table 17). The IC_{50} values for the other CYPs tested were > 100 µM. The IC_{50} for CYP2C8 is notable given that it is close to the observed clinical RDEA594 C_{max} of 7 µg/ml

Table 17. RDEA594 inhibitory effects on human CYP function in vitro (Study SR08-048).

P450 isoform	CYP1A2	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4 (testosterone)	CYP3A4 (midazolam)
IC_{50}* (µM)	>100	16.2 ± 4.15	40.7 ± 6.82	>100	>100	>100	>100

Substrates: CYP1A2 (phenacetin), CYP2C8 (amodiaquine), CYP2C9 (tolbutamide), CYP2C19 (S-mephenytoin), and CYP2D6 (bufuralol)

* Results are mean values ± standard error from three replicates (N = 3).

Time dependent inhibition

The potential for RDEA594 to function as a time-dependent inhibitor of CYP enzymes was tested at a concentration of 10 µM using a 30 minute pre-incubation at 37°C. Isoform specific probe substrates were then added and the incubations were left to continue for 5-30 minutes, according to the experimental conditions described above. Incubations containing 10 µM RDEA594 that were not subjected to preincubation were also included.

RDEA594 did not significantly inhibit any of the tested CYP enzymes in a time-dependent manner.

Reference ID: 3815621

SR12-043: In vitro evaluation of RDEA594 as an inhibitor of human cytochrome P450 2B6 isozyme

This study was carried out in human liver microsomes using similar methodology to that used in Study SR08-48 (above). RDEA594 was tested at concentrations from 0.016 – 100 µM. Buproprion was used as the CYP2B6 substrate.

RDEA594 did not inhibit CYP2B6 function at concentrations us to 100 µM.

SR10-002: Qualitative reaction phenotyping of UGT isozymes responsible for glucuronidation of RDEA594

The objective of this experiment was to identify the UGT isoforms responsible for glucuronidation of RDEA594. Two approaches were taken:

(1) Glucuronidation of RDEA594 by human UGT isozymes 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17
(2) Inhibition of RDEA594 glucuronidation in human liver microsomes by selective enzyme inhibitors

Methods:

(1) UGT supersomes expressing one of the above mentioned enzymes were incubated with 10 µM RDEA594 for 60 mins at 37°C. The reaction was stopped by addition of acetonitrile. Precipitated proteins were removed by centrifugation and the supernatant analyzed by LC/MS/MS

(2) UGT 1A1, 1A3, and 2B7 were selected as targets based on the results of Part 1. Therefore bilirubin, imipramine, and fluconazole were selected as chemical inhibitors for this experiment conducted in pooled human liver microsomes.

    Microsomes were incubated with ([^{3}H]RDEA594 + unlabeled RDEA594 (20 µM final concentration) plus the respective chemical inhibitor at 37°C for 60 mins. The reaction was stopped by the addition of acetonitrile. Precipitated proteins were removed by centrifugation and the supernatant was analyzed by HPLC with mass spectrometric detection and radioactivity detection.

Results:

RDEA594 glucuronide formation is low in human liver microsomes. A total of 4 glucuronides were detectable. The radioactivity of these metabolites combined was equal to approximately 3% of total radioactivity.

The enzymes **UGT1A1** and **UGT2B7** were identified as the major contributors to RDEA594 glucuronidation.

Reference ID: 3815621

SR10-001: In vitro evaluation of RDEA594 as an inhibitor of human UDP-glucuronosyltransferase isozymes 1A1 and 2B7
UGT1A1 and UGT2B7 enzymes were determined to be responsible for glucuronidation of RDEA594 in human liver microsomes (Study SR10-002).

Pooled human liver microsomes were used in the current study. β – estradiol (50 μM) and zidovudine (AZT, 1,000 μM) were used as the substrates for 1A1 and 2B7 respectively. RDEA594 was tested at concentrations from 5 – 1250 μM. Several other compounds, including probenecid, were also examined for their inhibitory potency at these enzymes. Reactions were carried out in a manner consistent with previous enzyme inhibition studies such as SR08-048.

RDEA594 inhibited UGT1A1 and 2B7 with IC_{50} values of 148 and 384 μM respectively (Table 18). While these IC_{50} values are lower than that for the approved URAT1 inhibitor probenecid, they are still well above the expected maximum plasma concentration of RDEA594 at the 200 mg MRHD.

Table 18. Summary of inhibitory effects of selected compounds on the function of UGT1A1 and UGT2B7.

UGT Isoform	Substrate (μM)	Test Compound	IC_{50} (μM)
UGT1A1	β-Estradiol (50 μM)	RDEA594	148
		Probenecid	1460
		Bilirubin	24
UGT2B7	Zidovudine (1000 μM)	RDEA594	384
		Fluconazole	3250
		Diclofenac	74.6
		Ibuprofen	508
		Indomethacin	395
		Probenecid	3050

Study SR10-063: In vitro evaluation of RDEA594 as an inducer of cytochrome P450 2B and 2C expression in cultured human hepatocytes.
The experiment tested the ability of RDEA594 to act as an inducer of human CYP2B6, CYP2C8, CYP2C9, and CYP2C19. The effects of treatment on enzyme RNA expression as well as specific enzyme function were measured in cultured human hepatocytes.

Methods: Cultured human hepatocytes were treated once per day for 3 consecutive days with RDEA594 at 10, 30, or 100 μM (experiment carried out in triplicate with hepatocytes cultured from 3 separate donors). Phenobarbital and rifampin (known CYP inducers) were included as positive controls.

24 hours after the final treatment, hepatocytes were either incubated with probe substrates (30 min at 37°C) to measure enzymatic function, or lysed for RNA isolation.

Reference ID: 3815621

Metabolites were quantified in functional experiments using LC/MS/MS methods. RNA levels in hepatocyte lysates were measured using quantitative RT-PCR.

Results:

Enzyme function

CYP2B6 and CYP2C19 function were increased aby ~2-3 fold over controls at all RDEA594 concentrations tested (Table 19). The maximal effect was observed at 30 µM. RDEA594 treatment increased CYP2C8 function at 10 and 30 µM, but was inhibitory at 100 µM.

The function of **CYP2C9**, the enzyme responsible for conversion of RDEA594 to M3 & M3c, was decreased by RDEA594 at both 30 and 100 µM. This is consistent with the evidence from Study SR08-048.

Table 19. CYP enzyme activity in cultured human hepatocytes after 3 consecutive days of treatment with RDEA594 (SR10-063). Values are presented as fold increase over negative controls.

Treatment	Concentration	Fold increase [a]			
		Bupropion hydroxylation (CYP2B6)	Amodiaquine *N*-dealkylation (CYP2C8)	Diclofenac 4′-hydroxylation (CYP2C9)	*S*-Mephenytoin 4′-hydroxylation (CYP2C19)
Dimethyl sulfoxide	0.1% (v/v)	1.00 ± 0.46	1.00 ± 1.05	1.00 ± 0.40	1.00 ± 0.99
RDEA594	10 µM	2.31 ± 0.58	2.38 ± 0.57	1.04 ± 0.15	1.98 ± 0.42
RDEA594	30 µM	3.15 ± 0.82	2.21 ± 0.57	0.638 ± 0.108	3.25 ± 0.52
RDEA594	100 µM	2.53 ± 0.59	0.482 ± 0.028	0.413 ± 0.116	2.04 ± 0.93
Phenobarbital	750 µM	8.77 ± 1.00	NA	NA	NA
Rifampin	10 µM	NA	9.94 ± 0.70	8.59 ± 10.68	6.04 ± 0.48

a Values are the mean ± standard deviation of three determinations (human hepatocyte preparations H989, H990 and H991).

NA Not applicable

Data are shown graphically in Figure 9. Individual data are shown in Appendix 3.

Enzyme mRNA expression

Treatment with RDEA594 at up to 30 µM increased the expression of CYP2B6, CYP2C8 and CYP2C9 mRNA by approximately 2 – 4 fold (Table 20). mRNA expression of these enzymes was lower in cultures treated with 100 µM than 30 µM RDEA594. mRNA expression of CYP2C19 was not changed at any RDEA594 dose level.

The evidence for RDEA594 induced increases in **CYP2C9** expression raises a potential concern given the established role of this enzyme in the metabolism of RDEA594 to the M3 and M3c (epoxide) metabolites. This concern appears to be mitigated by the fact that RDEA594 acts as a direct inhibitor of CYP2C9 activity at concentrations >30 µM (Table 19, Study SR08-048).

Reference ID: 3815621

Table 20. Effects of RDEA594 treatment on CYP enzyme mRNA expression in cultured human hepatocytes. Values expressed as fold-increases over controls.

Treatment	Concentration	Fold increase [a]			
		CYP2B6	CYP2C8	CYP2C9	CYP2C19
Dimethyl sulfoxide	0.1% (v/v)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
RDEA594	10 µM	1.74 ± 0.13	2.69 ± 0.55	2.13 ± 0.44	0.918 ± 0.196
RDEA594	30 µM	3.04 ± 1.01	4.18 ± 1.02	3.46 ± 0.93	1.01 ± 0.24
RDEA594	100 µM	1.27 ± 0.65	1.66 ± 0.69	2.57 ± 0.79	1.36 ± 0.27
Phenobarbital	750 µM	9.82 ± 5.56	NA	NA	NA
Rifampin	10 µM	NA	5.14 ± 2.28	3.93 ± 1.51	1.24 ± 0.61

a Values are relative to vehicle control, normalized to GAPDH and are the mean ± standard deviation of three determinations (human hepatocyte preparations H989, H990 and H991).

NA Not applicable

Data are shown graphically in Figures 2, 4, 6, 8 and 9. Individual data are shown in Appendix 3.

In vivo metabolism

Humans

Study RDEA594-112: An AME (Absorption, Metabolism and Excretion) study of [^{14}C]RDEA594 orally administered to healthy adult male volunteers.

Clinical study RDEA594-112 examined lesinurad metabolite formation in humans by administering a single oral dose of 600 mg [^{14}C]lesinurad to 6 healthy adult males. Blood, urine and feces samples were collected through 144 hours after dosing.

As seen in Table 21, plasma samples taken at 3 hours post-dose were comprised of mainly unchanged RDEA594 (93.1%). The major route of elimination was via urinary excretion (63.4%, Table 22).

Table 21. Lesinurad metabolites detected in human plasma, urine, and feces samples after a single 600 mg oral dose of [^{14}C]lesinurad. Values represent % total radioactivity. From Study RDEA594-112.

Matrix	Time (hr)	Radioactivity %										
		M1	M2	M3	M3b	M4	M5	M5b	M16	Others	Lesinurad	Total
Plasma	3	ND	ND	2.2	ND	2.0	ND	ND	ND	ND	93.1	97.3
Urine	0-24	2.4	0.4	18.9	1.5	24.8	ND	ND	0.8	1.9	49.3	100
Faeces	24-48	ND	15.7	0.9	7.9	17.2	9.2	25.7	5.1	11.4	6.9	100
Faeces	48-72	ND	14.5	0.7	4.9	14.9	12.2	23.5	2.5	23.3	3.5	100

Source: Pharmacokinetic Report RDEA594-112-MET (Appendix 16.2.5.3, Table 3)
ND = Not detected

While unchanged RDEA594 comprises the majority of the recovered radioactivity, there are 2 disproportionate human metabolites (>10% of total systemic exposure of parent compound): **M3** (hydroxyl, 12.3%) and **M4** (dihydrodiol, 20.7%). The formation of M4 implicates the presence of an epoxide (M3c), as described in the review of Study SR11-031 above.

Table 22. Lesinurad metabolites detected in human urine and feces over 6 days after a single 600 mg oral dose of [^{14}C]lesinurad . Values represent % initial dose. From Study RDEA594-112-MET.

Matrix	Time (hr)	Dose %										
		M1	M2	M3	M3b	M4	M5	M5b	M16	Others	RDEA594	Total
Urine	0-144	1.5	0.3	12.0	1.0	15.7	ND	ND	0.5	1.2	31.3	63.4
Feces	0-144	ND	4.8	0.3	1.9	5.0	3.6	7.8	1.1	7.5	1.5	33.5

Reference ID: 3815621

Mice

Study SR11-037: Evaluation of the metabolic profile of RDEA594 in urine and feces in wild type CBYB6F1-Tg(Hras)2JIC mice following a single oral administration of [^{14}C]RDEA594 at 100 mg/kg (60 µCi/kg)

Methods: Male and female wild-type TgRasH2 mice (*n* = 3/sex) were administered a single oral gavage dose of 100 mg/kg [^{14}C]RDEA594. Urine and feces were collected and analyzed by HPLC with radioactive detection for metabolite profiling and by LC/MS/MS for structural confirmation. Urine, and feces samples were taken at the following time points:

	Time after dosing (h)
Urine	-18 – 0 (predose), 0 - 24, 24 – 48, 48 – 72, 72 -96
Feces	-18 – 0 (predose), 0 - 24, 24 – 48, 48 – 72, 72 -96

Urine samples were analyzed directly by HPLC without further sample preparation. Feces samples were homogenized and extracted with ethanol/water. Supernatants were analyzed by HPLC.

Radioactivity in urine and feces samples was quantified using liquid scintillation counting.

Results: Recovery of most of the [^{14}C]RDEA594 related radioactivity occurred during the 0 – 24 hr time interval. Urine and feces samples from this period were subjected to metabolite profiling. The most abundant components included unchanged RDEA594 along with metabolites M2, M3, and other unidentified metabolites (Table 23). **Metabolite M4** was not detectable in significant quantities in excreta of males or females.

Table 23. Metabolite profile of RDEA594 in WT TgRasH2 mice over the course of 24 hours after a single oral dose (Study SR11-037).

Gender	Matrix	Time (hr)	% of Dose							
			M1	M2	M3	M4	M5	Other	RDEA594	Total
Male	Urine	0-24	NA[a]	0.7	0.9	Trace	0.1	0.1	11.8	13.6
	Feces	0-24	NA	26.4	1.2	NA	NA	4.2	15.5	47.3
Female	Urine	0-24	1.1	1.8	2.6	NA	NA	0.3	11.5	17.3
	Feces	0-24	NA	19.0	0.9	NA	NA	26.2	22.6	68.7

[a] NA = not applicable

Over the course of 96 hours after dosing, the majority of the [^{14}C]RDEA594 related radioactivity was eliminated by fecal excretion in males and females (69.3% and 71.3% respectively, Table 24).

Reference ID: 3815621

Table 24. Recovery of [^{14}C]RDEA594-related radioactivity in urine and feces after a single oral dose to WT TgRasH2 mice (SR11-037).

Group/Sex	Matrix	Interval Excretion (% of Dose)				Cumulative Excretion (% of Dose)			
		0–24 hr	24–48 hr	48–72 hr	72–96 hr	0–24 hr	24–48 hr	48–72 hr	72–96 hr
1/Male	Urine	13.6	2.1	0.52	0.06	13.6	15.8	16.3	16.4
	Feces	47.3	17.1	4.7	0.28	47.3	64.3	69.0	69.3
	Total	60.9	19.2	5.2	0.34	60.9	80.1	85.3	85.7
2/Female	Urine	17.3	0.36	0.11	0.15	17.3	17.7	17.8	17.9
	Feces	68.7	1.5	0.38	0.69	68.7	70.2	70.6	71.3
	Total	86.0	1.9	0.49	0.84	86.0	87.9	88.4	89.2

Reference ID: 3815621

Rats

Study SR08-120: Evaluation of the metabolic profile of [^{14}C]RDEA594 in rat plasma, urine, and feces following oral administration to male and female Sprague-Dawley rats

Methods: Male and female rats were administered a **single** IV (20 mg/kg) or oral (20 mg/kg) dose of [^{14}C]RDEA594. Plasma, urine, and feces samples were taken at the following time points:

Table 25. Times of sample collection from male and female rats after single dose of 20 mg/kg [^{14}C]RDEA594.

	Time after dosing (h)	
	IV	Oral
Plasma	0.5, 4	1, 4
Urine	0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120	
Feces	0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120	

Urine and feces samples from individual rats were pooled from 0 – 24 hours. The 0-24 hour pooled samples were then pooled again for rats of the same gender and route of administration. Separation of RDEA594 and metabolites were achieved using HPLC. Radioactivity was detected using an IN/US β-RAM detector coupled with an AIM X Flow ™ System.

Results: Unchanged RDEA594 was the only compound detected in plasma at 0.5 or 1 hr plasma samples (**Table 26**). The metabolite profile in urine and feces was generally consistent between males and females and between IV and oral dosing. Metabolites M2 and M3 were detected in urine and feces while metabolite M5 was only detected in feces. Metabolite M4 was not detected.

Reference ID: 3815621

Table 26. Metabolic profile of [^{14}C]RDEA594 administered PO or IV in SD rats (Study SR08-120).

Species	Gender	Route	Matrix	Time (hr)	Concentration %				
					M2	M3	M5	RDEA594	Total
Rat	M	PO	Plasma	1	0.0	0.0	0.0	100.0	100.0
			Urine	0-24	13.1	47.7	0.0	38.4	99.2
			Feces	0-24	34.6	9.8	30.1	24.2	98.7
		IV	Plasma	0.5	0.0	0.0	0.0	100.0	100.0
			Urine	0-24	10.4	55.0	0.0	26.9	92.3
			Feces	0-24	35.5	8.0	38.0	16.3	97.8
	F	PO	Plasma	1	0.0	0.0	0.0	100.0	100.0
			Urine	0-24	5.3	18.1	0.0	76.6	100.0
			Feces	0-24	43.7	2.3	13.7	32.6	92.3
		IV	Plasma	0.5	0.0	0.0	0.0	100.0	100.0
			Urine	0-24	4.1	10.2	0.0	85.4	99.7
			Feces	0-24	47.2	2.4	16.0	33.6	99.2

<u>Study SR10-021</u>: Plasma, urine and fecal metabolite profile, urinary and fecal excretion evaluation in male and female Sprague Dawley rats following a single oral dose of [^{14}C]RDEA594 at 300 mg/kg after <u>repeated</u> daily doses of RDEA594

<u>Methods:</u> Animals were given once daily oral doses of 300 mg/kg RDEA594 or vehicle for 35 days. On day 36, a total of 15 rats (n = 7 male, 8 female) were dosed with 300 mg/kg [^{14}C]RDEA594 (50 µCi/kg)

	Time after dosing (h)
Plasma	1, 4
Urine & feces	-18 – 0, 0-6, 6-12, 12-24, 24-48, 48-72, 72-96

Radioactivity was quantified in urine and feces samples by liquid scintillation counting.

For metabolite profiling: urine samples and feces homogenates from 0-24 hours were pooled and centrifuged. Supernatant was analyzed directly. RDEA594 and its metabolites were separated using HPLC. An IN/US β-RAM detector coupled with an AIM x Flow™ System was used in the metabolite profiling analysis.

Reference ID: 3815621

Results:
The majority of radioactivity was eliminated via the feces in male and female rats (71.2% and 60.7% respectively, Table 27).

Table 27. Recovery of [^{14}C]RDEA594-related radioactivity over the course of 96 hrs after a single 300 mg/kg oral dose to male and female rats. Dose was administered on day 36 after 35 consecutive days of unlabeled 300m/kg RDEA594 dosing (SR10-021).

Gender	Sample	Interval Excretion of Radioactivity (% of Dose)				
		0-6hr	6-12 hr	12-24 hr	0-24 hr	0-96 hr
Male	Urine	1.71	3.10	2.35	7.13	10.0
	Feces	0.56	1.40	39.1	41.1	71.2
	Total	2. 90	5.37	42.4	50.7	84.4
Female	Urine	2.60	7.38	6.42	16.4	19.9
	Feces	1.03	1.72	36.0	38.8	60.7
	Total	4.26	10.2	43.3	57.7	83.7

Unchanged RDEA594 was the main component observed in the plasma at 1 and 4 hours after dosing (Table 28). Unchanged RDEA594 was also the main component in feces in males and females after 24 hours. Metabolites M2 and M3, detected in urine and feces of males and females, were also observed in the single dose metabolism study (SR08-120). Metabolite M6 (S-dealkylated), also detected in urine and feces of male and female rats, represents a new metabolite that was not observed in the previous single dose rat metabolism study. This suggests that M6 formation may be mediated by an enzyme that is induced by repeated dosing with RDEA594. Metabolite M7 (methylated M6) was detected at low levels in the urine of male rats only.

Table 28. Metabolite profile of [^{14}C]RDEA594 in rat plasma, urine, and feces over 24 hours after a single dose of [^{14}C]RDEA594. Radiolabeled dose was administered on day 36 after 35 consecutive days of unlabeled 300m/kg RDEA594 dosing (SR10-021).

Matrix	Gender	Time (hr)	% of Total Radioactivity in Samples					
			M2	M3	M6	M7	RDEA594	Total
Plasma	Male	1	0	0	0	0	100	100
	Female	1	0	0	0	0	100	100
	Male	4	2.0	5.7	0	0	92.3	100
	Female	4	0.3	0	0	0	99.7	100
Urine	Male	0-24	2.46	53.7	2.24	1.0	40.0	100
	Female	0-24	1.86	18.0	0.63	0	79.5	100
Feces	Male	0-24	2.69	12.7	21.3	0	63.3	100
	Female	0-24	3.0	6.74	10.2	0	80.1	100

Reference ID: 3815621

Study SR09-070-MET: Exploratory non-GLP plasma metabolic profiling of TK samples in 24 month rat carcinogenicity study

Methods: Blood samples taken for TK analysis from HD animals on Day 1 (6 hrs post-dose), week 26 (0, 1, 3, 6, 12, 24 hrs post-dose) and week 52 (same as week 26) were assayed for detection of RDEA594 metabolites. Metabolites were identified based on HPLC retention time, mas-to-charge ratio, and MS/MS fragmentation. Metabolite profiles in urine and feces were not determined in this study.

Results: The parent compound, RDEA594, is the major component in plasma on day 1 and after 26 or 52 weeks of dosing. Metabolites identified at the 6 hr time point (T_{max}) on day 1 were M2 and M3. At weeks 26 and 52, metabolites M6, M8, M13, and M20 were also identified (Figure 7).

The most notable finding from this analysis is that the major human metabolite, M4, was not detected in vivo in rats. Detection of this metabolite in the rat carcinogenicity study could have contributed to safety qualification of this metabolite with respect to carcinogenicity. The safety qualification of M4 is discussed in the complete nonclinical review of study SR09-070 (Whittaker, 7/29/15).

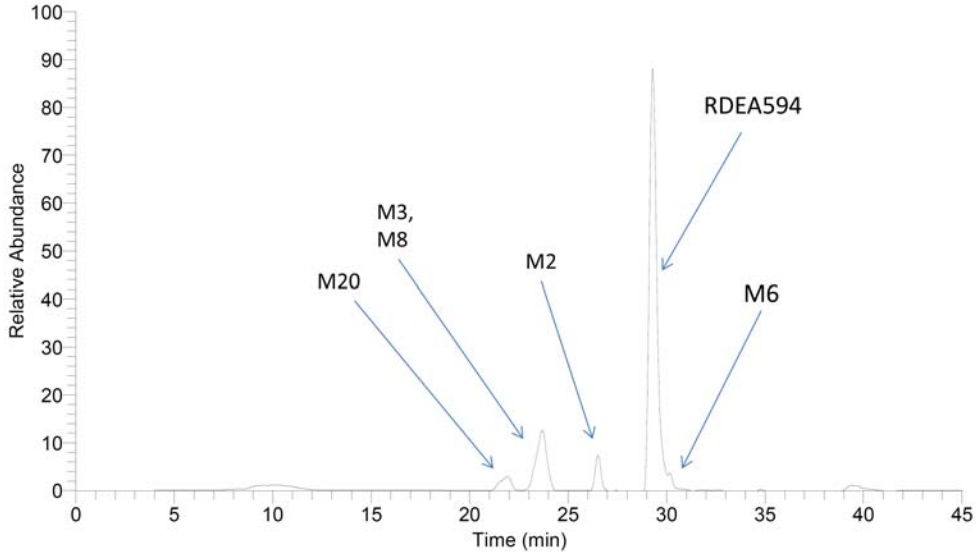


Figure 7. Representative ion chromatogram form LC-MS analysis of plasma metabolites in male rats at 6 hours post dose in week 52 of study SR09-070.

Reference ID: 3815621

Monkeys

Study SR08-119: In vivo metabolism study of RDEA594 in monkey plasma, urine and feces following single intravenous or oral administration to male cynomolgus monkeys.

Methods: Cynomolgus monkeys were treated with a single IV (10 mg/kg) or oral (20 mg/kg) dose of [^{14}C]RDEA594.

Samples were collected at the following time points

	Time after dosing (h)	
	IV	Oral
Plasma	1, 4	2, 4
Urine	0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120	
Feces	0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120	

For metabolic profiling, urine and feces samples from individual monkeys were pooled from 0 – 24 hours. Separation of RDEA594 and metabolites were achieved using HPLC. Radioactivity was detected using an IN/US β-RAM detector coupled with an AIM X Flow ™ System.

Results: Unchanged RDEA594 is the major component in plasma and urine after IV or oral dosing (Table 29). Metabolite M2 (debrominated RDEA594) was found to be the major component in feces after dosing by either route. Metabolite M3 was detected at low levels in urine and feces after IV or oral dosing.

Table 29. Metabolic profile of [^{14}C]RDEA594 administered in a single PO or IV dose to male cynomolgus monkeys (Study SR08-119).

Route	Matrix	Time (hr)	Concentration %					
			M1	M2	M3	M4	RDEA594	Total
PO	Plasma	2	0	0	0	0	100	100
	Urine	0-24	<1	4.1	0.9	0.5	89.9	98.9
	Feces	0-24	<1	69.6	1.4	0	24.4	95.4
IV	Plasma	1	0	0	0	0	100	100
	Urine	0-24	0	1.6	1.9	0	96.5	100
	Feces	0-24	<1	77.4	<1	0	12.3	91

Reference ID: 3815621

Study SR10-029-MET: [^{14}C]RDEA594 metabolite profile of radioactivity in plasma, excreta and bile of male cynomolgus monkeys following 28-day repeated oral gavage doses of 300 mg/kg RDEA594

Methods:
Monkeys (*n* =12) were dosed once daily with 300 mg/kg/day RDEA594 (oral) for 25 days. 300 mg/kg/day [^{14}C]RDEA594 was administered during days 26 – 28 to allow for a total dosing period of 28 days. Monkeys were divided into 4 groups of 3 (groups A, B, C, and D). On day 28, monkeys were sacrificed at 2 (Group A), 6 (Group B), 12 (Group C), and 24 hr (Group D) post dose.

Samples collected for metabolite profiling included plasma, urine, feces, bile and liver. Samples were collected as follows:

Sample type	Group	Sampling times
Blood	A, B, C, D	Day 28: pre –terminal & post-terminal
Urine & Feces	D	Day 28: 0 – 6, 6 - 12, 12 – 24 h post dose
Bile & Liver	A, B, C, D	Day 28: 2, 6, 12, 24 h post dose

Bile present in the gall bladder was aspirated with a syringe and needle and immediately acidified with formic acid. Liver homogenates were prepared with liver samples + acetonitrile:water (1:1 ratio). Samples from each animal were pooled for each respective time point prior to analysis.

Liquid scintillation counting was conducted to measure radioactivity. RDEA594 and its metabolites were separated and identified using HPLC-MS/MS.

Results:
RDEA594 was eliminated primarily in feces in monkeys (62.5%, Table 30). Unchanged parent compound was the major component detected in plasma, urine, feces, and liver.

The M1 glucuronide was the major component observed in bile (Table 30, Figure 8). The observation that RDEA594 was the major component in feces suggests that M1 secreted into bile was likely metabolized back to RDEA594 in the intestine allowing for enterohepatic recirculation of RDEA594.

The M9a and M9b metabolites (Cysteine conjugates of M3c epoxide; Figure 8) were detected in monkeys, but not in mice, rats, or humans. M9 comprised approximately 8.85% of the total radioactivity detected, and was observed mainly in bile and feces. Ardea proposes that RDEA594 is metabolized by CYP2C9 to the M3c epoxide intermediate in monkeys. M3c subsequently undergoes cysteine conjugation to form metabolite M9.

Reference ID: 3815621

Table 30. Distribution of metabolites in male cynomolgus monkeys at the conclusion of 28 days of 300 mg/kg RDEA59 treatment ([^{14}C]RDEA594 was administered on days 26 – 28, Study SR10-029-MET).

Matrix	Time (hr)	*Mean Tissue Weights (g)	*Mean Concentration (µg eq/gram)	% of Dose In Matrix*	% of Dose								
					M1	M2	M3	M4	M9	M10	Others	RDEA594	Total
Bile	2	2.338	12800	NA	43.7	1.43	4.47	0.09	7.93	0.65	2.92	23.4	84.6
	6	1.199	14500	NA									
	12	1.055	5500	NA									
	24	1.214	5940	NA									
Urine	0-24	NA	NA	11.0	0.12	0.50	1.50	0.26	0.03	0.04	1.84	6.69	11.0
Feces	0-24	NA	NA	62.49	0.35	3.18	0.76	0.00	0.54	0	1.57	56.1	62.5
Liver	2	32.95	265	1.142	0.23	0.12	0.042	0.005	0.055	0	0.013	0.72	1.19
	6	31.82	131	0.5713	0.050	0.032	0	0	0.026	0.008	0	0.45	0.57
	12	41.00	49.2	0.2720	0.039	0.046	0.005	0.004	0	0	0.007	0.18	0.28
	24	35.67	39.4	0.1844	0.026	0.028	0.002	0	0	0	0	0.12	0.18

The mean body weight of animals was 2.5 kg, the mean daily dose 736 mg.
*Data source: reference [2] and Appendix 1.
Others include M3a, M3b, M4a, M5, M5b, M16, M17, M18 and unidentified metabolites

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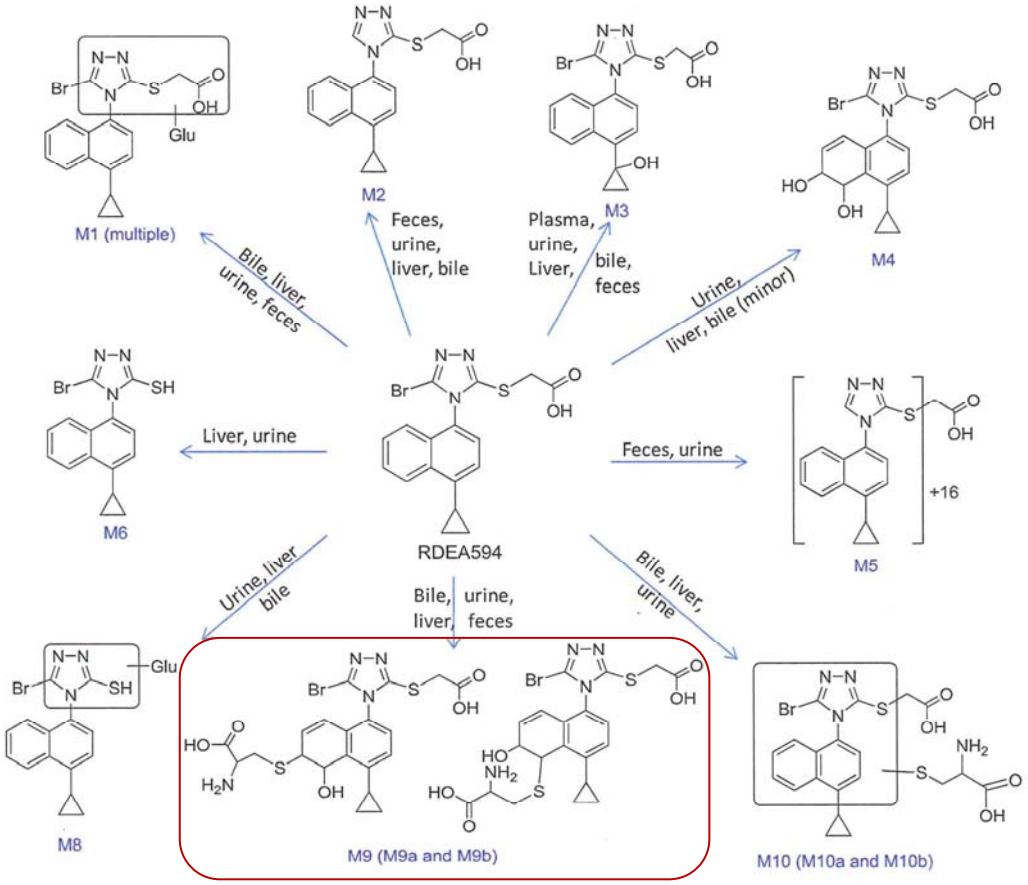

Figure 8. Chemical structures of all RDEA594 metabolites in monkeys identified after 28 days of treatment. Note the M9a and M9b metabolites. According to the sponsor, these represent derivatives of the M3c epoxide intermediate (Study SR10-029).

<u>Study SR08-094-MET</u>: RDEA594: A 12-month repeat dose toxicity and toxicokinetic study with a 3-month recovery period and a 6-month pull in cynomolgus monkey

This study was reviewed previously (IND 102128, nonclinical review dated 12/24/12). Briefly, plasma samples from animals dosed at 600 mg/kg/day (6 males, 6 females) taken at day 1 week 3, and week 50 were analyzed by LC/MS/MS. In addition, TK samples from a single 600 mg/kg male (#051049) were taken at day 1, week 3, week 12, week 24, week 37 and week 50.

On day 1, RDEA594, M2 and M3 were the only compounds detected. By week 50, multiple Phase I and Phase II metabolites of RDEA594 were detectable in plasma samples, most notably the S-dealkylated metabolite M6 (Figure 8). Additional metabolites detected were sequential metabolites of M6 (Table 31). These included derivatives resulting from M6 glucuronidation (M8), debromination (M13), desulfuration (M15), and oxidation (M14).

Reference ID: 3815621

Plasma RDEA594 exposures decreased markedly over time after multiple doses. Starting at week 24 and continuing to week 50, significant amounts of M6 and M8 were observed in monkey plasma. These increases were accounted for by a concurrent decrease in systemic exposure of RDEA594 (Figure 9).

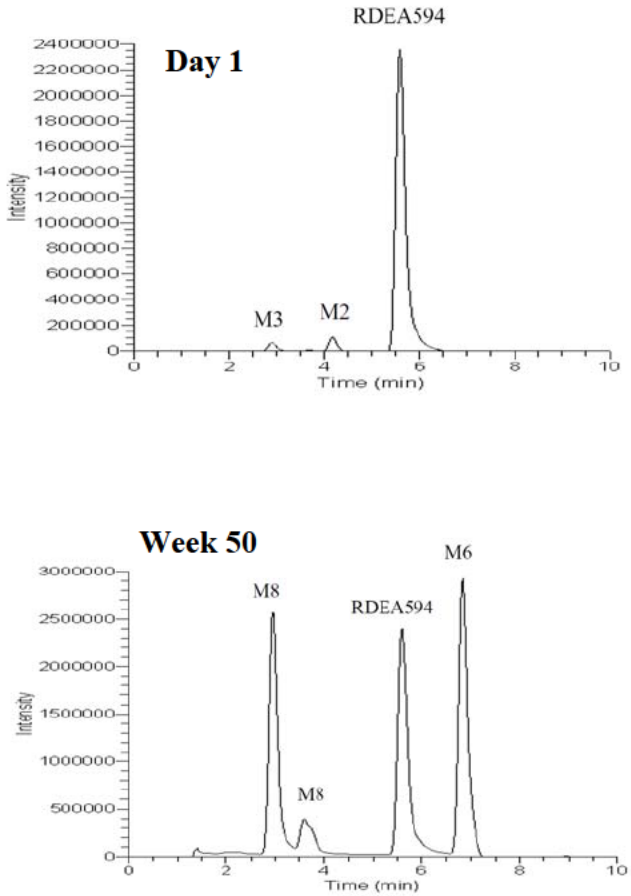

Figure 9. LC/MS ion chromatogram of plasma samples from monkeys at 1 hour after dosing with 600 mg/kg RDEA594 at day 1 (*top*) and week 50 (*bottom*).

Reference ID: 3815621

Table 31. Sponsor's summary of plasma RDEA594 and metabolite levels in cynomolgus monkeys after a single dose and after daily dosing for **50 weeks** (SR08-094-MET).

Metabolite	Molecular Ion (m/z)	Retention Time (min)	Day 1	Week 50
RDEA594	404	5.8	major	major
M2	326	4.3	minor	minor
M3	420	2.9	minor	ND
M6	346	6.9	trace	major
M8	522	3.0	ND	moderate
M11	362	4.4	ND	minor
M12	538	2.2	ND	moderate
M13	268	5.1	ND	minor
M14	284	2.6	ND	minor
M15	314	6.6	ND	minor

The safety of M6 and M8 metabolites in monkeys is not considered to be toxicologically relevant as humans do not synthesize these metabolites of RDEA594.

Reference ID: 3815621

Disproportionate Metabolite Qualification

Metabolites M3 and M4 are disproportionate human metabolites. They were detected at 12.3% and 20.7% of the initial dose, respectively, in humans (Study RDEA594-112). According to the CDER *Guidance for Industry: Safety Testing of Drug Metabolites* (February 2008), human metabolites that are formed at greater than 10% of parent drug systemic exposure at steady state can raise a safety concern. Adequate safety qualification of metabolites is generally achieved by demonstrating metabolite exposure at the NOAEL in a study of sufficient duration with a single nonclinical species that exceeds the clinical metabolite exposure at the maximum recommended human dose.

It is noted that neither the M3 nor the M4 is metabolite is pharmacologically active at URAT1 or OAT4 (Study SR11-039).

M3 Metabolite

The M3 metabolite has been qualified for safety on the basis of its exposure at the NOAEL in the 12 month monkey toxicology study (SR08-094). M3 exposure in study SR08-094 was calculated using data from in vivo monkey metabolism study SR10-029-MET. Briefly, the percentage of the initial dose detected as M3 (6.73%, urine + feces + bile) in study SR10-029-MET was multiplied by the NOAEL of RDEA594 in study SR08-094 to establish the M3 exposure at the NOAEL (Table 32). M3 exposure at the monkey NOAEL exceeds the predicted clinical M3 exposure at the MRHDD by a factor of 5.4.

The M3 metabolite has also been qualified for safety with respect to carcinogenicity based on its exposure at the 200 mg/kg dose in the 2 year rat carcinogenicity study (SR09-070) in which RDEA594 was found to be non-tumorigenic. M3 exposure was calculated using the percentage of the initial RDEA594 dose detected as M3 in rat bile + urine (8.86) by the maximum RDEA594 dose in study SR09-070 (Table 30).

Table 32. Exposure margin calculation for the **M3 metabolite** at the NOAEL dose in monkeys and rats vs. calculated M3 exposure in humans at the MRHDD.

Species	Study Type	Study #	Lesinurad NOAEL (mg/kg)	Lesinurad NOAEL (mg/m^2)	Total M3 %	M3 exposure (mg/m^2)	Safety Margin
Monkey	General Tox (12 month)	SR08-094	100	1200[a]	6.73[b]	80.8	5.4
Rat	Carcinogenicity (2 year)	SR09-070	200	1200[c]	8.86[d]	106.3	7.0
Human	Single dose [^{14}C]lesinurad ADME study	RDEA594-112	3.33[e] (max clinical dose)	123[f]	12.3[g]	15.1	-

Reference ID: 3815621

[a] 100 mg/kg * 12 kg/m^2 (monkey k_m value) = 1200 mg/m^2
[b] Total (bile + feces + urine) monkey M3 (% of initial dose) reported in study SR10-029-MET
[c] 200 mg/kg * 6 kg/m^2 (rat k_m value) = 1200 mg/m^2
[d] Total (bile + urine) rat M3 (% of initial dose) reported in study SR12-032
[e] 200 mg (Maximum clinical RDEA594 dose) / 60 kg (average human body weight) = 3.33 mg/kg
[f] 3.33 mg/kg * 37 kg/m^2 (human k_m value) = 123 mg/m^2
[g] Total (urine + feces) human M3 (% of initial dose) reported in study RDEA594-112

M4 Metabolite

The M4 metabolite (didhydrodiol metabolite of RDEA594) raises concern due to its formation via an epoxide intermediate, termed M3c (Figure 6). The epoxide represents a structural alert for mutagenicity. The M3c epoxide metabolite is unstable and is not detectable *in vivo* in any species tested (human, rat, monkey).

The M3c metabolite is not qualified for safety with respect to carcinogenicity based on the low level of exposure in the 2 year carcinogenicity study in Sprague Dawley rats (Table 33, see nonclinical review dated 7/29/15). Concerns with regard to the potential carcinogenic effects of M3c may be mitigated by its transient nature and the fact that, in humans, this molecule is detoxified to a didhydrodiol (M4).

The M3c metabolite has been qualified for safety with respect to general toxicity based on the calculated exposure to metabolites M4 and M9 (derivatives of M3c, see SR10-029-MET) at the NOAEL in the 12 month monkey toxicology study (SR08-094).

Table 33. Exposure margin calculation for **M3c metabolite (epoxide intermediate)** at the NOAEL dose in monkeys vs. the calculated M3c exposure in humans at the MRHD.

Species	Study Type	Study #	Lesinurad NOAEL (mg/kg)	Lesinurad NOAEL (mg/m^2)	Total M3c %	M3c exposure (mg/m^2)	Safety Margin
Monkey	General Tox (12 month)	SR08-094	100	1200 [a]	8.85[b]	106	4.2
Rat	Carcinogenicity (2 year)	SR09-070	200	1200 [c]	0.13[d]	1.6	0.06
Human	Single dose [^{14}C]lesinurad ADME study	RDEA594-112	3.33[e] (max clinical dose)	123[d]	20.7	25.5	-

[a] 100 mg/kg * 12 kg/m^2 (monkey k_m value) = 1200 mg/m^2
[b] Monkey M3c% value equivalent to M4 + M9 levels in bile and feces (study SR10-029-MET)
[c] 200 mg/kg * 6 kg/m^2 (rat k_m value) = 1200 mg/m^2
[d] Rat M3c is based on Total (urine + feces) rat M4 (% of dose) reported in study SR12-032. Assumption is that M4 is formed via M3c intermediate in rats
[e] 200 mg (Maximum clinical RDEA594 dose) / 60 kg (average human body weight) = 3.33 mg/kg
[f] 3.33 mg/kg * 37 kg/m^2 (human k_m value) = 123 mg/m^2
[g] Total (urine + feces) human M4 (% of initial dose) reported in study RDEA594-112

Reference ID: 3815621

In vitro evidence suggests that, in humans, all M3c that is formed is converted to M4 by microsomal epoxide hydrolase (SR11-031). Given that M3c is qualified with respect general toxicity, exposure to M4 (a less toxic metabolite than M3c) at the MRHD is also considered safe from the nonclinical perspective.

Reference ID: 3815621

Elimination

The RDEA594 excretion profiles in rats and monkeys differ somewhat from that of humans. In rats and monkeys, the majority of orally administered RDEA594 is eliminated via the feces, while in humans the majority of orally administered RDEA594 (~63%) is eliminated in urine.

Rats

SR08-028: Pharmacokinetic, blood to plasma ratio, and mass balance of [^{14}C]RDEA594 administered to Sprague-Dawley rats following single intravenous and oral doses

Methods

Male and female Sprague-Dawley rats received a single 20 mg/kg dose (50 µCi/kg) of [^{14}C]RDEA594 via either the IV or oral route of administration.

Mass balance

Radioactivity was measured in samples of blood, plasma, urine & feces, cage rinse, and the animal carcass over the time course of 0 – 120 h after dosing. Mass balance determination was assessed in groups of 3 rats/sex/route.

PK profile

Blood samples were collected from groups of 3 rats per sex per time point (t = 0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 25 36, 48, 60, 72 h postdose) for analysis of the pharmacokinetic profile of RDEA594.

Blood: plasma ratio

Radioactivity was quantified in both whole blood and in plasma by liquid scintillation counting. Calculation of the blood: plasma ratio allows for an evaluation of the amount of drug bound to blood cells vs. the amount in plasma. Extensive binding to RBCs may indicate the potential for hematotoxicity.

Results

Consistent with the results of Study SR08-060, the blood and plasma concentration of [^{14}C]RDEA594 after oral dosing peaked at 0.25 h. Radioactivity in blood was below the limit of quantitation by the 24-36 hour time points.

Mean radioactivity concentrations in urine were highest at t = 6 hours in males and females after IV or oral administration. Radioactivity concentration in feces was highest at t = 12 hours in males and females after IV or oral administration.

Blood: plasma ratio values ranged from approximately 0.5 – 1.0 in male and female rats over the course of 24 hours after oral or IV dosing. This evidence suggests that RDEA594 does not preferentially associate with red blood cells in rats.

The distribution of radioactivity to urine and feces was similar whether [^{14}C]RDEA594 was administered via the oral or IV route (Table 34). The large majority of radioactivity in males was detected in feces (~ 75 – 92%), suggesting that biliary excretion is a major elimination route. In females, radioactivity was split essentially evenly between urine and feces. Nearly all radioactivity was eliminated from rats by the 120 h time point.

Reference ID: 3815621

Table 34. Mean cumulative excretion of [^{14}C]RDEA594 – derived radioactivity in the excreta and carcass of male and female SD rats over the course of 120 hours after a single 20 mg/kg IV (*left*) or oral (*right*) dose.

Intravenous

Matrix	% Dose (Mean ± SD, *n*= 3/dose route)	
	Male	Female
Urine	8.14 ± 1.60	34.11 ± 9.91
Cage Rinse	2.71 ± 0.53	16.00 ± 7.16
Feces	92.37 ± 3.64	38.58 ± 0.98
Carcass	3.00 ± 3.31	5.69 ± 4.59
Total	**106.23 ± 2.42**	**94.38 ± 1.62**

Oral

Matrix	% Dose (Mean ± SD, *n*= 3/dose route)	
	Male	Female
Urine	11.89 ± 7.68	36.33 ± 1.49
Cage Rinse	1.35 ± 0.64	2.58 ± 0.58
Feces	75.27 ± 9.79	44.15 ± 4.07
Carcass	0.00 ± 0.00	0.14 ± 0.24
Total	**88.50 ± 1.49**	**83.19 ± 2.06**

Reference ID: 3815621

Study SR11-068: RDEA594 excretion to rat milk
Milk samples from lactating rats treated with RDEA594 in this pre- and postnatal reproduction toxicology study were obtained at 4 hours post-dose on lactation day 10. RDEA594 was detected in milk at similar concentrations to what was observed in plasma.

See Section 9.3 of this review for a more detailed discussion of the presence of RDEA594 in rat milk.

Study SR09-056: Evaluation of biliary excretion, enterohepatic circulation, and metabolism of [^{14}C]RDEA594 after a single oral dose followed by intraduodenal readministration in rats
Methods
This study was comprised of 2 parts. In part I, a single 20 mg/kg oral dose of [^{14}C]RDEA594 was administered to bile duct cannulated male Sprague-Dawley rats (n = 4). Bile from each rat was collected and pooled over time = 0-6 h after dosing. This bile was then used for duodenal dosing in part II.

Samples from bile, urine, and feces were taken over the course of 24 hours after oral and intraduodenal dosing to examine the elimination pathways of [^{14}C]RDEA594 related radioactivity. RDEA594 metabolites present in rat bile were quantified after oral and intraduodenal dosing.

Results
The total radioactivity absorbed was equivalent to 70.4% of the initial dose (recovery in urine + bile), with the vast majority recovered in bile (Table 35). This is consistent with the evidence in male rats in study SR08-028 in which 75% of the dose was recovered in feces.

Table 35. [^{14}C]RDEA594 related radioactivity recovered over the course of 24 hours after a single oral dose (Part I) or an intraduodenal dose of bile collected during t = 0-6 h in Part I (Part II).

		Mean % of initial dose recovered			
	Route	Bile	Urine	Feces	Total
Part I	Oral	65.2	5.2	13.0	83.3
Part II	Intraduodenal (pooled bile from Part I)	38.4	6.1	37.7	82.2

Approximately 45% (bile + urine) of the RDEA594-derived radioactivity present in the bile that was intraduodenally administered in Part II was absorbed. RDEA594 was found to be the major component detected in the bile in Part II of the study, thus indicating that there is substantial enterohepatic circulation of RDEA594 in male rats (Table 36).

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Table 36. Metabolite profile in rat bile after a single oral dose of [^{14}C]RDEA594 (Part I) and after an intraduodenal dose of pooled bile collected during t = 0-6 h in Part I.

		Metabolic profile of rat bile (% initial radioactivity)					
	Route	M1	M2	M3	M5	RDEA594	Total
Part I	Oral	17.0	1.7	3.5	16.3	52.7	91.2
Part II	Intraduodenal (pooled bile from Part I)	16.0	6.6	0.0	14.7	53.4	90.7

Study SR12-032: Evaluation of the metabolic profile of RDEA594 in bile and urine in male bile-duct cannulated Sprague-Dawley rats following a single oral administration of [^{14}C]RDEA594 at a dose of 200 mg/kg (100 µCi/kg)

Methods: Male bile duct cannulated (BDC) rats (*n* = 3) received a single oral dose of 200 mg/kg [^{14}C]RDEA594. Bile, urine, and feces were collected out to 24 h post-dose. Sample analysis included HPLC with radioactive detection for metabolite profiling. Samples were analyzed by LC-MS/MS for metabolite structural confirmation. The sponsor did not evaluate feces samples due to only ~5% of the initial dose being recovered in this matrix after 24 hours.

Results:
The majority of the [^{14}C]RDEA594 dose was recovered in bile (Table 37). RDEA594 was the major component of the radioactivity detected in urine and bile with minor contributions from M1, M2, M3, and M4. It is noted that the major human metabolite, M4, is detected in rat urine. Metabolite chemical structures are seen in Figure 10.

Table 37. Metabolite profiles in bile and urine in bile duct cannulated rats after a single oral dose of 200 mg/kg [^{14}C]RDEA594. Values are expressed as % of initial dose.

Matrix	Time (hr)	% of Dose						
		M1	M2[a]	M3	M4	Others[b]	RDEA594	Total
Bile	0-24	3.63	0.74	2.99	ND	0.92	37.7	46.0
Urine	0-24	0.11	2.48	5.87	0.13	0.78	11.8	21.2

[a] M2 was not separated well from one of M1 metabolites (a group of glucuronides of RDEA594). M2 contribution was estimated based on mass spectrometric data.
[b] Others include one metabolite with mass shift +16 and other unidentified metabolites.
ND= not detected by the reported radioactivity detection method (estimation of limit of radioactivity detection 279 ng/mL with 100 µL injection).

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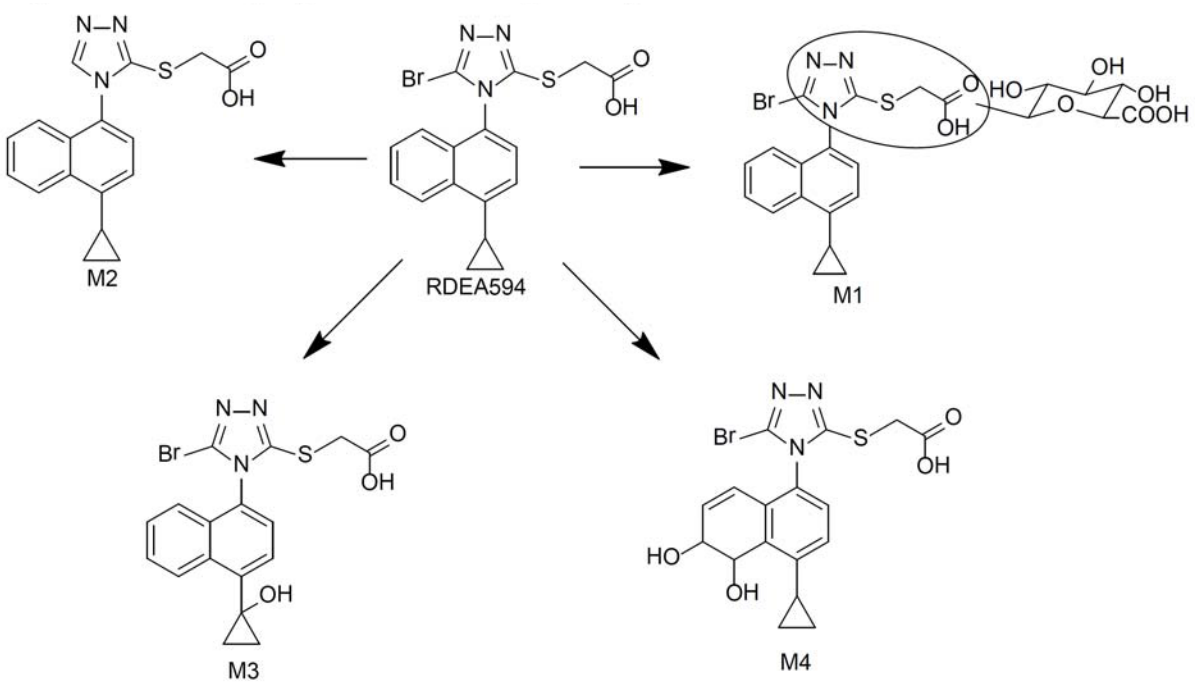

Figure 10. Sponsor's representation of chemical structures of RDEA594 metabolites detected in rats after oral administration.

Monkeys

SR08-017: [^{14}C]RDEA594: Mass balance and blood to plasma ratio of radioactivity in male cynomolgus monkeys following single oral and intravenous doses

Methods

The study was conducted in 2 phases. In phase 1, 3 male cynomolgus monkeys were treated with a single **IV** dose of 10 mg/kg (20 µCi/kg) [^{14}C]RDEA594. Phase 2 of the study examined the elimination of RDEA594 after oral administration. A single **oral** gavage dose of [^{14}C]RDEA594 was administered to the same 3 monkeys that were used in phase 1 (after a 3 week washout period) at 20 mg/kg (20 µCi/kg).

Blood: plasma ratio

Blood samples were collected during both Phase 1 and Phase 2 for quantitation of radioactivity in whole blood and in plasma. Samples were collected from the femoral vein pre-dose and at the following time points: 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours post dose. An aliquot of whole blood (~0.5 ml) was collected and stored refrigerated until analysis. The remaining blood was centrifuged at 4°C to obtain plasma. Samples were analyzed via liquid scintillation counting.

Mass balance study

Urine, feces, and cage residue were collected from animals during Phase 1 and Phase 2 at time points out to 120 hours postdose for quantitation of radioactivity. Samples were analyzed via liquid scintillation counting.

Results

Consistent with study SR08-059, maximum concentrations of radioactivity in blood and plasma were observed at 1 – 2 hours after oral administration.

After IV dosing, radioactivity in whole blood and plasma decreased rapidly between 5 minutes and 2 hours after dosing. Radioactivity was not measurable in whole blood beyond 72 hours post-dose, but was detectable in plasma for up to 120 hours post dose.

Blood:plasma ratios were approximately 0.50 – 0.68 over the first 48 hours after IV dosing. After oral dosing, ratios ranged from 0.61 – 0.84. Collectively, the evidence shows that RDEA594 does not preferentially associate with red blood cells in rats.

RDEA594 related radioactivity distributed to feces (42 – 45%) to a slightly greater extent than urine (26 – 33%) after both Oral and IV administration (Table 38).

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Table 38. Summary of [^{14}C]RDEA594 excretion data over the course of 120 hours after a single dose of [^{14}C]RDEA594 to male cynomolgus monkeys (*n* = 3). Study SR08-017.

	Mean % of initial dose (*n* = 3)	
	Oral	**IV**
Urine	32.6	25.7
Feces	41.7	45.0
Cage rinse	16.9	13.2
Total recovery	91.3	83.8

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6 General Toxicology

6.1 Single-Dose Toxicity

Single dose toxicity studies were not conducted in support of NDA 207988.

6.2 Repeat-Dose Toxicity

Ardea has conducted repeated dose toxicity studies in rats and monkeys. The monkey was chosen as the non-rodent species based on the lack of M4 metabolite formation in dogs.

Oral toxicology studies of 14 and 28 day conducted in rats and monkeys have been reviewed previously (see IND 102128 nonclinical review dated 6/18/10).

The pivotal nonclinical toxicology studies to support the marketing approval of lesinurad are chronic toxicology studies conducted in rats (26 weeks, Study SR08-095) and monkeys (12 months, SR08-094). The importance of these studies is based on the proposed chronic administration of lesinurad in the clinical setting. These studies have been reviewed in detail previously (see IND 102128 nonclinical review dated 12/24/12) and are discussed further in Section 11.

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7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

There is no evidence for mutagenic potential of RDEA594 in bacterial reverse mutation assays. See nonclinical review dated 6/23/11 for evaluation of Studies **SR08-022** and **SR09-015**.

7.2 *In Vitro* Assays in Mammalian Cells

RDEA594 did not induce chromosomal aberrations in in vitro chromosome aberration assays conducted in Chinese Hamster Ovary (CHO Cells). See nonclinical review dated 6/23/11 for evaluation of Studies **SR06-086** and **SR08-023**.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

RDEA594 was negative for induction of micronuclei formation in rat bone marrow after single oral doses up to 1000 mg/kg. See nonclinical review dated 6/23/11 for evaluation of Study **SR08-024**.

7.4 Other Genetic Toxicity Studies

Computational toxicology assessment of metabolites M3c and M4 (September 26, 2014)
RDEA594 and metabolites M3c and M4 were evaluated by CDER/OTS/OCP/DARS for bacterial mutagenicity using quantitative structure-activity relationship (QSAR) models. Three software programs were used: *Derek Nexus* 4.0.5 (*DX*), *Leadscope Model Applier* 1.8.3-1 (*LMA*), and *CASE Ultra* 1.4.6.6 (*CU*). To maximize sensitivity and negative predictivity, a positive prediction from any one software program was used to justify a positive study call.

M3c was predicted to be positive for both *Salmonella* and *E. coli* mutagenicity based on the presence of the epoxide group in the structure. Metabolite M4 was predicted to be negative for *Salmonella* mutagenicity. No call could be made for *E. coli* mutagenicity due to inadequate coverage of the structural features of M4 in the model training sets.

The M3c metabolite raises potential clinical concerns due to the chronic duration of RDEA594 dosing. As described in 5.1 above (Metabolism), rats synthesize the M4 metabolite at low levels *in vivo*. It is assumed that all of the M4 metabolite present in rats is formed via the M3c intermediate (and not via other metabolic pathways). The level of M3c exposure at the NOAEL in the 2 year rat carcinogenicity study (Study SR09-070) is well below the human exposure to M3c at the maximum recommended human dose (Exposure margin: 0.06; see nonclinical review dated July 29, 2015). Therefore, M3c is not considered to be qualified for safety with respect to carcinogenicity.

From a technical perspective, it would not be possible for the sponsor to directly administer M3c to rats in a stand-alone study based on the unstable nature of epoxides. Therefore, no further studies are required for safety qualification of the M3c epoxide intermediate with respect to carcinogenicity (See ECAC Meeting Minutes dated May 26, 2015). Some concern with regard

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to the potential carcinogenic effects of M3c may be mitigated by its transient nature and the fact that, in humans, this molecule is detoxified to a didhydrodiol (M4).

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8 Carcinogenicity

RDEA594 was not tumorigenic in rats or mice. See Review dated July 29, 2015 for summaries and evaluations along with the associated Executive Carcinogenicity Assessment Committee (ECAC) meeting minutes for carcinogenicity studies conducted with RDEA594 in SD rats (2 years) and TgRasH2 mice (26 weeks)

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9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

See IND 102128 nonclinical review dated June 22, 2011 for summaries and evaluations of Study **SR10-007**, a Fertility and Early Embryonic Development study in rats.

9.2 Embryonic Fetal Development

See IND 102128 nonclinical review dated June 22, 2011 for summaries and evaluations of Studies **SR10-008** and **SR10-009**, Embryofetal Development toxicity studies conducted in rats and rabbits respectively.

9.3 Prenatal and Postnatal Development

Study title: A Developmental and Perinatal/Postnatal Reproduction Study by Oral Gavage in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	SR11-068
Study report location:	Electronic submission, SD 203
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 16, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RDEA594 Sodium salt 17JL01.HQ00001 100% purity

Key Study Findings

- **Pregnant F0 Females**
- Rats were dosed orally with RDEA 594 at 0, 100, 200, or 300 mg/kg/day from gestational day 7 (GD 7) – lactation day 20 (DL 20)
- RDEA 594 at ≥ 200 mg/kg/day was highly maternally toxic:
  - RDEA 594 at ≥ 200 mg/kg/day caused premature deaths during the gestation period
    - 4/25 females at 200 mg/kg/day died prematurely
    - 10/25 females at 300 mg/kg/day died prematurely
    - Deaths were preceded by numerous clinical signs including dehydration, hunched posture, decreased motor activity and marked body weight loss
    - Target organs included the stomach, kidney, and liver.
  - Body weight gain was significantly reduced (≥10%) vs. controls at <u>all RDEA 594 dose levels</u> during the <u>gestation period</u>

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        - Magnitude of effect size increased with dose
    - Mean body weight gain increased in a dose-proportional manner during the lactation period.
        - Absolute body weights of rats in all dose groups were comparable to control by DL 20.

- Mean RDEA 594 plasma and milk concentrations increased proportionally with dose between 100 and 200 mg/kg/day. RDEA 594 levels plateaued at the 200 mg/kg/day dose both in plasma and milk.
    - The milk: plasma ratio was ≥ 1 in all treatment groups, showing that RDEA594 distributes approximately evenly between plasma and milk.
        - Individual milk: plasma ratios ranged between 0.7 – 2.9, with no clear effect of RDEA 594 dose on milk: plasma partitioning

- There is no NOAEL with respect to maternal toxicity based on statistically significant, dose-dependent decreases in body weight gain >10% at all doses.

- **F1 generation rats**
- 200 mg/kg and 300 mg/kg RDEA 594 treatment to F0 females evoked multiple adverse effects on F1 offspring
    - 200 mg/kg
        - 200 mg/kg/day RDEA594 treatment resulted in significantly reduced viability index, mean number of surviving pups per litter at postnatal day (PND) 21, and mean pup weight at birth & PND 21 vs. controls
        - Litter size at PND 21 value and mean pup weight values were outside of the range observed in historical control animals.
    - 300 mg/kg
        - Viability index (# of live pups on PND 4/ # of live pups on PND 1) and lactation index (# of live pups on PND 21/ # of live pups on PND 4) were significantly lower in the 300 mg/kg dose group vs. controls.
        - The mean number of surviving pups at PND 21 and mean pup weight at birth & PND 21 were also significantly reduced in the 300 mg/kg group vs. controls.
        - Litter size at PND 21 value and mean pup weight values were outside of the range observed in historical control animals.
- After weaning, 25 rats/sex/group were selected for continued evaluation
- 3/25 HD F1 males died prematurely, a statistically significant increase vs. the control group
    - 2 HD animals were found dead (d 49 and d 102) while the third animal was euthanized on day 24 (shortly after weaning).
    - These deaths are attributed to RDEA594 administration to F0 females.
- HD F1 males and females showed clinical signs of: dehydration (mild – moderate), thin body condition and scant feces
- F1 males and females in the maternal 200 mg/kg and 300 mg/kg dose groups had significantly lower body weights vs. controls at the time of weaning (PND 22)
    - Body weights in all dose groups recovered to control levels by PND 92 in males and PND 71 in females

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- Mean time to vaginal patency was significantly delayed in MD and HD F1 females
  - Duration was outside of the historical control range in both dose groups

- The NOAEL for fetal developmental toxicity was 100 mg/kg
  - Viability index was significantly decreased vs. controls at ≥ 200 mg/kg/day
  - Mean pup weight at d 1 and d 21 were significantly decreased vs. controls at ≥ 200 mg/kg/day

- **F2 generation fetuses**
- RDEA 594 treatment did not induce gross external changes in F2 generation fetuses.

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Methods

Doses: F0 generation rats: 0, 100, 200, 300 mg/kg/day

Frequency of dosing: Once per day

Dose volume: 10 ml/kg

Route of administration: Oral gavage

Formulation/Vehicle: RO water
0.1N HCl to pH 6.8 ± 0.2

Species/Strain: Crl:CD(SD) Sprague Dawley rats

Number/Sex/Group:
- F0 females: 25 per dose group
- F1 rats: 25/sex/dose group

Satellite groups: None

Study design: F0 generation rats**:**
- 100 mated female rats were assigned to 1 of 4 dose groups (25 rats per group) and housed individually
- Lesinurad or vehicle was administered via oral gavage once per day from gestational day 7 (GD 7) – day 20 of lactation (DL 20) or GD 24 (for rats that did not deliver a litter).
- DL 1 is defined as the day of birth
- All F1 generation rats were weaned on postnatal day 21 (PND 21)

F1 generation rats**:**
- After weaning on PND 21, 25 male and 25 female pups per dose group were randomly selected for further evaluation.
- This resulted in a total of 200 F1 generation rats (100 per sex) chosen for continued evaluation. At least 1 male pup and 1 female pup per litter were selected, when possible.
- These rats were individually housed prior to cohabitation
- Measures of learning and memory were conducted with each rat (passive avoidance, water maze; see "Parameters Evaluated" below)
- Reproductive performance was also evaluated
  - Cohabitation included 1 male rat & 1 female rat
  - See "Parameters Evaluated" below

Deviation from study protocol: There were no notable deviations from the study protocol.

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Text Table 3
Experimental Design - F0 Generation Rats

Group No.	Test Material	Dose Level[a] (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	Assigned F0 Generation Rat Numbers
1	Vehicle Control Article	0	0	10	3501 - 3525
2	RDEA594	100	10	10	3526 - 3550
3	RDEA594	200	20	10	3551 - 3575
4	RDEA594	300	30	10	3575 - 3600

[a] A correction factor of 1.2 was used for the purpose of dose calculations.

Text Table 4
Experimental Design - F1 Generation Rats

Group No.	Test Material	Maternal Dose Level (mg/kg/day)	Maternal Concentration (mg/mL)	Maternal Dose Volume (mL/kg)	Assigned F1 Generation Rat Numbers	
					Male	Female
1	Vehicle Control Article	0	0	10	3701 - 3725	3801 - 3825
2	RDEA594	100	10	10	3726 - 3750	3826 - 3850
3	RDEA594	200	20	10	3751 - 3758, 17[a], 3760 - 3775	3851 - 3862, 18[a], 3864 - 3875
4	RDEA594	300	30	10	3776 - 3800	3876 - 3900

[a] On the day of randomization, prior to the start of F1 generation evaluations, male rat 3759 and female rat 3863 were excluded from study due to premature deaths and therefore, replaced with male rat 17 and female rat 18, respectively, from litter 3573.

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Parameters Evaluated

F0 generation rats

- Viability (twice daily)
- Clinical observations (daily)
- Maternal behavior (DL 1, 4, 7, 14, 18, 21)
- Body weight (daily during dosing period)
- Food consumption (GD 0, 7, 10, 12, 15, 18, 20, 25 & days 1, 4, 7, 10 and 14 post-partum)
- Natural delivery observations
    - Adverse clinical signs
    - Duration of gestation
    - Litter size
    - Pup viability at birth

Blood and milk sample collection:

- 5 dams/group were selected for sample collection
- Blood (1.0 ml, tail vein) and milk (0.02 – 0.10 ml, after IV injection of oxytocin) sample collection was conducted approximately 4 hours after dosing on DL 10
- Blood was processed to plasma using standard methodologies

Plasma and milk sample analysis:

- Plasma and milk samples were frozen and shipped to Ardea
- Samples were analyzed to quantify RDEA594 concentration in plasma and milk by Ardea using non-GLP methods. RDEA594 quantitation was achieved using LC/MS/MS methods.

Terminal procedures

- F0 female rats were euthanized by CO_2 asphyxiation after completion of the 21-day lactation period. (Those dams that did not deliver pups were euthanized on GD 25)
- The reproductive tract was dissected out from the abdominal cavity. The number and distribution of implantation sites were recorded.
- Gross necropsy of the thoracic, abdominal and pelvic viscera was performed for each rat
- Tissue samples were taken from all gross lesions and masses present in all F0 females and preserved in 10% neutral buffered formalin. Samples were also taken from the organs listed in Table 39 depending upon the nature of the rat's death and/or its pregnancy status.

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Table 39. List of tissues collected at necropsy from F0 female rats.

Tissue	Collected	Comment
Cervix	X	Collected with uterus. All nonpregnant rats.
Esophagus	X	Infused with 10% neutral buffered formalin. Rats found dead or euthanized before scheduled termination.
Gross lesions/masses	X	All rats.
Heart	X	Rats found dead or euthanized before scheduled termination.
Kidneys	X	Rats found dead or euthanized before scheduled termination.
Liver	X	Rats found dead or euthanized before scheduled termination.
Lungs	X	Infused with 10% neutral buffered formalin. Rats found dead or euthanized before scheduled termination.
Ovaries	X	All nonpregnant rats.
Spleen	X	Rats found dead or euthanized before scheduled termination.
Stomach	X	Rats found dead or euthanized before scheduled termination.
Trachea	X	Infused with 10% neutral buffered formalin. Rats found dead or euthanized before scheduled termination.
Uterus	X	All nonpregnant rats.

X = Procedure conducted

- A summary of the terminal procedures conducted on F0 females is seen in Table 40.

Table 40. Summary of terminal procedures conducted on F0 female rats.

Group No.	No. of Rats	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights		
1	25	DL 21	Full Exam	X	X	-	-	-
2	25						-	-
3	25						-	-
4	25						-	-
Dams That Did Not Deliver		DG 25	Full Exam	X	X	-	-	-
Dams With No Surviving Pups		a	Full Exam	X	X	-	-	-
Unscheduled Deaths			Full Exam	X	X	-	-	-

X = Procedure conducted; - = Not applicable.
[a] On the day the observation was made.

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F1 generation rats

Pre-weaning (all F1 pups)

- Viability (twice daily)
- Clinical observations (daily)
- Body weight (weight at birth & days 4, 7, 14, 18, 21 postpartum)

Post-weaning (25 rats/sex/group)

- Viability (twice daily)
- Clinical observations (daily)
- Body weight (once per week during the post-weaning period)
  - Note that in female rats only (i.e. pregnant female mothers of F2 generation pups), body weights were measured on: GD 0, 7, 10, 14, 17, 21
- Food consumption (once per week except during cohabitation)
  - Female rats only (i.e. pregnant female mothers of F2 generation pups),: GD 0, 7, 10, 14, 17, 21
- Evaluate sexual maturation (daily)
  - Males: Start on d 39 postpartum
  - Females: Start on d 28 postpartum

- Learning and memory parameters**:**
  - *Passive avoidance evaluation*: Initial test at postnatal day (PND) 23 - 25
    - Each rat was tested twice. Sessions separated by a 1 week interval
  - *Water maze*: (M Maze): Initial test on PND 68 - 72
    - Each rat was tested twice. Sessions separated by a 1 week interval
- Reproductive capacity*:*
  - At **PND 94 - 98**, rats were assigned to cohabitation (1 M and 1 F per cage)
  - Cohabitation limit was 21 days
  - Females with evidence of mating were considered to be at GD 0 and assigned to individual housing

Terminal procedures

- Pups that were found dead prior to examination of the litter for pup viability on PND 1 were evaluated for vital status at birth by removing the lungs and immersing them in water. Lungs that did not sink were identified as live born and to have died shortly after birth.
- All F1 pups weaned on PND 21 that were not selected for continued evaluation were euthanized by CO_2 asphyxiation and examined for gross lesions.
  - Gross lesions were preserved in neutral buffered 10% formalin
  - Necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly

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Rats selected for continued evaluation

- *Male rats*: After completion of the cohabitation period (PNDs 127 -130), F1 male rats were euthanized and examined for gross lesions. Organ weights were recorded for the organs listed in Table 41. Representative samples were retained from each tissue and preserved in 10% neutral buffered formalin.

Table 41. List of tissues collected and weighed at necropsy from F1 male and female rats

Tissue	Weighed	Collected	Comment
Cervix	-	X	Collected with uterus. All nonpregnant females.
Epididymides	X	X	Paired weight. All males.
Gross lesions/masses	-	X	All animals.
Heart	-	X	Animals found dead or euthanized before scheduled termination.
Kidneys	-	X	Animals found dead or euthanized before scheduled termination.
Liver	-	X	Animals found dead or euthanized before scheduled termination.
Lungs	-	X	Animals found dead or euthanized before scheduled termination.
Ovaries	-	X	All nonpregnant animals.
Spleen	-	X	Animals found dead or euthanized before scheduled termination.
Stomach	-	X	Animals found dead or euthanized before scheduled termination.
Testes	X	X	Paired weight. Fixed in Bouin's solution for 48 to 96 hours before being retained in neutral buffered 10% formalin. All males.
Uterus	-	X	Collected with cervix. All nonpregnant animals.

X = procedure conducted; - = not applicable.

- *Female rats:*
  - Pregnant female F1 rats were euthanized on GD 21
  - Rats without a confirmed mating date were euthanized on GD 25
  - The reproductive tract was dissected from the abdominal cavity.
    - The contents of the uterus were examined
    - Fetuses were removed and placed in individual containers
    - Ovaries and uterus were examined for the following:
      - Number & distribution of corpora lutea
      - Implantation sites
      - Placentae (size, color, shape)
      - Live and dead fetuses
      - Early & late resorptions
  - Gross necropsy of the thoracic, abdominal, and pelvic viscera was performed for each rat
  - The weights the organs listed in Table 41 were recorded.
  - Representative samples were retained from each tissue and preserved in 10% neutral buffered formalin.

F2 fetuses

- Fetuses were examined for sex and for gross abnormalities
- Body weight of each fetus was recorded
- 1/2 of the fetuses were retained in Bouin's solution and 1/2 were retained in alcohol for possible future evaluation.

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Observations and Results

F0 Dams

Mortality

A dose-related increase in mortality was observed in pregnant female rats at 200 and 300 mg/kg/day. No deaths were observed in the 100 mg/kg/day treatment group. Table 42 lists the animals that were either found dead or euthanized due to adverse clinical observations.

Table 42. Summary of premature deaths in F0 dams in rat PPND study SR11-068. Individual animal ID numbers are listed below the total values.

RDEA 594 (mg/kg/day)	0	100	200	300
Total *n*	25	25	25	25
Total Mortalities	**1**	**0**	**4**	**10**
Euthanized – gestation period	0	0	2 3558 3574	1 3597
Found dead – gestation period	0	0	2 3570 3571	9 3577 3585 3578 3588 3580 3589 3581 3591 3584
Euthanized – lactation period	1 3510	0	0	0

Table 43 summarizes the relevant clinical signs along with body weight change in the days prior to death as well as gross necropsy observations in animals that died prematurely. Dehydration was observed in all animals that died prematurely. Decreased motor function, hunched posture, thin body condition, and changes in breathing parameters were additional clinical signs common to the majority of animals that died prematurely. Marked body weight decreases were observed in the 2 – 4 days prior to death in all animals with the exception of HD animal #3589. The most common gross lesions observed were in the stomach. Black areas on the mucosal surface of the stomach (fundic, cardiac, pyloric) were detected in MD and HD animals. It is notable that gross findings were not observed in all animals that died prematurely.

The observed clinical signs along with the gross pathology findings in the stomach in animals that died prematurely are highly consistent with observations made in premature deaths in

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Sprague-Dawley rats dosed with ≥ 300 mg/kg/day RDEA594 in study SR08-095, a 6 month general toxicology study (see nonclinical review, 12/24/12, Whittaker). It is clear that high doses of RDEA594 (≥ 200 mg/kg/day), when administered by oral gavage, can act as a direct toxicant in the stomach in rats.

The cause of death in the single control animal (#3510) is not known at this time, but it does not appear to be due to gavage error based on the absence of gross pathologic findings.

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Table 43. Summary of relevant observations in pregnant F0 females that died prematurely in Study SR11-068. Euth = euthanized due to adverse clinical signs. FD = found dead.

RDEA 594 (mg/kg/day)	Animal ID #	Day of Death	Clinical signs	Body weight change (g)	Necropsy observations
0	3510	DL 15 (euth)	Dehydration Thin body condition Scant feces	-118 (DL 13 – DL 15)	All tissues normal
200	3558	GD 20 (euth)	Decreased motor activity Hunched posture Dehydration Thin body condition Scant feces	-41 (GD 17 – GD 20)	All tissues normal
	3570	GD 22 (FD)	Decreased motor activity Rales Bradypnea Dehydration Thin body condition Pale extremities	-11 (GD 20 – GD 22)	**Stomach**, fundic, pyloric regions, mucosal surface: numerous black areas
	3571	GD 15 (FD)	Decreased motor activity Hunched posture Ptosis Bradypnea Dehydration Thin body condition Pale extremities Piloerection Scant feces	-68 (GD 10 – GD 14)	**Stomach**, fundic region, mucosal surface: numerous black areas
	3574	GD 22 (euth)	Decreased motor activity Ataxia Ptosis	-25 (GD 20 – GD 22)	**Stomach**, fundic region, mucosal surface: numerous black areas

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RDEA 594 (mg/kg/day)	Animal ID #	Day of Death	Clinical signs	Body weight change (g)	Necropsy observations
			Bradypnea Dehydration Pale extremities		**Kidneys,** bilateral, pelvis: slight dilatation
300	3577	GD 21 (FD)	Decreased motor activity Chromorhinorrhea Hunched posture Dehydration Thin body condition Scant feces	-49 (GD 18 – GD 20)	All tissues normal
	3578	GD 17 (FD)	Decreased motor activity Impaired righting reflex Hunched posture Dyspnea Dehydration Thin body condition Pale extremities Piloerection	-26 (GD 15 – GD 16)	**Stomach,** fundic region, mucosal surface, numerous black areas **Liver**, all lobes, numerous tan areas
	3580	GD 15 (FD)	Dehydration Pale extremities Scant feces	-51 (GD 7 – GD 14)	All tissues normal
	3581	GD 18 (FD)	Decreased motor activity Ptosis Chromorhinorrhea Excess salivation Bradypnea Dehydration Thin body condition Pale extremities Piloerection	-42 (GD 13 – GD 18)	All tissues normal

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RDEA 594 (mg/kg/day)	Animal ID #	Day of Death	Clinical signs	Body weight change (g)	Necropsy observations
	3584	GD 21 (FD)	Decreased motor activity Chromorhinorrhea Hunched posture Dehydration Thin body condition Scant feces	-53 (GD 17 – GD 20)	All tissues normal
	3585	GD 12 (FD)	Decreased motor activity Impaired righting reflex Hunched posture Bradypnea Dehydration Thin body condition Piloerection Scant feces	-64 (GD 7 – GD 12)	**Stomach,** cardiac region, serosal surface, numerous black areas
300	3588	GD 15 (FD)	Hunched posture Ptosis Hyperpnea Dehydration Thin body condition Pale extremities	-63 (GD 11 – GD 15)	All tissues normal
	3589	GD 22 (FD)	Decreased motor activity Hunched posture Chromorhinorrhea Ptosis Hyperpnea Dehydration Thin body condition	-7 (GD 17 – GD 21)	All tissues normal

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RDEA 594 (mg/kg/day)	Animal ID #	Day of Death	Clinical signs	Body weight change (g)	Necropsy observations
300	3591	GD 14	Decreased motor activity Hunched posture Aggressive Chromorhinorrhea Ptosis Bradypnea Dehydration Thin body condition Piloerection Scant feces	-67 (GD 7 – GD 13)	All tissues normal
	3597	GD 18	Decreased motor activity Hunched posture Chromorhinorrhea Chromodacryorrhea Bradypnea Dehydration Thin body condition Piloerection Scant feces	-51 (GD 11 – GD 18)	**Stomach**, pyloric & fundic regions, numerous black areas

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Clinical signs

There were no relevant clinical signs observed in controls or in animals dosed at 100 mg/kg/day. Table 44 summarizes all of the clinical observations made in all animals in the study (premature deaths as well as those that survived to study completion combined). Many of the same clinical signs observed in animals that died prematurely (Table 43) were also seen in animals in the 200 mg/kg/day and 300 mg/kg/day groups that survived to study completion. These signs included: dehydration (mild-moderate), thin body condition, and hunched posture. The number of surviving animals showing these clinical signs was increased in the 300 mg/kg group compared to the 200 mg/kg group. Many of the observations made during the gestation period were absent in the lactation period due to the fact that these signs were observed in animals that died during the gestation period.

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Table 44. Summary of all clinical signs observed in F0 generation female rats. Values are listed as: total observations / # of animals with the observation. The table includes observations made in animals that died prematurely as well as those that survived to study completion.

	Clinical signs							
	Gestation Period				Lactation period			
RDEA 594 (mg/kg/day)	0	100	200	300	0	100	200	300
Rats tested	25	25	25	25	25	25	25	25
Thin body condition	0/0	0/0	7/4	38/13	1/1	0/0	19/5	40/7
Dehydration								
mild	0/0	0/0	17/6	37/11	0/0	0/0	14/3	24/5
moderate	0/0	0/0	15/7	25/13	1/1	0/0	9/2	3/2
severe	0/0	0/0	0/0	7/5	0/0	0/0	1/1	0/0
Total	0/0	0/0	31/9	58/14	1/1	0/0	23/4	27/7
Hunched posture	0/0	0/0	3/2	20/12	1/1	0/0	2/1	7/4
Cold to touch	0/0	0/0	3/3	12/11	0/0	0/0	2/1	0/0
Scant feces	0/0	0/0	8/5	18/10	1/1	0/0	0/0	0/0
Chromorhinorrea	0/0	0/0	1/1	19/10	1/1	1/1	1/1	0/0

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Clinical signs								
	Gestation Period				Lactation period			
RDEA 594 (mg/kg/day)	0	100	200	300	0	100	200	300
Rats tested	25	25	25	25	25	25	25	25
Decreased motor activity	0/0	0/0	5/4	14/9	1/1	0/0	2/1	0/0
Ptosis	0/0	0/0	2/2	12/7	1/1	0/0	2/1	0/0
Pale extremities	0/0	0/0	6/4	6/6	0/0	0/0	0/0	0/0
Piloerection	0/0	0/0	1/1	9/6	0/0	0/0	0/0	0/0
Red or pink substance on fur	0/0	0/0	1/1	8/6	0/0	0/0	0/0	0/0
Bradypnea	0/0	0/0	3/3	8/5	0/0	0/0	0/0	0/0
Hyperpnea	0/0	0/0	0/0	4/3	0/0	0/0	0/0	0/0
Excess salivation - slight	0/0	0/0	0/0	4/4	0/0	0/0	1/1	0/0
Both ears – pale	0/0	0/0	6/4	3/3	0/0	0/0	0/0	0/0
Animal pale	0/0	0/0	0/0	2/2	0/0	0/0	0/0	0/0
Rales	0/0	0/0	4/2	1/1	0/0	0/0	1/1	3/2

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Body weight

Note that animals were dosed from GD 7 – DL 20. Pups were born on DL 1. Therefore, body weight comparisons for the gestational period are from GD 7 – GD 20. Body weight comparisons for the lactation period are from DL 1 – DL 20.

Gestation period

Body weight gains were significantly reduced vs. controls in all treatment groups over the course of GD 7 – GD 20 (Table 45, Figure 11). The magnitude of the effect size increased with dose. Body weight gain in HD treated animals during the treatment period of GD 7 – GD 20 was 35.4% of controls.

Table 45. F0 maternal body weight comparison during the gestation period (GD 7 – GD 20).

	Mean Body Weight (g)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
Rats tested	25	25	25	25
Rats pregnant	25	24[a]	25	24[b]
GD 7	279.0 [25]	280.8 [24]	278.1 [25]	277.8 [24]
GD 20	402.3 [25]	383.8** [24]	343.3** [24]	321.4** [17]
Difference from GD 7 (g)	123.3	103.0**	65.2**	43.6**
BW gain relative to control (%)	100.0%	83.5%	52.9%	35.4%

** $p \leq 0.01$ vs. control value

[a] Rat 3530 was found to be not pregnant. Body weight values for this animal not included in mean calculation

[b] Rat 3596 was found to be not pregnant. Body weight values for this animal not included in mean calculation

[ ] Number of animals used in mean calculation. Animals euthanized or found dead during the treatment period were not included in body weight calculation.

Lactation period

RDEA594 was administered from the day of litter delivery (DL 1) to DL 20. Mean body weight values during the lactation period were calculated using body weights from animals that delivered a litter on DL 1 only. Additionally, F0 females found dead, euthanized, or for which no surviving pups were observed (e.g. due to cannibalism), were excluded from mean body weight calculation.

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Table 46 provides data from DL1 – DL14 in order to maintain consistency with the reporting of food consumption data (Table 47). Pups are assumed to begin to consume maternal food after day 14 of lactation.

RDEA594 treated animals started the lactation period with lower mean body weights than control animals. Mean body weight gains, normalized to initial body weight on DL 1 were found to increase in a dose-dependent manner during the lactation period. By DL20, mean final absolute body weights were comparable between control rats and all RDEA594 treated groups (Table 46, Figure 11).

Table 46. F0 maternal body weight comparison during the lactation period (DL 1 – DL 20).

	Mean Body Weight (g)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
Rats tested	25	25	25	25
Delivered a litter	25	24	21	13
DL 1	299.5 [25]	290.3 [24]	261.1 [21]	250.8 [13]
DL 14	344.5 [25]	341 [24]	323.7 [19]	319.3 [10]
BW gain (g)	45.0	50.7	62.6	68.5
BW gain (% initial BW)	+15.0%	+17.5%	+24.0%	+27.3%
BW gain relative to control (%)	100.0%	116%	159.6%	181.8%
DL 20	336.8 [24]	337.0 [24]	327.7 [19]	331.4 [9]
BW Gain DL 1 - 20 (g)	37.3	46.7	66.6	80.6
BW gain DL 1 - 20 (% initial BW)	+12.5%	+16.1%	+25.5%	+32.1%
BW gain (DL 1 – DL 20) relative to control (%)	100.0%	129.2%	205%	258.2%

** $p \leq 0.01$ vs. control value
[ ] Number of animals used in mean calculation. Animals euthanized or found dead during the treatment period not included in body weight calculation. Also, animals with no surviving pups excluded from body weight calculation.

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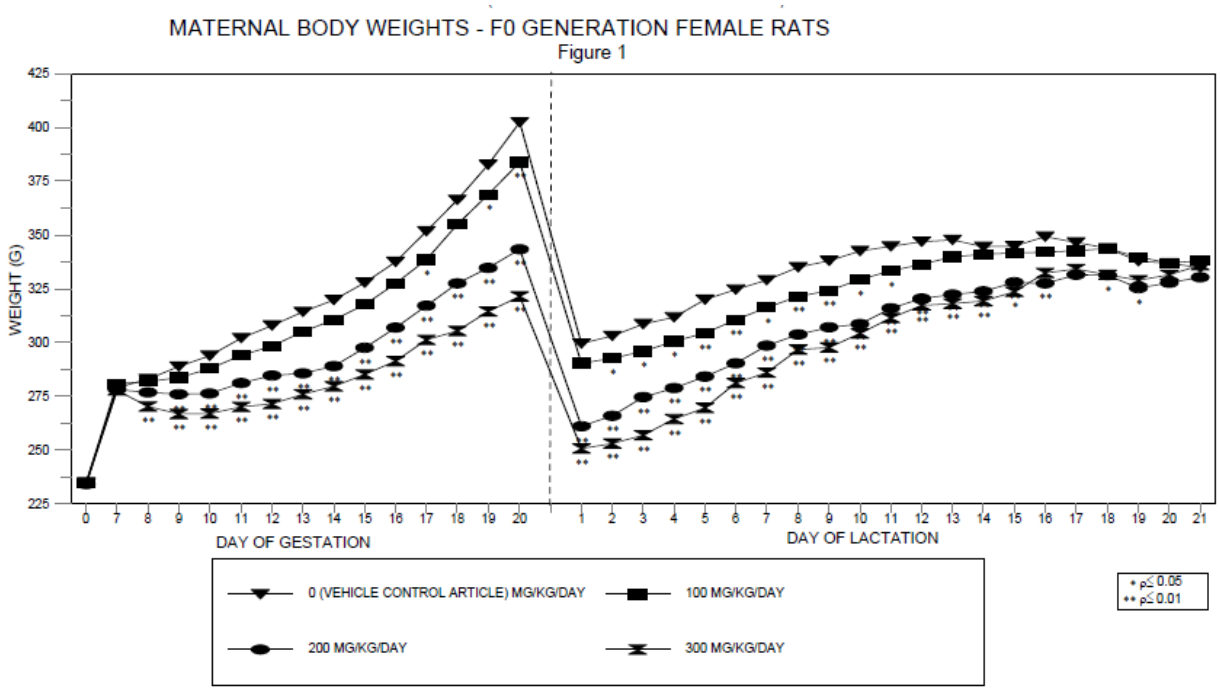

Figure 11. Mean maternal body weight values in pregnant F0 female rats during the gestation period and lactation period in study SR11-068.

Maternal food consumption

Food consumption comparisons are made over the gestation and lactation periods in the same manner as body weight.

Gestation period

Mean food consumption values were significantly reduced vs. controls in all treatment groups over the course of GD 7 – GD 20 (Table 47). The magnitude of the effect size increased with dose. The observed decreases in food consumption generally correlated with the observed decreases in body weight gain in all treatment groups.

Table 47. F0 maternal food consumption during the gestation period (GD 7 – GD 20).

	Mean food consumption (g/day)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
Rats tested	25	25	25	25
Rats pregnant	25	24	25	24
GD 7 - 20	26.0 [25]	23.5** [25]	19.5** [24]	17.8** [17]
Food consumption relative to control (%)	100.0%	90.4%	75.0%	68.5%

** $p \leq 0.01$ vs. control value
[ ] Number of animals used in mean calculation. Animals euthanized or found dead during the treatment period were not included in food consumption calculations.

Lactation period

Comparisons of food consumption values during the lactation period are limited to DL 1 – DL 14 even though dosing continued to DL 20. The rationale behind this limitation is the presumption that pups begin to consume maternal food after day 14 of lactation.

Absolute and relative food consumption values were significantly reduced in all treatment groups vs. controls during DL1 – DL 14 (Table 48). However, **body weight gains** were increased in these animals relative to controls over this time period (Table 46). These observations can be explained by the markedly decreased initial body weights in RDEA594 treated animals at the start of the lactation period.

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Table 48. F0 maternal food consumption during days 1 - 14 of the lactation period (DL 1 – DL 14).

	Mean **absolute** food consumption (g/day)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
Rats tested	25	25	25	25
Delivered a litter	25	24	21	13
DL 1 - 14	56.5 [25]	52.0* [24]	47.8** [19]	38.3** [10]
Food consumption relative to control (%)	100.0%	92.0%	84.6%	67.8%
	Mean **relative** food consumption (g/kg/day)			
DL 1 - 14	172.2	163.8	160.6*	132.4**
Food consumption relative to control (%)	100.0%	95.1%	93.3%	76.9%

* $p \leq 0.05$ vs. control value
** $p \leq 0.01$ vs. control value
[ ] Number of animals used in mean calculation. Animals euthanized or found dead during the treatment period were not included in food consumption calculations. Also, animals with no surviving pups were excluded from food consumption calculations.

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Natural Delivery and Litter Observations

There were 25, 24, 25, and 24 pregnant F0 females in the 0, 100, 200, and 300 mg/kg/day dose groups respectively. RDEA594 at ≥ 200 mg/kg/day showed signs of marked toxicity. As described in the Survival section, there were 4/25 and 10/24 females that died prematurely in the 200 and 300 mg/kg/day dose groups respectively. Additionally, HD animal #3593 had a litter with 17 early resorptions. Therefore, there were a total of 25, 24, 21 and 13 animals that delivered litters in each dose group.

Table 49 lists animals that delivered litters but were euthanized due to the loss of all surviving pups (not due to adverse clinical signs). This effect appeared to be dose-related. Fetal tissue was detected in the stomach in 2/2 animals in the 200 mg/kg group and 2/4 animals in the 300 mg/kg group thus providing evidence for apparent infanticide and cannibalism. This behavior may be reflective of conditions of stress in these animals as a result of RDEA594 treatment.

Table 49. Summary of pregnant F0 dams euthanized due to loss of all pups in rat PPND study SR11-068. Individual animal ID numbers are listed below the total values. Day of euthanasia is listed in parentheses.

RDEA 594 (mg/kg/day)	0	100	200	300
Total *n*	25	25	25	25
Euthanized – no surviving pups	0	0	2 3554 (DL 2)* 3560 (DL 3)*	4 3579 (DL 1)* 3590 (DL 1) 3595 (DL 3)* 3599 (DL 14)

*Fetal tissue present in stomach

The gestation index (# of rats with live offspring / # of pregnant rats) was significantly reduced in HD animals vs. controls (Table 50). There was no apparent effect of RDEA594 treatment on duration of gestation, number of implantation sites per dam, or the percentage of dams with stillborn pups.

Viability index (# of live pups on PND 4/ # of live pups on d 1) and lactation index (# of live pups on PND 21/ # of live pups on PND 4) were significantly lower in the 300 mg/kg dose group vs. controls. The mean number of surviving pups at PND 21 and mean pup weight at birth & PND 21 were also significantly reduced in the HD group vs. controls. The litter size at PND 21 values as well as the mean pup weight values were all outside of the range observed in historical control animals.

200 mg/kg/day RDEA594 treatment resulted in similar adverse effects on litter observations. Viability index, mean number of surviving pups per litter at PND 21, and mean pup weight at birth & PND21 were all significantly reduced in the MD group vs. controls. As with the 300

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mg/kg animals, the litter size at PND 21 values as well as the mean pup weight values were all outside of the range observed in historical control animals.

There were no apparent effects of 100 mg/kg/day RDEA594 on any of the observed natural delivery and litter parameters.

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Table 50. Summary of natural delivery and litter observations in SD rats treated with RDEA 594 in PPND study SR11-068.

RDEA 594 (mg/kg/day)	0	100	200	300	Historical control[a]
F0 Generation Females (*n*)	25	25	25	25	
Pregnant (Pregnancy rate)	25 (100%)	24 (96%)	25 (100%)	24 (96%)	88.4%
Delivered a litter (%)	25 (100%)	24 (100%)	21 (84%)	13 (54.2%)	98.1%
Duration of gestation (days)	23.0 ± 0.4	22.8 ± 0.4	22.8 ± 0.4	23.0 ± 0.4	22.2 - 24.0
Gestation index (#of rats with live offspring/ # of pregnant rats)	25/25 (100%)	24/24 (100%)	21/25 (84%)	13/24 (54.2%)**	50.0 – 100.0
Implantation sites (mean per litter)	398 (15.9)	361 (15.0)	329 (15.7)	194 (14.9)	7.8 – 16.3
Dams with stillborn pups (%)	5 (20%)	6 (25%)	4 (19%)	2 (15.4%)	0 – 33.3%
F1 generation litters					
Viability index (# of live pups on d 4/ # of live pups on d 1)	99.2% (362/365)	94.9 (318/335)	80.6 (246/305)**	68.5 (113/165)**	50.0 – 100.0
Lactation index (# of live pups on d 21/ # of live pups on d 4)	95.8% (347/362)	99.0 (315/318)*	98.8 (243/246)	90.3 (102/113)**	92.5 – 100.0
Litter size					
Day 1	14.6	14.0	14.5	12.7	7.5 – 15.7
Day 21	14.4	13.1	11.6*	7.8**	12.3 – 14.2
Pup weight (g)					
Day 1	6.8	6.6	5.5**	5.3**	6.2 – 7.5
Day 21	40.9	39.4	33.5**	31.1**	39.3 – 43.5

* $p \leq 0.05$ vs. control; ** $p \leq 0.01$ vs. control
[a] Historical control data from Charles River Laboratories PCS-PA site, June 2008 – 2010 (*n* = 29 studies)

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Necropsy observation

Gross necropsy of the thoracic, abdominal and pelvic viscera was performed for each rat. Tissue samples were taken from all gross lesions and masses present in all F0 females and preserved in 10% neutral buffered formalin. Samples were also taken from the organs listed in Table 39 depending upon the nature of the rat's death and/or its pregnancy status.

There were no gross pathology findings observed in any animals treated at the 100 mg/kg/day dose level. Treatment related adverse findings were observed primarily in the stomach of a subset of MD and HD animals (Table 51). These observations were made in animals that were euthanized due to adverse clinical findings or were found dead prior to study completion.

Table 51. Gross necropsy findings in F0 females treated with RDEA 594 in study SR11-068.

RDEA 594 (mg/kg/day)	0	100	200	300
F0 Generation Females (*n*)	25	25	25	25
Appeared normal	25	25	20**	20**
Stomach				
Fetal tissue present	0	0	2	2
Black areas (fundic, pyloric, or cardiac)	0	0	3	3
Liver				
Tan areas, all lobes	0	0	0	1
Kidney				
Bilateral, pelvis, slight dilatation	0	0	1	0

* Significantly different from control group ($p \leq 0.01$)

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Toxicokinetics

5 dams/group were selected for sample collection. Blood (1.0 ml, tail vein) and milk (0.02 – 0.10 ml, after IV injection of oxytocin) sample collection was conducted at a single time point, 4 hours after dosing on **DL 10.** The t = 4 h time point is close to the reported T_{max} of 3.0 h in the 6 month rat toxicology study (SR08-095).

Blood was processed to plasma using standard methodologies. Plasma and milk samples were frozen (-80 ± 15°C) and shipped to Ardea for analysis. Plasma and milk samples were analyzed for RDEA 594 quantitation using an LC/MS/MS method. The limit of quantitation was 15 ng/ml in plasma and milk. Individual and summary statistics for milk and plasma RDEA 594 concentrations were tabulated using WinNonlin software version 5.2.

All RDEA 594 concentrations in plasma and milk samples from the control group were below the limit of quantitation. RDEA 594 concentrations in plasma and milk are listed in Table 52 below. Mean RDEA 594 plasma and milk concentrations increased proportionally with dose between 100 and 200 mg/kg/day. RDEA 594 levels plateaued at the 200 mg/kg/day dose both in plasma and milk. Comparable concentrations were observed in animals dosed with 300 mg/kg/day. The milk: plasma ratio was ≥ 1 in all treatment groups, showing that RDEA594 distributes approximately evenly between plasma and milk. Individual milk: plasma ratios ranged between 0.7 – 2.9, with no clear effect of RDEA 594 dose on milk: plasma partitioning.

Table 52. Summary of mean ± SD RDEA 594 concentrations in milk and plasma at t = 4 hrs post-dose on lactation day 10 in Study SR11-068.

RDEA 594 dose (mg/kg/day)	*n*	Mean milk concentration (µg/ml)	Mean plasma concentration (µg/ml)	Mean milk: plasma ratio
100	5	91.2 ± 15.8	93.3 ± 25.2	1.02 ± 0.25
200	5	275 ± 190	208 ± 54.6	1.38 ± 0.91
300	5	240 ± 108	194 ± 75.1	1.23 ± 0.25

Dose formulation analysis

RDEA594 solutions were analyzed by Ardea using reversed phase HPLC. Samples were taken from solutions prepared on the first and last days of preparation. Samples ranged from 99.7 – 103.3% of the claimed RDEA 594 concentration.

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F1 Generation Rats

After weaning on PND 21, 25 male and 25 female pups of F0 dams per dose group were randomly selected for further evaluation. At least 1 male pup and 1 female pup per litter were selected, when possible. These rats represented the F1 generation that was retained for further evaluation.

F1 rats were housed individually until PND 94 – 98, when rats were assigned to cohabitation for assessment of reproductive capacity. The cohabitation limit was 21 days.

Mortality

3/25 HD F1 males died prematurely, a statistically significant increase vs. the control group (Table 53). 2 HD animals were found dead (d 49 and d 102) while the third animal was euthanized on day 24 (shortly after weaning). These deaths are attributed to RDEA594 administration to F0 females. No deaths were observed in males in the LD or MD treatment groups.

Premature deaths in 1/25 control and 1/25 100 mg/kg females respectively were not attributable to RDEA594 treatment.

Table 53. Summary of premature deaths in F1 rats in PPND study SR11-068. Individual animal ID numbers are listed below the total values. FD = found dead. Euth = euthanized.

	F1 males				F1 Females			
Maternal RDEA 594 dose (mg/kg/day)	0	100	200	300	0	100	200	300
Total *n*	25	25	25	25	25	25	25	25
Total Mortalities	0	0	0	3**	1	1	0	0
				#3783 (FD, d49) #3800 (FD, d102) # 8797 (euth, d24)	#3812 (euth, d14)	#3846 (euth, after delivering pups on GD20)		
	** $p \leq 0.01$ vs. controls							

Additional details pertaining to observations in the 3 HD males that died prematurely are presented in Table 54. Animals 3783 and 3800 both had gross lesions in structures of the urinary tract including slight-moderate dilatation of the ureters as well as urinary bladder filled with calculi and/or brown cloudy fluid. Animal 3797 did not have evidence of tissue damage at

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necropsy. However, it is noted that this animal was small in size & failed to thrive after weaning. This animal was selected as a male representative of its litter.

Table 54. Summary of relevant observations of HD F1 males that died prematurely in study SR11-068.

Maternal RDEA 594 (mg/kg/day)	Sex	Animal ID #	Date	Clinical signs	Body weight change (g)	Necropsy observations
300	Male	3783	49 (FD)	None	+69 (d 36 – 43)	▪ Ureters: slight – moderate dilatation ▪ Urinary bladder: numerous calculi and/or filled with brown cloudy fluid
	Male	3797	24 (euth)	Days 22-24: Ataxia, ▼ motor activity, hunched posture, bradypnea, pale extremities, cold to touch	NA (euthanized 3 d after weaning)	▪ All tissues normal
	Male	3800	102 (FD)	None	-51 (d 94 – 99)	▪ Ureters: slight – moderate dilatation ▪ Urinary bladder: numerous calculi and/or filled with brown cloudy fluid ▪ Kidney (pelvis): slight – marked dilatation, numerous calculi present

Clinical signs (surviving animals)

Adverse clinical signs observed in HD F1 male and female rats attributed to RDEA594 treatment are listed in Table 55. A single HD male (#3796) showed signs of dehydration, ptosis, thin body condition, scant feces, and lenticular opacity.

In HD females a significantly higher incidence of ptosis, mild-moderate dehydration, thin body condition, and scant feces relative to controls were observed. These observations were made during the pre-cohabitation period and did not persist to the gestation period.

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Table 55. Summary of clinical observations in F1 male and female rats. Values are expressed as: Total number of observations / # of animals with the observation.

	F1 males				F1 Females			
Maternal RDEA 594 dose (mg/kg/day)	0	100	200	300	0	100	200	300
Total *n*	25	25	25	25	25	25	25	25
Ptosis	0/0	0/0	0/0	2/1	0/0	0/0	0/0	5/3**
Dehydration								
mild	0/0	0/0	0/0	4/1	0/0	0/0	0/0	9/3**
moderate	0/0	0/0	0/0	2/1	0/0	0/0	0/0	3/3**
Thin body condition	0/0	0/0	0/0	1/1	0/0	0/0	0/0	3/3**
Scant feces	0/0	0/0	0/0	1/1	0/0	0/0	0/0	3/3**
Lenticular opacity	0/0	0/0	0/0	1/1	0/0	0/0	0/0	20/2
	** $p \leq 0.01$ vs. controls							

Body weight

Mean body weights were significantly reduced in male and female MD and HD F1 rats vs. controls at the initial day of body weight measurement (PND 22). For MD and HD males, body weights remained significantly lower than controls through PND 85 (Figure 12). By PND 92, body weights were comparable in males in all dose groups (
Table 56). The recovery to control body weight levels in MD and HD F1 males over the course of the pre-cohabitation period (PND 22 – PND 92) was a result of increased body weight gain (119 – 120% vs. controls) in these animals over this time period.

In MD and HD females, mean body weights remained significantly lower than controls through PND 50 (Figure 12). By PND 92, body weights were comparable in females in all dose groups (Table 57).

The recovery to control body weight levels in MD and HD F1 females over the course of the pre-cohabitation period (PND 22 – PND 92) was a result of increased body weight gain (128 – 139% vs. controls) in these animals over this time period.

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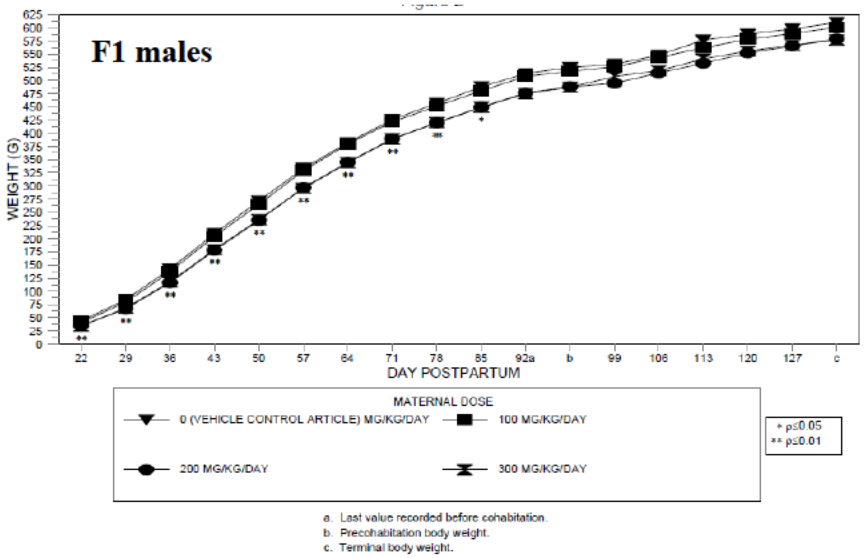

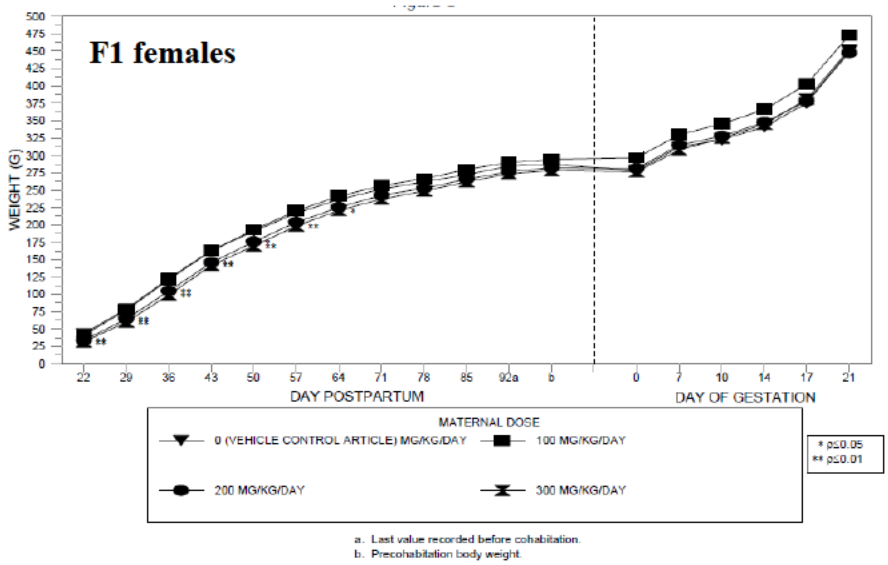

Figure 12. Mean body weight values over time in F1 male and female rats in Study SR11-068.

Table 56. F1 male body weight comparison during the PND 22 – PND 92 (precohabitation period).

	Mean Body Weight (g)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
n =	25	25	25	25
PND 22	44.3	41.1	35.0**	34.5**
PND 92	513.4	507.8	476.6	475.4
BW gain PND 22 - 92 (g)	469.1	466.7	441.6	440.9
BW gain PND 22 – 92 (% initial BW)	1059%	1136%	1262%	1278%
BW gain relative to control (%)	100.0%	107.2%	119.2%	120.7%

** $p \leq 0.01$ vs. control value

Table 57. F1 female body weight comparison during the PND 22 – PND 92 (precohabitation period).

	Mean Body Weight (g)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
n =	25	25	25	25
PND 22	43.3	41.4	33.9**	31.4**
PND 92	284.5	289.7	276.4	273.8
BW gain PND 22 - 92 (g)	241.2	248.3	242.5	242.4
BW gain PND 22 - 92 (% initial BW)	557%	600%	715%	772%
BW gain relative to control (%)	100.0%	107.7%	128.4%	138.6%

** $p \leq 0.01$ vs. control value

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Food Consumption

Mean weekly food consumption values in F1 male rats were significantly lower in the MD and HD groups vs. controls during PND 22 – 29, 29 – 36, and 36 – 43, consistent with the observed decreased body weights observed in these animals during this time period. The magnitude of the difference (approximately - 8.0% vs. controls) was equivalent in the MD and HD groups. Mean weekly food consumption values were approximately equal across all groups by PNDs 50 – 57. There was no difference in overall mean weekly food consumption values during PNDs 22 – 92 (Table 58).

Table 58. F1 male food consumption comparison during the PND 22 – PND 92 (precohabitation period).

	Mean weekly food consumption (g)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
n =	25	25	25	25
PND 22 - 92	27.1	27.1	26.0	25.8
Mean food consumption relative to control (%)	100.0%	100.0%	95.9%	95.2%

Mean weekly food consumption values in F1 female rats were significantly lower in the MD and HD groups vs. controls during PND 22 – 29 only. The magnitude of the difference (approximately - 11.0% vs. controls) was comparable in the MD and HD groups. Mean weekly food consumption values were approximately equal across all groups after PND 29. There was no difference in overall mean weekly food consumption values during PNDs 22 – 92 (Table 59).

Table 59. . F1 female food consumption comparison during the PND 22 – PND 92 (precohabitation period).

	Mean weekly food consumption (g)			
	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day
n =	25	25	25	25
PND 22 - 92	27.1	27.1	26.0	25.8
Mean food consumption relative to control (%)	100.0%	100.0%	95.9%	95.2%

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Sexual maturation

There was not a significant effect of RDEA 594 treatment on mean number of days to preputial separation in F1 male rats.

Vaginal patency was significantly delayed in MD and HD F1 female rats. The mean times to vaginal patency for females in both the MD and HD groups were outside of the historical control range. These delays are concurrent with observed decreased mean body weights and body weight gains in the early post-weaning period. It is noted that these doses were maternally toxic to F0 generation dams.

Table 60. Mean number of days to vaginal patency in F1 female rats.

RDEA 594 dose	0 mg/kg/day	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day	
n =	25	25	25	25	Historical control
Days to vaginal patency	33.6 ± 1.7	34.0 ± 1.7	35.7 ± 2.1**	36.3 ± 2.8**	32.8 [30.1 – 35.3]
BW on day of sexual maturity (g)	105.9	108.0	102.6	98.4	105.3 [92.4 -120.8]

**p ≤ 0.01 vs. control

Neurological assessment

Passive avoidance performance

Beginning at PNDs 23 to 25, 1 male rat and 1 female rat from each litter, where possible, were evaluated in a passive avoidance test for learning, short-term retention, and long-term retention.

The passive avoidance apparatus was comprised of a 2-compartment chamber. One compartment was lit with a bright light and had a Plexiglass floor. The other compartment ("dark compartment") had a grid floor. For each trial, the rat was placed into the light compartment and allowed to explore the apparatus until it entered the dark compartment. The door between compartments was then closed and a brief pulse of current was delivered to the grid floor. The rat was then removed from the apparatus and placed into a holding cage for 30 seconds before the start of the next trial. Trials were repeated until the rat remained in the bright compartment for 60 seconds on 2 consecutive trials (this is the criterion for learning). The maximum number of trials allowed was 15. The latency to enter the dark compartment was recorded for each trial.

Each rat was tested twice. The test sessions were separated by a one-week interval. The learning criterion was the same for both days of testing.

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There were no observed effects of RDEA 594 treatment in males or females on the number of trials required to achieve the learning criterion on either testing day. There were no significant differences in males or females in the latency to enter the dark compartment in trial 1 or trial 2 on either test day.

Watermaze performance
During PND 68 – 72, 1 male and 1 female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, and learning & memory.

On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the 2 arms) and required to swim to 1 of the 2 goals of the M-maze, in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal and then removed from the water. A 15-second inter-trial interval separated each trial. Each rat was required to reach a criterion of 5 consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15.

Latency to choose the correct goal was recorded for each trial. The number of errors (incorrect turns in the maze) was also recorded for each trial.

Each rat was tested twice, with test sessions separated by a 1-week interval. The following parameters were compared between dose groups:

Parameter	Measurement
The number of trials to criterion on the first day of testing	Compared groups for overall learning performance
The average number of errors (incorrect turns in the maze) for each trial on the first day of testing	Compared groups for overall learning performance
The latency (in seconds) to reach the correct goal on trial 2 of the first day of testing	Compared groups for short-term retention
The number of trials to criterion on the second day of testing	Compared groups for long-term retention
The average number of errors for each trial on the second day of testing	Compared groups for long-term retention
The latency (in seconds) to reach the correct goal on trial 1 of day 2 of testing	long-term retention

The watermaze performance data is summarized in the modified sponsor's table below (Table 61). There were no observed effects of RDEA 594 treatment on watermaze performance in F1 males. HD F1 females had a statistically significant increase in the mean number of errors per trial relative to controls during session 2 (memory retention phase). However this value is within the historical control range of 146 such studies. Considering the high degree of variability in the individual values for errors/trial and the fact that delays were not observe in male rats, the finding is not considered to be treatment related.

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Table 61. Sponsor's table summarizing the results of watermaze testing of F1 male and female rats.

MATERNAL GROUP		1	2	3	4	
TEST MATERIAL		VEHICLE CONTROL ARTICLE	RDEA594	RDEA594	RDEA594	
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	100	200	300	
MALE RATS:						
SESSION 1a	N	24	24	19	9	
TRIALS TO CRITERION	MEAN±S.D.	9.8 ± 3.1	8.8 ± 2.5	8.2 ± 1.6	9.2 ± 3.1	
ERRORS PER TRIAL	MEAN±S.D.	0.38 ± 0.23	0.38 ± 0.22	0.38 ± 0.17	0.41 ± 0.27	
LATENCY TRIAL 2b	MEAN±S.D.	13.2 ± 11.5	14.9 ± 8.9	18.1 ± 13.8	16.2 ± 9.7	
FAILED TO LEARN c	N(%)	4(16.7)	1(4.2)	0(0.0)	1(11.1)	
SESSION 2a	N	20	23	19	8	
TRIALS TO CRITERION	MEAN±S.D.	6.4 ± 2.0	6.2 ± 2.2	7.8 ± 3.6	5.2 ± 0.5	
ERRORS PER TRIAL	MEAN±S.D.	0.08 ± 0.15	0.10 ± 0.17	0.19 ± 0.21	0.04 ± 0.08	
LATENCY TRIAL 1b	MEAN±S.D.	7.9 ± 4.7	7.8 ± 6.0	8.5 ± 4.9	9.9 ± 5.7	
FEMALE RATS:						
SESSION 1a	N	24	24	19	10	
TRIALS TO CRITERION	MEAN±S.D.	9.8 ± 2.8	9.3 ± 3.2	9.3 ± 3.4	9.3 ± 2.3	
ERRORS PER TRIAL	MEAN±S.D.	0.43 ± 0.25	0.39 ± 0.15	0.43 ± 0.23	0.41 ± 0.10	
LATENCY TRIAL 2b	MEAN±S.D.	17.9 ± 14.3	16.0 ± 7.9	14.2 ± 9.8	16.8 ± 14.2	
FAILED TO LEARN c	N(%)	0(0.0)	3(12.5)	2(10.5)	0(0.0)	
SESSION 2a	N	24	21	17	10	Historical control
TRIALS TO CRITERION	MEAN±S.D.	7.0 ± 2.9	8.0 ± 3.7	7.7 ± 3.2	8.5 ± 3.6	
ERRORS PER TRIAL	MEAN±S.D.	0.12 ± 0.15	0.19 ± 0.22	0.16 ± 0.18	0.36 ± 0.27**	0.15 [0.06 – 0.45]
LATENCY TRIAL 1b	MEAN±S.D.	10.0 ± 4.6	9.3 ± 6.8	10.4 ± 5.2	12.6 ± 7.8	

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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Mating and Fertility

On PND 94 – 98, F1 rats were assigned to cohabitation. 1 male rat and 1 female rat were housed per cage for a maximum of 21 days. Females observed with spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at GD 0 and assigned to individual housing.

There was no effect of F0 maternal doses of RDEA594 up to 300 mg/kg/day on mating or fertility in F1 generation males or females (Table 62).

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Table 62. Mating and fertility data in F1 generation males and females.

RDEA 594 (mg/kg/day)	0	100	200	300	Historical control
F1 Generation Males					
Rats in cohabitation	25	25	25	23	
Rats included in analysis	25	25	24[a]	23	
Fertility index (# of pregnancies/ # of rats that mated)	22/24 (91.7%)	23/23 (100%)	22/22 (100%)	22/22 (100%)	93.9% [78.3 – 100%)
# of pregnancies / # of rats in cohabitation	22/25 (88.0%)	23/25 (92.0%)	22/24 (91.7%)	22/23 (95.6%)	91.5% [75.0 – 100%]
F1 Generation Females					
Rats in cohabitation	25	25	25	25	
Total rats that mated	24 (96.0%)	25 (100%)	24 (96.0%)	24 (96.0%)	98.6% [75.0 – 100%]
Fertility index (# of pregnancies/ # of rats that mated)	22/24 (91.7%)	25/25 (100%)	24/24 (100%)	24/24 (100%)	93.7 % [80.0-100%]
Mated by second male (d 15 – 21)	0	2	1	0	
Total # of pregnancies / # of rats in cohabitation	22/25 (88.0%)	25/25 (100%)	24/25 (96.0%)	24/25 (96.0%)	92.3% [75.0 – 100%)

[a] Rat 3775 removed due to sibling pairing

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Gross pathology findings

There was no evidence for treatment related gross pathology findings in F1 male or female rats that survived to study completion. Gross findings in F1 males that died prematurely are discussed in the Mortality section.

Male Reproductive organs

There was no evidence for treatment related effects on male reproductive organs in F1 rats.

Caesarean Sectioning and Litter Observations – F1 females

There were no biologically meaningful effects of RDEA 594 treatment to F0 dams on the uterine parameters of pregnant F1 females or on the litter parameters of pups delivered by F1 females (Table 63). Mean F2 fetal body weights were significantly higher in the 300 mg/kg dose group, but the values are within the historical control range.

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Table 63. F1 female Caesarian section data (GD 21) and F2 litter evaluation.

RDEA 594 (mg/kg/day)	0	100	200	300	Historical control
F1 Female rats examined (*n*)	25	25	25	25	
Pregnant	22/25 (88%)	25/25 (100%)	24/25 (96.0%)	24/25 (96.0%)	94.4% [80.0 – 100%]
Rats C-sectioned on GD 21	21	24	24	24	
Implantations	14.8 ± 1.8	15.8 ± 2.1	14.5 ± 3.6	13.9 ± 3.4	14.5 [13.0 – 16.4]
Litter size	14.2 ± 2.0	14.8 ± 2.6	13.9 ± 3.5	13.2 ± 3.5	13.8 [12.4 – 16.0]
Resorptions	0.6 ± 0.9	1.0 ± 1.3	0.6 ± 0.7	0.7 ± 0.9	0.7 [0.2 – 1.2]
Litters with 1 or more viable fetuses	21/21 (100%)	24/24 (100%)	24/24 (100%)	24/24 (100%)	99.9% [95.4 -100%]
F2 generation litters					
Live fetuses (male/female)	298 (154/144)	355 (150/205)	334 (164/170)	318 (159/159)	
Average litter size	14.2 ± 2.0	14.8 ± 2.6	13.9 ± 3.5	13.2 ± 3.5	13.8 [12.4 – 16.0]
Mean fetal body weight (g)	5.32 ± 0.3	5.30 ± 0.28	5.37 ± 0.38	5.59 ± 0.37*	5.52 [5.30 – 5.75]
*$p \leq 0.05$ vs. controls					

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F2 generation fetuses

Fetal gross external examination

RDEA 594 treatment did not induce gross external changes in F2 generation fetuses.

Summary

A NOAEL was not identified for maternal toxicity due to dose-dependent decreased body weight gain >10% vs. controls at all doses tested. Additionally, RDEA 594 at ≥200 mg/kg/day caused premature deaths of pregnant females with target organs identified as the stomach and kidney. These target organs are consistent with observations in multiple toxicology studies conducted in SD rats with comparable RDEA 594 doses.

100 mg/kg has been identified as the NOAEL for RDEA 594 with respect to fetal growth and development. Viability index and mean fetal pup weight at birth and at weaning were significantly reduced in the offspring of dams treated with ≥ 200 mg/kg/d RDEA 594. These effects were observed in the presence of maternal toxicity. The RDEA 594 milk: plasma ratio was ≥ 1 in all treatment groups, showing that RDEA594 distributes approximately evenly between plasma and milk.

Mean time to vaginal patency was significantly delayed in F1 females of dams treated with ≥ 200 mg/kg/d RDEA 594. The duration was outside of the historical control range in both dose groups. It is noted that there was no effect of RDEA 594 on neurobehavioral function of F1 rats on passive avoidance assay performance or watermaze performance. Furthermore, there were no observed effects of RDEA594 at any dose tested on mating and fertility parameters, gross pathology findings, or litter observations in F1 animals. There was no evidence for an effect of RDEA 594 treatment on gross external parameters in F2 generation fetuses.

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10 Special Toxicology Studies

Ardea has proposed to administer lesinurad in combination with an XO inhibitor in order to mitigate the risk of uric acid microcrystal formation in the renal tubules in gout patients.

Ardea is not pursuing the inclusion of lesinurad as a fixed dose combination product with an XO inhibitor. Nonetheless, Ardea conducted two separate 13 week toxicology studies in rats to evaluate the effects of lesinurad + allopurinol (SR11-002) and lesinurad + febuxostat (SR13-051). Nonclinical support is not required for co-administration of lesinurad plus an FDA approved XO inhibitor at its recommended dose. Therefore, evaluations of these studies were not included in the NDA review.

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11 Integrated Summary and Safety Evaluation

Lesinurad (RDEA594) is under development by Ardea for the treatment of hyperuricemia associated with gout. Its proposed mechanism of action is via inhibition is to lower serum uric acid levels by inhibiting the reabsorption of uric acid from the renal proximal tubules. Lesinurad is to be administered once per day at 200 mg, in combination with a xanthine oxidase inhibitor.

Ardea has submitted nonclinical studies conducted with lesinurad that include pharmacology, safety pharmacology, ADME, general toxicology, genetic toxicology, carcinogenicity, and reproductive toxicology in support of marketing approval.

The applicant's nonclinical pharmacology and toxicology package for lesinurad is complete. The available data supports the safety of the proposed clinical dose of 200 mg/day for the (b) (4) treatment of hyperuricemia associated with gout, to be used in combination with a xanthine oxidase inhibitor.

Drug Substance Development

Ardea has chosen to proceed with a crystalline free acid form of the lesinurad drug substance for commercial development. Nonclinical studies were conducted with free acid and (b) (4) sodium salt forms of lesinurad (Table 64).

Table 65 provides a comparison of lesinurad systemic exposure values and target organs of toxicity in rats and monkeys treated with both the free acid and sodium salt forms of lesinurad. The target organs (kidney, GI tract) were consistent between both forms of the drug substance.

Lesinurad sodium salt had comparable systemic exposure to the free acid at the 100 mg/kg/day NOAEL dose in rats ($AUC_{0\text{-}24h}$ ~400 µg*hr/ml). In monkeys, systemic exposure was comparable between the two DS forms after 28 days of dosing at the NOAEL dose of 300 mg/kg/day ($AUC_{0\text{-}24h}$ 300 - 400 µg*hr/ml). It is noted that these findings in rats and dogs were recapitulated in humans in Clinical Study RDEA594-109. Healthy volunteers were given a single dose of the free acid tablet (200, 400, or 600 mg) or the sodium salt capsule (400 mg). At 400 mg, C_{max} and AUC values for the free acid and sodium salt were nearly identical. Major human metabolite M4 was also formed to a similar extent after administration of either form of lesinurad.

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Table 64. Summary of RDEA594 drug substance forms and the studies conducted with each form.

Drug Substance Form	Synthesis route	Lots	Studies conducted
(b) (4)	(b) (4)	10454-139-3 10454-154-4 10461-41-1 0804610	▪ Nonclinical ▪ Initial clinical studies
(b) (4) Sodium Salt		0804648 A10001-122-5 0904697 A10063-23-20 A10063-28-8 A10060-64-1 A10060-70-3 A10063-80-20 A10060-65-1 A10060-72-3 0904728 0904759 NE-023025-Batch-01-2009 NE-023025-Batch-02-2009 NE-023025-Batch-03-2009 NE-023025-Batch-04-2009	▪ Nonclinical ▪ Ph1 and Ph2 clinical studies
		17JL01.HQ00001	▪ Nonclinical development
Crystalline free acid		1004864 1004897	▪ Ph1 clinical studies
		1104966 17JL02.HE00002 17JL02.HE00004 17JL02.HE00006 17JL02.HE00007	▪ Ph 1, 2 & 3 clinical trials
		17JL02.HE00008 17JL02.HE00009 17JL02.HE000010 17JL02.HE000011 17JL02.HE000012 17JL02.HE000014 17JL02.HE000015 17JL02.HE000016	▪ Some Ph1 and Ph2 clinical trials ▪ All phase 3 clinical trials ▪ **Selected form for commercial production**

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Table 65. Summary of results of toxicology studies conducted in rats and monkeys with RDEA594 free acid and (b) (4) sodium salt forms.

Study #	RDEA 594 Form	Study Type	Species	Doses (mg/kg/day)	NOAEL (mg/kg/day)	Target organs	Relevant AUC (µg*hr/ml)
SR08-015 (GLP)	Free acid [10461-41-1]	14 day oral	Rat	0, 30, 100, 300, 1000	100	▪ Kidney: tubular degen. ▪ GI tract: epithelial cell necrosis	100 mg/kg M: 267 F: 454
SR08-034 (GLP)	Free acid [10454-139-3]	28 day oral	Rat	0, 10, 30, 100, 300	100	▪ Liver: hypertrophy ▪ Kidney: tubular degen.	100 mg/kg M: 433 F: 369
SR08-016 (GLP)	Free acid [10461-41-1]	14 day oral	Monkey	0, 30, 100, 300, 1000	300	▪ GI tract: neutrophil infiltration	300 mg/kg M: 249 F: 153
SR08-035 (GLP)	Free acid [10454-154-4]	28 day oral	Monkey	0, 10, 30, 100, 300	300	No target organs	300 mg/kg M: 408 F: 210
SR08-095 (GLP)	Sodium Salt [multiple lots]	6 months oral	Rat	0, 10, 30, 100, 300, 600	100	▪ Kidney: tubular dilatation ▪ Stomach: erosion/ hemorrhage	Day 92: 100 mg/kg M: 388 F: 373
SR08-094 (GLP)	Sodium Salt [multiple lots]	12 months oral	Monkey	0, 30, 100, 300, 1000	100	▪ GI tract: erosion ▪ Bile duct hyperplasia	Day 28: 300 mg/kg M: 295 F: 326 Week 50: 100 mg/kg (NOAEL) M: 81.4 F: 83.7 {▲ metabolism of Lesinurad over time in monkeys}

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Pharmacology

In vitro
URAT1 is considered to be the transporter responsible for the majority of the re-absorption of uric acid from the proximal tubule in humans[4]. Ardea has identified URAT1 as the principal target for lesinurad. In vitro studies were conducted using *Xenopus* oocytes and HEK-293 cells expressing specific transporters. The inhibitory potency of lesinurad was evaluated by measurement of transport of radiolabeled substrate in the presence of varying lesinurad concentrations. The evidence from these studies shows that lesinurad acts with similar potency (3 – 7 μM) at four different carrier proteins (URAT1, OAT4, OAT1, OAT3) that transport uric acid. The potency of lesinurad is comparable to that of the approved product probenecid.

Secondary pharmacology
Lesinurad was tested at 100 μM to evaluate its inhibition of specific substrate binding to a panel of 169 different receptors. Lesinurad inhibited ligand binding of 3 such receptors with an IC_{50} of less than 50 μM. These included the Prostanoid D2 (IC_{50} = 41.8 μM), angiotensin AT2 (IC_{50} = 31.2 μM), and the thromboxane A2 (Prostanoid TP) receptor with an IC_{50} of 4.9 μM. This receptor is involved in thrombosis/hemostasis as well as modulation of the immune response.

Ardea tested the functional effects of lesinurad (e.g. agonist or antagonist activity) at TP receptors in a separate in vitro functional assay with TP receptors expressed in HEK-293 cells. At a concentration of 100 μM, RDEA594 has no agonist effects at this receptor. Lesinurad (100 μM) showed minimal antagonism of this receptor as it inhibited the reference agonist response by approximately 15%.

In vivo
In vivo pharmacodynamic assessment of the serum uric acid (sUA) lowering ability of lesinurad in rodents and monkeys is considered to be inconclusive. Animal species express the uricase enzyme, which converts uric acid to allantoin. Humans do not express this enzyme. For this reason, baseline sUA levels are low in animals relative to humans.

Lesinurad demonstrated efficacy in a rat air pouch model of crystal-induced arthropathy, a rat model of monosodium urate induced gouty arthritis and a rat carrageenan induced paw edema model. RDEA594 treatment was not efficacious in the OVA induced airway inflammation model in mice or the rat adjuvant induced arthritis model.

Pharmacologic classification

The pharmacologic activity of lesinurad is comparable to that of the currently approved drug probenecid. Lesinurad is to be defined as a uricosuric, which is consistent with the classification of probenecid.

[4] Hediger M, et al. (2005) Molecular physiology of urate transport. *Physiology*. 20, 125-133.

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Safety Pharmacology

A complete battery of safety pharmacology studies was conducted with lesinurad. These studies included assessments of cardiovascular, respiratory, neurologic, renal/urinary, and gastrointestinal effects.

Cardiovascular
RDEA594 inhibition of the I_{Kr} potassium channel current was investigated in HEK 293 cells expressing the human ether-a-go-go related gene (hERG) in study SR08-021. Cells were held at -80 mV. Onset and steady state inhibition of hERG potassium current by RDEA594 were measured using a pulse pattern with fixed amplitudes (conditioning prepulse: +20 mV for 1 sec; repolarizing test ramp to –80mV (-0.5 V/s) repeated at 5 s intervals. RDEA594 was tested at 10, 30, 100, and 200 µM. RDEA594 inhibited the hERG current with a calculated IC_{50} of 198 µM.

Rabbit Purkinje fiber assays were conducted with and without human serum albumin (SR08-054, SR08-085). RDEA594 was tested at 0, 1, 5, 50, and 200 µM in each study. A slight increase in action potential duration was observed at the 200 µM dose in the absence of HSA. The NOAEL was defined at 50 µM. There were no RDEA594 related effects at any dose tested in the presence of HSA.

An in vivo assessment of cardiovascular and respiratory system function was conducted in telemetered male cynomolgus monkeys (SR08-020). Assessment of respiratory function was carried out after 2 oral doses of RDEA594 at 0, 30, 100, or 300 mg/kg administered 2 days apart (n = 4/dose level). Respiratory rate, tidal volume, and minute volume were collected pre-test, for at least 2 h prior to dose administration, and for 24 h post-dose. Cardiovascular parameters included heart rate, PR, QRS, RR, QT, and QTc intervals, as well as systolic, diastolic, and mean arterial pressure. Measurements were made pre-test, for at least 2 h prior to dose administration, and for 24 h post-dose.

Vomiting occurred in 1/4 animals at the 100 mg/kg dose and 3/4 monkeys at the 300 mg/kg dose within 2 hours of dosing. There were no treatment related effects on respiratory or cardiovascular parameters observed at any dose tested.

Central nervous system
Study SR08-027 tested neurologic function via functional observational battery in SD rats after a single dose of RDEA594 at 0, 30, 100, or 300 mg/kg. Functional observational battery parameters and motor activity was assessed at pre-dose, 2.5 and 24 h post-dose. There were no treatment-related effects observed at any dose tested.

Renal/urinary system function in male rats
Male SD rats (n = 8/group) were treated with a single oral dose of RDEA594 at 0, 30, 100, 300, or 1000 mg/kg. Clinical chemistry and urinary excretion parameters were measured. Urine samples were collected from t = 0 – 3 h post dose and during 3 – 24 h post dose. Blood samples for clinical pathology assessment were collected pre-dose and at 24 h post dose.

Serum sodium and chloride levels were decreased at 1000 mg/kg and ≥ 300 mg/kg respectively. The NOAEL for effects on clinical chemistry parameters was 100 mg/kg.

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In urine: sodium and chloride excretion was increased at ≥100 mg/kg. Urine osmolality was increased in animals at ≥ 300 mg/kg at 0 – 3 h after dosing. The following components were increased in urine at ≥ 300 mg/kg: uric acid, calcium, phosphorus, protein and potassium. The NOAEL for urinary excretion changes was 30 mg/kg. The NOAEL for clinical chemistry changes was 100 mg/kg.

Gastrointestinal system
A single oral dose of RDEA594 was administered to SD rats at 0, 30, 100, 300, or 1000 mg/kg. Approximately 2.5 h after dosing, 5% charcoal suspension in 10% acacia in deionized water was administered via oral gavage at a volume of 10 ml/kg.

The 1,000 mg/kg dose slowed GI motility by approximately 12 – 17% vs. control. Additionally, findings of watery feces were observed at the 1,000 mg/kg dose level. The NOAEL was established at 300 mg/kg.

ADME

Details pertaining to the absorption, distribution, metabolism, and elimination of lesinurad have been examined in both in vitro and in vivo studies conducted in mice, rats, dogs, monkeys, and humans. The current review focuses mainly on evidence in rats, monkeys, and humans.

Absorption
In vitro studies conducted in Caco-2 monolayers identified efflux ratios that ranged from 2.92 - 3.50, indicating active transport of RDEA594.

Oral bioavailability was determined in single dose studies conducted in rats and monkeys. Animals received a single 20 mg/kg [^{14}C]RDEA594 dose via the oral or intravenous route of administration. RDEA594 absorption was more rapid in rats than in monkeys as indicated by the shorter mean T_{max} (0.25 h vs. 1.33 hrs). RDEA594 oral bioavailability was greater in rats than in monkeys (73% vs. 41%)

Distribution
RDEA594 percent plasma protein binding values exceeded 94% in mouse, rat, dog, monkey, or human plasma. Plasma protein binding can affect the pharmacokinetic properties of a drug given that only unbound drug is available for passive diffusion to sites outside of the vasculature. Furthermore, only unbound drug is available for glomerular filtration. This is an important consideration based on the proposed mechanism of action for RDEA594 (i.e. inhibiting uric acid reabsorption from the kidney proximal tubule lumen).

Quantitative tissue distribution studies conducted in male rats found that RDEA594 distribution into tissues is limited after a single oral dose of 20 mg/kg [^{14}C]RDEA594. Volume of distribution values for rat (0.546 - 0.931 L/kg) and monkey (0.289 L/kg) suggest distribution into total body water. The kidney and liver were the only tissues in which tissue:blood ratios exceeded 2 at 24 h post-dose. The thyroid gland showed an increase in tissue:blood ratio over the 4-24 h post dose period, providing evidence of slower distribution to, and elimination from, this tissue. Maximum radioactivity levels in urine were detected at 2 h post-dose.

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Additional studies were conducted in monkeys to quantify the extent of [^{14}C]RDEA594 distribution to whole blood, bile, kidneys, and liver. Plasma radioactivity levels were greatest between 2 – 6 hours after dosing. The whole blood:plasma ratio ranged from 0.52 – 0.57, indicating that there was little distribution into red blood cells. The highest levels of radioactivity were detected in bile at the 2 h time point. Only a small fraction of the initial dose was recovered in kidney and liver tissue (0.2% and 1.6% of initial dose respectively).

[^{14}C]RDEA594 distribution was also examined in monkeys after 28 days of dosing. The results were consistent with those observed after a single dose.

*Metabolism*The major metabolites observed in humans were also observed in at least one nonclinical species. Metabolites M3, a hydroxylated metabolite, and M4, a dihydrodiol, represent two disproportionate human metabolites (12.3% and 20.7% of the initial dose). M3 is formed at low levels in both rats and monkeys, while M4 is observed at low levels in monkeys only.

The M3 metabolite has been qualified for safety with respect to general toxicity on the basis of its exposure at the NOAEL dose in the 12 month monkey toxicology study. It has also been qualified for safety with respect to carcinogenicity based on its exposure at the high dose in a 2 year rat carcinogenicity study in which there was no evidence for treatment-related tumor formation.

The M4 metabolite, a dihydrodiol, is formed from the action of microsomal epoxide hydrolase on the epoxide intermediate M3c. M3c is formed from the action of CYP2C9 on the parent molecule, RDEA594. The M3c epoxide metabolite raises concern as a structural alert for mutagenicity. M3c is unstable and is not detectable *in vivo* in any species tested (human, rat, monkey). However, monkeys metabolize M3c to M9, a cysteine conjugate of M3c. Measurement of M4 and M9 levels in monkey bile, urine, and feces provides an indication of exposure to M3c. M3c is qualified for safety with respect to general toxicity based on its calculated exposure at the NOAEL in the 12 month monkey toxicology study. The M3c metabolite is not qualified for safety with respect to carcinogenicity due to the low/absent levels of exposure in rodents. From a technical perspective, it would not be possible for the sponsor to directly administer M3c to rats in a stand-alone carcinogenicity study based on the unstable nature of epoxides. Some concerns with regard to the potential carcinogenic effects of M3c may be mitigated by its transient nature and the fact that, in humans, this molecule is detoxified to a didhydrodiol (M4).

Elimination
Mass balance studies conducted in rats and monkeys indicated that the majority of orally administered RDEA594 is eliminated via feces (Rats: 44 – 75%, Monkeys: 42%). In humans, the majority of orally administered RDEA594 (~63%) is eliminated in urine. RDEA594 was detected in the milk of lactating female rats at similar concentrations to concentrations observed in plasma.

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Ardea provided evidence for enterohepatic circulation of RDEA594 in male rats. In this study, bile duct cannulated rats were given a single dose of [^{14}C]RDEA594. Bile from each rat was collected and pooled from t = 0 – 6 h. The bile samples were then administered intraduodenally to rats. Approximately 45% of the RDEA594-derived radioactivity present in the bile that was intraduodenally administered was absorbed. RDEA594 was found to be the major component detected in the bile in Part II of the study, thus indicating that there is substantial enterohepatic circulation of RDEA594 in male rats.

General toxicology

Given that lesinurad will be administered chronically for the treatment of hyperuricemia associated with gout, the most relevant toxicology studies in support of marketing approval are considered to be the chronic studies conducted in rats (26 weeks) and monkeys (12 months). Ardea chose the monkey as the non-rodent species based on the lack of M4 metabolite (major human metabolite) formation in dogs. Evidence obtained in in vitro metabolism studies suggests that monkeys produce both the M3c epoxide intermediate and the M4 metabolite.

Rats

In the 6 month repeat-dose rat toxicology study, animals were dosed orally with RDEA594 at 0, 10, 30, 100, 300, and 600 mg/kg/d. An interim sacrifice was conducted at the 3 month time point. 13/60 high dose animals died within the first 3 weeks of dosing. These animals showed clear evidence of toxicity in the kidney and gastrointestinal tract. The remaining HD animals were euthanized on study day 23.

Mean body weight was decreased by 10-12% vs. controls in 300 mg/kg males at the 3 month time point and both males and females at this dose level at the 6 month time point. Decreased body weight values persisted through 4 and 8 week recovery periods respectively.

Pancreas (single cell necrosis) and stomach (erosion, hemorrhage) appeared to be potential target organs of toxicity at the 300 mg/kg dose at the 3 month time point, but not at the 6 month time point. Neither incidence nor severity of these findings increased between the 3 and 6 month time points. More specifically, the pancreas single cell necrosis and stomach erosion findings were each limited to an incidence of 1/14 (separate animals) with a severity grading of minimal after 6 months of treatment.

Hepatocellular hypertrophy observed at doses ≥ 100 mg/kg/day at the 3 month and 6 month time points was considered to be an adaptive response and not regarded as a dose-limiting toxicity. Indicators of potential treatment-related kidney toxicity at 300 mg/kg/d included increased water consumption, increased urine volume (decreased ability to concentrate urine), increased serum creatinine, and decreased BUN:creatinine ratio. However, there was limited microscopic evidence for overt kidney pathology at either the 3 month or 6 month time points (limited to increased incidences of tubular dilation for males and females at 300 mg/kg/day).

There was evidence for RDEA594 induced metabolic enzyme induction as systemic exposure to RDEA594 was decreased in 100 mg/kg and 300 mg/kg dose M & F at 3 months and 6 months vs. day 1 (accumulation ratio = 0.6 – 0.9). The NOAEL was judged to be 100 mg/kg/day based

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on mortality observed at the 600 mg/kg dose and histopathological findings at 300 mg/kg/day in the pancreas (single cell necrosis) and stomach (erosion, hemorrhage) at the 3-month time point and kidneys at the 6-month time point. This dose was associated with an AUC_{0-24h} of 415 µg*hr/ml.

Monkeys
In the 12 month repeat dose monkey toxicology study, animals were dosed orally with RDEA594 at 0, 30, 100, 300, or 600 mg/kg/day. There were 5 premature deaths in HD animals (600 mg/kg/d). 4/5 deaths were due to treatment related severe diarrhea, emesis, and decreased food consumption. In animals that survived to study completion, clinical signs of diarrhea, emesis and soft stool increased with dose and persisted throughout the study. Red discolored urine was observed primarily in animals in the 300 mg/kg/d and 600 mg/kg/d groups. This finding reversed upon cessation of treatment. The source of the discoloration was not identified. There is no evidence to suggest that blood or red blood cells are the source of discoloration. Increased liver and kidney weights were observed in males and females receiving ≥ 100 mg/kg/d RDEA594.
Systemic exposure of RDEA594 decreased over time, indicative of enzyme induction. Decreased RDEA594 exposure was accompanied by a concurrent increase of metabolite M6. The observed bile duct hyperplasia in 300 mg/kg/d males and 600 mg/kg/d M & F with correlating increase in gamma glutamyltransferase levels in affected animals was considered to be dose limiting. The NOAEL was judged to be 100 mg/kg/day. This dose was associated with an AUC_{0-24h} of 82.6 µg*hr/ml.

Common target organs of RDEA594 in the rat and monkey include the kidney and GI tract. Premature deaths related to toxicities in these organs were observed in HD animals (600 mg/kg/d) in both rats and monkeys. Evidence for autoinduction was also observed in both species, and systemic exposure was markedly reduced in monkeys receiving ≥ 300 mg/kg/d after 12 weeks of treatment. The monkey is the more sensitive species, with an AUC_{0-24h} of 82.6 µg*hr/ml. This NOAEL supports the maximum clinical RDEA594 exposure at the proposed 200 mg/day dose level (Table 66).

Table 66. Exposure margins between AUC_{0-24h} at rat and monkey NOAELs in chronic toxicology studies and the clinical exposure at the 200 mg dose.

Species	**NOAEL (mg/kg/day)**	**AUC_{0-24h} (µg*h/mL)**		
Rat	100	415		
Monkey	100	82.6		
			Exposure margin	
	Clinical Dose (mg)		Rat	Monkey
Human[1]	200	28.0	15	3.0

[1] Mean AUC_{0-24} (M & F combined, n = 12) for 200 mg RDEA594 administered 1x/d for 14 days in healthy adults (Study RDEA594-105)

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Genetic toxicology

The mutagenic and clastogenic potential of RDEA594 was assessed in the standard battery of genotoxicity assays.

In the bacterial reverse mutation assay, revertant colony counts for *Salmonella typhimurium* strains TA 1535, TA1537,TA98, and TA100 and *E. coli* strain WP2 *uvrA* were unaffected following treatment with RDEA594 at doses up to 5000 µg/plate with or without metabolic activation (Aroclor1254-induced rat liver S9), indicating no evidence of mutagenic potential under the conditions of the assay.

In the *in vitro* mammalian chromosome aberration test, the percent of cells with structural or numerical aberrations in RDEA594-treated groups was not significantly increased above controls and was also within the range of historical values. Therefore, RDEA594 was considered to be negative for induction of chromosomal aberrations under the conditions of the study.

RDEA594 was found to be negative in the *in vivo* clastogenicity assay in rats. There was no evidence for induction of micronuclei formation in bone marrow of male or female rats after single oral doses up to 1000 mg/kg.

Carcinogenicity

RDEA594 was not tumorigenic in SD rats or TgRasH2 mice.

Rats
RDEA594 was administered to SD rats at doses of 0, 25, 75, or 200 mg/kg/day by oral gavage in a 2 year carcinogenicity study. These doses received concurrence from the ECAC (See meeting minutes: IND 102128, 12/22/09). The 200 mg/kg/day dose showed evidence for achieving maximum tolerated doses in males and females based upon decreased body weights. There were no dose groups that survived to the planned 104-week study duration. However, there was no evidence for a treatment-related effect of RDEA594 on survival. RDEA594 was not tumorigenic in rats at doses up to 200 mg/kg/day. Treatment related non-neoplastic lesions in the GI tract, liver, kidney, pancreas and thyroid were generally consistent with findings in the 6 month toxicology study in SD rats.

Mice
RDEA594 was administered to hemizygous TgRasH2 mice at doses of 0, 15, 45, or 125 mg/kg/day (males) and 0, 30, 90, or 250 mg/kg/day (females) by oral gavage for a duration of 26 weeks. These doses received concurrence from the ECAC (See meeting minutes: IND 102128, 4/28/11). The high doses in males and females represented maximum tolerated doses (MTDs) based on deaths in males at ≥ 500 mg/kg/day and females at ≥750 mg/kg/day in a 1-month dose range finding study. Positive control animals in the current study received a single intraperitoneal injection of 75 mg/kg N-methyl-N-nitrosourea (MNU) on study day 1. The study design is consistent with established methods for carcinogenicity testing in TgRasH2 mice[5]

[5] Takaoka M. et al. (2003) Interlaboratory comparison of short-term carcinogenicity studies using CB6F$_1$-*ras*H2 transgenic mice. *Toxicologic Pathology*. 32, 191-199.

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There was no effect of RDEA594 on survival. RDEA594 was not tumorigenic in males at up to 125 mg/kg/day or females at up to 250 mg/kg/day (highest doses tested). Neoplastic lesions in MNU-treated mice were detected in the hematopoietic system (malignant lymphosarcoma), skin, and nonglandular stomach. These target organs are consistent with established results in MNU-treated TgHras2 mice and provide support that the study could detect a tumorigenic response to a carcinogen.

RDEA594-treatment related non-neoplastic lesions in the liver (hepatocyte hypertrophy) observed in MD & HD males and HD females were consistent with findings in the 1 month dose-range finding study in WT mice as well as 6 month toxicology and 2 year carcinogenicity studies conducted in SD rats (Study SR08-095 and SR09-2168 respectively). Additional non-neoplastic lesions identified in the glandular stomach were consistent with evidence observed in long-term studies in SD rats.

The results of the 2-year oral carcinogenicity study with rats and 26-week oral carcinogenicity study conducted in TgRasH2 mice were discussed with the Executive Carcinogenicity Assessment Committee (ECAC) on May 19, 2015 and June 30, 2015, respectively. In both cases, the ECAC concurred that the studies were acceptable, and that there was no evidence for treatment-related neoplasms in rats or mice. No further studies were required for the safety qualification of the M3c epoxide intermediate. It was considered technically unfeasible to administer the unstable epoxide intermediate to rodents in a carcinogenicity study. There is an undefined risk to human subjects from exposure to the epoxide intermediate. Some concern with regard to the potential carcinogenic effects of M3c may be mitigated by its transient nature and the fact that, in humans, this molecule is detoxified to a dihydrodiol (M4).

Reproductive toxicology

RDEA594 showed adverse effects on several reproductive parameters, including prolonged estrous cycles, a slight increase in mean number of days to mate, a slight decrease in number of corpora lutea and implantation sites in rats that received an oral dose of 300 mg/kg/day, which also caused overt parental toxicity in females and males. There was no evidence of teratogenicity or any other embryo-fetal developmental toxicity in rats and rabbits that received oral doses up to 300 and 75 mg/kg/day, respectively, during the period of organogenesis.

Fertility and early embryonic development

RDEA594 was tested at oral doses of 0, 75, 150, and 300 mg/kg/day. Male rats were dosed for at least 10 weeks prior to scheduled sacrifice, including 4 weeks prior to mating and throughout the cohabitation period through the day prior to scheduled sacrifice. Female rats were dosed for 2 weeks prior to mating and throughout the cohabitation period, and Gestation Days 0 through 7.

Female rats at 300 mg/kg/day exhibited overt toxicity, including mortality, adverse clinical signs, and decrease in mean body weight gain and mean food consumption. Male rats at this dose also exhibited adverse clinical signs and decrease in mean body weight gain and mean food consumption. Males at 150 mg/kg/day and females at 75 and 150 mg/kg/day also exhibited slightly decreased mean body weight gain.

Female rats at 300 mg/kg/day exhibited prolonged estrous cycles and a slight increase in mean number of days to mate, compared to the control group. In addition, slightly decreased mean

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numbers of corpora lutea and implantation sites were observed in this dose group in the presence of overt toxicity.

Embryonic fetal development (EFD)
In the rat EFD study, time-mated female rats received RDEA594 at oral doses of 0, 75, 150, and 300 mg/kg/day from Gestation Day (GD) 6 through GD 17. Rats at 300 mg/kg/day exhibited maternal toxicity, including mortality, adverse clinical signs, decrease in mean food consumption and mean body weight gains. There were no test article-related effects on post-implantation loss or fetal parameters including body weight, sex ratio, external, visceral or skeletal observations. The NOAEL for maternal toxicity was 150 mg/kg/day, whereas the NOAEL for embryofetal developmental toxicity was 300 mg/kg/day. Thus, RDEA594 was not teratogenic in rats at oral doses up to 300 mg/kg/day.

In the rabbit EFD study, time-mated female rabbits received RDEA594 at oral doses of 0, 25, 75, and 125 mg/kg/day from GD 7 through GD 20. Rabbits at 125 mg/kg/day exhibited maternal toxicity, including mortality, adverse clinical signs, a marked decrease in food consumption and body weight loss. These findings led to the early termination of all remaining rabbits in this group. In addition, two and one dams at 75 and 25 mg/kg/day, respectively, were also preterminally sacrificed due to severe toxicity similar to that observed at 125 mg/kg/day.

There were no observed test article related effects on post-implantation loss and fetal parameters including body weight, sex ratio, external, visceral or skeletal observations in animals at the 25 and 75 mg/kg/day dose levels. Due to the early termination of all animals in the 125 mg/kg/day dose group, there were no available Caesarean section or fetal data from these animals.

A NOAEL for maternal toxicity was not identified due to mortality in all RDEA594-dosed groups. The NOAEL for embryo-fetal developmental toxicity was 75 mg/kg/day, the highest dose that was examined for fetal parameters. Thus, RDEA594 was not teratogenic in rabbits at oral doses up to 75 mg/kg/day.

Pre- and postnatal development
Pregnant female rats were dosed orally with RDEA 594 at 0, 100, 200, or 300 mg/kg/day from gestational day 7 (GD 7) – lactation day 20 (DL 20). RDEA594 was highly maternally toxic at ≥ 200 mg/kg/day, with target organs including the stomach, kidney, and liver. The milk: plasma ratio was ≥ 1 in all treatment groups, showing that RDEA594 distributes approximately evenly between plasma and milk. There was no NOAEL identified with respect to maternal toxicity based on statistically significant, dose-dependent decreases in body weight gain >10% at all doses.

RDEA594 at ≥ 200 mg/kg evoked multiple adverse effects on F1 offspring. 200 mg/kg/day RDEA594 treatment resulted in significantly reduced viability index, mean number of surviving pups per litter at PND 21, and mean pup weight at birth & PND 21 vs. controls. The litter size at PND 21 value and mean pup weight value were both outside of the range observed in historical control animals.

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Viability index (# of live pups on d 4/ # of live pups on d 1) and lactation index (# of live pups on d 21/ # of live pups on d 4) were significantly lower in the 300 mg/kg dose group vs. controls. The mean number of surviving pups at day 21 and mean pup weight at birth & day 21 were also significantly reduced in the 300 mg/kg group vs. controls. Litter size at day 21 value and mean pup weight values were outside of the range observed in historical control animals.

Mean time to vaginal patency was significantly delayed in F1 females in the 200 mg/kg and 300 mg/kg groups. There were no treatment-related effects on learning and memory or mating and reproduction performance in the F1 generation. The NOAEL for fetal developmental toxicity was 100 mg/kg. RDEA594 did not induce gross external changes in F2 generation fetuses.

Qualification of M3c metabolite
No further studies were required for the safety qualification of the M3c epoxide intermediate with respect to reproductive toxicology. It was considered technically unfeasible to administer the unstable epoxide intermediate to rodents and/or rabbits in reproductive toxicology studies. There is an undefined risk to human subjects from exposure to the epoxide intermediate. Some concern with regard to the potential adverse effects of M3c may be mitigated by its transient nature and the fact that, in humans, this molecule is detoxified to a dihydrodiol (M4).

Unresolved toxicology issues (if any):

None

Recommendations:

NDA 207988 is recommended for approval from the nonclinical pharmacology and toxicology perspective.

Evaluation of labeling:

Ardea submitted proposed labeling in general conformance with the Physician Labeling Rule (PLR, 21 CFR Parts 201, 314, and 601) "Requirements on Content and Format of Labeling for Human Prescription Drug and Biological Products" (Final Rule: January 24, 2006) as well as the Pregnancy and Lactation Labeling Rule (PLLR) "Content and Format of Labeling for Human Prescription Drug and Biological Products; Requirements for Pregnancy and Lactation Labeling" (Final Rule: December, 2014).

Recommended changes from the nonclinical perspective were made to the language used in the following sections: Indications and Usage, Section 8 (Use in Specific Populations), Section 12.1 (Mechanism of Action), and Section 13 (Nonclinical Toxicology). The revised labeling in the relevant nonclinical sections containing the recommended labeling changes is shown below with "track changes" visible. Written explanations of the proposed changes follow.

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---------- **INDICATIONS AND USAGE** ----------

ZURAMPIC is a uricosuric agent ~~URAT1 inhibitor~~ indicated for the treatment of hyperuricemia associated with gout in combination with a xanthine oxidase inhibitor. (1)

8.1 Pregnancy

Risk Summary

There are no available data on use of Zurampic in pregnant women to inform a drug-associated risk. No teratogenicity was observed in embryo-fetal development studies with oral administration of lesinurad to pregnant rats and rabbits during organogenesis at doses that produced maternal exposures up to approximately 45 and 10 times, respectively, the exposure at the maximum recommended human dose (MRHD). No adverse developmental effects were observed in a pre- and postnatal development study with administration of lesinurad to pregnant rats from organogenesis through lactation at a dose approximately 5 times the MRHD.

[*see* (b) (4) *Data*]

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data

Animal Data

In an embryo-fetal development study in pregnant rats dosed during the period of organogenesis from gestation days 6-17, lesinurad was not teratogenic at exposures up to approximately 45 times the MRHD (on an AUC basis at maternal oral doses up to 300 mg/kg/day). In an embryo-fetal development study in pregnant rabbits dosed during the period of organogenesis from gestation days 7-20, lesinurad was not teratogenic at exposures up to approximately 10 times the MRHD (on an AUC basis at maternal oral doses up to 75 mg/kg/day). Severe maternal toxicity, including mortality, was observed in rats and rabbits at exposures equal to or greater than approximately 45 and 4 times the MRHD (on an AUC basis at maternal oral doses of 300 mg/kg/day in rats and 25 mg/kg/day and higher in rabbits) respectively.

In a pre- and post-natal development study in pregnant female rats dosed from gestation day 7 through lactation day 20, lesinurad had no effects on delivery or growth and development of offspring at a dose approximately 5 times the MRHD (on a mg/m^2 basis at a maternal dose oral dose of 100 mg/kg/day). (b) (4)

(b) (4)

(b) (4)

8.2 Lactation

Risk Summary

There is no information regarding the presence of Zurampic in human milk, the effects on the breastfed infant, or the effects on milk production. Lesinurad is present in the milk of rats *[see Use in Specific Populations (8.1)]*. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Zurampic and any potential adverse effects on the breastfed infant from Zurampic or from the underlying maternal condition. (b) (4)

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

The carcinogenic potential of lesinurad was evaluated in Sprague-Dawley rats and TgRasH2 mice. No evidence of tumorigenicity was observed in male or female rats that received lesinurad for 91 to 100 weeks at oral doses up to 200 mg/kg/day (approximately 35 times the MRHD on an AUC basis). No evidence of tumorigenicity was observed in TgRasH2 mice that received at lesinurad for 26 weeks at oral doses up to 125 and 250 mg/kg/day in male and female mice, respectively. (b) (4)

(b) (4)

Lesinurad tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* chromosomal aberration assay in Chinese hamster ovary ~~(CHO)~~ cells, and *in vivo* rat bone marrow micronucleus assay ~~in rat bone marrow~~.

(b) (4)

Fertility and reproductive performance were unaffected in male or female rats that received lesinurad at oral doses up to 300 mg/kg/day (approximately 15 times the MRHD on a mg/m^2 basis).

(b) (4)

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Indications and Usage

There is currently no Established Pharmacologic Class (EPC) for inhibitors of uric acid reabsorption. Ardea proposes to use "URAT1 inhibitor" as the EPC for lesinurad.

Probenecid is the only marketed drug product in the US that acts to lower serum uric acid levels by inhibiting the reabsorption of uric acid from the urinary filtrate in the renal proximal tubule. Lesinurad and probenecid both inhibit multiple carrier proteins that transport uric acid: specifically URAT1, OAT4, OAT1 and OAT3. Lesinurad and probenecid act with comparable potency at each of these receptors (Table 2). The language used in the "Description" section the prescribing information for probenecid states that "Probenecid is a uricosuric and renal tubular transport blocking agent".

Associate Director for Pharmacology & Toxicology Dr. Paul Brown and medical officers Drs. Sarah Yim and Rosemarie Neuner were consulted for input on the proposed sponsor's proposed language in this section. The conclusion was that the EPC used for lesinurad should be "uricosuric" rather than "URAT1 inhibitor". The rationale behind this conclusion is that allowing for the use of "URAT1 inhibitor" might present a misleading impression that lesinurad is more specific and/or acts via a different mechanism than probenecid when in fact it is very similar.

8. Use in Specific Populations

Recommended changes to the language in Sections 8.1 (Pregnancy), 8.2 (Lactation) and (b) (4) [redacted] were made in collaboration with the Pediatric and Maternal Health Staff. Sections 8.1 and 8.2 were structured to be consistent with the PLLR.

Section 8.1

The nonclinical review team is generally in agreement with the sponsor's interpretation of the reproductive toxicology studies referenced in this section of the label. Recommended changes were made to (b) (4) [redacted]. Exposure multiples referencing data from the pre-and postnatal development study in rats were calculated on a mg/m^2 basis (Table 67).

It is also noted that the sponsor (b) (4) [redacted] are not required for prescribing decisions and are recommended to be removed from the sections 8.1 and 15.

Section 8.2

The sponsor's proposed language was adjusted to establish consistency with language in currently approved products.

(b) (4) [redacted]

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12.1 Mechanism of Action

Ardea's proposed description of lesinurad as a "[redacted] (b) (4) [redacted]" was removed based on the *in vitro* pharmacology data that shows relatively comparable selectivity between 4 different carrier proteins that transport uric acid: URAT1, OAT1, OAT3, and OAT4 (Table 2).

13. Nonclinical Toxicology

The nonclinical review team is generally in agreement with the sponsor's interpretation of the reproductive toxicology studies referenced in this section of the label. Changes were made to exposure multiple values for the rat carcinogenicity data and the rat fertility data. Ardea's proposed labeling in the *Impairment of Fertility* subsection calculated exposure multiples based on [redacted] (b) (4) was not measured in the rat fertility and early embryonic development study. Therefore exposure multiples were calculated on a mg/m^2 basis (Table 67).

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Table 67. Lesinurad exposure multiples from rodent carcinogenicity studies and reproductive toxicology studies, calculated on a mg/m^2 basis.

Drug: **Lesinurad**

	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m^2
Pediatric	6-11			0	18	0.0000	25	0.00
Adult	>12	200	1	200	60	3.3333	37	123.33

		route	mg/kg/d	conv. factor	mg/m^2	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
	Carcinogenicity:								
Male	mouse	oral	125	3	375	3.04	---	3	---
Female	mouse	oral	250	3	750	6.08	---	6	---
	rat	oral	200	6	1200	9.73	---	10	---
	Reproduction and Fertility:								
Rat FEED	rat	oral	300	6	1800	14.59	N/A	15	N/A
Rat PPND	rat	oral	100	6	600	4.86	N/A	5	N/A
Rat PPND	rat	oral	200	6	1200	9.73	N/A	10	N/A
	Teratogenicity:								
Rat EFD	rat	Oral	300	6	1800	14.59	N/A	15	N/A
Rat EFD	rat	Oral	300	6	1800	14.59	N/A	15	N/A
Rabbit EFD	rabbit	Oral	25	12	300	2.43	N/A	2	N/A
Rabbit EFD	rabbit	Oral	75	12	900	7.30	N/A	7	N/A

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12 Appendix/Attachments

Appendix 1: Nonclinical Review of IND 102128. June 18, 2010.
Appendix 2: Nonclinical Review of IND 102128, April 29, 2011.
Appendix 3: Nonclinical Review of IND 102128. June 22, 2011.
Appendix 4: Nonclinical Review of IND 102128. June 23, 2011.
Appendix 5: Nonclinical Review of IND 102128. December 24, 2012.

Appendix 1: Nonclinical Review of IND 102128. June 18, 2010.

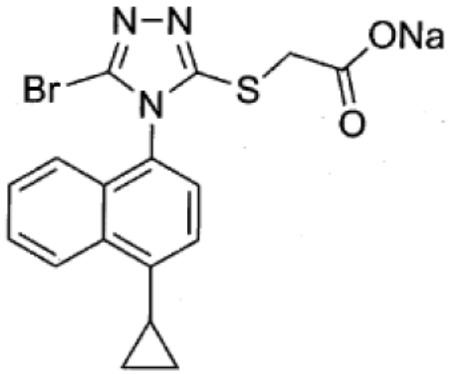
APPEARS THIS WAY ON ORIGINAL

FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Rheumatology Products
10903 New Hampshire Avenue, Silver Spring, MD 20993

30-DAY INITIAL SAFETY REVIEW
MEMO TO FILE

IND number:	102,128
Sponsor:	Ardea Biosciences, Inc.
Submission Type:	Original IND
Supporting Doc Number:	1
Submission Date/Receipt:	September 30, 2009 / October 2, 2009
Drug Substance:	
Code name	RDE594
Chemical name:	Sodium, [5-Bromo-4-(4-cyclopropyl-napthalen-1-yl)-4H-[1, 2, 4]triazol-3-yl-sulfanyl]-acetate
CAS registry number:	1151516-14-1
Molecular formula/molecular weight:	$C_{17}H_{13}BrN_3NaO_2S$ / 426.26 Daltons (404.28 for the free acid)

Structure:



Reviewer name:	Kathleen Young, Ph.D.
Division name:	Division of Anesthesia, Analgesia, and Rheumatology Products
Review completion date:	10/30/2009

Recommendation: The proposed protocol is considered reasonably safe to proceed from a pharmacology and toxicology perspective.

Background/Prior Regulatory History:

(b) (4)

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(b) (4)

the Sponsor was encouraged to submit an IND using the RDEA594 compound. The present IND 102,128 represents that effort.

RDEA806

- **Nonclinical Studies on RDEA806**
  - **See Appendix 1 for listing of nonclinical findings**
- **Clinical Studies on parent compound RDEA806**
  - Include repeated-dose clinical studies at doses of up to 500 mg/day for 14 days (RDEA594 AUC 83 mcg.h/ml, Cmax 5 mcg/ml)
- **PIND** 102,128 meetings of April 1, 2008 and June 2, 2008 (**RDEA594** for hyperuricemia in patients with gout):
  - **Recommendations to Sponsor**:
  - 28-d GLP studies in rat and monkey adequate to support 3-d dose escalation clinical trial of up to 28 days with adequate safety margins (≥10X NOAEL for starting dose in First in Human study, and dose escalation limited by nonclinical toxicokinetic values at NOAEL of most sensitive species using safety margin of ≥1
  - Genotoxicity support using RDEA806 (parent) may be included if data supports significant exposure to RDEA594
  - (b) (4) not sufficient to support chronic RDEA594 study in gout patients with hyperuricemia of 6-8 weeks duration; however, the 28-day studies on RDEA594 in rat could support 1-month trial and the chronic rat study (RDEA594) could support chronic clinical dosing pending adequate safety margins
  - RDEA806 reproductive toxicity studies in rats and rabbits can support RDEA594 development if bridging toxicity studies demonstrate appropriateness, there is adequate dosing demonstrated by measured levels of RDEA594, and RDEA594 dose levels exceed human blood levels
  - RDEA806 carcinogenicity studies may support registration of RDEA594 pending results of RDEA806-RDEA594 bridging studies (equivalent toxicity profiles and RDEA594 exposures ≥over clinical exposure at highest recommended dose level, unless MTD is demonstrated; post-approval carcinogenicity testing will not be considered to satisfy requirements for RDEA594 registration, by the Division
  - An ADME package should be submitted for RDEA806, RDEA594 and other relevant metabolites for all species evaluated
  - Monkey may not be appropriate non-rodent nonclinical test species for RDEA594 or RDEA806, based on ADME and absence of toxicity in this species

RDEA 594

- **Studies on RDEA594 in other countries**:
  - 3 Phase 1 clinical studies in 125 healthy adult male subjects in United Kingdom at single doses from **5-600 mg**, or once daily from **100-600 mg for 7-10 days**

Reference ID: 3815621

- 1 Phase 2a clinical study in Canada in 21 gout patients given doses of 200 mg/day for 7 days followed by 400 mg/d for 7 days, vs. placebo and allopurinol (300 mg for 14 days)
- Phase 2 Study in Patients with Gout (RDEA594-501) in 35 patients at doses of 400 and 800 (400 mg b.i.d.) mg/d for 4 weeks

Relevant INDs/NDAs/DMFs:

IND/NDA/DMF	Drug Name	Division	Status	Indication	Company
				(b) (4)	Ardea
					Ardea

Hypothesis for use: RDEA594 is the major metabolite of RDEA806, a non-nucleoside reverse transcriptase inhibitor (NNRTI) in animals and humans. The subjects in the clinical studies on RDEA806 for the treatment of HIV showed significantly decreased urinary urate levels, correlating with urinary RDEA594 concentrations (R2 = 0.9858), (b) (4) This observation led to multiple studies resulting in isolation of RDEA594 as the responsible component and to development of this metabolite for the potential treatment of gout via its uricosuric properties in blocking reuptake of uric acid in the kidney by URAT1 transporter inhibition.

Clinical trial proposed: ***A Phase 1, Single Dose, Pharmacokinetic and Pharmacodynamic Study of RDEA594 in Adult Volunteers with Various Degrees of Renal Insufficiency (Study RDEA594-104)***:

- In-patient (72 hours after dosing), multi-center
- 4 cohorts (1 healthy and 3 with renal insufficiency to include mild, moderate and severe) of 8 male and female subjects each; total **32 subjects**
- **Single-dose 400 mg (4x100mg capsules, Lot 09JM-250) in fed state**
- PK samples from 0-72 hours after dosing
- Safety measures for up to 72 hours after dosing prior to discharge, and 8 days after dosing at time of Follow-up visit
- Efficacy (uricosuric effects) measured by urinary uric acid and creatinine, serum uric acid and creatinine from 6 through 72 hours after dosing

Additional Clinical Studies in the Investigational Plan (all using immediate relase capsule formulation):

- RDEA594 at up to 600 mg/day for up to 28 days with and without xanthine oxidase inhibitors febuxostat and/or allopurinol (drug-drug interaction study)
- Phase 2b dose response study (200, 400, 600 mg/d) for 28 days in hyperuricemic gout patients with normal or mild renal insufficiency (ongoing in Europe and Canada)
- Phase 1 drug-drug interaction study in healthy adults in Canada at 200 and 400 mg/d for 14 days with febuxostat (40 mg/d)

Reference ID: 3815621

- Phase 2b efficacy, safety and drug interaction study in Europe and North America at 100-400 mg/d for 28 days with allopurinol (300 mg) in gout patients with normal or mild renal insufficiency
- Phase 2b study in hyperuricemic gout patients with renal insufficiency , with and without allopurinol in Belgium

Previous Clinical Experience:

Studies on RDEA594 in other countries:

- 3 Phase 1 clinical studies in 125 healthy adult male subjects in United Kingdom at single doses from **5-600 mg**, or once daily from **100-600 mg for 7-10 days**
    - Most frequent AE: headache in 3/28 subjects (11%), dizziness in 2 subjects (7%), with no treatment-related changes of concern in clinical laboratory and cardiovascular parameters (including QT interval) at up to 600 mg single dose, in first study (RDEA594-101)
    - In second (10-day) study (RDEA594-102), diarrhea (1-2 subjects at each dose level) attributed to study conditions, headache in 2/6 subjects at **200 mg IR** capsule (fed state), dizziness in 2/6 subjects at **400 mg/d IR capsules** (asted), dry mouth in 2/6 subjects at 600 mg ER (fed state)
    - In Study RDEA594-103 (Single dose food effect), AEs included back pain, upper respiratory tract infection in 2/18 subjects at 50 mg single dose
- Phase 2a clinical study in Canada (RDEA594-201) in 21 gout patients given doses of 200 mg/day for 7 days followed by **400 mg/d for 7 days**, vs. placebo and allopurinol (300 mg for 14 days)
    - Mild to moderate transient treatment-related increased serum creatinine in 2/11 subjects, flatulence in 1/11 subjects
- Phase 2 Study in Patients with Gout (RDEA594-501) in 35 patients at doses of **400 and 800 (400 mg b.i.d.) mg/d for 4 weeks**: preliminary data showed arthralgia, gout flare, nasopharyngitis in 3 patients, increased lipase, joint stiffness, dizziness an dhypertension in 2 patients, with no adverse treatment-related findings in the clinical laboratory and ECG measurements

Reference ID: 3815621

PK/TK

Single Dose PK: Clinical Study RDEA594-103

Dose (mg)/Form	n	C_{max} (mcg/ml)	T_{max} (h)	AUC_{0-24h} (mcg.h/ml)	AUC_{0-inf} (mcg.h/ml)	$T_{1/2}$ (h)
50 mg IR capsule (fasted)	6 M	2.26	1.00	7.99	8.01	6.00
50 mg IR capsule (fed)	9 M	1.91	2.00	7.59	7.62	5.06
50 mg (b) (4) tablet (fasted)	9 M	0.623	3.00	3.65	3.65	7.44
50 mg (b) (4) tablet (fed)	9 M	0.916	5.00	5.84	5.84	6.52
50 mg (b) (4) tablet (fasted)	8 M	0.320	3.00	1.97	1.97	6.06
50 mg (b) (4) tablet (fed)	9 M	0.526	4.00	3.19	3.19	5.56
200 mg (b) (4) tablet (fasted)	8 M	1.72	3.00	11.0	11.0	7.08
200 (b) (4) tablet (fed)	9 M	3.49	5.00	20.5	20.5	6.39

Single Dose PK: Clinical Study RDEA594-101 (n=4/group): Reproduced in part from the original IND submission

Food State	Dose (mg)	C_{max} (mcg/ml)	AUC_{0-24h} (mcg.h/ml) Normalized*	AUC_{0-inf} (mcg.h/ml) Normalized*	AUC_{0-24h} (mcg.h/ml)	AUC_{0-inf} (mcg.h/ml)	T_{max} (h)
Fasted	5	3.97	10.3	10.2	0.787	0.779	0.75
Fasted	25	5.18	10.5	10.6	3.12	3.15	0.50
Fasted	100	5.34	12.4	12.4	17.5	17.9	0.50
Fasted	200	4.56	12.5	12.5	31.9	32.2	0.50
Fed (low fat)	100	2.54	11.7	8.89	10.9	11.2	1.50
Fed (low fat)	400	3.64	15.7	11.7	56.4	56.9	0.25
Fed (low fat)	600	3.21	8.79	15.7	106	108	8.75

*For dose and body weight (mg/kg)

Repeated Dose PK: Clinical Study RDEA594-102 (n=6/group): Reproduced in part from the original IND submission

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Day	Form	Dose (mg)	C_{max} (mcg/ml)	AUC_T (mcg.h/ml)	$AUC_{0\text{-}inf}$ (mcg.h/ml)	T_{max} (h)
1	(b) (4) IR Tablet	100 (fed)	4.74	14.2	14.2	0.75
		200 (fasted)	10.8	28.7	28.9	0.75
		400 (fasted)	23.1	70.5	71.2	1.00
		200 (fed)	5.54	29.8	30.2	5.00
	(b) (4) Capsule	200 (fed)	8.18	26.1	26.3	3.00
		400 (fed)	13.8	51.8	52.3	3.00
		600 (fed)	20.1	73.5	74.4	2.50
	(b) (4) Tablet	200 (fed)	6.16	20.9	21.1	1.75
10	(b) (4) IR Tablet	100 (fed)	4.02	12.1	Not provided	0.625
		200 (fasted)	11.7	33.1	Not provided	0.75
		400 (fasted)	21.9	89.8	Not provided	2.00
		200 (fed)	6.51	30.3	Not provided	4.50
	(b) (4) Capsule	200 (fed)	7.95	25.9	Not provided	3.50
		400 (fed)	15.7	53.9	Not provided	2.50
		600 (fed)	20.8	84.9	Not provided	3.50
	(b) (4) Tablet	200 (fed)	5.57	21.2	Not provided	4.50

Formulation/Impurities-Degradants/Excipients:

Formulation:

(b) (4) **Capsule containing the following Ingredients**:
RDEA594 Na salt (Drug Substance **Lot NE-023025-Batch-01-2009**) (b) (4)
(b) (4)

Total Fill Weight

(b) (4)

Excipients:

All excipients USP/NF grade

Impurities:

Potential Impurities

(b) (4)

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(b) (4)

Impurities Identified in the Nonclinical and Clinical Study Batches (Reproduced from the original IND submission):

Impurity (drug-related and process-related)	Acceptance Criteria[1,2]	Results				
		10461-41-1 14-d DRF studies in rat and monkey (RDEA594 free acid)	10454-139-3 28-d GLP toxicology in rat, safety pharmacology, genetox (Ames, Chrom Ab, Micronucleus) (RDEA594 free acid)	10454-154-4 28-d GLP toxicology in monkey (RDEA594 free acid)	NE-023025-Batch-01-2009 Clinical (RDEA594 Sodium salt)	Lot 0904759 Clinical (RDEA594 Sodium salt), Ames Test (impurity)

(b) (4)

[1]Acceptance criteria (**bolded**) have been tightened subsequent to release of lot NE-123025-Batch-01-2009. Lot 0904759 was released against current acceptance criteria provided in section 3.2.S.4.1 of the IND submission.
[2]Acceptance criteria applicable to GMP batches only.

Qualification of the Impurities (Reproduced from the original IND submission):

Impurity (Specification Limit)	Qualifying Toxicology Study	Drug Lot	% Impurity	RDEA594 NOAEL (mg/kg)	RDEA594 NOAEL HED[1] (mg/kg)	Maximum Permissible Impurity Level[2] (mg/kg)	Level of Impurity at Maximum Human Dose[3] (mg/kg)
(b) (4)	14-day Rat DRF	10461-41-1					(b) (4)
	14-day Rat DRF	10461-41-1					
	14-day Rat DRF	10461-41-1					
	28-day Rat	10454-139-3					

[1]RDEA594 NOAEL ÷ (b) (4) surface area conversion factor = human equivalent dose (HED)
[2]RDEA594 NOAEL x % impurity = Maximum Permissible Impurity Level

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[3]Level of Impurity at Maximum Human Dose = Impurity % x maximum clinical dose in mg/kg ((b) (4) mg/kg based on 60 kg body weight)

- RDEA594 free acid Lot 10454-139-3 evaluated in the Ames, Chromosomal Aberration and In vivo Micronucleus Studies: Negative
- RDEA594 Sodium (Lot 0904697 evaluated in the Ames Assay: Negative
- No structural alert for genotoxicity, for the (b) (4) impurity, according to the CMC review

Studies Conducted and Planned for the IND:

Nonclinical Pharmacology:

Primary Pharmacodynamics:

***In vitro*:**

No Effect of RDEA594 on the Enzymatic and Cellular Activity of Purine Nucleoside Phosphorylase (SR08-007)

Lack of Effect of RDEA594, RDEA806 and its other Metabolites on Xanthine Oxidase Conversion of Xanthine and Hypoxanthine to Uric Acid (SR08-08)

Significant Inhibition of Uric Acid Transport by RDEA594, RDEA806 and its other Metabolites on the Human URAT1 Transporter (SR08-012)

Evaluation of RDEA594 Effects on Additional Uric Acid Transporters (SR09-019)

RDEA594 Exhibits no Effect on Uric Acid Uptake Activity by Human SLC2A9 Transporters Expressed in Xenopus Laevis Oocytes

RDEA594 Exhibits no Effect on Uric Acid Uptake Activity by SLC2A9v2 in Stably-Transfected HEK293 Cells

Effects of RDEA594 on Uric Acid Transport by Human hOAT4 (SLC22A11)

***In vivo* and *Ex vivo*:**

Inhibitory Activity against Rat Homolog of URAT1 (RST-1)

Serum Uric Acid and Uricase in the Cebus Model (SR07-117)

Secondary Pharmacodynamics:

Weak Inhibition in Receptor Cross-Reactivity Screen by RDEA594 (SR08-086)

No Effects on Nuclear Receptors in Cross-Reactivity Screen (SR08-072)

Lack of Significant Anti-HIV-1 Activity (SR08-025)

Lack of Mitochondrial Toxicity of RDEA594 (SR09-060)

Safety Pharmacology:

in vitro

hERG Assays (Studies PR2005-061, SR08-021)

Rabbit Purkinje Fiber Assays (SR08-054, SR08-085)

in vivo

Central Nervous System (SR08-027)

Respiratory System (SR08-020)

Cardiovascular system (SR08-020)

Renal/Urinary System (SR08-088)

Autonomic Nervous System

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Gastrointestinal System (SR08-033)

Others:

Pharmacologic Profiles of Metabolites
Pharmacologic Profile of Stereoisomers
Pharmacologic Profile of Impurities
Pharmacodynamic Drug Interactions

Pharmacokinetics and Drug Metabolism:

Absorption:

Single Dose of RDEA594 to Mice (SR09-018)
Single Dose of RDEA594 to Sprague Dawley Rats (SR08-028, SR08-060)
Dose Proportionality Study Following Single Dosing of RDEA594 to Sprague Dawley Rats (SR08-071)
Single Dose of RDEA594 to Beagle Dogs (SR08-030, SR08-058)
Single Dose of RDEA594 to Cynomolgus Monkeys (SR08-017, SR08-059)
Multiple Dosing of RDEA594 to Rats (SR08-015, SR08-034)
Multiple Dosing of RDEA594 to Monkeys (SR08-016, SR08-035)
Effect of Food on Absorption of RDEA594 (SR08-071, SR08-034)

Distribution:

Tissue Distribution Studies
Quantitative Tissue Distribution of RDEA504-Derived Radioactivity in Rats (SR08-046)
In Vivo Partition between Plasma and Blood (SR08-028, SR08-17)
In Vitro Protein Binding (SR08-045, SR08-132)
In Vitro Permeability Evaluation in Caco-2 Monolayer Assay (8ARDEP3R1)
Placental Transfer Studies

Metabolism (interspecies comparison):

Chemical Structures and Quantities of Metabolites in Biological Samples
Possible Metabolic Pathways
Metabolic Profiles in Rat Plasma, Urine, and Feces (SR08-120)
Metabolic Profiles in Monkey Plasma, Urine, and Feces (SR08-119)
Metabolic Profiling in Liver Microsomes and Cryopreserved Hepatocytes (SR08-056)
P450 Phenotyping (SR08-038)
Enzyme Inhibition and Induction (SR08-048, SR08-026)

Excretion:

Urinary and Fecal Excretion (SR08-028, SR08-017)
Urinary and Biliary Excretion
Enterohepatic Circulation

Pharmacokinetic Drug Interactions:

In Vitro Assessment of Potential Interactions with hOAT1 and hOAT3 (SR08-018)
In Vivo Assessment of Potential Interactions with hOAT1 and hOAT3 (SR-9-047)
In Vivo Assessment of Potential Interactions with Febuxostat (SR09-012)
In Vivo Assessment of Potential Interactions with Allopurinol/Oxypurinol in Monkey

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Toxicology:

Single Dose:

Single-dose toxicity in Rats
Single-dose toxicity in Dogs or Monkeys
Other Single-dose toxicity studies

Repeat-Dose:

14-Day dose-range-finding (DRF) study with RDEA594 in rats (SR08-015)
28-Day Toxicity study with RDEA594 in rats (SR08-034)
13-Week Interim evaluation in the 26-week Toxicity study in rats (SR08-095)
14-Day Repeated Dose DRF Toxicity study with RDEA594 in Monkeys (SR08-016)
28-Day Repeated Dose Toxicity study with RDEA594 in Monkeys (SR08-035)
52-Week Chronic Toxicity study in Monkeys with 26-Week Interim (SR08-094)

Genotoxicity:

In Vitro Non-Mammalian Cell System: Bacterial Reverse Mutation Assay using RDEA594 Free acid(SR08-22, GLP)
In Vitro Mammalian Cell System: Exploratory Mammalian Chromosomal Aberration Assay with Free Acid RDEA594 (SR06-086, non-GLP)
In Vitro Mammalian Cell System: Mammalian Chromosomal Aberration Assay in Chinese Hamster Ovary Cells with Free Acid RDEA594 (SR08-23, GLP)
In Vivo Mammalian System: Mammalian Erythrocyte Micronucleus Assay in Sprague Dawley Rats with Free Acid RDEA594 Administered by Oral Gavage (SR08-024, GLP)
Other Systems

Carcinogenicity: Planned oral gavage studies using RDEA594 Na Salt

Carcinogenicity Study in Rats
Carcinogenicity Study in Transgenic(T*gras*H2) Mice: Model chosen based on negative results in the genotoxicity studies

Reproductive and Developmental Toxicology:

Fertility and Early Embryonic Development Oral Gavage Study with RDEA806 in Rats (SR07-078, GLP)
Fertility and Early Embryonic Development: **Planned** GLP Study in Rats using RDA594 Na Salt
Embryo-Fetal Development Oral Gavage Study with RDEA806 in Sprague Dawley Rats
Embryo-Fetal Development Oral Gavage Study with RDEA806 in Pregnant Rabbits (SR07-061)
Embryo-Fetal Development: **Planned** GLP oral gavage studies in Sprague Dawley rats and New Zealand Albino Rabbits using the RDEA594 Na salt
Prenatal and Postnatal Development, Including Maternal Function: **Planned** oral gavage studies in Sprague Dawley rats using the RDEA594 Na salt

Local Tolerance: Not applicable

Other Studies:

In Vitro Study on Impurities: Reverse Mutation Assay in *S. tymphimuriu*m and *E. Coli* using the RDEA594 Na Salt (SR09-015, GLP)

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Exploratory Acute Cardiovascular Safety Assessment in Sprague Dawley Rats using the RDEA594 Na Salt by Intravenous Route

Pharmacology/Nonclinical efficacy

Summary of Primary Pharmacodynamics:

- RDEA594, via concentration-dependent **inhibition of the uric acid transporter (URAT1) in the kidney proximal tubule** (responsible for up to 80% reabsorption of uric acid), increases urinary excretion of uric acid which should reduce formation of uric acid crystals that accumulate in, and increase inflammation in joints and other tissues in gout patients, as well as increase the incidence of kidney stones

- ***In vitro* Studies**:
  - **No blockade of uric acid synthesis**;
    - No inhibition of purine nucleoside phosphorylase (PNP, metabolizes purine nucleosides to purine base and ribose phosphate) by RDEA806 or RDEA594 (Study SR08-007)
    - No inhibition of xanthine oxidase (XA, converts xanthine and hypoxanthine to uric acid) by RDEA806 or RDEA594 (Study SR08-08), vs. allopurinol control, the latter which inhibited conversion of xanthine to uric acid (IC_{50} = 0.71mcM) and conversion of hypoxanthine to uric acid (IC_{50} = 1.8 mcM)
  - Significantly **inhibited uric acid transport via inhibition of the human URAT1 transporter**
    - (Study SR08-012) in human URAT1-expressing *Xenopus* oocytes in concentration-dependent manner (IC_{50} = 52.5 mcM), vs. positive control benzbromarone (IC_{50} = 0.1 mcM)
    - RDEA594 blocked URAT1 uptake of 14C-uric acid in stably transfected HEK-293 cells with an IC_{50} of 7.3 µM
  - The results of the evaluation of RDEA594 effects on additional uric acid transporters other than URAT1 (Study SR09-019) showed the following:
    - **Decreased uric acid uptake activity by human hOAT4** (SLC22A11, EC_{50} = 3.7 mcM) in stably-expressing HEK293 cells in concentration-dependent manner, comparable to the reference compounds benzbromarone (EC_{50} = 1.84 mcM) and probenecid (EC_{50} = 11.1 mcM)
    - **No effect on uric acid uptake by SLC2A9v2** in stably transfected HEK-293 cells (EC_{50} >50 microM)
    - **No effect on uric acid uptake activity by human OAT4 and SLC2A9 transporters** expressed in *Xenopus laevis* oocytes (EC_{50} >100 mcM for both variants).

- ***In vivo* Studies**:
  - Presence of uricase in animals but not humans precludes using most animal models of hyperuricemia

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- Low levels of uric acid detected in Cebus monkeys
  - Cebus monkeys tested for potential use as a model for testing uricosuric agents against rat homolog of URAT1 (RST-1) serum uric acid and uricase (Study SR07-117)
  - Uric acid excretion increased (vs. baseline) by RDEA594 (plasma uric acid 1.34 mg/dL after treatment vs. 13.4 mg/dL at baseline)
  - However, reliable interpretation of results not possible due to low baseline serum uric acid and presence of uricase

Secondary Pharmacodynamics:

- **High specificity for the URAT1** transporter (Study SR08-086):
  - Weak or no inhibition of radio-labeled ligand binding to 169 pharmacological target in a binding assay, except for 10 pharmacological targets (very weak inhibition, with binding inhibited >50%)
  - Only the human prostanoid, thromboxane A2 receptor showed inhibition at <30 mcM (IC_{50} = 5 mcM)
  - No agonism at up to 100 mcM, in which 15% inhibition of agonist observed
  - In light of high level of protein binding, off target interactions not expected
- **Weak or no effects on nuclear receptors** in a cross-reactivity screen (Study SR08-072)
  - Human nuclear receptors tested: peroxisome proliferator-activated receptor α, β, γ (PPARα, β, γ) Retinoid X receptor- β (RXRB), liver X receptor-α, β (LXRα, β), farnesol X receptor (FXR), vitamin D receptor (VDR), and thyroid hormone receptor-α (TRα)
  - Only interactions found: 2X activation of PPAR γ and inhibition of TRα at 100 mcM
  - No activity at any nuclear receptor at 25 mcM and below
- **No significant anti-HIV-1 activity** (Study SR08-025) in cell based assays and against HIV-1 reverse transcriptase under physiological conditions (with 45 mg/ml human serum albumin); suggests no antiviral activity
- **No mitochondrial toxicity** by RDEA594 in human HepG2 cell line (Study SR09-060), which was observed by the hepatotoxic drug benzbromarone via interaction with mitochondrial functions in liver that resulted in apoptosis, prompting withdrawal from marketing

Safety pharmacology

In vitro

hERG Assays (Studies PR2005-061 [Exploratory, Non-GLP], SR08-021 [GLP])

- Voltage-clamped human embryonic kidney cells (HDK293) expressing human ether-a-go-go-related gene (hERG)
- Concentrations 0, 10, 30, 100, 200 mcM
- No serum used, because protein binding 98% in monkey and human serum

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  - therefore results presented below can be considered related to free drug, not total drug in clinical exposure
- Results: Inhibition of hERG potassium current (IC_{50} = 198 mcM [80 mcg/ml RDEA594)
- Protein-adjusted IC_{50} ≈9,910 mcM (≈ 4000 mcg/ml total free circulating drug in humans when adjust for protein binding) drug
- Positive control terfenadine (60 nM) inhibited hERG potassium current 84%

Rabbit Purkinje Fiber Assays with and without Human Serum Albumin (HSA) (GLP Study SR08-054 [w/o HSA], SR08-085 [w/HSA])

In absence of HSA

- RDEA concentrations 0, 1, 5, 50, 200 mcM
- Slight ↑action potential duration at highest concentration of 200 mcM (81 mcg/ml)
- ↓maximum rate of depolarization without concentration response (suggested blockade of sodium channels)
- No effects on resting membrane potential, action potential amplitude
- NOAEL 50 mcM

In presence of HSA (35 mg/ml, approximating human plasma level)

- RDEA concentrations 0, 1, 5, 50, 200 mcM
- No RDEA594-related effects
- NOAEL 200 mcM (81 mcg/ml)

<u>In vivo</u>

Central Nervous System Function in Conscious Rats (GLP Study SR08-027):

- M & F rats
- Doses 0, 30, 100, 300 mg/kg PO RDEA594
- No treatment-related effects by RDEA594 at up to HD of 300 mg/kg (NOAEL)

Respiratory System Function in Conscious Monkeys (GLP Study SR08-020):

- Single RDEA594 doses 0, 30, 100, 300 mg/kg PO given 2 days apart (n=4/dose level)
- No RDEA594 treatment-related effects (including respiratory rate, tidal volume, minute volume) at up to the HD of 300 mg/kg
- Exposure at the NOAEL estimated from results of Day 1 TK evaluation in the 28-day oral gavage toxicology study in monkeys at the dose of 300 mg/kg:
  - $AUC_{0\text{-}24}$ ≈ 453 (F) – 687 (M) mcg.h/ml (mean 570 mcg.h/ml)
  - C_{max} ≈ 76.0 (F) – 117 (M) mcg/ml (mean 96.5 mcg/ml)

Cardiovascular Function in Conscious Implanted Telemetered Male Cynomolgus Monkeys (GLP Study SR08-020)

- Single RDEA594 doses 0, 30, 100, 300 mg/kg PO given 2 days apart (n=4/dose level)
- Vomiting in ¼ monkeys at 100 and ¾ monkeys at 300 mg/kg within 2h of dosing

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- Decreased blood pressure and heart rate at all doses vs. controls, sporadic at LD and MD, no relationship to time post-dose or plasma levels at any dose, and no differences from pr-test values, considered not to be treatment-related
- Significant ↑QT interval, but no differences when corrected for HR (QTcR) vs. controls; therefore attributed to reduced HR
- No other RDEA594 treatment-related effects on ECG parameters at up to the HD of 300 mg/kg (NOAEL)
- Exposure at the NOAEL estimated from results of Day 1 TK evaluation in the 28-day oral gavage toxicology study in monkeys at the dose of 300 mg/kg:
    - $AUC_{0\text{-}24} \approx$ 453 (F) – 687 (M) mcg.h/ml (mean 570 mcg.h/ml)
    - $C_{max} \approx$ 76.0 (F) – 117 (M) mcg/ml (mean 96.5 mcg/ml)

Renal/Urinary System Function in Male Rats (GLP Study SR08-088):

- Parameters included urine volume, water intake, free water clearance, electrolyte excretion (sodium ion, potassium ion, chloride ion, total calcium, phosphorus), osmolality, pH, blood dipstick, quantitative urinary analysis (creatinine, urea nitrogen, protein, glucose, uric acid), renal clearance (sodium ion, potassium ion, chloride ion, total calcium, phosphorus, creatinine, urea nitrogen, protein, glucose), fractional electrolyte clearance (sodium ion, potassium ion, chloride ion, total calcium, phosphorus), relative clearance for uric acid and clinical chemistry (osmolality, sodium, potassium chloride, creatinine, albumin, urea nitrogen, glucose, bicarbonate, phosphorus, calcium total protein)
- Single RDEA594 doses of 0, 30, 100, 300, 1000 mg/kg PO
- Changes in urinary excretion parameters at 1000 mg/kg PO RDEA594
- Minimal changes in serum clinical chemistry at 1000 mg/kg PO RDEA594
    - Minimal ↓sodium (1000 mg/kg), chloride (≥300 mg/kg)
    - Minimal ↑serum creatinine (≥300 mg/kg), BUN (1000 mg/kg), glucose and CO_2 (1000 mg/kg)
- Urinary excretion changes without effects on serum clinical chemistry at ≥300 mg/kg PO
    - ↑urine osmolality (≥300 mg/kg) at 0-3h after dosing
    - ↓urine volume (1000 mg/kg) at 0-3h after dosing, but ↑urine volume (1000 mg/kg) at 3-24h after dosing
    - ↑uric acid excretion at 300 and 1000 mg/kg (pharmacologic effect)
    - ↑calcium and phosphorus excretion at (≥300 mg/kg) from 0-3h after dosing and at 1000 mg/kg from 3-24h after dosing
    - ↑protein and potassium excretion (≥300 mg/kg), sodium and chloride excretion (≥100 mg/kg, minimal)
- NOAEL for urinary excretion changes 30 mg/kg PO RDEA594
- NOAEL for clinical chemistry effects 100 mg/kg PO RDEA594

Autonomic Nervous System: No studies conducted

Gastrointestinal System (Motility) in Rats (GLP Study SR08-033):

- Single RDEA594 doses of 0, 30, 100, 300, 1000 mg/kg
- N=10 males/group

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- Watery feces at 1000 mg/kg
- Statistically significant reduction at 1000 mg/kg RDEA594 (-12% to -17% vs. controls)
- NOAEL 300 mg/kg

Others: Not applicable

ADME:

Absorption:

Single Dose RDEA594:

- T_{max}
  - approximately 3h in M and 1h in F mice
  - 2.33-16.5 h (at 20-1000 mg/kg PO) in rats, increased with dose
  - 0.438 h in dogs, 1.33h in monkeys
- Dose proportionality:
  - Dose proportional increase in exposure without gender difference in mice
  - Greater than dose proportional increase in exposure at lower doses and less than dose proportional increase in exposure at higher doses in rats
- Plasma $t_{1/2}$
  - for total radioactivity 6.5 h in M and 3 h in F rats (IV)
  - for RDEA594 7.4h in M and 5h in F rats (IV), 7.5h in M dogs
- Cl
  - 0.2 (M) and 0.17 (F) L/h/kg in rats
  - 0.13 L/h/kg in dogs
  - 0.173 L/h/kg in M monkeys
  - lower than hepatic blood flow of 3.3 L/h/kg in rats, 1.8 L/h/kg in dogs, 2.6 L/h/kg in monkeys
- Vd
  - 0.9 L/kg in M and 0.55 in F rats
  - 0.36 L/kg in dogs
  - 0.289 L/kg in monkeys
  - approximated body fluid volume
  - little tissue distribution across species
- Ratio of radioactivity attributed to RDEA594 in circulation:
  - 83% in rats
  - 68% (IV) to 74% (oral) in monkeys
  - Stable in systemic circulation
- Oral bioavailability
  - 75.3% in M and 71.1% in F rats
  - 100% in dogs
  - 41% in monkeys
  - Suggests minimal to moderate (latter in monkey) first-pass metabolism

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Repeated Oral Dose RDEA594:

- Exposure (AUC values)
  - higher in M than in F rats at lower doses, approximately equivalent at higher doses
  - linear, greater than dose-proportional increase in monkeys
- No meaningful gender differences in exposure in monkeys
- Accumulation:
  - similar AUC values on Days 15 and 28 vs. Day 1 after dosing in monkeys (at 30-1000 mg/kg/d): no accumulation
  - lower exposures at higher doses following multiple dosing: suggests auto-induction at higher doses
- No food effects at lower doses (≤30 mg/kg/d), but higher exposures under fasted conditions at higher doses (≥100 mg/kg/d) in rats

Oral Absorption (reproduced from the original IND submission):

Table 9: Mean Pharmacokinetic Parameters for Total Radioactivity and RDEA594Following Oral or Intravenous Administration of [^{14}C]RDEA594 to Male Monkeys

Analyte	Parameter	Monkey (N=3)	
		PO	IV
		20 mg/kg	10 mg/kg
Total ^{14}C	T_{max} (hr)	1.33	0.0833
	C_{max} (µg-eq/mL)	17.8	90.3
	AUC_{last} (µg-eq·hr per g or mL)	80.4	82.0
	AUC_{inf} (µg-eq·hr per g or mL)	111	93.1
	$t_{1/2}$ (hr)	61.5	40.6
	Absorption (%)	45.9	NE
RDEA594	T_{max} (hr)	1.33	0.0833
	C_{max} (µg/mL)	16.2	97.8
	AUC_{last} (µg·hr/mL)	50.0	59.4
	AUC_{inf} (µg·hr/mL)	50.1	59.5
	F (%)	41.1	NA
	$t_{1/2}$ (hr)	21.8	43.8
	CL (L/hr/kg)	NE	0.173
	V_{ss} (L/kg)	NE	0.289
(RDEA594/^{14}C)	C_{max} (%)	90.0	100
	AUC_{last} (%)	68.4	73.9

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Single Dose of RDEA594 to Mice (Study SR09-018, table reproduced from the original IND submission)

Gender (n=3/sex/dose)	Dose RDEA594 (mg/kg)	Tmax (h)	Cmax (mcg/ml)	AUC0-24h (mcg.h/ml)
M	60	3.0	22.3	214
	200	3.0	77.9	700
	600	3.0	144	1950
F	60	1.0	45.4	220
	200	1.0	114	1210
	600	1.0	177	1940

Single Dose of RDEA594 to Sprague Dawley Rats (SR08-028, SR08-060, table reproduced from the original IND submission):

Analyte	Parameter	M		F	
		20 mg/kg PO	20 mg/kg IV	20 mg/kg PO	20 mg/kg IV
Total ^{14}C	T_{max} (h)	0.25	0.0833	0.25	0.833
	C_{max} (mcg-eq/ml)	34.2	45.2	26.0	71.6
	AUC_{last} (mcg-eq h/ml or g)	96.9	114	94.7	137
	AUC_{inf} (mcg-eq h/ml or g)	99.5	116	96.6	138
	$t_{1/2}$ (h)	6.44	6.46	1.96	3.06
	Absorption (%)	85.8	Not estimated	70.0	Not estimated
RDEA594	T_{max} (h)	0.25	0.0833	0.250	0.0833
	C_{max} (mcg/ml)	33.3	60.9	28.7	62.3
	AUC_{last} (mcg h/ml)	73.6	97.7	81.7	115
	AUC_{inf} (mcg h/ml)	73.6	97.8	81.8	115
	F (%)	75.3	Not estimated	71.1	Not estimated
	$t_{1/2}$ (h)	3.39	7.40	3.98	5.06
	Clearance (L/h/kg)	Not estimated	0.205	Not estimated	0.174
	Vss (L/kg)	Not estimated	0.931	Not estimated	0.546
RDEA594/^{14}C	C_{max} (%)	97.4	100	100	87.0
	AUC_{last} (%)	76.0	85.7	86.3	83.9

Effect of Food on Absorption of RDEA594 (SR08-071, SR08-034, table reproduced from the original IND submission):

Table 12: Mean Pharmacokinetic Parameters for RDEA594 in Rats Following Single Oral of RDEA594

API	Study	Food	N	Dose (mg/kg)	C_{max} (µg/mL)	AUC_{0-24hr} (µg·hr/mL)
Free acid	PK	Fasted	3	20	9.78	64.9
			3	100	83.7	767
			3	300	165	2820
			3	1000	286	5380
	28-Day Tox (Day 1)	Fed	3/time point	10	3.28	31.2
			3/time point	30	9.66	81.4
			3/time point	100	61.0	444
			3/time point	300	214	2030

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Distribution:

Quantitative Tissue Distribution of RDEA504-Derived Radioactivity in Rats (SR08-046)

- Similar pattern of distribution after oral and IV dosing
- Distribution primarily to GI tract after oral dosing (small intestine early, large intestine after 24h), stomach, kidney, small amount in liver (possibly representing bile), lung, thyroid, urine (2-4h after oral dosing)
- Nearly completely cleared by 24-72 hours from body
- Similar distribution in albino and pigmented rats: no binding to melanin
- No RDEA594 detected in eye humor or lens
- Distribution to brain minimal

***In Vivo* Partition between Plasma and Blood (SR08-028, SR08-17)**

- Blood to plasma ratio [^{14}C]RDEA594-derived radioactivity 0.5-0.8 over 12h after dosing in rats
- Blood to plasma ratio [^{14}C]RDEA594-derived radioactivity 0.5-0.8 over 48 hours after dosing in monkeys
- No preferential distribution of [^{14}C]RDEA594-derived radioactivity between blood and plasma, in rat and monkey

***In Vitro* Protein Binding (SR08-045, SR08-132, table reproduced from the original IND submission):**

Table 16. Protein Binding of RDEA594 in Plasma

Species	% Protein Binding (Mean ± SD, N=3)					
	1 µM	10 µM	50 µM	200 µM	500 µM	1000 µM
Mouse	95.2 ± 0.45	94.9 ± 0.35	94.0 ± 0.15	NM	NM	NM
Rat	97.7 ± 0.34	98.1 ± 0.09	97.7 ± 0.01	97.6 ± 0.11	96.4 ± 0.06	95.1 ± 0.18
Dog	98.3 ± 0.06	98.2 ± 0.11	98.1 ± 0.13	NM	NM	NM
Monkey	98.2 ± 0.15	98.3 ± 0.03	98.2 ± 0.10	97.8 ± 0.06	97.3 ± 0.02	NM
Human	98.5 ± 0.06	98.4 ± 0.02	97.9 ± 0.17	NM	NM	NM

NM: Not measured

- RDEA594 highly protein bound across species and in humans
- Protein binding ≥97.7% in rat, dog, monkey and human plasma at RDEA594 concentrations at and below 50 mcM, but higher than 94% in mouse plasma
- Plasma protein binding decreased with increasing RDEA594 concentrations of 200 mcM and above in rat and monkey

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***In Vitro* Permeability Evaluation in Caco-2 Monolayer Assay (8ARDEP3R1)**

- No evidence was found that RDEA594 is a P-gp substrate, in an assay that measured permeability through Caco-2 monolayers with and without P-gp inhibitor, cyclosporine

Placental Transfer Studies are planned

Metabolism

***In vitro*:**

- RDEA594 stable when incubated with rat, dog, monkey and human liver microsomes and hepatocytes *in vitro*; ≥92% parent drug remained
- The main metabolites in monkey liver microsomes and monkey and human hepatocytes were formed by addition of an oxygen atom to form M3 and addition of 2 hydroxy groups to form M4

***In vivo*:**

- RDEA594 metabolized by glucuronidation (to M1), oxidation (to M3, M4, and M5) and debromination (to M2 and M5) in rat and monkey

Chemical Structures of Metabolites in Biological Samples (reproduced from pIND Pharmacology and Toxicology Presentation by Dr. Gary Bond, April 1 and June 2, 2008).

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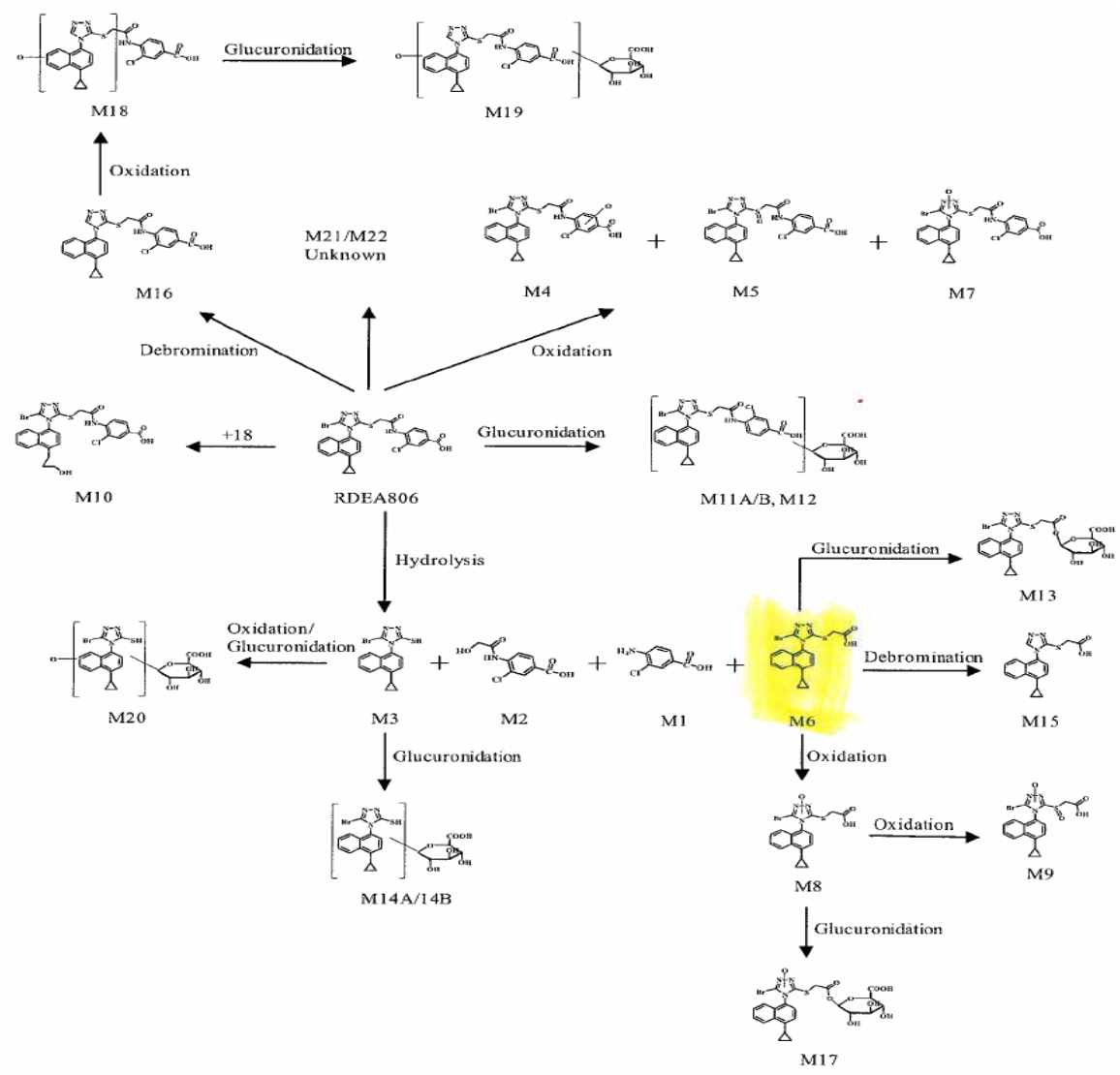

Five metabolites of RDEA594 have been detected: M1-M5.

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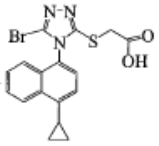
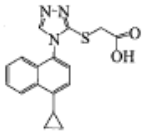
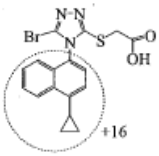
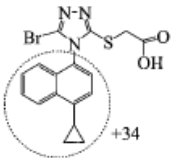
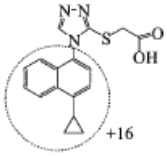
Chemical Names and Structures for RDEA594 and Its Metabolites (reproduced from the original IND submission):

Table 19 Chemical Names and Structures for RDEA594 and Its Metabolites

Compound	Structure	Occurrence	Assignment
RDEA594 $C_{17}H_{14}BrN_3O_2S$	 2-(5-bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-ylthio)acetic acid	Microsome (r, d, m, h) Hepatocyte (r, d, m, h) Plasma (r, d, m, h) Urine (r, m, h) Feces (r, m)	Final HPLC RT MS
M1 $C_{23}H_{22}BrN_3O_8S$		Urine (m) Feces (m)	Tentative MS
M2 $C_{17}H_{15}N_3O_2S$	 2-(4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-ylthio)acetic acid	Urine (r, m) Feces (r, m)	Final HPLC RT MS
M3 $C_{17}H_{14}BrN_3O_3S$	 +16	Microsome (m) Hepatocyte (m, h) Urine (r, m) Feces (r, m)	Tentative MS
M4 $C_{17}H_{16}BrN_3O_4S$	 +34	Hepatocyte (m, h) Urine (m)	Tentative MS
M5 $C_{17}H_{14}BrN_3O_3S$	 +16	Urine (r) Feces (r)	Tentative MS

r (rat), d (dog), m (monkey), h (human)

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Possible Metabolic Pathways

- 5 metabolites identified in the *in vitro* and *in vivo* evaluations
- Phase I hydrolysis, oxidation and reduction
- Phase II glucuronidation
- Metabolic profiles similar in monkey and human

Comparative *In Vitro* and *In Vivo* Metabolites (table reproduced from the original IND submission):

Table 18. *In Vitro* and *In Vivo* Metabolites

Species	*In Vitro* Metabolites	*In Vivo* Metabolites
Rat	ND	M2, M3, M5
Dog	ND	NA
Monkey	M3, M4	M1, M2, M3, M4
Human	M3, M4	NA

ND: not detected; NA: not available

Interspecies comparison (reproduced from the original IND submission):

Table 22 Metabolic Profile of RDEA594 in Microsomes and Hepatocytes

Matrix	Species	% Radioactivity			
		M3	M4	RDEA594	Total
Microsome	Rat	0	0	100	100
	Dog	0	0	100	100
	Monkey	7.9	0	92.1	100
	Human	0	0	100	100
Hepatocytes	Rat	0	0	100	100
	Dog	0	0	100	100
	Monkey	1.45	0.47	98.1	100
	Human	2.24	5.69	92.1	100

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Metabolic Profiles in Rat Plasma, Urine, and Feces (SR08-120, reproduced from the original IND submission):

Table 20 Metabolic Profile of RDEA594 in Plasma, Urine and Feces Following Oral and Intravenous Dosing to Rats

Gender	Route	Matrix	Time (hr)	Concentration %				
				M2	M3	M5	RDEA594	Total
M	PO	Plasma	1	0.0	0.0	0.0	100	100
		Urine	0-24	13.1	47.7	0.0	38.4	99.2
		Feces	0-24	34.6	9.8	30.1	24.2	98.7
	IV	Plasma	0.5	0.0	0.0	0.0	100	100
		Urine	0-24	10.4	55.0	0.0	26.9	92.3
		Feces	0-24	35.5	8.0	38.0	16.3	97.8
F	PO	Plasma	1	0.0	0.0	0.0	100	100
		Urine	0-24	5.3	18.1	0.0	76.7	100
		Feces	0-24	43.7	2.3	13.7	32.7	92.2
	IV	Plasma	0.5	0.0	0.0	0.0	100	100
		Urine	0-24	4.1	10.2	0.0	85.4	99.6
		Feces	0-24	47.2	2.4	16.0	33.6	99.2

Metabolic Profiles in Monkey Plasma, Urine, and Feces (SR08-119, table reproduced from the original IND submission):

Table 21 Metabolic Profile of RDEA594 in Plasma, Urine and Feces Following Oral and Intravenous Dosing to Monkeys

Route	Matrix	Time (hr)	% Radioactivity					
			M1	M2	M3	M4	RDEA594	Total
PO	Plasma	2	0	0	0	0	100	100
	Urine	0-24	<1	4.1	0.9	0.5	89.9	98.9
	Feces	0-24	<1	69.6	1.4	0	24.4	95.4
IV	Plasma	1	0	0	0	0	100	100
	Urine	0-24	0	1.6	1.9	0	96.5	100
	Feces	0-24	<1	77.4	<1	0	12.3	91

<Click Here then Paste Figure>

P450 Phenotyping (SR08-038)

- Methods:
  - Incubation of RDEA594 at 1 mcM with cDNA-expressed human P450 isozymes (100 pmol/ml), with monitoring of parent compound depletion using LC-MS/MS
- Results:
  - Only CYP2C9 metabolism detected after 60 minute incubation

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Enzyme Inhibition and Induction in Liver Microsomes and in Human Hepatocytes (SR08-048, SR08-026)

Human Liver Microsomes:

- Methods:
  - Incubation of human liver microsomes incubated with marker substrates for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, with and without RDEA594 at 0.0305-200 mcM
- Results:
  - RDEA594 inhibition of CYP2C8 (IC_{50} = 16.2 mcM) and CYP2C9 (IC_{50} = 40.7 mcM)
  - IC_{50} for CYP1A2, CYP2C19, CYP2D6, and CYP3A4 >100 mcM

Human Hepatocytes

- Methods:
  - Cultured human liver hepatocytes treated with RDEA594 (0.1, 1, 10 mcM) or human CYP450 liver enzyme inducers (e.g., omeprazole at 100 mcM, rifampin at 10 mcM) for 3 days
  - Microsomes analyzed for 7-ethoxyresorufin *O*-dealkylation (CYP1A2) activity and testosterone 6-beta0hydroxylation (CYP3A4/5 activity)
- Results:
  - No increase in 7-ethoxyresorufin *O*-dealkylation activity
  - 3-fold induction in testosterone 6-beta0hydroxylation (CYP3A4/5) activity at 10 mcM RDEA594 (67% that by rifampin)

<u>Excretion</u>:

Urinary and Fecal Excretion (SR08-028, SR08-017)

- **Rats:**
  - Male rats: Excretion **predominantly in feces** (92.4% total radioactivity), with minor amounts found in urine (8.04%) after IV dosing (20 mg/kg)
  - Female rats: Excretion to similar extent in feces (44.2% after PO and 42.7% after IV dosing) and urine (36.3% after PO and 39.3% after IV dosing)
  - Results suggest **extensive biliary excretion**
- **Monkeys**:
  - Male monkeys: **Excretion somewhat higher in feces** (41.7% after PO and 49.9% after IV dosing) than in urine (32.6% after PO and 25.7% after IV dosing)
  - Female monkeys not evaluated

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Interspecies Comparison of Excretion Patterns (table reproduced from the original IND submission):

Table 24. Excretion Pattern in Animals Following a Single Dose of [^{14}C]RDEA594

Species	Sex	Route	Duration (hr)	Dose (mg/kg)	Recovery of Dose (%)				
					Urine	Feces	Other [a]	Carcass	Total
Rat	M	PO	120	20	11.9	83.2	1.34	0.34	96.8
Rat	M	IV	120	20	8.04	92.4	2.68	3.00	106
Rat	F	PO	120	20	36.3	44.2	2.58	0.34	83.5
Rat	F	IV	120	20	39.3	42.7	16.0	5.69	104
Monkey	M	PO	120	20	32.6	41.7	15.7	NA	90.0
Monkey	M	IV	120	10	25.7	49.9	12.2	NA	87.8

[a] Cage wash/wipe; PO = oral; IV = intravenous; NA = data not available since animals were not sacrificed for radioactivity counting

Enterohepatic Circulation: Study planned, for evaluation of biliary excretion, enterohepatic reabsorption, and metabolic RDEA594 profiles in bile

Pharmacokinetic Drug Interactions:

- ***In Vitro* Assessment of Potential Interactions with hOAT1 and hOAT3 (SR08-018)**
  - Methods: *Xenopus* oocyte cell lines expressing hOAT1 and hOAT3 transporters incubated with [^{14}C]RDEA594 at 5 and 25 mcM and uptake and inhibition of uptake evaluated vs. aminohippurate positive control for hOAT1 and estrone-3-sulfate positive control for hOAT3
  - Results:
    - Relative RDEA594 uptake ratio approximately 2X negative control, lower than positive control values of 81X at hOAT1 and 13X at hOAT3
    - Inhibition of uptake by RDEA594 was 36% to 64% at the low concentration and 87%-95% at the high concentration
  - Conclusions:
    - Interactions between RDEA594 and hOAT1/hOAT3 minimal
    - RDEA594 weak inhibitor of hOAT1 and hOAT3 (IC_{50} <5 mcM) vs. RDEA594 human plasma concentration of 50 mcM at 600 mg (clinical study RDEA594-102) Note that 50 µM total drug with 98% protein binding would be equivalent to 2 µM free.

- ***In Vivo* Assessment of Potential Interactions with hOAT1 and hOAT3 (SR-9-047)**
  - Methods:
    - Male SD rats administered tenofovir disoproxil fumarate (tenofovir prodrug, excreted via OAT1 transporter mediation) at 25 mg/kg (target tenofovir target dose) IV, followed by urine collection for 72 hours post-dose

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        - After 2-d washout, rats administered RDEA594 at 80 mg/kg/d for 3 days, and then tenofovir disoproxil fumarate at 25 mg/kg (tenofovir target) IV and urine collected again for 72 hours and analyzed for tenofovir and RDEA594 by LC-MS/MS
    - Results:
        - Mean recovery of tenovir in urine similar (51.2% vs. 56.4%) with and without RDEA594
    - Conclusions:
        - Minimal effect on hOAT1 and hOAT3 transporters by RDEA594 in vivo in rat

- ***In Vivo* Assessment of Potential Interactions with Febuxostat (SR09-012)**
    - Methods:
        - Male SD rats administered RDEA594 at 30 mg/kg PO given alone, febuxostat (xanthine oxidase inhibitor) at 10 mg/kg PO given alone and at 5 mg/kg PO in combination with RDEA594 (15 mg/kg PO)
        - Febuxostat and RDEA594 plasma exposure measured
    - Results:
        - No statistically significant changes in plasma exposure (AUC, Cmax and Tmax values) for either RDEA594 or febuxostat
    - Conclusions: No pharmacokinetic interactions between RDEA594 and febuxostat

- ***In Vivo* Assessment of Potential Interactions with Allopurinol/Oxypurinol in Monkey**
    - Methods:
        - Male Cynomolgus monkeys administered allopurinol (12 mg/kg PO) followed by 1wk washout
        - The monkeys received RDEA594 (25 mg/kg PO) and either benzebromarone (4.5 mg/kg PO) or probenecid (40 mg/kg)
    - Results:
        - No effect on plasma allopurinol and oxypurinol pharmacokinetics by RDEA594
        - In comparison: probenecid increased allopurinol and oxypurinol exposure significanly
    - Conclusions:
        - No pharmacokinetic interaction by RDEA594 with allopurinol and oxypurinol, commonly administered drugs for the proposed indication

Other Pharmacokinetic Studies: Planned evaluation of drug-drug interactions with drugs excreted by active renal secretion, and other co-administered drugs including aspirin, colchicine, zidovudine, nonsteroidal anti-inflammatory drugs.

Reference ID: 3815621

Toxicology Program (reproduced from the original IND submission):

Table 28. Toxicology Program

Study Type and Duration	Compound Administered	Route of Administration	Species
Single-dose toxicity	-	-	-
Repeated-dose toxicity			
2 weeks*	RDEA594 Free acid	Oral Gavage	Sprague Dawley Rats Cynomolgus Monkeys
4 weeks*	RDEA594 Free acid	Oral Gavage	Sprague Dawley Rats Cynomolgus Monkeys
26 weeks with 13 weeks interim* (ongoing)	RDEA594 Na salt	Oral Gavage	Sprague Dawley Rats
52 weeks with 26 weeks interim* (ongoing)	RDEA594 Na Salt	Oral Gavage	Cynomolgus Monkeys
Genotoxicity			
In vitro reverse mutation assay*	RDEA594 Free acid	*In Vitro*	*S. tymphimurium* and *E. Coli*
Exploratory *in vitro* chromosome aberration assay	RDEA594 Free acid	*In Vitro*	Chinese Hamster Ovary Cells
In vitro chromosome aberration assays*	RDEA594 Free Acid	*In Vitro*	Chinese Hamster Ovary Cells
In vivo micronucleus assay*	RDEA594 Free acid	Oral Gavage	Sprague Dawley Rats
Carcinogenicity (planned)		-	-
2-year	RDEA594 Na salt	Oral Gavage	Rat
6-month Transgenic	RDEA594 Na salt	Oral Gavage	Tg*ras*H2 mouse
Reproductive and developmental toxicity			
Fertility and early embryonic development*	RDEA806	Oral Gavage	Sprague Dawley Rats
Embryo-fetal development*	RDEA806	Oral Gavage	Sprague Dawley Rats New Zealand Albino Rabbits
Fertility and early embryonic development* (planned)	RDEA594 Na salt	Oral Gavage	Sprague Dawley Rats

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Embryo-fetal development* (planned)	RDEA594 Na salt	Oral Gavage	Sprague Dawley Rats New Zealand Albino Rabbits
Prenatal and postnatal development, including maternal function (planned)	RDEA594 Na salt	Oral Gavage	Sprague Dawley Rats
Other Studies			
Studies on Impurities *In vitro* reverse mutation assay*	RDEA594 Na salt	*In Vitro*	*S. tymphimurium* and *E. Coli*
Other Studies Exploratory Acute Cardiovascular Safety Assessment	RDEA594 Free acid	Intravenous	Sprague Dawley Rats

* Denotes GLP Study; Na salt – sodium salt

[a] Screening assay not described in section 2.6.6 or 2.6.7 but the report is provided in Module 4.

Acute Toxicology

Studies to evaluate single-dose toxicity were not conducted; acute treatment-related effects were evaluated using Day 1 measurements in the repeat-dose toxicology studies.

Repeat-Dose Toxicology Summary: All Studies Used Oral Gavage Administration

14-Day dose-range-finding (DRF) study with RDEA594 in rats (Study SR08-015, GLP, (b) (4))

- 0 (phosphate buffered saline), 30, 100, 300, & 1000 mg/kg/d RDEA594 (n=8/sex/dose)
- Deaths in 6/8 M and 6/8 F within 5-8 days, preceded by reduced body weights and food consumption at 1000 mg/kg/d
  - Increased BUN, creatinine (≥300 mg/kg/d)
- Target organs of toxicity:
  - Kidneys: tubular epithelium degeneration (≥300 mg/kg/d)
  - Gastrointestinal tract: epithelial cell necrosis (1000 mg/kg/d)
- NOAEL 100 mg/kg/d
- MTD 300 mg/kg/d
- Exposure at the NOAEL: AUC (D14) = 267 (M) - 454 (F) mcg.h/ml (mean 360 mcg.h/ml M+F combined, approximately **6X** anticipated exposure at the proposed clinical dose of 400 mg)

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Exposure Findings (AUC in mcg.h/ml) in the 14-Day Oral Gavage Range Finding Study in Rats (Steady State Values on Day 14, Data unavailable for the 1000 mg/kg/d dose level due to mortality in that group)

Dose RDEA594 (mg/kg/d PO)	Males	Females
30	116	72.2
100	267	454
300	1160	1480

RDEA594: A 28-Day Repeated Oral Dose Toxicity and Toxicokinetic Study Followed by a 28-Day Recovery Period in Sprague-Dawley Rats with a 14-Day Pull (GLP Study SR08-034)

- **Methods**:
  - Study Initiation 10 April 2008
  - RDEA594 free acid at oral gavage doses of 0 (phosphate buffered saline), 10, 30, 100, 300 mg/kg/d PO
  - n:
    - 24/sex/ Control, 100, and 300 mg/kg/d
    - 14/sex/dose at 10 and 30 mg/kg/d
    - 4/sex/dose 14-d interim examination (necropsy with full histopathology)
    - 10/sex/group sacrificed and evaluated at SD29
    - 10/sex/control, 100, and 300 mg/kg/day evaluated after 28-day recovery at SD57
    - 6/sex/dose TK (3/sex/control)
    - 3M/dose evaluation of liver microsomal protein & total cytochrome P450 content at termination (all doses) and recovery (100 & 300 mg/kg/d)
  - rats ages 7-8 weeks at initiation
- **Key study findings (% values are vs. controls)**:
  - ↓BW (-1% to -6% vs. controls, across all doses)
  - ↓BWG (-8% to -10% at LD, -13% to -14% at 300)
- **Target organs**: **Liver & kidneys**
  - **Liver: (reversible)**
    - **Increased liver weights** across doses, statistically significant at 100 (+26% in M on D15, +22% in M and +16% in F on D29)
    - **Cellular hypertrophy** in 4 HDM on Day 15 and 2 HDM on Day 29
    - **No evidence of hepatic enzyme induction** (P450 content or microsomal protein levels), no clear reduction in exposure with repeated dosing except in HD males
  - **Kidney (reversible)**
    - **tubular degeneration** in 4 HDM and 3 HDF on D15
    - **Kidney nephropathy** in 4 M at 100 mg/kg/d (minimal severity), and 2M (bilateral, mild) and 1F (bilateral, moderate) at 300 mg/kg/d on D29; Control group had 1 male with unilateral minimal nephropathy on SD29. Recovery group males from Control and 300 mg/kg/day had identical

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observations of nephropathy (1/10 with unilateral nephropathy of minimal severity and 1/10 with bilateral nephropathy of minimal severity)

Notable findings in the 28-Day Toxicology Study on RDEA594 in Rats (reproduced from the original IND submission)

Daily Dose:	**0 (Control)**		**10**		**30**		**100**		**300**	
Number of Animals:	**M: 24**	**F: 24**	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 24**	**F: 24**	**M: 24**	**F: 24**
Toxicokinetics: AUC_{last} (µg·hr/mL)										
Day 1	nd	nd	26.0	9.36	75.1	35.4	422	498	1800	1270
Day 14	nd	nd	20.1	11.5	75.4	55.3	447	389	1250	1200
Day 28	nd	nd	27.2	12.4	91.5	68.7	433	369	1540	1560
Noteworthy Findings										
Died or sacrificed moribund	0	0	0	0	1[a]	0	0	0	0	0
Body Weight (%)[b]	374 g	236 g	- 4	- 2	+ 1	- 3	- 3	0	- 6	- 4

Daily Dose:	**0 (Control)**		**10**		**30**		**100**		**300**	
Number of Animals:	**M: 24**	**F: 24**	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 24**	**F: 24**	**M: 24**	**F: 24**
Body Weight gain (%)[c]	165 g	69 g	- 10	- 8	- 1	- 8	- 5	- 1	- 13	- 14
Food Consumption (%)	-	-	-	-	-	-	-	-	-	-
Clinical Observations:	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy:	-	-	-	-	-	-	-	-	-	-
Hematology:										
Number Evaluated **Day 15:**	4	4	4	4	4	4	4	4	4	4
White Blood Cells (10^3/µL)	9.448	7.090	9.588	5.973	9.075	7.675	14.193*	7.938	12.128	11.440**
Absolute Lymphocytes (10^3/µL)	6.848	5.275	7.413	4.243	6.838	5.565	10.958**	5.230	9.770	8.868**
Number Evaluated **Day 29:**	10	10	10	10	9	10	10	10	10	10
White Blood Cells (10^3/µL)	9.683	-	10.122	-	9.947	-	10.651	-	12.183*	-
Absolute Neutrophils (10^3/µL)	1.549	-	1.697	-	2.046	-	2.345*	-	2.523**	-
Absolute Monocytes (10^3/µL)	0.224	-	0.268	-	0.262	-	0.338**	-	0.325**	-
Serum Chemistry: [d]										
Number Evaluated **Day 15:**	4	4	4	4	4	4	4	4	4	4
Cholesterol (mmol/L)	1.543	1.605	1.973	1.753	1.865	1.938	2.470**	2.035	2.140	2.635**
Calcium (mmol/L)	2.830	-	2.938	-	3.110	-	3.273**	-	3.350**	-
Phosphorus (mmol/L)	3.23	-	3.53	-	3.60	-	3.70*	-	3.65*	-
Number Evaluated **Day 29:**	10	10	10	10	9	10	10	10	10	10
Cholesterol (mmol/L)	1.589	1.971	1.710	2.230	1.911	2.078	2.079*	2.439*	2.311**	2.611**
Chloride (mmol/L)	105.19	104.54	106.29	105.07	106.28	106.81*	108.23**	107.47**	109.39**	109.43**
Calcium (mmol/L)	2.453	2.408	2.517	2.384	2.679**	2.419	2.751**	2.630**	2.844**	2.586**
Phosphorus (mmol/L)	2.76	-	2.97*	-	3.22**	-	3.17**	-	3.19**	-

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Daily Dose:	0 (Control)		10		30		100		300	
Number of Animals:	**M: 24**	**F: 24**	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 24**	**F: 24**	**M: 24**	**F: 24**
Urinalysis:	-	-	-	-	-	-	-	-	-	-
Organ Weights: [e]										
Liver **Day 15** (n=4)	9.338g	6.981g	+ 3	+ 8	+ 8	0	+ 26*	+ 1	+ 22	+ 16
Liver **Day 29** (n=9 or 10)	11.09g	7.175g	+ 2	+ 9	+ 8	+ 2	+ 8	+ 9	+ 25**	+ 16*
Gross Pathology:	-	-	-	-	-	-	-	-	-	-
Histopathology [f]:										
Number Evaluated **Day 15**:	4	4	4	4	4	4	4	4	4	4
Kidney: Tubular degeneration	0	0	0	0	0	0	0	0	4	3
Bilateral; minimal	0	0	0	0	0	0	0	0	4	2
Bilateral; mild	0	0	0	0	0	0	0	0	0	1
Kidney: Nephropathy	0	0	1	0	0	0	1	0	1	0
Unilateral; minimal	0	0	0	0	0	0	1	0	0	0
Bilateral; minimal	0	0	1	0	0	0	0	0	1	0
Liver: cellular hypertrophy (minimum)	0	0	0	0	0	0	0	0	4	0
Number Evaluated **Day 29**:	10	10	10	10	9	10	10	10	10	10
Kidney: Nephropathy	1	0	2	1	2	0	4	0	2	1
Unilateral; minimal	1	0	1	1	2	0	4	0	0	0
Bilateral; minimal	0	0	1	0	0	0	0	0	0	0
Bilateral; mild	0	0	0	0	0	0	0	0	2	0
Bilateral; Moderate	0	0	0	0	0	0	0	0	0	1
Liver: cellular hypertrophy (mild)	0	0	0	0	0	0	0	0	2	0
Additional Examinations:										

Daily Dose:	0 (Control)		10		30		100		300	
Number of Animals:	**M: 24**	**F: 24**	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 24**	**F: 24**	**M: 24**	**F: 24**
Coagulation	-	-	-	-	-	-	-	-	-	-
Number Evaluated	3	0	3	0	3	0	3	0	3	0
Hepatic microsomal protein	-	n/a	-	n/a	-	n/a	-	n/a	-	n/a
Total cytochrome P450 content	-	n/a	-	n/a	-	n/a	-	n/a	-	n/a
Postdose Evaluation:	10	10	0	0	0	0	10	10	10	10
Histopathology										
Kidney: Nephropathy	2	0	n/a	n/a	n/a	n/a	0	0	2	1
Unilateral; minimal	1	0	n/a	n/a	n/a	n/a	0	0	1	0
Bilateral; minimal	1	0	n/a	n/a	n/a	n/a	0	0	1	0
Bilateral; mild	0	0	n/a	n/a	n/a	n/a	0	0	0	1

- No noteworthy findings n/a: Not applicable nd: not determined Dunnett's Test: * - p<0.05 ** - p<0.01

[a] One animal was found dead on Day 16. There were no clinical signs prior to the death. At necropsy, white frothy liquid was found in the trachea and mild, red, diffuse discoloration of the lungs were noted, which correlated microscopically with mild, multifocal congestion. Although the definitive cause of death for this animal could not be determined by histopathological examination, most likely the death was caused by a gavage error based on the gross necropsy findings, and was not test article related, because there were no deaths in the higher dose groups. In addition, there were no histopathological changes in any tissue for animals dosed up to 300 mg/kg/day, the character or severity of which would suggest RDEA594 was directly related to the cause of death.

[b] Day 29 (day of necropsy). For controls, group means are shown. For treated groups, percent differences from controls are shown.

[c] Absolute body weight gain from Day 1 to Day 29. For controls, group means are shown. For treated groups, percent differences from controls are shown.

[d] The changes in clinical chemistry were considered to be test article related, but not toxicological relevant.

[e] Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent difference for the absolute organ weights. Statistical significance is based on actual data (not on the percent differences). Statistically significant changes in liver to percent body weight were also seen on Days 15 and 29 in Groups 4 and 5 for males and Group 5 for females.

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- **NOAEL:** 100 mg/kg/d
- **Exposure at the NOAEL**:
  - NOAEL $AUC_{0\text{-}24h}$ = 433/369(M/F) mcg.h/ml (401 mcg.h/ml average) approximately **6X** anticipated exposure at the proposed clinical dose of 400 mg);
  - NOAEL C_{max} is 71 µg/mL which represents **4.5X** human C_{max}.

Reference ID: 3815621

Exposure Findings (AUC_{0-24hr} in mcg.h/ml) in the 28-Day Oral Gavage Toxicity Study in Rats
(Steady State Values on Day 28)

Dose RDEA594 (mg/kg/d PO)	Males	Females
10	27	12
30	92	69
100	433	369
300	1540	1560

13-Week Interim evaluation in the Ongoing Chronic 6-month Rat study (GLP Study Methods:
Reviewer note: This data was not provided in the IND; summary noted in Investigator Brochure only

-
  - *RDEA594 Free Acid Na salt at doses of 0, 30, 100, 300, 600 mg/kg/d PO (gavage)*
  - *600 mg/kg/d exceeded MTD; groups sacrificed at 4 weeks*
- ***Key Study Findings:***
  - *Mortality at 600 mg/kg/d (Wks 1 & 2)*
  - *↓BW at 600 mg/kg/d, due predominantly to enteropathy*
  - *Hepatocellular hypertrophy at 100 and 300 mg/kg/d, considered enzyme induction related and adaptive*
  - *No treatment-related effects on kidney*
  - *600 mg/kg/d exceeded MTD, due to mortalities and severe BW loss with enteropathy*
- ***NOAEL:*** *Tentatively 300 mg/kg/d when considering observed hepatocellular hypertrophy as adaptive, and due to enzyme induction*
- ***Exposure at the NOAEL:*** *Not provided*

14-Day dose-range-finding study with RDEA594 in Cynomolgus Monkeys (GLP Study SR08-016)

- **Methods**:
  - RDEA594 Free acid at doses of 0 (phosphate buffered saline), 30, 100, 300, and 1000 mg/kg/d PO (gavage, n=2/sex/dose)
- **Key Study Findings**
  - The 2 monkeys given 1000 mg/kg/d sacrificed *in extremis* showed ↓BW, food consumption, emesis, necrotizing lung inflammation from test article aspiration, and in 1 monkey neutrophilic inflammation in ileal villi
  - Other monkeys at 1000 mg/kg/d showed mild-moderate neutrophilic inflammation in lamina propria in ileum and duodenum
- **NOAEL: 300 mg/kg/d**
- **Exposure at the NOAEL:** AUC at steady state (D14) = 153 (F) – 249 (M) mcg.h/ml (mean approximately 200 mcg.h/ml; 3.6X exposure at the proposed clinical dose of 400 mg)

Reference ID: 3815621

Exposure Findings (AUC in mcg.h/ml) in the 14-Day Oral Gavage Range Finding Study in Monkeys (Steady State Values on Day 14, data unavailable for the 1000 mg/kg/d group males due to excessive mortality)

Dose RDEA594 (mg/kg/d PO)	Males	Females
30	137	241
100	304	313
300	249	153
1000	-	1080

RDEA594: A 28-Day Repeated Oral Dose Toxicity and Toxicokinetic Study Followed by a 28-Day Recovery Period in Cynomolgus Monkeys (GLP Study SR08-035)

- **Methods:**
  - Study Initiation April 30, 2008
  - RDEA594 Free acid at doses of 0 (phosphate buffered saline), 10, 30, 100, and 300 mg/kg/d
  - 6/sex/dose controls and HD (3/sex/group main study, 3/sex/group recovery animals) including evaluation of microsomal protein and total cytochrome P450 content
  - 3/sex/dose all other RDEA594 groups
  - Animal ages 2-4 years
- **Key Study Findings:**
  - Death in 1 F at 300 mg/kg/d (gavage error)
  - Increased total cytochrome P450 content in M at 300 mg/kg/d (Day 28, +213% vs. controls)

Notable findings in the 28-Day Toxicology Study on RDEA594 in Monkeys (reproduced from the original IND submission)

Daily Dose:	0 (Control)		10		30		100		300	
Number of Animals:	M: 6	F: 6	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 6	F: 6
Toxicokinetics: $AUC_{0\text{-}24hr}$ (µg·hr/mL)										
Day 1	nd	nd	18.9	20.8	64.3	39.9	210	168	687	453
Day 15	nd	nd	19.3	16.8	57.3	41.1	137	147	338	250
Day 28	nd	nd	19.9	20.3	61.6	50.7	77.6	128	408	210
Noteworthy Findings										
Died or sacrificed moribund	0	0	0	0	0	0	0	0	0	1[a]
Body Weight (%)	-	-	-	-	-	-	-	-	-	-
Food Consumption (%)	-	-	-	-	-	-	-	-	-	-
Clinical Observations:	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy:	-	-	-	-	-	-	-	-	-	-
Hematology:	-	-	-	-	-	-	-	-	-	-

Reference ID: 3815621

Daily Dose:	0 (Control)		10		30		100		300	
Number of Animals:	M: 6	F: 6	M: 3	F: 3	M: 3	F: 3	M: 6	F: 6	M: 3	F: 3
Serum Chemistry:	-	-	-	-	-	-	-	-	-	-
Urinalysis:	-	-	-	-	-	-	-	-	-	-
Organ Weights:	-	-	-	-	-	-	-	-	-	-
Gross Pathology:	-	-	-	-	-	-	-	-	-	-
Number Evaluated:	3	3	3	3	3	3	3	3	3	3
Histopathology:	-	-	-	-	-	-	-	-	-	-
Number Evaluated:	3	3	3	3	3	3	3	3	3	3
Additional Examinations:										
Blood Pressure:	-	-	-	-	-	-	-	-	-	-
Body Temperature:	-	-	-	-	-	-	-	-	-	-
Hepatic microsomal protein										
Coagulation:	-	-	-	-	-	-	-	-	-	-
Number Evaluated:	3	3	0	0	0	0	0	0	3	3
Hepatic microsomal protein	-	-	n/a	n/a	n/a	n/a	n/a	n/a	-	-
Total cytochrome P450 content	-	-	n/a	n/a	n/a	n/a	n/a	n/a	213%[a]	-
Postdose Evaluation:										
Number Evaluated:	3	3	0	0	0	0	0	0	3	2
All observations	-	-							-	-

[b] Group mean expressed as a percentage of the corresponding control. Not considered of biological or toxicological significance.

- **No target organs identified** assuming hepatocellular hypertrophy and increased cytochrome P450 content is adaptive
- **NOAEL: 300 mg/kg/d**
- **Exposure at the NOAEL on D28:**
  - $AUC_{0\text{-}24h}$ 309 mcg.h/ml (approximately **4.5X** clinical exposure of RDEA594 at 400 mg);
  - C_{max} 43 µg/mL (**2.7X** clinical C_{max})

Exposure Findings (AUC in mcg.h/ml) in the 28-Day Oral Gavage Toxicity Study in Monkeys (Steady State Values on Day 28)

Dose RDEA594 (mg/kg/d PO)	Males	Females
10	19.9	20.3
30	61.6	50.7
100	77.6	128
300	408	210

Reference ID: 3815621

Genetic Toxicology Studies on RDEA594

Summary: All studies conducted at (b) (4)

Study (Study No.)	Administration Method	Dose or Concentration	GLP	Results
Ames/*S.typhimurium* and *E. Coli* (Study SR08-22)	*In Vitro*	0, 1.5-5000 mcg/plate	Yes	Negative
Chromosome Aberration in CHO Cells (Exploratory) (Study SR06-086)	*In Vitro*	0, 0.005-50 mcg/ml	No	Negative
Chromosome Aberration in CHO Cells (Study SR08-23)	*In Vitro*	0, 10-120 mcg/ml (up to limit of solubility and 50% growth inhibition)	Yes	Negative
Micronucleus assay in Sprague Dawley Rats (Study SR08-024)	Oral Gavage *In Vivo* (1 day)	0, 250, 500, 1000 mg/kg (up to MTD based on mortality)	Yes	Negative (AUC =4440 mcg.h/ml at HD)

Reproductive Toxicology

Summary: All studies oral gavage

- **Fertility and early embryonic development (RDEA806) in SD Rat (Study SR07-078)**
  - n=25/sex/group
  - RDEA806 doses: 0, 60, 200, 600 mg/kg/d
    - 28 days prior to pairing, through mating until sacrifice in M
    - 14 days prior to pairing through mating to gestation day (GD) 7in F
  - Results: (b) (4)
  - RDEA594 exposure at the RDEA806 NOAEL
    - RDEA594 $AUC_{0\text{-}24h}$ =
      - 248 (D59) – 311 (D1) mcg.h/ml in M
      - 200 (GD7) – 311 (D1) mcg.h/ml in F
    - RDEA594 C_{max} at 600 mg/kg/d RDEA806 22-25 mcg/ml (Day 1), 33.5 mcg/ml (Day 59 in M), 14.9 mcg/ml (DG 7 in F)
    - RDEA594 AUC similar at 600 mg/kg/d RDEA806 vs. RDEA594 given alone at 100 mg/kg/d in the 28-d rat toxicology study
    - T_{max} 1.00-6.33h in M&F

Reference ID: 3815621

RDEA594 Exposure (AUC in mcg.h/ml) in the Fertility and Early Embryonic Development to Implantation Study in Rats Administered RDEA 806 (Steady State Values on Day 57 in males and Day 7 in Females)

Dose RDEA594 (mg/kg/d PO)	Males	Females
60	75.8	35.8
200	182	53.5
600	248	200

- **Fertility and early embryonic development (RDEA594) in Rat: Planned**

Embryofetal Development (RDEA806) in SD Rat (Study SR07-062)

- RDEA806 doses: 0, 150, 300, 600 mg/kg/d
  - GD6-17
    (b) (4)
- Results:
  (b) (4)
- RDEA594 exposure at the RDEA806 NOAEL
  - RDEA594 AUC_{0-t} = 425 (GD6) and 334 (GD17) mcg.h/ml (mean 380 mcg.h/ml)
  - RDEA594 C_{max} = 27.6 (GD6) - 27.7 (GD17) mcg/ml at 600 mg/kg/d RDEA806
  - T_{max} 1.00-3.00 h (GD6) and 0.50-1.00 h on GD17
  - (b) (4) no RDEA594 accumulation

RDEA594 Exposure (AUC in mcg.h/ml) in the Embryofetal Development Study in Rats Administered RDEA806 (Steady State Values on Gestation Day 17)

Dose RDEA594 (mg/kg/d PO)	Maternal Rats
150	54.9
300	112
600	334

Reference ID: 3815621

Embryofetal Development (RDEA806) in NZ Albino Rabbit (Study SR07-061)

- RDEA806 doses: 0, 50, 100, 400 mg/kg/d
  - N=22F/group
  - GD 6-19
  - [redacted] (b) (4)
- Results:
  [redacted] (b) (4)
- RDEA594 exposure [redacted] (b) (4)
  - RDEA594 AUC_{0-t} = 135 (GD19) – 186 (GD6) mcg.h/ml (mean 161 mcg.h/ml)
  - RDEA594 C_{max} = 19.4 (GD19) – 29.3 (GD6) mcg/ml
  - RDEA594 T_{max} 2.33-8.00 h

RDEA594 Exposure (AUC in mcg.h/ml) in the Embryofetal Development Study in Rabbits Administered RDEA806(Steady State Values on Gestation Day 19)

Dose RDEA594 (mg/kg/d PO)	Maternal Rabbits
50	65.5
100	135
400	947

Embryo-fetal Development (RDEA594 Na salt) in SD Rat: Planned

Embryo-fetal Development (RDEA594 Na salt) in NZ Albino Rabbit: Planned

Pre- and post-natal development, including maternal function (RDEA594 Na salt) in SD Rat: Planned

Reference ID: 3815621

Special Toxicology Studies

Bacterial Reverse Mutation Assay to Evaluate Effect of (b) (4) impurity (Study SR09-015):

- Impurity found in RDEA594 (b) (4) Lot 0904697 at (b) (4)%, exceeding amount in Lot 10454-139-3 used in the genotoxicity battery studies ((b) (4)%)
- *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. Coli* strain WP2uvrA +/- S9, using plate incorporation method at up to (b) (4) mcg/plate
- Results negative for precipitate, cytotoxicity and mutagenicity
- NOAEL for RDEA594 Lot 0904697 with (b) (4)% (b) (4) impurity = (b) (4) mcg/plate

Exploratory Acute Cardiovascular Safety Assessment with RDEA594 in Rats (Study SR08-61)

- Male SD rats (n=6/dose group + 6/dose for satellite toxicology including clinical pathology, and TK groups) administered RDEA594 at 0 (phosphate buffer), 50, 100, and 150 mg/kg IV over 2 minutes via indwelling femoral catheter
- ECG recorded continuously via implanted (SC) leads for 1h pre-dose through 2h post dose
- **Results:**
  - Mortality in 2/6 rats at 150 mg/kg within 10 minutes of dosing, with slow breathing, partially closed eyelids
  - Slight ↓total protein, albumin at 100 and 150 mg/kg, globulin at 150 mg/kg, Ca, Cl at 100 and 150 mg/kg
  - Hypotension at all doses
  - AV block at 150 mg/kg IV, possibly disrupting atrioventricular conduction and cause of death in the 2 rats
- **RDEA594 NOAEL for adverse cardiovascular effects = 100 mg/kg IV**
- **RDEA594 Exposure:**

Dose:	**50**	**100**	**150**
Cmax (mcg/ml):	109	352	427
C0 (mcg/ml)	127	409	456
AUC0-t (mcg.h/ml)	116	464	613

Reference ID: 3815621

Safety/Exposure Margin Table

Study	RDEA594 (RDEA806 where indicated) Doses (mg/kg/d) / concentrations (mcM)	NOAEL (mg/kg/d PO)	RDEA594 AUC0-24 at the NOAEL (mcg.h/ml)	RDEA594 Cmax (mcg/ml) at the NOAEL	Safety Margin vs. Clinical Dose (AUC basis)*
Reference: Clinical PK*	400 mg single dose, fed state	-	≈70	≈11-12	-
hERG assay	0, 1, 5, 50, 200 mcM	200 mcM (81 mcg/ml)	-	-	6.75X (based on Cmax)
Rabbit Purkinje Fiber Assay	0, 1, 5, 50, 200 mcM	200 mcM (81 mcg/ml in presence of human serum albumin)	-	-	6.75X (based on Cmax)
CV & Respiratory Function in Monkey	0, 30, 100, 300 mg/kg single dose	300	570	96.5	8X (AUC) 8X (Cmax)
Exploratory CV in Rat	0, 50, 100, 150 IV	100 IV	464	352	6.6X (AUC) 29X (Cmax)
14-d DRF in Rat	0, 30, 100, 300, 1000	100	(267♂, 454♀) 360	30(♂) 92(♀)	5X
14-d DRF in Monkey	0, 30, 100, 300, 1000	300	200	-	2.9X
28-d Tox in Rat	0, 10, 30, 100, 300	100	401	-	5.7X
28-d Tox in Monkey	0, 10, 30, 100, 300	300	309	-	4.4X
Chrom.Ab. in CHO cells	0, 10-120 mcg/ml	120 mcg/ml	-	-	10X (up to limit of solubility)
In vivo micronucleus in rat	0, 250, 500, 1000	1000	4440	-	63X
Fertility in rat (RDEA806)	0, 60, 200, 600 (RDEA806)	600 (RDEA806)	RDEA594: 248 in M 200 in F	-	3.5X in M 2.8X in F
EFD in Rat (RDEA806)	0, 150, 300, 600 (RDEA806)	600 (RDEA806)	RDEA594: 334 (GD17)	-	4.8X
EFD in Rabbit (RDEA806)	0, 50, 100, 400	100 (RDEA806)	RDEA594: 186 (GD1)	-	2.7X

*Based on estimates by the Clinical Pharmacology Reviewer, Sheetal Agarwal, Ph.D. (personal communication) for clinical exposure at 400 mg single dose under the fed state in the proposed protocol: Cmax ≈ 11-12 mcg/ml, Tmax ≈ 5-8 h, and AUC ≈ 70 mcg.h/ml.

Reference ID: 3815621

Internal Comments/Recommendations

From the nonclinical perspective, the proposed clinical study is considered reasonably safe to proceed.

1. No concerns at this time regarding the excipients, all of which are USP/NF grade.

2. The impurities identified in the clinical study batches appear to be adequately qualified by inclusion in the nonclinical study (including DRF, 28-day toxicology in rat and monkey, genetic toxicity and safety pharmacology) batches at this time. There are no structural alerts for genetic toxicity by the (b) (4) impurity, according to personal communication from the CMC reviewer.

3. Additionally, the results were negative in an investigation conducted specifically to evaluate the potential for genetic toxicity by the (b) (4) impurity, found at (b) (4)% and exceeding the amount paresent in the RDEA594 Lot used in the pivotal battery of genotoxicity studies.

4. The following target organs were identified in the nonclinical studies on RDEA594, and RDEA806 where TK results were available for exposure to RDEA594, a primary metabolite of RDEA806:
    a. GI:
        i. **Decreased GI motility**, watery feces, at high oral doses in rat
        ii. **Epithelial cell necrosis** (14-day study) in rat
        iii. **Neutrophilic inflammation in ileal villi** (14-day study) and lamina propria in ileum and duodenum (14-day study) in monkey at highest oral dose
    b. Kidney:
        i. **Urinary excretion changes** without effects on serum clinical chemistry, including increased osmolality, and calcium, phosphorus, protein, potassium, sodium and chloride excretion, and decreased urine volume for 3h after dosing in rat
        ii. **Tubular epithelial degeneration** at high oral doses (14-day and 28-day studies)
        iii. **Nephropathy** at high oral doses (28-day study) in rat
    c. Liver:
        i. **Increased liver weights** across doses, statistically significant at high oral doses in rat (28-day study)
        ii. **Hepatocellular hypertrophy** at high oral doses
            1. in rat (28-day study, 13-week interim evaluation in the ongoing 6-month study)
            2. in monkey (interim 6-month evaluation in ongoing 9-month toxicology study)
        iii. Increased total cytochrome P450 content in monkey at highest dose (28-day study)
    d. Cardiovascular:

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i. **Inhibition of hERG** potassium current at the protein-adjusted IC_{50} of approximately 9,910 mcM (4000 mcg/ml, approximately 222X the clinical exposure [Cmax] at the proposed clinical dose); NOAEL = 81 mcg/ml without adjustment for protein binding, representing approximately 4.5X the clinical Cmax
ii. Slight increase in action potential duration at 81 mcg/ml, and decreased maximum rate of depolarization without a concentration response (1-200 mcM) in rabbit Purkinje Fiber Assays in the absence of human serum albumin (HAS), suggesting blockade of sodium channels; however, no RDEA504-related effects at up to 200 mcM (81 mcg/ml) in the presence of HAS
iii. **Decreased blood pressure and heart rate** at all doses (30-300 mg/kg PO) in conscious telemetered monkeys, with significant increases in QT interval prior to correction for heart rate; however, no treatment-related effects on corrected QT interval (QTcR) in this assay
iv. **Hypotension** at all doses, and **AV block** at 150 mg/kg IV, possibly disrupting atrioventricular conduction in 2 rats that died in exploratory cardiovascular safety assessment in rats

e. Embryo-fetal toxicity: **Decreased fetal weights** and **marginally delayed ossification** at the highest oral dose (maternally toxic based on decreased food consumption, BW loss, decreased BWG, abortions) in rabbits

5. The safety margins for the treatment-related toxicity observed in the nonclinical studies are acceptable to support the safety of the proposed clinical study, based on clinical experience in previous Phase 1 studies at doses and treatment durations exceeding those proposed in this submission.

6. The treatment-related toxicity observed in the nonclinical studies was reversible in the recovery periods, and is monitorable in the proposed in-patient clinical study.

7. The patients should be monitored for potential treatment-related gastrointestinal upset and irritation, adverse changes in urinary excretion parameters, clinical chemistry and hematology changes that might reflect adverse effects on liver function, and potential changes in cardiovascular function (e.g., hypotension).

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APPENDIX 1

Treatment-related toxicity observed in previous studies on RDEA806:

(b) (4)

Human:

- RDEA594 Exposure with 14-day RDEA806 administration at 500 mg:
  - Cmax 5 mcg/ml
  - AUC 83 mcg.h/ml
- Urinary Uric Acid changes correlated with urinary RDEA594 concentrations

Nonclinical:

- No Xanthine oxidase inhibition or purine nucleoside phosphorylase activity by RDEA806
- Extensive metabolism of RDEA806 to RDEA594
- (b) (4)
- 5 detected RDEA594 metabolites

Single Dose:

- Mortality in rat at ≥1000 mg/kg PO and monkey at 2000 mg/kg PO
- Emesis, salivation, abnormal fecal observations in dog at ≥100 mg/kg PO
- Vomiting and liquid feces in monkey at 2000 mg/kg PO

Repeated Dose:

- Mortality at ≥1000 mg/kg/d PO in rat
- Lethargy, decreased body T, at 1000 mg/kg/d in rat
- Vomiting and loose feces in monkey at 200 mg/kg/d (14-d study)
- ↓RBCs, Hb, Hct, MCV and MCH at 1000 mg/kg/d in rat
- ↑ALP, BUN/Creatinine at ≥300 mg/kg/d in rat and ALP, ALT, gamma-GT, AG ratio, bilirubin, phosphorus at ≥1000 mg/kg/d in rat
- ↓cholesterol and Ca at 800 mg/kg/d in monkey (14-d study)
- **GI toxicity**
  - Local irritation at ≥100 mg/kg/d in rat (14-d study, NOAEL 30)
  - mucosal lesions including vacuolation in squamous epithelium, eosinophil infiltration at ≥200 mg/kg/d and discoloration of glandular stomach, duodenum, jejunum, ileum at ≥1000 mg/kg/d) in rat (NOAEL 60)
- **Renal effects**:
  - **Tubular degeneration at** ≥100 mg/kg/d in rat (14-d, NOAEL 30)

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- **Liver**:
  - increased absolute and relative weights at ≥200 mg/kg/d in rat
  - cytotoxic damages at 1000 mg/kg/d in rat (14-d, NOAEL 300 mg/kg/d)
  - adaptive changes at ≥300 mg/kg/d in rat
- **Lung**: diffuse/multifocal discoloration, at ≥100 mg/kg/d in rat
- **Thymus:** diffuse/multifocal discoloration at ≥100 mg/kg/d in rat, decreased organ wt. at ≥1000 mg/kg/d in rat

Genotoxicity:

- Negative in Ames, Chromosomal Aberrations Assay *in vitro* with HSA2, mouse lymphoma assay, *in vivo* Micronucleus Test, and rat liver UDS test
- Positive in Chromosomal Aberrations Assay *in vitro* in absence of HSA2

Reproductive toxicity:

- Negative in Segment I in rat
- Negative in Segment II at up to 300 mg/kg/d in rat
- ↑fetal mortality (late resorptions) and ↓fetal weights at 600 mg/kg/d in rabbit Segment II

Reference ID: 3815621

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-102128	ORIG-1	ARDEA BIOSCIENCES INC	RDEA594

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ADAM M WASSERMAN

06/18/2010

I concur the initial clinical study is safe to proceed and have submitted this review on behalf of Dr. Young.

Appendix 2: Nonclinical Review of IND 102128. April 29, 2011.

APPEARS THIS WAY ON ORIGINAL

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	IND 102,128
Supporting document/s:	SPA-2 SDN 51
Sponsor's letter date:	March 17, 2011
CDER stamp date:	March 18, 2011
Product:	RDEA594
Indication:	Hyperuricemia in patients with gout
Sponsor:	Ardea Biosciences, Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Kathleen Young, Ph.D.
Supervisor/Team Leader:	Molly Topper, Ph.D.
Division Director:	Badrul A. Chowdhury, M.D., Ph.D.
Project Manager:	Michelle Y. Jordan Garner

Reference ID: 2916021

1 Executive Summary

1.1 Introduction

RDEA594 is the major metabolite of RDEA806, a non-nucleoside reverse transcriptase inhibitor (NNRTI) [redacted] (b) (4) RDEA594 is under development for the treatment of patients with gout (original IND submitted September 30, 2009). RDEA594 blocked reuptake of uric acid in the kidney proximal tubule (responsible for 80% reabsorption of uric acid) by URAT1 transporter inhibition, at doses of up to a maximum of 600 mg/day in clinical studies. RDEA594 showed no inhibition of purine nucleoside phosphorylase and xanthine oxidase, suggesting no inhibition of uric acid synthesis.

This amendment was submitted to seek execCAC concurrence for a proposed carcinogenicity study protocol in 001178-T (hemizygous) CByB6F1-Tg (HRAS) 2Jic (TgrasH2) wild-type mice administered RDEA594 at doses of 0, [redacted] (b) (4) and 125 mg/kg/day in males and 0, [redacted] (b) (4), and 250 mg/kg/day in females by oral gavage, once daily for 26 weeks. The vehicle is water and the proposed Positive Control article will be N-ethyl-N-nitrosurea (MNU) in acidified physiological saline (150 mM sodium chloride and 15 mM sodium citrate in reverse osmosis/deionized water (75 mg/kg single Day 1 dose in 10 ml/kg). The Sponsor proposed these doses based on maximum tolerated doses (MTD) observed in a 28-day dose range finding study in TgrasH2 (wild type) mice.

Review of the supportive data from a 28-day oral repeat dose mouse toxicity study is completed herein. Information previously submitted to this IND on pharmacokinetics and genetic toxicity is also considered in the recommendations to the Sponsor for the proposed carcinogenicity study in mice.

1.2 Brief Discussion of Nonclinical Findings in the 28-day Dose Range Finding Study in TgrasH2 Mice

The results of the clinical and nonclinical PK/TK evaluations of RDEA594 showed rapid absorption, with a Tmax of approximately 3 hours in male and 1 hour in female mice. There were dose-proportional increases in exposure in mice, although exposure was generally higher in the females than in the males. Distribution patterns were similar after oral and IV dosing, and included distribution primarily to the GI tract (stomach, small and large intestine) and kidney, with small amounts in liver, lung, thyroid and urine 2-4 hours after oral dosing. Protein binding was similar across species, and ranged from 94.0%-95.2% in mice and 97.9%-98.5% in humans.

Reference ID: 3046023

Comparative metabolism studies showed RDEA594 stability when incubated with mouse, rat, dog, monkey and human liver microsomes and hepatocytes. The main metabolites are formed by addition of an oxygen atom to form M3 and addition of 2 hydroxy groups to form M4. *In vivo* investigation showed RDEA594 metabolism by glucuronidation to M1, oxidation to M3, M4 and M5, and debromination to M2 and M5 in the rat and monkey. Only CYP2C9 metabolism was detected after the 60 minute incubation of RDEA 594 (1 mcM) with cDNA-expressed human P450 isozymes (100 mmol/ml) followed by monitoring of parent compound depletion using LC-MS (Study SR08-038). In human liver microsomes incubated with marker substrates for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, with and without RDEA594; at 0.0305-200 mcM, RDEA594 inhibited CYP2C8 (IC_{50} = 40.7 mcM). The IC_{50} for CYP1A2, CYP2C19, CYP2D6, and CYP3A4 was >100 mcM. Cultured human liver hepatocytes treated with RDEA594 (0.1-10 mcM) or human CYP450 liver enzyme inducers (e.g., omeprazole at 100 mcM, refampin at 10 mcM) for 3 days, following by analysis of microsomes for 7-ethoxyresorufin *O*-dealkylation (CYP1A2) activity and testosterone 6-beta-*O*-hydroxylation (CYP3A4/5 activity) at 10 mcM RDEA594 (67% of that by rifampin) but no increase in 7-ethoxyresorufin *O*-dealkylation activity. CYP2C9 is the major enzyme responsible for RDEA594 in humans, and possible CYP3A4/5 induction was suggested by increased testosterone 6beta-hydroxylase activity by 3-fold.

The results of a standard battery of genotoxocity studies on RDEA594 (Ames assay, 2 chromosome aberrations assays, and an *in vivo* rat micronucleus study) were negative.

A 1-month dose range-finding (DRF) study was conducted in TgrasH2 mice to determine the dose selection for the proposed 26-week carcinogenicity evaluation in this strain. In the first of two phases of the study, the mice were administered RDEA594 at the doses of 0, 500, 750 and 1000 mg/kg/day by oral gavage once daily. RDEA594 was lethal in most of the male (M) and female (F) mice at 750 and 1000 mg/kg/day and the remaining, surviving animals in these groups were sacrificed early. One additional male and 2 toxicokinetic study males given 500 mg/kg/day test article were sacrificed *in extremis* on Dosing Day 4. Based on the high mortality in Phase 1 of the DRF study, additional groups were added (Phase 2) at the doses of 125 and 250 mg/kg/day. Treatment-related clinical signs in the animals that died or were sacrificed early included hunched posture, ataxia, recumbency, opaque or squinted eyes, labored and/or irregular breathing, cold to touch, swollen head, and rough haircoat. The surviving animals showed rough haircoat and swollen head at doses of 500 mg/kg/day and above.

Body weights (BW) and body weight changes (BWG) increased with RDEA594 treatment, although the BW increase was not dose-related in the males. Thus, there appears to be no adverse treatment-related effects on body weights. The results of the clinical pathology evaluations at terminal sacrifice showed treatment-related increases in neutrophils, basophils, and large unstained cell counts (lymphocytes), and increased total protein (at 250 mg/kg/day and above) and globulin (at 250 and above) in the M . These effects are likely a result of dehydration, in agreement with the Sponsor's interpretation. Increased ALT was observed in the M and F given 500 mg/kg/day.

Reference ID: 2916021

Other clinical pathology effects included increased cholesterol (M & F) and decreased triglycerides (M) at all doses tested.

Treatment-related organ weight changes were observed, at all dose levels. Increased absolute and relative (to BW and brain weight) liver and gallbladder weights were found in both the M and F at 125 mg/kg/day and above, correlating with histopathology findings of hepatocellular hypertrophy. Kidney weights (relative to BW) were reduced in F given 250 mg/kg/d and above and in the M at the 500 mg/kg/d level, and correlated with cortical tubular regeneration and degeneration/necrosis in the M. Also, there was a treatment-related decrease in brain weights in the M at 500 mg/kg/d, without histopathology findings.

Microscopic examination at scheduled sacrifice supported identification of the liver and kidney as target organs of toxicity. Increased (vs. controls) hepatocellular hypertrophy that was dose-related in incidence and severity, with enlarged hepatocytes with pale to eosinophilic cytoplasm was found in the M and F at 125 mg/kg/day. This effect was of minimal severity at 125 mg/kg/d, minimal to slight at 250 mg/kg/day and slight to moderate at 500 mg/kg/d. Single cell degeneration and necrosis was found in 2/10 M at the 125 mg/kg/d dose level and 1 F at the 500 mg/kg/d level, with severity ratings from minimal to slight. The single cell hepatocyte necrosis and degeneration and necrosis was found in all males at 250 mg/kg/day with a severity rating of minimal, and in the high dose groups (500 mg/kg/d) to be of slight to moderate severity in the M and F. The histopathology findings in liver could be due to treatment-related toxicity and/or to adaptive changes commonly found following treatment with xenobiotics due to proliferation of smooth endoplasmic reticulum upon increases in drug metabolizing enzyme activity. There was a correlation of the findings in liver and increased ALT at the 500 mg/kg/d dose level. The histopathology findings in kidney included minimal to moderate severity regeneration of cortical tubules in 1 M at 250 and 5 M at 500 mg/kg/d, that was also found in 1 Control M, and degeneration and/or cortical tubule necrosis in the M at 500 mg/kg/d but not in the F or controls.

MTDs of 125 mg/kg/day in the male and 250 mg/kg/d in the female mice were used for the high dose selection for the 6-month carcinogenicity study, based on deaths in males at dose levels ≥ 500 mg/kg/day and in the females at ≥ 750 mg/kg/day. These doses produced minimum toxicity that is not expected to affect survival or confound the interpretation of the carcinogenicity bioassay, and therefore in agreement with the Sponsor, can be considered for the high dose level in the carcinogenicity study. The anticipated multiples of the RDEA594 MRHD represented by the doses proposed by the Sponsor for the 6-month carcinogenicity study in TgrasH2 mice are 2.5X, 4.9X and 8.8X in M and 7.8X, 14X, and 21.5X in F at the proposed low, mid- and high doses, respectively. At the recommended dose levels of 15, 45, and 125 mg/kg/d in M and 30, 90, and 250 mg/kg/day in F mice, the anticipated multiples of the RDEA594 MRHD are approximately 1.25X, 4X and 8.8X in the males and 4X, 11.5X and 21.5X in the females, based on extrapolation of AUC data available.

Reference ID: 2916021

1.3 Recommendations

Doses recommended by Reviewer: 0, 15, 45, and 125 30, 90, and 250

Basis for Recommendation:

The recommended high doses in the proposed 6-month carcinogenicity study in TgrasH2 mice, in agreement with the Sponsor are 125 mg/kg/day in the males based on the mortality at 500 mg/kg/day in the M and 750 mg/kg/day in the F, and on the histopathology finding of kidney and liver toxicity (single cell necrosis and degeneration at higher doses). These doses produced minimal toxicity, but no treatment-related effects were identified that might be expected to confound the interpretation of the results or produce excessive mortality in the 6-month carcinogenicity study. The recommended low (15 and 30 mg/kg/day in M and F, respectively) and mid-dose (45 and 90 mg/kg/day in M and F, respectively) levels are based on approximately 1/3 dose intervals. The anticipated multiples of the proposed clinical high dose (600 mg/day) at the LD, MD and HD levels in the proposed carcinogenicity study are adequate at 1.25X, 4X and 8.8X in the M, and 4X, 11.5X and 21.5X in the F.

2 Drug Information

2.1 Drug

CAS Registry Number: 1151516-14-1

Generic Name: None

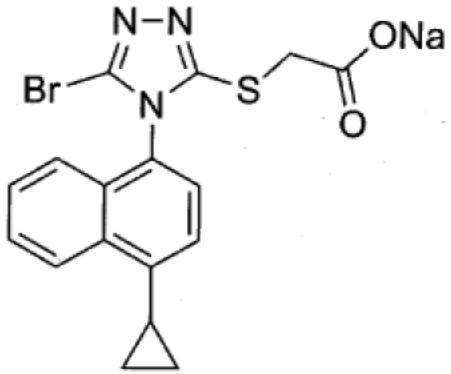
Code Name: RDEA594

Chemical Name: Sodium, [5-Bromo-4-(4-cyclopropyl-napthalen-1-yl)-4H-[1, 2, 4]triazol-3-yl-sulfanyl]-acetate

Molecular Formula/Molecular Weight: $C_{17}H_{13}BrN_3NaO_2S$ / 426.26 Daltons (404.28 for the free acid)

Structure or Biochemical Description:

Reference ID: 3916023



Pharmacologic Class: Uricosuric URAT1 transporter inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND/NDA/DMF	Drug Name	Division	Status	Indication	Company
[redacted] (b) (4)					Ardea
					Ardea

2.3 Drug Formulation

[redacted] (b) (4) **Capsule containing the following Ingredients**:

RDEA594 Na salt (Drug Substance **Lot NE-023025-Batch-01-2009**) [redacted] (b) (4)

[redacted] (b) (4)

Total Fill Weight

[redacted] (b) (4)

2.4 **Comments on Novel Excipients**: All excipients USP/NF grade

Reference ID: 2946023

2.5 Comments on Impurities/Degradants of Concern: Addressed in the original IND review.

3.0 Studies Submitted:

(b) (4) **Protocol**: RDEA594: 26-Week Oral Gavage Oncogenicity Study with RDEA594 in 001178-T (hemizygous) Tg*ras*H2 Mice

Bioanalysis and Pharmacokinetics of RDEA594: A Single Dose Oral Gavage Pharmacokinetic Study in Male TgHras2 (Wild Type) Mice (Non GLP) (Study SR10-40)

Bioanalysis and Toxicokinetics of RDEA594: A 28-Day Oral Gavage Toxicokinetic Study in TgHras2 (Wild Type) Mice (Non-GLP) (Study SR10-060)

RDEA594: 4-Week Dose Range Finding Oral Gavage Toxicity and Toxicokinetic Study in TgHras2 (Wild Type) Mice (Study SR10-018)

3.1 Studies Reviewed

(b) (4) Protocol: RDEA594: 26-Week Oral Gavage Oncogenicity Study with RDEA594 in 001178-T (hemizygous) Tg*ras*H2 Mice

Bioanalysis and Pharmacokinetics of RDEA594: A Single Dose Oral Gavage Pharmacokinetic Study in Male TgHras2 (Wild Type) Mice (Non GLP) (Study SR10-40)

Bioanalysis and Toxicokinetics of RDEA594: A 28-Day Oral Gavage Toxicokinetic Study in TgHras2 (Wild Type) Mice (Non-GLP) (Study SR10-060)

RDEA594: 4-Week Dose Range Finding Oral Gavage Toxicity and Toxicokinetic Study in TgHras2 (Wild Type) Mice (Study SR10-018)

3.2 Studies Not Reviewed

Evaluation of the Plasma Protein Binding of RDEA594 in Pre-Dose **Human** Plasma by Equilibrium Dialysis from: “A Phase 1, Single Dose, Pharmacokinetic and Pharmacodynamic Study of RDEA594 in Adult Volunteers with Various Degrees of Renal Insufficiency” (Study RDEA594-104)

Reference ID: 2916023

3.3 Previous Reviews Referenced:

Evaluation of the plasma Protein Binding Potential of RDEA594 in Mouse, Rat, Dog, Monkey and Human Plasma by Equilibrium Dialysis (Study SR08-045)

Bacterial Reverse Mutation Assay (Study SR08-022)

In Vitro Mammalian Chromosome Aberration Screening Test (Study SR06-086) (Amendment to Study SR08-023 to correct typographical error in the final report for Study SR08-023)

RDEA594: *In Vitro* Mammalian Chromosome Aberration Test (Study SR08-023)

Rat Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of RDEA594 (study SR08-024)

Bacterial Reverse Mutation Assay (Study SR09-015) (to confirm negative results in the original Bacterial Reverse Mutation Assay # SR08-023)

4 Pharmacology

RDEA594, via concentration-dependent **inhibition of the uric acid transporter (URAT1) in the kidney proximal tubule** (responsible for up to 80% reabsorption of uric acid), increases urinary excretion of uric acid which can form uric acid crystals that accumulate in, and increase inflammation in joints and other tissues in gout patients, as well as increase the incidence of kidney stones. The results of in vitro primary pharmacology studies showed no blockade of uric acid synthesis. RDEA594 did not inhibit purine nucleoside phosphorylase which metabolizes purine nucleosides to purine base and ribose phosphate, and no inhibition of xanthne oxidase, which converts xanthine and hypoxanthine to uric acid. No other uric acid transporters (human h4 and SLC2A9) other than URAT1 were inhibited by RDEA594.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

In support of the proposed SPA doses, the Sponsor conducted a two phase 28-day oral toxicity study in TgrasH2 mice. This study is reviewed under the toxicology section of this IND review. The first Phase of the study, animals were dosed with 500-1000 mg/kg/day. Due to significant mortality, a second Phase was added where the animals were dosed with 125 and 250 mg/kg/day. No TK data was available in the first 4-week dose finding study for Day 28 in TgrasH2 mice at the dose levels of 750-1000

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mg/kg/day due to the high mortality incidence and early termination of these groups. The results of the TK evaluation in the 28-day dose range finding study in TgrasH2 mice are presented in the following table:

TK Results from the 1st Phase of the 28-Day DRF Study in TgrasH2 Mice

Day	Dose (mg/kg/d)	Male		Female	
		AUC0-24 (mcg.h/ml)	Cmax (mcg/ml)	AUC0-24 (mcg.h/ml)	Cmax (mcg/ml)
1	500	2590	144	2000	177
	750	2220	190	2420	179
	1000	2590	211	2340	218
28	500	2710	238	3450	270
	750	NA	NA	NA	NA
	1000	NA	NA	NA	NA
28/1	500	1.05	1.65	1.73	1.53
	750	NA	NA	NA	NA
	1000	NA	NA	NA	NA

NA: Not available; dosing discontinued before Day 28

TK Results from the 2nd Phase of the 28-Day DRF Study in TgrasH2 Mice

Dose (mg/kg/d)	Male		Female	
	AUC0-24h (mcg.h/ml)	Cmax (mcg/ml)	AUC0-24h (mcg.h/ml)	Cmax (mcg/ml)
60	409	72.7	647	145
125	737	125	1170	148
250	1660	161	1800	183
500	2710	238	3450	270

At steady-state, the results of the TK analyses showed rapid absorption on Day 1 (Tmax = 0.5-1.0 h), but a moderate rate of absorption on Day 28 (Tmax = 6h) at 500 mg/kg/day in the male and female mice The higher exposure found in Week 28 when compared to that in Week 1 suggests accumulation of RDEA594 in mice with repeated dosing. The AUC levels for the first (Days 1 and 28 measurements) and second (steady state measurements) 28-day dose-range finding studies in mice are presented in the following tables (from the original submission):

The highest proposed clinical dose for the management of hyperuricemia in gout patients is considered by the Sponsor not to exceed 600 mg/day. At that dose, exposure in the single dose clinical study (RDEA594-101, fed state) was 106 mcg.h/ml (AUC0-24) and 108 mcg.h/ml (AUC0-inf). The PK evaluation after repeated administration in capsule form for 10 days (Clinical Study RDEA594-102) showed exposure in the fed state of **84.9 mcg.h/ml** (AUC0-t). The Cmax values at 600 mg/day

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in the single and repeated dose clinical studies were 3.12 and 20.8 mcg/ml, respectively, and Tmax values were 8.75 and 3.50 h, respectively.

The results of the ADME evaluations for the original IND are summarized below:

Absorption: Absorption by the oral route was rapid with Tmax occurring approximately 3 hours in male and 1 hour in female mice. There was a dose-proportional increase in exposure in mice, with generally greater exposure in the females than in males. The Tmax was 2.33-16.5 hours (at 20-1000 mg/kg PO) in rats, 0.438 hours in dogs and 1.33 hours in monkeys. The plasma t1/2 was 6.5 hours in male and 3 hours in female rats for total radioactivity, and 7.4 hours in male and 5 hours in female rats for RDEA594, after IV dosing. In male dogs, the t1/2 was 7.5 hours. A greater than dose proportional increase in exposure was found at lower doses and less than dose proportional increase in exposure at higher doses in rats. Clearance was lower than blood flow (3.3 L/h/kg in rats, 1.8 L/h/kg in dogs, and 2.6 L/h/kg in monkeys) at 0.2 (male) and 0.17 (female) L/h/kg in rats, 9,13 L/h/kg in dogs, 0.173 L/h/kg in monkeys. The volume of distribution was 0.9 in M and 0.55 in female rats, 0.36 L/kg in dogs, 0.289 L/kg in monkeys, which approximated body fluid volume, and thus little tissue distribution was seen across these species. Oral bioavailability was 75.3% in male and 71.1 % in female rats, 100% in dogs, and 41% in monkeys. After repeated oral dose exposure, the AUC values were higher in the male than in female rats at lower doses, and approximately equivalent at higher doses. AUC values were similar on Days 15 and 28 vs. Day 1 after oral dosing in monkeys, suggesting no accumulation at the doses of 30-1000 mg/kg/day. However, lower exposures at high doses suggest autoinduction at the higher dose levels. There were no food effects at lower doses (≤30 mg/kg/d) in rats, but higher exposures under fasted conditions at doses of ≥100 mg/kg/d.

Distribution: RDEA594 shows a similar pattern of distribution after oral and IV dosing, and is primarily distributed to the GI tract (stomach, small intestine early, and large intestine after 24 hours), kidneys, with a small amount in the liver (possibly representing bile), lung, thyroid, and urine (2-4 hours after oral dosing). RDEA594 is nearly completely cleared by 24-72 hours from the body. A similar distribution was found in albino and pigmented rats, suggesting no binding to melanin. No RDEA594 was detected in eye humor or lens and distribution to brain was minimal. Evaluation of in vivo partition between plasma and blood showed blood to plasma ratios of 0.5-0.8 over 12 hours in rats and over 48 hours after dosing in monkeys.

Protein Binding:

The results of the plasma protein binding studies showed that RDEA594 is highly protein bound across species and in humans; rat, dog, monkey and human plasma, at plasma concentrations at and below 50 mcM. Protein binding was ≥94.0% in C57BL/6 mouse plasma. Protein binding decreased with increasing RDEA594 concentrations of

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200 mcM and above in rats. Interspecies comparison of protein binding is provided in the following table (from the original IND submission, Study #s SR08-045, SR08-132):

In Vitro Protein Binding (SR08-045, SR08-132, table reproduced from the original IND submission):

Table 16. Protein Binding of RDEA594 in Plasma

Species	% Protein Binding (Mean ± SD, N=3)					
	1 µM	10 µM	50 µM	200 µM	500 µM	1000 µM
Mouse	95.2 ± 0.45	94.9 ± 0.35	94.0 ± 0.15	NM	NM	NM
Rat	97.7 ± 0.34	98.1 ± 0.09	97.7 ± 0.01	97.6 ± 0.11	96.4 ± 0.06	95.1 ± 0.18
Dog	98.3 ± 0.06	98.2 ± 0.11	98.1 ± 0.13	NM	NM	NM
Monkey	98.2 ± 0.15	98.3 ± 0.03	98.2 ± 0.10	97.8 ± 0.06	97.3 ± 0.02	NM
Human	98.5 ± 0.06	98.4 ± 0.02	97.9 ± 0.17	NM	NM	NM

NM: Not measured

RDEA594 is highly protein bound across species and in humans, at ≥97.7% in rat, dog, monkey and human plasma at RDEA594 concentrations at and below 50 mcM, but higher than 94% in mouse plasma. Plasma protein binding decreased with increasing RDEA594 concentrations of 200 mcM and above in rat and monkey.

Metabolism

In an *in vitro* evaluation, RDEA594 was stable when incubated with mouse, rat, dog, monkey and human liver microsomes and hepatocytes; ≥92% parent drug remained. The main metabolites in monkey liver microsomes and monkey and human hepatocytes were formed by addition of an oxygen atom to form M3 and addition of 2 hydroxy groups to form M4. *In vivo* investigation showed RDEA594 metabolism by glucuronidation to M1, oxidation to M3, M4 and M5, and debromination to M2 and M5 in the rat and monkey.

***In vitro*:**

- RDEA594 stable when incubated with rat, dog, monkey and human liver microsomes and hepatocytes *in vitro*; ≥92% parent drug remained
- The main metabolites in monkey liver microsomes and monkey and human hepatocytes were formed by addition of an oxygen atom to form M3 and addition of 2 hydroxy groups to form M4

Only CYP2C9 metabolism was detected after the 60 minute incubation of RDEA 594 (1 mcM) with cDNA-expressed human P450 isozymes (100 mmol/ml) followed by monitoring of parent compound depletion using LC-MS (Study SR08-038). In human

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liver microsomes incubated with marker substrates for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, with and without RDEA594; at 0.0305-200 mcM, RDEA594 inhibited CYP2C8 (IC_{50} = 40.7 mcM). The IC_{50} for CYP1A2, CYP2C19, CYP2D6, and CYP3A4 was >100 mcM.

***In vivo*:** RDEA594 is metabolized by glucuronidation (to M1), oxidation (to M3, M4, and M5) and debromination (to M2 and M5) in rat and monkey

Gender differences in metabolic profile of RDEA594 were found in rat (Study SR08-120), with higher levels of M3 found in urine and feces, and higher levels of M5 found in feces over 24 hours post-dose in the male rats than in the female rats. The results of the metabolic profile evaluation for this study are presented in the following table:

Table 20 Metabolic Profile of RDEA594 in Plasma, Urine and Feces Following Oral and Intravenous Dosing to Rats

Gender	Route	Matrix	Time (hr)	Concentration %				
				M2	M3	M5	RDEA594	Total
M	PO	Plasma	1	0.0	0.0	0.0	100	100
		Urine	0-24	13.1	47.7	0.0	38.4	99.2
		Feces	0-24	34.6	9.8	30.1	24.2	98.7
	IV	Plasma	0.5	0.0	0.0	0.0	100	100
		Urine	0-24	10.4	55.0	0.0	26.9	92.3
		Feces	0-24	35.5	8.0	38.0	16.3	97.8
F	PO	Plasma	1	0.0	0.0	0.0	100	100
		Urine	0-24	5.3	18.1	0.0	76.7	100
		Feces	0-24	43.7	2.3	13.7	32.7	92.2
	IV	Plasma	0.5	0.0	0.0	0.0	100	100
		Urine	0-24	4.1	10.2	0.0	85.4	99.6
		Feces	0-24	47.2	2.4	16.0	33.6	99.2

Only CYP2C9 metabolism was detected after the 60 minute incubation of RDEA 594 (1 mcM) with cDNA-expressed human P450 isozymes (100 mmol/ml) followed by monitoring of parent compound depletion using LC-MS (Study SR08-038). In human liver microsomes incubated with marker substrates for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, with and without RDEA594; at 0.0305-200 mcM, RDEA594 inhibited CYP2C8 (IC_{50} = 40.7 mcM). The IC_{50} for CYP1A2, CYP2C19, CYP2D6, and CYP3A4 was >100 mcM.

Excretion: Evaluation in male rats showed excretion predominantly in feces (92.4% total radioactivity), with minor amounts found in urine (8.04%) after IV dosing (20 mg/kg). Excretion was found at a similar extent in female rats, (44.2% in feces after PO and 42.7% after IV dosing, and 36.3% in urine after PO and 39.3% in urine after IV

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dosing). These results suggest extensive biliary excretion. Excretion was somewhat higher in feces (41.7% after PO and 49.9% after IV dosing) than in urine (32.6% after PO and 25.7%) after IV dosing in male monkeys (females not evaluated). An interspecies comparison of excretion patterns is presented below (from the original IND submission):

Table 24. Excretion Pattern in Animals Following a Single Dose of [^{14}C]RDEA594

Species	Sex	Route	Duration (hr)	Dose (mg/kg)	Recovery of Dose (%)				
					Urine	Feces	Other [a]	Carcass	Total
Rat	M	PO	120	20	11.9	83.2	1.34	0.34	96.8
Rat	M	IV	120	20	8.04	92.4	2.68	3.00	106
Rat	F	PO	120	20	36.3	44.2	2.58	0.34	83.5
Rat	F	IV	120	20	39.3	42.7	16.0	5.69	104
Monkey	M	PO	120	20	32.6	41.7	15.7	NA	90.0
Monkey	M	IV	120	10	25.7	49.9	12.2	NA	87.8

[a] Cage wash/wipe; PO = oral; IV = intravenous; NA = data not available since animals were not sacrificed for radioactivity counting

For comparison, the results of the clinical PK evaluations are presented in the following tables:

Repeated Dose PK: Clinical Study RDEA594-102 (n=6/group): Reproduced in part from the original IND submission

Day	Form	Dose (mg)	C_{max} (mcg/ml)	AUC_T (mcg.h/ml)	$AUC_{0\text{-}inf}$ (mcg.h/ml)	T_{max} (h)
1	(b) (4)	100 (fed)	4.74	14.2	14.2	0.75
	IR Tablet	200 (fasted)	10.8	28.7	28.9	0.75
		400 (fasted)	23.1	70.5	71.2	1.00
		200 (fed)	5.54	29.8	30.2	5.00
	(b) (4) Capsule	200 (fed)	8.18	26.1	26.3	3.00
		400 (fed)	13.8	51.8	52.3	3.00
		600 (fed)	20.1	73.5	74.4	2.50
	(b) (4) Tablet	200 (fed)	6.16	20.9	21.1	1.75
10	(b) (4)	100 (fed)	4.02	12.1	Not provided	0.625
	IR Tablet	200 (fasted)	11.7	33.1	Not provided	0.75
		400 (fasted)	21.9	89.8	Not provided	2.00
		200 (fed)	6.51	30.3	Not provided	4.50
	(b) (4) Capsule	200 (fed)	7.95	25.9	Not provided	3.50
		400 (fed)	15.7	53.9	Not provided	2.50
		600 (fed)	20.8	84.9	Not provided	3.50
	(b) (4) Tablet	200 (fed)	5.57	21.2	Not provided	4.50

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6 General Toxicology

6.1 Single-Dose Toxicity

Studies to evaluate single-dose toxicity were not conducted; acute treatment-related effects were evaluated using Day 1 measurements in the repeat-dose toxicology studies.

6.2 Repeat-Dose Toxicity

Study title: ***RDEA594: 4-Week Dose Range Finding Oral Gavage Toxicity and Toxicokinetic Study in TgHras2 (wild type) Mice* And *Bioanalysis and Toxicokinetics of RDEA594: a 28-Day Oral Gavage Toxicokinetic Study in TgHras2 (Wild Type) Mice (TK study was Non-GLP, Report # SR10-060)***

Study no.:	SR10-018 ((b) (4) Study # 8226465)
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	25 May 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RDEA594 sodium salt, Lot NE-023025-Batch 03-2009, 84.5% purity (Analytical Standards for TK analysis: RDEA594, Lot 10454-161-2, purity 94.3%, [D_6]RDEA594, Lot L0002, Purity 96.0%)

Key Study Findings

Study Findings

- **Target organs: liver and kidneys**
- **RDEA594 lethal in most M and F mice at 750 and 1000 mg/kg/day**, and in 1 main study M and 2 TK study M at 500 mg/kg/d
- Treatment-related clinical signs were hunched posture, ataxia, recumbency, opaque/squinted eyes, labored/irregular breathing, cold to touch, swollen head, rough haircoat in decedents at ≥500, and rough haircoat and swollen head in surviving mice at ≥500
- Treatment-related clinical pathology findings suggested dehydration and treatment-related toxicity in liver (e.g., increased ALT ≥ 500)
- Treatment-related organ weight changes:
  - At ≥ 125 mg/kg/day:

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        - ↑ **liver/gallbladder** weights (abs and rel to BW & brain wt) in M & F (correlated w/hepatocellular hypertrophy)
    - At ≥ 250 mg/kg/day:
        - ↓ rel (to BW) **kidney** wts in F
    - At ≥ 500 mg/kg/day:
        - ↑ abs & rel (to BW) **kidney** wts in M (correlated w/ cortical tubular regeneration and degeneration/necrosis)
        - ↓ **brain weights** in M (w/o correlating histopathology findings)
    - At ≥ 750 mg/kg/day:
        - ↑ rel (bo BW) **kidney** wts in F
        - ↓ absolute **kidney** wts in M
        - ↑ rel (to BW) **brain** wts in F
        - ↓ **heart weights** in M and rel (to brain wt & BW) in F
        - ↓ **epididymis and pituitary weights** in M
        - ↑ **pituitary** (rel to BW and brain wt) in F
        - ↓ abs and rel (to brain wt) **testis** wts in M at
        - ↓ abs and rel (to brain wt) **ovary** wt in F
    - At 1000 mg/kg/d:
        - ↓ **brain weights** in F (w/o correlating histopathology findings)
        - ↓ **adrenal weights** in F
        - ↓ rel (to BW) **ovary** wt in F
- Histopathology Findings:
- **Kidney**
    - **Regeneration of cortical** tubule in controls (2/10 M, 0/10 F) and at 500 mg/kg/day in M (5/10, minimal to moderate) and F (1/10, minimal)
    - **Degeneration/necrosis of cortical** tubule in M (minimal to slight) at 500 mg/kg/day but no females or controls
- **Liver**
    - **Hepatocellular hypertrophy** in all male groups (minimal at 125 (10/10 M & F mice), slight at 250 (3/10 M, 5/10 F) and moderate at 500 mg/kg/d (4/10 M)
    - **Single cell hepatocyte degeneration and necrosis** minimal in 1/10 M mice at 125 mg/kg/d 10/10 M at 250 mg/kg/d) and 1/10 F mice at 500 mg/kg/d, , slight in 1/10 M at 125 mg/kg/day and 8/10 M at 500 mg/kg/d, and 9/10 F at 500 mg/kg/d, and moderate in 1/10 M at 500 mg/kg/d

- **NOAEL** values:
    - **Not determined**: the findings at the lowest dose tested (125 mg/kg/d) produced increased liver weights, hepatocellular hypertrophy and single cell degeneration and necrosis (2/10 males) at 125 mg/kg/d

- **MTD**: the MTD for the Carcinogenicity study is considered to be 125 mg/kg/d in the M and 250 mg/kg/d in the F, due to deaths observed at doses of ≥500 mg/kg/day in males and ≥750 mg/kg/day in females. Additionally, dose-limiting kidney findings were observed in males at ≥250 mg/kg/day and in females at ≥500 mg/kg/day. Liver toxicity was observed at all doses, but had low indicences

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at the identified MTD. The MTD doses are not expected to affect survival or confound the interpretation of the 6-month carcinogenicity study in the transgenic mice.

Methods

Doses:	Phase 1: 0, 500, 750, and 1000 mg/kg/day; Phase 2: 0, 125, and 250 mg/kg/day for 29 days
Frequency of dosing:	Once daily
Route of administration:	Oral by gavage
Dose volume:	10 ml
Formulation/Vehicle:	Test article in reverse osmosis/deionized water
Species/Strain:	001178-W (wild type), CByB6F1-Tg(HRAS)2Huc[TgHras2] mice (b) (4)
Number/Sex/Group:	10/sex/dose
Age:	8 weeks (M) and 9 weeks (F)
Weight:	22-31.6 g M and 17.7-24.3 g F
Satellite groups:	N=3/sex/dose group/time point: Plasma samples from groups receiving 60 (TK study only), 125, 250, 500, 750, and 1000 mg/kg/day (6/sex controls)) for 28 days
Unique study design:	Phase 1: 1000 mg/kg group terminated on Day 3, 750 mg/kg M group terminated on Day 4, and 750 mg/kg F terminated on Day 15 due to high mortality. Therefore, the Sponsor initiated Phase 2 with lower doses of 125 and 250 mg/kg/day.
Deviation from study protocol:	Early termination of higher dose groups (750-1000 mg/kg)

Observations and Results

Mortality

Methods: Twice daily; All main study animals including those that died or were sacrificed early were examined after deaths and at time of scheduled sacrifice

Results:

- Deaths in the Phase 1 animals are presented in the following table. No controls died prior to scheduled sacrifice.

Unscheduled Deaths

	500 mg/kg/d		750 mg/kg/d		1000 mg/kg/d	
	Main Study	**TK Mice**	**Main Study**	**TK Mice**	**Main Study**	**TK Mice**
Males	1 (Day4)	2 (Day 4)	6 (Day 3)	12 (Day 4)	9 (Day 2	13 (Day 2)
Females	0	0	1 (Day 9)	11 (Day 3)	1 (Day 2)	3 (Day 4

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- All animals at 125 - 500 mg/kg/day (from Phases 1 and 2) survived except 1 main study M at 500 mg/kg/day sacrificed *in extremis* on Day 4
- Surviving animals for Phase 1 dosing at 750 and 1000 mg/kg/day terminated early
- Deaths associated with hunched posture, hypoactivity, squinted eyes, labored breathing, rough haircoat, cold to touch

Clinical Signs

Methods: Twice daily for pain and distress signs, daily cageside observations, detailed observations at pre-dose and on Days 1, weekly during dosing phases, and day of sacrifice

Results:

- Hunched posture, ataxia, recumbency, opaque and/or squinted eyes, labored and/;or irregular breathing, cold to touch, swollen head, rough haircoat at ≥500 mg/kg/day (in unscheduled deaths), most appearing by 2-3 days after initiation of treatment
- Rough haircoat and swollen head in surviving animals at ≥500 mg/kg/day
- The results of the clinical signs observations are presented in the following table:

Clinical Signs

	Dose RDEA594 (mg/kg/day)					
	0	**125**	**250**	**500**	**750**	**1000**
Males (n=10/dose)						
Hunched	0	0	0	**2**	**4**	**5**
Swollen head	0	0	0	**2**	**1**	**1**
Squinted eyes	0	0	0	**1**	**1**	**3**
Irregular respiration	0	0	0	0	0	**5**
Skin cold to touch	0	0	0	0	**1**	**4**
Females (n=10/dose)						
Swollen head	0	0	0	0	**1**	**8**
Irregular respiration	0	0	0	0	0	**1**
Cold to touch	0	0	0	0	0	**1**

Body Weights

Methods: At pre-dose, prior to dosing on Day 1, and weekly during dosing

Results:

- ↑ BWG in M & F at 250 and 500 mg/kg/d vs. controls (Days 1-8)
- ↓ BWG in F at 750 mg/kg/day and 1000 mg/kg/day vs. controls (Days 1-8)

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- No correlation with ↑ food consumption at different intervals during dosing periods
- The results of the body weight measurements at the time of sacrifice (Day 29) are presented in the following tables:

Mean Body Weights (BW)

	Dose RDEA594 (mg/kg/d)					
	0	**125**	**250**	**500**	**750**	**1000**
Males						
Mean BW (g)	27.5	28.8	27.8	26.9	NA	NA
BW difference vs. controls (%)	Reference	+ 5%	+ 1%	- 2%	NA	NA
Mean BW Change (D1-D29, g)	+1.4	+1.9	+1.7	+1.6	NA	NA
BW Change difference vs. controls (%)	Reference	+36%	+ 21%	+ 14%	NA	NA
Females						
Mean BW (g)	23.4	23.9	24.1	24.0	NA	NA
BW difference vs. controls (%)	Reference	+2%	+ 3%	+ 3%	NA	NA
Mean BW Change (D1-D29, g)	2.9	3.6	2.3	3.6	NA	NA
BW Change difference vs. controls (%)	Reference	+ 24%	- 81%	+ 24%	NA	NA

NA: Not applicable

The body weights for the Phase 1 (0 & 500 mg/kg/day only) and Phase 2 (125 and 250 mg/kg/day) of the study are presented in the following graphs (from the IND submission):

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Figure 3
Mean Body Weights - Males

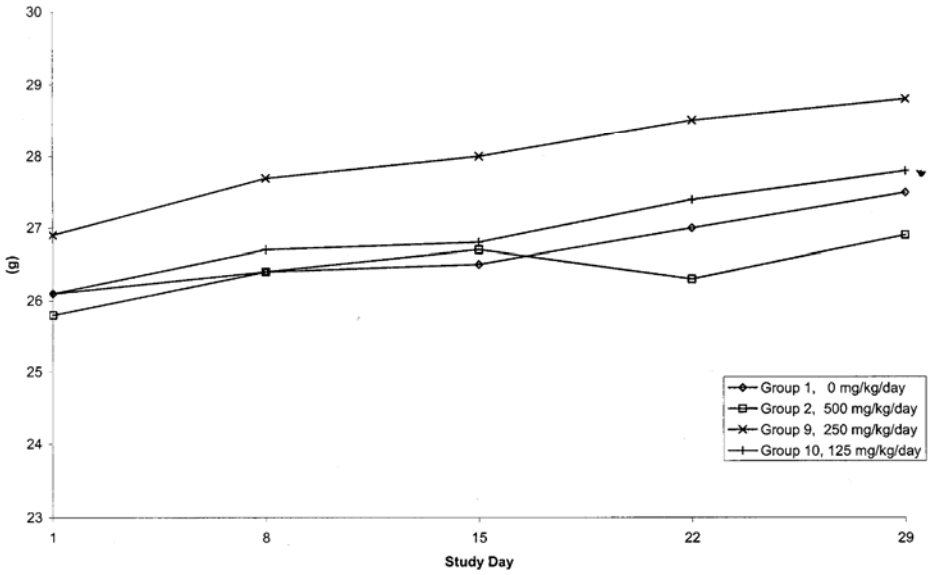
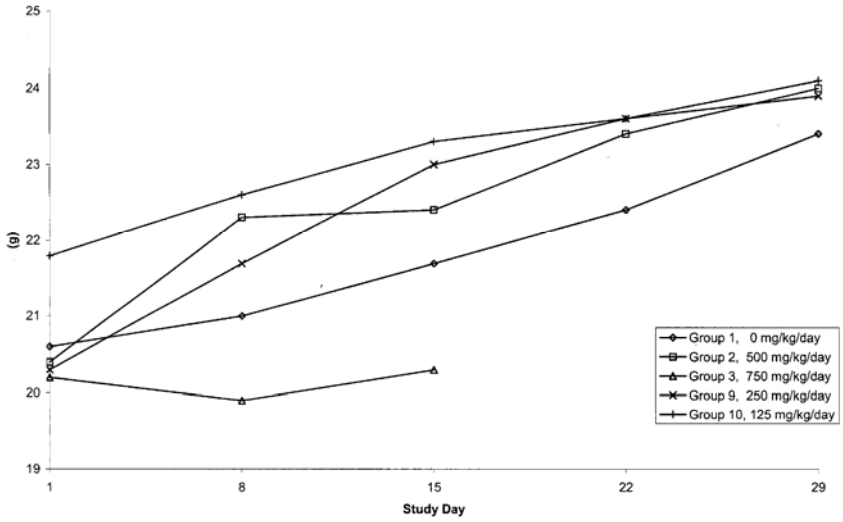


Figure 4
Mean Body Weights - Females



Food Consumption

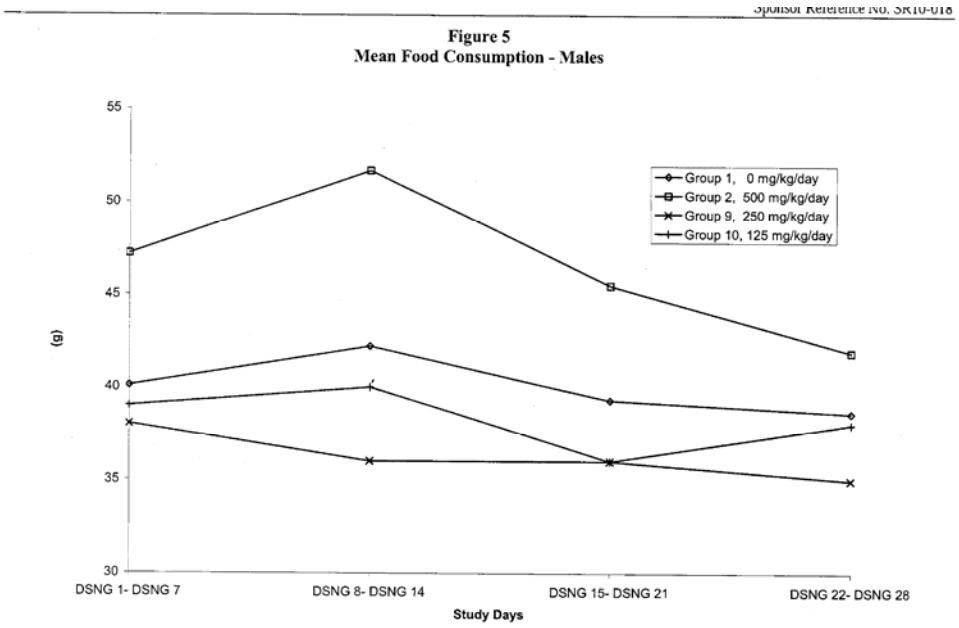
<u>Methods</u>: Weekly during dosing phases

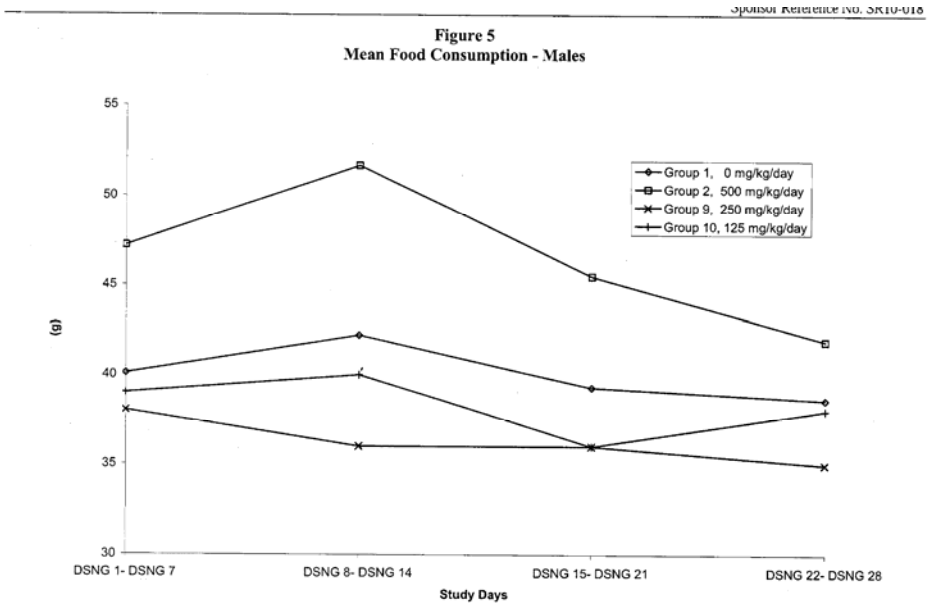
<u>Results</u>:

Reference ID: 2916093

- ↑food consumption in M and F without correlation to BWG changes (different weeks than seen in the BW gains)

The results of the food consumption measurements are presented in the following graphs (from the IND submission):





Clinical Pathology:

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Methods: No clinical pathology data was collected from the mice given 750 and 1000 mg/kg/day (Phase 1) that died or were sacrificed prior to the end of the dosing period. Blood samples were collected via cardiac puncture from non-fasted surviving animals (≤500 mg/kg/day M and < 750 mg/kg/d F) at scheduled sacrifice.

Results: Day 30

- ↑ (slight) **neutrophils, basophils, large unstained cell counts** (↑ lymphocytes) in M at 500 mg/kg/day
- ↑ **ALT** at 500 mg/kg/d in 1 M and 1F, associated with hepatocellular degeneration and necrosis in M at 125, 250, 500 mg/kg/d and F at 500 mg/kg/d
- ↑ **total protein and globulin** (both minimal) w/ ↑ **albumin** (minimal) at 250 and 500 mg/kg/d in M, both suggest dehydration
- ↑ **cholesterol** in M & F (minimal) at ≥ 125 mg/kg/d
- ↓ **triglycerides** (minimal) in M at ≥ 125 mg/kg/d
- Most clinically relative changes were increases in the hematology parameters suggesting dehydration, and increased ALT correlating with the hepatotoxicity observed in the necropsy.

The clinical pathology findings at the end of the dosing period are presented in the following table (statistically significant findings in Bold, % change is vs controls):

Dose RDEA594 (mg/kg/d)	**0**	**125**	**250**	**500**
		Males		
Glucose (mg/dL)	154	185	176	127
Total Protein (g/dL)	5.2	**5.8 (+ 11.5%)**	**5.9 (+ 13.5%)**	5.3
ALB (g/dL)	3.5	3.6	**3.8 (+ 8.6%)**	**3.8 (+ 8.6%)**
Globulin (g/dL)	1.5	**2.9 (+ 93%)**	**2.0 (+33%)**	**1.4 (-7%)**
AGR	2.3	**1.8 (-22%)**	**1.9 (-17%)**	**2.8 (+22%)**
Chol (mg/dL)	124	**170 (+ 37%)**	**178 (+ 43%)**	**157 (+ 27%)**
Trig (mg/dL)	138	105	**93 (-33%)**	**108 (- 22%)**
ALT (U/L)	41	59	57	**91 (+122%)**
		Females		
Total Protein (mg/dL)	5.0	**5.7 (+ 14%)**	**5.6 + 12%)**	5.3
GLOB (g/dL)	1.0	**1.9 +90%)**	**1.8 (+ 80%)**	1.2
AGR	2.9	**2.0 (- 31%)**	**2.1 (- 28%)**	3.3

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Ophthalmoscopy, ECG, Urinalyses Not conducted

Gross Pathology

Methods: At scheduled sacrifice in surviving mice (Phases 1) and all Phase 2 mice, with specialized necropsy of oral, cervical, thoracic cavities, and stomach contents in main study animals found dead or sacrificed before end of dosing period (Phase 1)

Results: The only treatment-related macroscopic finding in the mice with unscheduled deaths and those that survived until scheduled sacrifice was discolored stomach in 0, 0, 0, 1, 2, and 5 males at 0, 125, 250, 500, 750, and 1000 mg/kg/day.

Organ Weights

Methods: At scheduled sacrifice (Phase 1 surviving mice, and all Phase 2 mice)

Results:

- ↑ absolute, relative to BW and relative to brain weight **liver-gallbladder weights** in M & F at 125, 250, 500, and 750, and in F at 1000 mg/kg/day
    - Correlated with hepatocellular hypertrophy of liver
- ↑ absolute & relative to BW **kidney weights** in M at 500 mg/kg/day (correlated with cortical tubular regeneration and degeneration/necrosis in the males), ↑ kidney weights relative to BW in F at 750 and 1000 mg/kg/day
- ↓ absolute **kidney weights** in M at 750 mg/kg/day, and kidney to BW in F at 250 and 500 mg/kg/day
- ↓ **brain weights** in M at 500 mg/kg/day (without correlating histopathology findings) and F at 1000, ↑ brain weights relative to BW in F at 750 mg/kg/d
- ↓ **adrenal weights** in F at 1000 mg/kg/day
- ↓ **heart weights** in M at 750 mg/kg/day, and heart weights relative to brain weight in F at 750 and 1000 mg/kg/day and heart weights relative to BW in F at 750 mg/kg/day
- ↓ **epididymis and pituitary weights** in M at 750 mg/kg/day, ↑ pituitary to BW and pituitary to brain weights in F at 750 and ↓ pituitary relative to brain weights in F at 1000 mg/kg/day
- ↓ **testis** and testis relative to brain weights in M at 750 mg/kg/d
- ↓ **ovary** weights and ovary to brain weights in F at 750 and 1000 mg/kg/day, and ovary to BW in F at 1000 mg/kg/day
- **NOAEL not identified** due to increased relative liver-gallbladder weights in the M & F at 125 mg/kg/day.

The results of the group mean organ weight measurements are presented in the following table:

Organ	Dose RDEA594 (mg/kg/d)					
	0	125	250	500	750	1000

Reference ID: 2916023

Males						
Brain Absolute wt. Rel. to BW	0.5056	0.5065	0.5010	**0.4747***	0.4806	0.4657
Kidney Rel. to BW	1.7556	1.6153	**1.5678***	1.8762*	1.6120	1.4621
Liver Absolute Rel. to BW	1.5006 5.4849	**1.8134*** **6.6545***	**2.1804*** **7.6820***	**2.3928*** **9.1466***	**2.4169*** **9.8117***	2.7750 10.8398 (Not analyzed)
Epidimis Absolute Rel. to BW	0.1328 0.4883	0.1312 0.4828	0.1299 0.4583	0.1186 0.4534	**0.3654*** **0.3654**	0.0687 0.2684 (not analyzed)
Pituitary Absolute Rel to BW	0.0023 0.0085	0.0029 0.0107	0.0031 0.0111	0.0028 0.0109	**0.0007*** **0.0029***	0.0007 0.0027 (not analyzed)
Testis Absolute Rel to BW	0.2898 1.0635	0.2962 1.0881	0.2919 1.0322	0.2739 1.0458	**0.2402*** 0.9818	0.2077 0.8113 (not analyzed)
Females						
Brain Absolute Rel to BW	0.5075 2.2001	0.5133 2.1605	0.5094 2.1333	0.4891 2.0719	0.4887 **2.4008***	**0.4552*** 2.2616
Heart Absolute Rel to BW	0.1687 0.7270	0.1750 0.7334	0.1756 0.7345	0.1505 **0.6358***	**0.1354*** 0.6615	**0.1209*** 0.5842
Kidney Absolute Rel to BW	0.3444 1.4881	0.3527 1.4789	0.332 **1.3788***	0.3286 **1.3887***	0.3493 **1.7099***	0.3541 **1.7226***
Liver Absolute Rel to BW	1.1876 5.1373	**2.0976*** **8.8521***	**1.7318*** **7.2461***	**2.0976*** **8.8521***	**2.0902*** **10.2161***	**1.9239*** **1.9239***
Pituitary Absolute Rel to BW	0.0022 0.0096	0.0029 0.0121	0.0027 0.0114	0.0028 0.0119	**0.0034*** **0.0164***	**0.0010*** **0.0046***
Ovary Absolute Rel to BW	0.0333 0.1458	0.0308 0.1294	0.0318 0.1336	0.0272 0.1145	**0.0232*** 0.1135	**0.0152*** **0.0746***

*Statistically significant difference from controls

Histopathology

Reference ID: 2916023

Methods: At scheduled sacrifice in surviving mice administered 0, 125, 250 and 500 mg/kg/day RDEA594. The following organs were examined: brain and spinal cord, adrenals, pituitary, nerve (sciatic), trachea, esophagus, thyroid, parathyroid, heart, aorta, tongue, muscle (femoris), liver, gallbladder, spleen, lung, thymus, kidney, urinary bladder, stomach, duodenum, ileum, colon, cecum, jejunum, rectum, lymph node (mesenteric), salivary gland (mandibular), pancreas, Harderian gland, nerve optic, eye, skin/subcutis, seminal vesicle, prostate, testis, epididymis, mammary (female), ovary, uterus, cervix, vagina, bone (femur), marrow (femur), tone (sternum), marrow (sternum)

Adequate Battery: Yes
Peer Review: No

Results: Histological Findings

- ↑ **Hepatocellular hypertrophy** (dose-related in severity and incidence), enlarged hepatocytes with pale to eosinophilic cytoplasm
  - Minimal in 10/0 M and F at 125 mg/kg/day
  - Minimal to slight in (10/10) M at 250 mg/kg/day and (9/9) M at 500 mg/kg/day
  - Slight to moderate in 9/9 M and 500 mg/kg/day and 10/10 F at 500 mg/kg/day
  - May be treatment-related and/or adaptive due to effect of xenobiotic treatment with and proliferation of smooth endoplasmic reticulum upon increased drug metabolizing enzyme activity
  - Correlated with ↑ ALT in the 500 mg/kg/day animals
  - ↑ liver glycogen in controls (diffuse) and treated mice (mainly in portal and periportal areas)
  - The liver findings are presented in the following table (from the Sponsor):

Incidence and Severity of Test Article-Related Microscopic Findings in the Liver (n=10/sex/dose)

Dose RDEA594 (mg/kg/d)	Males				Females			
	0	**125**	**250**	**500**	**0**	**125**	**250**	**500**
Hepatocellular Hypertrophy								
Not present	10	0	0	**1**	10	0	0	0
Minimal	0	**10**	**7**	0	0	**10**	**5**	**1**
Slight	0	0	**3**	**5**	0	0	**5**	**9**
Moderate	0	0	0	**4**	0	0	0	0
Average Severity Score	0	**1.0**	**1.3**	**2.2**	0	**1.0**	**1.5**	**1.9**
Degeneration/Necrosis, individual hepatocyte								
Not Present	**10**	**8**	0	1	10	10	10	0
Minimal	0	**1**	**10**	0	0	0	0	**1**
Slight	0	**1**	0	**8**	0	0	0	**9**
Moderate	0	0	0	**1**	0	0	0	0
Average Severity Score	0	**1.3**	**1.0**	**1.9**	0	0	0	**1.9**

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- **Kidney**
  - **Regeneration of cortical** tubule in controls (2/10 M, 0/10 F) and at 250 (1F) 500 mg/kg/day in M (5/10, minimal to moderate) and F (1/10, minimal)
  - **Degeneration/necrosis of cortical** tubule in M (minimal to slight) at 500 mg/kg/day but no females or controls

Additional sporadic microscopic findings were not observed at higher levels, were present in the controls, or were of low incidence (e.g. 1/10 at the highest dose), except for tubule cell regeneration in the males, and are presented in the following table:

Additional Microscopic Findings in the Unscheduled Deaths

	Dose RDEA594 (mg/kg/d)			
	0	**125**	**250**	**500**
Males				
Unscheduled Deaths				
Skin erosion/ulcer with necrotic cell debris, epidermal surface inflammation, chronic	0	0	0	1
Scheduled Sacrifice				
Adenal Cortex: hyperplasia	0	2	4	4
Kidney				
Tubule Cell regeneration	2	0	1	5
Tubule degeneration/necrosis	0	0	0	1
Pelvis dilatation	0	0	0	1
Chronic inflammation	0	0	0	1
Females				
Unscheduled Deaths: no treatment-related findings				
Scheduled Sacrifice				
Adenal Cortex: hyperplasia	0	0	0	1

Toxicokinetics

Methods:

Plasma samples (100 mcL) were collected from 3/sex/dose group/timepoint (all treated groups at 60, 125, and 250 mg/kg/day) from the tail vein (1st time point) and by cardiac puncture (2nd time point), at 0.5, 1, 3, 6, 12, and 24 hours after dosing, and analyzed using LC/MS/MS.

Results:

Reference ID: 2916093

- 5 deaths on Day 28, after first TK samples drawn (2LDM, 1MDM, 2HDF): attributed to prolonged isoflurane anesthesia
- Increased exposure with dose in M & F
- Exposure higher in F than in M
- No increase in exposure at doses above 500 mg/kg/day, and between Day 1 and Day 28 in M, but slightly increased with duration of dosing in F
- Tmax increased with dose in M, range 0.5-3 h in males, 1-3 h in F

The results of the TK evaluation for the Phase 2 animals (60, 125, and 250 mg/kg/day) are presented in the following tables (from the Sponsor's submission):

Dose (mg/kg/d)	Males			Females		
Mean TK Parameters						
	AUC0-24/D (mcg.h/ml)	**Cmax (mcg/ml)**	**Tmax (h)**	**AUC0-24 (mcg.h/ml)**	**Cmax (mcg/ml)**	**Tmax (h)**
60	409	72.7	0.5	647	145	1.0
125	737	125	1.0	1170	148	3.0
250	1660	161	3.0	1800	183	3.0
Dose-normalized Mean TK Parameters						
	AUC0-24 (mcg.h/ml)/(mg/kg)	**Cmax (mcg/ml)**	**Tmax (h)**	**AUC0-24 (mcg.h/ml)/(mg/kg)**	**Cmax (mcg/ml)**	**Tmax (h)**
60	6.82	1.21		10.8	2.42	1.0
125	5.90	1.00	0.5	9.36	1.18	3.0
250	6.64	0.64	1.0	7.20	0.73	3.0

The results of the TK evaluation for the Phase 1 animals (500, 750, and 1000 mg/kg/d) are presented in the following table (from the Sponsor's submission):

Day	Dose (mg/kg/d)	Males		Females	
		AUC0-24h (mcg.h/ml)	**Cmax (mcg/ml)**	**AUC0-24h (mcg.h/ml)**	**Cmax (mcg/ml)**
1	**500**	2590	144	2000	177
	750	2220	190	2420	179
	1000	2590	211	2340	218
28	**500**	2710	238	3450	270
	750	NA	NA	NA	NA
	1000	NA	NA	NA	NA
28/1	**500**	1.05	1.65	1.73	1.53
	750	NA	NA	NA	NA
	1000	NA	NA	NA	NA

NA: Not Applicable

Stability and Homogeneity

Methods: Evaluated for up to (b) (4) concentrations verified on Study Days 1 and 28 (Day 28 data not available for the 750

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and 1000 mg/kg dose groups due to early termination); analysis Reports provided, acceptable concentration specification 90%-110%

Results: Test article solution found stable (within 90%-110% nominal) and homogenous (variations < 5%). No evidence of RDEA594 was found in the control vehicle samples.

7 Genetic Toxicology

RDEA was negative in a standard battery of genotoxicity studies. The results are presented in the following table (from the original IND review):

Summary: All studies conducted at (b) (4)

Study (Study No.)	Administration Method	Dose or Concentration	GLP	Results
Ames/*S.typhimurium* and *E. Coli* (Study SR08-22)	*In Vitro*	0, 1.5-5000 mcg/plate	Yes	Negative
Chromosome Aberration in CHO Cells (Exploratory) (Study SR06-086)	*In Vitro*	0, 0.005-50 mcg/ml	No	Negative
Chromosome Aberration in CHO Cells (Study SR08-23)	*In Vitro*	0, 10-120 mcg/ml (up to limit of solubility and 50% growth inhibition)	Yes	Negative
Micronucleus assay in Sprague Dawley Rats (Study SR08-024)	Oral Gavage *In Vivo* (1 day)	0, 250, 500, 1000 mg/kg (up to MTD based on mortality)	Yes	Negative (AUC =4440 mcg.h/ml at HD)

10 Special Toxicology Studies

Bacterial Reverse Mutation Assay to Evaluate Effect of (b) (4) impurity (Study SR09-015):

- Impurity found in RDEA594 (b) (4) Lot 0904697 at (b) (4) %, exceeding amount in Lot 10454-139-3 used in the genotoxicity battery studies (b) (4) %)
- *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. Coli* strain WP2uvrA +/- S9, using plate incorporation method at up to 5000 mcg/plate
- Results negative for precipitate, cytotoxicity and mutagenicity
- RDEA594 Lot 0904697 with (b) (4) % (b) (4) impurity = (b) (4) mcg/plate was negative for inducing genetic toxicity

11 Summary

RDEA594, the major metabolite of RDEA806 (non-nucleoside reverse transcriptase inhibitor (NNRTI) (b) (4) is under development for the treatment of patients with gout (original IND submitted September 30, 2009). RDEA594 has been shown to block reuptake of

Reference ID: 2946023

uric acid in the kidney proximal tubule (responsible for 80% reabsorption of uric acid) by URAT1 transporter inhibition, at doses of up to a maximum of 600 mg/day in clinical studies.

This submission is to seek execCAC concurrence for a proposed carcinogenicity study protocol in mice administered RDEA594 by oral gavage. The proposed carcinogenicity assessment in TgrasH2 mice (wild type) once daily treatment with RDEA594 for 26 weeks at the proposed dose levels of 0, 030, 60 and 125 mg/kg/day in males and 0. 60, 125, and 250 mg/kg/day in females based on MTD, using positive control article N-ethyl-N-nitrosurea (MNU). Supportive data from a 28-day oral repeat dose mouse toxicity study was used to determine the recommended doses, also considering the results of previous studies on RDEA594 pharmacokinetics and genetic toxicity.

A 1-month dose range-finding (DRF) study was conducted in TgrasH2 mice to determine the dose selection for the proposed 6-month carcinogenicity evaluation in this strain. In the first study Phase 1, the mice (n=10/sex/dose group, with 3/sex/dose group/timepoint satellite animals for TK analysis) were administered RDEA594 in osmosis/deionized water at the doses of 0, 500, 750 and 1000 mg/kg/day by oral gavage once daily. Additional groups were added (Phase 2) at the dose of 125 and 250 mg/kg/day due to high mortality at the two highest doses (750 and 1000 mg/kg/day in Phase 1). RDEA594 was lethal in most of the male (M) and female (F) mice at 750 and 1000 mg/kg/day and the remaining, surviving animals in these groups were sacrificed early. One additional M and 2 TK study males given 500 mg/kg/day test article were sacrificed *in extremis* on Dosing Day 4. Treatment-related clinical signs in the animals that died or were sacrificed early included hunched posture, ataxia, recumbency, opaque or squinted eyes, labored and/or irregular breathing, cold to touch, swollen head, and rough haircoat. The surviving animals showed rough haircoat and swollen head at doses of 500 mg/kg/day and above.

Body weights and body weight changes increased with RDEA594 treatment, although not in a dose-related manner in the M. Thus, there appears to be no adverse treatment-related effects on body weights. The results of the clinical pathology evaluations at terminal sacrifice showed treatment-related increases in neutrophils, basophils, and large unstained cell counts (lymphocytes), and increased total protein (at 250 mg/kg/day and above) and globulin (at 250 and above) in the M . These effects are likely a result of dehydration, in agreement with the Sponsor's interpretation. Increased ALT was observed in the M and F given 500 mg/kg/day. Other clinical pathology effects included increased cholesterol (M&F) and decreased triglycerides (M) at all doses tested.

Treatment-related organ weight changes were observed, at all dose levels. Increased absolute and relative (to BW and brain weight) liver and gallbladder weights were found in both the M and F at 125 mg/kg/day and above. This finding correlated with hepatocellar hypertrophy. Kidney weights (relative to BW) were reduced in F given 250 mg/kg/d and above and in the males at the 500 mg/kg/d level, and correlated with cortical tubular regeneration and degeneration/necrosis. Also, there was a treatment-

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related decrease in brain weights in the M at 500 mg/kg/d with no correlating histopathology findings.

Microscopic examination at scheduled sacrifice supported identification of the liver and kidney as target organs of toxicity. Increased (vs. controls) hepatocellular hypertrophy that was dose-related in incidence and severity, with enlarged hepatocytes with pale to eosinophilic cytoplasm was found in the M and F at 125 mg/kg/day. This effect was considered to be of minimal severity at 125 mg/kg/d, minimal to slight at 250 mg/kg/day and slight to moderate at 500 mg/kg/d. Single cell degeneration and necrosis was found in 2/10 M at the 125 mg/kg/d dose level and 1 F at the 500 mg/kg/d level, with severity ratings o minimal to slight. The single hepatocyte degeneration and necrosis was found in all males at 250 mg/kg/day with severity rating of minimal,, and in the high dose groups (500 mg/kg/d) to be slight to moderate in the M and F. The histopathology findings in liver may be direct treatment-related toxicity and/or an adaptive response commonly found following treatment with xenobiotics due to proliferation of smooth endoplasmic reticulum upon increases in drug metabolizing enzyme activity. There was a correlation of the findings in liver and increased ALT at the 500 mg/kg/d dose level. The histopathology findings in kidney included minimal to moderate regeneration of cortical tubules in 1M at 250 and 5M at 500 mg/kg/d that was also found in 1 Control M, and degeneration and/or cortical tubule necrosis in M at 500 mg/kg/d but not in the females or controls.

Although a NOAEL was not determined in the 1-month DRF study, an MTD for the 6-month carcinogenicity study could be considered 125 mg/kg/day in the male and 250 mg/kg/d in the F mice based on the low incidence and or severity of treatment-related toxicity at those dose levels. These doses produced minimum toxicity that is not expected to affect survival or confound the interpretation of the carcinogenicity bioassay, and therefore in agreement with the Sponsor, can be considered for the high dose level in the carcinogenicity study.

The anticipated multiples of the RDEA594 MRHD represented by the doses proposed by the Sponsor for the 6-month carcinogenicity study in TgrasH2 mice are presented in the following table:

Parameter	**Male**			**Female**		
Mouse dose (mg/kg/day)	30	60	125	60	125	250
Mouse AUC (mcg.h/ml)	205*	409	737	647	1170	1800
Multiple of Human AUC**	2.5X	4.9X	8.8X	7.8X	14X	21.5X

* Projected based on systemic exposure at 60 mg/kg/day dose
** Human exposure at 600 mg/day (AUC = 83.4 mcg.h/ml)

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The anticipated multiples of the RDEA594 MRHD (600 mg/day, AUC basis using extrapolation) represented by the doses recommended in this review for the 6-month carcinogenicity study are presented below:

Parameter	**Male**			**Female**		
Mouse dose (mg/kg/day)	15	45	125	30	90	250
(Mouse AUC (mcg.h/ml)	100* (approximate)	300* (approximate)	737	325* (approximate)	900* (approximate)	1800
Multiple of Human AUC**	1.25X* (approximate)	4X* (approximate)	8.8X	4X* (approximate)	11.5X* (approximate)	21.5X

* Approximate, based on extrapolation using systemic exposures measured at 60, 125 and 250 mg/kg/day dose

** Human exposure at 600 mg/day (AUC = 83.4 mcg.h/ml)

Conclusions and Recommendations:

The proposed 26-week carcinogenicity study protocol on RDEA594 and supporting 28-day dose-range finding study results in Tgras H2 mice were presented to the Executive Carcinogenicity Assessment Committee (Executive CAC) on April 26, 2011 for Agency concurrence with the carcinogenicity study protocol. The ECAC agreed with the reviewers recommended doses. The Recommendations and Conclusions by the executive CAC are presented below:

- The Committee recommended doses of 0, 15, 45, and 125 mg/kg/day in males and 0, 30, 90, and 250 mg/kg/day in females, based on the MTD (mortality in males at 500 mg/kg/day and in females at 750 mg/kg/day, with toxicity seen in the kidneys and liver). Dose spacing was based on AUC data.

- The Committee noted that organ weights are not needed for FDA at the end of the study.

- The Committee agreed with the selection of N-ethyl-N-nitrosurea (MNU) for the positive control article in the 26-week carcinogenicity study in TgrasH2 mice.

- For the 26-week carcinogenicity study in transgenic mice, histopathological examination should be conducted in all dose groups.

The ECAC Meeting Minutes were sent to the Sponsor on April 28, 2011.

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

KATHLEEN A YOUNG
04/29/2011

MOLLY E TOPPER
04/29/2011
I concur.

Appendix 3: Nonclinical Review of IND 102128. June 22, 2011.

APPEARS THIS WAY ON ORIGINAL

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	102,128
Supporting document/s:	SDN 41
Sponsor's letter date:	November 2, 2010
CDER stamp date:	November 3, 2010
Product:	Lesinurad (RDEA594)
Indication:	Hyperuricemia in patients with gout
Sponsor:	Ardea Biosciences, Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Grace S. Lee, Ph.D.
Supervisor/Team Leader:	Timothy W. Robison, Ph.D., D.A.B.T.
Division Director:	Badrul Chowdhury, MD, Ph.D.
Project Manager:	Michelle Y. Jordan-Garner

Template Version: September 1, 2010

Reference ID: 2965720

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1 Executive Summary

1.1 Introduction

1.2 Brief Discussion of Nonclinical Findings in Reproductive and Developmental Studies

RDEA594 showed adverse effects on several reproductive parameters, including prolonged estrous cycles, a slight increase in mean number of days to mate, a slight decrease in number of corpora lutea and implantation sites in rats that received an oral dose of 300 mg/kg/day, which also caused overt parental toxicity in females and males. There was no evidence of teratogenicity or any other embryo-fetal developmental toxicity in rats and rabbits that received oral doses up to 300 and 75 mg/kg/day, respectively, during the period of organogenesis.

2 Drug Information

2.1 Drug

CAS Registry Number: 1151516-14-1

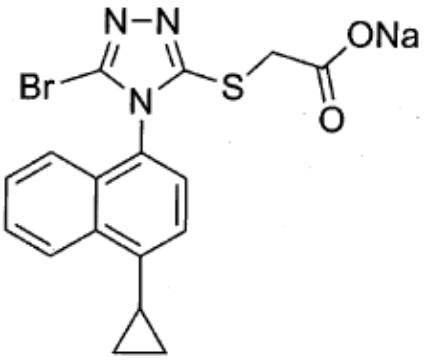
Generic Name: Lesinurad

Code Name: RDEA594

Chemical Name: Sodium, [5-Bromo-4-(4-cyclopropyl-napthalen-1-yl)-4H-[1, 2, 4]triazol-3-yl-sulfanyl]-acetate

Molecular Formula/Molecular Weight: $C_{17}H_{13}BrN_3NaO_2S$ / 426.26 Daltons (404.28 for the free acid)

Structure:



Pharmacologic Class: Uricosuric URAT1 transporter inhibitor

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2.2 Relevant INDs, NDAs, BLAs and DMFs

					Company
(b) (4)					Ardea
					Ardea

3 Studies Submitted

3.1 Studies Reviewed

RDEA594: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation in Rats (Study SR10-007)

RDEA594: Rat Oral Gavage Embryo-Fetal Developmental Toxicity and Toxicokinetic Study (Study SR10-008)

RDEA594: Rabbit Oral Gavage Embryo-Fetal Developmental and Toxicokinetic Study (Study SR10-009)

3.2 Studies Not Reviewed:

RDEA594: Rabbit Oral Gavage Dose Range-Finding Developmental Toxicity Study (Study SR09-068): The findings from this study are summarized under Rabbit Oral Gavage Embryo-Fetal Developmental and Toxicokinetic Study (Study SR10-009) in this review.

The following studies conducted with RDEA806, which were included with the initial submission, were not reviewed since RDEA806 is a prodrug of RDEA594 and RDEA594 is being used for clinical development.

Study of Fertility and Early Embryonic Development to Implantation in Rats Following Oral Administration of RDEA806 (Study SR07-078)

RDEA806: An Oral Embryo-Fetal Toxicity and Toxicokinetic Study in Rats (Study SR07-062)

RDEA806: An Oral Embryo-Fetal Toxicity and Toxicokinetic Study in Rabbits (Study SR07-061)

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9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: RDEA594: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation in Rats

Study no.:	(b) (4) SN 8216783 (Sponsor Reference No. SR10-007)
Study report location:	SDN 41, Volume 2, Pages 1-289
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 19, 2010
GLP compliance:	Yes
QA statement:	Yes, however, in-life inspection was limited to body weights, clinical observations and food consumption. Some other critical activities such as dose formulation preparation, dosing procedure, estrous cycle check, mating, and C-section were not inspected.
Drug, lot #, and % purity:	RDEA594, Lot # 0904728, 80.6% purity as free acid (85.0% purity as sodium salt); dose concentrations were based on purity of RDEA594 free acid and a correction factor of 1.24 was used.

Key Study Findings

- Effects of RDEA594 on fertility and early embryonic development were assessed in rats that received oral doses of 0, 75, 150, and 300 mg/kg/day. Male rats were dosed for at least 10 weeks prior to scheduled sacrifice, including 4 weeks prior to mating and throughout the cohabitation period through the day prior to scheduled sacrifice. Female rats were dosed for 2 weeks prior to mating and throughout the cohabitation period, and Gestation Days 0 through 7.
- Female rats at 300 mg/kg/day exhibited overt toxicity, including mortality, adverse clinical signs, and decrease in mean body weight gain and mean food consumption. Male rats at this dose also exhibited adverse clinical signs and decrease in mean body weight gain and mean food consumption. Males at 150 mg/kg/day and females at 75 and 150 mg/kg/day also exhibited slightly decreased mean body weight gain.
- Female rats at 300 mg/kg/day exhibited prolonged estrous cycles and a slight increase in mean number of days to mate, compared to the control group. In addition, slightly decreased mean numbers of corpora lutea and implantation sties were observed in this dose group, where caused overt toxicity.

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- Mating and fertility indices were unaffected in male and female rats that received the test article at doses up to 300 mg/kg/day.

Methods

Doses:	0, 75, 150 and 300 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Reverse osmosis/deionized water; test article dose formulation was adjusted to pH 6.8
Species/Strain:	Crl:CD (SD) rats
Number/Sex/Group:	22
Satellite groups:	None
Study design:	Males were dosed for at least 10 weeks prior to sacrifice, including 4 weeks prior to mating and throughout the cohabitation period through the day prior to termination. For males, the 4-week dosing period prior to cohabitation were judged to be acceptable based upon a 28-day toxicology study with rats that received doses up to 300 mg/kg/day and there were no test article-related histopathological findings in male reproductive organs. Females were dosed for at least 2 weeks prior to mating, throughout the cohabitation period and through Gestation Day (GD) 7. Females that did not confirm mating were dosed for an additional 7 days after the 3-week cohabitation period.
Deviation from study protocol:	None that could affect the integrity of data interpretation

Observations and Results

Mortality

Viability was checked twice daily.

Three female rats in the high dose group were preterminally sacrificed during the premating phase (Day 3, 8 or 12) due to moribund condition. Prior to the sacrifice, these females exhibited hunched appearance, hypoactivity, ataxia, squinted eyes, few or no feces, yellow haircoat, being difficult to dose, and/or not eating dietary enrichment. These rats lost body weight (15-18% relative to their respective body weight on Day 0). The necropsy finding was limited to the depressed area in the stomach in one rat. The histopathologic evaluation was performed for these rats to determine the cause of the death. All three rats exhibited similar histpathologic findings, including lymphoid depletion/atrophy in the spleen, lymphoid depletion/necrosis in the thymus, depletion of

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zymogen granules in the pancreas, dilatation in the tubular kidneys, and villous atrophy in the small and large intestines. In addition, one animal also had erosion/ulceration in the glandular stomach, which was correlated with the necropsy finding of the depressed area in the stomach, and another rat exhibited congestion in the glandular stomach without necropsy findings. It is stated that one additional female rat (animal No. B62582) in the high dose group was sacrificed without necropsy on Premating Day 3 due moribund condition (hunched posture, hypoactivity, and few feces), and this rat was replaced with another female with same animal identification number. All data presented in the tables for animal No. B62582 appear to be the data collected from the replaced animal, but none from the rat preterminally sacrificed. Thus, the findings of animal No. B62582 that was sacrificed on Day 3 could not be verified.

All other females and all males in the study survived until scheduled sacrifice.

Clinical Signs

Twice daily check for abnormalities and signs of pain or distress, postdose observations (at ~2 hour postdose) during the first two weeks of the dosing period, and detailed observations at days of the body weight measurement were performed.

In addition to the females described in the Mortality section, a small number of remaining females in the high dose group also exhibited a few incidences of similar clinical signs, including hunched posture, thin appearance, hypoactivity, squinted eyes, irregular respiration, few or no feces, brown/yellow haircoat, rough haircoat, being difficult to dose, and/or not eating dietary enrichment during the premating period. Single or a few incidences of hunched posture, thin appearance, yellow haircoat, rough haircoat and/or being difficult to dose were also noted during the gestation period.

A small number of males in the high dose group also exhibited a few incidences of being difficult to dose, not eating dietary enrichment, rough haircoat and/or few or no feces.

Body Weight

Body weight was measured at least once during pre-dosing period, prior to dosing on the first day of dosing, twice weekly and at scheduled necropsy for males, and twice weekly prior to and during cohabitation period for females. For non-confirmed females, twice weekly body weights continued after the cohabitation period. For confirmed females, body weight was also measured on presumed GDs 0, 3, 7, 10 and 13.

In male rats, there was a decrease in mean body weight gain in the mid and high dose groups (-9.4% and -15.8%, respectively, relative to the control group (277.9g)) during the entire dosing period (Days 0 to 71). Thus, mean terminal body weights in these groups were lower than the control group (-4.3% and -7.4% in the mid and high dose groups, respectively, relative to the control group (594.1g)).

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In female rats, a decrease in mean body weight gain was observed in all RDEA594-dosed groups (-10.7, -11.0 and -53.3% in the low, mid and high dose groups, respectively, relative to the control group (33.6 g)) during the premating period (Days 0-14) and in mid and high dose groups (-6.4 and -17.7%, respectively, relative to the control group (36.2 g)) between GD 0 and GD 7.

Feed Consumption

Food consumption was measured weekly during the premating dosing period for both males and females and also on presumed GDs 0, 3, 7, 10 and 13 for confirmed females.

In males in the high dose group, a slight decrease in mean food consumption was observed (-10.9%; 29.4 g/day in the HD group vs. 33.0 g/day in the control group) during the first week of the dosing period, but there was no decrease for the remainder of the premating dosing period. Similarly, females in the high dose group also exhibited a slight decrease in mean food consumption (-11.3%; 18.9 g/day in the HD group vs. 21.3 g/day in the control group) during the first week of the premating dosing period.

There were no other test article-related changes in food consumption.

Estrous Cycle Determination

Vaginal smears were daily evaluated for estrous stage during the premating period.

In the high dose group, there was an increase in number of females with prolonged diestrus stage (at least 4 days of diestrus stage) for estrous cyclicity (6 females). All these females were confirmed to mate, but one female was not pregnant and five females were pregnant with viable fetuses.

There were a few number of females with prolonged diestrus stage in other RDEA 594-dosed groups (1 and 2 in the low and mid dose groups, respectively, compared to 1 in the control group), and these observations were considered incidental due to the small number of affected animals.

Sperm Analysis

At the scheduled sacrifice, the first 10 males from each dose group were evaluated for sperm motility and total sperm count (from the right vas deferens and right epididymis). Total sperm count reports were adjusted for caudal epididymis weight. Sperm motility evaluation images were analyzed for percent motility. Two slides of sperm from the total count suspension were prepared from each male for possible future analysis of sperm morphology.

A slight decrease in mean percent motility of sperm was observed in the high dose group (87.3% vs. 96% in the control group), but the decrease was mainly due to one

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male, which had 39% motility sperm. The female partner of the male did not exhibit adverse effects on C-section data. In addition, the mean percent motility in the high dose group was within the range of historical control data from the conducting facility (87-99.8% for studies conducted in 2008-2009). Thus, the finding was not considered test article-related, but within the normal variability.

The mean tissue weight in the high dose group (0.320 g) for sperm count was lower than the weight from the control group (0.365 g). The smaller tissue weight was due to the decrease in body weight in the high dose group as described in organ weights under Necropsy Section.

Dosing Solution Analysis

Sample from control and test article formulations prepared on weeks 1, 3, 7 and 10 were analyzed for concentration. The test article formulations were solution and thereby were not analyzed for homogeneity.

The achieved concentrations were in the range from 98.3 to 101.4% of the nominal concentrations.

Reviewer's Note: In the study procedures described in the main body of the study report, it states that samples were also collected from formulations prepared on week 4, however, there were no data on week 4 samples in the dose formulation analysis report.

Necropsy

Male rats were necropsied and epididymis (left and right), prostate, seminal vesicles (with coagulating gland) and testis (left and right) were weighed at the male scheduled sacrifice and preserved. Paired organs were weight separately.

For confirmed female rats that were sacrificed on GD 13 (scheduled C-section), the uterus was examined for evidence of pregnancy and any grossly abnormal cervical, thoracic or abdominal viscera were noted. The placenta or amniotic sac was also examined for any abnormalities. For pregnant rats, the number of corpora lutea, implantation, resorptions (early or late) and fetuses (live or dead) were counted. For the non-confirmed female, any grossly abnormalities of the thoracic, abdominal and pelvic viscera were examined and the uterus and ovaries were examined. For animals that were preterminally sacrificed, except for one female rat in the high dose group as described in the Mortality Section, any grossly abnormalities of the cervical, thoracic, and abdominal viscera were examined, and these tissues were collected. For three female rats that were preterminally sacrificed, the tissues were microscopically evaluated to determine the cause of the death (see Mortality section).

Besides the necropsy finding described in the Mortality section, there were no test article-related parental necropsy observations.

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The finding of mass in the epididymis in one male rat in the low dose group during the necropsy was considered incidental due to a single incidence only in the low dose group. There were statistically significant, but slight decreases in mean absolute weights of testes (5-8% decrease) and prostate (10-17% decrease) in the mid and high dose groups and also epididymis (right; 9% decrease) in the high dose group, compared to the values from the control group. However, after adjusted to final body weight, the relative weights of these organs in these groups were comparable to those in the control group, except for the prostate weight. The decreased mean relative weight of the prostate adjusted to the final body weight was observed in all RDEA594-dosed groups (-7.5, -4.8 and -10.2% in the low, mid and high dose groups, respectively, relative to the control), however, the finding was also considered incidental due to small changes without dose-response relationship.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Mating and fertility indices were unaffected by test article, however, there was a slight increase in mean number of days to mate in the high dose group (2.84 days vs. 2.29 days in the control group). In addition, there was a slight decrease in number of corpora lutea (mean of 13.6) in the high dose group, compared to the control group (mean of 15.6). Consequently, number of implantation site was also slightly decreased in the high dose group (mean of 12.8 vs. 15.1 in the control group). These values in the high dose group were outside of the range of historical control data from the conducting laboratory facility (a range of 14.9-17.7 for number of corpora lutea; a range of 14.1 -16.5 for number of implantation sites from studies conducted from 2005-2009). There were no other test article-related effects on C-section parameters.

9.2 Embryonic Fetal Development

Study title: RDEA594: Rat Oral Gavage Embryo-Fetal Developmental Toxicity and Toxicokinetic Study

Study no.:	(b) (4) SN 8216778 (Sponsor Reference No. SR10-008)
Study report location:	SDN 41, Volume 3, Pages 1-394
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 4, 2010
GLP compliance:	Yes
QA statement:	Yes, however, only in-life inspection was food consumption
Drug, lot #, and % purity:	RDEA594, Lot # 0904728, 80.6% purity as free acid (85.0% purity as sodium salt); dose concentrations were based on purity of RDEA594 free acid and a correction factor of 1.24 was used.

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Key Study Findings

- In an embryo-fetal development study, time-mated female rats received RDEA594 at oral doses of 0, 75, 150, and 300 mg/kg/day from Gestation Day (GD) 6 through GD 17.
- Rats at 300 mg/kg/day exhibited maternal toxicity, including mortality, adverse clinical signs, decrease in mean food consumption and mean body weight gains.
- There were no test article-related effects on post-implantation loss and fetal parameters including body weight, sex ratio, external, visceral or skeletal observations.
- NOAEL for maternal toxicity was 150 mg/kg/day, whereas NOAEL for embryo-fetal developmental toxicity was 300 mg/kg/day. Thus, RDEA594 was not teratogenic in rats at oral doses up to 300 mg/kg/day.

Methods

Doses:	0, 75, 150 and 300 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Reverse osmosis/deionized water; test article dose formulation was adjusted to pH 6.8
Species/Strain:	Crl:CD (SD) rats
Number/ Group:	25
Satellite groups:	3 for the control group; 9/group for test article-dosed groups
Study design:	Time-mated rats were dosed from Gestation Day (GD) 6 to 17 and scheduled C-section was performed on GD 21.
Deviation from study protocol:	None that could affect the integrity of data interpretation

Observations and Results

Mortality

Viability was checked twice daily.

In the high dose group, five dams (3 main and 2 TK) were either found dead or preterminally sacrificed on GDs 12-18. These dams exhibited adverse clinical signs including thin appearance, hunched posture, hypoactivity, squinted eyes, rough haircoat, and/or being cold to touch prior to death. These rats lost body weight, compared to their respective GD 6 weights (a range of loss from 25 to 46 g). At necropsy, one main study dam had depressed areas in the stomach with discoloration (tan).

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Clinical Signs

Abnormalities and signs of pain or distress were checked twice daily for each animal. Daily cageside observations in the morning, postdose observations (at ~2 hour postdose) during the dosing period, and detailed observations at days of the body weight measurement were performed for main study animals.

In addition to the dams described in the Mortality section, one dam in the high dose group also exhibited thin appearance, hunched posture and rough haircoat.

Body Weight

Body weight was recorded on GD 0, 4, 6, 8, 10, 12, 14, 16, 18 and 21. Values from non-pregnant animals were excluded from mean calculations.

During the dosing period (GD 6-18), a marked decrease in mean body weight gain was observed in main study animals in the high dose group (-40.9% relative to the control value (98.0 g)). Although an increase was observed in the high dose group (+75.5% relative to the control value (22.9 g)) after the dosing period (GD18-21), the mean body weight gain from GD 6 to 21 in the high dose group (98.1 g) was still lower, compared to the control group (121.0 g), consistent with a lower net change from GD 4 to 21 for correct body weight (after removed gravid uterus) in the high dose group. Similarly, a marked decreased mean body weight gain was also observed in TK animals in the high dose group during the dosing period.

Feed Consumption

Food consumption was measured on the days of body weight measurement, beginning on GD 4, for main study animals. Values from non-pregnant animals were excluded from mean calculations.

A decrease in mean food consumption was observed in dams in the high dose group (-10.6%; 26.2 g/animal/day vs. 29.3 g/animal/day in the control group) during the dosing period (GD 6-18) with a greater decrease during the first week of the dosing (-18%). After the dosing period (GD 18-21), an increase was observed in this group (+21.8%; 29.0 g/animal/day vs. 23.8 g/animal/day in the control group).

Toxicokinetics

Blood was collected from non-fasted TK animals via a jugular vein at designated time points (1, 2, 4, 8, 12 and 24 hours postdose for RDEA594-dosed rats; 2 hours postdose for vehicle-dosed rats) on GDs 6 and 17. Plasma concentrations of RDEA594 were evaluated using a validated LC/MS/MS method. The calibration curve was linear over the concentration range of 5.00 to 2000 ng/mL with a lower limit of quantification (LLOQ) of 5.00 ng/mL.

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TK analysis showed that $AUC_{0\text{-}24hr}$ and C_{max} of RDEA594 increased with dose on GD 6 and GD 17, with no accumulation.

Table 1: Mean (%CV) Toxicokinetic Parameters of RDEA594 in pregnant rats

Gestation Day	Group	Dose (mg/kg/day)	N/ time point	$AUC_{0\text{-}24hr}$ (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)
GD6	Group 6	75	3	252	45.6	1.00
	Group 7	150	3	834	107	4.00
	Group 8	300	3	1440	125	8.00
GD17	Group 6	75	3	301	47.6	4.00
	Group 7	150	3	569	67.2	2.00
	Group 8	300	3*	1300	75.4	4.00

Accumulation Ratio	Group	Dose (mg/kg/day)	N/ time point	$AUC_{0\text{-}24hr}$	C_{max}	T_{max}
GD17/GD6	Group 6	75	3	1.19	1.04	NA
	Group 7	150	3	0.682	0.628	NA
	Group 8	300	3*	0.903	0.603	NA

NA: Not applicable.
*For Group 8, N=2 for 8, 12 and 24 hr sampling.

Dosing Solution Analysis

Sample from control and test article formulations prepared on GDs 6 and 17 were analyzed for concentration. The test article formulations were solution and thereby were not analyzed for homogeneity.

The achieved concentrations were in the range from 98.4 to 100.9% of the nominal concentrations.

Necropsy

For animals that were sacrificed on GD 21, the uterus was examined for evidence of pregnancy, and any grossly abnormal cervical, thoracic or abdominal viscera were noted. For pregnant rats, the uterus was weighed. For animals (main and TK) that were either found dead or preterminally sacrificed, any grossly abnormalities of the cervical, thoracic, abdominal and/or pelvic viscera were examined, and pregnancy status was determined. The placenta or amniotic sac was examined for any abnormalities. For TK animals that were sacrificed on the scheduled day, pregnancy status was determined without necropsy.

Besides the necropsy finding described in the Mortality section, there were no test article-related necropsy findings in dams.

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One dam in the high dose group had pale and enlarged kidneys with the presence of cyst. Because of the single incidence, this finding was considered incidental.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

From main animals that were sacrificed on GD 21, the number of corpora lutea, implantation, resorptions (early or late) and fetuses (live or dead) were counted. For main animals found dead or preterminally sacrificed, the number of corpora lutea, implantation, and live and dead fetuses (early or late deaths) were evaluated to the extent possible. The litter size was used as the covariate.

There were no test article-related effects on reproductive parameters that were evaluated during the C-section.

In the high dose group, there were one dam delivered early and another dam with no viable fetuses. Because of the single litter with no viable fetuses, slightly increased mean postimplantation loss (10.4% vs. 2.0% in the control group) and slightly decreased mean live fetuses (89.6% vs. 98.0 % in the control group) were also observed in the high dose group. Since these findings were mainly due to one litter with no viable fetuses and only one dam delivered early, all these findings in the high dose group were considered incidental.

Offspring (Malformations, Variations, etc.)

From main animals that were sacrificed on GD 21, fetuses were sexed, weighed and examined for external anomalies. Approximately one-half of all fetuses from each litter were processed for visceral examination (using the Wilson's section technique for the head and modified Staple's technique for the internal organs of the thoracic and abdominal cavities). The remaining fetuses were stained with alizarin red S for skeletal examinations. For main animals found dead or preterminally sacrificed, external morphology of fetuses was evaluated to the extent possible. The litter size was used as the covariate.

There were no test article-related effects on fetal parameters including sex ratio, body weight, external, visceral and skeletal observations.

Fetal abnormal observations in this study were considered incidental since the incidences were low and/or the lack of dose-response relationship and most findings were also observed in the control group with similar or higher incidences. One fetus in the low dose group exhibited multiple skeletal malformations, including absence of bilateral ribs from 3rd to 13th and vertebrate from T3 to last caudal and fused arches between left and right T2, and these findings corresponded to external findings of filamentous tail, anal atresia and malrotated hindlimbs. Due to the single fetus in the low dose group, the findings were considered incidental.

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9.3 Embryonic Fetal Development

Study title: RDEA594: Rabbit Oral Gavage Embryo-Fetal Developmental and Toxicokinetic Study

Study no.:	(b) (4) SN 8216780 (Sponsor Reference No. SR10-009)
Study report location:	SDN 41, Volume 4, Pages 1-436
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 4, 2010
GLP compliance:	Yes
QA statement:	Yes, however, in-life inspection was limited to body weights, clinical observations and food consumption. Some other critical activities such as dose formulation preparation, dosing procedure, necropsy and fetal evaluation were not inspected.
Drug, lot #, and % purity:	RDEA594, Lot # 0904728, 80.6% purity as free acid (85.0% purity as sodium salt); Dose concentrations were based on purity of RDEA594 free acid and a correction factor of 1.24 was used.

Key Study Findings

- In an embryo-fetal development study, time-mated female rabbits received RDEA594 at oral doses of 0, 25, 75, and 125 mg/kg/day from Gestation Day (GD) 7 through GD 20.
- Rabbits at 125 mg/kg/day exhibited maternal toxicity, including mortality, adverse clinical signs, a marked decrease in food consumption and body weight loss, leading to early termination of all remaining rabbits in this group. In addition, two and one dams at 75 and 25 mg/kg/day, respectively, were also preterminally sacrificed due to severe toxicity similar to what were observed at 125 mg/kg/day.
- Due to the early termination of all animals at 125 mg/kg/day, there were no available C-section and fetal data in this dose group. There were no test article-related effects on post-implantation loss and fetal parameters including body weight, sex ratio, external, visceral or skeletal observations.
- A NOAEL for maternal toxicity was not identified in the study due to mortality occurred in all RDEA594-dosed groups, whereas the NOAEL for embryo-fetal developmental toxicity was 75 mg/kg/day, the highest dose that was examined for fetal parameters. Thus, RDEA594 was not teratogenic in rabbits at oral doses up to 75 mg/kg/day.

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Methods

Doses:	0, 25, 75 and 125 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Reverse osmosis/deionized water; test article dose formulation was adjusted to pH 6.8
Species/Strain:	New Zealand white rabbits (Hra:(NZW)SPF)
Number/ Group:	20/group
Satellite groups:	4/group for TK evaluation
Study design:	Time-mated rabbits were dosed from Gestation Day (GD) 7 to 20 and scheduled C-section was performed on GD 29.
Deviation from study protocol:	None that could affect the integrity of data interpretation

Justification for selection of doses

The dose levels were based the results from a dose range-finding developmental toxicity study in rabbits ([redacted] (b) (4) Study No. 8216779). In this dose range-finding study, nonpregnant rabbits (3 per group) were administered RDEA594 at oral (gavage) doses of 0, 100, 200, 300 and 400 mg/kg/day for 7 days, whereas pregnant rabbits (6 main and 3 TK per group) were administered oral doses of 0, 100, 150, 200 and 250 mg/kg/day between GD 7 and GD 20. All nonpregnant rabbits at ≥300 mg/kg/day and all pregnant rabbits at ≥200 mg/kg/day were preterminally sacrificed due to a marked decrease in food consumption and body weight loss along with abnormal fecal finding (no or few feces). Necropsy findings included rough surface of the stomach. In addition, three (2 main and 1 TK) and one (main) pregnant rabbits at 150 and 100 mg/kg/day, respectively, were also preterminally sacrificed due to similar toxicity. One surviving pregnant rabbit at 150 mg/kg/day also exhibited similar adverse effects during the dosing period. There were no apparent test article-related effects on endpoints of reproduction or fetal development (including survival, body weight, and external observations) at 100 and 150 mg/kg/day. Exposure to RDEA594 ($AUC_{0\text{-}24hr}$ and C_{max}) increased with dose on GD 7 (from 100 to 250 mg/kg/day) and GD 20 (at 100 and 150 mg/kg/day) in pregnant rabbits.

Observations and Results

Mortality

Viability check was performed twice daily.

In the high dose group, due to severe toxicity including mortality, adverse clinical signs, no food consumption, and body weight loss, all remaining animals (main study and TK) were preterminally sacrificed on GDs 23 and 24. Seven main study animals in this group were preterminally sacrificed on GDs 15-21, and these dams exhibited thin

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appearance, not eating supplements, and/or no or few feces prior to death. During the dosing period, dams in the high dose group exhibited decreased mean food consumption (-25.8% relative to the control; 105.7 g/animal/day vs. 142.4 g/animal/day in the control group for main study animals) and body weight loss (mean of 49.3 g loss, compared to mean of 174.9 g gain in the control group for main study animals). In addition to the high dose group, two and one rabbits in the mid and low dose groups, respectively, were also preterminally sacrificed due to moribund condition. As what were observed in dams in the high dose group, these three dams also exhibited adverse clinical signs, body weight loss (a range of 11 to 14 % loss relative to their respective GD 7 weights) and a marked decrease in food consumption (almost no consumption).

Five main study animals (two, two and one dams in the control, mid and high dose groups, respectively) were found dead due to dosing trauma. Three dams in RDEA594-dosed groups exhibited discolored and perforated lungs and/or fluid-filled thoracic cavity. The report states tissues from these dams were microscopically examined to determine cause of death and the microscopic findings in the lungs, such as pleural and septal disruptions, hemorrhage with mild, focal fibrin deposition with intraalveolar macrophages, were stated in the main body (Result Section) of the report. However, the actual data were not included in the study report. In addition to the main study animals, one TK animal in the low dose group was also found dead. The report stated that the death of this animal was also attributed to gavage trauma based on in-life observations and the timing of the death in relation to dose administration. However, the evidence for dosing trauma in this TK animal could not be verified from the limited data presented for TK animals in the study report.

Clinical Signs

Abnormalities and signs of pain or distress were checked twice daily for each animal. Daily cageside observations in the morning, postdose observations (at ~2 hours postdose) during the dosing period, and detailed observations on days of the body weight measurement were performed for main study animals.

In addition to the dams described in the Mortality section, a sight increase in incidences of few or no feces was observed in the main study dams surviving until schedule necropsy from the low and mid dose groups (3 and 5 rabbits, respectively, for 1 to 4 days), compared to the control group (1 rabbit for 5 days).

Body Weight

Body weight was recorded on GD 0, 4, 7, 9, 11, 13, 15, 18, 20 (TK animals only)/21 (main study animals only), 24, 27 and 29. Values from non-pregnant animals were excluded from mean calculations.

Besides the body weight loss described in the Mortality section, there were no other test article-related adverse effects on mean body weight or body weight gain.

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A higher body weight loss in the dams in the mid dose group (-41.8 g), compared to the control group (-22.4 g) between GD 7 and GD 9 was not considered adverse since the effect was transient that the mean body weights in this group were similar to those in the control group at the end of the dosing period (GD 21) and at the study termination (GD 29). A slight decrease in mean body weight gain in dams in the low dose group (-14.0% relative to the control group) during the dosing period (GD 7-21) was considered incidental since no test article-related change was observed during the first week of the dosing period, and there was an increase in mean body weight gain after the dosing period (GD 21-29) and thereby, at the study termination, mean body weight in the dams in the low dose group was comparable to the control group.

Feed Consumption

Beginning on GD 4, food consumption was measured on GDs 6, 7, 9, 11, 13, 15, 17, 18, 20, 21, 23, 24, 26, 27 and 29 for main study animals. Any main study animals with food consumption < 50 g/day/interval for 2 consecutive intervals was supplemented with vegetables and shredded wheat once daily until food consumption reached ≥ 50 g/day/interval. Similarly, any TK animals exhibiting qualitative signs of decreased food consumption for 2 consecutive intervals were also supplemented until food consumption appeared normal. Values from non-pregnant animals were excluded from mean calculations.

Besides decreased food consumption described in the Mortality section, there were no other test article-related adverse effects on mean food consumption.

A slight decrease in mean food consumption was observed in dams in the mid dose group between GD 7 and GD 15 (a range of 4-14%/ measurement interval lower relative to the control group) was not considered adverse since the effect was transient and minor in magnitude.

Toxicokinetics

Blood was collected from non-fasted TK animals via a jugular vein at designated time points (~1, 2, 4, 6, 8, 12 and 24 hours postdose for RDEA594-dosed animals; ~4 hours postdose for vehicle-dosed animals) on GDs 7 and 20. Plasma concentrations of RDEA594 were evaluated using a validated LC/MS/MS method. The calibration curve was linear over the concentration range of 5.00 to 2000 ng/mL with a lower limit of quantification (LLOQ) of 5.00 ng/mL. Due to the decreased number of main study animals available for litter evaluation, TK animals in Group 5 (control) and Group 7 (75 mg/kg/day) were subject for C-section and fetal evaluation on GD 29. TK animals in Group 6 (25 mg/kg/day) were sacrificed and discarded without necropsy on GD 29.

TK analysis showed that $AUC_{0\text{-}24hr}$ and C_{max} of RDEA594 exposure increased with dose on GDs 7 and 20. There was an accumulation at 125 mg/kg/day on an AUC value

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comparison (2.4-fold higher value on GD 20 relative to the value on GD 7), but there was no accumulation at doses of 25 and 75 mg/kg/day.

Table 2: Mean (%CV) Toxicokinetic Parameters of RDEA594 in pregnant rabbits

Gestation Day	Group	Dose (mg/kg/day)	N	AUC_{0-24hr} (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)
GD 7	6	25	3[a]	114 (21.6)	23.1 (30.6)	2.33 (65.5)
	7	75	3[a]	408 (17.7)	61.5 (24.2)	4.00 (0.00)
	8	125	4	1090 (35.4)	123 (35.9)	4.50 (22.2)
GD 20	6	25	2[a, b]	112 (NC)	25.2 (NC)	1.50 (NC)
	7	75	3[a]	306 (26.6)	43.0 (33.6)	4.00 (50.0)
	8	125	4	2460 (83.2)	165 (50.2)	7.00 (54.7)

Accumulation Ratio	Group	Dose (mg/kg/day)	N	AUC_{0-24hr}	C_{max}	T_{max}
GD 20/GD 7	6	25	2[a, b]	1.00 (NC)	1.10 (NC)	NA
	7	75	3[a]	0.745 (11.0)	0.727 (36.9)	NA
	8	125	4	2.41 (82.2)	1.48 (52.2)	NA

[a] one female was not pregnant and is excluded from summary TK data
[b] one animal died
NA: Not applicable.
NC: Not calculated; N=2

Dosing Solution Analysis

Sample from control and test article formulations prepared on GDs 7 and 20 were analyzed for concentration. The test article formulations were solution and thereby were not analyzed for homogeneity. The dose formulations were analyzed by HPLC.

The achieved concentrations were in the range from 98.1 to 100.3% of the nominal concentrations.

Necropsy

For main study animals that were sacrificed on scheduled necropsy day (GD 29), the uterus was examined for evidence of pregnancy, and any grossly abnormalities of the cervical, thoracic and abdominal viscera were examined. Two and three main study rabbits at the 25 and 75 mg/kg/day, respectively, were not pregnant. For pregnant rabbits, the uterus was weighed and the uterine contents were examined. Due to the decreased number of main study animals available for litter evaluation, postmortem data (necropsy, C-section, and fetal observations) were also collected from TK animals in Group 5 (control) and Group 7 (75 mg/kg/day) that were sacrificed on GD 29. TK animals in Group 6 (25 mg/kg/day) were sacrificed and discarded without necropsy on GD 29.
For animals (main and TK) that were either found dead or preterminally sacrificed, any grossly abnormalities of the cervical, thoracic and abdominal viscera were examined, and pregnancy status was determined. For animals that were found dead and one

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high-dose dam with the necropsy finding of discolored lungs, visceral tissues were microscopically examined to determine cause of death. Abnormal tissues were collected in 10% neutral-buffered formalin.

There were no test article-related necropsy findings in dams.

One dam that was preterminally sacrificed on GD 16 had discolored lungs, however, there was only single dam with discolored lungs in the high dose group, except for the dams that were found dead due to dosing trauma as described in the Mortality section. Therefore, this finding was not considered test article-related.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

From pregnant animals of all main study portion and TK portion (Group 5 (control) and Group 7 (75 mg/kg/day)) that were sacrificed on GD 29, the number of corpora lutea, implantation, resorption (early or late) and fetuses (live and dead) were counted. For main study dams found dead or preterminally sacrificed, the number of corpora lutea, implantation, resorption (early or late), and dead fetuses or apparently normally developing fetuses were evaluated to the extent possible. Placenta and amniotic sac were also examined. The litter size was used as the covariate.

Due to the early termination of the high dose group, C-section data were not available at this dose level. There were no test article-related effects on reproductive parameters that were collected during the C-section at ≤75 mg/kg/day.

A slight increase in mean postimplantation loss was observed in the mid dose group (12.4% vs. 2.2% in the control group), which was outside of the historical control range of the conducting laboratory facility from 2005 to 2009 (0.0-6.8%). However, this finding was considered incidental since the increase was mainly due to one liter with no viable fetus, and there was no evidence of adverse effects on any reproductive and fetal developmental endpoints, including postimplantation loss, at ≤150 mg/kg/day in the dose range-finding EFD study in rabbits.

Offspring (Malformations, Variations, etc.)

From all main study animals and TK animals in Group 5 (control) and Group 7 (75 mg/kg/day) that were sacrificed on GD 29, fetuses (live or dead) were examined for external anomalies and were sexed and weighed. A midcoronal slice was made in the head of each fetus for evaluation of contents of the cranium, and visceral organs of the thoracic and abdominal cavities were examined using the modified Staple's technique. After visceral examinations, fetuses were stained with alizarin red S for skeletal examinations. The litter size was used as the covariate.

There were no test article-related effects on fetal parameters, including sex ratio, body weight, external, visceral and skeletal observations.

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A slight decrease in mean number of live fetus in the mid dose group (7.8 vs. 9.0 in the control group) was considered incidental for the same reason as for the decrease in mean postimplantation loss at this dose group, namely, the decrease was mainly due to one litter with no viable fetuses and the magnitude of change was minor. All fetal abnormal observations in this study were also considered incidental since the incidences were low without dose-response relationship and most findings were also observed in the control group with similar or higher incidences.

11 Integrated Summary and Safety Evaluation

RDEA594 is the major metabolite of RDEA806, a non-nucleoside reverse transcriptase inhibitor (NNRTI) (b) (4) RDEA594 is under development for the treatment of patients with gout (original IND submitted September 30, 2009). RDEA594 blocked reuptake of uric acid in the kidney proximal tubule (responsible for 80% reabsorption of uric acid) by URAT1 transporter inhibition, at doses of up to a maximum of 600 mg/day in clinical studies. RDEA594 showed no inhibition of purine nucleoside phosphorylase and xanthine oxidase, suggesting no inhibition of uric acid synthesis.

Summaries and evaluations of reproductive toxicology studies conducted with RDEA594 are provided in this review. These reproductive toxicology studies include a fertility and early embryonic developmental toxicity study in the rat and embryo-fetal developmental toxicity and toxicokinetic studies in the rat and rabbit.

In the fertility and early embryonic developmental toxicity study, the effects of RDEA594 on fertility, pregnancy and early embryonic development was examined in rats that received RDEA594 at oral doses of 0, 75, 150 and 300 mg/kg/day. The dosing period encompassed 4 weeks prior to mating and continuously until the day before necropsy for males and 2 weeks prior to mating and through Gestation Day (GD) 7 for females. Female rats at 300 mg/kg/day exhibited overt toxicity, including mortality, adverse clinical signs, and decrease in mean body weight gain and mean food consumption. Male rats at this dose also exhibited adverse clinical signs and decrease in mean body weight gain and mean food consumption. Males at 150 mg/kg/day and females at 75 and 150 mg/kg/day also exhibited slightly decreased mean body weight gain. Female rats at 300 mg/kg/day exhibited prolonged estrous cycles and a slight increase in mean number of days to mate, compared to the control group. In addition, slightly decreased mean numbers of corpora lutea and implantation sties were observed in this dose group. Mating and fertility indices were unaffected in male and female rats that received the test article at oral doses up to 300 mg/kg/day.

In the embryo-fetal developmental toxicity study in the rat, time-mated female rats received RDEA594 at oral doses of 0, 75, 150 and 300 mg/kg/day from GD 6 through GD 17. Rats at 300 mg/kg/day exhibited maternal toxicity, including mortality, adverse clinical signs, decrease in mean food consumption and mean body weight gains. There were no test article-related effects on post-implantation loss and fetal parameters

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including body weight, sex ratio, external, visceral or skeletal observations. RDEA594 was not teratogenic in rats at oral doses up to 300 mg/kg/day.

In the embryo-fetal developmental toxicity study in the rabbit, time-mated female rabbits received RDEA594 at oral doses of 0, 25, 75 and 125 mg/kg/day from GD 7 through GD 20. Rabbits at 125 mg/kg/day exhibited maternal toxicity, including mortality, adverse clinical signs, a marked decrease in food consumption and body weight loss, leading to early termination of all remaining rabbits in this group. In addition, two and one dams at 75 and 25 mg/kg/day, respectively, were also preterminally sacrificed due to severe toxicity similar to what were observed at 125 mg/kg/day. Due to the early termination of all animals at 125 mg/kg/day, there were no available C-section and fetal data in this dose group. There were no test article-related effects on post-implantation loss and fetal parameters including body weight, sex ratio, external, visceral or skeletal observations. A NOAEL for maternal toxicity was not identified in the study due to mortality occurred in all RDEA594-dosed groups, whereas the NOAEL for embryo-fetal developmental toxicity was 75 mg/kg/day, the highest dose that was examined for fetal parameters. Thus, RDEA594 was not teratogenic in rabbits at oral doses up to 75 mg/kg/day.

Reference ID: 2965720

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/s/

GRACE S LEE
06/22/2011

TIMOTHY W ROBISON
06/22/2011
I concur

Appendix 4: Nonclinical Review of IND 102128. June 23, 2011.

APPEARS THIS WAY ON ORIGINAL

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	102128
Supporting document/s:	SDN1
Sponsor's letter date:	September 30, 2009
CDER stamp date:	October 2, 2009
Product:	RDEA594
Indication:	Gout
Sponsor:	Ardea Biosciences, Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Marcie Wood, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D., DABT
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Michelle Jordan Garner

Template Version: September 1, 2010

Reference ID: 2965826

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1 Executive Summary

1.1 Introduction

RDEA594 is a URAT1 inhibitor indicated for the (b) (4) treatment of gout.

RDEA594 is the major metabolite of RDEA806, a non-nucleoside reverse transcriptase inhibitor (NNRTI) (b) (4)

1.2 Brief Discussion of Nonclinical Findings

Genotoxicity studies with RDEA594 were reviewed in preparation for a planned EOP2 meeting with the sponsor to discuss the adequacy of the nonclinical program for registration.

1.3 Recommendations

None

2 Drug Information

2.1 Drug

CAS Registry Number: 878672-00-5

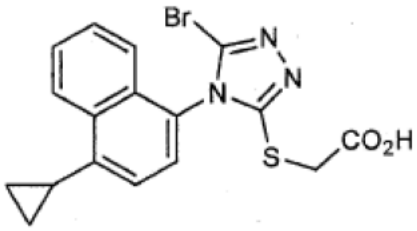
Generic Name: Lesinurad

Code Name: RDEA594 (previously known as VRX-495594)

Chemical Name: Acetic acid, 2-[[5-bromo-4-(4-cyclopropyl-1-naphthalenyl)-4H-1,2,4-triazol-3-yl]thio]-

Molecular Formula/Molecular Weight: $C_{17}H_{14}N_3O_2SBr$ / 404.28 (free acid)

Structure or Biochemical Description



Pharmacologic Class: URAT1 inhibitor

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2.2 Relevant INDs, NDAs, BLAs and DMFs

IND/NDA/DMF	Drug Name	Division	Status	Indication
(b) (4)				

3 Studies Submitted

3.1 Studies Reviewed

Study #	Study Title	Location
SR08-022	Bacterial Reverse Mutation Assay	SDN ?, Mod 4, Vol 21
SR06-086	In Vitro Mammalian Chromosome Aberration Screening Test	SDN ?, Mod 4, Vol 27
SR08-023	RDEA594: In Vitro Mammalian Chromosome Aberration Test	SDN ?, Mod 4, Vol 27
SR08-024	Rat Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of RDEA594	SDN ?, Mod 4, Vol 27
SR09-015	Bacterial Reverse Mutation Assay	SDN ?, Mod 4, Vol 27

3.3 Previous Reviews Referenced

Preliminary Safety Review by Dr. Kathleen Young (dated 6/18/2010, submitted by Dr. Adam Wasserman on behalf of Dr. Young)

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay (Draft Report)

Study no.:	SR09-015
Study report location:	SDN 1, Module 4, Volume 27
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 30, 2009
GLP compliance:	Yes, with the exception of the analytical report which was not included in the study report
QA statement:	No QA statement in study report (but stamped as QA audited draft)
Drug, lot #, and % purity:	RDEA594 (sodium salt), lot # 0904697, 82.1% pure*

* Dosing formulations were adjusted to compensate for the purity and free acid of the test article (82.1%) using a correction factor of 1.22.

Key Study Findings

Reference ID: 2965826

- A (b) (4) impurity was found in RDEA594 (b) (4) Lot 0904697 at (b) (4)%, which exceeded the amount ((b) (4)%) in Lot 10454-139-3 that was used in the Ames assay conducted as part of the genotoxicity battery of studies (Study SR08-022, see below); therefore, the sponsor conducted an additional Ames assay (Study SR09-015) with the RDEA594 Lot 0904697.
- Revertant colony counts for *Salmonella typhimurium* strains TA 1535, TA1537, TA98, and TA100 and *E. coli* strain WP2 *uvrA* were unaffected following treatment with RDEA594 at doses up to 5000 µg/plate with or without metabolic activation (Aroclor1254-induced rat liver S9), indicating no evidence of mutagenic potential under the conditions of the assay.

Methods

Strains:	*Salmonella typhimurium* strains TA98, TA1535, TA1537, and TA100 and *E. coli* strain WP2 *uvrA*
Concentrations in definitive study:	Doses of 50, 150, 500, 1500, and 5000 µg RDEA594/plate with or without metabolic activation (Aroclor 1254-induced rat liver S9)
Basis of concentration selection:	Doses of 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg RDEA594/plate with or without metabolic activation (10% Aroclor 1254-induced rat liver S9) were tested in an initial assay. No positive mutagenic responses, precipitate or toxicity were observed.
Negative control:	DMSO
Positive control:	See table below (all positive controls were diluted in DMSO, with the exception of sodium azide which was diluted in water)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plates were treated with RDEA594 or controls and incubated for 48 to 72 hrs at 37°C

Table 1: Positive controls for Bacterial Reverse Mutation Assay (SR09-015)

Positive control	Dose per plate (µg)	Bacterial strain	Metabolic activation
2-aminoanthracene	1.0	TA98, TA1535, TA1537	+
2-aminoanthracene	2.0	TA100	+
2-aminoanthracene	10	WP2 *uvrA*	+
2-nitrofluorene	1.0	TA98	-
Sodium azide	1.0	TA100, TA1535	-
9-aminoacridine	75	TA1537	-
Methyl methanesulfonate	1000	WP2 *uvrA*	-

Study Validity

Reference ID: 2965826

Both the initial and definitive studies utilized the plate incorporation method to examine the mutagenic potential of RDEA594 on four *S. typhimurium strains* and one *E. coli* strain. The sponsor examined all necessary strains for completion of the bacterial reverse mutation battery. In the initial and definitive studies, dosing was performed in duplicate and triplicate, respectively, and RDEA594 was tested up to the limit dose of 5000 µg/plate. A test article was considered to be mutagenic when the following conditions were met: 1) A dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article was observed, 2) For strains TA1535 and TA1537, the increase in revertants was ≥ 3-fold the mean vehicle control value, and 3) For strains TA98, TA100, and WP2 *uvrA*, the increase in revertants was ≥ 2-fold the mean vehicle control value. Positive controls produced the minimum mean increase in revertant colonies (3 times the concurrent solvent control) as required, and revertants in negative controls were within acceptable historical ranges. All criteria for a valid assay were met.

Results

No precipitate or cytotoxicity was observed in the initial or definitive studies up to 5000 µg/plate. Revertant colony counts for all strains were unaffected following treatment with RDEA594 with or without metabolic activation.

Study title: Bacterial Reverse Mutation Assay

Study no.:	SR08-022
Study report location:	SDN 1, Module 4, Volume 21
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 24, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RDEA594, lot # L10454-139-3 / Batch # 10454139-3, 89.5 % pure

* Dosing formulations were adjusted to compensate for the purity of the test article using a correction factor of 1.11.

Key Study Findings

- Revertant colony counts for *Salmonella typhimurium* strains TA 1535, TA1537, TA98, and TA100 and *E. coli* strain WP2 *uvrA* were unaffected following treatment with RDEA594 at doses up to 5000 µg/plate with or without metabolic activation (Aroclor1254-induced rat liver S9), indicating no evidence of mutagenic potential under the conditions of the assay.

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Methods

Strains:	*Salmonella typhimurium* strains TA98, TA1535, TA1537, and TA100 and *E. coli* strain WP2 *uvrA*
Concentrations in definitive study:	Doses of 50, 150, 500, 1500, and 5000 µg RDEA594/plate with or without metabolic activation (Aroclor 1254-induced rat liver S9) were tested in definitive study B3
Basis of concentration selection:	Doses of 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg RDEA594/plate with or without metabolic activation (10% Aroclor 1254-induced rat liver S9) were tested in an initial assay (B1). Due to unacceptable positive control values, strains TA100, TA1537, and WP2 uvrA with metabolic activation and TA100 without metabolic activation were not evaluated and were retested in an additional assay (B2). No positive mutagenic responses, precipitate or toxicity were observed in B1 or B2.
Negative control:	DMSO
Positive control:	See table below (all positive controls were diluted in DMSO, with the exception of sodium azide which was diluted in water)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plates were treated with RDEA594 or controls and incubated for 48 to 72 hrs at 37°C

Table 2: Positive controls for Bacterial Reverse Mutation Assay (Study SR08-022)

Positive control	Dose per plate (µg)	Bacterial strain	Metabolic activation
2-aminoanthracene	1.0	TA98, TA1535, TA1537, TA100	+
2-aminoanthracene	10	WP2 *uvrA*	+
2-nitrofluorene	1.0	TA98	-
Sodium azide	1.0	TA100, TA1535	-
9-aminoacridine	75	TA1537	-
Methyl methanesulfonate	1000	WP2 *uvrA*	-

Study Validity

Both the initial and definitive studies utilized the plate incorporation method to examine the mutagenic potential of RDEA594 on four *S. typhimurium strains* and one *E. coli* strain. The sponsor examined all necessary strains for completion of the bacterial reverse mutation battery. In the initial and definitive studies, dosing was performed in duplicate and triplicate, respectively, and RDEA594 was tested up to the limit dose of 5000 µg/plate. A test article was considered to be mutagenic when the following

Reference ID: 2965826

conditions were met: 1) A dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article was observed, 2) For strains TA1535 and TA1537, the increase in revertants was ≥ 3-fold the mean vehicle control value, and 3) For strains TA98, TA100, and WP2 *uvrA*, the increase in revertants was ≥ 2-fold the mean vehicle control value. In initial assay B1, positive control values for strains TA100, TA1537, and WP2 *uvrA* with metabolic activation and TA100 without metabolic activation were described as "unacceptable" in the study report (data not provided in study report). These strains were not evaluated and were retested in an additional assay (B2, data provided in study report), where positive controls produced the minimum mean increase in revertant colonies (3 times the concurrent solvent control) as required. Positive controls in confirmatory assay B3 also produced the minimum mean required increase in revertant colonies. Revertants in negative controls were within acceptable historical ranges. All criteria for a valid assay were met.

Results

No precipitate or cytotoxicity was observed in the initial (B1/B2) or definitive (B3) studies up to 5000 µg/plate. Revertant colony counts for all strains were unaffected following treatment with RDEA594 with or without metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Screening Test

Study no.:	SR06-086
Study report location:	SDN 1, Module 4, Volume 21
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 25, 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	VRX-495594, lot # and % purity were not provided

Key Study Findings

- The percent of cells with structural or numerical aberrations in the VRX-495594-treated groups was not significantly increased above controls and was also within the range of historical values; therefore, the sponsor considered that VRX-495594 was negative for induction of chromosomal aberrations under the conditions of the study. However, the concentrations of the test article used in the study were too low to achieve sufficient cytotoxicity and the study was judged to be inadequate to assess the clastogenic potential of the test article. The sponsor conducted a second study with higher concentrations of the test article. Further, the absence of a positive control precluded evaluation of test system viability for the screening study.

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Methods

Cell line:	Chinese Hamster Ovary (CHO) cells
Concentrations in definitive study:	N/A
Basis of concentration selection:	Doses of 0.0005, 0.015, 0.05, 0.15, 0.5, 1.5, 5, 15, and 50 µg VRX-495594/mL with and without metabolic activation (Aroclor-induced S9, % not provided) were tested in screening study
Negative control:	DMSO
Positive control:	Positive control was not used in screening study
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Without metabolic activation: CHO cells were treated for 4 and 20 hrs With metabolic activation: CHO cells were treated for 4 hrs All cells were harvested at 20 hrs after treatment initiation

Study Validity

Substantial toxicity (at least 50% cell growth inhibition versus solvent controls) was not observed at any dose level for any exposure group: in the 4 hr treatment group without metabolic activation, cell growth inhibition ranged from -4 to 12% (not dose-dependent); in the 4 hr treatment group with metabolic activation, cell growth inhibition ranged from -40 to 2% (not dose-dependent), in the 20 hr treatment group without metabolic activation, cell growth inhibition ranged from 6 to 20% (not dose-dependent). The top dose selected for analysis was 50 µg/mL in all treatment groups, as well as two additional lower doses 5 and 15 µg/mL. Slides were scanned at low magnification, and cells that had ~20 chromosomes were analyzed at high magnification and recorded as normal or aberrant. A minimum of 20 metaphase spreads from each dose level were examined and scored for structural aberrations. The percent polyploid and endoreduplicated cells (numerical aberrations) was evaluated per 100 cells for each dose level analyzed. The study design did not include a positive control.

Results

The percent of cells with structural or numerical aberrations in the VRX-495594-treated groups was not significantly increased above controls and was also within the range of historical values. The concentrations of the test article used in the study were too low to achieve sufficient cytotoxicity and the study was judged to be inadequate to assess the clastogenic potential of the test article. The sponsor conducted a second study with higher concentrations of the test article (see below). Positive controls ± S9 were not used in the study.

Reference ID: 2965826

Study title: RDEA594: In Vitro Mammalian Chromosome Aberration Test

Study no.:	SR08-023
Study report location:	SDN 1, Module 4, Volume 21
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 10, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RDEA594, lot # L10454-139-3 / 10454139-3, 90.3% pure (provided by sponsor), 89,5% pure (COA)

* Dosing formulations were adjusted to compensate for the purity of the test article using a correction factor of 1.11.

Key Study Findings

- The percent of cells with structural or numerical aberrations in the RDEA594-treated groups was not significantly increased above controls and was also within the range of historical values; therefore, RDEA594 was negative for induction of chromosomal aberrations under the conditions of the study.

Methods

Cell line:	Chinese Hamster Ovary (CHO) cells
Concentrations in definitive study:	Without metabolic activation, 4 hr treatment: 25, 50, 100, 120, 135, 150, and 200 µg/mL Without metabolic activation, 20 hr treatment: 25, 50, 100, 120, 135, 150, and 200 µg/mL With metabolic activation, 4 hr treatment: 10, 20, 40, 60, 80, 100, and 120 µg/mL
Basis of concentration selection:	Doses of 0.4043, 1.213, 4.043, 12.13, 40.43, 121.3, 404.3, 1213, and 4043 µg/mL with (4 hrs treatment) and without (4 and 20 hrs treatment) metabolic activation were tested in a preliminary toxicity test
Negative control:	DMSO
Positive control:	Without metabolic activation: Mitomycin C (0.1 and 0.2 µg/mL) With metabolic activation: Cyclosphsphamide (10 and 20 µg/mL)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Without metabolic activation: CHO cells were treated for 4 and 20 hrs With metabolic activation: CHO cells were treated for 4 hrs All cells were harvested at 20 hrs after

Reference ID: 2965826

treatment initiation

Study Validity

In the definitive assay, duplicate flasks were examined for each concentration of test article, as well as positive and negative controls, at each treatment condition. A concurrent toxicity test was also performed for the activated and non-activated conditions. Selection of dose levels for analysis was based on toxicity of test article. Mitotic index was also determined for each treatment group. In treatment groups with lack of substantial toxicity (50% reduction in cell growth), mitotic index was used for selection of dose levels for analysis (lowest dose with at least 50% reduction in mitotic index). When possible, 200 metaphase spreads (100 per duplicate flask) were scored per treatment. The test article was considered to induce a positive response when the percentage of cells with aberrations was increased in a dose-responsive manner with one or more concentrations being statistically significant. Values that were significant but did not exceed the range of historical solvent controls were judged not biologically significant. Appropriate mutagens were used as positive controls and produced statistically significant increases in the formation of chromosomal aberrations. The negative controls did not induce an increase in structural aberrations beyond background and were also within the range of historical solvent controls. All criteria for a valid assay were met.

Results

In the preliminary toxicity assay, visible precipitate was observed at the highest dose of 4043 µg/mL. Substantial toxicity (at least 50% cell growth inhibition versus solvent controls) was observed at doses ≥ 121.3 µg/mL in the non-activated treatment groups (4 hrs of exposure: 53, 150, 225, and 225% cell growth inhibition at 121.3, 404.3, 1213, and 4043 µg/mL, respectively; 20 hrs of exposure: 117, 207, 207, and 207% cell growth inhibition at 121.3, 404.3, 1213, and 4043 µg/mL, respectively) and at doses ≥ 40.43 µg/mL in the activated treatment groups (4 hrs of exposure: 84, 90, 169, 264, and 264% cell growth inhibition at 40.43, 121.3, 404.3, 1213, and 4043 µg/mL, respectively). Therefore doses ranging from 25 to 200 µg/mL were chosen for the non-activated treatment groups and doses ranging from 10 to 120 µg/mL were chosen for the activated treatment groups in the definitive study.

Definitive study results are as follows: In the 4 hr treatment group without metabolic activation, dose levels selected for analysis were 25, 50, and 120 µg/mL. At the highest dose of 120 µg/mL, % cell growth inhibition was 19% and % mitotic inhibition was 50% versus control (complete cell growth inhibition and mitotic inhibition data for the 4 hr treatment group without metabolic activation are presented in the table below). Mitotic index was used to select the highest dose level for analysis of aberrations, as there was no significant toxicity at any RDEA594 dose level. The percent of cells with structural or numerical aberrations in the RDEA594-treated groups was not significantly increased above controls and was also within the range of historical values.

Reference ID: 2965826

Table 3: Concurrent toxicity test using RDEA594 in the absence of metabolic activation (4 hr treatment, 16 hr recovery)

	RDEA594 (µg/mL)						
	25	50	100	120	135	150	200
Cell growth inhibition (%)	12	21	37	19	20	23	13
Mitotic inhibition (%)	19	22	21	50	59	73	NA

NA: Mitotic index was not calculated for this dose.

In the 4 hr treatment group with metabolic activation, dose levels selected for analysis were 10, 20, and 60 µg/mL. At the highest dose of 60 µg/mL, % cell growth inhibition was 48% and % mitotic inhibition was 51% versus control (complete cell growth inhibition and mitotic inhibition data for the 4 hr treatment group with metabolic activation are presented in the table below). The percent of cells with structural aberrations in the RDEA594-treated groups was not significantly increased above controls. The percent of cells with numerical aberrations was statistically significantly increased (8.5%) versus control but was within the historical solvent control range of 0 to 12.5%. Therefore, the increase was not considered biologically significant.

Table 4: Concurrent toxicity test using RDEA594 in the presence of metabolic activation (4 hr treatment, 16 hr recovery)

	RDEA594 (µg/mL)						
	10	20	40	60	80	100	120
Cell growth inhibition (%)	10	13	39	48	35	36	48
Mitotic inhibition (%)	-5	4	34	51	60	65	67

In the 20 hr treatment group without metabolic activation, dose levels selected for analysis were 25, 50, and 100 µg/mL. At the highest dose of 100 µg/mL, % cell growth inhibition was 34% and % mitotic inhibition was 55% versus control (complete cell growth inhibition and mitotic inhibition data for the 20 hr treatment group without metabolic activation are presented in the table below). The percent of cells with structural or numerical aberrations in the RDEA594-treated groups was not significantly increased above controls and was also within the range of historical values.

Table 5: Concurrent toxicity test using RDEA594 in the absence of metabolic activation (20 hr continuous treatment)

	RDEA594 (µg/mL)						
	25	50	100	120	135	150	200
Cell growth inhibition (%)	-8	-7	34	75	87	82	74
Mitotic inhibition (%)	-4	12	55	58	63	64	66

Reference ID: 2965826

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Rat Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of RDEA594

Study no:	SR08-024
Study report location:	SDN 1, Module 4, Volume 21
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 24, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RDEA594, lot # L10454-139-3 / 10454139-3, 90.3% pure (provided by sponsor), 89,5% pure (COA)

Key Study Findings

- RDEA594 was negative for induction of micronuclei formation in bone marrow after single oral doses up to 1000 mg/kg in male or female rats under the conditions of the assay.

Reference ID: 2965826

Methods

Doses in definitive study:	0, 250, 500, or 1000 mg/kg
Frequency of dosing:	Rats in 5 groups (neg and pos control and RDEA594 treatment groups) received a single dose and were euthanized at 24 hrs post dose. Rats in an additional 2 groups (neg control and high-dose RDEA594) received a single dose and were euthanized at 48 hrs post dose.
Route of administration:	Oral gavage
Dose volume:	20, 5, 10, 20, or 20 mL/kg for the vehicle control, low-, mid-, high-dose, and positive control groups, respectively
Formulation/Vehicle:	Phosphate buffer, pH 6.8
Species/Strain:	Sprague Dawley rat
Number/Sex/Group:	5/sex/group
Satellite groups:	An additional 5 rats/sex were received a single high-dose of RDEA495 and were included to be used as replacements for the high-dose group in the event of mortality. Four TK satellite groups (3 rats/sex/gp) were included in the definitive study for the neg control and RDEA594 treatment groups.
Basis of dose selection:	In a dose range-finding study, SD rats (3/sex/gp) received a single dose of RDEA594 at 1000, 1500, or 2000 mg/kg via oral gavage (10 mL/kg) and monitored for toxicity for 3 days
Negative control:	Phosphate buffer, pH 6.8
Positive control:	Cyclophosphamide, 40 mg/kg

Study Validity

In the definitive assay, micronucleus frequency was determined by analyzing the number of micronucleated polychromatic erythrocytes (PCEs) from 2000 PCEs per animal (n=5/sex from each treatment group). PCE:EC ratio was determined by scoring the number of PCEs and ECs observed while scoring at least 1000 erythrocytes per animal. The criterium for a positive response was the detection of a statistically significant, dose-responsive increase in micronucleated PCEs at one or more dose levels versus vehicle control. Evidence of dose-response, biological relevance, historical data, and potential confounding effects of toxicity were also considered. The number of micronucleated PCEs in the vehicle group did not exceed the historical control range, and the incidence of micronucleated PCEs in the positive control groups was statistically significant relative to the vehicle group. All criteria for a valid assay were met.

Results

Reference ID: 2965826

In the dose range-finding study, 2 rats (1 M, 1 F) in the 1500 mg/kg group and 2 rats (1 M, 1 F) in the 2000 mg/kg group were found dead on Day 1. Piloerection was observed in all rats at all doses. The maximum tolerated dose was determined to be 1000 mg/kg.

In the definitive study, 2 male rats and 3 female rats in the high-dose replacement group were found dead on Day 1. No deaths occurred in the main study groups. Piloerection was observed in all rats in the 1000 mg/kg group. No test article-related clinical signs were observed in rats in the 250 or 500 mg/kg groups. In male RDEA594 groups at 24 hours post dose, PCEs:ECs ratios were decreased up to 11% versus vehicle control, but without a dose-response relationship. PCEs:ECs ratios were unchanged in female RDEA594 groups at 24 hours post dose. In the high-dose RDEA594 group at 48 hrs post dose, PCEs:ECs ratios were decreased by 1 and 11% in males and females, respectively, versus controls. RDEA594 did not significantly increase micronucleated PCEs per 10,000 PCEs (total PCEs/group, 2000 PCEs/animal) in female groups versus vehicle control at 24 or 48 hrs post dose. RDEA594 significantly increased micronucleated PCEs per 10,000 PCEs in high-dose (1000 mg/kg) males at 24 hrs post dose; however, the number of micronucleated PCEs was within the range of historical control data and thus was not considered biologically relevant. For reference, cyclophosphamide statistically significantly increased micronucleated PCEs in both male and female rats.

Mean TK results following single dose oral administration of RDEA594 in male and female rats in the micronucleus assay are presented below.

Table 6: In Vivo rat micronucleus TK parameters

Dose (mg/kg)	Sex	T_{max} (hr)	C_{max} (µg/mL)	AUC (µg*hr/mL)	
				0-24 hrs	0-48 hrs
250	M	6.0	153	1950	NA
	F	3.0	206	2140	NA
500	M	12.0	200	3050	NA
	F	11.0	245	3580	NA
1000	M	48.0	327	4660	11600
	F	28.0	300	4220	7820

11 Integrated Summary and Safety Evaluation

The mutagenic and clastogenic potential of RDEA594 was assessed in the standard battery of genotoxicity tests. RDEA594 was negative in the *in vitro* bacterial reverse mutation assay, *in vitro* CHO cell chromosomal aberration assay, and *in vivo* rat micronucleus assay.

Reference ID: 2965826

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/s/

MARCIE L WOOD
06/23/2011

TIMOTHY W ROBISON
06/23/2011
I concur

Appendix 5: Nonclinical Review of IND 102128. December 24, 2012.

APPEARS THIS WAY ON ORIGINAL

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	IND 102,128
Supporting document/s:	**Supporting Document 16**: "A 6-month repeat oral dose toxicity and toxicokinetic study with a 2 month recovery period and a 3-month pull with a 28-day recover period in Sprague-Dawley rats" **Supporting Document 44: "**RDEA594: A 12-month repeat dose toxicity and toxicokinetic study with a 3-month recovery period and a 6-month pull in cynomolgus monkey"
Sponsor's letter date:	**Supporting Document 16**: 2/19/10 **Supporting Document 44**: 12/16/10
CDER stamp date:	**Supporting Document 16**: 2/22/10 **Supporting Document 44**: 12/20/10
Product:	RDEA594 (Lesinurad)
Indication:	Gout
Sponsor:	Ardea Biosciences, Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products (DPARP)
Reviewer:	Matthew Whittaker, PhD
Supervisor/Team Leader:	Timothy Robison, PhD
Division Director:	Badrul A. Chowdhury, MD, PhD
Project Manager:	Michelle Jordan Garner

Template Version: December 7, 2009

Reference ID: 3216021

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1 Executive Summary

1.1 Recommendations

1.1.1 Clinical Study (ies) Safe to Proceed: Yes

A no observed adverse effect level (NOAEL) of 100 mg/kg/d for RDEA594 was established in a 12 month repeat dose study in cynomolgus monkeys (most sensitive nonclinical species). This study supports clinical systemic exposure of up to $AUC_{0\text{-}24h}$ = 82.6 µg*hr/ml for the circulating parent drug, adequate to cover exposures at clinical doses to be used in Phase 3 studies.

1.2 Brief Discussion of Nonclinical Findings

In the 6 month repeat dose **rat** study (Study SR08-095, once daily, oral), high dose (600 mg/kg/d) RDEA594 treatment led to mortality in a total of 13/60 animals (7 M, 6 F) within 3 weeks of the start of dosing. The surviving animals were euthanized and main study animals were necropsied on Day 23. The target organs in the deceased animals included the **gastrointestinal (GI) tract and kidney**. These animals showed microscopic evidence of single cell necrosis in all organs of the GI tract in addition to single cell necrosis of renal tubule epithelial cells and tubular degeneration in the kidney. Lesions of the pancreas, stomach and kidney were observed in 300 mg/kg/d animals at the 3-month interim sacrifice; however these findings do not progress or were no longer evident at the 6-month sacrifice. Premature deaths that occurred at lower doses were considered unrelated to treatment (gavage error, TK bleed). Based on the deaths at 600 mg/kg/d and histopathological observations at 300 mg/kg/d in the pancreas and stomach at the 3-month time point and kidneys at the 6-month time point, the overall no observed adverse effect level (NOAEL) in the **rat** is judged to be **100 mg/kg/d.** Systemic exposure of RDEA594 was decreased at 3 months and 6 months in 100 mg/kg and 300 mg/kg treated rats relative to exposure at day 1, suggestive of drug induced metabolic enzyme induction. The rat NOAEL is associated with a mean systemic exposure (male & female) of $AUC_{0\text{-}24h}$ = 415 µg*hr/ml.

Study SR08-094 was a 12 month repeat dose study in cynomolgus **monkeys**. There were 5 (2M, 3F) premature deaths in HD animals (600 mg/kg/d), all within the first 183 days of the study. 4 deaths were due to severe diarrhea, emesis, and decreased food consumption and were considered to be due to RDEA594 treatment. The other death was due to gavage error. There was one premature death in the 100 mg/kg/d group on day 320 (# 0611016). This animal had signs of discoloration of the lungs and the mucosa of the stomach as well as a marked decrease in thymus size. The microscopic changes in this animal were not entirely consistent with what was observed in the premature deaths at the HD. Specifically, this animal possessed multifocal erosions of the stomach, a more severe observation than what was seen at higher doses. Given that there were no premature deaths among 20 monkeys at the next highest dose (300 mg/kg/day), this death is not considered to be treatment related. One male in the 30 mg/kg/d group (#0508173) was moribund sacrificed on day 217. The cause of death was determined to be sepsis secondary to proctitis/colitis. Inflammation of the large intestine/colitis is a relatively common finding in captive monkeys (Adler et al. 1993; Chamanza et al. 2010). Furthermore, this animal had generally more severe GI tract lesions than those observed at higher doses. Thus this death is not considered related to RDEA594 treatment. In animals that survived to study completion, clinical signs of diarrhea, emesis and soft stool increased with dose and persisted throughout the study. Evidence for metabolic enzyme autoinduction is seen with markedly decreased systemic exposure of RDEA594 in males and females

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starting after 3 months of treatment. The no observed adverse effect level in monkeys was determined to be **100 mg/kg**/day based on bile duct hyperplasia in males at doses ≥300 mg/kg/day. This dose level is associated with an $AUC_{0\text{-}24h}$ of 82.6 µg*h/mL (average of male and female AUC, week 50).

Based on pharmacokinetic data from a 10 day clinical study (RDEA594-102), the doses to be used in Phase 3 clinical trials (200 mg or 400 mg, once per day) are supported by the nonclinical data.

2 Drug Information

2.1 DRUG

2.1.1 CAS Registry Number (Optional): 1151516-14-1

2.1.2 Generic Name: Lesinurad

2.1.3 Code Name: RDEA594 Sodium

2.1.4 Chemical Name: Sodium, [5-Bromo-4-(4-cyclopropyl-napthalen-1-yl)-4H-[1, 2, 4]triazol-3-yl-sulfanyl]-acetate

2.1.5 Molecular Formula/Molecular Weight: $C_{17}H_{13}BrN_3NaO_2S$ / 426.26 Da (404.28 for free acid)

2.1.6 Structure

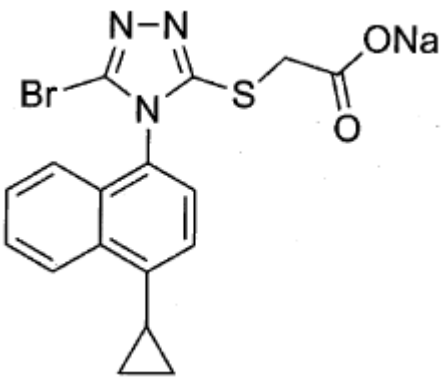

Figure 1. Chemical structure of RDEA594

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2.1.7 Pharmacologic class: URAT1 (human urate transporter) inhibitor

2.3 Clinical Formulation

2.3.1 Drug Formulation: RDEA594 (b) (4)

2.3.2 Comments on Novel Excipients: There are no novel excipients present in the drug formulation.

2.4 Proposed Clinical population and dosing regimen

The ongoing Phase 3 clinical studies with RDEA594 are listed in Table 1.

Table 1. Ongoing phase 3 studies with RDEA594

Protocol #	Description	RDEA 594 Doses	Duration	# of subjects
RDEA594-301	Evaluate the Efficacy and Safety of Lesinurad and Allopurinol Compared to Allopurinol Alone in Subjects with Gout who have had an Inadequate Hypouricemic Response to Standard of Care Allopurinol (CLEAR 1)	200 mg/d or 400 mg/d	6 -12 months	~600
RDEA594-302	Evaluate the Efficacy and Safety of Lesinurad and Allopurinol Compared to Allopurinol Alone in Subjects with Gout who have had an Inadequate Hypouricemic Response to Standard of Care Allopurinol (CLEAR 2)	200 mg/d or 400 mg/d	6 -12 months	~600
RDEA594-303	Lesinurad Monotherapy in Gout Subjects Intolerant to Xanthine Oxidase Inhibitors (LIGHT)	400 mg/d	6 months	~200
RDEA594-304	Evaluate the Efficacy and Safety of Lesinurad and Febuxostat Compared to Febuxostat Alone at Lowering Serum Uric Acid and Resolving Tophi in Subjects with Tophaceous Gout	200 mg/d or 400 mg/d	12 months	~315

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3 Studies Submitted

3.1 Studies Reviewed

Study no. SR08-095
RDEA594: A 6-month repeat oral dose toxicity and toxicokinetic study with a 2-month recovery period and a 3-month pull with a 28-day recovery period in **Sprague-Dawley rats**

Study no. SR08-094
RDEA594: A 12-month repeat dose toxicity and toxicokinetic study with a 3-month recovery period and a 6-month pull in **cynomolgus monkey.**

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetic data submitted from clinical studies includes a 10 day repeat dose study in healthy volunteers with once daily RDEA594 doses from 200 mg – 600 mg/day (Study RDEA594-102) as well as a single dose study in healthy volunteers at doses from 400 mg – 1600 mg/day (Study RDEA594-117).

Phase 1 and 2 studies were reported to be conducted with a (b) (4) sodium salt form of RDEA594. A crystalline free acid immediate release tablet is to be used in Phase 3 trials. The intended doses in Phase 3 clinical trials (RDEA594-301, RDEA594-302, RDEA594-303 and RDEA594-304) are 200 mg or 400 mg, once per day, with food. Clinical PK data from study RDEA594-102 was excerpted from the 30 day nonclinical safety review completed by Kathleen Young, Ph.D. on October 30, 2009. This study examined the PK parameters of different formulations of RDEA594 ((b) (4) immediate release tablet, (b) (4)) as well as the fasted vs. fed state. The most relevant data is from subjects that received the (b) (4) capsule formulation (200 – 600 mg) once daily for 10 days in the fed state (Table 2).

As discussed in the review below, the no observed adverse effect level (NOAEL) established in a 12-month study in cynomolgus monkeys (SR08-094) is 100 mg/kg/day. This NOAEL is associated with a mean systemic exposure of $AUC_{0\text{-}24h}$ of 82.6 µg*h/mL at the conclusion of dosing. Based on the data from clinical study RDA594-102, treatment with RDEA594 at the doses proposed in Phase 3 trials (200 mg or 400 mg) would result in systemic exposure levels that are lower than those observed in the monkey at the NOAEL. Therefore, these doses are supported by the existing nonclinical data.

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Table 2. Selected pharmacokinetic data from clinical study RDEA 594-102. Plasma AUC at conclusion of a 10 day repeat dose study (data excerpted from 30d nonclinical safety review, Kathleen Young, Ph.D., 10/30/2009)

RDEA 594 Dose	$AUC_{0\text{-}24\,h}$ (µg*h/mL)	C_{max} (µg/mL)
Clinical Study RDEA594-102 **Day 10** (b) (4) capsule 200 mg (fed) 400 mg (fed) 600 mg (fed)	 25.9 53.9 84.9	 7.95 15.7 20.8
Monkey NOAEL (100 mg/kg/d) Week 50	 82.6	 13.5

High systemic exposures were observed in study RDEA594-117, a single dose clinical study at supra-therapeutic RDEA594 doses. Exposures at the highest dose were similar to those observed in the first week of dosing at the NOAEL in the 12 month monkey study (Table 3).

Table 3. Plasma pharmacokinetic data for RDEA594 following a single dose of RDEA594 (clinical study RDEA594-117)

RDEA 594 Dose	$AUC_{0\text{-}24\,h}$ (µg*h/mL)		C_{max} (µg/mL)	
	Male	Female	Male	Female
Clinical Study RDEA594-117				
400 mg*	56.9	64.2	15.0	17.3
800 mg	120	147	30.6	40.9
1200 mg	212	268	50.3	59.9
1600 mg	386	536	70.2	84.0
Monkey NOAEL (100 mg/kg/d)				
Day 1	346	349	57.2	67
Week 50	81.4	83.7	13.6	12.5
*systemic exposure is for 0-23h				

Exposure increased in a close to linear fashion with respect to dose. For this reason, exposure data from this study was linearly extrapolated to estimate exposure levels at proposed Phase 3 clinical dose levels (

Table 4). This data provides further evidence that the proposed clinical doses are supported by the nonclinical NOAEL (i.e. human exposure at highest proposed clinical dose is less than the exposure in the monkey at the NOAEL).

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Table 4. Predicted human RDEA594 exposure at proposed clinical doses based on data from single dose clinical study in healthy volunteers (RDEA594-117)

RDEA 594 Dose	Predicted $AUC_{0\text{-}24\ h}$ (µg*h/mL)	
	Male[1]	Female[2]
Proposed clinical doses		
200 mg	-22.1	-53.5
400 mg	31.8	23.3

[1]Calculated using equation derived from exposure data in RDEA594-117: AUC = 0.269 (dose) -76.1
[2]Calculated using equation derived from exposure data in RDEA594-117: AUC = 0.384 (dose) -130.3

Study RDEA594-117 also measured plasma levels of the M6 metabolite of RDEA594 (Table 5). This was the major metabolite in the 12 month monkey study. Levels of M6 in human plasma were found to be very low relative to RDEA594 after a single dose. This appears to be an expected result as significant levels of this metabolite did not accumulate in the monkey until between 12 and 24 weeks of dosing (Table 40).

Table 5. Plasma pharmacokinetic data for RDEA594 M6 metabolite following a single dose of RDEA594 (clinical study RDEA594-117)

RDEA 594 Dose	M6 Metabolite $AUC_{0\text{-}24\ h}$ (µg*h/mL)		M6 Metabolite C_{max} (µg/mL)	
	Male	Female	Male	Female
Clinical Study RDEA594-117				
1200 mg	0.296	0.408	0.0235	0.0316
1600 mg	0.590	0.618	0.0387	0.0431
Monkey NOAEL (100 mg/kg/d)				
Day 1	1.87	1.65	0.132	0.124
Week 50	301	206	18.6	12.2

Distribution

In clinical study RDEA594-112, 6 healthy male volunteers received a single 600 mg dose of [^{14}C]Lesinurad (500 µCi). A mean of 63.4% of the dose was recovered in urine while 32.3% was recovered in feces. 61.1% of the dose was recovered in urine by 24 hour s post dose. 87.3% of the total initial dose was recovered within 72 hours. Volume of distribution was 40 L, showing distribution of the molecule throughout the total body water.

Reference ID: 3816021

6 General Toxicology

6.2 Repeat-Dose Toxicity

6.2.1 Study SR08-095. 6 month rat study

Study title: RDEA594: A 6-month repeat oral dose toxicity and toxicokinetic study with a 2-month recovery period and a 3-month pull with a 28-day recovery period in Sprague-Dawley rats

Study no.:	SR08-095 (Ardea's study number)
Study report location:	S0016, Vol 2, pg. 1-2676
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 16, 2009
GLP compliance:	Yes- signed Feb 5, 2010
QA statement:	Yes- signed Feb 5, 2010
Drug, lot #, and % purity:	RDEA594 Lot A10001-122-5/81.8% pure (Ardea) Lot 0904697/82.1% pure (Ardea) Lot NE-023025-batch-01-2009/ 83.0% pure (b) (4) Lot A10063-28-8/82.4% pure (Ardea) Lot A10063-23-20/81.7% pure (Ardea) Lot A10060-64-1/82.1% pure (Ardea) Lot A10060-65-1/82.4% pure (Ardea) Lot A10063-80-20/82.4% pure (Ardea)

Reference ID: 3216021

6.2.1.1 Key Study Findings

- Study no. SR08-095
- Rats were dosed daily with RDEA594 via oral gavage for up to 6 months
- 13/60 HD (600 mg/kg) died within 3 weeks of dosing – all had evidence of GI and kidney toxicity
    - All surviving HD animals were euthanized on study day 23.
    - The **kidney** and **GI tract** are target organs of toxicity in male and female rats receiving 600 mg/kg/d
- Mean body weight was decreased by 10-12% vs. controls in 300 mg/kg males at the 3 month time point and both males and females at the 6 month time point.
    - The magnitude of body weight decrease was similar at both the 3 month and 6 month time points
    - Decreased body weight values persisted through 4 and 8 week recovery periods respectively.
- **Pancreas** (single cell necrosis) and **stomach** (erosion, hemorrhage) appeared to be potential target organs of toxicity at the 3 month time point, but not at the 6 month time point.
    - Neither incidence nor severity of these findings increased between the 3 and 6 month time points.
    - At the 6 month time point the pancreas finding was confined to a single animal with a grading of minimal.
    - Stomach erosion/ulceration has been found to occur spontaneously in the rat with a percent incidence of ~10% (Gad & Chengelis, 1992).
    - The stomach finding was confined to a single animal at the 6 month time point with a grading of minimal.
- Evidence of hepatocellular hypertrophy at doses ≥ 100 mg/kg/day at the 3 month and 6 month time points is considered to be an adaptive response and is not regarded as a dose-limiting toxicity.
- Evidence for potential treatment-related kidney toxicity at 300 mg/kg/d includes increased water consumption, increased urine volume (decreased ability to concentrate urine), increased serum creatinine, and decreased BUN:creatinine ratio.
    - However, there is limited microscopic evidence for overt kidney pathology at either the 3 month or 6 month time points (limited to increased incidences of tubular dilation for males and females at 300 mg/kg/day).
    - The changes of urinalysis and clinical chemistry parameters might be attributed to the pharmacological properties of the test article.
- Follicular hypertrophy of the **thyroid** was seen in the 100 mg/kg and 300 mg/kg dose groups. This is considered to be a rat specific finding.

- There is evidence for RDEA594 induced metabolic enzyme induction
    - Systemic exposure of RDEA594 is decreased in 100 mg/kg and 300 mg/kg dose M & F at 3 months and 6 months vs. day 1 (accumulation ratio = 0.6 – 0.9)

- The **reviewer's** overall NOAEL is 100 mg/kg/day based on mortality observed at the 600 mg/kg dose and histopathological findings at 300 mg/kg/day in the pancreas and stomach at the 3-month time point and kidneys at the 6-month time point. This NOAEL is not in agreement with the sponsor's NOAEL (300 mg/kg/day). 100 mg/kg/day RDEA 594 treatment is associated with a mean systemic exposure (male & female) of $AUC_{0\text{-}24h}$ = 415 µg*hr/ml. RDEA59 has a steep dose-response curve based upon a NOAEL at 100 mg/kg/day and lethality at 600 mg/kg/day.

Reference ID: 3216021

6.2.1.2 Methods

Doses:	0 (DI water), 10, 30, 100, 300, 600 mg/kg/day
Frequency of dosing:	Once-daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/ Vehicle:	Milli-Q deionized water (DI)
Species/Strain:	Sprague-Dawley rats
Number/Sex/ Group:	See Table 6 below. Total animals used on study: Males: 244 Females: 245
Age:	7-8 weeks. Except group 4 interim recovery animals that were 8-9 weeks old
Weight:	M: 208.5-288.8 g F: 145.5-224.3 g
Satellite groups:	Toxicokinetic animals: 10/sex/group (groups 2, 3, and 6 are TK only animals)
Unique study design:	The study includes both a 3 month study with 4 week recovery animals as well as a 6 month study with 8 week recovery animals.
Deviation from study protocol:	All animals receiving the highest dose (600 mg/kg/d) were euthanized early (day 23) due to multiple premature deaths in this dose group.

Reference ID: 3816021

Table 6. RDEA494. Study SR08-095. Experimental design of repeat dose study in Sprague Dawley rats.

				Interim Study				**Terminal study**			
Group	Dose (mg/kg/day)	**Day 23**		3 months **(d 92, wk 14)**		3 months + 28 d rec. **(d 120, wk 18)**		6 months **(d 183, wk 27)**		6 months + 2 month rec. **(d 239, wk 35)**	
				IT (Interim)		IR (Interim rec.)		TN (Terminal)		TR (Terminal rec.)	
1	**0 (DI water)**	Male (8) 12471-12476 12478-12479	Female (9) 12481-12490	Male (10) 12001-12010	Female (10) 12046-12055	Male (10) 12011-12020	Female (10) 12056-12065	Male (15) 12021-12035	Female (15) 12066-12080	TK + Recovery	
										Male (10) 12036-12045	Female (10) 12081-12090
2	**10**	-		Male (10) 12091-12100	Female (10) 12126-12135	-		Male (15) 12101-12115	Female (15) 12136-12150	TK Only *No Histo	
										Male (10) 12116-12125	Female (10) 12151-12160
3	**30**	-		Male (10) 12161-12170	Female (10) 12196-12205	-		Male (15) 12171-12185	Female (15) 12206-12220	TK only *No Histo	
										Male (10) 12186-12195	Female (10) 12221-12230

Reference ID: 3215021

				Interim Study				**Terminal study**			
Group	Dose (mg/kg/day)	**Day 23**		3 months **(d 92, wk 14)**		3 months + 28 d rec. **(d 120, wk 18)**		6 months **(d 183, wk 27)**		6 months + 2 month rec. **(d 239, wk 35)**	
				IT (Interim)		IR (Interim rec.)		TN (Terminal)		TR (Terminal rec.)	
4	**100**	-		Male (10) 12231-12240	Female (10) 12266-12275	**Day 113, Wk 17** Male (10) 12451-12260	Female (10) 12461-12470	Male (15) 12241-12255	Female (15) 12276-12290	TK + Recovery Male (10) 12256-12265	Female (10) 12291-12300
5	**300**	-		Male (10) 12301-12310	Female (10) 12346-12355	Male (10) 12311-12320	Female (10) 12356-12365	Male (15) 12321-12335	Female (15) 12366-12380	TK + Recovery Male (10) 12336-12345	Female (10) 12381-12390
6	**600**	Male (20) 12391-12410	Female (20) 12421-12440	-		-		-		TK Only *No Histo Male (10) 12411-12420	Female (10) 12441-12450

Reference ID: 3815021

6.2.1.3 Observations and Results

Mortality

Cageside observations were made three times daily, prior to dose and at approximately 2 and 6 hours post-dose. There were 27 total early deaths in the study (Table 7 & Table 8). In the main study, 9/40 (4M, 5F) animals in the high-dose (HD) group (600 mg/kg/day) died or were sacrificed between Days 4 and 20 due to drug-related toxicity. Deaths were attributed to enteropathy and renal toxicity by the study pathologist. The gastrointestinal (**GI**) tract, **liver** and **kidney** were target organs of toxicity in HD animals. Microscopic observations of deceased animals included single cell necrosis (minimal – moderate), congestion, and hemorrhage in **stomach**, **duodenum**, **jejunum**, **ileum**, **cecum** and **colon**. Single cell necrosis (minimal – mild) and tubular degeneration (minimal – moderate) were observed in the **kidney** and **hepatocellular hypertrophy** (minimal – mild) was observed in the liver. Animals in this group that were sacrificed due to moribundity presented clinical signs of lethargy, rough coat or coarse coat, cold to touch, and hunched posture. An additional 4/20 HD animals (3M, 1F) in the toxicokinetic study (Table 8) died or were moribund sacrificed by study day 22. Necropsy was not performed on these animals.

The 14 total deaths in the 0, 30, 100 and 300 mg/kg/day groups were considered by the sponsor to be unrelated to study drug (Table 7,Table 8). All deaths in the 30 and 100 mg/kg/day groups were attributed to either gavage error or accidental death due to bleeding as a result of errors in blood sampling technique. Gavage error was supported in each case by microscopic and/or macroscopic evidence of pulmonary edema/congestion. All animals that died from bleeding showed evidence of hemorrhage in the thoracic area. 2/10 males in the 3 month study/300 mg/kg/day group were found dead (#12309: day 8; #12312: day 96) and 1/10 females in this dose group (#12365, day 27) was moribund sacrificed. Animal #12309 died from bacterial sepsis, while animal #12312 died from what the sponsor describes as an undetermined cause. This animal had evidence of multifocal congestion in the lung suggesting that gavage error may have contributed to its death. The death of animal #12365 was declared to be due to gavage error. This conclusion is supported by the presence of mild congestion in the lungs. However, this animal also showed the same GI toxicity (single cell necrosis of jejunum, duodenum, ileum and cecum) as that observed in the HD (600 mg/kg/day) group.

Table 7. Premature deaths in **main study of SR08-095**.

Dose group	Dose (mg/kg/d)	Sex	Animal no.	Mode of death	Day of death	Cause of death
1	Control	F	12069	Found dead	176	Accidental-TK bleed
		F	12075	Found dead	176	Accidental-TK bleed
3	30	M	12181	Found dead	158	Gavage accident
		F	12196	Found dead	29	Accidental-Tk bleed
		F	12212	Found dead	143	Gavage accident
		F	12216	Found dead	176	Accidental-Tk bleed
4	100	F	12464	Found dead	64	Gavage accident
5	300	M	12309	Found dead	8	Bacterial sepsis
		M	12312	Found dead	96	Undetermined/evidence for lung congestion
		F	12365	Moribund	27	Gavage accident but also showed

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Dose group	Dose (mg/kg/d)	Sex	Animal no.	Mode of death	Day of death	Cause of death
				sacrifice		**GI toxicity similar to 600 mg/kg/day animals**
6	600	M	12391	Moribund sacrifice	6	Enteropathy and renal toxicity
		M	12403	Moribund sacrifice	20	Enteropathy and renal toxicity
		M	12406	Moribund sacrifice	13	Renal toxicity, liver hypertrophy
		M	12409	Found dead	9	Enteropathy and renal toxicity
		F	12421	Moribund sacrifice	5	Enteropathy and renal toxicity
		F	12430	Moribund sacrifice	15	Enteropathy and renal toxicity
		F	12431	Found dead	7	Enteropathy and renal toxicity
		F	12436	Found dead	5	Enteropathy and renal toxicity
		F	12438	Found dead	4	Enteropathy and renal toxicity

Table 8. Premature deaths in toxicokinetic study of SR08-095.

Dose group	Dose (mg/kg/d)	Sex	Animal no.	Mode of death	Day of death	Cause of death
2	10	M	12117	Moribund sacrifice	66	Undetermined
3	30	F	12225	Found dead	111	Gavage accident
5	300	M	12341	Found dead	38	Gavage accident
		F	12386	Found dead	79	Gavage accident
6	600	M	12412	Found dead	20	Undetermined-TK only – no necropsy
		M	12414	Found dead	22	Undetermined-TK only – no necropsy
		M	12415	Moribund sacrifice	6	Undetermined-TK only – no necropsy
		F	12445	Found dead	12	Undetermined-TK only – no necropsy

Clinical Signs

Cageside observations were made three times daily, prior to dose and at approximately 2 and 6 hours post-dose. Detailed clinical observations were made prior to dosing on Day 1 and weekly thereafter and prior to each sacrifice. Relevant cageside observations are summarized in
Table 9. Findings including lethargy, rough coat, thin, moribund, and hunched posture were generally limited to males and females dosed at ≥ 300 mg/kg/day. Rales was observed in a subset of males receiving ≥ 100 mg/kg/day, but not in females at any dose.

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Table 9. Summary of cageside observations of main study animals over the course of study days 1-239 in rats. For 600 mg/kg group, data is from days 1-23.

	Males						Females					
RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n=	45	25	25	35	35	20	45	25	25	35	35	20
Lethargy												
Number of observations	-	-	-	-	8	18	-	-	-	-	4	7
Number of animals	-	-	-	-	2	7	-	-	-	-	1	5
Days (from – to)	-	-	-	-	5-38	6-21	-	-	-	-	26-27	4-11
Rough coat/coarse fur												
Number of observations	-	-	-	-	7	8	-	-	-	-	-	7
Number of animals	-	-	-	-	2	2	-	-	-	-	-	3
Days (from – to)	-	-	-	-	6-38	8-21	-	-	-	-	-	6-18
Thin												
Number of observations	-	-	-	-	-	30	-	-	-	-	-	4
Number of animals	-	-	-	-	-	6	-	-	-	-	-	2
Days (from – to)	-	-	-	-	-	6-21	-	-	-	-	-	15-18
Moribund												
Number of observations	-	1	-	-	-	4	-	-	-	-	1	3
Number of animals	-	1	-	-	-	4	-	-	-	-	1	3
Days (from – to)	-	66-66	-	-	-	6-20	-	-	-	-	27	5-18
Found dead												
Number of observations	-	-	1	-	3	3	2	-	4	1	1	4
Number of animals	-	-	1	-	3	3	2	-	4	1	1	4
Days (from – to)	-	-	158	-	8-96	9-22	176	-	29-176	64	79	4-12
Hunched posture												
Number of observations	-	-	5	-	7	9	-	-	-	-	4	2
Number of animals	-	-	1	-	2	4	-	-	-	-	1	2
Days (from – to)	-	-	27-28	-	6-38	8-21	-	-	-	-	26-27	15-18

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	Males						Females					
RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n=	45	25	25	35	35	20	45	25	25	35	35	20
Rales												
Number of observations	-	-	-	1	7	2	-	-	-	-	-	-
Number of animals	-	-	-	1	4	2	-	-	-	-	-	-
Days (from – to)	-	-	-	72	6-45	8-20	-	-	-	-	-	-
- = no observation												

Body Weights

Body weights (Table 10, Table 11) were measured prior to dosing on Day 1 and weekly thereafter. Animals were also weighed on the day prior to each sacrifice and on the day of sacrifice. Mean body weight as well as body weight gain (as % control) values were decreased in males and females at all dose levels after 3 months and 6 months of RDEA 594 treatment. Body weights were not reported for control males or females on day 23, in which animals in the 600 mg/kg treatment group were sacrificed. Therefore, only body weight data from the conclusion of the 3 month and 6 month study are presented in this review.

Mean body weight was **decreased** in males and females receiving 300 mg/kg/day at both the **3 month** and **6 month** time points. At 3 months, 300 mg/kg male mean body weight was 88% of controls. Decreased mean body weight persisted through a 4 week recovery period, as mean body weight was also 88% of controls in recovery males. Mean body weight of 300 mg/kg females at 3 months was 93% of controls. After a 4 week recovery period, mean body weight returned closer to control levels (97%).

At 6 months, RDEA 594 effects on body weight were generally similar to that at 3 months. 300 mg/kg male mean body weight was found to be 88% of mean body weight of control animals. Decreased mean body weight persisted in animals that were maintained through the 8 week recovery period, as mean body weight was 89% of controls in recovery males. In 300 mg/kg females, mean body weight was 90% of controls at the 6 month time point. Mean body weight of 300 mg/kg females decreased in recovery animals to 80% of controls

Body weight gain values were decreased by 21.1% and 20.5% vs. controls in 300 mg/kg/day males and females respectively at the conclusion of the 3 month study (Table 10). Males showed similar body weight gain to controls (+1.7%) during the recovery period (days 92-120), while females continued to show decreased body weight gain relative to controls (-21.6%). Body weight gain values were decreased (-20.5% and -24.2%) vs. controls in 300 mg/kg/day males and females respectively at the conclusion of the 6 month study (Table 11). Males showed increased body weight gain relative to controls (+60.6%) during the recovery period (days 183-239), while females continued to show decreased body weight gain relative to controls (-19.1%).

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Table 10. Study SR08-095- 3 month study. Body weight measurements of Sprague Dawley rats

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
Day		**Absolute body weight (g)**											
1		240.9 n=35	237.3 n=25	240.0 n=25	241.3 n=35	243.2 n=35	235.0 n=20	189.4 n=35	192.0 n=25	189.5 n=25	198.6 n=35	192.1 n=35	193.1 n=20
22		384.3 n=35	375.4 n=25	385.5 n=25	370.1 n=35	**357.3** n=34**	**302.7** n=16**	247.9 n=35	247.9 n=25	241.9 n=25	242.4 n=35	239.6 n=35	**216.9** n=15**
92		558.8 n=35	541.3 n=25	546.3 n=25	519.8** n=25	**494.0** n=34**	-	300.1 n=35	292.0 n=25	289.2 n=24	290.7 n=25	**280.1** n=34**	-
Day 92 % control		100%	97%	98%	93%	88%	-	100%	97%	96%	97%	93%	-
Recovery Day 120		605.3 n=10	NT	NT	NT	**534.4** n=9**	NT	302.0 n=10	NT	NT	NT	292.2 n=9	NT
Day 120 % control		100%	-	-	-	88%	-	100%	-	-	-	97%	-
		Body weight gain											
Days 1-22	**BW gain (g)**	143.4	138.1	145.5	128.8	114.1	67.7	58.5	55.9	52.4	43.8	47.5	23.8
	% chg from control	- n=35	-3.7 n=25	1.5 n=25	-10.2 n=35	-20.4 n=34	-52.8 n=16	- n=35	-4.4 n=25	-10.4 n=25	-25.1 n=35	-18.8 n=34	-59.3 n=16
Days 1-92	**BW gain (g)**	317.9	304.0	306.3	278.5	250.9	-	110.7	100.0	99.7	92.1	88.0	-
	% chg from control	- n=35	-4.4 n=25	-3.7 n=25	-12.4 n=25	-21.1 n=34	-	- n=35	-9.7 n=25	-9.9 n=25	-16.8 n=25	-20.5 n=34	-
Recovery													
Days 92-120	**BW gain (g)**	37.3	NT	NT	NT	37.9	NT	13.8	NT	NT	NT	10.8	NT
	% chg from control	- n=10	-	-	-	1.7 n=9	-	- n=10	-	-	-	-21.6 n=9	-

**p<0.01 vs. control, ANOVA, Dunnett's post hoc test
NT = Not tested

Reference ID: 3816021

Table 11. **Study SR08-095,** 6 month study**.** Body weight measurements of Sprague Dawley rats .

		Males						**Females**					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
		Absolute body weight (g)											
Day 1		242.3 n=15	243.2 n=15	242.0 n=15	231.6 n=15	245.1 n=15	NT	190.0 n=15	197.8 n=15	190.1 n=15	197.3 n=15	193.0 n=15	NT
Day 183		655.1 n=15	618.8 n=15	654.6 n=14	599.4 n=15	**573.4** n=15**	NT	343.6 n=13	334.8 n=15	**310.1* n=13**	329.2 n=15	**309.5* n=15**	NT
Day 183 % control		100%	94%	100%	91%	88%	NT	100%	97%	90%	96%	90%	NT
Recovery Day 239		715.3 n=10	NT	NT	676.5 n=10	**637.4* n=9**	NT	402.7 n=10	NT	NT	362.3 n=10	**322.2** n=9**	NT
Day 239 % Control		100%	-	-	95%	89%	-	100%	-	-	90%	80%	-
		Body weight gain											
Days 1-183	**BW gain (g)**	412.8 n=15	375.6 n=15	412.7 n=14	367.8 n=15	328.4 n=15	NT	153.7 n=13	137.0 n=15	120.0 n=15	131.9 n=15	116.5 n=15	NT
	% chg from ctrl	-	-9.0	0.0	-10.9	-20.5	NT	-	-10.8	-21.9	-14.2	-24.2	NT
Recovery													
Days 183-239	**BW gain (g)**	32.7 n=10	NT	NT	49.4 n=10	52.5 n=9	NT	27.0 n=10	NT	NT	15.8 n=10	21.9 n=9	NT
	% chg from ctrl	-	NT	NT	51.1	60.6		-	NT	NT	-41.6	-19.1	NT

*p<0.05; **p<0.01 vs. control. One-way ANOVA, Dunnett's post hoc test.
NT = not tested

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Feed Consumption

Rats were housed 1 per cage. **Food consumption** was measured once weekly and **water consumption** was measured for 3 consecutive days ending in Weeks 26 (6 months) and 34 (2 month recovery). While there were transient decreases in food consumption, there were no dose-related changes in overall food consumption in males or females in the 3 month study or 6 month study. From these observations, it can be concluded that observed decreases in body weights were not a result of decreased food intake.

Water Consumption

Water consumption was measured over 3 days at the conclusion of the 6 month study (d173 – d176) as well as in recovery animals from d229 – d232 (**Table 12**). Males and females in the 300 mg/kg/day group showed increased **total water consumption** vs. controls on days 173-176 (M: +47%, F: +50%). Increases were statistically significant vs. controls on each of the three individual days. Water consumption values returned to control levels during the 4 week recovery period. This finding may be of potential concern as increased water consumption could be secondary to renal pathology. It is well established that Sprague Dawley rats are highly susceptible to development of nephropathy, with males more susceptible than females to this disease (Rao, 2002). However, there is limited histopathological evidence of renal injury at the 6 month time point in the present study (Table 24). Increased water consumption may also be linked to the pharmacological mechanism of action of RDEA 594. RDEA 594 inhibits the reabsorption of uric acid from the renal tubule into the blood. A resultant increase in osmolarity of the glomerular filtrate may lead to a decrease in reabsorption of water into the blood. These events may trigger increased water consumption in rats.

Table 12. IND 102128. 6 month rat study. Water consumption at conclusion of 6 months treatment.

		Males					**Females**				
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**0**	**10**	**30**	**100**	**300**
	n	25	14	13	24	21	25	15	10	21	18
Water consumption (ml/animal)	Days 173-176	103.9	101.8	111.3	108.2	152.5	89.1	98.5	91.8	117.4	133.3
% change from control		-	-2.0	7.1	4.1	46.8	-	9.0	2.6	27.3	49.6
	n	10	NT	NT	10	9	10	NT	NT	10	9
Water consumption-Recovery (ml/animal)	Days 229-232	129.9	-	-	120.1	115.1	106.2	-	-	103.3	107.7
% change from control		-			-7.5	-11.4	-			-2.7	1.4

NT = not tested

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Ophthalmoscopy

Ophthalmology examination was conducted on all animals once prior to randomization, 1-week prior to interim for all groups 1-5 and 1-week prior to interim recovery sacrifice and 1 week prior to terminal sacrifice for the remaining animals.

Based on slit lamp and indirect ophthalmoscopy, there were no drug-related ophthalmic changes in RDEA594 treated males and females compared to controls.

ECG

ECG measurements were not recorded in this study.

Hematology

Blood samples (1.0 mL) were collected by jugular vein puncture or from the retro-orbital plexus from food fasted animals on Days 15, 23, 29, 92, 120 (3 month study, recovery animals), 176, and 232 (6 month study, recovery animals). A complete battery of hematology parameters was assessed. Blood samples (1.8 mL) were also collected for coagulation assessment at the above time points with the exception of day 15

Day 23
There were no clear drug related effects on hematology parameters between control and 600 mg/kg treated rats euthanized on day 23

Day 92
Statistically significant decreases vs. controls in red blood cells (RBCs), hematocrit and hemoglobin levels were observed in 300 mg/kg males and females vs. controls at day 92. Although statistically significant, the magnitude of these differences (-2.0% to -8.7%) is small and does not appear to be biologically relevant.

Day 176
Statistically significant decreases vs. controls in RBC, hematocrit, and hemoglobin levels were also observed in males and females on day 176 (M: 100 mg/kg, 300 mg/kg; F: 300 mg/kg). The magnitude of the effect size was less than 9.5% in all cases, thus limiting the biological significance of these effects. Females in the 100 mg/kg and 300 mg/kg dose groups also had statistically significant, but small (<10%) decreases in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), which might be suggestive of potential **anemia**.

No drug related effects were observed in the **coagulation** parameters activated partial thromboplastin time (APTT) or prothrombin time (PT) in males or females at any dose or time point.

Clinical Chemistry

Blood samples (1.2 mL) were collected by jugular vein puncture or from the retro-orbital plexus from food fasted animals on Days 15, 29, 92, 120 (interim recovery), 176, and 232 (terminal recovery animals). A complete clinical chemistry battery was evaluated.

Day 23
HD animals had markedly increased total bilirubin vs. controls (~+300%) in males and females (**Table 13**). Increased serum alanine aminotransferase (ALT) was also observed in males. Elevated serum creatine kinase (+62%) as well as blood urea nitrogen (+38%) in males provides evidence of **renal** toxicity which is consistent

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with findings of decreased kidney weight (**Table 19**) as well as tubular degeneration and single cell necrosis in HD animals (Table 22). Elevated triglycerides in 600 mg/kg F may be secondary to hepatic injury. Hepatocellular hypertrophy was observed in most of the HD animals at the time of necropsy. Further evidence of liver pathology included minimal single cell necrosis in 1/16 M (Table 22) and minimal (1/16 M) to mild (1/16 M) coagulative necrosis in single animals.

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Table 13. RDEA494. **Study SR08-095,** Day 23. Clinical chemistry findings in Sprague Dawley rats

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n	8	-	-	-	-	16	10	-	-	-	-	15
Alanine Aminotransferase (U/L)	Day 23	34.9	NT	NT	NT	NT	63.9	37.8	NT	NT	NT	NT	37.4
% change from control							83.1	0.0					-1.1
Creatine Kinase (U/L)	Day 23	997.9	NT	NT	NT	NT	1613.4	623.8	NT	NT	NT	NT	636.5
% change from control							61.7	0.0					2.0
Total Bilirubin (µM)	Day 23	0.5	NT	NT	NT	NT	1.948	0.56	NT	NT	NT	NT	2.37
% change from control							289.6	0.0					323.2
Blood Urea Nitrogen (mM)	Day 23	4.98	NT	NT	NT	NT	6.88	6.86	NT	NT	NT	NT	7.27
% change from control							38.2	0.0					6.0
Triglycerides (mM)	Day 23	0.464	NT	NT	NT	NT	0.39	0.106	NT	NT	NT	NT	0.261
% change from control							-15.9	0.0					146.2

NT = not tested

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Day 92

Clinical chemistry findings for the 3 month study are summarized in **Table 14**. Statistically significant decreases in serum glucose (~-15%) and triglyceride (-50 to -62%) levels, as well as increases in serum cholesterol (+31 to +43%), and alkaline phosphatase (+70 to +82%) in males and females at 300 mg/kg are all characteristic of **hepatic** toxicity, consistent with findings on day 23. Increases in alanine aminotransferase (ALT) and A:G ratio, also indicators of liver pathology, were seen in 300 mg/kg males only. Despite reaching statistical significance, the relatively small magnitude of effect sizes (all less than 2-fold change vs. controls) and lack of clear histopathological correlates in the liver (**Table 23**) outside of the observed hepatocellular hypertrophy (adaptive response) limit the biological significance of these findings.

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Table 14. RDEA494. Study SR08-095, 3 month study. Clinical chemistry findings in Sprague Dawley rats.

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n (recovery)		35 (10)	25	25	35 (10)	34 (9)	NT	35 (10)	25	24	34 (9)	34 (9)	NT
Alanine aminotransferase (U/L)	Day 92	33.9	31.5	33.2	38.8	**44.2****	NT	39.9	32.0	31.0	28.4	34.1	NT
% change from control		-	-7.1	-2.1	14.5	**30.4**		-	-19.8	-22.3	-28.8	-14.5	
Alanine aminotransferase Recovery (U/L)	Day 120	36.7	NT	NT	37.6	39.1	NT	35.1	NT	NT	31	29.4	NT
% change from control		-			2.5	6.5		-			-11.7	-16.2	
Alkaline phosphatase (U/L)	Day 92	83.7	90.8	96.4	116.5	**152.6****	NT	39.3	42.0	42.8	54.6	**66.7****	NT
% change from control		-	8.5	15.2	39.2	**82.3**			6.9	8.9	38.9	**69.7**	
Alkaline phosphatase Recovery (U/L)	Day 120	73.0	NT	NT	87.4	87.2	NT	41.4	NT	NT	49.4	38.1	NT
% change from control		-			19.7	19.5		-			19.3	-8.0	
A:G Ratio	Day 92	1.8	1.8	1.8	1.9	**2.4****	NT	2.8	2.6	2.7	2.8	2.9	NT
% change from control		-	-1.1	1.7	7.2	**30.9**		-	-5.9	-5.4	-1.2	4.6	
A:G Ratio Recovery	Day 120	1.9	NT	NT	2.0	2.3	NT	2.8	NT	NT	2.8	3.0	NT
% change from control		-			4.8	21.8		-			0.0	8.3	

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		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n (recovery)		35 (10)	25	25	35 (10)	34 (9)	NT	35 (10)	25	24	34 (9)	34 (9)	NT
Creatinine (µM)	Day 92	24.5	26.0	26.4	29.1	**31.7****	NT	28.7	29.3	29.9	33.9	31.1	NT
% change from control		-	6.1	7.8	18.8	**29.4**		-	2.1	4.2	18.1	8.4	
Creatinine Recovery (µM)	Day 120	25.3	NT	NT	25.4	25.0	NT	28.9	NT	NT	32.4	27.4	NT
% change from control		-			0.4	-1.2		-			12.1	-5.2	
Glucose (mM)	Day 92	6.4	6.2	6.2	5.9	**5.5****	NT	6.6	6.4	6.3	6.0	**5.6****	NT
% change from control		-	-3.4	-3.3	-9.2	**-14.1**		-	-3.6	-5.6	-10.3	**-15.5**	
Glucose Recovery (mM)	Day 120	7.0	NT	NT	6.6	**6.5***	NT	6.5	NT	NT	6.2	6.6	NT
% change from control		-			-5.7	**-7.1**					-4.3	1.6	
Cholesterol (mM)	Day 92	2.1	2.3	2.2	2.8	**3.0****	NT	2.3	2.3	2.4	2.7	**3.0****	NT
% change from control		-	7.9	5.6	32.3	**41.3**		-	0.0	5.7	18.2	**31.0**	
Cholesterol Recovery (mM)	Day 120	2.1	NT	NT	2.2	2.2	NT	2.1	NT	NT	2.6	2.3	NT
% change from control		-			2.8	5.1		-			23.0	10.5	
Triglycerides (mM)	Day 92	0.5	0.5	0.5	0.3	**0.2****	NT	0.2	0.2	0.1	0.1	0.1	NT
% change from control		-	6.0	6.0	-42.0	**-62.0**		-	31.3	-18.8	-31.3	-50.0	

Reference ID: 3836021

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n (recovery)		35 (10)	25	25	35 (10)	34 (9)	NT	35 (10)	25	24	34 (9)	34 (9)	NT
Triglycerides Recovery (mM)	Day 120	0.8	NT	NT	0.6	0.5	NT	0.2	NT	NT	0.3	0.2	NT
% change from control		-			-14.2	-30.3		-			27.9	-19.8	

NT = not tested
*p<0.05, **p<0.01 vs. control, ANOVA, Dunnett's post hoc test

Reference ID: 3236021

Day 176

As in the 23 day study and 3 month study, several clinical chemistry parameters provide evidence for **liver** toxicity (**Table 15**) after 6 months of RDEA594 treatment. Serum **alkaline phosphatase** levels were significantly increased vs. controls in males at doses ≥ **100 mg/kg** (+54 –+ 71%) as well as 300 mg/kg females (+53%). **Triglyceride** levels were dose dependently decreased in males and females receiving ≥ **100 mg/kg** (-33 to -70%). The magnitude of these effect sizes were both similar to changes seen in the 3 month study. **ALT** was dose dependently increased in males, as seen at the two previous time points. **A:G** ratio was dose dependently increased in males and females (+13 to +33%) to a similar degree as in the 3 month study. As with the 3 month study, the relatively small magnitude of changes in liver enzyme levels and lack of clear histopathological correlates in the liver (Table 24) outside of the observed hepatocellular hypertrophy (adaptive response) limit the biological significance of these findings.

Serum creatinine values are significantly increased vs. controls in 300 mg/kg males (+32%), an effect that reversed in recovery animals. Females showed no effect of drug treatment on creatinine levels. Elevated creatinine is consistent with decreased kidney weight findings (**Table 21**), and suggests that males may be more susceptible to kidney toxicity from RDEA594. Statistically significant decreases (-10 to -20%) were observed in BUN:creatinine ratio in males receiving doses ≥ **30 mg/kg**. Although these clinical chemistry values point toward evidence of kidney damage, there is limited histopathological evidence to support this at the 6 month time point (Table 24). Tubular dilatation was observed in a subset of males (1/15 100 mg/kg; 3/15 300 mg/kg) and females (4/15 300 mg/kg). However this finding may not be indicative of overt kidney damage. It is likely the result of obstruction of kidney tubules due to crystalline deposits. Crystal formations, considered by the sponsor to be salt forms of the test article or a metabolite, were observed in the urine of animals dosed at ≥ 100 mg/kg/d (see 'Urinalysis'), and may be the source of the observed tubular dilatation.

Reference ID: 3816021

Table 15. RDEA494. Study SR08-095, 6 month study. Clinical chemistry findings in Sprague Dawley rats.

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n (recovery)		25 (10)	15	14	25 (10)	24 (9)	NT	24 (10)	15	14	25 (10)	24 (9)	NT
Alanine aminotransferase (U/L)	Day 176	38.9	32.7*	33.9	41.6	**44.7***		52.0	46.9	48.8	37.8	**33.5***	
% change from control		-	-15.9	-12.9	6.9	**14.9**		-	-9.8	-6.2	-27.3	**-35.6**	
Alanine aminotransferase Recovery (U/L)	Day 232	41.4	NT	NT	39.6	41.9		43.0	NT	NT	56.7	33.8	
% change from control		-			-4.3	1.2		-			31.9	-21.4	
Alkaline phosphatase (U/L)	Day 176	69.6	68.2	75.6	**107.5****	**118.8 ****		31.9	31.3	33.9	40.7	**48.8****	
% change from control		-	-2.0	8.6	**54.5**	**70.7**		-	-1.9	6.3	27.6	**53.0**	
Alkaline phosphatase Recovery (U/L)	Day 232	70.3	NT	NT	82.5	64.7		28.2	NT	NT	32.6	32.1	
% change from control		-			17.4	-8.0		-			15.6	13.8	
A:G Ratio	Day 176	1.8	1.7	1.8	**2.0****	**2.4****		2.7	2.5	2.8	2.9	**3.1****	
% change from control		-	-4.9	0.5	**10.4**	**33.0**		-	-6.7	1.9	8.1	**13.7**	
A:G Ratio Recovery	Day 232	2.1	NT	NT	2.3	2.4*		2.9	NT	NT	2.9	3.0	
% change from control		-			11.6	15.7		-			-1.2	4.9	

Reference ID: 3236021

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n (recovery)		25 (10)	15	14	25 (10)	24 (9)	NT	24 (10)	15	14	25 (10)	24 (9)	NT
Creatinine (µM)	Day 176	27.4	27.2	29.5	**33.4****	**36.4****		28.1	28.7	31.4	**33.1****	31.2	
% change from control		-	-0.7	7.7	**21.9**	**32.8**		-	2.1	11.7	**17.8**	11.0	
Creatinine Recovery (µM)	Day 232	25.2	NT	NT	25.7	25.9		28.7	NT	NT	29.2	26.7	
% change from control		-			2.0	2.8		-			1.7	-7.0	
BUN: Creatinine Ratio	Day 176	20.7	20.2	**18.5****	**17.2****	**16.7****		21.9	22.2	21.8	20.0	21.8	
% change from control		-	-2.2	**-10.4**	**-16.9**	**-19.5**		-	1.4	-0.8	-9.1	-0.6	
BUN/Creatinine Ratio Recovery	Day 232	22.3	NT	NT	21.4	23.7		25.6	NT	NT	25.5	26.4	
% change from control		-			-4.1	6.3		-			-0.6	3.1	
Triglycerides (mM)	Day 176	0.8	0.7	0.6	**0.4****	**0.2****		0.2	0.1	0.1	**0.1****	**0.1****	
% change from control		-	-15.4	-24.4	**-51.3**	**-70.5**		-	-22.2	-22.2	**-33.3**	**-50.0**	
Triglycerides Recovery (mM)	Day 232	0.8	NT	NT	0.5	0.5		0.3	NT	NT	0.4	0.3	
% change from control		-			-34.5	-37.5		-			31.3	7.1	

NT = not tested
*p<0.05, **p<0.01 vs. control, ANOVA, Dunnett's post hoc test

Reference ID: 3236021

Urinalysis

Urine was collected from all animals in metabolic cages on Day 23, 29, 92, 120 (interim recovery), 176, and 232 (terminal recovery animals). Urine parameters assessed included: color, clarity, glucose, bilirubin, ketone, specific gravity, occult blood, pH, protein, urobilinogen, nitrite, leukocyte, urine volume and microscopic sediment assessment. When crystals were observed in urine sediment urine sample smears were prepared and evaluated by a board certified clinical pathologist.

Day 23
On day 23, 1/16 HD males (#12400) had urine that was turbid, along with increased bilirubin, occult blood, urobilinogen, leukocytes, and ketones in the urine. This animal had evidence of erosion of the stomach mucosa and mild necrosis of the cecum mucosa. The urine findings are consistent with observation of mild kidney tubular degeneration and tubular single cell necrosis (minimal) observed in this animal.

Day 92
No clear treatment related effect on urine parameters was observed in males or females after 3 months of RDEA594 treatment. Sporadic evidence of increased blood, protein levels, urobilinogen, and leukocytes were observed, but there was no evidence of dose-dependence on these observations.

Day 176
Evidence for occurrence of spontaneous nephropathy in Sprague-Dawley rats is seen in **control** males. 7/25 had measures of protein of greater than 1+. RDEA594 treated animals did not show evidence for exacerbation of this effect. As on day 92, sporadic evidence of increased blood, protein levels, urobilinogen, and leukocytes were observed, but there was no evidence of dose-dependence on these observations.

Urine volume

There was no clear effect of 600 mg/kg RDEA 594 treatment on urine volume at day 23 due to high variability in mean values. Mean values were also highly variable at day 92 and day 96, but differences between **300 mg/kg** males and females and their concurrent controls achieved statistical significance at both time points (**Table 16**). Data from recovery animal values are difficult to interpret, as urine volumes appear to return toward baseline levels in males, while remaining elevated in females. The observed increase in urine volume after 3 and 6 months of treatment is consistent with observations of increased water consumption (**Table 12**). Changes in urinalysis parameters such as increased urine volume might be attributed to renal pathology and/or decreased capacity to concentrate urine. There is limited histopathological evidence of overt renal injury at the 6 month time point in the present study (Table 24). Urine specific gravity values were examined to assess the ability of RDEA594 treated rats to concentrate urine. Decreased specific gravity values indicate urine that is more dilute. Mean urine specific gravity in 300 mg/kg males was slightly but not significantly decreased vs. controls (**Control**: 1.024 ± 0.006; **300 mg/kg**: 1.020 ± 0.006) at the 6 month time point. A similar effect was seen in 300 mg/kg females (**Control**: 1.024 ± 0.009; **300 mg/kg:** 1.018 ± 0.006). As a URAT1 inhibitor, RDEA 594 inhibits the reabsorption of uric acid from the renal tubule back into the blood. A resultant increase in osmolarity of the glomerular filtrate may lead to a decrease in reabsorption of water, and more dilute urine. Therefore, the observed increase in urine volume may be a consequence of the pharmacological mechanism of action of RDEA 594.

Reference ID: 3816021

Crystal formation

Photomicrographs of urine wet mount preparations as well as air-dried slide preparations of urine from females in the 100 mg/kg group (d 176), M & F in the 300 mg/kg group (d 92, d 176) and the 600 mg/kg group (d 23) showed evidence of 'unusual' grey – light brown, circular to oval shaped crystals, 20 – 40 µm in diameter. These were considered by the study pathologist to be salt compounds comprised of test article or a test article biotransformation product.

Reference ID: 3216021

Table 16. IND 102128. 6 month rat study. Urine volume measurements.

RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
		Day 23											
	n	8	NT	NT	NT	NT	16	10	NT	NT	NT	NT	15
Mean Urine Volume (ml)		19.5					13.31	9.15					11.47
% change from control							-31.7						25.4
		Day 92											
	n	35	25	25	35	34	NT	35	25	24	34	34	NT
Mean Urine Volume (ml)		10.41	11.26	12.68	14.77	**22.03****	-	8.78	7.94	7.53	12.18	**12.99****	-
% change from control		-	8.2	21.8	41.9	**111.6**			-9.6	-14.2	38.7	**47.9**	
	n	10	NT	NT	10	9	NT	10	NT	NT	9	9	NT
Mean Urine volume-Recovery (ml) [day 120]		13.85	-	-	12.85	21.89	-	5.00	-	-	5.33	9.22	-
% change from control					-7.2	58.1					6.6	84.4	
		Day 176											
	n	25	15	14	25	24	NT	25	15	14	25	24	NT
Mean Urine Volume (ml)		11.60	11.80	9.46	13.88	**16.79****	-	7.22	7.05	6.43	10.28	**11.35****	

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% change from control		-	1.7	-18.4	19.7	**44.7**		-	-2.4	-10.9	42.4	**57.2**	
	n	10	NT	NT	10	9	NT	10	NT	NT	9	9	NT
Mean Urine volume-Recovery (ml) [day 232]		14.60	-	-	11.27	11.03	-	4.04	-	-	6.11	8.02	-
% change from control					-22.8	-24.5					51.2	98.5	

NT = not tested
**p<0.01 vs. Control. One way ANOVA, Dunnett's post hoc test

Reference ID: 3215021

Gross Pathology

Gross examination included external surface area, orifices, cranial cavity, external surface of the brain, the cranial, thoracic, abdominal and pelvic cavities and their viscera, cervical areas, carcass and genitalia.

Day 23

Discoloration of **GI tract** organs (focal and multifocal) was observed occasionally in 600 mg/kg treated males and females. This appears likely to be the result of direct toxicity of the administered dose of the drug substance. The observation of pelvis dilatation in the **kidney** in 2/15 HD females is consistent with evidence of multiple microscopic pathologies observed in animals treated with 600 mg/kg/d (Table 22). Lung discoloration is seen in 1/16 HD M and 1/15 HD M. These observations are considered to be artifacts of the oral gavage delivery method, and not related to a direct effect of RDEA 594. The ovarian cyst found in 1/15 F is not considered drug related.

Table 17. RDEA 594 Study 08-095. Day 23. Gross pathology findings in Sprague-Dawley rats.

	Males						**Females**					
RDEA594 dose mg/kg/day	0	10	30	100	300	600	0	10	30	100	300	600
	n=8	NT	NT	NT	NT	*n*=16	*n*=8	NT	NT	NT	NT	*n*=15
Day 23												
Thymus Discoloration; red, focal	0	-	-	-	-	0	0	-	-	-	-	1
Lungs with mainstem bronchi Discoloration; red, multifocal	0	-	-	-	-	1	0	-	-	-	-	0
Discoloration, white, cranial, focal	0	-	-	-	-	0	0	-	-	-	-	1
Stomach Discoloration, red glandular, mucosa, *focal*	0	-	-	-	-	1	0	-	-	-	-	0
Discoloration, red glandular, mucosa, *multifocal*	0	-	-	-	-	0	0	-	-	-	-	1
Discoloration, dark red, glandular; *multifocal*	0	-	-	-	-	1	0	-	-	-	-	0
Discoloration, dark red, glandular; mucosa, *focal*	0	-	-	-	-	1	0	-	-	-	-	0
Discoloration, dark red glandular, mucosa, *multifocal*	0	-	-	-	-	0	0	-	-	-	-	2
Yellow material content	0	-	-	-	-	0	0	-	-	-	-	1
Jejunum Dark brown content	0	-	-	-	-	1	0	-	-	-	-	0
Cecum Discoloration, red, mucosa, *focal*	0	-	-	-	-	1	0	-	-	-	-	0

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Kidney Dilation right pelvis	0	-	-	-	-	0	0	-	-	-	-	2
Mass, white	0	-	-	-	-	1	0	-	-	-	-	0
Ovary Cyst, firm, right, *focal*	-	-	-	-	-	-	0	-	-	-	-	1
NT = not tested												

3 month study

Evidence for discoloration of the **stomach** mucosa (both focal and multifocal) was observed sporadically in 300 mg/kg males and females (**Table 18**). These observations are similar to those seen in the 600 mg/kg animals at day 23, and likely represent direct toxicity of the administered dose of the drug substance. These findings correlate with histopathological evidence of glandular erosion and hemorrhage/congestion of the stomach mucosa in 300 mg/kg animals (**Table 23**). Discoloration of the eye seen in 1/10 300 mg/kg F as well as discoloration of the ovary (1/10 300 mg/kg F) are considered incidental findings not related to RDEA594 administration.

Table 18. RDEA 594 Study 08-095**. 3 month** study**.** Gross pathology findings in Sprague-Dawley rats.

	Males						**Females**					
RDEA594 dose mg/kg/day	0	10	30	100	300	600	0	10	30	100	300	600
	n=10	*n*=10	*n*=10	*n*=10	*n*=9	-	*n*=10	*n*=10	*n*=9	*n*=10	*n*=10	-
Day 92 (3 months)												
Stomach Discoloration, dark, glandular, mucosa, multifocal	0	0	0	0	1	-	0	0	0	0	0	-
Discoloration, red, mucosa, glandular, multifocal	0	0	0	0	0	-	0	0	0	0	1	-
Discoloration, dark red, glandular, mucosa, focal	0	0	0	0	1	-	0	0	0	0	2	-
Discoloration, dark red, glandular, mucosa, multifocal	0	0	0	0	1	-	1	0	0	1	3	-

6 month study

There was no evidence of treatment-related gross pathology in any organ at the 6 month time point. The lack of evidence of gross pathology in the stomach may suggest that adaptive mechanisms developed over time after repeated administration of the study drug.

Reference ID: 3816021

Organ Weights

A complete battery of organs and tissues were collected and preserved. Organ weight was measured for: adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes with epididymides, thymus and uterus with cervix.

Day 23

A summary of organ weight findings at day 23 is found in **Table 19**.

Absolute **heart** weight, and heart: brain weight were **decreased** vs. controls (-18 to -28%) in males and females receiving 600 mg/kg/day. The significance of the observed decreases in heart weight is questionable, as there were no treatment related macroscopic or microscopic changes in the heart in males or females in the 600 mg/kg dose group.

Absolute **liver** weight, liver: Body weight and liver: Brain weights were all **increased** vs. controls **(+16 to +50%)** in males and females receiving 600 mg/kg/day. Increased liver weight is correlated with microscopic findings including hepatocellular hypertrophy (Table 22).

Finally, absolute **kidney** weight and kidney:brain weight were **decreased** vs. controls (-15 to -21%) in males and **increased** vs. controls (+13 to +28%) in females receiving 600 mg/kg/day. While the discrepancy between males and females is unclear, both males and females had microscopic evidence of tubular necrosis and degeneration (Table 22).

Reference ID: 3216021

Table 19. IND 102128. RDEA 594. Study SR08-095. Day 23. Organ weights.

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n	8	-	-	-	-	16	9	-	-	-	-	15
Liver	Absolute organ weight (g)	11.06	NT	NT	NT	NT	12.84	7.87	NT	NT	NT	NT	9.66
	% change from control	-					16.1	-					22.8
	Organ:Body weight ratio (%Body Wt)	3.14	NT	NT	NT	NT	4.72	3.33	NT	NT	NT	NT	4.60
	% change from control	-					50.6	-					37.9
	Organ:Brain weight ratio (% Brain Wt)	529.74	NT	NT	NT	NT	659.20	418.33	NT	NT	NT	NT	509.50
	% change from control	-					24.4	-					21.8
Kidney	Absolute organ weight (g)	3.13	NT	NT	NT	NT	2.47	2.05	NT	NT	NT	NT	2.33
	% change from control	-					-21.1	-					13.6
	Organ:Body weight ratio (%Body Wt)	0.89	NT	NT	NT	NT	0.91	0.87	NT	NT	NT	NT	1.12
	% change from control	-					2.6	-					28.7
	Organ:Brain weight ratio (% Brain Wt)	149.84	NT	NT	NT	NT	126.97	108.75	NT	NT	NT	NT	123.18

Reference ID: 3236021

		Males						Females					
RDEA594 (mg/kg/day)		**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n	8	-	-	-	-	16	9	-	-	-	-	15
	% change from control	-					-15.3	-					13.3

Reference ID: 3236021

Day 92/120

A summary of organ weight findings after 3 months of RDEA 594 treatment is found in **Table 20**. **Heart** weight was decreased in males and females but this finding was not associated with any treatment related macroscopic or microscopic changes.

Absolute **liver** weight, liver:body weight, and liver:brain weight were all significantly **increased** vs. controls (+17 to +33%) in 300 mg/kg males. This effect reversed in recovery animals, as recovery males had decreased liver weights compared to controls on day 120. In 300 mg/kg females, all three liver weight measures were also significantly increased vs. controls to a similar magnitude as males receiving the same dose of RDEA594 (+22 to +35%). Liver weights in recovery animals were similar to controls. As in the 23 day study, increased liver weight correlated with hepatocellular hypertrophy (**Table 23**). These findings in the liver are considered to be adaptive.

Similar to the results on day 23 in the 600 mg/kg males, **kidney** weight was **decreased** in 300 mg/kg males (-14 to -15%) on day 92. Absolute kidney weight and kidney:brain weights were significantly lower than concurrent controls. Kidney weight values were closer to controls after the 4 week recovery period, but were still lower. There was no clear effect of drug treatment on kidney weight in females at this time point. Evidence for microscopic correlates (**Table 23**) to these changes in kidney weight in males was limited to cyst, minimal (1/9 M) and mononuclear cell infiltrate, minimal (1/9). The significance of decreased kidney weights is unclear.

Reference ID: 3816021

Table 20. IND 102128. RDEA 594. SR08-095. 3 month study (d92). Organ weights

		Males						Females					
	RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n (recovery n)	10 (10)	10 (NT)	10 (NT)	10 (10)	9 (9)	NT	10 (10)	10 (NT)	10 (NT)	10 (9)	9 (9)	NT
Liver	Absolute organ weight (g)	14.68	14.64	14.93	15.48	**17.16****		8.2	7.7	8.25	8.83	**10.11***	
	% change from control		-0.3	1.7	5.4	**16.9**			-6.1	0.6	7.7	**23.3**	
	Recovery (d 120) Absolute organ weight (g)	16.23	NT	NT	14.65	**13.97***		7.46	NT	NT	7.71	7.85	
	% change from control	-			-9.7	**-13.9**		-			3.35	5.20	
	Organ:Body weight ratio (% body wt.)	2.66	2.69	2.78	**2.94***	**3.54****		2.65	2.69	2.71	**3.06****	**3.58****	
	% change from control	-	1.0	4.4	**10.2**	**33.0**		-	1.6	2.4	**15.3**	**35.1**	
	Recovery (d 120) Organ:Body weight ratio (% Body wt.)	2.68	NT	NT	2.65	2.62		2.48	NT	NT	2.60	2.69	
	% change from control	-			-1.3	-2.5		-			4.7	8.3	
	Organ:Brain weight ratio (%Brain wt)	663.99	659.62	678.02	714.26	**793.77****		415.95	391.33	415.09	441.19	**507.73****	
	% change from control	-	-0.7	2.1	7.6	**19.5**		-	-5.9	-0.2	6.1	**22.1**	
	Recovery (d120) Organ:Brain weight ratio (% Brain wt)	716.07			648.71	**639.66***		389.01			395.33	406.33	
	% change from control	-			-9.4	**-10.7**		-			1.6	4.5	

Reference ID: 3236021

		Males						Females					
	RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n (recovery n)	10 (10)	10 (NT)	10 (NT)	10 (10)	9 (9)	NT	10 (10)	10 (NT)	10 (NT)	10 (9)	9 (9)	NT
Kidney	Absolute organ weight (g)	4.08	3.88	3.88	**3.66****	**3.44****		2.28	2.18	2.25	2.18	2.29	
	% change from control	-	-4.9	-4.9	**-10.3**	**-15.7**		-	-4.69	-1.58	-4.73	0.07	
	Recovery (d 120) Absolute organ weight (g)	4.46	NT	NT	3.95	**3.95****		2.15	NT	NT	2.15	2.28	
	% change from control	-			-11.43	**-11.43**		-			-0.19	6.21	
	Organ:Body weight ratio (% body wt.)	0.74	0.72	0.73	0.70	0.71		0.74	0.76	0.74	0.75	**0.81****	
	% change from control	-	-3.6	-2.4	-6.4	-4.3		-	3.5	0.1	1.8	**10.0**	
	Recovery (d 120) Organ:Body weight ratio (% Body wt.)	0.73	NT	NT	0.71	0.74		0.71	NT	NT	0.72	**0.78***	
	% change from control	-			-3.1	0.8		-			1.3	**9.8**	
	Organ:Brain weight ratio (%Brain wt)	184.98	175.11	176.11	169.04	**159.16****		115.82	110.73	113.03	108.67	115.41	
	% change from control	-	-5.3	-4.8	-8.6	**-14.0**		-	-4.4	-2.4	-6.2	-0.4	
	Recovery (d120) Organ:Brain weight ratio (% Brain wt)	196.54	NT	NT	174.78	180.78		111.78	NT	NT	110.03	118.22	
	% change from control	-			-11.1	-8.0		-			-1.6	5.8	

NT = not tested
* p <0.05, **p<0.001; 1 way ANOVA, Dunnett's post hoc test

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Day 183/239

A summary of organ weight findings after 6 months of RDEA 594 treatment is found in **Table 21**. Absolute **heart** weight was significantly decreased in males (-11.9%) and females (-12.4%) treated at the 300 mg/kg dose level. However, as in the 23 day and 3 month studies, there was no correlating histopathology.

Absolute **liver** weight, liver:body weight, and liver:brain weight values were **increased** vs. controls in **300 mg/kg** males (+15 to +33%) and females (+30 to +43%) at day 183. These findings were associated with microscopic findings of hepatocellular hypertrophy in 300 mg/kg males and females (Table 24). The magnitude of these increases is similar to what was observed at days 23 and 92. Liver weights were similar to, or slightly decreased, vs. controls in recovery animals after an 8-week recovery period.

Absolute **kidney** weight and kidney:brain weight were significantly **decreased** in both the **100 mg/kg** (-12 to -17%) and **300 mg/kg** (-14 to -18%) males vs. controls after 6 months of RDEA594 treatment. The magnitude of the decrease is similar to what was seen after 3 months of treatment. There was no apparent effect of RDEA594 treatment on kidney weight in females at day 183. Microscopic evidence of tubular dilatation is seen in 1/15 100 mg/kg males and in both males (3/15) and females (4/15) in the 300 mg/kg dose groups (Table 24).

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Table 21. IND 102128. RDEA 594. 6 month rat study (d183) **Organ weights**

		Males						Females					
	RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n (recovery n)	15 (10)	15 (NT)	14 (NT)	15 (10)	15 (9)	NT	13 (10)	15 (NT)	13 (NT)	15 (10)	15 (9)	NT
Liver	Absolute organ weight (g)	16.52	15.72	16.63	16.85	**19.12****		8.63	8.98	8.27	9.5	**11.2****	
	% change from control		-4.8	0.7	2.0	**15.7**			4.1	-4.2	10.1	**29.8**	
	Recovery (d 239) Absolute organ weight (g)	18.07	NT	NT	16.23	15.91		10.17	NT	NT	9.40	9.03	
	% change from control	-			-10.2	-12.0		-			-7.5	-11.2	
	Organ:Body weight ratio (% body wt)	2.51	2.54	2.54	**2.81****	**3.33****		2.52	2.69	2.67	**2.89****	**3.62****	
	% change from control		1.1	0.9	**11.8**	**32.7**			6.8	6.0	**14.9**	**43.7**	
	Recovery (d 239) Organ:Body weight ratio (% body wt)	2.52	NT	NT	2.40	2.50		2.53	NT	NT	2.60	**2.81****	
	% change from control	-			-4.7	-0.7		-			2.8	**10.8**	

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		Males						Females					
	RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n (recovery n)	15 (10)	15 (NT)	14 (NT)	15 (10)	15 (9)	NT	13 (10)	15 (NT)	13 (NT)	15 (10)	15 (9)	NT
	Organ:Brain weight ratio (% Brain wt)	749.82	695.67	731.00	772.79	868.31		415.54	442.64	402.89	467.86	567.83	
	% change from control	-	-7.2	-2.5	3.1	15.8		-	6.5	-3.0	12.6	36.6	
	Recovery (d239) Organ:Brain weight ratio (% Brain wt)	783.30	NT	NT	724.37	694.48		505.77	NT	NT	451.38	447.64	
	% change from control	-			-7.5	-11.3		-			-10.8	-11.5	
Kidney	Absolute organ weight (g)	4.46	4.19	4.02	**3.69**	**3.66**		2.53	2.45	2.37	2.31	2.47	
	% change from control	-	-6.1	-9.9	**-17.3**	**-17.9**		-	-3.2	-6.3	-8.7	-2.5	
	Recovery (d 239) Absolute organ weight (g)	4.76	NT	NT	**4.21**	4.27		2.81	NT	NT	2.67	2.74	
	% change from control	-			**-11.6**	-10.4		-			-5.0	-2.6	

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	Males						Females					
RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
n (recovery n)	15 (10)	15 (NT)	14 (NT)	15 (10)	15 (9)	NT	13 (10)	15 (NT)	13 (NT)	15 (10)	15 (9)	NT
Organ:Body weight ratio (% Body wt)	0.65	0.68	0.62	0.62	0.64		0.74	0.74	0.77	0.70	0.80	
% change from control	-	4.2	-5.3	-5.2	-1.9		-	-0.5	4.1	-4.7	8.1	
Recovery (d 239) Organ:Body weight ratio (% Body wt)	0.67	NT	NT	0.62	0.67		0.70	NT	NT	0.74	**0.85****	
% change from control				-6.6	0.7					5.0	**20.7**	
Organ:Brain weight ratio (% brain wt)	193.52	185.19	**176.92***	**169.27****	**166.05****		121.74	120.82	115.56	113.49	124.78	
% change from control	-	-4.3	**-8.6**	**-12.5**	**-14.2**		-	-0.8	-5.1	-6.8	2.5	
Recovery (d239) Organ:Brain weight ratio (% brain wt)	206.82	NT	NT	188.01	186.35		139.98	NT	NT	128.14	135.66	
% change from control	-			-9.1	-9.9		-			-8.5	-3.1	

NT = not tested
* p <0.05, **p<0.001; 1 way ANOVA, Dunnett's post hoc test

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Histopathology

Adequate Battery

Yes. The histopathology study was conducted in compliance with GLP regulations. Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathological evaluation was performed on all tissues from animals sacrificed on day 23 [controls and Group 6 animals (600 mg/kg)]. All tissues from all animals in groups 1 (controls), 4 (100 mg/kg), and 5 (300 mg/kg) sacrificed at the 3 month (day 92) and 6 month (day 183) time points were examined. Histopathology was conducted on selected target tissues of main study animals in groups 2 (10 mg/kg) and 3 (30 mg/kg) as well as in recovery animals in these groups at each time interval. For the 3 month study, target tissues included stomach in groups 2 and 3 and liver in group 3 in both main study animals and recovery animals (day 120). For the 6 month study animals, liver and thyroid were examined in all animals from all dose groups. In recovery animals (Day 239), liver and thyroid were examined in all animals from all dose groups. Tissues which were not examined histopathologically are listed as 'NE' (not examined) in Table 23 and Table 24.

Peer Review

Yes. Two separate microscopic peer reviews were performed for this study. The first was completed and signed on September 16, 2009. In this peer review, all tissues from selected control and treated animals sacrificed on days 23, 92 were re-examined. Re-examination of liver and stomach tissue was carried out for all animals. The second peer review statement was signed on January 15, 2010. All tissues from selected animals in the control and 300 mg/kg dose groups of animals sacrificed on day 183 were re-examined. Liver and thyroid tissue of all animals was re-examined. All neoplasms were examined, as well as all tissues from animals which died during the study.

Histological Findings

Dead and sacrificed animals

See 'Mortality' section for discussion of histopathology findings in dead and sacrificed animals.

Day 23

The principal target organs of toxicity in rats treated with 600 mg/kg/d RDEA 594 were the **liver, stomach, thymus** and **kidney (**Table 22**)**. Consistent with observed increases in **liver** weight, hepatocellular hypertrophy was seen in 16/16 HD males and 12/15 HD F. Hepatocellular hypertrophy is viewed as an adaptive response to metabolize high levels of the drug in the liver and is generally not regarded as a dose-limiting toxicity. However, adverse findings in the liver included single cell necrosis (minimal) and minimal – mild coagulative necrosis in HD males euthanized on day 23. These findings were also observed in 1 M and 1 F in the 300 mg/kg group that suffered premature deaths.

Kidney pathology was evidenced by minimal-moderate tubular degeneration (M: 15/16; F: 15/15) and single cell tubular necrosis (M: 16/16; F: 15/15) in nearly all animals. These pathologies could all be potential causes of mortality in these animals. Benign nephroblastoma was an unusual finding observed in 1/16 HD males. Microscopic evidence of toxicity to the pancreas, spleen, lymph node, bone marrow, stomach and small intestine was observed sporadically. It is notable that observations of minimal- mild glandular erosion of the stomach in 600 mg/kg males (2/16) and females (2/15) were also seen in animals dosed at lower levels after 3 and 6 months of RDEA 594 treatment (Table 23, Table 24).

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Apoptotic necrosis of the thymus (minimal – mild) was seen in 10/16 HD males and 5/16 HD Females. Apoptotic necrosis (as opposed to oncotic necrosis) is often seen as a secondary response to stress (Elmore, 2006), and may not be related to a direct effect of RDEA594 on the thymus.

Table 22. Histopathology findings, Study 08-095. RDEA 594. Day 23. Sprague-Dawley Rat.

	Males						Females					
RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n=8	NT	NT	NT	NT	*n*=16	*n*=9	NT	NT	NT	NT	*n*=15
Thymus												
Hemorrhage												
Minimal	0	-	-	-	-	0	0	-	-	-	-	1
Necrosis, apoptotic												
Minimal	0					5	2					0
Mild	0	-	-	-	-	5	0	-	-	-	-	4
Moderate	0					0	0					1
Depletion, lymphoid												
Moderate	0	-	-	-	-	1	0	-	-	-	-	0
Liver												
Hypertrophy, hepatocellular												
Minimal	0	-	-	-	-	13	0	-	-	-	-	11
Mild	0					3	0					1
Necrosis, single cell												
Minimal	0	-	-	-	-	1	0	-	-	-	-	0
Necrosis, coagulative												
Minimal	0	-	-	-	-	1	0	-	-	-	-	0
Mild	0					1	0					0
Stomach												
Edema, submucosal, nonglandular												
Minimal	0	-	-	-	-	1	0	-	-	-	-	0
Erosion, glandular												
Minimal	0	-	-	-	-	1	0	-	-	-	-	0
Mild	0					1	0					2
Pancreas												
Atrophy, lobular												
Minimal	0	-	-	-	-	0	0	-	-	-	-	1
Kidney												
Basophilia tubular												
Minimal	0	-	-	-	-	1	0	-	-	-	-	0
Degeneration, tubular												
Minimal	0	-	-	-	-	8	0	-	-	-	-	5
Mild	0					5	0					9
Moderate	0					2	0					1

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	Males						Females					
RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n=8	NT	NT	NT	NT	*n*=16	*n*=9	NT	NT	NT	NT	*n*=15
Necrosis, single cell tubular												
Minimal	0	-	-	-	-	14	0	-	-	-	-	6
Mild	0					2	0					9
Dilatation pelvis, unilateral												
Minimal	0	-	-	-	-	0	0	-	-	-	-	1
Moderate	0					0	0					1
Nephropathy, chronic progressive												
Minimal	0	-	-	-	-	1	0	-	-	-	-	0
Nephroblastoma, benign	0	-	-	-	-	1	0	-	-	-	-	0
NT = Not tested												

3 Month study (Day 92)

All organs from all animals in control, 100 mg/kg, and 300 mg/kg dose groups were examined microscopically at the 3 month time point. Organs from 10 mg/kg and 30 mg/kg animals were generally not examined microscopically, with the exception of the stomach.

Hepatocellular hypertrophy was observed in 7/10 M and 1/10 F in the **100 mg/kg** group and 9/9 M and 8/10 F in the **300 mg/kg** group (**Table 23**). These findings correlated with increased liver weights in both males and females. Recovery animals showed no signs of hepatocellular hypertrophy after a 4-week dose-free period. 100 mg/kg and 300 mg/kg males and females had increased incidence of hepatocellular hypertrophy at **the 6 month** time point (Table 24) relative to the 3 month time point. Hepatocellular hypertrophy is considered to be an adaptive response to drug administration and this change is brought about by the need for increased metabolic activity (Lewis et al. 2002). It is generally not judged to be a dose-limiting finding.

Glandular erosion (minimal – mild), as well as hemorrhage/congestion of the **stomach** mucosa (minimal) was observed in males (2/9 300 mg/kg) and females (2/10 100 mg/kg; 3/10 300 mg/kg) at the conclusion of 3 months of dosing. These findings correlate with gross findings of mucosal discoloration. While mild glandular erosion was observed in 1/10 F dosed at 10 mg/kg, there were no such findings in males or females in the 30 mg/kg dose group. However, incidence increased to 2/10 and 3/10 in the 100 and 300 mg/kg F dose groups respectively. Glandular erosion of the stomach was also observed after **6 months** of dosing, but only in **1/15 300 mg/kg males** (Table 24) suggesting that stomach toxicity did not progress with time. Concern over the stomach findings is lessened in light of reports that erosion/ulceration of the stomach mucosa is a sporadic phenomenon observed in approximately 10% of young control rats (Gad and Chengelis, 1992)

Incidence of single cell necrosis (minimal) of the **pancreas** increased with dose in males after 3 months of treatment, but no pancreatic toxicity was observed in females. No NOAEL could be determined for this finding at the 3 month time point since the sponsor did not perform histopathological examination of pancreas tissue in males in the 10 or 30 mg/kg/d groups. It is noted that this finding was evident in only 1/15 300 mg/kg males at the 6-month sacrifice suggesting that pancreatic toxicity did not progress with time. There was no evidence in

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the available scientific literature to indicate that single cell necrosis of the pancreas occurs spontaneously in the rat. However, this finding was observed in 1/10 control males in the 3 month study.

Apparent **kidney** pathologies were also seen at after 3 months of dosing. Cysts were detected in 1/9 300 mg/kg males as well as 1/10 100 mg/kg and 3/10 300 mg/kg females. Additional findings in the 300 mg/kg group included single instances of tubular dilatation, mononuclear cell infiltrate, and chronic progressive nephropathy. The sponsor did not address these findings in the explanation of results. The kidney pathologies observed at day 92 were reduced as compared to findings observed in 600 mg/kg animals on day 23. Kidney tubular dilatation persisted in animals treated for **6 months** (1/15 M 100 mg/kg; 3/15 300 mg/kg M; 4/15 300 mg/kg F). While tubular dilation may be the result of obstruction due to crystalline deposits, the other findings are not likely to be due to drug treatment.

Recovery animals in the 3 month study (+4 weeks, day 120 [n = 10/sex/group; control, 100 mg/kg, 300 mg/kg]) were only examined histopathologically at selected organs. Furthermore, at these selected organs, not every animal was examined. Liver and stomach were the only organs examined in all recovery animals. There were no drug-related findings observed in liver and stomach of recovery animals.

Table 23. Histopathology findings, Study 08-095. RDEA 594. **3 month** (d 92) plus 28d recovery (d 120) study.

	Males						**Females**					
RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**600**	**0**	**10**	**30**	**100**	**300**	**600**
	n=10	*n*=10	*n*=10	*n*=10	*n*=9	NT	*n*=10	*n*=10	*n*=9	*n*=10	*n*=10	NT
Liver *Hypertrophy, hepatocellular* Minimal	0	NE	0	7	9	-	1	NE	1	1	8	-
Stomach *Erosion glandular* Minimal Mild	0 0	0 0	0 0	0 0	2 0	-	0 0	0 1	0 0	1 1	2 1	-
Hemorrhage/congestion, glandular, mucosa Minimal	0	0	0	0	2	-	0	0	0	0	2	-
Pancreas *Necrosis, single cell* Minimal	1	NE	NE	2	3	-	0`	NE	NE	0	0	-
Kidney *Cyst* Minimal	0	NE	NE	0	1	-	0	NE	NE	1	3	-
Dilation, tubular Minimal	0	NE	NE	0	0	-	0	NE	NE	0	1	-
Infiltrate, mononuclear cell, pelvis Minimal	0	NE	NE	0	1	-	0	NE	NE	0	0	-
Nephropathy, chronic progressive Minimal	0	NE	NE	0	0	-	0	NE	NE	0	1	-
NT= Not tested NE= Not examined												

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6 Month study (Day 183)

As with the 3 month study, all organs from all animals in the control, 100 mg/kg, and 300 mg/kg dose groups were examined microscopically (Table 24) in this section of the study. Organs from 10 mg/kg and 30 mg/kg animals were generally not examined microscopically, with the exception of the liver (all), thymus (subset), lungs (subset), and thyroid (all). In addition, not all recovery animals were subjected to microscopic examination. Liver and thyroid were the only organs that were examined in all recovery animals.

Hepatocellular hypertrophy (minimal) is the only treatment-related histopathology finding in the **liver** after 6 months of RDEA594 treatment (Table 24). This finding is seen in males receiving **30 mg/kg/d** (4/14), **100 mg/kg/d** (15/15) and **300 mg/kg/d** (15/15). No females in the 30 mg/kg dose group had evidence of this finding, however it was observed in nearly all females receiving higher doses (13/15 100 mg/kg; 15/15 300 mg/kg). Hepatocellular hypertrophy was associated with increased serum levels of ALT and alkaline phosphatase (**Table 15**) as well as increased liver weight in these animals (**Table 21**). The increase in liver weight is an expected consequence of hepatocellular hypertrophy, and serum levels of ALT and ALP are clinically monitorable. Hepatocellular hypertrophy is considered to be an adaptive response and is not judged to be dose-limiting. Hepatocellular hypertrophy was not observed in recovery males (+8 weeks) at any dose, but was observed in 1/10 recovery females in the 100 mg/kg group and 2/9 females in the 300 mg/kg group. The evidence suggests that this response is generally reversible after withdrawal of drug treatment.
Minimal focal hyperplasia was observed in the **adrenal** gland of 1/15 100 mg/kg F and 1/15 300 mg/kg F after 6 months of treatment. This is a potentially adverse finding. However, the lack of an increase in incidence between the 100 mg/kg and 300 mg/kg treatment groups despite an approximately 2-fold increase in exposure (Table 25) suggests that this may not be a treatment-related finding. The finding may be considered to be a stress-related toxicity, as adrenal hyperplasia is observed in rats subjected to stress (Greaves and Faccini 1992; Ulrich-Lai et al. 2006). Follicular hypertrophy of the **thyroid** (minimal) was seen with high frequency in 100 mg/kg (M: 8/15; F: 4/15) and 300 mg/kg (M: 12/15; F: 11/15) dose groups. This is considered to be a rat specific finding. Induction of liver cytochrome P450 enzymes can enhance the metabolism of the thyroid hormones, triiodothyronine (T3) and thyroxine (T4). Consequently, thyroid stimulating hormone (TSH) levels are increased in an effort to synthesize more T3 and T4. Elevated TSH levels lead to the observed follicular hypertrophy of the thyroid.

The overall incidence of **stomach** (glandular erosion, 1/15 300 mg/kg M) and **pancreas** (single cell necrosis; 1/15 300 mg/kg M) pathologies was decreased at the 6 month time point compared to the 3 month time point. Furthermore, the apparent potency of RDEA 594 to induce these effects was decreased in animals treated for 6 months (i.e. pathologies first appeared at higher doses in the 6 month study than in the 3 month study). Collectively, the histopathology evidence suggests that apparent RDEA 594 induced stomach and pancreas toxicities do not progress with extended RDEA594 administration. In the rat, systemic RDEA594 exposures were lower in 100 mg/kg or 300 mg/kg animals (Table 25) at both the 3 and 6 month time points compared to the respective exposures on day 1 (accumulation ratio: 0.62 – 0.92). This evidence suggests potential **liver enzyme induction** at doses ≥ 100 mg/kg, which is consistent with findings in the chronic monkey study (SR08-094, Table 40). However, systemic exposure in rats in the 100 mg/kg and 300 mg/kg dose groups remained consistent between the 3 and 6 month time points. Therefore, the decreased incidence of histopathology findings at the 6 month time point vs. the 3 month time point cannot be explained by a decrease in drug exposure. Concern over the stomach finding is further lessened by the observation that erosion/ulceration of the stomach is reported in ~10% of control rats (Gad and Chengelis 1992). The stomach and pancreas findings are not used to define the rat NOAEL due to their low incidence (1/15 300 mg/kg M), minimal severity, and lack of progression from 3 months to 6 months of dosing.

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Pathologies observed in other organs were also generally decreased in incidence and/or severity at 6 months compared to the 3 month time point. Mild tubular dilation of the kidney was observed in 300 mg/kg M (3/15) and F (4/15). Tubular dilation was also observed in 1/1 HD recovery males. This finding is likely the result of obstruction of tubules due to crystalline deposits rather than overt treatment-related kidney damage. Crystal formations were observed in the urine of animals dosed at ≥ 100 mg/kg/d (see 'Urinalysis'). These crystals were considered by the study pathologist to be salt compounds comprised of test article or a test article biotransformation product. Sporadic (≤2/group) findings in the eye, heart, ovaries and mandibular lymph node were considered to be incidental findings and not treatment-related.

Table 24. Histopathology findings, Study 08-095. 6 month study (d 183) plus 2-month recovery (d 239). RDEA 594, Sprague-Dawley Rat.

	Males					Females				
RDEA594 (mg/kg/day)	**0**	**10**	**30**	**100**	**300**	**0**	**10**	**30**	**100**	**300**
	n=15	*n* =15	*n* =14	*n* =15	*n* =15	*n* =13	*n* =15	*n* =13	*n* =15	*n* =15
Mammary gland *Adenocarcinoma; malignant*	-	-	-	-	-	0	0	0	0	1
Liver *Hypertrophy, hepatocellular* Minimal	1	0	4	15	13	0	0	0	13	15
Liver **Recovery (+56 days, Day 239)** *Hypertrophy hepatocellular, periportal* Minimal	0/10	NE	NE	0/10	0/9	0/10	NE	NE	1/10	2/9
Stomach *Erosion, glandular* Minimal	0	NE	NE	0	1	0	NE	NE	0	0
Pancreas *Necrosis, single cell* Minimal	0	NE	NE	0	1	0	NE	NE	0	0
Adrenal gland *Hyperplasia, focal* Minimal	0	NE	NE	0	0	0	NE	NE	1	1
Kidney *Dilation, tubular* Mild	0	NE	NE	1	3	0	NE	NE	0	4
Kidney **Recovery (+56 days, Day 239)** *Dilation, pelvis, unilateral* Minimal	0/1	NE	NE	0/1	1/1	NE	NE	NE	NE	NE
Thyroid *Hypertrophy, follicular epithelium* Minimal	2	1	1	8	12	0	0	0	4	11
NE= not examined										

Histopathology summary

The NOAEL for rats dosed with RDEA594 for 6 months is judged to be **100 mg/kg/day** based upon: (1) deaths at 600 mg/kg/d, (2) histopathological findings in the pancreas and stomach in the 300 mg/kg/d group at the 3-

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month time point and (3) histopathological findings in the kidneys (tubular dilation) at the 6-month time point. At the 6-month time point, the low incidence (1/15 M) and lack of progression lessens concern for apparent histopathology findings in the **stomach** and **pancreas** of rats dosed at 300 mg/kg/day. Potential findings in other tissues were judged to be either adaptive, rat specific, or incidental and did not contribute to NOAEL determination. Severe dose-limiting toxicity was evident at the high dose of 600 mg/kg/day. RDEA594 has a steep dose-response curve based upon a NOAEL at 100 mg/kg/d and death at 600 mg/kg/d.

Toxicokinetics

Blood (0.3 mL) was collected from the retro-orbital plexus of non-fasted toxicokinetic (TK) animals on Days 1, 14 (600 mg/kg only), 22 (600 mg/kg only), 28, 91 and 168 at time points pre-dose,1, 3, 6, 12 and 24 hours post-dose. The samples were processed to plasma, and plasma samples were analyzed for RDEA594 concentration via a validated LC-MS/MS method at [redacted] (b) (4). The lower limit of quantification was reported to be 5.0 ng/ml. TK parameters were determined using WinNonlin Professional software version 5.2 (Pharsight Corp., Mountain View, CA) using a non-compartmental method.

Systemic RDEA594 exposure increased with increasing dose at each time point, and T_{max} ranged from 1 – 6 hours (Table 25). At the two low doses (10 mg/kg/d and 30 mg/kg/d), males had higher systemic exposure than females at all time points. There were no differences in exposure between males and females at doses ≥ 100 mg/kg/d. There was evidence of slight accumulation (1.31 – 1.78) at the 10 mg/kg and 30 mg/kg doses in males and females after 3 and 6 months of treatment. However, systemic RDEA594 exposures were lower in 100 mg/kg or 300 mg/kg animals at both 3 months and 6 months compared to day 1 (accumulation ratio: 0.62 – 0.92). This evidence suggests potential **liver enzyme induction** at doses ≥ 100 mg/kg, which is consistent with findings in the chronic monkey study (SR08-094, Table 40). Systemic exposure in rats in the 100 mg/kg and 300 mg/kg dose groups remained consistent between the 3 and 6 month time points, suggesting that steady state exposure was achieved by 3 months of dosing.

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Table 25. IND 102128. Toxicokinetic parameters for RDEA 594 in 6 month rat study (SR08-095).

		Male			Female		
	Dose (mg/kg/day)	$AUC_{0\text{-}24\,h}$ (µg*h/mL)	Accumulation Ratio (vs. Day 1)	C_{max} (up/mL)	$AUC_{0\text{-}24h}$ (µg*h/mL	Accumulation Ratio (vs. Day 1)	C_{max} (up/mL)
Day 1	10	21.7	-	3.30	12.8	-	2.84
	30	82.9	-	15.6	47.8	-	12.3
	100	595	-	82.0	453	-	99.6
	300	1670	-	158	1360	-	166
	600	2430	-	178	2560	-	204
Day 22	600	2840	1.17	261	4200	1.54	315
Day 92 (3 months)	10	38.3	1.76	4.47	12.2	1.36	3.87
	30	126	1.52	17.9	63.5	1.50	18.5
	100	388	0.65	59.5	373	0.59	58.9
	300	1060	0.63	109	818	0.61	102
Day 176 (6 months)	10	33.9	1.56	5.57	18.1	1.95	5.54
	30	109	1.31	18.8	84.9	2.07	25.5
	100	371	0.62	40.9	459	0.66	65.7
	300	1060	0.63	116	973	0.92	153

Reference ID: 3215021

Conclusions

The NOAEL for RDEA 594 administration to Sprague-Dawley rats for 6 months is **100 mg/kg/day** based on premature deaths (within 3 weeks of start of dosing) associated with kidney and GI toxicities in animals dosed at 600 mg/kg/day and histopathological findings in the stomach and pancreas at the 3-month time point and kidneys at the 6-month time point. At the 6-month time point, concern over potential toxicities in the stomach (glandular erosion) at the 300 mg/kg/day dose level is lessened in light of reports of similar findings in approximately 10% of control rats (Cox & Chengelis 1992; Greaves & Faccini 1992). Apparent toxicities observed in stomach (glandular erosion) and pancreas (single cell necrosis) of animals dosed at 300 mg/kg/day did not increase in incidence or severity from 3 months to 6 months of dosing. The incidence of these findings was particularly low after 6 months of dosing (1/15 M). The NOAEL dose is associated with a mean steady state systemic exposure of $AUC_{0\text{-}24h}$ = 415 (µg*h)/ml. This exposure allows for adequate safety margins at the clinical doses (Table 26) in Phase 3 trials with RDEA594 (Table 1).

Table 26. Systemic exposure at rat NOAEL compared to human exposure at Phase 3 clinical doses.

Species	NOAEL (mg/kg)	$AUC_{0\text{-}24h}$ (µg*h/mL)	Safety Factor
Rat	300	415	-
	Clinical Dose (mg)		
Human[1]	200	25.9	16.0
	400	53.9	7.7

[1] Clinical data taken from study RDEA594-102 (10 day repeat dose study in healthy volunteers)

Stability and Homogeneity

Triplicate samples were taken from the middle position from dose formulations of 1 mg/mL and 60 mg/mL prepared on Week 1 for stability analysis. These samples were tested after (b) (4) Homogeneity was performed on 1 mg/mL and 60 mg/mL formulations prepared for Week 1. Triplicate samples from the top, middle and bottom were analyzed. In addition duplicate samples from top, middle and bottom of dose formulations prepared Weeks 1, 4, 13 and 24 for homogeneity and concentrations were verified.

Concentrations for all examined formulations at Weeks 1, 4, 13 and 24 were within acceptable ranges (+/- 10%) and the formulations were homogeneous. The formulations were stable at (b) (4)

Reference ID: 3236221

6.2.2 Study SR08-094. 12 month study in cynomolgus monkeys

The final report for the 12 month monkey study is in supporting **document #44**. This was received on 12/20/2010.

Study title: RDEA594: A 12-month repeat dose toxicity and toxicokinetic study with a 3-month recovery period and a 6-month pull in **cynomolgus monkey**

Study no.:	SR08-094 (Ardea's study number)
Study report location:	Vol 3., page 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 10, 2009
GLP compliance:	Yes- signed and dated Dec 15, 2010
QA statement:	Yes- signed and dated Dec 15, 2010
Drug, lot #, and % purity:	RDEA594 sodium salt Lot no. 0904697/82.1% Pure Lot no. NE-023025-batch-01-2009/83% Pure Lot no. A10063-28-8/82.4% pure Lot no. A10063-23-20/81.7% pure Lot no. A10060-64-1/82.1% pure Lot no. A10063-80-20/82.4% pure Lot no. A10060-70-3/83.4% pure Lot no. A10060-72-3/80.3% pure Lot no. 0904728/90.6% pure The dose of test article was adjusted to 100% based on purity of each lot. Dose formulations were prepared weekly.

Reference ID: 3216021

6.2.2.1 Key Study Findings

- There were 5 premature deaths in HD animals (600 mg/kg/d).
  - 4 deaths were due to treatment related severe diarrhea, emesis, and decreased food consumption,
  - The other death was due to gavage error.
- There were no premature deaths in the 300 mg/kg/d treatment group.
- One female in the 100 mg/kg/d group died prematurely on day 320
  - The microscopic changes in this animal were not entirely consistent with what was observed in the premature deaths at the HD.
- One male in the 30 mg/kg/d group was moribund sacrificed on day 217.
  - The cause of death was determined to be sepsis secondary to proctitis/colitis.
  - The death was considered unrelated to drug treatment.
- In animals that survived to study completion, clinical signs of diarrhea, emesis and soft stool increased with dose and persisted throughout the study.
- Red discolored urine was observed primarily in animals in the 300 mg/kg/d and 600 mg/kg/d groups.
  - This finding reversed upon cessation of treatment.
  - The source of the discoloration was not identified. There is no evidence to suggest that blood or red blood cells are the source of discoloration.
- Increased **liver** and **kidney** weights were observed in males and females receiving ≥ 100 mg/kg/d RDEA594.
- In the 12 month study, bile duct hyperplasia in 300 mg/kg/d males and 600 mg/kg/d M & F with correlating increase in gamma glutamyltransferase levels in affected animals is considered to be dose limiting.
- Systemic exposure of RDEA594 **decreased** over time in males and females.
  - Exposure in HD females at week 50 is ~12% of exposure at week 1.
- Systemic exposure of metabolite M6 **increased** over time.
  - Exposure of M6 was 3 – 6 fold greater than that of RDEA594 at week 50.
- The no observed adverse effect level is determined to be **100 mg/kg/**day with an $AUC_{0\text{-}24h}$ = 82.6 (µg*h)/ml (average of male and female AUC, week 50) based on bile duct hyperplasia at ≥ 300 mg/kg/day.

Reference ID: 3816021

6.2.2.2 Methods

Doses:	0 (DI water), 30, 100, 300 and 600 mg/kg/day
Frequency of dosing:	Once-daily
Route of administration:	Oral (per os)
Dose volume:	10 mL/kg; based on body weights
Formulation/Vehicle:	Prepared in vehicle of Milli-Q de-ionized water
Species/Strain:	Cynomolgus monkey (*Macaca fascicularis*) Supplied by (b) (4)
Number/Sex/Group:	6-month interim: 3/sex/group 12-month end of dosing phase: 4/sex/group 3-month recovery phase: 3/sex/group
Age:	Males and females: 2-5 years old
Weight:	M: 2.40-3.44 kg F: 1.92-3.24 kg
Satellite groups:	None.
Unique study design:	The proposed study dosed monkeys for 12-months with a six month interim sacrifice.
Deviation from study protocol:	The following dose errors were made as single incidences: • 6 males in the 100 mg/kg/day group were dosed with 300 mg/kg/day on Day 163 by mistake • 3 males in the 300 mg/kg/day group were dosed with 600 mg/kg/day on Day 314 by mistake

Reference ID: 3816021

Table 27. IND 102128. Study SR08-094. RDEA594, 12 month study in monkeys. Animal number and dose group assignments

		Interim Study		**Terminal study**			
Group	Dose (mg/kg/day)	6 months **(d 183, wk 27)**		12 months **(d 365, wk 53)**		12 months + 3 month recovery **(d 456, wk 66)**	
		Male	Female	Male	Female	Male	Female
1	0	0602187 0511159 0611043	0603082 0404216 0406034	0507121 0603323 0610039 0612021	0608252 0611210 0411200 0608608	0512091 0603351 0603351 0509175	0610034 0701088 0604052
2	30	0507105 0603329 0508093	0612152 0508102 0403232	0604075 0511137 0508173 (MS) 0602301	0701026 0510018 0608270 0607056	-	-
3	100	0606077 0511239 0504297	0601162 0701074 0605078	0602167 0601177 0608041 0504153	0606262 0608100 0611016 (MS) 0606270	-	-
4	300	0601187 0512169 0509085	0504274 0702006 0610030	0512121 0603327 0412047 0605141	0612020 0701086 0608244 0609258	0603081 0603341 0506275	0612146 0701072 0606004
5	600	0508175 0602121 0604277 (FD) 0603355 (MS)	0604002 0604208 0512218 (MS) 0504108 (FD) 0608118 (FD)	0510149 0506001 0605139 0612029	0610012 0610026 0606134 0604260	0512813 0601131	0701092 0701044

- = No animals
FD = found dead
MS=Moribund sacrifice

Reference ID: 3215021

6.2.2.3 Observations and Results

Mortality

Cageside observations were performed three times daily. There were a total of **7 premature deaths** in this study (Table 28) which occurred between **days 15 and 320**. 5 animals in the HD (600 mg/kg/day) group died prematurely. The causes of death in 4 of these animals (**0604277, 060335, 0512218, 0608118)** were determined to be secondary to severe diarrhea, emesis and decreased food consumption. These conclusions are supported by evidence of GI tract pathology, including discoloration of tissue, erosion in colon and rectum, and GI components being filled with gas or yellowish material. HD female number **0504108** was found dead on **Day 15** with pulmonary edema. The sponsor considered this death secondary to gavage error, a conclusion that is supported by additional evidence of red/pink foam in the trachea, nasal and oral cavities. This animal was replaced by animal number 0608118 on Day 18.Female number **0611016** (100 mg/kg/day) was moribund sacrificed on **Day 320**. This animal was noted to show signs of lethargy for 3 days prior to moribund sacrifice. On the day of sacrifice, the animal was prostrate and thin with low body temperature. Gross pathology analysis showed slight dark red discoloration of the mucosa in stomach and rectum, moderate red discoloration of the lungs, and marked decrease in thymus size. The microscopic changes in this animal were not entirely consistent with what was observed in the premature deaths at the HD. Specifically, this animal possessed multifocal erosions of the stomach, a more severe observation than what was seen at higher doses. Given that there were no premature deaths among 20 monkeys at the next highest dose (300 mg/kg/day), this death is not considered to be treatment related.

Male **0508173** (30 mg/kg/day) was moribund sacrificed on **Day 217**. This animal was observed to have poor food consumption, moderate lethargy, and was cold to the touch prior to sacrifice. Approximately 15 ml of clear fluid was noted in the thoracic cavity. Mild degeneration of the heart was noted. Specifically, multiple foci of hypereosinophilic fibers were observed in the ventricular myocardium along with increased numbers of cells in the interstitium. These findings in the heart are likely correlated with the buildup of fluid in the thoracic cavity. The cecum, colon and rectum showed evidence of minimal to mild crypt abscesses, while the colon and rectum both had evidence of mild erosion, minimal hemorrhage, and minimal neutrophilic inflammation. The spleen is reported in the text to have mild neutrophilic inflammation of the red pulp, but this observation is not noted in the individual line listing for this animal. Mild lymphocyte depletion of the periarteriolar lymphoid sheath of the spleen is reported. A marked increase in myeloid:erythroid precursor ratio (M:E ratio) in the bone marrow of the sternum was observed, indicative of an infection. The sponsor considered the death of this animal to be due to sepsis secondary to proctitis/colitis, a conclusion that appears to be supported by the available evidence. The death of this animal is not considered to be treatment-related for several reasons. First, there were no animals that died in this manner the 100 mg/kg or 300 mg/kg dose groups. Chronic idiopathic colitis, with symptoms similar to those seen in this animal, has been observed as a relatively common spontaneous finding in captive monkeys (Adler et al. 1993). Furthermore, Chamanza et al. (2010) report inflammation/colitis in 12/570 (2.1%) control cynomolgus monkeys, with an incidence range of 0-37.5% in individual studies, at Charles River Laboratories, Edinburgh Scotland. Finally, animal 0508173 had generally more severe GI tract lesions than those observed at higher doses, suggesting that these findings are not related to RDEA594 treatment.

Reference ID: 3216021

Table 28. IND 102128. Study SR08-094. RDEA594, 12 month study in monkeys. Summary of premature animal deaths.

Dose group	Dose (mg/kg/d)	Sex	Animal no.	Mode of death	Day of death	Cause of death	Findings
2	30	M	0508173	Moribund sacrifice	217	Sepsis secondary to proctitis/colitis (inflammation of colon)	>Colon: crypt abscesses (mild); crypt loss (mild); hemorrhage, neutrophilic inflammation of lamina propria (minimal) >Rectum: crypt abscesses (minimal); hemorrhage (minimal); erosion (mild); neutrophilic inflammation (mild) >Cecum: crypt abscesses (min) >Adrenal: decreased vacuolation (mild) >Bone marrow, sternum: M:E ratio increased (marked) >Thymus: lymphoid depletion >Heart- degeneration of ventricular myocardium (mild) >Spleen: lymphocyte depletion (mild) >Liver- mononuclear cell infiltration (min)
3	100	F	0611016	Moribund sacrifice	320	Undetermined	>Lethargy for 3 days prior to sacrifice >Thin and prostrate on day of sacrifice >Stomach multifocal erosion (minimal) >Rectum: neutrophilic infiltration (focal, minimal) >Pancreas: moderate zymogen

Reference ID: 3215021

Dose group	Dose (mg/kg/d)	Sex	Animal no.	Mode of death	Day of death	Cause of death	Findings
							granule depletion >Thymus: marked decrease in size; moderate lymphoid depletion >Liver inflammation, neutrophilic (min) >Adrenal: decreased vacuolation (mild) >Kidney: tubular cast (mild) >Mandibular lymph node: sinus histiocytosis
5	600 mg/kg/d	M	0604277	Found dead	86	Secondary to severe diarrhea, emesis, decreased food consumption.	>Red discoloration of all components of GI tract >Rectum-mild hemorrhage and erosion >Decreased thymus size >Lymphoid depletion of thymus (moderate- marked) >Spleen lymphoid depletion (minimal) >Increased adrenal size >Mild adrenal cortical hypertrophy >Kidney- minimal tubular degeneration
		M	0603355	Moribund sacrifice	71	Secondary to severe diarrhea, emesis, decreased food consumption.	>Gas filled GI tract components: duodenum, jejunum, ileum, cecum, colon >Red discoloration of colon >Decreased thymus size >Thymus - Lymphoid depletion (moderate- marked)

Reference ID: 3815021

Dose group	Dose (mg/kg/d)	Sex	Animal no.	Mode of death	Day of death	Cause of death	Findings
							>Adrenal – min cortical hypertrophy
		F	0608118 (replacement for 0504108)	Found dead	178	Secondary to severe diarrhea, emesis, decreased food consumption.	>Slight red or dark discoloration of mucosa of stomach, colon, rectum >Decreased thymus size >Marked lymphoid depletion of thymus >Moderate erosion in colon >Minimal erosion in rectum >Moderate atrophy of fat of the skin >Moderate atrophy of fat of epicardium of heart >Moderate zymogen granule depletion of pancreas >Decreased salivary gland secretions >Kidney – tubular basophilia (min)
		F	0512218	Moribund sacrifice	87	Secondary to severe diarrhea, emesis, decreased food consumption.	>Stomach, duodenum, jejunum, ileum, cecum, colon, rectum: filled with yellow fluid and whitish material >Red discoloration of rectum mucosa >Rectum- minimal hemorrhage >Thymus- mild lymphoid depletion >Sternum bone marrow – minimal hypocellularity

Reference ID: 3215021

Dose group	Dose (mg/kg/d)	Sex	Animal no.	Mode of death	Day of death	Cause of death	Findings
		F	0504108	Found dead	15	Gavage accident	>Red/pink foam in trachea, nasal cavity, oral cavity >Pulmonary edema-moderate >Thymus – lymphoid depletion (mild) >Kidney- mild congestion; min mononuclear cell infiltrate

Reference ID: 3215021

Clinical Signs

Cageside observations were performed twice daily. Detailed clinical observations were conducted prior to dose initiation on Day 1, once weekly during the study, and prior to necropsy.

Diarrhea, **soft stool** and **emesis** were observed in males and females at all doses as well as in controls throughout the dosing period (Table 29). The incidence of each finding, as well as the total number of animals presenting with each finding increased with increasing RDEA594 dose. These findings are consistent with the findings observed in many of the animals that died prematurely (Table **28**). However there was no evidence of treatment-related GI tract pathology in any of the animals that were dosed until study completion. Furthermore, there were no treatment related findings in males or females in the recovery phase.

Red discolored urine was observed in males and females receiving ≥100 mg/kg/d at later time points in the study (starting on day 188). Additional investigation into the cause of the discolored urine was undertaken, but unique impurities in discolored urine samples were not identified. Furthermore, there is no evidence to support blood in the urine as being the cause of discoloration (See Urinalysis section).

Reference ID: 3816021

Table 29. IND 102128. Study SR08-094. RDEA 594. 12 month monkey study. Summary of cageside observations.

		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
Diarrhea	# of observations	2	10	80	88	146	1	5	35	138	372
	# of animals	2	3	4	8	10	1	3	4	10	10
	Days from - to	132-188	16-213	4-354	1-356	1-359	126-126	14-280	1-179	1-355	1-362
Emesis	# of observations	5	1	7	46	126	2	1	5	25	92
	# of animals	3	1	3	9	10	2	1	4	7	10
	Days from - to	19-168	168	82-357	1-188	1-357	80-355	80-80	1-355	1-355	1-355
Soft stool	# of observations	7	3	52	43	72	6	6	36	51	95
	# of animals	5	3	6	9	10	2	4	5	10	10
	Days from - to	23-216	12-214	1-355	2-275	1-240	36-135	1-196	1-180	3-186	4-333

Reference ID: 3236021

		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
Red discolored urine	# of observations	0	0	1	31	84	0	0	2	4	2
	# of animals	-	-	1	6	5	-	-	1	3	2
	Days from - to	-	-	365	245-365	188-365	-	-	353-362	358-362	362-362

Reference ID: 3236021

Body Weights

Body weights were recorded prior to dosing on Day 1, weekly thereafter, and prior to necropsy.

There were **slight decreases** (0.2 – 0.3 kg, approximately -6 to -8% vs. controls) in mean **body weight** in HD males between days 183 – 204 and from 274 – 301. The differences were small and transient, and were not statistically significant. No treatment related changes in mean absolute body weight were observed in females.

There were no treatment-related effects on **body weight gain** in males at either the 6 month or 12 month time points. Females treated with doses ≥ 100 mg/kg had increased body weight change vs. concurrent controls (+61.5 to +96.3%) after 6 months of treatment. However, this effect was not seen after 12 months of dosing. The absence of this effect at the 12 month time point may be related to the observed increased metabolism of RDEA594, and subsequent decrease in systemic exposure over time (Table 39).

Food Consumption

Food consumption was **qualitatively** assessed daily. The rating scale was as follows: Good = less than 1/3 of feed left; Fair = 1/3 – 2/3 of feed left; Poor = more than 2/3 of feed left.

There were no observed treatment-related effects on food consumption.

Ophthalmoscopy

Examinations were performed prior to dosing, at Weeks 12, 24, and 51 by indirect ophthalmoscope and by slit lamp biomicroscope. Each eye was examined following administration of one drop of atropine (0.5 mg/mL) for mydriasis.

There were no treatment related effects on ophthalmic examinations.

ECG

A 9-lead electrocardiogram (**ECG**) was recorded in conscious, restrained monkeys prior to randomization (week -3) and during Weeks 12, 23, 39 (3.85 – 12.03 hrs post dose; but 4-6 hrs postdose for most monkeys), 52 (1 – 2.62 hrs post dose) and 65 (recovery animals, prior to necropsy). Systolic, diastolic, and mean arterial blood pressure parameters were measured at the same time points. The evaluated ECG parameters included heart rate (HR), QRS duration, PR, QT and RR intervals and P, R, and T wave amplitude. Corrected QT interval was calculated using Fridericia's formula [[$QTcF=QT/(RR)^{1/3}$].

There were **no treatment-related effects** of RDEA594 on any ECG parameter or blood pressure parameter at any time point in males or females.

Hematology

Blood samples (1.0 mL) were collected from the femoral vein for hematological analysis prior to dosing (Days -5 or -6) and during the dosing phase Days 23, 91, 176, 267, 358 and 449. On the same days, blood samples (1.8 mL) were collected for coagulation (activated partial thromboplastin time [APTT] and prothrombin time) evaluation. A complete hematological battery was evaluated.

Reference ID: 3816021

Measurement of serum hematology parameters was highly variable. Selected differences between treatment groups and controls achieved statistical significance at individual time points (e.g. HD M & F hematocrit and hemoglobin, day 358), but the magnitude of the difference in means (6 – 8%) was not considered to be biologically significant. Therefore, there is no apparent effect of RDEA594 on hematology parameters in cynomolgus monkeys after 6 or 12 months of daily treatment.

There were no treatment-related effects on activated partial thromboplastin time or prothrombin time (**coagulation** parameters) after either 6 or 12 months of daily RDEA594 administration.

Clinical Chemistry

Blood samples (1.2 mL) were collected from the femoral vein for clinical chemistry analysis prior to dosing (Days -5, -6) and during the dosing phase Days 23, 92, 176, 267, 358 and 449. Animals were food-fasted overnight prior to obtaining samples. A complete clinical chemistry battery was evaluated.

Day 176

Statistically significant **decreases** in mean serum **bilirubin** concentration (Table 30) vs. controls were observed in males at all doses tested (≥30 mg/kg/d) and in females receiving ≥ 100 mg/kg/d. The magnitude of the effect size (-23 to -58% vs. controls) increased with dose and plateaued at 300 mg/kg/d. There was no microscopic evidence of treatment related liver pathology at this time point. However, liver weight was significantly increased in 300 and 600 mg/kg males and in 600 mg/kg females. The mechanism behind the decrease in serum bilirubin is unclear. One potential explanation might be that bilirubin metabolism is enhanced in the presence of RDEA 594.

Increased mean serum **triglyceride** concentration was also observed in males and females after 6 months of RDEA594 treatment. Males receiving ≥ 100 mg/kg/d and females receiving ≥30 mg/kg/d had statistically significant increases vs. controls. In males, the effect size (+102 to +245%) increased with dose. In females, there was no clear increase in effect size (+95 to +118%) over a range of doses spanning from 30 mg/kg – 600 mg/kg. Animals were fasted overnight prior to obtaining blood samples for clinical chemistry testing, so altered triglyceride levels are not the result of food consumption. Increased serum triglycerides may represent pathologies secondary to pancreatic, hepatic, or renal disease. This finding is in contrast to what was observed in rats, as serum triglycerides were **decreased** in rats dosed up to 300 mg/kg for 6 months with RDEA594 (Study SR08-095). Other liver enzymes, including alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) and aspartate aminotransferase (AST) were not affected by RDEA594 treatment at the 6 month time point.

Additionally, uric acid was not measured in this study. It may have been useful to measure this parameter in order to detect a potential pharmacodynamic effect of RDEA594 given its proposed mechanism of action as a URAT1 inhibitor.-

Reference ID: 3216021

Table 30. IND 102128. Study SR08-094. RDEA 594, 12 month monkey study. Clinical chemistry findings in 6 month study.

		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n	10	7	7	10	8	10	7	7	10	8
Total bilirubin (µM)	Day 176	4.70	**3.30***	**2.48***	**2.14***	**1.98***	3.76	2.91	**2.41***	**1.62***	**1.70***
% change from control			**-29.7**	**-47.3**	**-54.5**	**-57.8**		-22.7	**-36.0**	**-57.0**	**-54.8**
Triglycerides (mM)		0.171	0.270	**0.346***	**0.412***	**0.590***	0.274	**0.534***	**0.523***	**0.511***	**0.598***
% change from control			57.9	**102.3**	**140.9**	**245.0**		**94.9**	**90.9**	**86.5**	**118.2**

Reference ID: 3236021

Day 358

Consistent with observations in the 6 month study, total serum **bilirubin** concentration was significantly, and dose-dependently **decreased** in M & F receiving ≥ 100 mg/kg/d RDEA594 treatment for 12 months (Table 31). The magnitude of the effect size on total bilirubin was slightly larger than observed after 6 months (-52.5% to -71.5%). Recovery animals showed a return toward baseline levels after a 3-month recovery period. Minimal bile duct hyperplasia was reported in 2/4 M in the 300 mg/kg and 3/4 M in the 600 mg/kg dose groups, along with 2/4 600 mg/kg F (
Table 38). An **increase** in total serum bilirubin might be expected if animals were experiencing blockage of the bile duct in response to bile duct hyperplasia. However, the current data shows overall treatment related **decreases** in serum bilirubin concentration. The observed decrease in serum bilirubin may be explained by enhanced bilirubin metabolism in the presence of RDEA 594. Alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) changes typically correlate with bile duct hyperplasia. Individual ALP and GGT values for animals in which bile duct hyperplasia was detected at necropsy are presented in Table 32. GGT values were consistently elevated above control values in these animals (except F #0604260), while ALP values were not clearly increased or decreased vs. controls..

Minimal, diffuse vacuolation was observed in the liver of 1/4 HD males. This finding is generally regarded as adverse. Mild eosinophilic inflammation was also observed in the liver in 1/4 HD males. Mean serum **triglyceride** concentration was significantly increased vs. controls in males (≥ 100 mg/kg/d) and females (≥ 300 mg/kg/d) treated with RDEA 594 for 12 months, providing support for the liver as a target organ of RDEA594. In males, the range of increases is similar to that observed at 6 months, although the increase at the HD is smaller in animals treated for 12 months. In females, the magnitude of the effect size is smaller after 12 months than after 6 months at all doses. This finding is congruent with the toxicokinetic data which shows that systemic RDEA 594 exposure is lower at 12 months than at 6 months (Table 39**).**

Reference ID: 3216021

Table 31. IND 102128. Study SR08-094. RDEA 594, 12 month monkey study. Clinical chemistry findings in 12month study + recovery.

		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n	10	7	7	10	8	10	7	7	10	8
	Recov n	3	NT	NT	3	2	3	NT	NT	3	2
Total bilirubin (µM)	Day 358	3.71	1.97	**1.76**	**1.44**	**1.13**	3.52	2.19	**1.50**	**1.27**	**1.00**
% change from control		-	-46.8	**-52.5**	**-61.3**	**-69.5**		-37.8	**-57.3**	**-63.8**	**-71.5**
Recovery - bilirubin (µM)	Day 449	3.967	-	-	4.763	4.185	4.777	-	-	4.147	3.54
% change from control					20.1	5.5				-13.2	-25.9
Triglycerides (mM)	Day 358	0.20	0.32	**0.45***	**0.56***	**0.53***	0.27	0.25	0.32	**0.43***	**0.56***
% change from control			58.3	**122.1**	**175.0**	**158.8**		-9.2	16.1	**57.9**	**105.9**
Recovery- Triglycerides (mM)	Day 449	0.32	-	-	0.34	0.23	0.35	-	-	0.30	0.36
% change from control					4.3	-28.8				-14.2	3.4

Reference ID: 3236021

Table 32. ALP and GGT values on day 358 for animals in which bile duct hyperplasia was detected in histopathology.

	Animal #	Dose (mg/kg)	**ALP (U/L)**	% control	**GGT (U/L)**	% control
Males	*Control (mean, n=7)*	*0*	*614*	*100%*	*68.6*	*100%*
	0512121	300	552	89.9%	83.4	122%
	0605141	300	463	75.4%	85.3	124%
	0506001	600	268	43.6%	73.9	108%
	0605139	600	722	117.5%	122	178%
	0612029	600	510	83.0%	142.5	208%
Females	*Control (mean, n=7)*	*0*	*224*	*100%*	*49.3*	*100%*
	0610026	600	280	125.0%	60.9	124%
	0604260	600	197	87.9%	24.7	50%

Urinalysis

Urine samples were collected from food-fasted monkeys on Days 23, 92, 176, 267, 358, 392 and 449. Samples were transferred to plastic tubes from the metabolic cage collection containers. Urinalysis parameters included color, clarity, glucose, bilirubin, ketones, specific gravity, urine volume, occult blood, pH, protein, urobilinogen, nitrite, leukocyte, and microscopic examination of sediment.

Unlike the rat study (SR08-095), urine volume was not affected by RDEA594 treatment. **Red discoloration** of urine was detected in males and females receiving ≥30 mg/kg/d. This finding appears to be consistent with the 6 month rat study, in which 'unusual' grey – light brown, circular to oval shaped crystals, 20 – 40 µm in diameter were observed upon microscopic examination of urine samples of animals receiving ≥ 100 mg/kg/d. The identity of these crystals was considered by the study pathologist to be salt compounds comprised of test article or a test article biotransformation product. The first report of red urine discoloration in monkeys was in a visual cageside observation reported on day 188 in HD Male # 0512813. There was increased incidence and frequency of this finding with increased dose and time, most clearly in the 300 mg/kg and 600 mg/kg male groups (Table 29). This finding appeared to reverse upon cessation of treatment, as red discolored urine was not observed in recovery animals. The sponsor was unable to identify the specific nature of the red discoloration in the urine. Given that the first appearance of urine discoloration did not occur until study day 188, the red discoloration may be caused by a metabolite of RDEA594. As discussed in the Toxicokinetics section below, marked accumulation of the M6 metabolite of RDEA594 was observed in plasma after 24 weeks of treatment (Table 40).

Another potential explanation could be the presence of blood in the urine. To examine this, a table of individual animal urinalysis data was established (Table 33). This table includes the days in which urine discoloration was

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determined by the urine analyzer as well as the days which occult blood and/or RBCs were detected in urine of those animals. The table excludes females whose urine was contaminated by menstrual blood (and where the portion of the urine without menstrual blood contamination was not red). There were only a select few occasions in which observation of red urine discoloration coincided with observation of occult blood and/or red blood cells in the urine. Therefore, it does not appear that blood is the source of the observed red discoloration in urine.

Table 33. Assessment of presence of occult blood or red blood cells in urine of animals in which red discoloration in urine was detected by urinalysis analyzer

	Animal #	Dose (mg/kg)	Red Urine Discoloration Day(s)	Occult blood Day (score)	RBCs Day (# per 40x field)
Males	0610039	0	267	-	
	0602301	30	267	-	
	0606077	100	91		
	0602167		91	267 (1+)	267 (0-3)
	0608041		267	-	
	0512121	300	23	-	
	0509085		91	-	
	0603327		267, 358	-	
	0603081		267, 358	-	267 (0-3)
	0412047		267, 358	-	
	0605141		267	-	
	0603341		267, 358	-	
	0506275		267	-	
	0508175	600	91	-	
	051049		91,176,267,358	176 (2+)	176 (0-3)
	0512183		91,176,267,358	267 (2+)	267 (0-3)
	0506001		267	358 (Trace)	
	0612029		267	267 (1+)	
Females	0605078	100	91	-6 (3+)	176 (0-3)
	0606262		267	-	23 (0-3)
	0611016		267	91 (2+), 176 (3+)	
	0504274	300	91	-6 (2+), 91 (Trace), 176 (1+)	
	0612146		176,267,358	-6 (1+), 176 (3+)	
	0609258		267	176 (1+) 267 (Trace)	176 (0-3)
	0604002	600	91	176 (Trace)	-
	0606134		91	176 (2+), 267 (Trace), 358 (Trace)	23 (0-3), 176 (4-6)

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In addition, urine samples collected prior to necropsy were screened for the presence of porphyrin (precursor of heme) via a 35 UV/Vis Spectrometer at wavelength 400-401 nm. 300 mg/kg male #0603327 and female #0608244 as well as 600 mg/kg male #061029 were the only samples to demonstrate UV/Vis absorbance greater than the negative control samples. In all cases, absorbance was minimal (0.1 – 0.5) compared with the positive control (2.7). The evidence suggests that porphyrin does not contribute significantly to the observed urine discoloration. The sponsor also carried out an HPLC analysis of selected urine samples (Control: 2 M, 2 F; 300 mg/kg: 2M, 2F; 600 mg/kg: 2M, 2F) for detection of RDEA594 as well as individual impurities. Yellow urine samples were compared with reddish urine samples within each dose group. There was no correlation between the color of the urine samples and the amount of RDEA594 in each sample. Furthermore, there was no evidence of levels of any single impurity being elevated in the reddish colored urine samples. It is noted that the impurity present at relative retention time (b) (4) s highly elevated in males and females treated with 300 mg/kg ((b) (4) **µg/ml**) and 600 mg/kg ((b) (4) **µg/ml**) vs. controls (b) (4) µg/ml).

There were no treatment related effects on any of the other urine parameters measured at any time point.

Water Consumption

Water consumption was measured daily on days (Days 173-176, 264-267, and 355-358). There were no treatment related effects on water consumption at any time point tested.

Gross Pathology

All animals from early deaths were subjected to full gross necropsies. Monkeys surviving until scheduled sacrifice were sacrificed by overdose with pentobarbital on Days 183 (interim sacrifice), 365 (end of dosing phase) and 456 (recovery) and full gross necropsy was conducted on each animal. Necropsy included examination of the external surface body, all orifices, cranial cavity, external surface of the brain, the cranial, thoracic, abdominal and pelvic cavities and their visceral, cervical areas, carcass and genitalia.

Unscheduled deaths
The gross pathology findings in animals that died prior to study termination are also discussed in the Mortality section. Evidence for GI tract pathology was clear in all animals, particularly in the HD males and females (Table 34) where evidence of discoloration and gas filling was seen in duodenum, jejunum, ileum and cecum.

Four of the 6 total premature deaths showed evidence of decreased thymus size. This is an indicator that these animals were under stress.

6 month study
There were no treatment-related gross pathology findings in monkeys sacrificed after 6 months of RDEA594 treatment.

12 month study
There were no treatment-related gross pathology findings in monkeys sacrificed after 12 months of RDEA594 treatment.

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Table 34. IND 102128. Study SR08-094. RDEA 594, 12 month monkey study. Gross pathology findings in **unscheduled sacrifice** animals.

	Males					Females				
RDEA594 (mg/kg/day)	0	30	100	300	600	0	30	100	300	600
	-	n=1		-	n=2	-	-	n=1	-	n=3
Eye										
Discoloration; white; unilateral	-	0	-	-	0	-	-	0	-	1
Thymus										
Decreased size	-	0	-	-	2	-	-	1	-	1
Stomach										
Filled with yellow fluid and white material	-	0	-	-	0	-	-	0	-	1
Discoloration; dark red; focal	-	0	-	-	1	-	-	0	-	0
Discoloration; dark red; multifocal	-	0	-	-	0	-	-	1	-	0
Discoloration, red, fundus, mucosa, focal	-	0	-	-	0	-	-	0	-	1
Filled with yellow food debris	-	0	-	-	1	-	-	0	-	0
Duodenum										
Discoloration, dark red, multifocal	-	0	-	-	1	-	-	0	-	0
Gas filled	-	0	-	-	2	-	-	0	-	0
Yellowish fluid content with white material	-	0	-	-	0	-	-	0	-	1
Yellow mucous	-	0	-	-	0	-	-	0	-	1
Jejunum										
Discoloration, red dark, multifocal	-	0	-	-	1	-	-	0	-	0
Gas filled	-	0	-	-	2	-	-	0	-	0
Yellow fluid content with white material	-	0	-	-	0	-	-	0	-	1
Yellow mucous	-	0	-	-	0	-	-	0	-	1
Colon										

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	Males					Females				
RDEA594 (mg/kg/day)	0	30	100	300	600	0	30	100	300	600
	-	n=1		-	n=2	-	-	n=1	-	n=3
Discoloration; red; focal	-	0	-	-	1	-	-	0	-	0
Discoloration; dark red; multifocal	-	1	-	-	1	-	-	0	-	0
Yellowish semi-fluid feces	-	0	-	-	1	-	-	0	-	0
Gas filled	-	0	-	-	2	-	-	1	-	0
Yellowish fluid content with material	-	0	-	-	0	-	-	0	-	1
Discoloration; mucosa; red; multifocal	-	0	-	-	0	-	-	0	-	1
Yellow white material and mucous	-	0	-	-	0	-	-	0	-	1
Ileum										
Discoloration; dark red; multifocal	-	0	-	-	1	-	-	0	-	0
Content dark red	-	0	-	-	1	-	-	0	-	0
Gas filled	-	0	-	-	2	-	-	0	-	0
Yellowish fluid content with white whitish material	-	0	-	-	0	-	-	0	-	1
Yellowish mucous	-	0	-	-	0	-	-	0	-	1
Cecum										
Discoloration; dark red; multifocal	-	0	-	-	1	-	-	0	-	0
Gas filled	-	0	-	-	2	-	-	0	-	0
Yellowish fluid content with white whitish material	-	0	-	-	0	-	-	0	-	1
Yellow white material and mucous	-	0	-	-	0	-	-	0	-	1
Ileocecal junction										
Discoloration gray multifocal	-	0	-	-	1	-	-	0	-	0
Rectum										
Discoloration; dark red; focal	-	0	-	-	0	-	-	1	-	0
Discoloration; red/dark red; multifocal	-	1	-	-	1	-	-	0	-	1
Discoloration; dark red; diffuse	-	0	-	-	0	-	-	0	-	1
Yellowish fluid content with white whitish	-	0	-	-	1	-	-	0	-	0

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	Males					Females				
RDEA594 (mg/kg/day)	0	30	100	300	600	0	30	100	300	600
	-	n=1		-	n=2	-	-	n=1	-	n=3
material										
Adrenal gland										
Discoloration dark red diffuse	-	0	-	-	0	-	-	0	-	1
Increased in size	-	0	-	-	1	-	-	0	-	0
Lungs with mainstem bronchi										
Congestion, minimal	-	0	-	-	0	-	-	1	-	0
Lymph node, mandibular										
Discoloration dark red diffuse	-	0	-	-	0	-	-	0	-	1
Lymph node, mesenteric										
Decreased in size	-	0	-	-	0	-	-	1	-	0
Thoracic cavity										
With clear fluid	-	1	-	-	0	-	-	0	-	0

- Not examined. No premature deaths in dose group

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Organ Weights

After necropsy, the following organs were weighed from all animals: adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary, spleen, thyroids with parathyroids, testes with epididymides, thymus and uterus with cervix.

6 months
Treatment related organ weight findings at the conclusion of 6 months of dosing are summarized in Table 35. Mean absolute **liver** weight and liver:body weights were dose-dependently increased in males and females. Liver:body weight was statistically significantly increased vs. controls in males receiving ≥ 300 mg/kg/d (+32%) and in HD females (+31%). There was no evidence of **histopathologic** changes to the liver at this time point in males or females.

Mean absolute **spleen** weight, spleen:body weight, and spleen:brain weights were all statistically significantly increased vs. controls in HD males, while there was no treatment related effect in females. Spleen weight parameters did not increase in a dose-dependent manner in males, and there is a sharp increase in spleen weight between the 300 mg/kg/d group and 600 mg/kg/d group. Consistent with increased spleen weight, microscopic evidence for **splenic hyperplasia** was observed in 1/3 males in both the 300 mg/kg and 600 mg/kg dose groups. Splenic hyperplasia is a relatively common background finding in cynomolgus monkeys, and was observed in 6% of all control animals in an analysis of 60 studies conducted at Charles River Laboratories Edinburgh. The same review reported that this finding was observed in up to 50% of control monkeys in individual studies (Chamanza et al. 2010). There was no difference in spleen weight parameters between controls and any treatment group in animals dosed for an additional 6 months (12 months total, Table 36).

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Table 35. IND 102128. RDEA 594. 12 month monkey study. Organ weights at Day 183.

		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n	3	3	3	3	2	3	3	3	3	2
Liver	Absolute organ weight (g)	69.86	64.52	82.27	86.02	88.34	64.52	65.80	65.47	66.50	82.40
	% change from control	-	-7.64	17.77	23.13	26.45	-	1.97	1.46	3.06	27.71
	Organ:Body weight ratio (%Body Wt)	2.00	1.82	2.25	**2.64****	**2.64****	2.37	2.50	2.36	2.47	**3.10***
	% change from control	-	-9.31	12.24	**31.65**	**31.84**	-	5.24	-0.76	3.93	**30.57**
	Organ:Brain weight ratio (% Brain Wt)	95.76	92.96	134.78	126.44	120.06	109.59	102.46	99.36	107.31	128.72
	% change from control	-	-2.92	40.75	32.05	25.37	-	-6.50	-9.33	-2.08	17.45
Spleen	Absolute organ weight (g)	3.48	3.37	4.64	3.79	**7.76***	3.14	4.40	3.89	3.55	3.39
	% change from control	-	-3.16	33.36	9.09	**123.04**	-	40.26	24.10	13.26	7.91
	Organ:Body weight ratio (%Body Wt)	0.10	0.09	0.13	0.12	**0.23****	0.12	0.17	0.14	0.13	0.13
	% change from control	-	-1.36	35.05	20.99	**144.49**	-	40.27	15.99	10.44	6.05
	Organ:Brain weight ratio (% Brain Wt)	4.73	4.87	7.63	5.72	**10.49****	5.39	6.79	5.85	5.54	5.30

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		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n	3	3	3	3	2	3	3	3	3	2
	% change from control	-	3.03	61.41	21.07	**121.81**	-	26.04	8.54	2.78	-1.71

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12 months

Liver weight was increased vs. controls in males (+40 to +83%) and females (~+30%) receiving doses ≥100 mg/kg/d at the 12 month time point, with males showing greater increases than females (Table 36). The magnitude of effect size for all three organ weight parameters is greater than observed at the 6 month time point, and significant differences from controls are seen at lower doses than in the 6 month animals. This evidence suggests that effects on the liver are cumulative. The increased liver weight may correlate with histopathologic evidence of **bile duct hyperplasia** in males and females. Minimal bile duct hyperplasia was reported in males in the 300 mg/kg (2/4) and 600 mg/kg (3/4) dose groups, along with 2/4 females in the 600 mg/kg group. Minimal, **diffuse vacuolation** was observed in the liver of 1/4 HD males. Both bile duct hyperplasia and diffuse vacuolation are considered to be adverse findings. There were no findings in the liver of any animals following a month recovery period indicating that these toxicities are alleviated upon cessation of treatment.

Thymus weight was decreased in males (~-60%) but increased in females (~+60%) at the 12 month time point. Thymus weights were highly variable between animals. Decreased thymus weight is a known indicator of stress (Michael et al. 2007), and the observed decreases in male monkey thymus weight may be attributable to stress rather than a direct treatment-related effect. **Adrenal** gland weights were increased in males and females with no histologic correlates. Like the thymus finding, this finding is likely attributed to stress.

Kidney weight increased in a dose-dependent fashion in males, with absolute kidney weight (+50%) and kidney:body weight (+41.7%) statistically significantly increased in HD males vs. controls after 12 months of treatment. HD females had apparently increased kidney weight vs. controls, but there was no evidence for dose-dependence of effect size in females. Kidney weight values returned toward control levels in recovery animals. Increased kidney weight may be a potential concern given that the molecular target (URAT-1) of this drug is in the kidney. The kidney is an apparent target organ in chronically dosed rats (Study SR08-095), however kidney weight was decreased in this species. There was no evidence of treatment related macroscopic or microscopic changes in the kidney in male or female monkeys after 12 months of dosing.

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Table 36. IND 102128. RDEA 594. 12 month monkey study. Organ weights at Day 365 & recovery, day 456.

		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n (recovery n)	4 (3)	3 (NT)	4 (NT)	4 (3)	4 (2)	4 (3)	4 (NT)	3 (NT)	4 (3)	4 (2)
Liver	Absolute organ weight (g)	65.36	79.49	**90.47***	**92.37****	**109.78****	59.75	59.10	72.07	75.48	**79.13***
	% change from control	-	21.63	**38.43**	**41.33**	**67.97**	-	-1.07	20.62	26.33	**32.44**
	Recovery (d 456) Absolute organ weight (g)	80.27	NT	NT	80.67	79.90	64.20	NT	NT	64.23	62.10
	% change from control	-			0.50	-0.46	-			0.04	-3.28
	Organ:Body weight ratio (% Body Wt.)	1.69	1.85	2.12	**2.33****	**2.69****	2.16	2.26	2.20	**2.75**	**2.82**
	% change from control	-	9.87	25.53	**38.40**	**59.47**	-	4.58	1.79	**27.32**	**30.68**
	Recovery (d 456) Organ:Body weight ratio (% Body Wt)	1.59	NT	NT	1.69	1.82	1.99	NT	NT	2.06	1.93

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		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n (recovery n)	4 (3)	3 (NT)	4 (NT)	4 (3)	4 (2)	4 (3)	4 (NT)	3 (NT)	4 (3)	4 (2)
	% change from control	-			6.81	14.67	-			3.43	-3.15
	Organ:Brain weight ratio (% Brain Wt)	88.95	119.03	**127.90***	**131.29****	**163.30****	87.84	93.92	113.07	**119.41***	**115.75***
	% change from control	-	33.82	**43.79**	**47.60**	**83.59**	-	6.93	28.73	**35.95**	**31.78**
	Recovery (456) Organ:Brain weight ratio (% Brain Wt)	114.77	NT	NT	112.43	121.46	102.21	NT	NT	101.43	89.75
	% change from control	-			-2.04	5.83	-			-0.76	-12.20
Thymus	Absolute organ weight (g)	4.58	3.00	2.77	**1.71***	**1.94***	1.44	1.83	2.20	2.19	2.34
	% change from control	-	-34.50	-39.63	**-62.66**	**-57.58**	-	27.26	53.20	51.95	62.38
	Recovery (d 456) Absolute organ weight (g)	2.22	NT	NT	2.14	3.03	2.27	NT	NT	3.86	4.50

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		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n (recovery n)	4 (3)	3 (NT)	4 (NT)	4 (3)	4 (2)	4 (3)	4 (NT)	3 (NT)	4 (3)	4 (2)
	% change from control				-3.60	36.26				70.40	98.50
	Organ:Body weight ratio (% Body Wt.)	0.12	0.07	0.07	**0.05****	**0.05****	0.05	0.07	0.06	0.08	0.08
	% change from control	-	-40.08	-44.30	**-61.77**	**-59.24**	-	33.84	14.15	47.80	60.23
	Recovery (d 456) Organ:Body weight ratio (% Body Wt)	0.04	NT	NT	0.05	0.07	0.07	NT	NT	0.11	0.14
	% change from control	-			8.04	66.67	-			60.56	96.48
	Organ:Brain weight ratio (% Brain Wt)	6.14	4.56	3.93	**2.46***	2.89	2.16	2.90	3.35	3.37	3.47
	% change from control	-	-25.71	-35.92	**-60.00**	-53.00	-	34.29	55.10	56.30	60.57
	Recovery (456) Organ:Brain weight ratio (% Brain Wt)	3.14	NT	NT	3.02	4.65	3.52	NT	NT	6.03	6.50

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		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n (recovery n)	4 (3)	3 (NT)	4 (NT)	4 (3)	4 (2)	4 (3)	4 (NT)	3 (NT)	4 (3)	4 (2)
	% change from control	-			-3.82	47.93	-			71.37	84.82
Kidney	Absolute organ weight (g)	13.76	17.12	17.89	18.52	**20.64***	11.71	10.12	12.12	10.95	14.52
	% change from control	-	24.41	30.01	34.55	**49.99**	-	-13.60	3.50	-6.51	23.99
	Recovery (d 456) Absolute organ weight (g)	16.39	NT	NT	16.30	17.17	12.87	NT	NT	12.88	11.57
	% change from control	-			-0.51	4.75	-			0.10	-10.08
	Organ:Body weight ratio (% Body Wt.)	0.36	0.40	0.42	0.46	**0.51****	0.42	0.39	0.37	0.39	0.52
	% change from control	-	12.58	17.99	29.57	**41.68**	-	-7.68	-10.74	-6.68	23.99
	Recovery (d 456) Organ:Body weight ratio (% Body Wt)	0.32	NT	NT	0.34	0.35	0.40	NT	NT	0.42	0.36

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		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n (recovery n)	4 (3)	3 (NT)	4 (NT)	4 (3)	4 (2)	4 (3)	4 (NT)	3 (NT)	4 (3)	4 (2)
	% change from control	-			6.22	8.33	-			3.99	-10.29
	Organ:Brain weight ratio (% Brain Wt)	18.69	25.71	25.36	26.25	30.65	17.11	16.04	19.02	16.90	21.21
	% change from control	-	37.59	35.69	40.44	64.00	-	-6.25	11.13	-1.23	23.94
	Recovery (456) Organ:Brain weight ratio (% Brain Wt)	23.41	NT	NT	22.84	25.92	20.36	NT	NT	20.46	16.76
	% change from control	-			-2.44	10.71	-			0.52	-17.67
Adrenal	Absolute organ weight (g)	0.45	0.51	0.56	0.55	**0.64****	0.51	0.46	0.58	0.57	0.58
	% change from control	-	15.28	25.39	23.15	**44.49**	-	-9.80	13.73	12.35	14.31
	Recovery (d 456) Absolute organ weight (g)	0.51	NT	NT	0.57	0.64	0.47	NT	NT	0.42	0.50

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		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n (recovery n)	4 (3)	3 (NT)	4 (NT)	4 (3)	4 (2)	4 (3)	4 (NT)	3 (NT)	4 (3)	4 (2)
	% change from control	-			11.70	23.78	-			-11.84	5.71
	Organ:Body weight ratio (% Body Wt.)	0.01	0.01	0.01	0.01	**0.02***	0.02	0.02	0.02	0.02	0.02
	% change from control	-	1.69	10.17	16.95	**33.90**	-	-4.37	2.19	16.39	12.02
	Recovery (d 456) Organ:Body weight ratio (% Body Wt)	0.01	NT	NT	0.01	0.01	0.02	NT	NT	0.01	0.02
	% change from control	-			16.50	40.78	-			-8.67	3.33
	Organ:Brain weight ratio (% Brain Wt)	0.61	0.78	0.79	0.78	**0.96***	0.75	0.73	0.92	0.90	0.85
	% change from control	-	27.80	29.11	27.96	**57.57**	-	-2.01	23.09	20.54	14.09
	Recovery (456) Organ:Brain weight ratio (% Brain Wt)	0.73	NT	NT	0.80	0.96	0.75	NT	NT	0.66	0.72

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		Males					Females				
RDEA594 (mg/kg/day)		**0**	**30**	**100**	**300**	**600**	**0**	**30**	**100**	**300**	**600**
	n (recovery n)	4 (3)	3 (NT)	4 (NT)	4 (3)	4 (2)	4 (3)	4 (NT)	3 (NT)	4 (3)	4 (2)
	% change from control	-			10.00	31.51	-			-11.95	-4.38

NT= not tested

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Histopathology

Adequate Battery
Yes- Tissues from all animals were collected and preserved in 10% neutral buffered formalin, with the exception of eyes (with optic nerve and lacrimal gland) and testes/epididymides that were preserved in Davidson's fixative. A complete standard battery of tissues was subject to histopathological examination by a board certified veterinary pathologist. Duplicate bone marrow smears were prepared from femur and stained with Wright-Giemsa and archived.

Peer Review
Yes- A pathology peer review was performed by a board certified veterinary pathologist at interim, terminal and recovery periods.

Histological Findings
Unscheduled deaths
There were seven deaths prior to scheduled sacrifice. Five (2 M, 3 F) were in the HD group, one M was in the 30 mg/kg group and one F was in the 100 mg/kg group. Of the five in the HD group, only four were included in gross and histopathology tables. Please see the **Mortality** section for further discussion of the causes of death

6 month study
At the conclusion of the six month study, apparent treatment related microscopic findings were observed only in males. There was an increase in severity of mononuclear cell infiltrates (from minimal to mild) in the **liver** of HD animals. Mononuclear cell infiltrates in the liver were also observed in control and low dose females, lessening the concern over this finding in HD males. Inflammatory cell infiltration of the liver is a common finding in monkeys (61% of untreated monkeys, Chamanza et al. 2010), and this finding is judged to be not adverse.

Lymphoid follicle hyperplasia of the **spleen** is seen in males at 300 mg/kg (1/3) and 600 mg/kg (1/2). This finding correlates with the increase in spleen weight observed at this time point (Table 35). Splenic lymphoid follicle hyperplasia is observed sporadically in untreated monkeys as a result of infection (Greaves, 2007). Chamanza et al. (2010) report observation of this finding in 24/570 (6.0%) of control monkeys (range = 0-50%).

Hemorrhage (minimal) of the lamina propria of the **colon** is observed in 1/2 HD males. While this is consistent with findings observed in the animals that died prematurely, the observation of hemorrhage of the gut associated lymphoid tissue of the colon in 1/3 **control** males lessens the concern over this finding.

1/2 HD males showed mild vacuolation in the **kidney** pelvis. While this may provide evidence of kidney pathology, it is difficult to determine if this is truly a treatment related effect given the small sample size. Furthermore, kidney fibrosis is observed in 1/3 **control** females, but not in treated animals.

The sternum **bone marrow** of 1/2 HD males showed evidence of lymphoid follicle formation as well as increased myeloid:erythroid ratio. These findings both point to signs of infection in this animal rather than to a direct effect of drug treatment.

The above mentioned findings in spleen, colon, kidney and bone marrow were not detected in monkeys dosed for an additional 6 months (12 months total RDEA 594 treatment). One interpretation of this scenario is that

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the findings are not related to RDEA 594 treatment. Alternatively, these pathologies may be treatment-related but are not detectable at the 12 month time point due to potential adaptive mechanisms. Furthermore, systemic RDEA 594 exposure decreased at all doses throughout the treatment period (Table 39) providing additional rationale for enhanced pathology at the 6 month time point relative to the 12 month time point. Despite the absence of these findings at the 12 month time point, they are still considered adverse.

Table 37. IND 102128. Study SR08-094. RDEA 594, 12 month monkey study. Microscopic pathology findings in 6 month time point (d 183).

	Males					Females				
RDEA594 (mg/kg/day)	0	30	100	300	600	0	30	100	300	600
	N=3	N=3	N=3	N=3	N=2	N=3	N=3	N=3	N=3	N=2
Spleen										
Hyperplasia, splenic follicle										
-Minimal	0	0	0	1	1	0	0	0	0	0
Colon										
Hemorrhage; lamina propria										
-Minimal	0	0	0	0	1	0	0	0	0	0
Kidney										
Vacuolation pelvis										
-Mild	0	0	0	0	1	0	0	0	0	0
Bone marrow, sternum										
Lymphoid follicle formation										
-Minimal	0	0	0	0	1	0	0	0	0	0
Myeloid:Erythroid ratio increased										
-Minimal	0	0	0	0	1	0	0	0	0	0

12 month study

Minimal lymphoid depletion of the **thymus** was observed in males dosed with ≥ 100 mg/kg RDEA594 (Table 38). Concern over this finding is lessened by the observation of lymphoid depletion of increased severity (mild vs. minimal) in 1 control recovery male. This finding is likely to be related to stress and not to an effect of treatment. Minimal gliosis and mineralization in the **brain** of 1/4 HD F are not considered treatment related as there is evidence for both of these findings (range = 0 – 50% and 0-40% respectively) in untreated cynomolgus monkeys (Chamanza et al. 2010). Furthermore, there were no clinical signs of cognitive or functional deficits in these animals. Mild eosinophilic inflammation of the **liver** is a common background finding in untreated monkeys (Greaves, 2007; Chamanza et al. 2010), and is not likely to be related to RDEA594 treatment. Minimal, diffuse vacuolation was observed in the liver of 1/4 HD males (#0506001). This finding is considered adverse.

Minimal **Bile duct** hyperplasia was observed in 2/4 males at 300 mg/kg (#0512121, #0605141) along with 3/4 HD (#0506001,#0605139,#0612029) males and 2/4 HD females (#0610026, #0604260). Alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) changes typically correlate with bile duct hyperplasia. Individual ALP and GGT values for animals in which bile duct hyperplasia was detected at necropsy are presented in Table 32. GGT values were consistently elevated above control values (Table 32) in these animals

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(except F #0604260), while ALP values were not clearly increased or decreased. The lack of correlation of ALP values may be related to the timing of sample collection (e.g. blood samples may have been taken too long after initiation of liver injury to measure changes caused by bile duct hyperplasia) and does not nullify the histopathologic evidence. Bile duct hyperplasia is observed in a range of 0-20% of untreated control monkeys (*n* = 285 M, 285 F; Chamanza et al. 2010). The results of the current study exceed this range, indicating that bile duct hyperplasia, is likely related to RDEA594 treatment. This is considered to be an **adverse** finding. Consequently, the NOAEL is judged to be **100 mg/kg/day**.

Table 38. IND 102128. Study SR08-094. RDEA 594, 12 month monkey study. Microscopic pathology findings in 12 month time point (d 365). Recovery data is only shown in cases where findings are observed in recovery animals.

	Males					**Females**				
RDEA594 (mg/kg/day)	0	30	100	300	600	0	30	100	300	600
n	4	3	4	4	4	4	4	3	4	4
Recovery n	3	NT	NT	3	2	3	NT	NT	3	2
Thymus										
Lymphoid depletion										
-Minimal	0	0	1	1	3	0	0	0	0	0
-Mild	0	0	0	0	0	0	0	0	1	0
Thymus (Recovery)										
Lymphoid depletion										
-Minimal	0	-	-	0	0	0	-	-	0	0
-Mild	1	-	-	0	0	0	-	-	0	0
Liver										
Inflammation, eosinophilic; portal										
-Mild	0	0	0	0	1	0	0	0	0	0
Vacuolation, diffuse										
-Minimal	0	0	0	0	1	0	0	0	0	0
Hyperplasia, bile duct										
-Minimal	0	0	0	2	3	0	0	0	0	2
Brain, cerebrum										
Mineralization										
-Minimal	0	0	0	0	0	0	0	0	0	1
Gliosis										
-Minimal	0	0	0	0	0	0	0	0	0	1

NT- Not tested, NA-Not applicable

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Toxicokinetics

Blood samples (1.0 mL) were collected from the peripheral veins from all animals on Day 1 and Weeks 3, 12, 24, 37 and 50 at time points: pre-dose, 1, 3, 6, 12 and 24 h post-dose. Plasma was prepared from whole blood and assessed for parent drug, RDEA594, and the major metabolite, M6 using a validated LC-MS/MS method. Toxicokinetic parameters were computed using a non-compartmental method via WinNonlin Professional software version 5.2 (Pharsight Corp)

Exploratory metabolic profiling was performed on selected TK samples in order to understand the decrease in systemic exposure of RDEA594 over the dosing period.

Toxicokinetic parameters for orally administered RDEA594 are presented in Table 39. T_{max} ranged from 1.0 h to 4.0 hrs. Systemic exposure and C_{max} were similar in males and females, and increased with increasing dose. Values for AUC decreased markedly over time (up to 88% decrease vs. Week 1 values) starting at week 3 and continuing to the end of the study. This evidence suggests treatment-induced liver enzyme induction (autoinduction). An exploratory study on plasma samples from animals dosed with 600 mg/kg/d was carried out to identify potential metabolites of RDEA594. The results off this study are discussed below.

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Table 39. IND 102128. Study SR08-094. 12 month monkey study. Mean toxicokinetic parameters for RDEA594.

		n=		$AUC_{0-24\ h}$ (µg*h/mL)		Accumulation Ratio (vs. Day 1 values)		C_{max} (µg/mL)		T_{max} (h)	
	Dose (mg/kg/day)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day 1	30	7	7	62.6	89	-	-	13.9	24.4	1.29	2.71
	100	7	7	346	349	-	-	57.2	67	1.57	1.29
	300	10	10	1060	915	-	-	146	130	2.20	2.70
	600	10	10	1410	2260	-	-	240	278	2.00	2.40
Week 3	30	7	7	45.3	53	0.72	0.60	9.69	16.5	1.00	1.0
	100	7	7	129	147	0.37	0.42	18.4	31.4	1.00	1.29
	300	10	10	295	326	0.28	0.36	36.2	48	2.40	2.00
	600	10	9	572	505	0.41	0.22	65.7	63.1	3.10	2.11
Week 12	30	7	7	46.4	50.2	0.74	0.56	7.61	16.9	1.29	1.29
	100	7	7	133	122	0.38	0.35	18.4	22.4	1.86	1.00
	300	10	10	205	163	0.19	0.18	26.7	27.5	1.50	1.40
	600	9^{A}	$10^{a,b}$	621	317	0.44	0.14	71.4	50.1	2.44	1.60
Week 24	30	7	7	34.1	34.3	0.54	0.39	6.36	7.76	1.57	1.29
	100	7	7	90.7	86.6	0.26	0.25	16.0	16.2	1.71	1.29
	300	10	10	157	186	0.15	0.20	23.3	29.5	1.60	2.50
	600	8^{B}	$9^{b,c}$	469	273	0.33	0.12	51.5	42.9	4.00	2.00
Week 37	30	$3^{C,D}$	4^{e}	29.2	34.4	0.47	0.39	3.58	9.38	3.33	1.00
	100	4^{D}	4^{e}	78.0	93.5	0.23	0.27	11.5	21.6	2.0	1.00
	300	7^{D}	7^{e}	143	152	0.13	0.17	22.9	27.5	1.29	1.29
	600	$6^{E,F}$	$6^{f,g}$	376	300	0.27	0.13	27.2	62.9	3.17	1.33
Week 50	30	$3^{C,D}$	4^{e}	31.7	33.3	0.51	0.37	3.60	7.35	1.67	1.00

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	n=		$AUC_{0-24\ h}$ (µg*h/mL)		Accumulation Ratio (vs. Day 1 values)		C_{max} (µg/mL)		T_{max} (h)	
Dose (mg/kg/day)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
100	4[D]	3[e,h]	81.4	83.7	0.24	0.24	13.6	12.5	1.00	1.00
300	7[D]	7[e]	148	128	0.14	0.14	22.3	18.4	1.57	1.29
600	6[E,F]	6[f,g]	378	263	0.27	0.12	44.7	51	1.67	1.00

A 1 male did not survive to Week 12
B Second male did not survive to week 24
C One male did not survive to week 37
D 3 animals were interim sacrificed for necropsy
E 2 animals were interim sacrificed for necropsy
F 2 males did not survive to week 37

a 1 female did not survive to Week 13
b 1 female replacement animal was added
c 1 female did not survive to Week 24
e 3 animals were interim sacrificed for necropsy
f 3 animals did not survive to Week 37, including replacement animal
g 2 animals were interim sacrificed for necropsy
h 1 female did not survive to Week 50

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RDEA594 Metabolite TK Data

An exploratory study (non-GLP) to examine RDEA594 metabolites in monkey plasma was completed on samples from day 1, week 3, and week 50 from 600 mg/kg/day dosed monkeys (6 M, 6 F). The metabolites were detected and identified using LC/MS/MS methods

Metabolites M2 (debrominated RDEA594) and M3 (hydroxylated RDEA594) were detected in minimal amounts on day 1. M2 was detected minimally at week 50, while M3 was not detected at this time point. The major metabolite at week 50 was found to be an S-dealkylated metabolite of RDEA594, termed RDEA594-M6. Both M6 and its glucuronidated conjugates(M8) were found to be markedly increased in the week 50 plasma samples. All additionally identified metabolites (M13, M14, M15, M11, M12) were determined to be sequential metabolites of M6. Samples from a single male monkey (#0510149) were analyzed at time points between weeks 3 and 37 to determine the time course of M6 formation. These data showed that M6 and M8 levels were increased after 6 months of RDEA594 dosing and beyond.

Analysis of LC/MS extracted ion chromatograms in plasma samples from monkey # 051049 at week 50 provided evidence that M6 is eliminated more slowly than RDEA594. At 12 hours post-dose, both RDEA594 and the M6 metabolite were detectable. However, at 24 hrs post-dose, RDEA594 is absent, while the M6 metabolite was still clearly present.

Based on the results of the exploratory metabolite study, the sponsor carried out an additional analysis to assess the plasma concentration of the M6 metabolite in all monkeys in study SR08-094 at all time points. M6 was detected using a validated method at Ardea Biosciences in San Diego, CA. The method involves the addition of [D_6]RDEA594-M6 as the internal standard, protein precipitation by acetonitrile, and final analysis by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). The lower limit of quantitation was reported to be 5.00 ng/ml. The TK parameters C_{max}, T_{max} and $AUC_{0\text{-}24h}$ were computed for M6 using a non-compartmental method as with the RDEA-594 data.

$AUC_{0\text{-}24h}$ and C_{max} of the RDEA594-M6 metabolite generally increased with increasing RDEA594 dose in males and females, with the exception of week 12 (Table 40). Exposure was minimal on day 1, and increased over time, with marked increases in plasma M6 concentration at all dose levels first being seen at week 12. M6 levels appeared to reach a maximum at week 37 in males and females, and remained at similar levels at week 50. Comparison of M6 $AUC_{0\text{-}24h}$ data from each time point with that on day 1 provides evidence of marked accumulation of M6 in animals chronically dosed with RDEA594. Accumulation ratios ranged from 133 to 445 at week 50. The ratio of M6:RDEA594 also increased with time, reaching a maximum (1.47 to 5.61) at week 37 (Table 41). The increase in M6 systemic exposure appears to be accounted for by a concurrent decrease in RDEA594 exposure in weeks 12-50 (Table 39, Table 40).

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Table 40. IND 102128. Study SR08-094. 12 month monkey study. Mean toxicokinetic parameters for RDEA594-M6 metabolite.

		n=		RDEA594-M6 $AUC_{0-24\ h}$ (µg*h/mL)		Accumulation Ratio (vs. Day 1 values)		RDEA594-M6 C_{max} (µg/mL)		RDEA594-M6 T_{max} (h)	
	RDEA 594 Dose (mg/kg/day)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day 1	30	7	7	0.443	0.504	-	-	0.0395	0.0467	3.14	2.43
	100	7	7	1.87	1.65	-	-	0.132	0.124	4.29	3.57
	300	10	10	3.91	4.15	-	-	0.307	0.340	4.30	4.00
	600	10	10	5.88	7.65	-	-	0.477	0.532	4.20	4.10
Week 3	30	7	7	0.336	0.572	0.770	1.08	0.0294	0.0494	3.29	2.57
	100	7	7	1.37	1.43	0.757	0.908	0.0873	0.108	4.86	3.86
	300	10	10	4.09	3.48	1.39	1.34	0.261	0.245	4.00	3.70
	600	10	9	6.66	5.87	1.65	1.09	0.428	0.387	6.30	3.44
Week 12	30	7	7	0.413	0.558	0.948	1.09	0.0372	0.0572	3.43	2.14
	100	7	7	66.3	13.0	35.1	8.51	4.09	1.12	9.86	1.14
	300	10	10	48.1	109	20.3	61.1	3.31	6.44	5.00	9.40
	600	9[A]	10[a,b]	28.4	29.6	10.3	5.71	1.91	3.22	5.56	1.80
Week 24	30	7	7	41.9	46.0	92.7	76.5	2.36	2.43	8.57	9.14
	100	7	7	188	200	106	136	12.5	11.3	4.71	19.3
	300	10	10	444	503	215	194	38.4	33.3	0.900	11.0
	600	8[B]	9[b,c]	625	547	172	92.1	43.2	31.9	2.38	10.7
Week 37	30	3[C,D]	4[e]	72.6	40.7	169	88.4	50.6	2.67	5.00	6.00
	100	4[D]	4[e]	294	243	154	144	22.9	17.8	9.25	7.50
	300	7[D]	7[e]	671	468	441	123	41.0	30.8	2.86	5.57
	600	6[E,F]	6[f,g]	747	632	248	129	42.3	40.2	18.5	9.17
Week 50	30	3[C,D]	4[e]	72.6	75.0	174	163	4.29	6.55	6.00	6.75
	100	4[D]	3[e,h]	301	206	168	133	18.6	12.2	6.00	10.3
	300	7[D]	7[e]	728	581	445	161	42.2	34.2	13.1	3.00

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	n=		RDEA594-M6 $AUC_{0-24\ h}$ (µg*h/mL)		Accumulation Ratio (vs. Day 1 values)		RDEA594-M6 C_{max} (µg/mL)		RDEA594-M6 T_{max} (h)	
RDEA 594 Dose (mg/kg/day)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
600	6[E,F]	6[f,g]	844	938	265	190	49.9	52.4	7.33	6.33

A 1 male did not survive to Week 12
B Second male did not survive to week 24
C One male did not survive to week 37
D 3 animals were interim sacrificed for necropsy
E 2 animals were interim sacrificed for necropsy
F 2 males did not survive to week 37

a 1 female did not survive to Week 13
b 1 female replacement animal was added
c 1 female did not survive to Week 24
e 3 animals were interim sacrificed for necropsy
f 3 animals did not survive to Week 37, including replacement animal
g 2 animals were interim sacrificed for necropsy
h 1 female did not survive to Week 50

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Table 41. IND 102128. Study SR08-094. 12 month monkey study. Ratio of exposure of RDEA594-M6 to RDEA594.

		***n*=**		**M6:RDEA 594 Ratio $AUC_{0\text{-}24\ h}$ (µg*h/mL)**	
	Dose (mg/kg/day)	Male	Female	Male	Female
Day 1	30	7	7	0.00843	0.00687
	100	7	7	0.00633	0.00558
	300	10	10	0.00546	0.00618
	600	10	10	0.00524	0.00526
Week 3	30	7	7	0.00898	0.0122
	100	7	7	0.0121	0.0115
	300	10	10	0.0151	0.0128
	600	10	9	0.0152	0.0151
Week 12	30	7	7	0.0107	0.0133
	100	7	7	0.667	0.136
	300	10	10	0.380	0.963
	600	9^{A}	$10^{a,b}$	0.0841	0.105
Week 24	30	7	7	1.55	1.48
	100	7	7	2.40	2.79
	300	10	10	3.60	3.97
	600	8^{B}	$9^{b,c}$	2.39	2.73
Week 37	30	$3^{C,D}$	4^{e}	2.93	1.47
	100	4^{D}	4^{e}	4.23	2.90
	300	7^{D}	7^{e}	5.61	3.65
	600	$6^{E,F}$	$6^{f,g}$	2.98	2.71
Week 50	30	$3^{C,D}$	4^{e}	2.65	2.59
	100	4^{D}	$3^{e,h}$	4.28	2.86
	300	7^{D}	7^{e}	5.77	5.46
	600	$6^{E,F}$	$6^{f,g}$	3.83	4.26

A 1 male did not survive to Week 12
B Second male did not survive to week 24
C One male did not survive to week 37
D 3 animals were interim sacrificed for necropsy
E 2 animals were interim sacrificed for necropsy
F 2 males did not survive to week 37

a 1 female did not survive to Week 13
b 1 female replacement animal was added
c 1 female did not survive to Week 24
e 3 animals were interim sacrificed for necropsy
f 3 animals did not survive to Week 37, including

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	n=		M6:RDEA 594 Ratio $AUC_{0-24\ h}$ (µg*h/mL)	
Dose (mg/kg/day)	Male	Female	Male	Female

replacement animal
g 2 animals were interim sacrificed for necropsy
h 1 female did not survive to Week 50

Conclusions

The NOAEL for RDEA 594 administration to cynomolgus monkeys for 12 months is **100 mg/kg/day** based on bile duct hyperplasia in males dosed at ≥ 300 mg/kg/day. The NOAEL dose is associated with a mean steady state systemic exposure of AUC_{0-24h} = 82.6 (µg*h)/ml. Based on systemic exposure data from clinical study RDEA594-102, the doses to be used in Phase 3 studies are supported by the nonclinical data (Table 42).

Table 42. Comparison of systemic exposure at NOAEL in 12 month monkey study with human systemic exposure at doses used in Phase 3 clinical studies.

Species	NOAEL (mg/kg)	AUC_{0-24h} (µg*h/mL)	Safety Factor
Monkey	100	82.6	-
	Clinical Dose (mg)		
Human[1]	200	25.9	3.19
	400	53.9	1.53

[1] Clinical data taken from study RDEA594-102 (10 day repeat dose study in healthy volunteers)

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Stability and Homogeneity

RDEA594 **stability** testing was carried out by sampling from the middle of 3 mg/ml and 60 mg/ml formulations. Different lots were used for testing of stability under different conditions. The established acceptance limit for concentration was within 10% of the measured concentration at time 0. All formulations were within this limit under the conditions tested. Therefore, RDEA594 formulations can be considered **stable** for at least (b) (4)

Table 43. IND 102128. Study SR08-094. Stability data. 3 samples were taken from the middle of each formulation and averaged for each condition.

Batch	Storage condition	Nominal Concentration (mg/ml)	Actual concentration (mg/ml)	Percentage change from time 0
RDEA594_030309_01	Time 0	3	(b) (4)	-
		60		-
RDEA594_030409_01	24 hours: Room Temperature	3		(b) (4)
		60		
RDEA594_030609_01	Day 3: 5 ± 3°C	3		
		60		
RDEA594_031309_01	Day 10: 5 ± 3°C	3		
		60		

The **homogeneity** of RDEA594 formulations was tested by measuring the RDEA594 concentration in 1 ml samples taken from the top, middle, and bottom of formulations prepared for dosing at selected time points during the course of the study (

Table 44). The established acceptance limit for precision was ≤10% and the limit for individual accuracy was within 10% of nominal concentration values. All formulations prepared met the established acceptance criteria and were therefore considered homogeneous.

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Table 44. IND 102128. Study SR08-094. Homogeneity data. Samples were taken from the top, middle, and bottom of each formulation and averaged.

Week	Nominal concentration (mg/ml)	Total n	Precision (% CV)	Accuracy (%)
1	3	9	1.91	102
	10	3	1.68	103
	30	3	1.92	102
	60	9	1.70	97.7
4	3	3	1.51	104
	10	3	2.24	103
	30	3	1.04	103
	60	3	2.05	102
13	3	3	0.186	104
	10	3	1.11	104
	30	3	0.182	106
	60	3	0.173	96.5
26	3	3	0.813	103
	10	3	1.14	101
	30	3	1.60	105
	60	3	0.617	102
39	3	3	0.852	104
	10	3	3.93	100
	30	3	1.22	103
	60	3	1.39	102
52	3	3	0.327	102
	10	3	2.20	100
	30	3	0.569	101
	60	3	0.423	99.3

11 Integrated Summary and Safety Evaluation

In the 6 month repeat-dose rat study (SR08-095), animals were dosed orally with RDEA594 at 0, 10, 30, 100, 300, and 600 mg/kg/d. High dose animals that died within the first 3 weeks of dosing showed clear evidence of toxicity in the kidney and gastrointestinal tract. The remaining HD animals were euthanized on study day 23.

Liver weight increased in a dose dependent manner in males and females at both the 3 month and 6 month time points. Increased liver weight was associated with microscopic evidence of hepatocellular hypertrophy, which was detected in males at doses ≥ 30 mg/kg at the 6 month time point. Hepatocellular hypertrophy is considered to be an adaptive response to drug administration, brought about by the need for increased metabolic activity (Lewis et al. 2002). It is generally not judged to be a dose-limiting finding.

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Potential treatment related toxicity in the **kidney** was seen in rats dosed at ≥ 300 mg/kg/d. Apparent findings included decreased kidney weight, increased urine volume and increased water consumption. In addition, altered serum levels of creatinine and BUN:creatinine ratio pointed to treatment related adverse effects on the kidney. However, there is limited histopathological evidence to support RDEA594 related kidney toxicity at the 6 month time point (Table 24). Some findings such as increased urine volume and water consumption might be attributable to the mechanism of action of the test article. While tubular dilatation was observed in a subset of males (1/15 100 mg/kg; 3/15 300 mg/kg) and females (4/15 300 mg/kg), this finding may not be indicative of overt kidney damage. It is likely the result of obstruction of kidney tubules due to crystalline deposits. In support of this hypothesis, crystal formations, proposed by the Sponsor to be salt forms of the test article or a metabolite, were observed in the urine of rats dosed at ≥ 100 mg/kg/d (see 'Urinalysis'), and may represent the source of the observed tubular dilatation.

Follicular hypertrophy of the **thyroid** was seen in the 100 mg/kg and 300 mg/kg dose groups. This is considered to be a rat specific finding. The **stomach** and **pancreas** represent potential target organs of toxicity in the rat. Glandular erosion (minimal-mild) as well as hemorrhage/congestion (minimal) of the stomach mucosa were observed in males and females at the 3 month (100 mg/kg and 300 mg/kg dose groups) time point. In addition, single cell necrosis of the pancreas was found to increase in an apparent dose-dependent fashion in males after 3 months of RDEA 594 treatment. However, the overall incidence of **stomach** (glandular erosion, 1/15 300 mg/kg M) and **pancreas** (single cell necrosis; 1/15 300 mg/kg M) pathologies was decreased at the 6 month time point compared to the 3 month time point. Furthermore, the apparent potency of RDEA 594 to induce these effects was decreased in animals treated for 6 months (i.e. pathologies first appeared at higher doses in the 6 month study than in the 3 month study). Concern over the findings in the stomach is further decreased due to evidence that stomach erosions and ulcerations are present in ~10% of young control rats (Gad & Chengelis, 1992).

Systemic exposure in rats in the 100 mg/kg and 300 mg/kg dose groups remained consistent between the 3 and 6 month time points (Table 25). Therefore, the decreased incidence of histopathology findings at the 6 month time point vs. the 3 month time point cannot be explained by a decrease in drug exposure. For true treatment related phenomena, increased duration of treatment would be expected to increase the incidence and/or severity of adverse findings. The NOAEL for RDEA594 treatment in the rat is judged to be **100 mg/kg/day**. This dose is associated with a mean steady state systemic exposure (male & female) of $AUC_{0\text{-}24h}$ = 415 µg*hr/ml after 6 months of dosing. RDEA594 is noted to have a steep dose-response curve based upon a NOAEL at 100 mg/kg/day and lethality at 600 mg/kg/day. Systemic exposure of RDEA594 was decreased at 3 months and 6 months in 100 mg/kg and 300 mg/kg animals relative to exposure at day 1 (accumulation ratio = 0.6 – 0.9), suggestive of potential drug induced induction of metabolic enzyme activity. In study SR08-094, cynomolgus **monkeys** were dosed orally with RDEA594 at 0, 30, 100, 300, and 600 mg/kg/d for a total of 12 months. There were 5 premature deaths (2M, 3F) in HD animals. 4 deaths were due to severe diarrhea, emesis, and decreased food consumption and were considered treatment-related. The other death was due to gavage error. The one premature death at the 100 mg/kg dose level may be explained by an elevated sensitivity to drug treatment in this animal. This explanation is based upon the increased severity of GI tract findings in this animal relative to those that received higher doses. Secondly there were no premature deaths in 20 animals at the next highest dose level (300 mg/kg).

One male in the 30 mg/kg/d group was moribund sacrificed at day 217. The animal's condition was determined by the sponsor to be due to sepsis secondary to proctitis/colitis and was considered unrelated to RDEA594 treatment. In animals that survived to study completion, clinical signs of diarrhea, emesis and soft stool increased with dose and persisted throughout the study. Red discolored urine was observed primarily in

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animals in the 300 mg/kg/d and 600 mg/kg/d groups and the sponsor was unable to identify the impurity causing this discoloration. The evidence does not support blood as the source of discoloration. RDEA594 treatment also induced changes in rat urine, with microscopic examination identifying 'unusual' grey – light brown, circular to oval shaped crystals, 20 – 40 µm in diameter. This finding reversed upon cessation of treatment in the monkey and is also monitorable in a clinical setting and is considered non-adverse.

The main apparent treatment-related finding in monkeys was minimal **bile duct hyperplasia** in 300 mg/kg males (2/4) and 600 mg/kg M (3/4) & F (2/4) after 12 months of treatment. This finding was associated with increases in GGT levels in affected animals. It is noted that bile duct hyperplasia was not observed in the chronic rat study (SR08-095).
Systemic exposure of RDEA594 decreased over time in male and female monkeys, suggesting treatment induced autoinduction of metabolic enzymes. The magnitude of the decrease in exposure was greater in the monkey than in the rat. This observation is explained by evidence for the increased formation of the M6 metabolite of RDEA 594 over time in the monkey. At week 50, systemic exposure to RDEA594 was reduced to 12 – 27% of exposure on day 1 in animals dosed ≥ 100 mg/kg/d. Furthermore, systemic exposure to the major metabolite of RDEA594 (RDEA594-M6) was 3 – 6 fold greater than that of RDEA594 at this time point. The NOAEL in the monkey is determined to be **100 mg/kg/**day with an $AUC_{0\text{-}24h}$ of 82.6 µg*h/mL (average of male and female AUC, week 50) based on bile duct hyperplasia in males at doses ≥ 300 mg/kg/day.

Common target organs of RDEA594 in the rat and monkey include the **kidney** and **GI tract**. Premature deaths related to toxicities in these organs were observed in HD animals (600 mg/kg/d) in both rats and monkeys. Changes in urinalysis parameters are observed in both species at doses above 100 mg/kg/d that could be attributed to the mechanism of action of the test article. Evidence for autoinduction is also observed in both species, and systemic exposure is markedly reduced in monkeys receiving ≥ 300 mg/kg/d after 12 weeks of treatment. The monkey is the more sensitive species, with a NOAEL of 100 mg/kg/day. This NOAEL supports a maximum clinical exposure of 82.6 µg*h/mL which provides adequate nonclinical support for doses in Phase 3 studies (Table 45).

Table 45. Systemic exposure at NOAEL in chronic rat and monkey toxicity studies compared to human exposure at doses to be used in Phase 3 studies.

Species	NOAEL (mg/kg)	$AUC_{0\text{-}24h}$ (µg*h/mL)		
Rat	100	415		
Monkey	100	82.6		
			Safety Factor	
	Clinical Dose (mg)		Rat	Monkey
Human[1]	200	25.9	16.0	3.19
	400	53.9	7.7	1.53
[1] Clinical data taken from study RDEA594-102 (10 day repeat dose study in healthy volunteers)				

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13 References

Adler, R. R., P. F. Moor, D. L. Schmucker, and L. J. Lowenstine. 1993. Chronic colitis, juvenile *Macaca mulatta*, p. 81-87. *In* T. Jones, U. Moher, and R. Hunt (ed.), Nonhuman primates. Springer-Verlag, New York, NY.

Chamanza et al. (2010). Incidence and range of spontaneous findings in control cynomolgus monkeys (Macaca fascicularis) used in toxicity studies. *Toxicologic Pathology* 38, 642-657.

Elmore S. (2006) Histopathology of the lymph nodes. *Toxciologic Pathology*, 35: 425-454.

Gad S. and Chengelis C. (1992) *Animal Models in Toxicology*. Marcel Dekker, Inc.
New York, NY.

Greaves P. and Faccini J. (1992) *Rat Histopathology*(2nd Ed,). Elsevier, Inc. Amsterdam.

Greaves P. (2007) *Histopathology of Preclinical Toxicity Studies* (3d Ed.). Elsevier Inc., New York, NY.

Lewis R., Billington R., Debrune E., et al. (2002) Recognition of adverse and nonadverse effects in toxicity studies. *Toxicologic Pathology* 30, 66-74.

Michael B., Yano B., Sellers R., et al. (2007) Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicologic Pathology* 35, 742-750.

Rao (2002) Diet and kidney diseases in rats. *Toxicologic Pathology* 30: 651-656.

Tucker M. (2003) *Diseases of the Wistar Rat*. Taylor & Francis Limited. London:

Ulrich-Lai Y., Figuerido H., Ostrander M., Choi D., Engeland W., Herman J. (2006). Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. *Am J Physyiol Endocrinol Metab*. 291: E965-E973.

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/s/

MATTHEW T WHITTAKER
12/24/2012
Pharm/Tox review of 6 month rat study and 12 month monkey study with RDEA594.

TIMOTHY W ROBISON
12/24/2012
I concur

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09/03/2015

TIMOTHY W ROBISON
09/03/2015
I concur

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA CHEMISTRY CONSULTATION

Application number:	NDA 207988
Supporting document/s:	1
Applicant's letter date:	12/22/14
CDER stamp date:	12/29/14
Product:	Zurampic (Lesinurad)
Indication:	Hyperuricemia associated with gout in combination with a xanthine oxidase inhibitor
Applicant:	Ardea Biosciences, Inc.
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products
Reviewer:	Matthew Whittaker, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D.
Division Director:	Badrul Chowdhury, Ph.D.
Project Manager:	Michelle Jordan Garner

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

NDA 207988 was submitted on 12/29/14 to support marketing approval for Zurampic (lesinurad) as an oral treatment for hyperuricemia associated with gout. Lesinurad is a uric acid transport protein inhibitor to be administered once per day at a maximum recommended human dose (MRHD) of 200 mg.

A nonclinical inquiry from CMC reviewer Joseph Leginus, Ph.D. was received via email on 4/20/15. The inquiry pointed out that the specification limits for 4 specified organic impurities in the lesinurad *drug substance* (DS) were above the 0.15% qualification threshold established in the ICH Q3A(R2) Guidance. An additional nonclinical inquiry from CMC reviewer Art Shaw, Ph.D. was received via email on 4/20/15. The inquiry observed that the specification limit for a specified degradant of the *drug product* (DP) was above the 0.2% qualification threshold established in the ICH Q3B(R2) Guidance. Neither the DS impurities nor the DP degradant were flagged as having structural alerts for mutagenicity.

A complete evaluation of the relevant nonclinical data to support the safety qualification of the 4 impurities and one degradant is included in the current review.

1.2 Brief Discussion of Nonclinical Findings

Safety qualification for the specification limits of impurities and degradants is generally achieved by calculating the exposure of the impurity (or degradant) at the no observed adverse effect level (NOAEL) in a toxicology study of $\geq$ 13 weeks in one nonclinical species. A safety margin of $\geq$ 2 (on a mg/m^2 basis) between the impurity exposure at the NOAEL and its exposure at the maximum recommended human dose (MRHD) is desired.

The specification limits for the 4 specified organic impurities in the lesinurad DS are acceptable based on their exposure levels at NOAELs in 26-week rat or 12-month monkey toxicology studies with lesinurad.

The specification limit for the lesinurad DP degradant (b) (4) is also considered to be acceptable based on the results of a 13-week toxicology study conducted with (b) (4) in Sprague-Dawley rats.

1.3 Recommendations

1.3.1 Approvability

The sponsor's proposed specification limits for the organic impurities (b) (4) (b) (4) in the lesinurad DS are considered safe from the nonclinical perspective. The specification limit for the lesinurad DP degradant (b) (4) is also considered safe from the nonclinical perspective.

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2 Drug Information

2.1 Drug

Generic Name: Lesinurad

Code Name: RDEA594

Chemical Name: 2-((5-bromo-4-(4-cyclopropylnaphthalen-1-yl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid

Molecular Formula/Molecular Weight: $C_{17}H_{14}BrN_3O_2S$

Structure or Biochemical Description

Pharmacologic Class:
Lesinurad is a uric acid reabsorption inhibitor. It inhibits the function of several carrier proteins that transport uric acid including URAT1, OAT1, OAT3, and OAT4. There is currently no established pharmacologic class (EPC) text phrase for inhibitors of uric acid reabsorption.

2.2 Relevant INDs, NDAs, BLAs and DMFs

Application	Relevance
IND 102128	Original IND for RDEA594

2.3 Drug Formulation

Drug Substance
Ardea has chosen to proceed with a crystalline free acid form of the lesinurad drug substance for commercial development. Nonclinical studies were conducted with the (b) (4) free acid and (b) (4) sodium salt forms of lesinurad. See section 11.1 for further discussion of these studies.

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A comparison of lesinurad systemic exposure values and target organs of toxicity in rats and monkeys treated with both forms of the DS demonstrated that the exposures and toxicological profiles of the two different forms of lesinurad are comparable (Table 11).

Drug Product
The maximum recommended human daily dose of lesinurad is 200 mg/day. Table 1 shows the

Appears this way on original

Table 1. Drug product composition of 200 mg lesinurad tablet.

Component Name	Quality Standard	Function	Quantity per Unit (mg)	% w/w
Lesinurad	In-house	Active ingredient	200.00	(b) (4)
Hypromellose 2910	USP, Ph Eur		(b) (4)	
Microcrystalline Cellulose	NF, Ph Eur			
Lactose Monohydrate	NF, Ph Eur			
Crospovidone	NF, Ph Eur			
Magnesium Stearate	NF, Ph Eur			
Opadry Blue (b) (4)	Supplier			
(b) (4)	(b) (4)			
		Total Target Weight:	(b) (4) **mg/tablet**	**100.0%**

drug product formulation for a single 200 mg tablet.

2.4 Comments on Novel Excipients

All excipients in the drug product are within the range of excipients in currently approved products.

2.5 Comments on Impurities/Degradants of Concern

2.5.1 Drug substance impurities

The qualification threshold for impurities in a drug substance (DS) is 0.15% according to the ICH Q3A(R2) Guidance. There are 4 specified organic impurities in the lesinurad drug substance ((b) (4) (b) (4) (b) (4) and (b) (4) with proposed specification limits greater than 0.15% (Table 2). The sponsor has classified each of these impurities (b) (4) Additionally, (b) (4) has been identified as a degradant of the lesinurad drug product (DP).

Each impurity contains (b) (4) (Table 2).

Ardea plans to control impurities (b) (4) . The maximum clinical exposure of each impurity or degradant is defined as the exposure at the Sponsor's proposed specification limit at the maximum recommended human dose (MRHD) of lesinurad (Table 2).

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Table 2. Organic impurities in lesinurad drug substance with specifications above the 0.15% qualification limit.

Impurity	Structure	Acceptance criteria (% w/w)	Lesinurad MRHD (mg/m^2)[a]	Max impurity dose at MRHD (mg/m^2)
(b) (4)	(b) (4)	NMT (b) (4) 0%	(b) (4)	(b) (4)
(b) (4)		NMT (b) (4) %		
(b) (4)		NMT (b) (4) %		
(b) (4)		NMT (b) (4) %		
(b) (4)				(b) (4)

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2.5.2 Drug Product Degradant

Ardea's specification for 200 mg lesinurad tablets includes a limit of NMT (b) (4)% w/w for the degradant (b) (4) This compound has also been identified as an organic impurity in the lesinurad DS (Table 2). The DP specification limit of (b) (4)% results in a maximum potential (b) (4) exposure of (b) (4) mg/m^2 at the lesinurad MRHDD (Table 3).

Table 3. Calculation of maximum exposure of drug product degradant (b) (4) at the lesinurad MRHDD.

Impurity	Structure	Acceptance criteria (% w/w)	Lesinurad MRHDD (mg/m^2)[a]	Max impurity dose at MRHDD (mg/m^2)
(b) (4)	(b) (4)	NMT (b) (4)%	(b) (4)	(b) (4)
(b) (4)				

2.6 Proposed Clinical Population and Dosing Regimen

Zurampic is to be administered to adults with gout at a dose of 200 mg once daily in combination with a xanthine oxidase inhibitor. Zurampic is not a fixed dose combination product as it is to be administered separately from the xanthine oxidase inhibitor.

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3 Studies Submitted

3.1 Studies Reviewed

Study #	Title
SR08-094	RDEA594: A 12-month repeat dose toxicity and toxicokinetic study with a 3-month recovery period and a 6-month pull in **cynomolgus monkey**
SR08-095	RDEA594: A 6-Month Repeat Oral Dose Toxicity and Toxicokinetic Study with a 2-Month Recovery Period and a 3-Month Pull with a 28-Day Recovery Period in Sprague-Dawley **Rats**
SR13-019	(b) (4) A 13-week oral gavage toxicity study in Sprague-Dawley Rats

3.3 Previous Reviews Referenced

Application	Review	Author	Date
IND 102128	Nonclinical review of 6 month rat and 12 month monkey studies with RDEA594	M. Whittaker	12/24/12

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6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: (b) (4) **A 13-week oral gavage toxicity study in Sprague-Dawley rats**

Study no.:	SR13-019
Study report location:	NDA 207988 Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/16/13
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lesinurad degradant (b) (4) Lot A10191-194-9 99.7% purity

Key Study Findings

- A single dose level of RDEA594 drug substance impurity and drug product degradant (b) (4) (b) (4) mg/kg/day) was tested in male and female rats in a 13 week oral toxicology study
- (b) (4) treated females had decreased body weight gain vs. controls at the 13 week time point (-12%)
- No treatment related histopathological findings were observed
- The NOAEL was identified at (b) (4) mg/kg/day

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Methods

Doses:	0, (b) (4) mg/kg
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	25 mM phosphate buffer (pH 6.8)
Species/Strain:	Sprague Dawley rat
Number/Sex/Group:	10/sex/group
Age:	7 – 8 weeks
Weight (at initial dose):	Males: 237.1 – 266.0 g Females: 191.4 – 215.2 g
Satellite groups:	None

Observations and Results

Table 4. Study design summary for Study SR13-019.

G		
	(b) (4)	(b) (4)
M: a:		

Mortality

There were no premature deaths in this study.

Clinical Signs

Cageside observations were made twice daily (general health, signs of toxicity). Detailed clinical observations were conducted prior to dosing on study day 1 and once weekly thereafter.

There were no treatment related clinical observations in this study.

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Body Weights

Body weights were measured prior to dosing on study day 1 and once weekly thereafter.

There was no effect of (b) (4) on absolute body weight or body weight gain in male rats over the course of 13 weeks of treatment. (b) (4) treated female rats had decreased body weight gain relative to controls (-12%).

Table 5. Body weight gain values in SD rats treated with (b) (4) for 13 weeks (Study SR13-019).

	Males		Females	
(b) (4) dose (mg/kg)	0	(b) (4)	0	(b) (4)
Week 1				
n	10	10	10	10
Body weight (g)	252.5	253.4	203.7	204.5
Week 13				
n	10	10	10	10
Body weight (g)	557.07	546	329.9	315
Absolute BW (% control)	100%	98%	100%	95%
Δ week 1 (g)	304.57	292.6	126.2	110.5
BW Gain (% Initial)	121%	115%	62%	54%
BW Gain (% control)	100%	96%	100%	88%

Feed Consumption

Food consumption was measured once weekly.

There was no effect of (b) (4) treatment on mean food consumption in males during any interval of the study. Mean total food consumption over the course of the 13 week study was slightly lower in (b) (4) treated females vs. controls (Table 6); however, there was no statistically significant difference in total food consumption from weeks 1 - 13 between control and (b) (4) treated animals. Differences vs. controls in females achieved statistical significance at weeks 6, 10 and 12.

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Table 6. Mean total food consumption values in rats treated with RDEA594 degradant (b) (4) for 13 weeks (SR13-019).

	Males		Females	
(b) (4) dose (mg/kg)	0	(b) (4)	0	(b) (4)
n	10	10	10	10
Total food consumption Days 1 – 91 (g)	2319.26	2303.59	1738.85	1626.56
% control	100%	99%	100%	94%

Ophthalmoscopy

Ophthalmoscopy examinations were conducted prior to initiation of treatment and prior to terminal sacrifice. There was no evidence for any effect of (b) (4) on ophthalmoscopy parameters.

Clinical Pathology

Blood and urine samples were collected from all animals at day 92 prior to scheduled sacrifice. A complete battery of clinical chemistry, hematology, coagulation, and urinalysis parameters were examined (Table 7).

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Table 7. Summary of clinical pathology parameters examined in study S13-019.

Activat

Gl
Bil
Sp
Oc
pH

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Hematology
There were no treatment-related changes in hematology parameters at day 92.

Coagulation
There were no treatment-related changes in APTT or PT at day 92.

Clinical Chemistry
There were no treatment-related changes in clinical chemistry parameters at day 92.

Urinalysis
There were no treatment-related changes in urinalysis parameters at day 92.

Necropsy

On day 92, all animals were euthanized by CO_2 inhalation, pneumothorax severance and exsanguinated prior to necropsy. A complete battery of tissues was collected for histopathologic examination (Table 8). Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were examined by a board-certified veterinary pathologist.

Table 8. List of tissues collected at necropsy from rats treated with RDEA594 degradant (b) (4) for 13 weeks (Study SR13-019)

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Gross Pathology
There were no observed gross pathology findings in any animals.

Organ Weights
There were no toxicologically significant changes in organ weight observed.

Histopathology

Peer Review

Microscopic examination was conducted by (b) (6) DVM, Ph.D. The study report does not include reference to peer review of histopathology slides.

Histological Findings

Mononuclear cell infiltrate was observed in the kidney in 1/10 males and 3/10 females that received (b) (4) treatment. This finding, in the absence of other histopathological findings, is not considered adverse (Table 9). Tubular mineralization of the kidney was observed in 1/10 females. This finding was not considered to be related to treatment given that it is a common background finding in female rats.

Lesions of the eye (retinal folds/rosettes), adrenal gland (cortical vacuolation), and brain (dilated ventricle) were observed as single incidences in a single sex (1/20 total). Alveolar macrophages were observed in the lungs of 1/10 males and 1/10 females. These findings were considered to be spontaneous in nature and unrelated to treatment. In summary, no treatment-related adverse histopathological findings were identified in animals treated with (b) (4) mg/kg/day (b) (4)

Table 9. Potential (b) (4) related histopathology findings in study SR13-019.

		Males		Females	
(b) (4) dose (mg/kg)		0	(b) (4)	0	(b) (4)
number of animals		10	10	10	10
EYE (# examined)		10	10	10	10
Retinal folds/rosettes					
	minimal	0	1 (#80627)	0	0
LACRIMAL GLAND (# examined)		10	10	10	10
Mononuclear cell infiltrate					
	minimal	0	1 (80633)	0	0
LUNGS WITH MAINSTEM BRONCHI (# examined)		10	10	10	10
Alveolar macrophages					
	minimal	0	1 (80630)	0	1 (80644)

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	Males		Females	
(b) (4) dose (mg/kg)	0	(b) (4)	0	(b) (4)
number of animals	10	10	10	10
ADRENAL GLAND (# examined)	10	10	10	10
Vacuolation; cortical				
minimal	0	1 (80634)	0	0
KIDNEY (# examined)	10	10	10	10
Mononuclear cell infiltrate				
minimal	0	1	0	3
Tubular mineralization				
minimal	0	0	0	1
BRAIN (# examined)	10	10	10	10
Dilated ventricles				
mild	0	1 (80634)	0	0

Toxicokinetics

Toxicokinetic analysis was not conducted in this study.

Dosing Solution Analysis

One set of duplicate dose formulation samples (~1 mL each) was taken from the middle layer of the dose formulations prepared for Weeks 1 and 13 dosing prior to aliquotting for daily usage.

The dose formulation samples were analyzed for test article concentration using a validated HPLC method ((b) (4)-594-057). The dose formulation was within the acceptable range (±15% of nominal concentration) based on the results in study SR13-02. Homogeneity was not tested in this study.

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Table 10. Dosing solution analysis for (b) (4) in study SR13-019.

(b) (4)

(b) (4)

(b) (4)

a

b

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11 Integrated Summary and Safety Evaluation

11.1 Evaluation of impurity levels in lesinurad drug substance

Lesinurad drug substance
Ardea has chosen to proceed with a crystalline free acid form of the lesinurad drug substance for commercial development. Nonclinical studies were conducted with the (b) (4) free acid and (b) (4) sodium salt forms of lesinurad. A comparison of RDEA594 systemic exposure values and target organs of toxicity in rats and monkeys treated with both forms of the DS demonstrated that the exposures and toxicological profiles of the two different forms of RDEA594 are comparable (Table 11).

Lesinurad sodium salt had comparable systemic exposure to the free acid at the NOAEL of 100 mg/kg/day in rats (AUC_{0-24h} ~400 µg*hr/ml). In monkeys, systemic exposure was comparable between the two DS forms after 28 days of dosing at the NOAEL of 300 mg/kg/day (AUC_{0-24h} 300 - 400 µg*hr/ml). It is noted that the comparable exposures observed in rats and dogs were recapitulated in humans in Clinical Study RDEA594-109. Healthy volunteers were given a single dose of the free acid tablet (200, 400, or 600 mg) or the sodium salt capsule (400 mg). At 400 mg, C_{max} and AUC values for the free acid and sodium salt were nearly identical. Major human metabolite M4 was also formed to a similar extent after administration of either form of lesinurad.

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Table 11. Summary of results of toxicology studies conducted in rats and monkeys with RDEA594 free acid and (b) (4) sodium salt forms.

Study #	RDEA 594 Form	Study Type	Species	Doses (mg/kg/day)	NOAEL (mg/kg/day)	Target organs	Relevant AUC (µg*hr/ml)
SR08-015 (GLP)	Free acid [10461-41-1]	14 day oral	Rat	0, 30, 100, 300, 1000	100	▪ Kidney: tubular degen. ▪ GI tract: epithelial cell necrosis	100 mg/kg M: 267 F: 454
SR08-034 (GLP)	Free acid [10454-139-3]	28 day oral	Rat	0, 10, 30, 100, 300	100	▪ Liver: hypertrophy ▪ Kidney: tubular degen.	100 mg/kg M: 433 F: 369
SR08-016 (GLP)	Free acid [10461-41-1]	14 day oral	Monkey	0, 30, 100, 300, 1000	300	▪ GI tract: neutrophil infiltration	300 mg/kg M: 249 F: 153
SR08-035 (GLP)	Free acid [10454-154-4]	28 day oral	Monkey	0, 10, 30, 100, 300	300	No target organs	300 mg/kg M: 408 F: 210
SR08-095 (GLP)	Sodium Salt [multiple lots]	6 months oral	Rat	0, 10, 30, 100, 300, 600	100	▪ Kidney: tubular dilatation ▪ Stomach: erosion/ hemorrhage	Day 92: 100 mg/kg M: 388 F: 373
SR08-094 (GLP)	Sodium Salt [multiple lots]	12 months oral	Monkey	0, 30, 100, 300, 1000	100	▪ GI tract: erosion ▪ Bile duct hyperplasia	Day 28: 300 mg/kg M: 295 F: 326 Week 50: 100 mg/kg (NOAEL) M: 81.4 F: 83.7 {▲ metabolism of Lesinurad over time in monkeys}

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DS impurity qualification

Impurities (b) (4)

The two principal toxicology studies examined for DS impurity qualification were a 6 month oral gavage study in Sprague-Dawley rats (SR08-095) and a 12 month oral gavage study in cynomolgus monkeys (SR08-094). Multiple RDEA594 lots were used in each of the studies. The impurity levels (as %) in each lot (derived from the sponsor's Drug Substance Batch Analysis data [NDA 207988 Module 3.2.S.4.4]) and their respective exposures (in mg/m^2) at the NOAEL are reported in Table 12.

Given that multiple RDEA594 lots were used in each toxicology study, the final safety assessment for each impurity is based on the impurity level in a lot that was used for ≥ 3 months. Ardea reports that lot 0904728 was administered for the final 6 months of the 12 month monkey study (Study SR08-094). Impurity levels in this lot were used for qualification assessment.

The NOAEL for lesinurad in the 12 month monkey study was established at 100 mg/kg/day (see Nonclinical Review, Whittaker, 12/24/12). Impurity levels for (b) (4) (b) (4) and (b) (4) in lot 0904728 at the NOAEL were calculated first on a mg/kg basis. These values were then converted to mg/m^2 by multiplying by the k_m of 12 for cynomolgus monkeys (Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers). The levels of (b) (4) (b) (4) and (b) (4) at the NOAEL in the monkey study were all greater than their respective exposures at the lesinurad MRHD (Table 12). These impurities are considered to be qualified at the proposed specification limits.

Impurity (b) (4)

There were no RDEA594 lots administered for ≥ 3 months in the 26 week rat study (SR08-094). Ardea provided the lots used and the duration of use for each lot over the first 13 weeks of dosing in NDA 207988 Module 3.2.S.3: Drug Substance Characterization. The DS lots used over the remaining 13 weeks of dosing were listed in the SR08-095 study report, but the duration of use of each individual lot was not provided. The NOAEL for lesinurad in the 26 week rat study was established at 100 mg/kg/day (see Nonclinical Review, Whittaker, 12/24/12).

The weighted mean exposure of (b) (4) in the 3 RDEA594 lots administered over the first 13 weeks of the study at the NOAEL dose was calculated to be (b) (4) mg/m^2 (Table 13). The mean (b) (4) exposure over the final 13 weeks of study SR08-094 was calculated to be (b) (4) mg/m^2. The overall mean (b) (4) exposure at the NOAEL for the entire 26 week study was calculated as the mean of (b) (4) and (b) (4) mg/m^2 = (b) (4) mg/m^2. This represents a (b) (4)-fold exposure margin over the (b) (4) exposure at the MRHD (Table 13). Impurity (b) (4) is considered to be qualified at the proposed specification limit.

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Table 12. Evaluation of nonclinical support for RDEA594 drug substance impurity acceptance criteria. This table presents data derived from the 12-month monkey study (SR08-094). Bolded entries represent the lots relevant for impurity qualification due to exposure for ≥ 3 months.

Impurity	Acceptance criteria (% w/w)	Impurity dose at MRHD (mg/m^2)	Qualifying Study	NOAEL (mg/kg)	Lots used	% Imp'ty	Impurity amt at NOAEL (mg/kg)	Impurity amt at NOAEL (mg/m^2)[a]	Exposure margin
(b) (4) (b) (4)	NMT (b) (4) %	(b) (4)	12 month monkey (SR08-094)	100	RDEA594				
					0904697	(b) (4)			
					NE-023025-batch-01-2009				
					A10063-28-8				
					A10063-23-20				
					A10060-64-1				
					A10063-80-20				
					A10060-70-3				
					A10060-72-3				
					0904728				
(b) (4) (b) (4)	NMT (b) (4) %		12 month monkey (SR08-094)	100	RDEA594				
					0904697				
					NE-023025-batch-01-2009				
					A10063-28-8				
					A10063-23-20				
					A10060-64-1				

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Impurity	Acceptance criteria (% w/w)	Impurity dose at MRHD (mg/m^2)	Qualifying Study	NOAEL (mg/kg)	Lots used	% Imp'ty	Impurity amt at NOAEL (mg/kg)	Impurity amt at NOAEL (mg/m^2)[a]	Exposure margin
		(b) (4)			A10063-80-20				(b) (4)
					A10060-70-3				
					A10060-72-3				
					0904728				
(b) (4) (b) (4)	NMT (b) (4) %		12 month monkey (SR08-094)	100	RDEA594				
					0904697				
					NE-023025-batch-01-2009				
					A10063-28-8				
					A10063-23-20				
					A10060-64-1				
					A10063-80-20				
					A10060-70-3				
					A10060-72-3				
					0904728				

[a] Monkey conversion factor (k_m) for mg/kg to mg/m^2 = 12

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Table 13. Evaluation of nonclinical support for DS impurity (b) (4) acceptance criteria derived from the 26-week rat toxicology study (SR08-095).

DS Imp'ty	Accept criteria (% w/w)	Imp'ty dose at MRHD (mg/m^2)	Qual-ifying study	NOAEL (mg/kg)	RDEA594 Lots used	%	(b) (4) amt at NOAEL (mg/kg)	# of weeks used	Weighted (b) (4) exposure at NOAEL (mg/kg)	Weighted avg (b) (4) exposure at NOAEL (mg/kg)	Weighted avg (b) (4) exposure at NOAEL (mg/m^2)	Mean (b) (4) exposure at NOAEL (mg/m^2)	Exposure margin
(b) (4) (b) (4))	NMT (b) (4) %	(b) (4)	26 week rat (SR08-095)	100	A10001-122-5								(b) (4)
					904697								
					NE-023025-batch-01-2009								
					A10063-28-8								
					A10063-23-20								
					A10060-64-1								
					A10060-65-1								
					A10063-80-20								

ND = Not determined
[a] Lots A10063-28-8, A10063-23-20, A10060-64-1, A10060-65-1 & A10063-80-20 were used over the final 13 weeks of treatment. The duration of use of each lot was not defined in the study report for SR08-095 or in NDA 207988 Module 3.2.S.3: Drug Substance Characterization. The mean (b) (4) value from all of these lots is used in the current calculations.
[b] Rat conversion factor (k_m) for mg/kg to mg/m^2 = 6

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The exposures of impurities (b) (4) (b) (4) and (b) (4) in lesinurad lot 0904728 at the NOAEL in the 12 month monkey toxicology study exceed the maximum potential clinical exposure of each impurity at the lesinurad MRHD of 200 mg/day.

The exposure of impurity (b) (4) at the lesinurad NOAEL dose in the 26 week rat study exceeds the maximum potential clinical exposure of (b) (4) at the lesinurad MRHD of 200 mg/day.

Therefore, the sponsor's proposed specifications for the organic impurities (b) (4) and (b) (4) are all considered safe from the nonclinical perspective.

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11.2 Evaluation of degradant (b) (4) levels in lesinurad drug product

12 month monkey study

The maximum potential clinical exposure to lesinurad DP degradant (b) (4) at the current specification limit of NMT (b) (4)% is (b) (4) mg/m^2 (Table 3). (b) (4) exposure was (b) (4) mg/m^2 at the lesinurad NOAEL dose (lot 0904728) in the 12 month monkey study (Table 14), providing an exposure margin of approximately (b) (4). An exposure margin of at least 2.0 is desired for safety qualification of a degradant.

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Table 14. Evaluation of nonclinical support for RDEA594 drug product degradant (b) (4) acceptance criteria derived from 12 month monkey study. Bolded entry represents the lot relevant for safety qualification due to exposure for ≥ 3 months.

Impurity	Acceptance criteria (% w/w)	Impurity dose at MRHDD (mg/m^2)	Qualifying Study	NOAEL (mg/kg)	Lots used	% Imp'ty	Impurity amt at NOAEL (mg/kg)	Impurity amt at NOAEL (mg/m^2)[a]	Exposure margin
(b) (4) (b) (4)	NMT (b) (4) %	(b) (4)	12 month monkey (SR08-094)	100	RDEA594				
					0904697				(b) (4)
					NE-023025-batch-01-2009				
					A10063-28-8				
					A10063-23-20				
					A10060-64-1				
					A10063-80-20				
					A10060-70-3				
					A10060-72-3				
					0904728				

[a] Monkey conversion factor (k_m) for mg/kg to mg/m^2 = 12

13 week study with (b) (4) *in rats*
Ardea Biosciences also conducted a stand-alone 13-week, GLP, oral gavage toxicity study in SD rats (Study SR13-019) with the (b) (4) impurity at a dose of (b) (4) mg/kg/day ((b) (4) mg/m^2) vs. controls (*see Section 6*).

A decrease in mean body weight gain (-12% vs. controls) was observed in (b) (4) treated females only. This was not considered to be a dose-limiting finding. The sporadic nature of the observed histopathological findings suggests that they are spontaneous lesions that are unrelated to treatment. The NOAEL was identified at (b) (4) mg/kg/day. The (b) (4) exposure at the NOAEL was approximately (b) (4)-fold higher than the clinical (b) (4) exposure at the lesinurad MRHD (Table 15).

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Table 15. Evaluation of nonclinical support for RDEA594 drug product degradant (b) (4) acceptance criteria derived from 13 week rat study with (b) (4)

Impurity	Acceptance criteria (% w/w)	Impurity dose at MRHDD (mg/m^2)	Qualifying Study	NOAEL (mg/kg)	Lots used	% Imp'ty	Impurity amt at NOAEL (mg/kg)	Impurity amt at NOAEL (mg/m^2)[a]	Exposure margin
(b) (4) (b) (4)	NMT (b) (4) %	(b) (4)	13-week rat study with (b) (4) (SR13-019)	(b) (4)	Impurity (b) (4)				
					Lot A10191-194-9	(b) (4)			
[a] Rat conversion factor (k_m) for mg/kg to mg/m^2 = 6									

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11.3 Overall Recommendation

Ardea's proposed specification limits for the 4 specified organic impurities in the lesinurad DS are considered safe from the nonclinical perspective. Additionally, the proposed specification limit of NMT (b) (4) % for the lesinurad DP degradant (b) (4) is also considered safe from the nonclinical perspective.

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/s/

MATTHEW T WHITTAKER
09/01/2015

TIMOTHY W ROBISON
09/01/2015
I concur

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Applicant's letter date:	December 29, 2014
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1 Executive Summary

1.1 Introduction

Ardea Biosciences, Inc. is developing lesinurad (RDEA594) as an oral therapy for treatment of hyperuricemia associated with gout, to be used in combination with a xanthine oxidase inhibitor. The current review evaluates the results of a 2 year oral carcinogenicity study conducted in Sprague-Dawley rats (Study SR09-070) and a 26 week oral carcinogenicity study conducted in TgRasH2 mice (Study SR10-019). Both studies were included with the initial submission of NDA 207988 on 12/29/14.

A mass balance study in humans (study RDEA594-112) identified a disproportionate human metabolite (20.7% of initial dose) of RDEA594, a didhydrodiol metabolite termed M4. The M4 metabolite is formed in humans via an epoxide intermediate termed M3c. Epoxide functional groups are known structural alerts for mutagenicity. M3c formation may represent a potential clinical concern given that RDEA594 is to be administered chronically. The M4 metabolite is formed in rats, but not in mice. Therefore, the 2 year rat carcinogenicity study is the only relevant study for the assessment of the carcinogenic potential of the M3c metabolite. A major question to be addressed is whether M3c exposure in rats is sufficient to qualify this metabolite for safety with respect to carcinogenicity.

1.2 Brief Discussion of Nonclinical Findings

RDEA594 was found to be negative in a standard battery of genetic toxicology studies (Ames test, *in vitro* chromosome aberration test, and rat *in vivo* micronucleus test).

<u>Rat carcinogenicity study</u>
RDEA594 was administered to SD rats at doses of 0, 25, 75, or 200 mg/kg/day by oral gavage. These doses received concurrence from the ECAC (See meeting minutes: IND 102128, 12/22/09). The 200 mg/kg/day dose showed evidence for achieving the maximum tolerated dose in males and females. There were no dose groups that survived to the planned 104-week study duration. However, there was no evidence for a treatment-related effect of RDEA594 on survival. RDEA594 was **not tumorigenic** in rats at doses up to 200 mg/kg/day. Treatment related non-neoplastic lesions in the GI tract, liver, kidney, pancreas and thyroid were generally consistent with findings in the 6 month toxicology study in SD rats (Study SR08-095).

The M3c and M4 metabolites are not considered to be qualified for safety with respect to carcinogenicity based on the data in this study. However, given the unstable nature of the M3c metabolite, it is not possible to test the effects of this molecule in a stand-alone carcinogenicity study. Concern over potential carcinogenic effects of the epoxide is mitigated by its transient nature, and the fact that this molecule is further metabolized to a didhydrodiol.

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Mouse carcinogenicity study

RDEA594 was administered to hemizygous TgRasH2 mice at doses of 0, 15, 45, or 125 mg/kg/day (males) and 0, 30, 90, or 250 mg/kg/day (females) by oral gavage for a duration of 26 weeks. These doses received concurrence from the ECAC (See meeting minutes: IND 102128, 4/28/11). The high doses in males and females represented maximum tolerated doses (MTDs) based on deaths in males at ≥ 500 mg/kg/day and females at ≥750 mg/kg/day in a 1-month dose range finding study (Study SR10-018). Positive control animals in the current study received a single intraperitoneal injection of 75 mg/kg N-methyl-N-nitrosourea (MNU) on study day 1. The study design is consistent with established methods for carcinogenicity testing in TgRasH2 mice[1]

There was no effect of RDEA594 on survival. RDEA594 was **not tumorigenic** in males at up to 125 mg/kg/day or females at up to 250 mg/kg/day (highest doses tested). Neoplastic lesions in MNU-treated mice were detected in the hematopoietic system (malignant lymphosarcoma), skin, and nonglandular stomach. These target organs are consistent with established results in MNU-treated TgHras2 mice and provide support that the study could detect an expected characteristic tumorigenic response to a known carcinogen.

RDEA594-treatment related non-neoplastic lesions in the liver (hepatocyte hypertrophy) observed in MD & HD males and HD females were consistent with findings in the 1 month dose-range finding study in WT mice as well as 6 month toxicology and 2 year carcinogenicity studies conducted in SD rats (Study SR08-095 and SR09-2168 respectively). Additional non-neoplastic lesions identified in the glandular stomach were consistent with evidence observed in long-term studies in SD rats.

1.3 Recommendations

The results of the 2-year carcinogenicity study with rats and 26-week oral carcinogenicity study conducted in TgRasH2 mice were discussed with the ECAC on May 19, 2015 and June 30, 2015, respectively.

Executive CAC Recommendations and Conclusions for the 2-year carcinogenicity study with rats (May 19, 2015)

- The Committee concurred that the study was acceptable, noting prior Executive CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female rats.
- The Committee concurred that no further studies were required for the safety qualification of the M3c epoxide intermediate with respect to carcinogenicity.

- **Executive CAC Recommendations and Conclusions for the 26 week oral carcinogenicity study conducted in TgRasH2 mice (June 30, 2015)**The Committee

[1] Takaoka M. et al. (2003) Interlaboratory comparison of short-term carcinogenicity studies using CB6F$_1$-*ras*H2 transgenic mice. *Toxicologic Pathology*. 32, 191-199.

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concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms.

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2 Drug Information

2.1 Drug

Tradename: ZURAMPIC

Generic Name: Lesinurad

Code Name: RDEA594

Chemical Name: 2-((5-bromo-4-(4-cyclopropylnaphthalen-1-yl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid

Molecular Formula/Molecular Weight: $C_{17}H_{14}BrN_3O_2S$

Structure:

Pharmacologic Class:
Lesinurad is a uric acid reabsorption inhibitor. Its target is Uric Acid Transport Protein 1 (URAT1). There is currently no established pharmacologic class (EPC) text phrase for URAT1 inhibitors.

2.2 Relevant INDs, NDAs, BLAs and DMFs

Application	Relevance
IND 102128	Original IND for RDEA594

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2.3 Drug Formulation

The maximum recommended human daily dose of lesinurad is 200 mg/day. Table 1 shows the drug product formulation for a single 200 mg immediate release tablet.

Table 1. Clinical formulation of a single 200 mg immediate release lesinurad tablet.

Component Name	Quality Standard	Function	Quantity per Unit (mg)	% w/w
Lesinurad	In-house	Active ingredient	200.00	(b) (4)
Hypromellose 2910	USP, Ph Eur			(b) (4)
Microcrystalline Cellulose	NF, Ph Eur			
Lactose Monohydrate	NF, Ph Eur			
Crospovidone	NF, Ph Eur			
Magnesium Stearate	NF, Ph Eur			
Opadry Blue (b) (4)	Supplier			
(b) (4)	(b) (4)			
		Total Target Weight:	(b) (4) **mg/tablet**	**100.0%**

2.4 Comments on Novel Excipients

All excipients in the drug product are within the range of excipients in currently approved oral products.

2.5 Proposed Clinical Population and Dosing Regimen

Lesinurad is to be administered as an immediate release tablet to adults with gout at a dose of 200 mg once daily in combination with a xanthine oxidase inhibitor. The therapeutic goal is to lower serum uric acid (sUA) levels to a target of <6 mg/dL.

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2.6 Regulatory Background

2.6.1 Previous Clinical Experience

Ardea reports in their Clinical Overview (12/29/14) that 3,010 subjects have been included in the global clinical development program for lesinurad, with 2,586 exposed to lesinurad. Subjects with gout have been exposed to lesinurad for up to 3 years. The Phase 3 clinical development program has included 3 x 12 month studies that tested 200 mg and 400 mg lesinurad in combination with an XO inhibitor vs. an XO inhibitor alone.

Studies 301 (US Study) and 302 (Global study) tested lesinurad or placebo + allopurinol in patients with gout (≥2 gout flares in prior year, currently on allopurinol, elevated sUA levels). Study 304 has tested subjects with tophaceous gout and elevated sUA. The study tested lesinurad (200 mg) + febuxostat (80 mg) vs. lesinurad (400 mg) + febuxostat (80 mg) vs. placebo + febuxostat (80 mg).

2.6.2 History of Regulatory Submission

<u>Rat carcinogenicity study</u>
The initial IND application for IND 102,128 was submitted to the FDA on 9/22/09. The special protocol assessment for the 2 year rat carcinogenicity study was submitted to the Agency on 11/23/09. The FDA Executive Carcinogenicity Assessment Committee recommended revisions to the sponsor's proposed doses for this study on 12/22/09. The FDA-proposed doses were as follows:

0 mg/kg/day
25 mg/kg/day
75 mg/kg/day
200 mg/kg/day

Ardea agreed to conduct the study with the FDA-recommended doses.

FDA provided advice to Ardea on three occasions (9/9/11, 10/25/11 and 12/1/11) in response to specific inquiries concerning when to terminate selected treatment groups in the 2 year rat carcinogenicity study.

Several communications were made between FDA and Ardea related to the statistical analysis of tumor data in the 2 year rat carcinogenicity study, summarized in Table 2.

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Table 2. Summary of correspondence between FDA and Ardea regarding statistical analysis of tumor data in 2 year rat carcinogenicity study SR09-2168.

Received/ Communication Date	Source	Description
3/5/12	Ardea	Sponsor inquiry regarding tumor analysis
1/11/13 (in DARRTS 4/17/12)	FDA (M. Jackson)	▪ Males: Peto method is appropriate ▪ Females: Sponsor should use Poly-*k* analysis with *k* = 3
7/25/13	Ardea	Response to FDA recommendation from 1/11/13. Ardea proposes the following: ▪ Males: Standard Peto analysis ▪ Females: (b) (4)
11/1/13	FDA (S. Thomson)	FDA re-emphasizes its position that poly-k analysis should be used for females
1/7/14	Ardea	▪ Ardea agreed to conduct the poly-k statistical analysis on female dose groups using a *k* value of 3 ▪ Ardea requested a confirmatory response from FDA by 2/7/14
2/20/14	Ardea	Ardea confirmed that they will continue with the statistical analysis outlined in their 1/7/14 submission

The final study report for the 2 year rat carcinogenicity (Study SR09-2168) was submitted to IND 102,128 on 8/19/14. NDA 207988 was submitted on 12/29/14. The study report was also included in the NDA submission.

Mouse carcinogenicity study

The special protocol assessment for the proposed dose selection and draft protocol for the 6 month TgRasH2 mouse carcinogenicity study was submitted to the Agency on 3/17/11. The FDA Executive Carcinogenicity Assessment Committee recommended revisions to the sponsor's proposed doses for this study on 4/28/11. The FDA-proposed doses were based on maximum tolerated dose levels established in a 28 day dose-range finding study (Study SR10-018). The recommended doses are listed in Table 3.

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Table 3. ECAC recommended doses in male and female TgRasH2 mice in a 26 week carcinogenicity study

Males (mg/kg/day)	Females (mg/kg/day)
0	0
15	30
45	90
125	250

Ardea agreed to conduct the carcinogenicity study with the FDA-recommended doses. The final study report for Study SR10-019 was submitted to IND 102128 on 4/7/14. The study report was also included in the initial NDA submission on 12/29/14.

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3 Studies Submitted

3.1 Studies Reviewed

Study #	Title	Submission Date
SR09-070	RDEA594: An oral gavage 24-month carcinogenicity study in Sprague-Dawley rats	8/9/14
SR10-019	RDEA594: 26-week Oral Gavage Carcinogenicity and Toxicokinetic Study in CByB6F1-Tg(HRAS)2Jic Mice	4/7/14
SR12-032	Evaluation of the metabolic profile of RDEA594 in bile and urine in male bile-duct cannulated Sprague Dawley rats following a single oral administration of [^{14}C]RDEA594 at a dose of 200 mg/kg	5/2/13

3.3 Previous Reviews Referenced

Date	Review	Author
Reviews related to 2 year rat carcinogenicity study		
12/22/09	Special Protocol Assessment Review: 2 year rat carcinogenicity study with RDEA594	K. Young
9/9/11	Pharmacology/Toxicology Memo to file: Response to sponsor inquiry regarding termination of 75 mg/kg female group	M. Topper
10/25/11	Pharmacology/Toxicology Memo to file: Response to sponsor inquiry regarding termination of 200 mg/kg female group	M. Topper
12/1/11	Pharmacology/Toxicology Memo to file: Response to sponsor inquiry regarding termination of all surviving males once control n = 20	M. Topper
4/17/12	Biometrics Review: Statistician's comments on Ardea's planned statistical analysis of 2 year rat carcinogenicity study	M. Jackson
10/30/13	Biometrics Review: Statistician's comments on Ardea's planned statistical analysis of 2 year rat carcinogenicity study	S. Thomson
Reviews related to 26 week mouse carcinogenicity study		
4/28/11	Special Protocol Agreement: 26 week Tg mouse carcinogenicity study with RDEA594	K. Young
4/29/11	Special Protocol Assessment Review: 26 week carcinogenicity study with RDEA594 in CByB6F1-Tg(HRAS)2Jic Mice	K. Young

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8 Carcinogenicity

8.1 RDEA594: An Oral Gavage 24-month Carcinogenicity Study in Sprague-Dawley Rats

Study no.:	SR09-070
Study report location:	IND 102,128 Supporting document 222
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 4, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RDEA594 Sodium Salt Lot # NE-023025-Batch 03-2009 (b) (4) Purity: 99.8% Lot # NE-023025-Batch 04-2009 (b) (4) Purity: 99.6% Lot # 17JL01.HQ00001 (b) (4) Purity: 100.0%
CAC concurrence:	Yes

Key Study Findings

- In a 2 year carcinogenicity study, male and female rats (*n* = 60/sex/group) received RDEA594 by oral gavage at doses of 0 (water), 25, 75, or 200 mg/kg/day.
- None of the treatment groups were retained on study for the entire 104 week study duration
  - All surviving males were sacrificed at week 97 due to the control male population decreasing to *n* = 20.
  - HD females were sacrificed at week 97 (*n* = 15)
  - MD females sacrificed at week 91 (*n* = 15)
  - LD females sacrificed at week 100 (*n* = 15)
  - Control females sacrificed at week 100 (*n* = 20)
- There was no apparent effect of RDEA594 on mortality rate. Mortality rates in males and females were within the historical control range of SD rats in carcinogenicity studies.
- Mean absolute body weight was decreased by >10% vs. controls in males and females at the 200 mg/kg/day dose level by the conclusion of the dosing period.
- **RDEA594 was not tumorigenic in male or female rats at doses up to 200 mg/kg/day.**

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- Non-neoplastic findings in the thyroid that may correlate with observed adenomas and/or carcinomas include apparent dose-dependent increases in incidence or severity of thyroid C-cell hyperplasia in males and females at ≥25 mg/kg/day.
- Additional non-neoplastic target organs of toxicity included: GI tract, liver, kidney, and pancreas
    - GI tract findings appear likely to be a direct irritant effect of RDEA594 formulation
    - Liver, kidney, and pancreas findings were seen in males and females primarily at doses ≥ 75 mg/kg/day
        - Target organs are consistent with observations in chronic (6 month) rat toxicology study
    - Selected kidney lesions were observed in female rats at all doses (≥ 25 mg/kg/day)
- Plasma concentrations of RDEA594 in male and female rats increased with elevating doses during weeks 1, 4, 13, 26, 39, 52 and 72
    - AUC_{0-24} increased approximately proportionally with dose
    - Accumulation ratios of < 1 in males at ≥ 75 mg/kg/day and females at 200 mg/kg/day suggest potential liver enzyme autoinduction
- The investigators were **not** able to detect the M4 metabolite of RDEA594 in rat plasma at Week 1, 26, or 52. The M4 metabolite is a disproportionate human metabolite formed via an epoxide intermediate (M3c).

Adequacy of Carcinogenicity Study

- No treatment groups were retained for the entire planned duration of the study (104 weeks); however, the times of sacrifice used were considered acceptable based on established FDA ECAC guidelines. Prior concurrence for early sacrifice times was obtained from the Division in consultation with the ECAC.
- It appears that a maximum tolerated dose was achieved (200 mg/kg/day) in males and females based on decreased absolute body weights of greater than 10% vs. controls. Therefore, the high dose selection in this study appears adequate. Further, concurrence for doses was obtained from the ECAC.

Appropriateness of Test Models

- The 2 year carcinogenicity study was conducted in male and female Sprague-Dawley (SD) rats. The SD rat is an established model for assessment of carcinogenic potential. The oral gavage route of administration models the clinical route of administration.
- Concurrence for the general study design was obtained from the ECAC. Ardea obtained concurrence from the Division in consultation with the ECAC regarding early termination of specific dose-groups.

Evaluation of Tumor Findings

- There were no RDEA594-related neoplastic findings.
    - The numerical incidence of combined C-cell adenomas and carcinomas in males was increased vs. controls at RDEA594 doses ≥ 25 mg/kg/day
        - However, there was no statistically significant trend effect with increasing RDEA594 dose.
        - There were no statistically significant differences between any of the dose groups vs. controls

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- The observed incidences of C-cell adenomas and carcinomas in all treatment groups were within the historical control range of SD rats (Charles River Laboratories: 1992-2002 and 2002 – 2011).

Methods

Doses:	0, 25, 75, 200 mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	RDEA 594 Distilled water 0.1 N HCl (to adjust pH) pH 6.8 ± 0.2 ▪ Dose solutions were prepared weekly and stored at [redacted] (b) (4).
Basis of dose selection:	The FDA-recommended doses used by the sponsor (see Special Protocol Agreement, 12/22/09) were based on an established MTD of 200 mg/kg/day in the 13 week interim sacrifice portion of a 6 month toxicology study in SD rats (SR08-095). The 25 and 75 mg/kg/day doses were selected to provide information on the dose-relationship of findings.
Species/Strain:	Rats Albino Rats (Outbred) VAF/Plus CD (Sprague-Dawley derived) [Crl:CD (SD) IGS BR]
Number/Sex/Group:	60/sex/group (See Table 4)
Age:	Approximately 6 weeks at initiation of dosing
Animal housing:	Animals were individually housed in elevated stainless steel, wire mesh cages with the exception of the first week of the stabilization period (doubly housed)
Paradigm for dietary restriction:	Not applicable
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	▪ 6 male & 6 female control rats were included for toxicokinetic assessment ▪ 12 rats/sex/RDEA594 treatment group were included for toxicokinetic assessment.
Deviation from study protocol:	Deviations from the study protocol were minor in nature and did not affect the study outcome or interpretation of the results.

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Observations and Results

Table 4. Experimental outline for 2 year rat carcinogenicity study with RDEA594.

Table 5. Animal number assignments for 2 year rat carcinogenicity study.

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Mortality

Animals were observed in their cages twice daily for mortality and signs of severe toxic or pharmacologic effects.

Males
The number of surviving control males decreased to 20 at week 97. This led to the sacrifice of all surviving males in all treatment groups at that time point. This action is consistent with the "Criteria for when to terminate groups due to reduced survival in an ongoing rodent carcinogenicity study" established by the FDA Executive Carcinogenicity Assessment Committee (ECAC, Updated: 9/11/13).

The number of surviving males in each treatment group at **week 97** is summarized in the sponsor's table below (Table 6). There is no evidence for a RDEA594 treatment-related effect on mortality in males. In addition, the percent survival values are consistent with the values listed for control male SD rats from Charles River Laboratories (20.0 – 62.9%) in a total of 30 different 2 year carcinogenicity studies conducted from 1992 – 2002[2].

Table 6. Number of surviving males (with % of initial total) per treatment group at study termination of 2 year carcinogenicity study with RDEA594.

Females
In females, dosing in the HD group was stopped at week 90, when the number of animals in this group decreased to 20 (Table 7). All animals in this group were sacrificed at week 97, when the number of surviving animals decreased to 15. All animals in the MD female group were sacrificed at week 91, when the number of surviving animals decreased to 15. Dosing was stopped in LD females at week 97 when surviving animal number reached 20, and all animals in the LD female group were sacrificed at week 100, when the number of surviving animals decreased to 15. Finally, all members of the control group were sacrificed at week 100, when the number of surviving animals decreased to 20. All of these actions are consistent with the rodent carcinogenicity study termination guidelines of the FDA ECAC. There was no apparent dose-related effect of RDEA594 on female survival.

[2] Giknis M. and Clifford, C. (2004) Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD® (SD) Rats from Control Groups.
http://www.criver.com/files/pdfs/rms/cd/rm_rm_r_lesions_survival_crlcd_sd_rats.aspx

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The overall percent survival values in females are consistent with the values listed for control female SD rats from Charles River Laboratories (20.0 – 62.2%) in a total of 31 different 2 year carcinogenicity studies conducted between 1992 - 2002[2].

Table 7. Numbers of surviving females (with % of initial total) per treatment group at study termination of 2 year carcinogenicity study with RDEA594.

For males and females in all treatment groups, animal deaths began to increase notably during weeks 53-56 and continued throughout the remainder of the study (Figure 1). There was no apparent dose dependent effect of RDEA594 treatment on mortality in males or females.

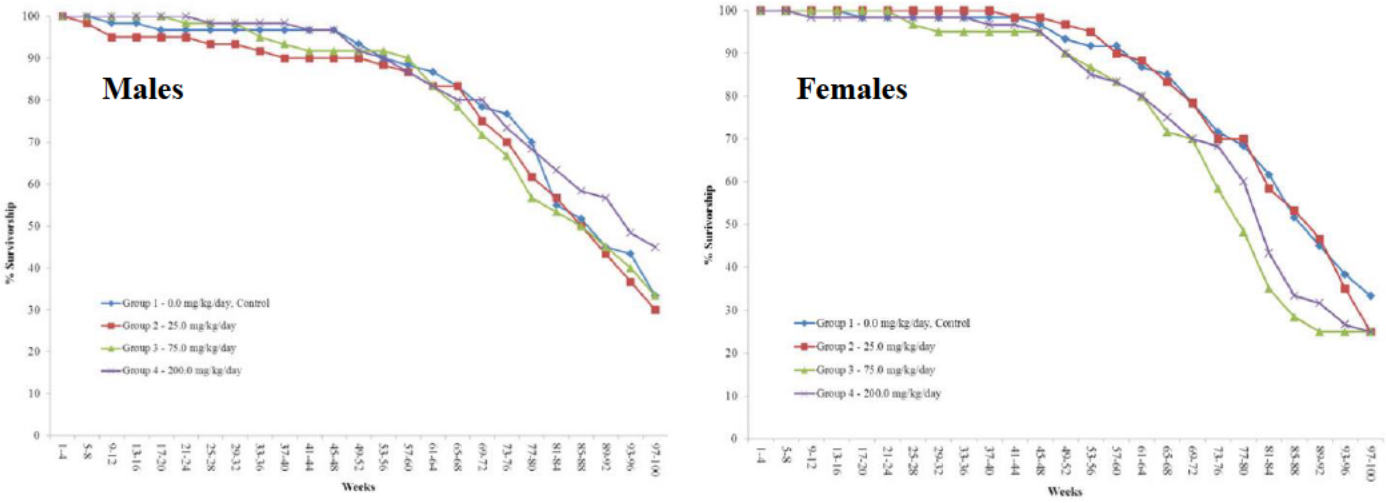

Figure 1. Percentage survivorship in male and female rats over the course of the 2 year carcinogenicity study with RDEA594.

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Clinical Signs

During the Dosing Phase, observations were made before and after dosing for any signs of poor health or toxic or pharmacologic effects such as abnormalities in general condition, appearance, activity, behavior and respiration. Physical examinations were conducted on main study animals twice prior to initiation of dosing and once weekly during the study period. The examinations included observation of general condition, skin and fur, eyes, nose, oral cavity, abdomen,

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external genitalia, respiration and palpation for tissue masses. TK animals were examined twice prior to initiation of dosing and once per month during the dosing phase.

There was evidence for increased incidence of thin appearance in HD males and females (Table 8, Table 9). This is consistent with the observed decreased mean body weight in these animals (Figure 2).

Incidence of decreased food consumption was also elevated relative to controls in MD males. Decreased mean food consumption did not result in decreased mean body weight in these animals. There was no clear signal in the actual measured food consumption data (in g/day) that there was an overall effect of RDEA594 on food consumption in males.

Increased incidence of swollen limbs/tail was observed in HD males and females. These findings may correlate with the observed impaired locomotion in these animals. Incidence of alopecia of the limbs was increased in HD females relative to all other dose groups (Table 9). Increased incidence of alopecia of the head/neck was observed in HD males only.

Table 8. Summary of potential treatment related effects on clinical signs detected in daily observations. Values are listed as "total # of observations"/ "total # of animals with finding".

	Males				Females			
RDEA594 dose (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
Decreased food consumption	70/8	79/9	112/14	82/14	196/21	291/26	169/16	146/28
Impaired locomotion	24/5	60/4	31/4	102/6	18/3	11/2	11/6	97/5
Thin appearance	85/4	87/6	73/9	103/11	280/11	292/13	211/10	307/16
Swollen limbs/tail	42/6	50/4	31/6	54/5	1/1	0	0	85/2

Table 9. Summary of potential treatment related effects on clinical signs detected in weekly physical examinations. Values are listed as "total # of observations"/ "total # of animals with finding".

	Males				Females			
RDEA594 dose (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
Alopecia, limbs	440/17	774/30	704/26	766/21	739/34	618/28	776/35	1286/42
Alopecia, Head/Neck	8/4	0	6/3	77/3	73/6	22/3	59/5	61/5
Thin appearance	21/7	7/3	25/8	52/13	53/9	40/9	45/10	51/11

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Palpable masses
There was no apparent effect of RDEA594 treatment on the incidence of palpable masses observed in males or females (Table 10). The overall increased incidence of masses observed in females is attributed by the sponsor to the high incidence of mammary tumors commonly seen in female rats.

Table 10. Sponsor's table summarizing the total number of animals in each treatment group with a palpable mass in the 2 year rat carcinogenicity study.

Body Weights

Carcinogenicity study animals were removed from their cages and weighed twice pretest, weekly during the first 14 weeks, every 2 weeks from Week 14 to Week 78, and in Weeks 82, 86, 91, 95 and 99 and terminally (after fasting), where applicable. Terminal, fasted body weights were obtained just prior to necropsy.

Mean absolute body weights were decreased in HD males vs. controls, with effects becoming evident at approximately week 7 and persisting throughout the remainder of the study (Figure 2).

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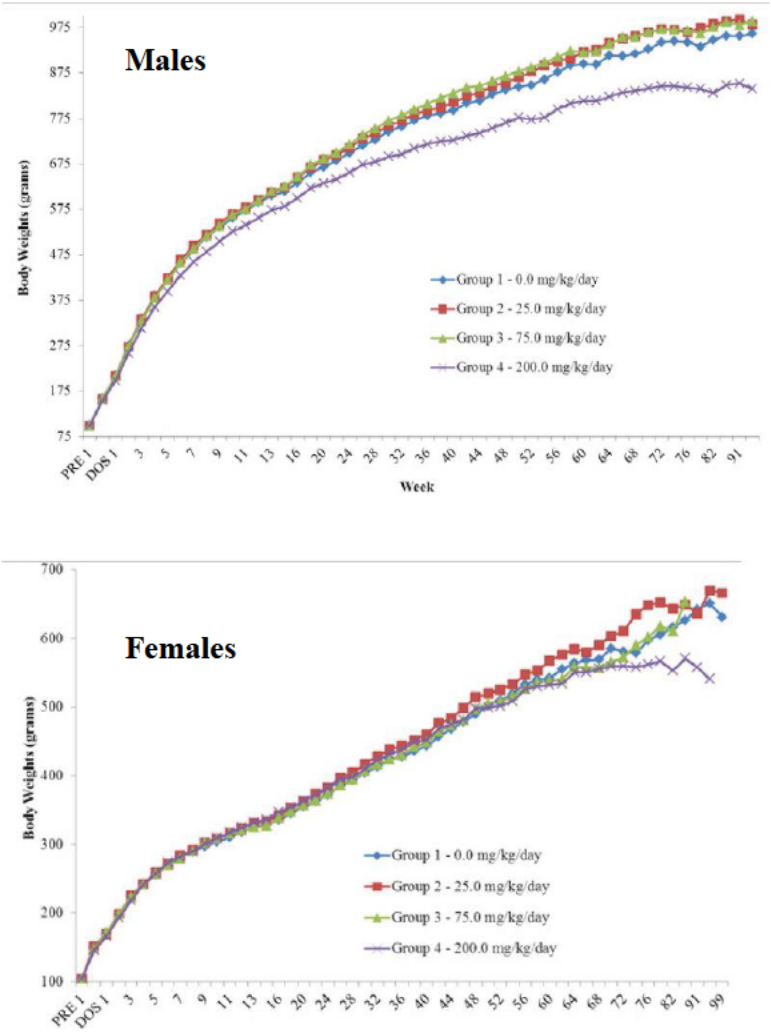

Figure 2. Mean body weight values in male and female rats over the course of the 2 year carcinogenicity study with RDEA594.

The magnitude of the difference in mean body weights between HD and control males increased over time. Mean absolute body weight was decreased by >10% vs. controls beginning at week 52, indicating that the maximum tolerated dose had been achieved (Table 11). Mean body weights were also decreased vs. controls in HD females beginning at approximately week 76, with the difference achieving statistical significance at week 91.

There were no changes of food consumption that corresponded with decreased body weights observed for males and females at 200 mg/kg/day. However, it is possible that decreased mean body weight values correlated with evidence for treatment induced lesions of the GI tract which included minimal – moderate single cell necrosis of the mucosa in the cecum, colon, ileum, jejunum and duodenum, as well as ulceration and hemorrhage of the cecum (Table 20). These

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findings are all consistent with lesions detected in rats in the 6 month general toxicology study (Study SR08-094).

Table 11. Changes in absolute body weights and body weight gains during the treatment period of the 2 yr rat carcinogenicity study.

	Males				Females			
RDEA594 dose (mg/kg)	0	25	75	200	0	25	75	200
Week 1								
n	60	60	60	60	60	60	60	60
Body weight (g)	209.7	208.4	207.7	198.6**	168.7	169.9	171.9	166.9
Week 26								
n	58	57	59	60	59	60	60	59
Body weight (g)	715.2	730	738.6	673.2**	386	396.7	386.2	394.4
Absolute BW (% control)	100%	102%	103%	94%	100%	103%	100%	102%
Δ from week 1 (g)	505.5	521.6	530.9	474.6	217.3	226.8	214.3	227.5
BW Gain (% Initial)	241%	250%	256%	239%	129%	133%	125%	136%
BW Gain (% control)	100%	103%	105%	94%	100%	104%	99%	105%
Week 52								
n	56	54	55	55	56	58	54	55
Body weight (g)	848	877.8	887.2	772.5**	509.5	525.4	507.7	501.5
Absolute BW (% control)	100%	104%	105%	91%	100%	103%	100%	98%
Δ from week 1 (g)	638.3	669.4	679.5	573.9	340.8	355.5	335.8	334.6
BW Gain (% Initial)	304%	321%	327%	289%	202%	209%	195%	200%
BW Gain (% control)	100%	105%	106%	90%	100%	104%	99%	98%
Week 78								
n	45	39	39	42	43	42	33	39
Body weight (g)	932.6	974.4	961.8	840.0**	605.4	652	617.8	566.6

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	Males				Females			
RDEA594 dose (mg/kg)	0	25	75	200	0	25	75	200
Absolute BW (% control)	100%	104%	103%	**90%**	100%	108%	102%	94%
Δ from week 1 (g)	722.9	766	754.1	641.4	436.7	482.1	445.9	399.7
BW Gain (% Initial)	345%	368%	363%	323%	259%	284%	259%	239%
BW Gain (% control)	100%	106%	104%	89%	100%	110%	102%	92%
Week 95								
n	27	25	24	30	25	21	0[a]	18
Body weight (g)	961.1	981.2	989.4	840.0**	650.9	669.3	NA	540.9*
Absolute BW (% control)	100%	102%	103%	**87%**	100%	103%	NA	**83%**
Δ from week 1 (g)	751.4	772.8	781.7	641.4	482.2	499.4	NA	374
BW Gain (% Initial)	358%	371%	376%	323%	286%	294%	NA	224%
BW Gain (% control)	100%	103%	104%	85%	100%	104%	NA	78%

[a] All 75 mg/kg F were sacrificed at week 91 due to population decrease to $n = 15$
** $p \leq 0.01$; * $p \leq 0.05$ vs. control

Feed Consumption

Food was presented to animals in full feeders of known weight. Food consumption was calculated by subtracting the feeder weight at a specified time interval from the initial weight. Food consumption was measured prior to the initiation of dosing, weekly through week 14, every 2 weeks for weeks 14 – 78 and in weeks 82, 86, 91, 95, and 99 for main study animals. Food consumption was measured over a total of 50 intervals. Food consumption values for each interval were reported in the study report in g/day. Mean food consumption, in g/day, was calculated by the reviewer and reported for each of 4 approximately 6 month intervals covering the entire course of the study

Mean food consumption was slightly increased in 200 mg/kg treated males vs. controls over the first 6 months of the study (Figure 3, Table 12). Mean body weight gain was slightly decreased in these animals over this same time period (Table 11), providing evidence for RDEA594 toxicity in male rats at this dose. There were no notable differences in mean food consumption in males at any dose over the remainder of the study.

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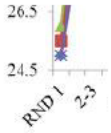
200 mg/kg RDEA594 treatment resulted in increased mean daily food consumption in females over the course of weeks 1 – 79 of the study ((Figure 3, Table 12). There was no concomitant effect on mean body weight in these animals.

Males



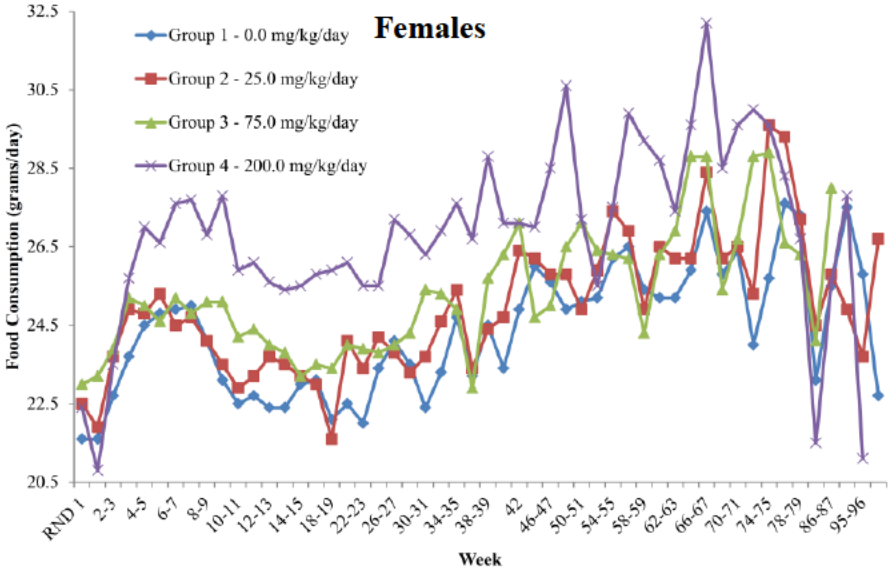


Figure 3. Mean food consumption values in male and female rats over time during 2 year carcinogenicity study with RDEA594.

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Table 12. Mean ± SEM food consumption values in rats over approximately 6 month intervals in a 2 year carcinogenicity study.

	Males				Females			
RDEA594 dose (mg/kg)	0	25	75	200	0	25	75	200
Weeks 1 - 27								
number of measurements	20	20	20	20	20	20	20	20
Mean food consumption (g/day)	33.5 ± 0.4	33.7 ± 0.4	34.8 ± 0.3	35.6 ± 0.6	23.2 ± 0.2	23.7 ± 0.2	24.2 ± 0.2	25.9 ± 0.4
Weeks 28 - 53								
number of measurements	13	13	13	13	13	13	13	13
Mean food consumption (g/day)	32.3 ± 0.2	32.5 ± 0.2	34.0 ± 0.2	33.8 ± 0.2	24.4 ± 0.3	25.0 ± 0.3	25.5 ± 0.3	27.4 ± 0.4
Weeks 54 - 79								
number of measurements	13	13	13	13	13	13	13	13
Mean food consumption (g/day)	32.4 ± 0.3	33.1 ± 0.3	34.3 ± 0.4	33.1 ± 0.5	26.0 ± 0.3	27.0 ± 0.4	26.9 ± 0.4	29.0 ± 0.4
Weeks 82-96								
number of measurements	4	4	4	4	4	4	2	4
Mean food consumption (g/day)	33.3 ± 0.8	32.8 ± 0.7	34.5 ± 1.0	32.0 ± 0.7	25.5 ± 0.9	24.7 ± 0.4	26.1 ± 2.0	24.0 ± 1.6

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Clinical pathology

Complete hematology, clinical chemistry, and urinalysis parameters were not measured in main study animals in this study. These measures are not required in carcinogenicity studies, as outlined in *OECD TG 451: Draft OECD Guideline for the Testing of Chemicals (November, 2008).*

Interim analysis of liver pathology

Liver –related clinical chemistry values (AST, ALT, ALP, Total bilirubin, direct bilirubin, indirect bilirubin) were measured in TK animals at the conclusion of the TK dosing period (week 72). Definitive conclusions regarding evidence for treatment related effects are difficult to make based on the relatively low number of animals and high variability in these measures. Two parameters of note are alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Values for these parameters generally increased vs. controls at ≥ 75 mg/kg/day in males and females (Table 13).

These findings correlate with histopathology evidence in both TK animals (Table 14) and main study animals (Table 20) for increased incidence of biliary hyperplasia in rats that received ≥75 mg/kg/day RDEA594. This phenomenon appears to represent an exacerbation of a background effect given the large number of control animals with this finding.

A single male and a single female in the MD group had very high ALT values relative to the rest of the animals in its dose group thus increasing overall mean values. Specifically, these outlier values were from MD male 3071 (ALT: 239 U/L) and MD female 3561 (ALT: 630 U/L). Each animal had correlating liver histopathology findings including biliary hyperplasia (#3071 & #3561), multifocal necrosis (#3071), and hepatocellular vacuolation (#3561). Additionally, HD female 4572 had disproportionately high ALT (285 U/L) and ALP (371) values relative to the rest of the animals in its dose group which correlated with microscopic findings of hepatocellular vacuolation.

Table 13. Liver-related clinical chemistry values in TK rats at week 72.

	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	5	9	10	6	5	9	10	7
Alanine aminotransferase (U/L)	52	86	84	51	46	50	119	81
% Control	100%	165%	162%	98%	100%	109%	259%	176%
Alkaline Phosphatase (U/L)	82	63	116*	130*	41	28	43	61
% Control	100%	77%	141%	159%	100%	68%	105%	149%

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Table 14. Incidence of biliary hyperplasia in TK animals at week 72 in 2 year rat carcinogenicity study. Percentage incidence values are included to allow comparison between groups with differing numbers of animals examined.

	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	6	12	12	12	6	12	12	12
LIVER (# examined)	5	10	10	8	5	10	10	7
Biliary hyperplasia								
Minimal	0	3	3	2	0	3	1	2
Slight	2	0	3	3	1	2	1	2
Moderate	0	0	0	0	0	0	1	0
Total	**2**	**3**	**6**	**5**	**1**	**5**	**3**	**4**
% Incidence	**40%**	**30%**	**60%**	**63%**	**17%**	**50%**	**30%**	**57%**

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Anatomic Pathology

Terminal necropsy was performed o main study animals/sex/group at early sacrifice time points following concurrence from the Division in consultation with the ECAC (Table 6, Table 7). Euthanasia was conducted by exsanguination following carbon dioxide inhalation. Animals were fasted overnight prior to necropsy.

Unscheduled necropsy was conducted on carcinogenicity animals that were found dead or killed in moribund condition.

Gross Pathology

Complete macroscopic examinations were performed on all carcinogenicity animals. The macroscopic examination included evaluation of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass for the presence of macroscopic morphologic abnormalities.

Treatment related macroscopic findings were identified in the kidney (irregular surface, cysts) in HD males and females (Table 15). Small size and irregular shape of the kidney were also observed in HD females only. An additional potential treatment-related gross finding was observed in the thyroid, where 3/60 HD females had evidence of a mass compared to 1/60 control animals.

Table 15. Treatment related macroscopic findings in rats in the 2 year carcinogenicity study.

	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
KIDNEY (# examined)	60	60	60	60	60	60	60	60
Irregular surface	0	0	0	6	0	0	1	18
Irregular shape	0	0	0	0	0	1	0	3
Small	0	0	0	0	0	0	0	3
Cyst	0	3	2	13	1	1	0	7
THYROID (# examined)	60	60	60	60	60	60	60	60
Mass	1	1	0	0	1	0	1	3

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Histopathology

The tissues listed in Table 16 were preserved in 10% neutral buffered formalin. Smear preparations of bone marrow were air dried and fixed in absolute methanol. After fixation, tissues and organs from all animals were routinely processed, embedded in paraffin, cut at 4-7 µm, mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy. The list of tissues examined microscopically is considered to be adequate and complete.

Table 16. List of tissues examined microscopically in carcinogenicity animals in 2 year rat study.

ORGAN NAME	PRESERVED	EXAMINED MICROSCOPICALLY
adrenal glands	X	X
aorta (thoracic)	X	X
bone marrow smear (femur)	X	
bone (sternum, distal femur)	X	X
bone marrow (sternum, distal femur)	X	X[a]
brain (medulla, pons, cerebrum and cerebellum)	X	X
epididymides	X	X
esophagus	X	X
eyes	X	X
Harderian gland	X	X
heart	X	X
kidneys	X	X
lacrimal glands	X	X
large intestine (cecum, colon, rectum[b])	X	X
liver	X	X
lungs (with mainstem bronchi)	X	X
lymph nodes (mesenteric, mediastinal)	X	X
mammary gland	X	X
nasal cavity	X	
nerve (sciatic)	X	X
optic nerve	X	
ovaries	X	X
pancreas	X	X
pituitary gland	X	X
prostate gland	X	X
salivary glands (submandibular)	X	X
seminal vesicles	X	X
skeletal muscle (*rectus femoris*)	X	X
skin (dorsal – base of tail)	X	X

Peer Review

A peer review of the microscopic findings reported by the study pathologist was performed by the reviewing pathologist as follows:

(1) Re-examination of all tissues from 10% of the control and high dose group per sex selected randomly.
(2) Re-examination of all hyperplasias and neoplasias diagnosed by the study pathologist in all groups in both sexes
(3) Re-examination of the following target tissues from all animals in all groups: kidney, stomach, duodenum, jejunum, ileum, cecum, colon.

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The sponsor states that the tables contained in the final study report reflect the mutually agreed upon diagnoses.

Neoplastic Lesions

Slight increases in incidence of neoplastic lesions in HD males and/or females relative to concurrent controls were seen in the lymphatic/reticular system, vascular tissue, pancreas, pituitary and skin (Table 17). However incidence levels were very low and there was no evidence for statistically significant trend effects of RDEA594 dose or effects between specific dose groups and controls (see Appendices 1 & 2).

Neoplastic lesions were observed in the thyroid in male rats (Table 17). C-cell *adenomas* were observed in 4/60 HD males (6.7%) vs 0/60 controls. C-cell *carcinomas* were observed in 0/60 HD males (0%) and 1/59 controls (1.7%). Statistical analysis found no significant trend effect of RDEA594 dose on tumor (adenoma + carcinoma) incidence (Table 18). Furthermore there were no significant differences in tumor incidence between any dose groups and controls. Female control and drug-treated animals were observed with comparable incidences of C-cell adenomas + carcinomas.

Thyroid C-cell adenomas and carcinomas are relatively common spontaneous lesions in control SD rats according to Giknis and Clifford[2] in their report of neoplasms detected in control animals in 2-year carcinogenicity studies conducted from 1992 – 2002 (Table 19).

The sponsor also included more recent historical control data for neoplasms in "Charles River Rats from 2 year Studies" in the current study report (Table 19). It is assumed that the sponsor is referring to studies with SD rats. The incidence of thyroid C-cell adenoma and carcinoma in the present study falls within the range reported in both datasets of historical control animals.

Given the lack of statistically significant differences in tumor incidence between any dose group and controls, along with incidence values falling within an established historical control range for SD rats, the observed tumor findings in the thyroid are not considered to be related to RDEA594 treatment. **RDEA594 was not tumorigenic in rats at doses up to 200 mg/kg/day**.

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Table 17. Potential treatment related neoplastic findings in 2 year RAT carcinogenicity study with RDEA 594 (NDA 207988).

	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
THYROID (# examined)	59	60	60	60	60	60	60	60
Benign Follicular cell adenoma	3	2	3	4	1	1	2	0
Malignant Follicular cell carcinoma	0	0	0	0	0	0	0	1
Adenoma + Carcinoma	3	2	3	4	1	1	2	1
Benign C-cell adenoma	0	2	2	4	4	3	2	3
Malignant C-cell carcinoma	1	1	0	0	1	0	1	1
Adenoma + Carcinoma	1	3	2	4	5	3	3	4
LYMPHATIC/RETICULAR SYSTEM (# examined)	60	60	60	60	60	60	60	60
Malignant granulocytic leukemia	0	0	0	1	0	0	0	0
Malignant lymphoma	0	0	0	0	0	0	0	1
VASCULAR TISSUE (# examined)								
Hemangioma	0	0	1	0	1	0	0	1
Hemangiosarcoma	1	0	2	0	0	0	0	1
Hemangioma + hemangiosarcoma	1	0	3	0	1	0	0	2

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	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
PANCREAS (# examined)	60	60	60	60	60	60	60	60
Benign islet cell adenoma	2	5	4	2	0	0	0	2
Malignant islet cell carcinoma	1	1	3	1	2	2	1	0
Adenoma + carcinoma	3	6	7	3	2	2	1	2
PITUITARY (# examined)	60	60	60	60	60	60	60	60
Benign pars distalis adenoma	31	23	30	28	43	41	41	49
Malignant pars distalis carcinoma	0	0	0	0	0	3	4	1
Adenoma + carcinoma	31	23	30	28	43	44	45	50
SKIN (other) [# examined]	10	11	12	10	38	41	35	35
Malignant Myxosarcoma	0	0	0	2	0	0	0	0

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Table 18. Statistical evaluation of effects of RDEA594 treatment on incidence of thyroid tumor formation in male rats (from Statistics Reviewer Steven Thomson, Ph.D.)

THYROID	C-CELL ADENOMA	P-value of test of trend or comparison	.0482	.2337	.2403	.0640
		Poly-3 adjusted number of animals at risk	39.7	38.0	39.0	41.8
		Number of animals reported with tumor	0	2	2	4
		Unadjusted number of animals at risk	59	60	60	60
	C-CELL CARCINOMA	P-value of test of trend or comparison	.9371	.7400	1	1
		Poly-3 adjusted number of animals at risk	39.8	37.9	37.8	41.4
		Number of animals reported with tumor	1	1	0	0
		Unadjusted number of animals at risk	59	60	60	60
	C-Cell Adenoma/Carcinoma	P-value of test of trend or comparison	.1571	.2977	.4901	.1955
		Poly-3 adjusted number of animals at risk	39.8	38.1	39.0	41.8
		Number of animals reported with tumor	1	3	2	4
		Unadjusted number of animals at risk	59	60	60	60

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Table 19. Incidence of thyroid neoplasms in male rats in the current study vs. historical control data from SD rat carcinogenicity studies conducted between 1992 – 2011.

	Males (current study)				Historical control data (males)	
RDEA594 dose (mg/kg)	0	25	75	200	Giknis & Clifford (1992-2002)	Charles River (2002-2011)
Thyroid C cell Adenoma						
Total/ No. examined	0/59	2/60	2/60	4/60	168/2141	50/843
% Incidence	0%	3.3%	3.3%	6.7%	Overall Mean: 7.9% Range: 1.4 – 14.8% 28/30 studies	Overall Mean: 5.9% Range: 1.4 – 16.9% 13/13 studies
Thyroid C cell Carcinoma						
Total/ No. examined	1/59	1/60	0/60	0/60	30/2141	6/843
% Incidence	1.7%	1.7%	0%	0%	Overall Mean: 1.4% Range: 1.4 – 14.8% 16/30 studies	Overall Mean: 0.71% Range: 1.4 – 2.0% 5/13 studies
Thyroid C cell Carcinoma + Adenoma						
Total/ No. examined	1/59	3/60	2/60	4/60	198/2141	56/843
% Incidence	1.7%	5.0%	3.3%	6.7%	Overall Mean: 9.2% Range: 1.7 – 17.0% 29/30 studies	Overall Mean: 6.6% Range: 1.4% – 18.6% 13/13 studies

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Non Neoplastic Lesions

The principal non-neoplastic target organs of toxicity identified in main study animals in the current study are the GI tract, liver, kidney, pancreas and thyroid (Table 20).

GI Tract
Evidence for treatment induced lesions of the GI tract included minimal – moderate single cell necrosis of the mucosa in the cecum, colon, ileum, jejunum and duodenum in male and female rats treated with ≥ 75 mg/kg/day (Table 20). There was evidence for increased incidence of hemorrhage of the stomach as well as ulcer, hyperplasia, and erosion of the nonglandular stomach in treated males and females vs. controls. Although findings in the nonglandular stomach are not relevant to humans, the overall body of evidence in the GI tract suggests a direct irritant effect of RDEA594 treatment administered via oral gavage.

The GI findings in the current study are generally consistent with the evidence for mucosal hemorrhage and erosion of the stomach in male and female rats receiving ≥100 mg/kg/day RDEA594 in the 6 month general toxicology study (Study SR08-095).

Liver
Biliary hyperplasia (minimal – moderate) was observed with increased incidence in males and females that received ≥ 75 mg/kg/day vs. controls. This evidence is consistent with the histopathology findings in the liver of TK animals assessed at week 72. Given the high incidence of this effect in control animals, it appears that RDEA594 treatment is exacerbating a background phenomenon present in aged rats. It is noted that there was no evidence for a dose-dependent progression from biliary hyperplasia to biliary neoplasia.

Kidney
Multiple RDEA594-related changes were observed in the kidneys of male and female rats at the 200 mg/kg dose level. These included tubular degeneration, tubular casts, cortical interstitial inflammation, tubular dilatation, papillary inflammation, papillary necrosis/loss, urothelial hyperplasia, cystic hyperplasia of collecting ducts and cysts. Severity scores generally ranged from minimal – marked for all findings. It is well understood that aged rats suffer from chronic progressive nephropathy (CPN). However, the dose-dependence of the kidney findings in the present study provides strong evidence that RDEA594 has pathologic effects in the kidney. It is noted that females appeared to be more sensitive to RDEA594-related kidney pathology as there was evidence for dose-related increases in incidence and severity of tubular degeneration, papillary necrosis, and urothelial hyperplasia at doses ≥ 25 mg/kg/day.

Pancreas
There was an apparent dose-response relationship of RDEA594 on exocrine hyperplasia of the pancreas in male rats only. Incidence and severity of this finding increased with dose at ≥75 mg/kg/day. The pancreas was also an apparent target organ (single cell necrosis) in male rats at doses ≥ 100 mg/kg/day at 3 months and at 6 months in study SR08-095.

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Thyroid

Focal C-cell **hyperplasia** of the thyroid was observed in males and females with an apparent dose-response relationship in females only. Diffuse C-cell hyperplasia was observed in LD and HD females only. These findings are notable given the apparent increase in thyroid C-cell adenomas in males (Table 17). C-cell hyperplasia may potentially represent a preneoplastic lesion that is part of the continuum toward tumor formation.

The thyroid was also identified as a target organ of toxicity for RDEA594 in rats in the 6 month toxicology study (SR08-095). In this study, male and female rats treated with ≥ 100 mg/kg/day had increased incidence of **hypertrophy** of the thyroid follicular epithelium. Incidences of follicular cell adenoma and carcinoma were comparable in male and female control and drug-treated animals.

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Table 20. Potential treatment related non-neoplastic findings in 2 year RAT carcinogenicity study with RDEA 594 (NDA 207988).

	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
CECUM (# examined)	49	50	47	49	56	56	55	53
Single cell necrosis, mucosa								
Minimal	0	0	0	0	0	0	0	1
Slight	0	0	0	1	0	0	0	0
Moderate	0	0	1	0	0	0	0	2
Marked	0	0	0	1	0	0	0	1
Total	**0**	**0**	**1**	**2**	**0**	**0**	**0**	**4**
Ulcer								
Minimal	0	0	0	0	0	1	0	1
Slight	0	0	0	1	0	0	0	1
Marked	0	0	0	0	0	0	0	1
Total	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**3**
Hemorrhage								
Minimal	0	0	0	0	0	1	0	1
Slight	0	0	0	0	0	0	1	2
Moderate	0	0	0	0	0	0	0	1
Total	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**4**
COLON (# examined)	60	56	56	58	58	60	59	55
Single cell necrosis, mucosa								
Slight	0	0	1	1	0	0	0	0
Moderate	0	0	0	0	0	0	0	3
Total	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**3**
DUODENUM (# examined)	59	57	57	58	60	60	60	58
Single cell necrosis, mucosa								
Minimal	0	0	0	1	0	0	0	1
Slight	0	0	1	3	0	0	1	4
Moderate	0	0	1	0	0	0	1	4

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	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
Total	**0**	**0**	**2**	**4**	**0**	**0**	**2**	**9**
ILEUM (# examined)	56	52	52	51	55	57	54	58
Single cell necrosis, mucosa								
Minimal	0	0	0	0	0	0	0	2
Slight	0	0	0	2	0	0	0	3
Moderate	0	0	1	1	0	0	1	1
Marked	0	0	0	0	0	0	0	1
Total	**0**	**0**	**1**	**3**	**0**	**0**	**1**	**7**
JEJUNUM (# examined)	59	54	52	52	56	58	54	58
Single cell necrosis, mucosa								
Minimal	0	0	0	1	0	0	0	2
Slight	0	0	2	2	0	0	0	4
Moderate	0	0	0	1	0	0	1	4
Total	**0**	**0**	**2**	**4**	**0**	**0**	**1**	**10**
KIDNEY (# examined)	60	60	60	60	60	60	60	60
Tubular degeneration								
Minimal	18	16	16	20	7	9	9	17
Slight	8	4	1	10	0	3	3	8
Moderate	4	2	3	6	0	0	1	14
Marked	0	0	0	8	0	0	1	10
Total	**30**	**22**	**20**	**44**	**7**	**12**	**14**	**49**
Tubular casts								
Minimal	22	14	15	19	8	5	4	14
Slight	2	2	2	6	0	0	1	5
Moderate	2	0	0	5	0	0	0	8
Marked	0	0	0	0	0	0	1	1
Total	**26**	**16**	**17**	**30**	**8**	**5**	**6**	**28**
Inflammation, interstitial, cortical								
Minimal	17	22	20	21	8	8	7	14

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	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
Slight	7	5	1	6	0	1	2	10
Moderate	2	1	3	6	0	0	0	10
Marked	0	0	0	7	0	0	1	11
Total	**26**	**28**	**24**	**40**	**8**	**9**	**10**	**45**
Tubular dilatation								
Minimal	2	1	3	13	0	1	10	15
Slight	0	1	1	9	1	1	2	12
Moderate	2	1	0	6	0	0	0	6
Marked	0	0	0	3	0	0	0	6
Total	**4**	**3**	**4**	**31**	**1**	**2**	**12**	**39**
Necrosis/loss, papilla								
Minimal	0	1	1	2	0	0	2	2
Slight	0	0	0	2	0	0	1	8
Moderate	0	0	0	4	0	0	0	4
Marked	0	0	0	2	0	0	0	3
Total	**0**	**1**	**1**	**10**	**0**	**0**	**3**	**17**
Inflammation, papilla								
Minimal	3	0	1	0	0	0	0	4
Slight	0	0	0	4	0	0	1	6
Moderate	0	0	0	5	0	0	0	13
Marked	0	1	0	0	0	0	0	0
Total	**3**	**1**	**1**	**12**	**0**	**0**	**1**	**23**
Hyperplasia, urothelium								
Minimal	2	1	5	10	2	7	10	10
Slight	3	1	2	10	0	3	3	24
Moderate	0	0	0	4	0	1	2	13
Total	**5**	**2**	**7**	**24**	**2**	**11**	**15**	**47**
Hyperplasia, cystic, collecting ducts								
Minimal	0	0	0	3	0	0	0	7

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	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
Slight	0	0	0	1	0	0	0	5
Moderate	0	0	0	2	0	0	0	4
Total	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**16**
Cyst								
Minimal	5	0	1	4	1	0	1	4
Slight	0	0	0	1	0	0	0	1
Marked	0	0	0	1	0	0	0	0
Total	**5**	**0**	**1**	**6**	**1**	**0**	**1**	**5**
LIVER (# examined)	60	60	60	60	60	60	60	60
Biliary hyperplasia								
Minimal	15	14	21	23	13	15	14	11
Slight	16	22	26	30	23	21	29	31
Moderate	2	3	4	2	1	3	0	4
Marked	1	0	0	0	0	0	0	0
Total	**34**	**39**	**51**	**55**	**37**	**39**	**43**	**46**
Cystic degeneration								
Minimal	8	4	10	15	4	4	1	1
PANCREAS (# examined)	60	60	60	60	60	60	60	60
Hyperplasia, exocrine								
Minimal	0	0	2	0	0	1	0	0
Slight	2	2	1	5	1	0	2	0
Moderate	1	0	1	2	0	0	0	0
Marked	0	0	1	1	0	0	0	0
Total	3	2	5	8	1	1	2	0
THYROID (# examined)	59	60	60	60	60	60	60	60
Hyperplasia, C-cell, Focal								
Minimal	1	0	0	0	0	1	1	0
Slight	1	1	5	1	2	5	6	6
Moderate	0	1	3	1	1	3	1	5

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	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
Marked	1	0	0	0	2	0	0	0
Total	3	2	8	2	5	9	8	11
Hyperplasia, C-cell, Diffuse								
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	2	0	3
Total	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**4**
STOMACH (#examined)	60	60	60	60	60	60	60	60
Hemorrhage								
Minimal	0	1	1	1	0	1	0	0
Slight	0	0	1	1	0	0	0	0
Moderate	0	0	0	0	0	0	1	0
Total	**0**	**1**	**2**	**2**	**0**	**1**	**1**	**0**
STOMACH, NONGLANDULAR (# examined)	60	60	60	60	60	60	60	60
Ulcer								
Minimal	2	0	0	0	1	1	3	2
Slight	2	0	1	2	1	1	2	3
Moderate	0	1	2	3	0	3	1	1
Marked	0	1	1	0	1	0	1	2
Total	**4**	**2**	**4**	**5**	**3**	**5**	**7**	**8**
Hyperplasia								
Minimal	0	0	4	0	3	2	5	2
Slight	0	3	5	6	3	5	2	6
Moderate	0	1	0	0	2	0	0	0
Total	**0**	**4**	**9**	**6**	**8**	**7**	**7**	**8**
Erosion								
Minimal	0	0	0	1	0	0	2	1

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	Males				Females			
RDEA 594 (mg/kg)	0	25	75	200	0	25	75	200
number of animals	60	60	60	60	60	60	60	60
Slight	0	0	0	0	0	1	0	1
Total	**0**	**0**	**0**	**1**	**0**	**1**	**2**	**2**

Reference ID: 3799181

Toxicokinetics

Main toxicokinetic study

6 male and 6 female control rats were maintained on study as TK animals, along with 12 rats/sex/group for all RDEA594-treated groups. TK animals were maintained on study for 72 weeks. Blood samples were obtained at day 1 and weeks 4, 13, 26, 39, 52 and 72 according to the schedule outlined in Table 21. Approximately 0.5 ml of whole blood was collected from each animal at each time point via the jugular vein.

Table 21. Sponsor's table outlining the schedule of TK sampling in 2 year rat carcinogenicity study.

C_{max} and systemic exposure to RDEA594 increased in an approximately dose-proportional manner in males and females through week 72 (Figure 4, Table 22). The observed values are generally consistent with the expected results based on the data from the 6 month rat study (SR08-095). There was decreased systemic exposure relative to week 1 (Accumulation ratio = 0.52 – 0.75) in males at ≥75 mg/kg starting at week 26, suggesting potential liver enzyme autoinduction. A similar decrease in systemic exposure was observed in females at 200 mg/kg at week 72. T_{max} ranged from 1.0 – 6.0 hrs. $T_{1/2}$ was not reported. Systemic exposure values remained generally consistent at all doses between day 1 – week 4 and throughout the remainder of the 72 weeks of data collection, suggesting that steady state is achieved quickly.

There were no marked differences between males and females in any toxicokinetic parameters reported.

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Males

Females

Figure 4. RDEA 594 concentration vs. time graphs in male and female rats over 24 hours after dosing during **week 72**.

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Table 22. Summary of toxicokinetic data in male and female rats in 2 year carcinogenicity study with RDEA594.

Males

	Dose (mg/kg/day)	AUC_{0-24hr} (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	Ratio to Day 1	
					AUC_{0-24hr}	C_{max}
Day 1	25	134	17.6	3.00	NA	NA
	75	621	70.5	3.00	NA	NA
	200	1740	174	3.00	NA	NA
Week 4	25	109	15.1	3.00	0.813	0.858
	75	614	76.0	3.00	0.989	1.08
	200	1470	142	6.00	0.845	0.816
Week 13	25	137	18.3	3.00	1.02	1.04
	75	654	78.1	3.00	1.05	1.11
	200	1650	173	6.00	0.948	0.994
Week 26	25	132	14.1	3.00	0.985	0.801
	75	465	47.8	3.00	0.749	0.678
	200	1210	127	6.00	0.695	0.730
Week 39	25	106	11.7	3.00	0.791	0.665
	75	477	63.8	3.00	0.768	0.905
	200	1560	147	6.00	0.897	0.845
Week 52	25	114	14.0	1.00	0.851	0.795
	75	454	55.0	3.00	0.731	0.780
	200	1300	127	6.00	0.747	0.730
Week 72	25	123	15.3	3.00	0.918	0.869
	75	431	48.6	3.00	0.694	0.689
	200	909	107	3.00	0.522	0.615

NA: Not applicable

Females

	Dose (mg/kg/day)	AUC_{0-24hr} (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	Ratio to Day 1	
					AUC_{0-24hr}	C_{max}
Day 1	25	94.9	13.2	3.00	NA	NA
	75	422	66.3	3.00	NA	NA
	200	1620	177	3.00	NA	NA
Week 4	25	87.4	15.6	3.00	0.921	1.18
	75	396	53.3	1.00	0.938	0.804
	200	1540	179	6.00	0.951	1.01
Week 13	25	109	20.6	1.00	1.15	1.56
	75	375	62.1	1.00	0.889	0.937
	200	1230	161	3.00	0.759	0.910
Week 26	25	114	19.5	1.00	1.20	1.48
	75	404	57.9	3.00	0.957	0.873
	200	1870	238	6.00	1.15	1.34
Week 39	25	79.1	13.7	1.00	0.834	1.04
	75	491	75.9	3.00	1.16	1.14
	200	1700	205	6.00	1.05	1.16
Week 52	25	113	14.1	6.00	1.19	1.07
	75	582	88.2	6.00	1.38	1.33
	200	1720	207	6.00	1.06	1.17
Week 72	25	104	14.0	3.00	1.10	1.06
	75	679	95.9	3.00	1.61	1.45
	200	1040	111	3.00	0.642	0.627

NA: Not applicable

Dosing Solution Analysis

During the study, RDEA594 dose solutions were prepared weekly and stored at [redacted] (b) (4) The sponsor points out that the test article mixtures (RDEA594 + water) are true solutions; therefore, no homogeneity analysis was performed.

All formulations in the dosing solution analysis report were prepared in distilled, deionized water at [redacted] (b) (4) and shipped to Ardea Biosciences [redacted] (b) (4). The concentration of RDEA594 in solution was determined by HPLC with UV detection. Dose confirmation analyses were conducted at weeks 1, 4, 8, 13, 26, 39, 52, 65, 78, and 91. At each time point, replicate samples were tested at each of the 3 dose concentrations used in the study: 0, 2.5, 7.5, and 20 mg/ml. In all cases, dose samples were within ± 10% of the target concentration. Secondly, replicate analysis showed less than 5% variation (Table 23).

Table 23. Results of RDEA594 dosing solution analysis at time points throughout the 2 year rat carcinogenicity study.

Sample Date	Analysis Date	Label Claim (mg/ml)	Replicate	Dilution factor	% Label Claim	Average (%)	Difference (%)
3-Feb-2010	12-Feb-2010	0	A	1.00	0.00[a]		
3-Feb-2010	12-Feb-2010	0	B	1.00	0.00[a]	0.00[a]	NA[b]
3-Feb-2010	12-Feb-2010	2.5	A	12.51	99.5		
3-Feb-2010	12-Feb-2010	2.5	B	12.60	100.7	100.1	1.2
3-Feb-2010	12-Feb-2010	7.5	A	37.93	100.3		
3-Feb-2010	12-Feb-2010	7.5	B	38.30	100.4	100.3	0.1
3-Feb-2010	12-Feb-2010	20	A	100.59	100.2		
3-Feb-2010	12-Feb-2010	20	B	101.45	100.4	100.3	0.2
24-Feb-2010	2-Apr-2010	0	A	1.00	0.00[a]		
24-Feb-2010	2-Apr-2010	0	B	1.00	0.00[a]	0.00[a]	NA[b]
24-Feb-2010	2-Apr-2010	2.5	A	12.86	100.8		
24-Feb-2010	2-Apr-2010	2.5	B	12.69	101.3	101.1	0.5
24-Feb-2010	2-Apr-2010	7.5	A	38.52	101.0		
24-Feb-2010	2-Apr-2010	7.5	B	37.88	99.8	100.4	1.2
24-Feb-2010	2-Apr-2010	20	A	102.97	100.2		
24-Feb-2010	2-Apr-2010	20	B	100.37	100.2	100.2	0.0
23-Mar-2010	2-Apr-2010	0	A	1.00	0.00[a]		
23-Mar-2010	2-Apr-2010	0	B	1.00	0.00[a]	0.00[a]	NA[b]
23-Mar-2010	2-Apr-2010	2.5	A	12.83	97.7		
23-Mar-2010	2-Apr-2010	2.5	B	12.88	98.6	98.1	0.9
23-Mar-2010	2-Apr-2010	7.5	A	38.91	100.5		
23-Mar-2010	2-Apr-2010	7.5	B	38.44	100.2	100.4	0.3
23-Mar-2010	2-Apr-2010	20	A	102.26	99.9		
23-Mar-2010	2-Apr-2010	20	B	101.48	100.1	100.0	0.2

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Sample Date	Analysis Date	Label Claim (mg/ml)	Replicate	Dilution factor	% Label Claim	Average (%)	Difference (%)
27-Apr-2010	13-May-2010	0	A	1.00	0.00[a]		
27-Apr-2010	13-May-2010	0	B	1.00	0.00[a]	0.00[a]	NA[b]
28-Apr-2010	13-May-2010	2.5	A	12.85	100.1		
28-Apr-2010	13-May-2010	2.5	B	12.69	100.1	100.1	0.0
28-Apr-2010	13-May-2010	7.5	A	38.19	100.2		
28-Apr-2010	13-May-2010	7.5	B	38.33	100.6	100.4	0.4
28-Apr-2010	13-May-2010	20	A	102.76	101.3		
28-Apr-2010	13-May-2010	20	B	99.28	100.7	101.0	0.6
27-Jul-2010	4-Aug-2010	0	A	1.00	0.00[a]		
27-Jul-2010	4-Aug-2010	0	B	1.00	0.00[a]	0.00[a]	NA[b]
28-Jul-2010	4-Aug-2010	2.5	A	12.74	100.5		
28-Jul-2010	4-Aug-2010	2.5	B	12.53	99.6	100.1	0.9
28-Jul-2010	4-Aug-2010	7.5	A	38.45	95.8		
28-Jul-2010	4-Aug-2010	7.5	B	38.26	96.5	96.2	0.7
28-Jul-2010	4-Aug-2010	20	A	101.00	95.2		
28-Jul-2010	4-Aug-2010	20	B	100.63	95.1	95.2	0.1
26-Oct-2010	4-Nov-2010	0	A	1.00	0.00[a]		
26-Oct-2010	4-Nov-2010	0	B	1.00	0.00[a]	0.00[a]	NA[b]
26-Oct-2010	4-Nov-2010	2.5	A	12.81	98.8		
26-Oct-2010	4-Nov-2010	2.5	B	12.85	99.4	99.1	0.6
26-Oct-2010	4-Nov-2010	7.5	A	37.85	97.4		
26-Oct-2010	4-Nov-2010	7.5	B	38.27	98.5	97.9	1.1
26-Oct-2010	4-Nov-2010	20	A	99.61	99.2		
26-Oct-2010	4-Nov-2010	20	B	102.85	99.0	99.1	0.2
25-Jan-2011	11-Mar-2011	0	A	1.00	0.00[a]		
25-Jan-2011	11-Mar-2011	0	B	1.00	0.00[a]	0.00[a]	NA[b]
25-Jan-2011	11-Mar-2011	2.5	A	12.78	98.3		
25-Jan-2011	11-Mar-2011	2.5	B	12.64	97.6	98.0	0.7
25-Jan-2011	11-Mar-2011	7.5	A	37.59	100.3		
25-Jan-2011	11-Mar-2011	7.5	B	38.20	99.3	99.8	1.0
25-Jan-2011	11-Mar-2011	20	A	98.41	99.2		
25-Jan-2011	11-Mar-2011	20	B	101.86	99.6	99.4	0.4
26-Apr-2011	15-Jun-2011	0	A	1.00	0.00[a]		
26-Apr-2011	15-Jun-2011	0	B	1.00	0.00[a]	0.00[a]	NA[b]
26-Apr-2011	15-Jun-2011	2.5	A	12.43	100.2		
26-Apr-2011	15-Jun-2011	2.5	B	12.60	99.2	99.7	1.0
26-Apr-2011	15-Jun-2011	7.5	A	37.82	99.3		
26-Apr-2011	15-Jun-2011	7.5	B	38.39	99.2	99.3	0.1
26-Apr-2011	15-Jun-2011	20	A	100.32	99.0		
26-Apr-2011	15-Jun-2011	20	B	102.22	99.2	99.1	0.2

[a]

End of study Stability Testing of RDEA594 Sodium Salt drug substance
Drug substance lots NE-023025-A— (b) (4) #1, NE-023025-Batch-04-2009, and 17JL01.HQ00001 were tested to evaluate their stability over the duration of its use in a 2 year carcinogenicity study. These lots were stored at RT and shipped from (b) (4) to Ardea Biosciences, Inc. at the conclusion of the carcinogenicity study. Both lots were found to meet all specifications.

Stability of RDEA594 sodium salt in water (Study SR09-016)
RDEA594 included data that they obtained upon stability testing of RDEA594 sodium salt (Lot 0904697) in water under varying storage conditions. RDEA594 in water (0.8 or 72 mg/mL) was found stable (b) (4) (Table 24).

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Table 24. Sponsor's tables (Study SR09-016) showing stability of RDEA594 sodium salt (lot 0940967) in water at concentrations of 0.8 mg/ml or 72 mg/ml.

(b) (4)

(b) (4)

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8.2 RDEA594: 26-week Oral Gavage Carcinogenicity and Toxicokinetic Study in CBYB6F1-Tg(Hras)2Jic Mice

Study no.:	SR10-019
Study report location:	IND 102128 Supporting Document 203 NDA 207988 Supporting document 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 2, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RDEA594 Sodium salt Batch #17JL01.HQ00001 (b) (4) 87.6% purity
CAC concurrence:	Yes

Key Study Findings

- In a 26 week carcinogenicity study, male and female TgRasH2 mice (25/sex/group) received RDEA594 by oral gavage at the following doses
  - Males: 0 (water), 15, 45, 125 mg/kg/day
  - Females: 0, 45, 90, 250 mg/kg/day
- There was no effect of RDEA594 treatment on survival
  - Premature deaths were increased in HD Females vs. vehicle controls (4/25 vs. 1/25)
  - Mortality was within a historical control range for female TgRasH2 mice
  - The effect is not considered to be related to RDEA594 treatment
- RDEA594 was not tumorigenic in male or female TgRasH2 mice
  - Males: up to 125 mg/kg/day
  - Females: up to 250 mg/kg/day
- MNU (75 mg/kg/day)- treated positive control mice had neoplastic lesions in the hematopoietic system (malignant lymphosarcoma), skin, and nonglandular stomach. These target organs are consistent with established results in MNU-treated TgRrasH2 mice.
- Non-neoplastic target organs of toxicity included the liver and glandular stomach
  - Liver findings in males ≥ 45 mg/kg/day; females at 250 mg/kg/day
  - Glandular stomach: females ≥ 30 mg/kg/day
- Toxicokinetic evaluation was conducted at the conclusion of the dosing period
  - RDEA594 C_{max} and AUC_{0-24} increased approximately proportionally with dose in both males and females

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Adequacy of Carcinogenicity Study

- The 26-week treatment duration was considered adequate for carcinogenicity assessment in TgrasH2 mice.
- Concurrence for all doses was obtained from the ECAC.
- There was no early termination of any study group due to mortality.

Appropriateness of Test Models

- The 26-week carcinogenicity study was conducted in male and female Hemizygous TgRasH2 mice. This study design represents an established model for assessment of carcinogenic potential[1]. The oral gavage route of administration models the clinical route of administration.
- Concurrence for the general study design was obtained from the ECAC.

Evaluation of Tumor Findings

- There were no RDEA-594 related neoplastic findings.
- MNU treated positive control animals had evidence of tumor formation in the following organs:
    - Whole body: benign hemangioma & malignant hemangiosarcoma
    - Nonglandular stomach: Squamous cell: benign papilloma & malignant carcinoma
    - Skin/Subcutis:
        - Squamous cell: benign papilloma
        - Zymbal's gland: malignant carcinoma
    - Uterus: benign polyp, endometrial
    - Vagina: Squamous cell: benign papilloma & malignant carcinoma

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Methods	
Doses:	▪ Males: 0, 15, 45, 125 mg/kg/day ▪ Females: 0, 30, 90, 250 mg/kg/day ▪ Positive control: Intraperitoneal N-methyl-N-nitrosourea (MNU): 75 mg/kg/day (M & F)
Frequency of dosing:	Once per day Positive control: MNU administered IP on study day 1
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Reverse osmosis water
Basis of dose selection:	The FDA-recommended doses used by the sponsor (see Special Protocol Agreement, 4/28/11) were based on established MTDs of 125 mg/kg/day and 250 mg/kg/day in male and female WT mice (TgRasH2 background), respectively, in a 28 d dose-range finding study.
Species/Strain:	▪ Carcinogenicity: Mouse: 001178-T (hemizygous), CByB6F1-Tg(HRAS)2Jic ▪ Toxicokinetic: Mouse: 001178-W (wild-type), CByB6F1-Tg(HRAS)2Jic ▪ Positive control: Mouse: 001178-T (hemizygous), CByB6F1-Tg(HRAS)2Jic
Number/Sex/Group:	▪ Carcinogenicity: 25/sex/group ▪ Toxicokinetic: o Vehicle: 3/sex/group o Treated groups: 18/sex/group ▪ Positive Control: 20 M & 20 F ▪ See Table 25
Age:	6 – 7 weeks old at initiation of dosing
Animal housing:	Animals were individually housed
Paradigm for dietary restriction:	No
Dual control employed:	No
Interim sacrifice:	Not applicable
Satellite groups:	▪ 3 male & 3 female control WT mice were included for toxicokinetic assessment 18 Tg mice/sex/RDEA594 treatment group were included for toxicokinetic assessment.
Deviation from study protocol:	There were no deviations that affected the study outcome or interpretation.

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Observations and Results

Male and female transgenic mice were obtained from (b) (4)
Animals were dosed daily with RDEA594 from study day 1 – 184. MNU treated positive controls were dosed on day 1 only.

Table 25. Experimental outline for 26 week TgRasH2 mouse study with RDEA594.

(b) (4)

d

Mortality

Animals were observed in their cages twice daily for mortality, abnormalities, and signs of pain or distress.

RDEA594 treated mice
There was no effect of RDEA594 treatment on mortality in male mice (Figure 5). There was an increase in premature deaths in HD females vs. controls (4/25 vs. 1/25; Table 26). Statistical analysis identified a significant trend effect of RDEA594 dose on mortality in females (p =0.0166). Pairwise comparison of mortality values in HD females vs. vehicle controls resulted in a p value of 0.0549 (Table 27). The cause of death was undetermined in 3/4 HD females that died prior to study completion. It is notable that there were no neoplastic findings in any of these animals. The premature death of HD Female #A59709 was reported to be due to complications related to gavage error. The histopathology data reported for this animal appear to support this explanation.

MNU treated positive control mice
Males and females in the MNU treated group (Group 5) showed significantly increased mortality vs. controls, as expected. Premature deaths occurred in 14/20 males and 14/20 females (Table 26). The most common cause of death in these animals was malignant lymphosarcoma. Deaths were first observed in MNU treated animals at study day 75.

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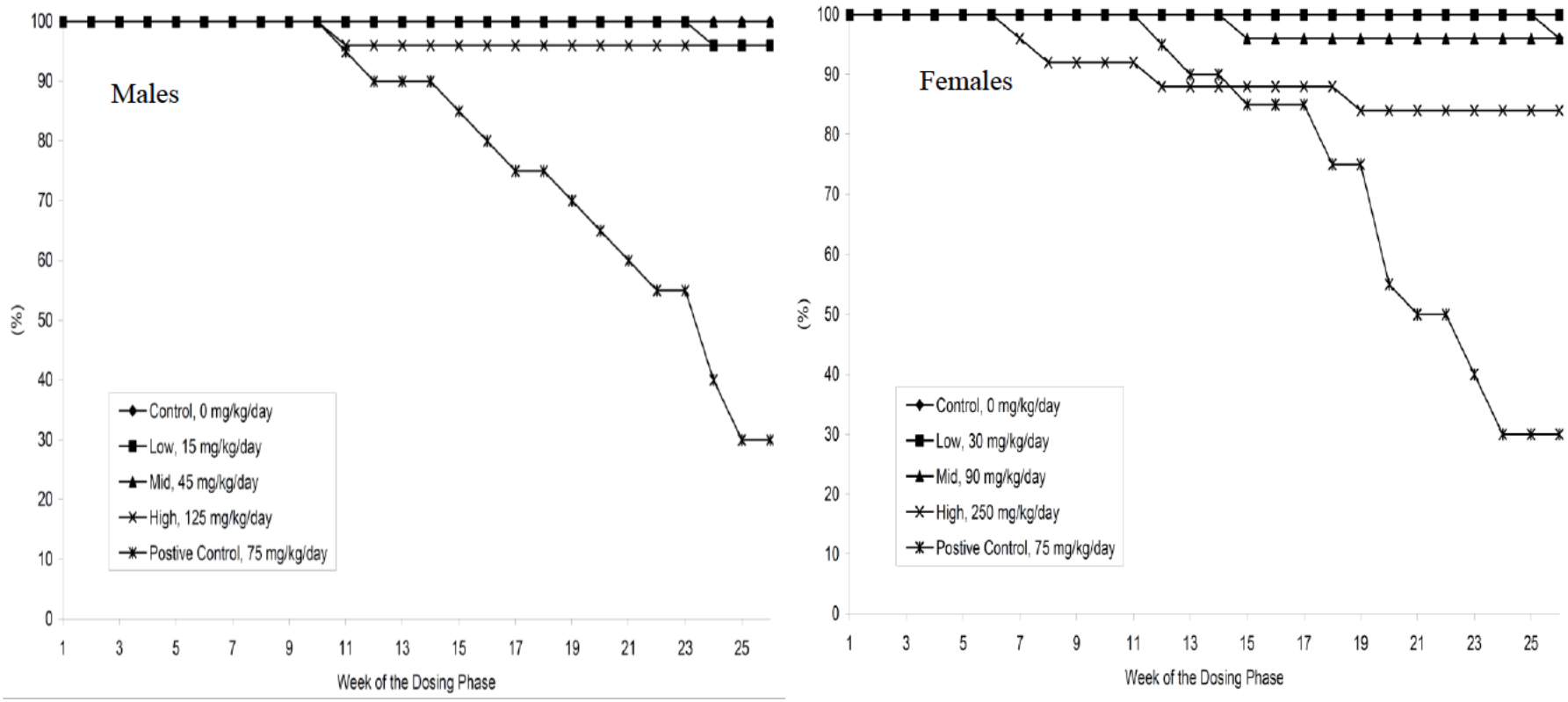

Figure 5. Survival graphs for male and female TgRasH2 mice treated with RDEA594 in a 26 week carcinogenicity study.

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Table 26. Premature deaths in male and female RDEA594-treated TgRasH2 mice in 26 week carcinogenicity study.

Sex	RDEA594 dose group (mg/kg/d)	Premature deaths (# deaths / total animals in group)	Animal ID #	Study day of death	Description
Male	Vehicle	0/25	NA	NA	-
	15	1/25	A59448	168	**Found dead.** Undetermined cause of death. Thoracic cavity contains grey fluid
	45	0/25	NA	NA	-
	125	1/25	A59525	77	**Moribund sacrifice**. Undetermined cause of death.
	MNU	14/20	A59553	115	**Found dead.** Undetermined
			A59554	165	**Moribund sacrifice.** Malignant lymphosarcoma
			A59556	162	**Moribund sacrifice.** Malignant lymphosarcoma
			A59557	127	**Moribund sacrifice.** Malignant lymphosarcoma
			A59559	75	**Found dead.** Malignant lymphosarcoma.
			A59561	141	**Moribund sacrifice.** Malignant lymphosarcoma
			A59562	169	**Found dead.** Undetermined
			A59563	162	**Moribund sacrifice.** Malignant lymphosarcoma
			A59565	101	**Found dead.** Malignant squamous cell carcinoma, stomach.
			A59566	107	**Moribund sacrifice.** Undetermined.
			A59567	135	**Moribund sacrifice.** Malignant lymphosarcoma

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Sex	RDEA594 dose group (mg/kg/d)	Premature deaths (# deaths / total animals in group)	Animal ID #	Study day of death	Description
			A59568	82	**Found dead.** Malignant squamous cell carcinoma, stomach
			A59571	153	**Found dead.** Malignant lymphosarcoma
			A59572	170	**Moribund sacrifice.** Undetermined
Female	Vehicle	1/25	A59590	182	**Found dead**. Thoracic cavity dark red; contains fluid. Gavage-related death.
	30	0/25	NA	NA	-
	90	1/25	A59665	99	**Found dead**. Death attributed to spleen hemangiosarcoma with rupture of mass and blood loss into abdominal cavity
	250	4/25	A59695	47	**Moribund sacrifice.** Undetermined cause of death. No evidence of neoplasms.
			A59699	83	**Moribund sacrifice.** Undetermined cause of death. No evidence of neoplasms.
			A59700	56	**Moribund sacrifice.** Undetermined cause of death. No evidence of neoplasms.
			A59709	127	**Moribund sacrifice.** Gavage-related death suggested by focal areas of periacinar macrophage infiltrates in one lobe of lung + bronchial chronic – active inflammation
	MNU	14/20	A59731	134	**Found dead.** Undetermined.
			A59732	160	**Moribund sacrifice.** Malignant lymphosarcoma
			A59733	167	**Found dead.** Malignant lymphosarcoma
			A59735	165	**Found dead.** Undetermined.
	MNU		A59737	100	**Moribund sacrifice.** Squamous cell carcinoma, stomach
			A59738	88	**Found dead.** Malignant lymphosarcoma

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Sex	RDEA594 dose group (mg/kg/d)	Premature deaths (# deaths / total animals in group)	Animal ID #	Study day of death	Description
Female			A59740	141	**Moribund sacrifice.** Malignant lymphosarcoma
			A59741	78	**Found dead.** Malignant lymphosarcoma
			A59742	138	**Found dead.** Malignant lymphosarcoma
			A59743	123	**Found dead.** Malignant hemangiosarcoma
			A59745	161	**Moribund sacrifice.** Malignant lymphosarcoma
			A59746	134	**Moribund sacrifice.** Malignant lymphosarcoma
			A59747	140	**Found dead.** Malignant lymphosarcoma.
			A59749	120	**Moribund sacrifice.** Malignant lymphosarcoma

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Table 27. Statistical analysis of the effect of RDEA594 on TgRasH2 mouse survival in a 26 week carcinogenicity study (Table provided by Steven Thomson, Ph.D., FDA Office of Biostatistics).

Hypotheses	Males		Females	
	Logrank	Wilcoxon	Logrank	Wilcoxon
Homogeneity over all groups 1-4.	1.00		0.0663	
Pairwise Vehicle vs High (i.e., groups 1 vs 4)	0.50		0.0549	
Trend over groups 1 to 4	0.4774	0.4730	0.0166	0.0167
Homogeneity over all groups 1-5	0.0		0.0	
Pairwise Vehicle vs Positive Control (i.e., groups 1 vs 5)	0.0		0.0	

Clinical Signs

Detailed observations were conducted on carcinogenicity animals at least once during the predose phase, before dosing on Day 1, and weekly thereafter during the dosing phase as well as on the day of scheduled sacrifice. For each grossly visible palpable mass: time of onset, location, size, appearance and/or progression was recorded weekly.

Clinical signs of hunched posture, hypoactive behavior, and irregular respiration were observed in many of the animals that died prior to study completion (Table 28). HD females were the only RDEA594 treated group with evidence for treatment-related adverse clinical signs.

Table 28. Incidence values for potential RDEA594-related clinical observations in TgRasH2 mice in a 26 week carcinogenicity study.

	Males					Females				
RDEA594 dose (mg/kg)	0	15	45	125	MNU	0	30	90	250	MNU
number of animals	25	25	25	25	20	25	25	25	25	20
Appearance										
Hunched	0	1 A59448*	0	1 A59525*	9	1 A59590*	0	1 A59654	6 A59688 A59689 A59695* A59696 A59700* A59709*	6
Behavior										
Hypoactive	0	1 A59448*	0	1 A59525*	2	0	0	1 A59654	4 A59688 A59695* A59699* A59700*	2
Excretion										

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	Males					Females				
RDEA594 dose (mg/kg)	0	15	45	125	MNU	0	30	90	250	MNU
number of animals	25	25	25	25	20	25	25	25	25	20
Feces, few	0	1 A59448*	0	0	4	2 A59581 A59590*	0	1 A59655	4 A59696 A59700* A59709* A59710	4
Respiration										
Irregular	0	0	0	1 A59525*	6	1 A59590*	0	0	5 A59688 A59689 A59699* A59700* A59709*	4
Labored	0	1 A59448*	0	1 A59525*	3	0	0	0	3 A59689 A59699* A59709*	0
Skin and pelage										
Cold to touch, entire body	0	1 A59448*	0	1 A59525*	2	0	0	0	3 A59699* A59700* A59695*	0

* Denotes RDEA594 treated animal that died prior to study completion

Palpable masses

There was no evidence for RDEA594-evoked palpable masses in males or females. Palpable masses were detected in a total of 12/20 MNU treated males and 16/20 MNU-treated females over the course of the study period. Specific mass locations and incidence values are listed in Table 29.

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Table 29. Incidence of palpable masses detected in TgRasH2 mice in a 26 week carcinogenicity study.

	Males					Females				
RDEA594 dose (mg/kg)	0	15	45	125	MNU	0	30	90	250	MNU
number of animals	25	25	25	25	20	25	25	25	25	20
Left inguinal	0	0	0	0	2	0	0	0	0	2
Left lumbar	0	0	0	0	1	0	0	0	0	0
Left Sacral	0	0	0	0	0	0	0	0	0	3
Left scrotum	0	0	0	0	5	0	0	0	0	0
Midline lumbar	0	0	0	0	1	0	0	0	0	0
Oral mucosa	0	0	0	0	1	0	0	0	0	2
Penis	0	0	0	0	2	-	-	-	-	-
Perineal area	0	0	0	0	0	0	1	0	0	9
Right hind foot	0	0	0	0	0	0	1	0	0	0
Right inguinal	0	0	0	0	0	0	0	0	0	1
Right scrotum	0	0	0	0	6	0	0	0	0	0
Vagina	-	-	-	-	-	0	1	0	0	8

Body Weights

Body weights were recorded for all animals at least once during the pre-dose phase, before dosing on day 1, and weekly thereafter.

There was no effect of RDEA594 on body weight at any dose tested (Figure 6). MNU treated males and females had statistically significant increases in mean body weight vs. vehicle control mice (Table 30).

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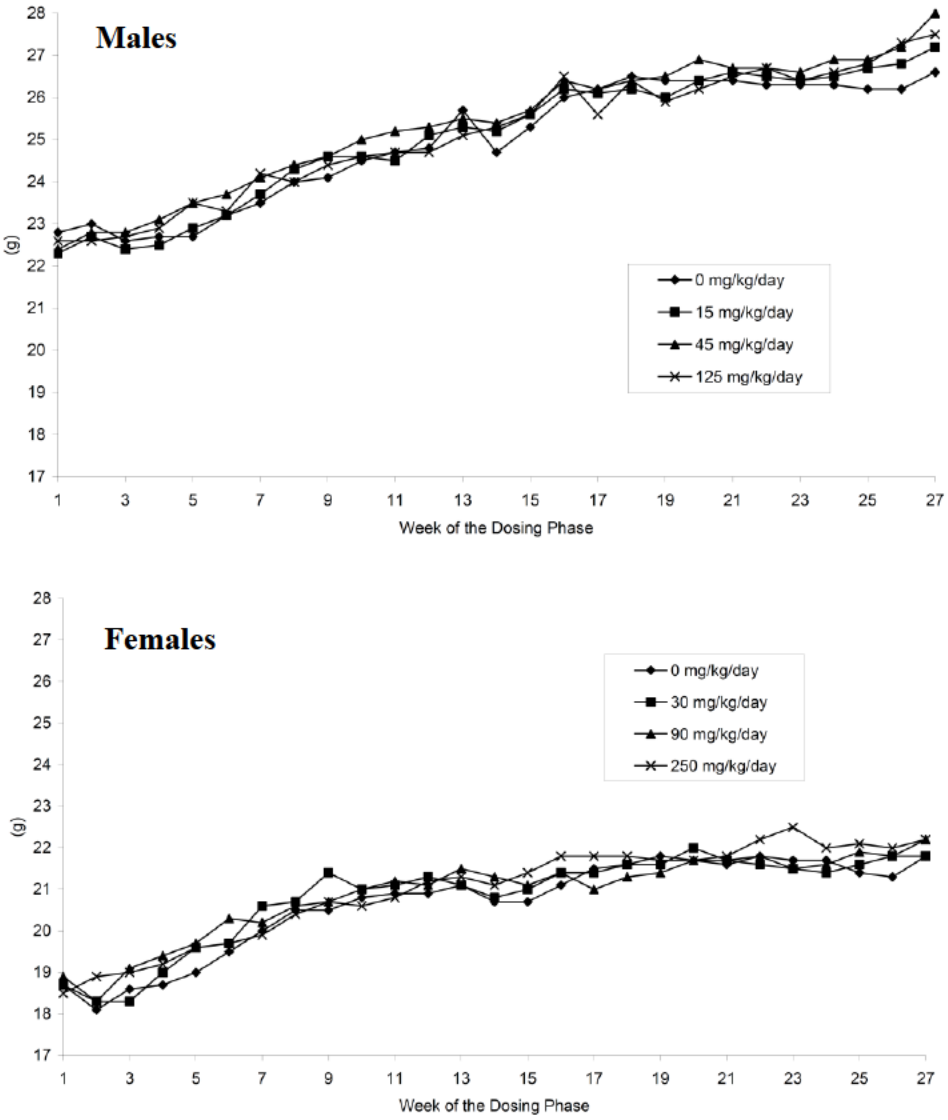

Figure 6. Mean body weight values in male (upper panel) and female (lower panel) TgRasH2 mice treated with RDEA594 for 26 weeks.

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Table 30. Changes in mean absolute body weights and body weight gains during the treatment period of the 26 week TgRasH2 mouse carcinogenicity study.

	Males					Females				
RDEA594 dose (mg/kg)	0	15	45	125	MNU	0	30	90	250	MNU
Week 1										
n	25	25	25	25	20	25	25	25	25	20
Body weight (g)	22.8	22.3	22.4	22.6	22.2	18.7	18.7	18.9	18.5	18.2
Week 13										
n	25	25	25	24	18	25	25	25	22	19
Body weight (g)	25.7	25.3	25.5	25.1	27.9*	21.1	21.1	21.5	21.3	21.9
Absolute BW (% control)	100%	98%	99%	98%	**109%**	100%	100%	102%	101%	104%
Δ from week 1 (g)	2.9	3	3.1	2.5	5.7	2.4	2.4	2.6	2.8	3.7
BW Gain (% Initial)	13%	13%	14%	11%	26%	13%	13%	14%	15%	20%
BW Gain (% control)	100%	103%	107%	86%	197%	100%	100%	108%	117%	154%
Week 27										
n	25	24	25	24	6	24	25	24	21	6
Body weight (g)	26.6	27.2	28	27.5	30.4*	21.8	21.8	22.2	22.2	22.9*
Absolute BW (% control)	100%	102%	105%	103%	**114%**	100%	100%	102%	102%	**105%**
Δ from week 1 (g)	3.8	4.9	5.6	4.9	8.2	3.1	3.1	3.3	3.7	4.7
BW Gain (% Initial)	17%	22%	25%	22%	37%	17%	17%	17%	20%	26%
BW Gain (% control)	100%	129%	147%	129%	216%	100%	100%	106%	119%	152%

$p \leq 0.05$ vs. vehicle (ANOVA with Dunnett's post test)

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Feed Consumption

Food consumption was measured quantitatively once per week during the dosing phase in carcinogenicity animals. There was no effect of RDEA594 treatment on mean food consumption at any dose tested (Figure 7).

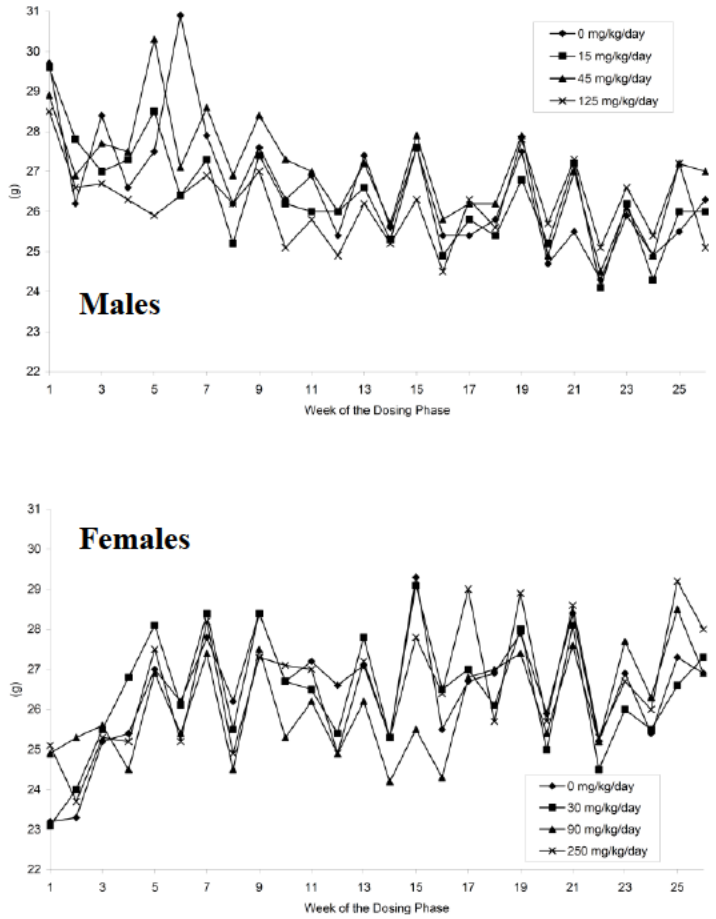

Figure 7. Mean food consumption values in male (upper panel) and female (lower panel) TgRasH2 mice treated with RDEA594 for 26 weeks.

Reference ID: 3799181

Clinical pathology

Blood samples for hematology assessment were collected via cardiac puncture from all surviving animals on study day 184. Potassium EDTA was used as the anticoagulant. Animals were not fasted for blood collection. Complete clinical chemistry and urinalysis parameters were not measured in this study. These measures are not required in carcinogenicity studies per FDA ECAC policy, and as outlined in *OECD TG 451: Draft OECD Guideline for the Testing of Chemicals (November, 2008).*

Hematology

Hematology parameters collected included:

Red blood cells	Monocytes
White blood cells	Eosinophils
Neutrophils	Basophils
Lymphocytes	Large unstained cells

There were no notable RDEA594-related changes in hematology parameters in males or females.

A single MNU treated female (# A59744) had very high WBC counts (attributed to increases of neutrophils, lymphocytes: ~10x – 200x higher than the mean of the values of other surviving animals) likely indicative of an infection.

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Gross Pathology

On day 184, carcinogenicity animals were anesthetized with sodium pentobarbital, exsanguinated and necropsied. The necropsy was comprised of an examination of the external features of the carcass, external body orifices, abdominal, thoracic, and cranial cavities; organs and tissues.

There was no evidence for RDEA594-related macroscopic findings. There were numerous macroscopic findings identified in MNU-treated positive control animals (Table 31). The most common recorded observations included large size, discoloration, mass, and raised area. Gross findings in the skin/subcutis, stomach, and uterus appeared to correlate with evidence for neoplasms in these organs.

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Table 31. Macroscopic findings observed in TgRasH2 mice in 26 week carcinogenicity study.

	Males					Females				
RDEA 594 (mg/kg/day)	0	15	45	125	MNU	0	30	90	250	MNU
number of animals	25	25	25	25	20	25	25	25	25	20
KIDNEY (# examined)	25	25	25	25	20	25	25	25	25	20
Discolored	0	0	0	0	3	0	0	0	0	3
Large	0	0	0	0	3	0	0	0	0	2
LIVER (# examined)	25	25	25	25	20	25	25	25	25	20
Discolored	0	0	0	0	0	0	0	0	0	3
Large	0	0	0	0	1	0	0	0	0	2
LYMPH NODE, MANDIBULAR (# examined)	25	25	25	25	20	25	25	25	25	20
Large	0	0	0	0	2	0	0	0	0	6
LYMPH NODE, MESENTERIC (# examined)	25	25	25	25	20	25	25	25	25	20

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	Males					Females				
RDEA 594 (mg/kg/day)	0	15	45	125	MNU	0	30	90	250	MNU
Large	0	0	0	0	2	0	0	0	0	4
LYMPH NODE, OTHER (# examined)	25	25	25	25	20	25	25	25	25	20
Large	0	0	0	0	5	0	0	0	0	9
SKIN/SUBCUTIS (# examined)	25	25	25	25	20	25	25	25	25	20
Mass	0	0	0	0	4	0	0	0	0	6
Raised area	0	0	0	0	4	0	0	0	0	1
SPLEEN (# examined)	25	25	25	25	20	25	25	25	25	20
Discolored	0	0	0	1	0	0	0	0	0	3
Large	0	0	0	0	5	0	0	0	0	8
Mass	0	0	0	0	0	0	0	0	0	1
STOMACH (# examined)	25	25	25	25	20	25	25	25	25	20

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	Males					Females				
RDEA 594 (mg/kg/day)	0	15	45	125	MNU	0	30	90	250	MNU
Mass	0	0	0	0	3	0	0	0	0	3
Raised area	1	0	0	1	10	3	0	0	1	12
THYMUS (# examined)	25	25	25	25	20	25	25	25	25	20
Large	0	0	0	0	1	0	0	0	0	2
Mass	0	0	0	0	1	0	0	0	0	2
UTERUS (# examined)	0	0	0	0	0	25	25	25	25	20
Cyst	-	-	-	-	-	0	0	1	1	8
VAGINA (# examined)	0	0	0	0	0	25	25	25	25	20
Mass	-	-	-	-	-	0	2	0	0	3

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Organ weights

The following organ weights were recorded at the time of scheduled sacrifice.

There was evidence for a dose-dependent increase in liver/gallbladder weight in males and females (Table 32). This effect correlated with microscopic findings of hepatocellular hypertrophy in males at ≥ 45 mg/kg/day and females at 250 mg/kg/day (Table 35). MNU treatment did not affect liver weight to the same degree as RDEA594.

Table 32. Summary of relevant RDEA594-related effects on organ weight in TgRasH2 mice in a 26 week carcinogenicity study. Note that values in treated groups are expressed as percentage of control value.

	Males					Females				
RDEA 594 (mg/kg/day)	0	15	45	125	MNU	0	30	90	250	MNU
number of animals	25	25	25	25	20	25	25	25	25	20
Liver/gall bladder										
Absolute mean weight (g)	1.3554	105%	119%*	141%*	119%*	1.1915	104%	116%*	127%*	109%
Body weight (%)	5.2044	102%	114%*	135%*	103%	5.5302	103%	114%*	123%*	105%
Brain weight (%)	276.266	103%	119%*	139%*	116%*	225.6203	105%	118%*	135%*	113%*

$p \leq 0.05$ vs. control group

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Histopathology

A complete battery of tissues (Table 33) from all carcinogenicity animals were preserved in 10% neutral buffered formalin (except eyes, Harderian gland, optic nerve & testes: collected in modified Davidson's fixative & stored in 10% neutral buffered formalin).

Table 33. List of tissues collected in carcinogenicity animals in 26 week carcinogenicity study in Tg(HRas2)Jic mice.

adrenal (2)	muscle, biceps femoris
aorta	optic nerve (2)[a]
brain	ovary (2)
cecum	pancreas
cervix	pituitary gland
colon	prostate
duodenum	rectum
epididymis (2)	salivary gland [mandibular (2)]
esophagus	sciatic nerve
eye (2)[a]	seminal vesicle
femur with bone marrow (articular surface of the distal end)	skin/subcutis
	spinal cord (cervical, thoracic, and lumbar)
gall bladder (drained)	spleen
Harderian gland[a]	sternum with bone marrow
heart	stomach
ileum	testis (2)[a]
jejunum	thymus
kidney (2)	thyroid (2 lobes) with parathyroid
lesions	tongue
liver	trachea
lung with large bronchi	urinary bladder
lymph node (mandibular)	uterus
lymph node (mesenteric)	vagina
mammary gland (females)	

a Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin

Microscopic evaluation was performed on protocol-required tissues from all animals administered the vehicle control or test article (RDEA594). Microscopic evaluation of tissue samples from MNU-treated animals was limited to macroscopic lesions & thymus.

Tissues for microscopic evaluation were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were shipped to the principal investigator for anatomic pathology and examined microscopically.

Peer Review

A pathology peer review was performed at (b) (4) by the sponsor's designee. The study report represented a consensus between the study and peer review pathologists.

Neoplastic Lesions

There were no RDEA594-related neoplastic findings in this study (Table 34). The slight increase in incidence of Whole Body hemangioma + hemangiosarcoma in HD males vs. controls (2/25 vs. 1/25) did not achieve statistical significance (p[Trend] = 0.228, p[HD vs. Veh] = 0.745).

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There was evidence for multiple neoplastic lesions in MNU-treated positive control animals, confirming that the study could detect an expected characteristic tumorigenic response to a known carcinogen. Neoplastic lesions in MNU-treated mice were detected in the hematopoietic system (malignant lymphosarcoma), skin, and nonglandular stomach (Table 34). These target organs are consistent with established results in MNU-treated TgHras2 mice[1]. Neoplastic lesions were also identified in the uterus and vagina of MNU-treated female mice in the current study.

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Table 34. Summary of neoplastic lesions detected in 26 week carcinogenicity study with TgRasH2 mice.

	Males					Females				
RDEA 594 (mg/kg/day)	0	15	45	125	MNU	0	30	90	250	MNU
number of animals	25	25	25	25	20	25	25	25	25	20
BODY, WHOLE/CAVITY (# examined)	25	25	25	25	20	25	25	25	25	20
Benign hemangioma	0	0	0	0	0	0	0	1	0	0
Malignant hemangiosarcoma	1	1	0	2	0	1	2	1	2	3
Hemangioma + hemangiosarcoma	1	1	0	2	0	1	2	2	2	3
Malignant lymphosarcoma	0	0	0	0	11	0	0	0	0	14
SKIN/SUBCUTIS (# examined)	25	25	25	25	9	25	25	25	25	9
Benign papilloma, squamous cell	0	0	0	0	7	0	0	0	0	9
Malignant carcinoma, Zymbal's gland	0	0	0	0	0	0	0	0	0	2
STOMACH, NONGLANDULAR (# examined)	25	25	25	25	14	25	25	25	25	15
Benign papilloma, squamous cell	1	0	0	1	9	0	0	0	0	13

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	Males					Females				
RDEA 594 (mg/kg/day)	0	15	45	125	MNU	0	30	90	250	MNU
Malignant carcinoma, squamous cell	0	0	0	0	3	0	0	0	0	1
Papilloma + Carcinoma	**1**	**0**	**0**	**1**	**12**	**0**	**0**	**0**	**0**	**14**
THYROID (# examined)	25	25	25	25	1	255	25	25	25	0
Malignant carcinoma, squamous cell	0	0	0	0	1	0	0	0	0	0
UTERUS (# examined)	0	0	0	0	0	25	25	25	25	8
Benign polyp, endometrial, stromal	-	-	-	-	-	0	0	0	0	6
VAGINA (# examined)	0	0	0	0	0	25	25	25	25	4
Benign papilloma, squamous cell	-	-	-	-	-	0	0	0	0	2
Malignant carcinoma, squamous cell	-	-	-	-	-	0	0	0	0	1
Papilloma + Carcinoma	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**0**	**3**

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Non-neoplastic Lesions

RDEA594-related non-neoplastic lesions were observed in the liver and in the glandular stomach (Table 35). These target organs are consistent with findings in the 1 month dose-range finding study in WT mice (SR10-018) as well as the 6 month toxicology and 2 year carcinogenicity studies conducted in SD rats (Study SR08-095 and SR09-2168 respectively).

Non-neoplastic lesions of the thymus were observed in a small number of male and female MNU-treated animals only. The thymus is an established target organ of MNU-treated TgRasH2 mice[1].

Liver

Hepatocellular hypertrophy was seen in males at ≥45 mg/kg and females at 250 mg/kg. This is considered to be an adaptive response to RDEA594 dosing. Focal hepatocyte necrosis increased in a dose dependent manner in males at ≥45 mg/kg. This finding was also observed in females, but there was not a clear dose-effect relationship.

Glandular stomach

The incidence of epithelial hyperplasia as well as mucosal inflammation increased with RDEA594 dose in females only. For both findings, increased incidence vs. controls was observed at all RDEA594 doses. Epithelial hyperplasia and mucosal inflammation were also observed in males, but there was no evidence of a dose-effect relationship.

Thymus

Thymus findings were observed in MNU-treated animals included moderate – marked atrophy as well as atypical lymphocyte hyperplasia (minimal – marked).

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Table 35. Treatment related non-neoplastic findings in 26 week carcinogenicity study in TgRasH2 mice with RDEA 594 (NDA 207988).

	Males					Females				
RDEA 594 (mg/kg)	0	15	45	125	MNU	0	30	90	250	MNU
number of animals	25	25	25	25	20	25	25	25	25	20
LIVER (# examined)	25	25	25	25	0	25	25	25	25	0
Hepatocellular hypertrophy										
minimal	0	0	9	11	-	0	0	0	23	-
slight	0	0	0	12	-	0	0	0	0	-
Total	**0**	**0**	**9**	**23**	**-**	**0**	**0**	**0**	**23**	**-**
Hepatocyte necrosis, focal										
minimal	0	0	2	5	-	3	0	2	1	-
slight	0	0	0	1	-	0	0	0	1	-
Total	**0**	**0**	**2**	**6**	**-**	**3**	**0**	**2**	**2**	**-**
Mineralization, focal/multifocal										
minimal	0	0	0	1	-	0	0	0	1	-
moderate	0	0	0	1	-	0	0	0	0	-
Total	**0**	**0**	**0**	**2**	**-**	**0**	**0**	**0**	**1**	**-**
GLANDULAR STOMACH (# examined)	25	25	25	25	0	25	25	25	25	0
Inflammation, submucosa, muscularis mucosa										
minimal	4	4	6	1	-	2	6	9	4	-

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	Males					Females				
RDEA 594 (mg/kg)	0	15	45	125	MNU	0	30	90	250	MNU
slight	2	1	0	0	-	2	4	3	12	-
moderate	0	0	0	0	-	1	1	0	1	-
Total	**6**	**5**	**6**	**1**	**-**	**5**	**11**	**12**	**17**	**-**
Epithelium, hyperplasia										
minimal	2	2	2	0	-	2	3	4	3	-
slight	1	0	0	1	-	3	5	5	13	-
moderate	0	0	0	0	-	1	0	0	0	-
Total	**3**	**2**	**2**	**1**	**-**	**6**	**8**	**9**	**16**	**-**
THYMUS (# examined)	**24**	**25**	**23**	**25**	**19**	**25**	**24**	**24**	**24**	**20**
Atrophy										
moderate	0	0	0	0	0	0	0	0	0	3
marked	0	0	0	0	1	0	0	0	0	0
Total	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**3**
Hyperplasia, lymphocytes, atypical										
minimal	0	0	0	0	1	0	1	0	0	0
slight	0	0	0	0	1	0	0	0	0	1
moderate	0	0	0	0	0	0	0	0	0	0
marked	0	0	0	0	0	0	0	0	0	1
Total	**0**	**0**	**0**	**0**	**2**	**0**	**1**	**0**	**0**	**2**

Reference ID: 3799181

Toxicokinetics

A total of 18 wild-type TgrasH2 mice per sex per RDEA594 treatment group were included as toxicokinetic animals (Table 25). The vehicle control TK group was comprised of 3 males and 3 females. Blood samples were collected at the end of the dosing period (day 181) via cardiac puncture from up to 3 animals/sex/group/time point. Samples from control animals were collected approximately 3 hours post dose. Samples from RDEA594-treated animals were collected at approximately 0.5, 1, 3, 6, 12, and 24 hours post dose. Animals were anesthetized by carbon dioxide inhalation prior to blood collection.

Plasma analysis was conducted using a validated liquid chromatography/tandem mass spectrometry method. Toxicokinetic analysis of RDEA594 data was conducted using WinNonlin (5.2) and included peak concentration (C_{max}), time to peak concentration (T_{max}), and area under the concentration-time curve from time zero to 24 hours postdose ($AUC_{0\text{-}24hr}$).

The mean toxicokinetic parameters in this study are summarized in Table 36. Systemic exposure values increased approximately proportionally with dose in males and females. C_{max} values also increased proportionally with dose in males. This was not the case in females at the 250 mg/kg dose, as C_{max} increased less than proportionally with dose in these animals. Values for C_{max} and $AUC_{0\text{-}24}$ at the HD levels in males and females were consistent with what was observed at these same doses in the 4-week dose-range finding study (Study SR10-018).

Table 36. Summary of RDEA594 toxicokinetic parameters in TgRasH2 mice on day 181 of a 26-week carcinogenicity study.

Reference ID: 3799181

Dosing Solution Analysis

RDEA594 dosing solutions were analyzed using HPLC with ultraviolet detection. Duplicate samples were taken from each dose concentration (0, 1.5, 3.0, 4.5, 9.0, 12.5, 25.0 mg/ml) for testing at weeks 1, 4, 13, and 26 of the dosing phase. RDEA594 concentration verification samples were within 97 – 101% of the nominal concentration at each time point.

Duplicate samples of MNU solution were measured on day 1. MNU concentration was measured to be at 95.8% of its target concentration of 7.5 mg/ml.

Stability

Stability of the RDEA594 sodium salt form was previously evaluated by the sponsor for concentrations of 0.8 mg/ml and 72 mg/ml for up to (b) (4) (Study SR09-016).

The stability of N-methyl-N-Nitrosourea (MNU) was confirmed for the following conditions:

- 5.0 & 9.0 mg/ml for 12 days at 2 – 8°C
- 5.0 & 9.0 mg/ml for 4 hrs on wet ice followed by 4 hrs at RT + 24 hr at 2 – 8°C + 6 hrs at RT

Homogeneity

The sponsor stated that the RDEA594 and MNU formulations were true solutions; therefore no homogeneity testing was performed in this study.

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11 Integrated Summary and Safety Evaluation

Rat carcinogenicity study

RDEA594

There were no treatment-related effects on survival in the 2 year rat study with oral RDEA594. A maximum tolerated dose was achieved (200 mg/kg) in both males and females based on reductions of absolute body weights of >10% compared to controls. RDEA594 was not tumorigenic in rats at doses up to 200 mg/kg. The systemic exposure at this dose provides safety margins of >32 vs. the exposure at the clinical dose of 200 mg/day (Table 37).

Table 37. Exposure margin calculation for RDEA594 at the tumorigenicity NOAEL in the rat based on systemic exposure values at the latest time point of TK sample collection (week 72).

Species	RDEA594 Dose	Sex	RDEA594 AUC_{0-24} (µg*hr/ml)	Exposure margin
Human	200 mg (maximum proposed human dose)	M & F	28.0[a]	-
Rat	200 mg/kg/day (NOAEL)	Male	909 (Week 72)	32.5
		Female	1040 (Week 72)	37.1

[a] Mean AUC_{0-24} (M & F combined, n = 12) for 200 mg RDEA594 administered 1x/d for 14 days in healthy adults (Study RDEA594-105)

Non-neoplastic target organs in both males and females included the GI tract, liver, kidney, pancreas and thyroid at doses ≥25 mg/kg/day. The findings in these organs were generally consistent with evidence in the previously conducted chronic (6 month) toxicology study in SD rats (Study SR08-095).

Evaluation of the Carcinogenic Potential of the M3c and M4 Metabolites

The M4 metabolite of RDEA594 is a disproportionate metabolite in humans, as it is present at 20.7% of the total RDEA594 dose administered (Study RDEA594-112). The structure of M4 does not raise significant clinical concerns. However, M4 is formed in humans via an epoxide intermediate termed M3c (Figure 8). The epoxide metabolite raises potential clinical concerns due to its recognized structural alert for mutagenicity and the chronic duration of RDEA594 dosing.

Ardea has determined in an in vitro study that RDEA594 is oxidized to form M3c via CYP2C9 activity (Study SR11-031). M3c is subsequently converted to the didhydrodiol M4 via the activity of microsomal epoxide hydrolase (mEH). The M3c epoxide metabolite is unstable and is not detectable *in vivo* in any species tested (human, rat, monkey).

Reference ID: 3799181

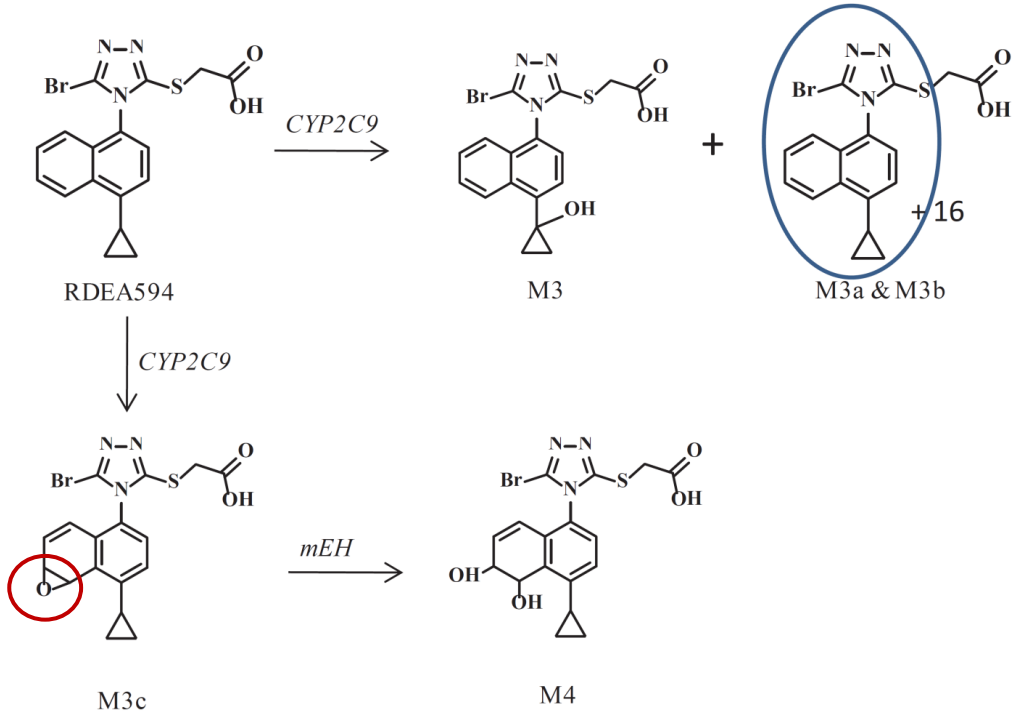

Figure 8. Sponsor's proposed metabolic pathway for formation of the M4 metabolite in humans. The M3c epoxide intermediate is formed via oxidation of RDEA594 by CYP2C9. M3c is then converted to M4 via the activity of microsomal epoxide hydrolase (mEH). *From* Study SR11-031.

An exploratory non-GLP plasma metabolic profiling study was included in the 2 year rat carcinogenicity study to establish metabolite exposures and provide evidence for safety qualification with respect to carcinogenicity (SR09-070-MET). Analyses were performed using the toxicokinetic samples collected from HD rats on Day 1 (6-hours post dose [corresponding to T_{max}]) and at 0, 1, 3, 6, 12, and 24 hours post-dose at Weeks 26 and 52. For each time point, samples from all males were pooled and samples from all females were pooled to ensure sufficient sample volume. The mass responses of parent compound (RDEA594) and its metabolites in plasma were detected using mass spectrometry.

Representative LC-MS traces from male and female plasma samples are shown in Figure 9 and Figure 10. The M4 metabolite was not detectable in rat plasma samples on Day 1, Week 26, or Week 52. Urine and feces samples were not assayed for metabolite detection in this study.

Reference ID: 3799181

Figure 9. Representative LC-MS chromatogram from male rat plasma at week 52, t = 6 hrs post-dose.

Figure 10. Representative LC-MS chromatogram from female rat plasma at week 52, t = 6 hrs post-dose.

Reference ID: 3799181

Ardea has investigated RDEA594 metabolism in rats in other in vitro (*SR08-056*: rat liver microsomes, hepatocytes; *Study SR12-026*: rat liver microsomes and S9 fractions) and in vivo (SR12-032) studies. There was no evidence for M4 formation in in vitro studies. However, study SR12-032 provided evidence that, in vivo, rats synthesize the M4 metabolite at low levels. In this study, male bile duct cannulated rats (*n*=3) were treated with a single oral dose of 200 mg/kg [^{14}C]RDEA594. M4 was detected in urine only (not detected in bile or feces) over the course of 24 hours after dosing at a level equivalent to 0.13% of the total RDEA594 dose. Metabolites M1, M2 and M3 were also identified in urine and bile. Ardea has not delineated the metabolic pathways and metabolizing enzymes involved in RDEA594 metabolism in the rat. Figure 11 illustrates the structures of the major RDEA594 metabolites formed in the rat. While M3c was not detected in this study, it is likely that M4 was formed via the M3c epoxide intermediate.

Figure 11. Structures of the major RDEA594 metabolites detected in vivo in the rat (Study SR12-032).

The evidence from Study SR12-032 can be used to estimate the rat exposure to M3c and M4 in the 2 year carcinogenicity study. It is assumed that all of the M4 metabolite present in rats is formed via the M3c intermediate (and not via other metabolic pathways). The estimated M4 exposure at the NOAEL in the rat carcinogenicity study is 1.6 mg/m^2 (see Table 38 for calculation). This exposure is well below the predicted clinical M4 exposure of 25.5 mg/m^2 at the RDEA594 dose of 200 mg/day (exposure margin: 0.06).

Reference ID: 3799181

Table 38. Exposure margin calculation for the M4 metabolite of RDEA594 in the rat 2 year carcinogenicity study.

Species	Study Type	Study #	RDEA594 dose (mg/kg)	RDEA594 dose (mg/m^2)	Total M4 %	M4 exposure (mg/m^2)	Exposure Margin
Rat	Carcinogenicity (2 year)	SR09-070	200 (NOAEL)	1200 [b]	0.13[d]	1.6	0.06
Human	Single dose [^{14}]C lesinurad ADME study*	RDEA594-112	3.33[a] (max clinical dose)	123[c]	20.7[e]	25.5	-

[a] 200 mg (Maximum proposed clinical RDEA594 dose)/ 60 kg (Mean human body weight) = 3.33 mg/kg
[b] Rat k_m for conversion of dose from mg/kg to mg/m^2 = 6
[c] Human k_m for conversion of dose from mg/kg to mg/m^2 = 37
[d] Total (urine + feces) rat M4 (% of dose) reported in study SR12-032
[e] Total (urine + feces) human M4 (% of dose) reported in study RDEA594-112
*600 mg lesinurad dose in liquid formulation was used in study RDEA594-112. Sponsor reports disproportionately higher exposure with 600 mg liquid formulation vs. 200 mg in immediate release capsule. Current calculations do not account for this observation.

The M3c and M4 metabolites are not considered to be qualified for safety with respect to carcinogenicity based on the data in the current study. However, there are several points to consider when deciding whether this evidence should impede the approval of RDEA594.

(1) Evidence in monkeys (Study SR10-029-MET) suggests that these animals synthesize the M3c metabolite to a greater extent than rodents. In monkeys, CYP2C9 oxidizes RDEA594 to M3c. M3c can then be conjugated with a cysteine molecule to form M9 (unique to monkeys), or it can be hydrolyzed by mEH to form M4 (as in humans). This process is illustrated in Figure 12. Total M3c exposure can be estimated as the sum of M4 and M9 exposures in urine and feces. Study SR10-029-MET found M3c to be present at 8.85% of the initial RDEA594 dose.

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(A)

Lesinurad —*CYP2C9*→ M3c* (not detected in vivo) —*mEH*→ M4 (major metabolite in human urine)

M3c* —*cysteine*→ M9* (key metabolite in monkey bile)

(B)

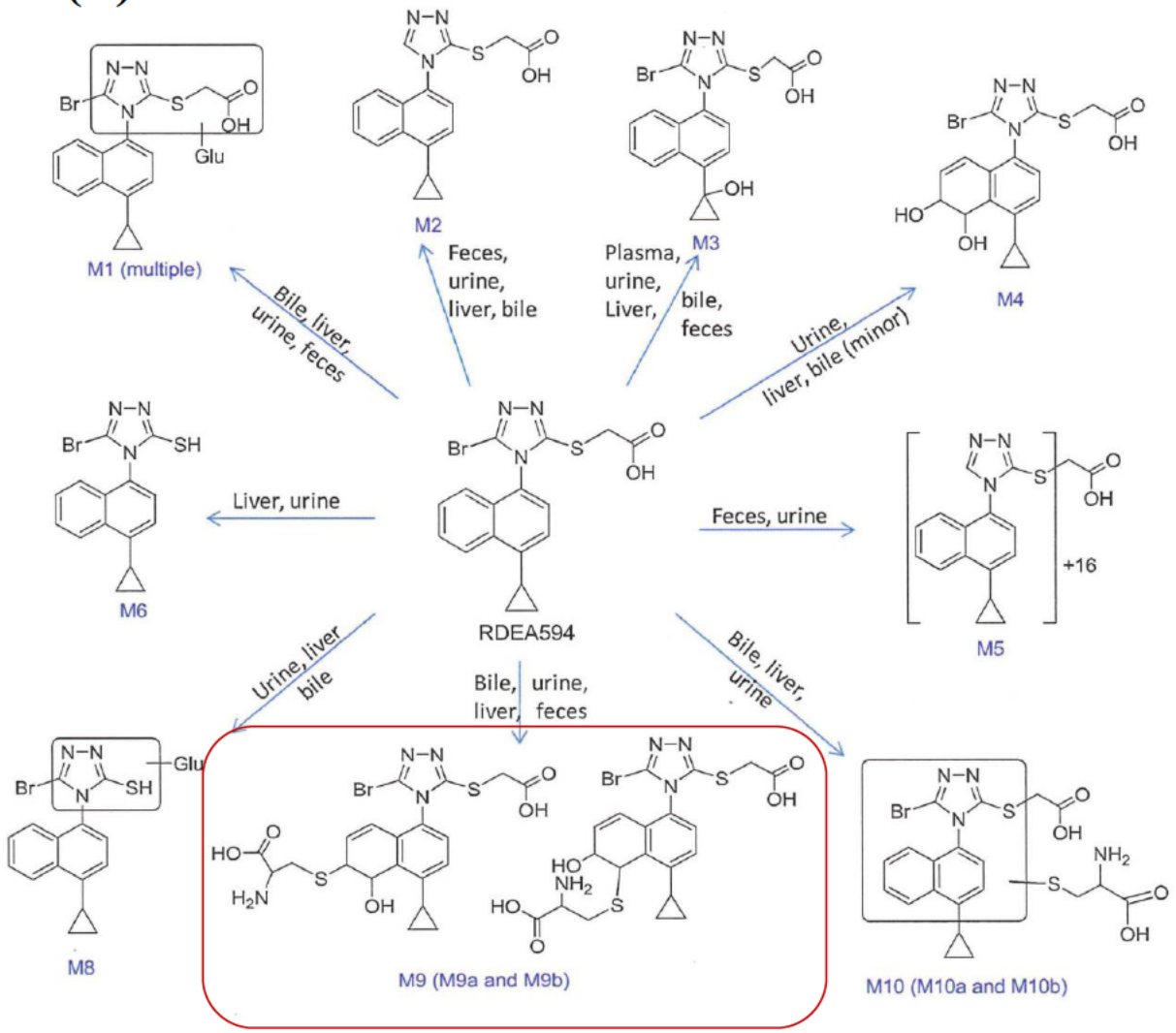

Figure 12. (A) Text description of proposed metabolic pathway of metabolite M4 formation in **monkeys**. **(B)** Chemical structures of all RDEA594 metabolites in monkeys. Note the M9a and M9b metabolites. According to the sponsor, these represent derivatives of the M3c epoxide intermediate. *From* Study SR10-029.

Reference ID: 3799181

(2) Based on the estimated M3c exposure at the NOAEL in the 12 month monkey toxicology study (106 mg/m^2, Study SR08-094) and the estimated M3c exposure at the maximum recommended clinical RDEA594 dose (25.5 mg/m^2), the M3c metabolite is qualified with respect to general toxicity (exposure margin: 4.2).

(3) There was no evidence for pre-neoplastic lesion formation in the 12 month monkey study. Although this treatment duration is short relative to the average monkey lifetime, it may provide supportive evidence for qualification of the M3c metabolite with respect to carcinogenicity.

(4) As a practical matter, it would not be possible for the sponsor to directly administer M3c to rats in a stand-alone study based on the unstable nature of epoxides.

(5) A risk: benefit assessment from the clinical review team may be necessary to weigh the potential risk of carcinogenicity from exposure to metabolite M3c vs. the expected clinical benefit of RDEA594 in gout patients.

ECAC conclusions

The ECAC had the following conclusions regarding the results of the rat carcinogenicity study (see meeting minutes: NDA 207988, 5/25/15):

- The Committee concurred that the study was acceptable, noting prior Executive CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female rats.
- The Committee concurred that no further studies were required for the safety qualification of the M3c epoxide intermediate with respect to carcinogenicity.

Reference ID: 3799181

Mouse carcinogenicity study

There were no treatment-related effects on survival in the 26 week TgRasH2 mouse study with oral RDEA594. While there was a numerical increase in premature deaths in HD females relative to vehicle controls (4/25 vs. 1/25) that approached statistical significance, these deaths were not related to treatment with RDEA594. The cause of 1 of the 4 deaths was gavage error, while the cause of death in the other 3 animals is unknown. Evaluation of the microscopic data from these animals failed to identify a common target organ or lesion type. The 4 premature deaths observed in the current study is within the mortality range for females reported in a published analysis of control data from 26 carcinogenicity studies conducted with TgRasH2 mice[3]. The totality of the data suggests that the premature deaths observed in HD females are not related to RDEA594 treatment. Mortality was markedly increased in MNU treated males and females, an expected effect of treatment.

RDEA594 was not tumorigenic in male TgRasH2 mice at doses up to 125 mg/kg/day and female TgRasH2 mice at doses up to 250 mg/kg/day. The assay serves for hazard identification only (yes/no response). Exposure margins are shown in the table below for informational purposes, but will not be used in the product labeling if approved.

Table 39. Exposure margin calculation for RDEA594 at the tumorigenicity NOAEL in TgRasH2 mice based on systemic exposure values at study day 181.

Species	RDEA594 Dose	Sex	RDEA594 AUC_{0-24} (µg*hr/ml)	Exposure margin
Human	200 mg (maximum proposed human dose)	M & F	28.0[a]	-
Mouse (TgRasH2)	125 mg/kg/day (NOAEL)	Male	926	33.1
	250 mg/kg/day (NOAEL)	Female	1760	62.9

[a] Mean AUC_{0-24} (M & F combined, n = 12) for 200 mg RDEA594 administered 1x/d for 14 days in healthy adults (Study RDEA594-105)

There was evidence for multiple neoplastic lesions in MNU-treated positive control animals, confirming expected, characteristic tumorigenic response to a known carcinogen. Neoplastic lesions in MNU-treated mice were detected in the hematopoietic system (malignant lymphosarcoma), skin, and nonglandular stomach (Table 34). These target organs are consistent with established results in MNU-treated TgRasH2 mice[1]. Neoplastic lesions were also identified in the uterus and vagina of female mice in the current study.

[3] Paranjpe, M. et al. (2013) Historical Control Data of Spontaneous Tumors in Transgenic CByB6F1-Tg(HRAS)2Jic (Tg.rasH2) Mice. *International Journal of Toxicology*. 32, 48 – 57.

Reference ID: 3799181

Non-neoplastic target organs in both males and females included the liver and glandular stomach. Liver pathology is consistent with observations in the 1 month dose-range finding study in WT TgRasH2 mice. The glandular stomach has previously been identified as a target of RDEA594-related toxicity in long term toxicity studies in SD rats.

Collectively, the data in the 26 week carcinogenicity study in TgrasH2 mice suggests that RDEA594, at up to 125 mg/kg/day in males and 250 mg/kg/day in females, has no effect on survival or tumor formation.

ECAC conclusions

The ECAC had the following conclusions regarding the results of the mouse carcinogenicity study (see meeting minutes: NDA 207988, 7/1/15):

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms.

Reference ID: 3799181

12 Appendices

Appendix 1: Executive CAC meeting minutes for rat carcinogenicity study. Meeting date: 5/19/15.

Appendix 2: Executive CAC meeting minutes for mouse carcinogenicity study. Meeting date: 6/30/15.

Reference ID: 3799181

Executive CAC
Date of Meeting: May 19, 2015

Committee: Karen Davis Bruno, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Tim McGovern, Ph.D., ONDIO, Member
Timothy Robison, Ph.D., DPARP, Pharm Tox Supervisor
Matthew Whittaker, Ph.D., DPARP, Presenting Reviewer

Author of Draft: Matthew Whittaker, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 209788
Drug Name: Lesinurad
Sponsor: Ardea Biosciences, Inc.

Background
Lesinurad is a urate transporter 1 (URAT1) inhibitor under development for the treatment of gout.

Rat Carcinogenicity Study
The sponsor conducted a 2-year bioassay in Sprague-Dawley rats (60 rats/sex/group) with lesinurad administered by oral gavage at doses of 0 (distilled water), 25, 75, or 200 mg/kg/day. All doses received concurrence from the Executive Carcinogenicity Assessment Committee.

Mortality in the vehicle control group in males led to the termination of all surviving males at week 97 following consultation with the Division and Executive CAC. Female rats were terminated between weeks 91 – 97 due to mortality following consultation with the Division and Executive CAC. There was no evidence of a treatment-related effect on survival in rats.

There were no treatment-related neoplastic findings based on the lack of statistical significance. Lesinurad was not tumorigenic in Sprague-Dawley rats at doses up to 200 mg/kg/day.

The disproportionate human metabolite M4 (20.7% of initial dose) is not synthesized to the same extent in rats (0.13% of initial dose). The M4 metabolite is a dihydrodiol metabolite of lesinurad, and does not raise significant safety concerns. However, M4 is formed via a transient M3c epoxide intermediate. The epoxide functional group represents a potential structural alert for mutagenicity. The estimated maximum exposure to M3c in the current rat study was 0.06-fold the exposure at the maximum recommended clinical dose. Given the unstable nature of the M3c metabolite, it was not possible to test the effects of this molecule in a stand-alone carcinogenicity study. Concern over potential carcinogenic effects of the epoxide was mitigated by its transient nature, and the fact that this molecule is further metabolized to a dihydrodiol.

Executive CAC Recommendations and Conclusions

- The Committee concurred that the study was acceptable, noting prior Executive CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female rats.
- The Committee concurred that no further studies were required for the safety qualification of the M3c epoxide intermediate with respect to carcinogenicity.

Karen Davis Bruno, Ph.D.
Chair, Executive CAC

cc:\

/Division File, DPARP
/Timothy Robison, DPARP
/Matthew Whittaker, DPARP
/Michelle Jordan Garner, DPARP
/ASeifried, OND IO

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/s/

MATTHEW T WHITTAKER
05/22/2015

KAREN L DAVIS BRUNO
05/25/2015

Executive CAC
Date of Meeting: June 30, 2015

Committee: Karen Davis Bruno, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Todd Bourcier, Ph.D., DMEP, Alternate Member
Timothy Robison, Ph.D., DPARP, Pharm Tox Team Leader
Matthew Whittaker, Ph.D., DPARP, Presenting Reviewer

Author of Draft: Matthew Whittaker, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 207988
Drug Name: Lesinurad
Sponsor: Ardea Biosciences, Inc.

Background
Lesinurad is a urate transporter 1 (URAT1) inhibitor under development for the treatment of gout.

Tg.rasH2 Mouse Carcinogenicity Study
Ardea Biosciences, Inc. conducted a 26-week bioassay in Tg.rasH2 mice (25 mice/sex/group) with lesinurad administered by oral gavage. Males were treated with doses of 0 (distilled water), 15, 45, or 125 mg/kg/day while females received doses of 0, 30, 90, or 250 mg/kg/day. All doses received concurrence from the Executive Carcinogenicity Assessment Committee (see minutes dated April 28, 2011). Positive control mice received a single intraperitoneal injection of 75 mg/kg N-methyl-N-nitrosourea (MNU) on study day 1.

There was no effect of lesinurad on survival. Lesinurad was not tumorigenic in Tg.rasH2 mice at doses up to 125 mg/kg/day in males and 250 mg/kg/day in females. MNU-treated positive control mice had neoplastic lesions in the hematopoietic system (malignant lymphosarcoma), skin (squamous cell papilloma/carcinoma), and nonglandular stomach (squamous cell papilloma/carcinoma). These target organs and lesions are consistent with established results in MNU-treated Tg.rasH2 mice.

Reference ID: 3790583

Executive CAC Recommendations and Conclusions

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms.

Karen Davis Bruno, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DPARP
/Timothy Robison, DPARP
/Matthew Whittaker, DPARP
/Michelle Jordan Garner, DPARP
/ASeifried, OND IO

Reference ID: 3790583

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/s/

ADELE S SEIFRIED
07/01/2015

KAREN L DAVIS BRUNO
07/01/2015

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/s/

MATTHEW T WHITTAKER
07/29/2015

TIMOTHY W ROBISON
07/29/2015
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA Number: 207988 **Applicant:** Ardea Biosciences **Stamp Date:** 12/29/14

Drug Name: Zurampic (Lesinurad) **NDA Type:** New Medical Entity 505(b)(1)

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		Nonclinical studies were submitted in paper format from 10/1/09 – 5/2/13. After 5/2/13, all studies were submitted electronically.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		All required studies have been completed and submitted at this time. However, the safety qualification of the M3c metabolite will be a review issue. Additional studies related to assessment of the carcinogenicity risk of this metabolite may be required.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		The commercial form of the lesinurad drug substance is the free acid. This form was used in early toxicology studies, including in 14- and 28-day toxicity studies conducted in rats and monkeys. Lesinurad sodium salt was used in all subsequent general toxicity, reproductive toxicity, and carcinogenicity studies. Comparison of systemic exposure values, target organs of toxicity, and No Observed Adverse Effect Levels (NOAELS) between the free acid (14- and 28-day toxicity studies) and sodium salt (chronic toxicity studies) forms in rats and monkeys found no apparent differences. Therefore, safety data obtained with lesinurad sodium salt appears to be applicable to lesinurad free acid.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		All toxicology studies were conducted using the oral gavage route of administration.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		The sponsor states in Module 2.4 (Nonclinical Overview, Section 1) that the pivotal safety pharmacology, toxicology, and supporting TK studies were GLP-compliant.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		In response to Question 9 of the Sponsor's pre-NDA Meeting Questions (pre NDA Meeting Minutes, 10/24/14), the nonclinical review team stated: "Provide justification in your NDA that the M3c/M4 metabolites have been adequately assessed and are qualified with respect to general toxicity, genetic toxicity, carcinogenicity, and reproductive toxicity. This should include information that the M3c epoxide intermediate was formed in a nonclinical species at sufficient levels to permit assessments of potential toxicity" The Sponsor's toxicity assessment of M3c and M4 is presented in Module 2.4 (Nonclinical Overview) of the initial NDA submission. Section 4.7.1 provides justification for the safety of the M3c and M4 metabolites. The safety qualification of the M3c metabolite (contains epoxide structural alert for mutagenicity) with respect to general toxicology and carcinogenicity will be a review issue. The level of concern with regard to the safety of M4 is lower than for M3c based on the absence of structural alerts for mutagenicity.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not			The assessment of issues related to impurities will be determined in

Reference ID: 3703366

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
	be needed.)			consultation with the reviewing chemist. There are currently 4 drug substance impurities with acceptance criteria greater than the ICH Q3A(R2) qualification threshold of 0.15%. The acceptance criteria appear to be acceptable based upon impurity exposures at the NOAEL dose in chronic rat or monkey studies. (b) (4) impurities that were found to be positive in the Ames test are controlled at levels below the threshold of concern of 1.5 µg/day (b) (4) ppm based on 200 mg/day proposed clinical dose).
11	Has the applicant addressed any abuse potential issues in the submission?	NA		There appear to be no issues regarding abuse potential.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	NA		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

(1) There is currently no Established Pharmacologic Class for URAT1 specific inhibitors (http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/ucm162549.htm). Therefore, the suitability of the proposed language in the Indications and Usage Section of the labeling will be a review issue.

(2) We acknowledge your explanation regarding the safety qualification of the M3c epoxide intermediate and the M4 metabolite in section 4.7.1 of the Nonclinical Overview. However, the safety qualification of the M3c metabolite with respect to carcinogenicity will be a review issue. We note that nonclinical exposure multiples over calculated human M3c exposures are computed on a mg/m^2 basis.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

Matthew Whittaker	2/17/15
Reviewing Pharmacologist	Date
Timothy Robison	2/17/15
Team Leader/Supervisor	Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

MATTHEW T WHITTAKER
02/17/2015
Potential nonclinical review issues are noted at the bottom of the document

TIMOTHY W ROBISON
02/17/2015
I concur