CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	202057
Supporting document/s:	SD-2
Applicant's letter date:	9/25/2011
CDER stamp date:	9/26/2011
Product:	Vascepa (icosapent ethyl) capsules
Indication:	Hypertriglyceridemia (≥500 mg/dL)
Applicant:	Amarin Pharmaceuticals Ireland Limited
Review Division:	Metabolism and Endocrinology Products
Reviewer:	Stephanie Leuenroth-Quinn, Ph.D.
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1 Executive Summary

1.1 Introduction

Under NDA 202057 the Applicant, Amarin Pharmaceuticals is proposing a clinical dose of 4 g/day Vascepa, icosapent ethyl for the treatment of hypertriglyceridemia. Icosapent ethyl, or ethyl-EPA/ ethyl - eicosapentaenoic acid, is rapidly converted to the omega-3 fatty acid EPA by pancreatic lipases. EPA is then incorporated into triglycerides, cholesteryl ester and phospholipids in addition to a small fraction remaining as unesterified or free fatty acid before passing into the lymph from the small intestine. From the lymph, EPA is then distributed systemically where it can act in numerous tissues to compete for other fatty acids such as arachidonic acid and thereby influence cellular signaling. The mechanism for triglyceride lowering, the intended pharmacologic effect, is through the promotion of fatty acid degradation resulting in a reduction of substrate needed for VLDL synthesis, inhibition of lipogenesis in the liver and increased clearance of triglycerides from the plasma. Nonesterified fatty acids such as EPA can modulate nuclear receptors such as PPARs as well as G-protein coupled receptors such as GPR119 or GPR120 which also have an impact on cellular functions such as fatty acid oxidation or lipid synthesis.

The Applicant has filed a 505(b)(2) application as they rely on published literature to support NDA 202057. There is extensive scientific literature on the pharmacology and pharmacokinetics of ethyl-EPA, EPA and omega-3 polyunsaturated acids in general, as mixtures of marine derived fatty acids have been used extensively in the human population. The Applicant has completed nonclinical toxicology studies including a 1-month rat, 1-month mouse, 9-month dog, 6-month Tg.rasH2 mouse for carcinogenicity, 2-year rat for carcinogenicity, a Seg II reproductive toxicology study in the rat, an Ames mutagenesis assay, three chromosomal aberration assays in CHO cells, and an *in vivo* mouse micronucleus assay. The Applicant has also submitted literature references that were sponsored by Mochida Pharmaceuticals in support of Japanese marketing of Epadel (ethyl-EPA). These literature references include single dose toxicology in the mouse and rat, 3- and 12- month rat repeat dose toxicology studies, a Seg I fertility study in the rat, a Seg II reproductive toxicology study in the rat, a Seg II reproductive toxicology study in the rabbit and a Seg III peri- and post-natal study in the rat. A bridging study was conducted so that the Applicant could rely on published literature for Epadel primarily for the use of reproductive toxicology data. This was a 1-month toxicology and pharmacokinetics study in rats comparing Epadel to AMR101. Results from this bridging study supported that AMR101 and Epadel were similar for the PK and toxicology profiles at a dose of 2000 mg/kg/day in the rat for 28 days. It was therefore reasonable to conclude that reproductive toxicology data from Epadel (ethyl-EPA) could be used to support NDA 202057 as a 505(b)(2) application.

1.2 Brief Discussion of Nonclinical Findings

The main sites of toxicity across multiple species including the rat, mouse and dog were the gastrointestinal tract, the hematopoietic system including WBCs and RBCs, the liver, and the skin. Multiple species had oily discharge from the anus at high doses

combined with localized skin irritation and fibrosis as well as skin or fur staining. Two dogs (non-dose dependent) had to be sacrificed due to blockages between the small and large intestines. Aside from skin irritation due to direct contact with metabolized or oxidized ethyl-EPA near the anus or abdomen, omega-3 fatty acids can compete for and deplete omega-6 fatty acids in the skin causing an omega-6 deficiency resulting in scaly skin or tails. WBCs showed mild elevations but may have been attributed to skin irritation and inflammation as omega-3 fatty acids generally have an anti-inflammatory effect. RBC parameters showed mild declining levels that may have indicated hemolysis and clotting parameters were infrequently elevated. Liver enzymes such as ALT and ALP (also possibly GI or corticosteroid related) increased in the dog and rat. In the dog, the adrenal gland (where EPA has been shown to accumulate in radiolabeled rat studies) was affected, as histopathology showed adrenal gland vacuolar degeneration of the inner cortex, increased serum ALP (that may have been attributed to corticosteroid-ALP), and a decrease in cortisol suggesting an effect on the hypothalamus-pituitary-adrenal axis.

A full panel of genotoxicity assays was completed and was negative for the Ames assay as well as the *in vivo* mouse micronucleus assay. Multiple replicates of the clastogenicity assay were completed where ethyl-EPA and EPA were shown to be positive in the presence and absence of metabolic activation in Chinese Hamster Ovary (CHO) cells. Two carcinogenicity assays were performed: 1) a 6-month transgenic rasH2 mouse model where male mice had an increased incidence of papillomas at the proximal tail at a high dose of 4600 mg/kg/day. Given the known incidence of oily leakage from the anus at high AMR101 doses in rodents, it is not clear if this result is relevant to humans. 2) a 2-year carcinogenicity study in the Wistar rat where females had an increased incidence of hemangiomas + hemangiosarcomas at the mesenteric lymph node (the site of drug absorption before systemic distribution). As a note, the incidence of hemangiomas + hemangiosarcomas at all sites combined was not statistically significant. At this same site in the 6-month transgenic mouse, there was an increased incidence of thrombosis and inflammation but no hemangiomas. In the 9-month dog study, erythrophagocytosis at the mesenteric lymph node was noted non-dose dependently at both 3- and 9- months. The relevance of the risk of MLN hemangiomas and hemangiosarcomas to humans is unclear as: 1) Wistar rats may be genetically predisposed to developing mesenteric lymph node hemangiomas due to an unknown mechanism, 2) the normal rodent diet consists of ~5% total fat while a healthy human diet consists of 20-35% fat (*i.e.* humans may be better adapted at processing fats), and 3) the mechanism of action of hemangioma formation in rodents is unknown.

Reproductive toxicology studies, most of which are relied upon from the scientific literature published as Mochida sponsored studies supporting Epadel marketing in Japan, indicated that the reproductive risk of ethyl-EPA and therefore Vascepa cannot be ruled out due to nonclinical findings. Visceral or skeletal abnormalities such as increased cervical rib, 13^{th} reduced ribs, and incomplete ossification of skeletal bones was observed in the rat. An increased incidence of absent optic nerves and unilateral testis atrophy were observed in rats at human systemic exposure following an oral dose of 4 g/day, and pups from high dose treated dams had decreased copulation rate,

delayed estrus and decreased implantations. In rabbits, there were increased dead fetuses at human systemic exposure levels and at higher exposure maternal toxicity was noted. Ethyl-EPA did not affect fertility when administered for 9 weeks before mating to male rats and 14 days before mating to female rats.

1.3 Recommendations

1.3.1 Approvability

Pharm/Tox recommends approval of Vascepa for the proposed indication of severe hypertriglyceridemia (≥500 mg/dL).

1.3.2 Additional Non Clinical Recommendations

The nonclinical studies are generally adequate to support the recommended dose of 4 g/day. These include Amarin sponsored and literature references for ethyl-EPA, on which the Applicant relies on for their 505(b)(2) NDA application. No further nonclinical studies are required.

1.3.3 Labeling

The Applicant's proposed labeling for sections 8.1 (Pregnancy), 8.2 (Labor and Delivery), 8.3 (Nursing Mothers), 12.1 (Mechanism of Action) and 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility) are below with reviewer's recommendations noted:

<u>8.1 Pregnancy</u>

"Pregnancy Category (b) (4): There are no adequate and well-controlled studies in pregnant women. It is unknown whether VASCEPA can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. VASCEPA should be used during pregnancy if the potential benefit to the patient justifies the potential risk to the fetus.

(b) (4)

(b) (4)

Reviewer's Recommended Changes:

Pregnancy Category (b) (4) **C**: There are no adequate and well-controlled studies in pregnant women. It is unknown whether VASCEPA can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. VASCEPA should be used during pregnancy if the potential benefit to the patient justifies the potential risk to the fetus.

(b) (4)

In pregnant rats given oral gavage doses of 0.3, 1 and 2 g/kg/day ethyl-EPA from gestation through organogenesis all drug treated groups had visceral or skeletal abnormalities including: 13th reduced ribs, additional liver lobes, testes medially displaced and/or not descended at human systemic exposures following a maximum oral dose of 4 g/day based on body surface comparisons. Variations including incomplete or abnormal ossification of various skeletal bones were observed in the 2g/kg/day group at 5 times human systemic exposure following an oral dose of 4 g/day based on body surface area comparison.

In a multigenerational developmental study in pregnant rats given oral gavage doses of 0.3, 1, 3 g/kg/day ethyl-EPA from gestation day 7-17, an increased incidence of absent optic nerves and unilateral testes atrophy were observed at ≥0.3 g/kg/d at human systemic exposure following an oral dose of 4 g/d based on body surface area comparisons across species. Additional variations consisting of early incisor eruption and increased percent cervical ribs were observed at the same exposures. Pups from high dose treated dams exhibited decreased copulation rates, delayed estrus, decreased implantations and decreased surviving fetuses (F2) suggesting multigenerational effects of ethyl-EPA at 7 times human systemic exposure following 4 g/d dose based on body surface area comparisons across species.

In pregnant rabbits given oral gavage doses of 0.1, 0.3, 3 g/kg/day from gestation through organogenesis there were increased dead fetuses at 0.1 g/kg/day at human systemic exposure at 4 g/day based on a comparison of body surface area across species. At higher exposures evidence of maternal toxicity was seen.

In pregnant rats given ethyl-EPA from gestation day 17 through lactation day 20 at 0.3, 1, 3 g/kg/day complete litter loss was observed in 2/23 litters at the low dose and 1/23 mid-dose dams by post-natal day 4 at human exposures based on a maximum dose of 4 g/day comparing body surface areas across species.

Reviewer's Proposed Labeling:

Pregnancy Category C: There are no adequate and well-controlled studies in pregnant women. It is unknown whether VASCEPA can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. VASCEPA should be used during pregnancy if the potential benefit to the patient justifies the potential risk to the fetus.

In pregnant rats given oral gavage doses of 0.3, 1 and 2 g/kg/day ethyl-EPA from gestation through organogenesis all drug treated groups had visceral or skeletal abnormalities including: 13th reduced ribs, additional liver lobes, testes medially displaced and/or not descended at human systemic exposures following a maximum oral dose of 4 g/day based on body surface comparisons. Variations including incomplete or abnormal ossification of various skeletal bones were observed in the 2g/kg/day group at 5 times human systemic exposure following an oral dose of 4 g/day based on body surface area comparison.

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In pregnant rabbits given oral gavage doses of 0.1, 0.3, 3 g/kg/day from gestation through organogenesis there were increased dead fetuses at 0.1 g/kg/day at human systemic exposure at 4 g/day based on a comparison of body surface area across species. At higher exposures evidence of maternal toxicity was seen.

In pregnant rats given ethyl-EPA from gestation day 17 through lactation day 20 at 0.3, 1, 3 g/kg/day complete litter loss was observed in 2/23 litters at the low dose and 1/23 mid-dose dams by post-natal day 4 at human exposures based on a maximum dose of 4 g/day comparing body surface areas across species.

Justification for Changes:

- The pregnancy category should be (b) (4) C as the risk to pregnancy cannot be ruled out due to findings in nonclinical toxicology studies. It is also of

note that the Epadel package insert, which also relied on the majority of reproductive toxicology as reported here, states that the safety of administration during pregnancy has not been confirmed. (b) (4)

- Exposure multiples were calculated by this reviewer where human body weight was 60 kg (Applicant used 70 kg), therefore accounting for lower dose exposures.
- This reviewer disagrees with the interpretation that studies did not show teratogenicity at any dose. The absence of an optic nerve during development is not a common finding; however some evidence exists that degeneration of the optic nerve with greater than 1 year of age in the rat is more common. While this finding was not dose dependent, other studies in the rat have shown that exposure is not dose dependent and does not increase above 1.0 g/kg and as TK data was not collected in any reproductive toxicology study, exposure levels cannot be used to discount these findings. Given the requirement of DHA in the developing brain including the retina, the relevance of this finding is unknown. Also in multiple studies there was a developmental variant reported for the rib (cervical rib, 13th vestigial or reduced rib).
- Additional study results were added so that all nonclinical reproductive toxicology results are represented.

(b) (4)

8.3 Nursing Mothers

(b) (4) studies with omega-3-acid ethyl esters have demonstrated excretion in human milk. The effect of this excretion is unknown; caution should be exercised when VASCEPA is administered to a nursing mother."

Reviewer's Recommended Changes:

(b) (4) Studies with omega-3-acid ethyl esters have demonstrated excretion in human milk. The effect of this excretion is unknown; caution should be exercised when

VASCEPA is administered to a nursing mother. **In lactating rats, given oral gavage ^{14}C-ethyl EPA, drug levels were 6-14 times higher in milk than in plasma.**

Reviewer's Proposed Labeling:

Studies with omega-3-acid ethyl esters have demonstrated excretion in human milk. The effect of this excretion is unknown; caution should be exercised when VASCEPA is administered to a nursing mother. In lactating rats, given oral gavage ^{14}C-ethyl EPA, drug levels were 6-14 times higher in milk than in plasma.

Justification for Changes:
Any available animal data should follow human data in labeling. As data were available for ethyl-EPA distribution in milk of lactating rats from a submitted literature reference, this information is included.

12.1 Mechanism of Action

"Studies suggest that EPA reduces hepatic very low-density lipoprotein triglycerides (VLDL-TG) synthesis and/or secretion and enhances TG clearance from circulating VLDL particles. Potential mechanisms (b) (4) include increased β-oxidation; inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase (DGAT); decreased lipogenesis in the liver; and increased plasma lipoprotein lipase activity. (b) (4)

Reviewer's Recommended Changes:
Studies suggest that EPA reduces hepatic very low-density lipoprotein triglycerides (VLDL-TG) synthesis and/or secretion and enhances TG clearance from circulating VLDL particles. Potential mechanisms (b) (4) **of action** include increased β-oxidation; inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase (DGAT); decreased lipogenesis in the liver; and increased plasma lipoprotein lipase activity. (b) (4)

Reviewer's Proposed Labeling:

Studies suggest that EPA reduces hepatic very low-density lipoprotein triglycerides (VLDL-TG) synthesis and/or secretion and enhances TG clearance from circulating VLDL particles. Potential mechanisms of action include increased β-oxidation; inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase (DGAT);decreased lipogenesis in the liver; and increased plasma lipoprotein lipase activity.

Justification for Changes:
Labeling should include only scientifically established and not theoretical mechanisms of action. (b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Reviewer's Recommended Changes:

In a 2-year rat carcinogenicity study with oral gavage doses of (b) (4) (b) (4) 0.09, 0.27, and 0.91 g/kg/day icosapent ethyl (b) (4) **males did not exhibit drug-related neoplasms. Hemangiomas and hemangiosarcomas of the mesenteric lymph node, the site of drug absorption were observed in females at clinically relevant exposures based on body surface area comparisons across species relative to the maximum clinical dose of 4g/day. Overall incidence of hemangiomas and hemangiosarcomas in all vascular tissues did not increase with treatment.** (b) (4)

In a 6-month carcinogenicity study in Tg.rasH2 transgenic mice **with** oral gavage doses of 0.5, 1, 2, and 4.6 g/kg/day icosapent ethyl**, drug-related** (b) (4) incidence**s** of benign squamous cell papilloma in the skin and subcutis of the tail was observed in **high dose** male mice. (b) (4) The papillomas were considered to develop secondary to chronic irritation of the proximal tail associated with fecal excretion of oil. (b) (4)

(b) (4) **Drug-related neoplasms were not observed in female mice.**

Icosapent ethyl was **not mutagenic with or without metabolic activation in the bacterial mutagenesis (Ames) assay or in the *in vivo* mouse micronucleus assay. A chromosomal aberration assay in** (b) (4) Chinese Hamster Ovary (CHO) cells **was positive for clastogenicity with and without metabolic activation.** (b) (4)

In a**n oral gavage** rat fertility study, **ethyl-EPA** (b) (4) was administered at doses of 0.3, 1, and 3 g/kg/day to male rats for 9 weeks before mating and to female rats for 14 days before mating through day 7 of gestation **increased anogenital distance in female pups and increased cervical ribs were observed at 3 g/kg/day (7 times** (b) (4) human systemic exposure with 4 g/day **clinical dose** based on a body surface area comparison**)**.

Reviewer's Proposed Labeling:

In a 2-year rat carcinogenicity study with oral gavage doses of 0.09, 0.27, and 0.91 g/kg/day icosapent ethyl respectively, males did not exhibit drug-related neoplasms. Hemangiomas and hemangiosarcomas of the mesenteric lymph node, the site of drug absorption were observed in females at clinically relevant exposures based on body surface area comparisons across species relative to the maximum clinical dose of 4g/day. Overall incidence of hemangiomas and hemangiosarcomas in all vascular tissues did not increase with treatment.

In a 6-month carcinogenicity study in Tg.rasH2 transgenic mice with oral gavage doses of 0.5, 1, 2, and 4.6 g/kg/day icosapent ethyl, drug-related incidences of benign squamous cell papilloma in the skin and subcutis of the tail was observed in high dose male mice. The papillomas were considered to develop secondary to chronic irritation of the proximal tail associated with fecal excretion of oil. Drug-related neoplasms were not observed in female mice.

Icosapent ethyl was not mutagenic with or without metabolic activation in the bacterial mutagenesis (Ames) assay or in the *in vivo* mouse micronucleus assay. A chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells was positive for clastogenicity with and without metabolic activation.

In an oral gavage rat fertility study, ethyl-EPA was administered at doses of 0.3, 1, and 3 g/kg/day to male rats for 9 weeks before mating and to female rats for 14 days before mating through day 7 of gestation increased anogenital distance in female pups and increased cervical ribs were observed at 3 g/kg/day (7 times human systemic exposure with 4 g/day clinical dose based on a body surface area comparison).

Justification for Changes:

- Carcinogenesis findings for the rat were taken from Executive CAC committee findings, where a statistically significant increased incidence of hemangiomas and hemangiosarcomas in female rats was observed at the mesenteric lymph node.

- Exposure multiples were calculated by this reviewer where human body weight was 60 kg (Applicant used 70 kg), therefore accounting for lower dose exposures.
- While an Ames assay and an *in vivo* mouse micronucleus assay were negative for mutagenicity, multiple chromosome aberration assays were positive for both icosapent ethyl and EPA and were reported as such in the study reports.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)
73310-10-8

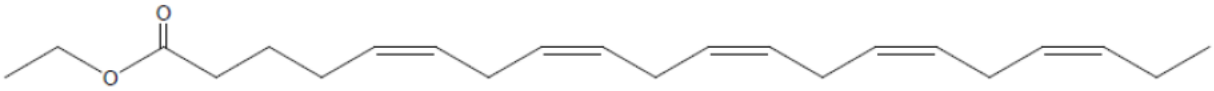
Generic Name
icosapent ethyl, ethyl-eicosapentaenoate (ethyl-EPA), eicosapentaenoic acid ethyl ester, ethyl icosapent

Code Name
AMR101, EPA-E

Chemical Name
Ethyl (5Z,8Z,11Z,14Z,17Z)-5,8,11,14,17-icosapentaenoate

Molecular Formula/Molecular Weight
$C_{22}H_{34}O_2$ / 330.51 Daltons

Structure or Biochemical Description



Pharmacologic Class
Lipid-lowering agent, Omega-3 fatty acid

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 102,457 (AMR101, ethyl-EPA)
(b) (4)
DMF 015062 (Nisshin Pharma Inc. Type II Drug Master File)

2.3 Drug Formulation

1 gram soft gelatin capsules for oral administration that contain icosapent ethyl and 0.2% tocopherol (b) (4).

2.4 Comments on Novel Excipients

Tocopherol is added (b) (4) at a 0.2% concentration. The capsule (b) (4) excipients are gelatin, (b) (4) sorbitol, glycerin and maltitol. (b) (4)

2.5 Comments on Impurities/Degradants of Concern

The majority of impurities and/ or degradants present may be from the drug substance as impurities or from degradation products formed from manufacturing or storage. These may include (b) (4) related substances such as the following:

(b) (4)

Summary of Drug Substance Batches Used in Non-Clinical Toxicology Studies

Related Substances[a] (all data reported as %w/w)	Drug Substance Batch Number					
	EE171GQ	EE050JR (LAX101-20050)[b]	U-99A-D4-K (Nu-Check Prep Inc.)[c]	EE070IX	EE141IS	EE030HU
(b) (4)						
Type of Study (Study or Report No.)	Genotox: Ames (01-0680-G1) Genotox: Chrom abb (01-0680-G2) Genotox: micronucleus (01-0680-G3)	Genotox: CHO cells (21548 and 21549)	Genotox: CHO cells (21882 and 21883)	4-wk mouse (459549) 4-wk rat (ZOC0001) 14-d DRF dog (515147) 39-wk dog (515194) Developmental tox rat (494981) 26-wk carc in transgenic mouse (8222196)	104-wk carc in rat (23040)	104-wk carc in rat (23040)

[a] All data reported as %w/w. Data are from Nisshin Certificate of Analysis, unless otherwise noted.
[b] Test Article was provided in gelatin capsules
[c] (b) (4) EPA contains no added tocopherol
[d] Listed as "Impurity 1" on CoA.
n.d. = Not detected
- Not determined. Test was not part of the specification at the time of testing. See DMF 015062 for more information.

[Reproduced table from Module 2.3.S, NDA 202057]

Summary of Related Substances Qualification Data Used in Non-Clinical Studies

Related Substance	Proposed Specification (%w/w)	Maximum Clinical Intake[a] (mg/kg/day)	Amount Present in Drug Batch Used in 39-wk Dog Study[b] (%w/w)	Daily Exposure to Related Substance at NOAEL Dose of 39 wk-Dog Study[c] (mg/kg/day)	Animal to Human Safety Margin[d]
(b) (4)					

[a] Maximum clinical exposure based on maximum daily icosapent ethyl dose of 4 g/day and assumes human body weight of 60 kg.
[b] Based on Nisshin Certificate of Analysis for drug substance batch EE070IX.
[c] Daily exposure to dogs based on NOAEL dose of 1000 mg/kg.
[d] Daily exposure to dogs divided by the maximum clinical exposure on a mg/kg body weight basis.

[Reproduced table from Module 2.3.S, NDA 202057]

2.6 Proposed Clinical Population and Dosing Regimen

Patients with very high triglycerides (≥500 mg/dL)

2.7 Regulatory Background

The sponsor initially opened pre-IND 102,457 with the Division of Metabolism and Endocrinology products on May 9, 2008 with a meeting request to discuss the use of AMR101 (ethyl-EPA) for the treatment of hyperlipidemia. The sponsor wanted to rely on published toxicology data that supported the marketing of the ethyl-EPA Japanese product Epadel (Mochida), for the initiation of clinical studies. The sponsor was informed that they would have to establish that a reliance on safety data provided in the literature was scientifically justified, that a repeat dose toxicology study would need to be completed in a second species (non-rodent), a carcinogenicity study would need to be completed in a second species and that a toxicology study with an Epadel comparator group would be required. At the time of filing NDA 202057, the Applicant relies on literature references predominantly for nonclinical reproductive toxicology studies and therefore is a 505 (b)(2) application.

3 Studies Submitted

3.1 Studies Reviewed

Study Number	Study Title
AMR-NC-11-01	Inhibition of Human Cytochromes P450 by Eicosapentaenoic Acid
AMR-NC-11-02	Induction of Human Cytochromes P450 by Eicosapentaenoic Acid
789867	Determination of the In Vitro Binding of EPA (Free Acid) to the Plasma Proteins and Partitioning into the Blood Cells of Rat, Dog and Human
OPT-2010-157 OPT-2010-158	Assessment of EPA as a potential inhibitor of human P-gp, BCRP, OAT1, OAT3, OCT2, OATP1B1 and OATP1B3-mediated transport, and AMR101 as a potential inhibitor of human P-gp
309365	Validation of an Analytical Method for the Determination of AMR101 and EPA in Dog, Rat and Mouse Plasma by LC-MS/MS
314194	Validation of an Analytical Method for the Determination of Total Eicosapentaenoic Acid (EPA) in Rat Plasma by LC-MS/MS
N/A	The Effects of Ethyl-EPA in P-gp Knockout Mice
8222196	26-Week Oral Gavage Oncogenicity Study with AMR101 in 001178-T (Hemizygous) Mice
456223	EPA-E (5, 8, 11, 14, 17-Eicosapentaenoic Acid Ethyl-Ester) 104 Week Carcinogenicity Study in Rats with Administration by Gavage
522093	A 4 Week Study of AMR101 and Epadel by Oral Gavage Administration in Rats

N/A: study number not provided

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

IND 102,457
(Pharm/Tox Review No. 1, S. Leuenroth-Quinn; Pharm/Tox Review No. 2, B.T. Hummer)

4 Pharmacology

4.1 Primary Pharmacology

Vascepa is highly purified icosapent ethyl (ethyl-eicosapentaenoic acid, ethyl-EPA, AMR101), an omega-3 fatty acid derived from fish oil. The formulation of Vascepa is purified ethyl-EPA oil with (b) (4) tocopherol. (b) (4)

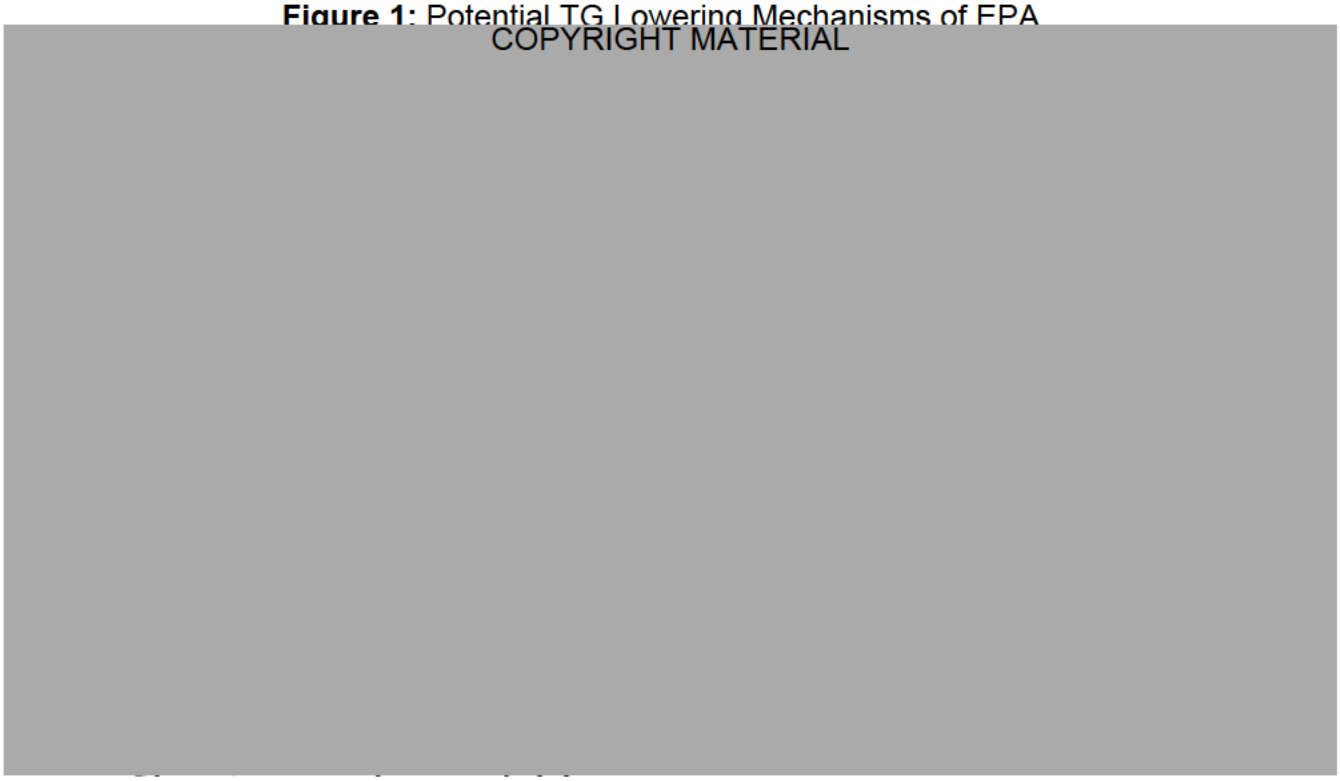
(b) (4)
or may be degradants formed during manufacturing or storage. There is extensive published literature for ethyl-EPA describing the primary pharmacology of this omega-3 fatty acid. Although icosapent ethyl is not naturally occurring, it is rapidly converted to EPA primarily by pancreatic lipase; therefore the pharmacologic effect is due to biologically active EPA. EPA is a long chain, 20 carbon, omega-3 polyunsaturated fatty acid (PUFA) found in the diet such as in fish oil. Following demethylation of ethyl-EPA to EPA, enterocyte uptake is followed by re-esterification, chylomicron formation and secretion into the lymph before systemic distribution. Once EPA has been incorporated into cell membranes, it can affect membrane fluidity, regulate cellular signaling and alter the production of such mediators as prostaglandin, leukotriene and platelet activating factor. Polyunsaturated fatty acids such as EPA can also serve as endogenous ligands for PPARs (peroxisome proliferator-activated receptors) and certain G-protein coupled receptors (GPR) such as GPR119 and GPR120 expressed in the gut.

The mechanism of action for the triglyceride lowering effect of EPA is through the promotion of fatty acid degradation (peroxisomal or mitochondrial β-oxidation) resulting in a reduction of substrate needed for very-low-density lipoprotein (VLDL) synthesis, inhibition of lipogenesis in the liver and increasing clearance of triglycerides from the plasma. At least four different nuclear receptor families are modulated by nonesterified fatty acids and include peroxisome proliferator-activated receptors (PPARs), farnesol X receptor (FXR), liver X receptor (LXR) and hepatocyte nuclear factor-α (HNF-4α), with omega-3 fatty acids being particularly potent regulators when compared to other fatty acids. Multiple gene products are affected that are downstream of receptor activation by fatty acids such as EPA. For example, sterol receptor element binding protein-1c levels are reduced resulting in decreased lipid synthesis, genes that stimulate fatty acid oxidation such as PPARs are upregulated and binding of polyunsaturated fatty acids to HNF-4α inhibits gene transcription that will result in glucose to glycogen conversion. Therefore the net effect is a metabolic shift from triglyceride production and storage to oxidation and elimination.

Literature references have reported that ethyl-EPA can lower plasma triglycerides (TG) and cholesterol in nonclinical species. Studies in rats fed a high cholesterol diet or administered corn oil to induce a hypertriglyceridemic state showed that ethyl-EPA could significantly reduce cholesterol and TG levels in these models. Administration of ethyl-EPA increases EPA incorporation into phospholipids, cholesteryl esters and triglycerides, increases LDL hepatic uptake and decreases hepatic triglyceride synthesis. Furthermore, studies in rats have also demonstrated that ethyl-EPA can inhibit intestinal cholesterol absorption. Mochida Pharmaceutical Company conducted pharmacology studies in cholesterol fed rabbits, where ethyl-EPA decreased total cholesterol in VLDL, IDL and LDL without change in HDL. These studies also showed that the n-3 PUFA content of LDL was increased leading to an enhanced binding to hepatic cell membranes and subsequent uptake and clearance.

Nonclinical toxicology studies conducted with AMR101 in normolipidemic mice, rats and dogs showed variable decreases in cholesterol, triglycerides and lipoprotein levels depending on the dose used. In a 28-day mouse study, reduced cholesterol levels reached statistical significance at ≥ 1.0 g/kg/day (HED ≥ 0.081 g/kg/day or 4.9 g/day for a 60 kg human). In a 28-day rat study, a dose related decrease in cholesterol was observed in both sexes at ≥ 1.0 g/kg/day (HED ≥ 0.16 g/kg/day or 9.6 g/day for a 60 kg human). In a 39-week dog study, cholesterol levels decreased in both sexes and at all doses at ≥ 0.3 g/kg/day (HED ≥ 0.167 g/kg/day or 10 g/day for a 60 kg human).

Figure 1: Potential TG Lowering Mechanisms of EPA



[Figure reproduced from Applicant's NDA 202057, Module 2.7.2]

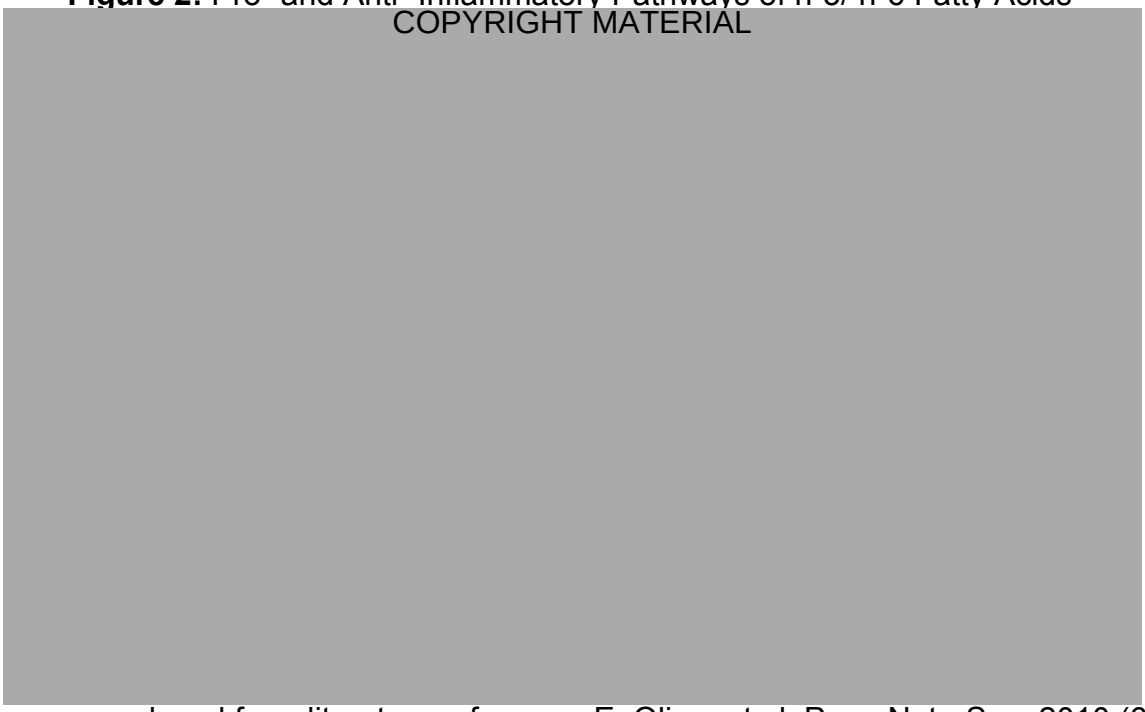
4.2 Secondary Pharmacology

Published literature has described secondary pharmacological effects of ethyl-EPA on platelets, the cardiovascular system, and WBCs (anti-inflammatory). Ethyl-EPA can reduce platelet aggregation through incorporation into platelet membranes and can compete with arachidonic acid (AA), an omega-6 fatty acid. This shift in the n-3/n-6 fatty acid ratio in cell membranes leads to an increase in membrane fluidity, increased eicosanoid synthesis, vasodilation and inhibition of platelet aggregation and thrombus formation due to omega-3 fatty acid accumulation in phospholipids over time. As the incorporation of omega-3 fatty acids such as EPA into phospholipid membranes has been well established, the concern is that inhibition of platelet aggregation could result in an increased risk of hemorrhage especially if given in combination with anti-clotting medication.

There are literature references that also describe an effect of ethyl-EPA on the cardiovascular system, such as maintenance of elasticity of the aorta in rabbits fed a high cholesterol diet. Literature references have also described a protective effect of ethyl-EPA in that it may prevent ventricular arrhythmias as described in dog studies, and in a rabbit model, ethyl-EPA administration decreased the duration of atrial fibrillation. Ethyl-EPA has also been attributed to the suppression of atherosclerotic lesions, and has been reported to increase migration of vascular endothelial cells while decreasing binding to vascular endothelial growth factor (VEGF). A protective effect in preventing atherosclerosis by omega-3 fatty acids is also attributed to decreased macrophage reactivity to pro-inflammatory cytokines (*e.g.* attenuation of cytokine release and adhesion molecule expression).

This mechanism of out-competing other fatty acids for cellular membrane incorporation also accounts for EPA's general anti-inflammatory effect but may also be associated with an increased risk of infection. Omega-3 fatty acids such as EPA will incorporate into white blood cells as well as in platelets and red blood cells. Conversion of omega-3 (versus omega-6) fatty acids into such mediators as resolvin, neuroprotectin and a subset of leukotrienes and prostaglandins are anti-inflammatory. The effects can include resolution of inflammation such as through increased phagocytosis of apoptotic neutrophils, and inhibition of neutrophil and eosinophil chemotaxis. Also, omega-3 fatty acids can inhibit inflammation as they compete with arachidonic acid membrane incorporation which leads to prostaglandin D_2 production and COX-1 and -2 activities. There are however studies in nonclinical species that have also demonstrated that a diet high in fish oil (*i.e.* high omega-3 fatty acids) can also impair the immune response to viral infection. While inhibition of cytokine secretion, upregulation of adhesion molecules and migratory behavior of white blood cells can be beneficial for attenuating inappropriate inflammation leading to disease, there is a possibility for decreased immune response to primary infection.

Figure 2: Pro- and Anti- Inflammatory Pathways of n-3/ n-6 Fatty Acids



[Figure reproduced from literature reference: E. Oliver et al. Proc. Nutr. Soc. 2010 (69)]

4.3 Safety Pharmacology

There were no dedicated safety pharmacology studies conducted by the Applicant due to the abundant published literature studies on ethyl-EPA, and the extensive experience of omega-3 fatty acid intake in the human population. Literature references on ethyl-EPA or studies conducted with AMR101 did not have specific safety pharmacology concerns other than already specified such as potential for increased bleeding risk due to the known mechanism of action of omega-3 fatty acids on platelets. A large randomized trial with 18,000 patients was conducted where the 1.8 g/day ethyl-EPA group showed a reduction in major coronary events and no evidence of sudden death. During the pre-IND meeting for IND 102,457, it was discussed that as there was favorable-to-neutral data on cardiac arrhythmias and sudden death that routine ECG assessments in clinical trail would be sufficient to reaffirm the electrocardiographic safety of ethyl-EPA. Furthermore, multiple clinical trials have been conducted with AMR101, and ethyl-EPA has been publicly available since 1991 as Epadel in the Japanese market, without known safety pharmacology issues. Due to the extensive human data, dedicated nonclinical safety pharmacology studies would not provide additional information over what is already known in the human population and what has been completed within published nonclinical toxicology studies.

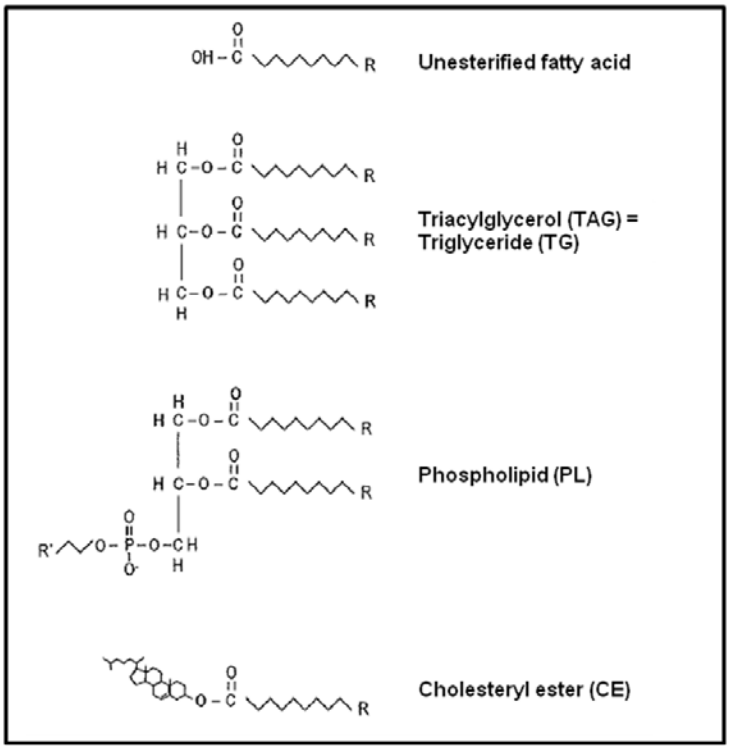
5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Limited PK studies were conducted for Vascepa with the majority of ethyl-EPA pharmacokinetics data published within the scientific literature and were sponsored by Mochida Pharmaceutical Co. in support of Epadel marketing. These Mochida sponsored studies were conducted in the nonclinical species of rat and dog. Amarin Pharma has sponsored *in vitro* protein binding, an *in vivo* rat metabolism study and *in vitro* CYP induction, inhibition and transporter assays. Toxicokinetic data is provided from Amarin sponsored studies in the rat, mouse and dog and were part of repeat dose toxicology studies. When considering PK data, AMR101 (ethyl-EPA) was usually below the level of detection due to the rapid conversion to EPA. Fatty acids may either be free (unesterified) or can be incorporated into triacylglycerol, phospholipids, cholesteryl esters or are part of cellular membranes as shown in Figure 3 below. The PK data provided is therefore presented as unesterified EPA, as assessed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), unless otherwise indicated. It should be noted however that the unesterified free fatty acid fraction is a small percentage of the total pool of fatty acid, with the majority being incorporated into triglycerides, phospholipids and cholesteryl esters.

In the 28-day rat toxicology study, AMR101 detection was only present in nine samples near the LLOQ=20 ng/mL, indicating that AMR101 was almost completely converted to EPA before systemic exposure. Exposure of unesterified EPA in rats increased more than dose proportional between 0.3 and 1.0 g/kg/day and less than dose proportional between 1.0 and 2.0 g/kg/day. There was some evidence of accumulation between days 1 and 28 in the rat. Following repeat dosing with AMR101 in a 28-day mouse study, exposure of unesterified EPA increased less than dose proportionally with some increase in systemic exposure between days 1 and 28 at doses of 1.0 and 2.0 g/kg/day. In the 9-month dog toxicology study, exposure of AMR101 was low but detectable and increased more than dose proportionally between 0.3 – 1.0 g/kg/day. Between a dose of 1.0 – 2.0 g/kg/day AMR101, there was no further increase in systemic AMR101 exposure. EPA exposure in the dog increased more than dose proportionally across all doses and in both sexes. In the literature references sponsored by Mochida Pharma in support of Epadel marketing, no TK data was published in any repeat dose toxicology study in rats.

Figure 3: Structure of Fatty Acids (Unesterified) and Lipids that Carry Fatty Acids in an Esterified Form



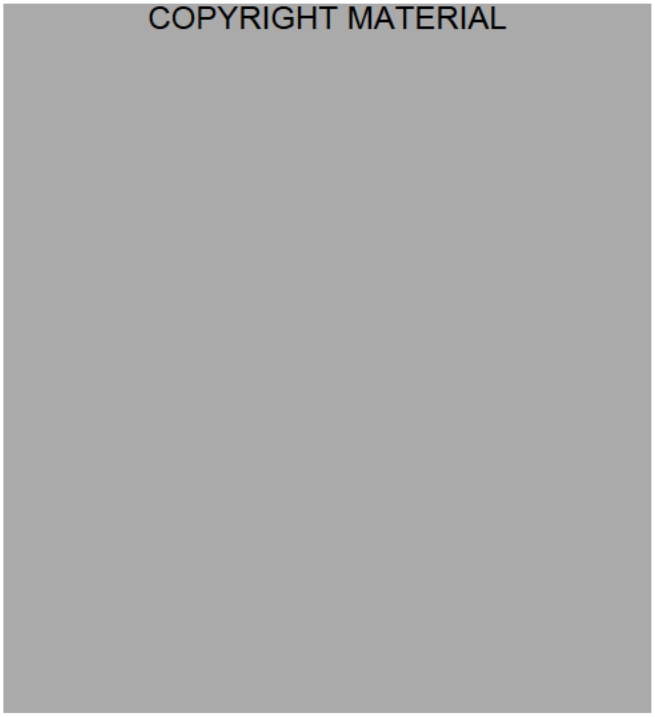
[Figure reproduced from applicant's NDA 202057, Module 2.6.4]

Following absorption from the small intestine and distribution to the systemic circulation via the lymphatic system, steady state levels are reached within 7-10 days. In rats, the highest concentrations of radiolabeled EPA were found in the GI tract, brain, liver, skin, fat and muscle. As part of a study (submitted by Amarin) in mice to assess ethyl-EPA levels in different tissues in P-gp knockout animals, it was observed that in WT mice, ethyl EPA increased over baseline levels to the greatest extent in adipose, spleen, intestines, kidney, liver and lung. Literature references have also observed that EPA is unique from other fatty acids in that following intravenous administration in the rat, EPA levels in the adrenal cortex remained high 18-hours after injection. EPA can be further metabolized to docosapentaenoic acid (DPA) or docosahexaenoic acid (DHA) or by cell membrane incorporation. Elimination of EPA is primarily through beta-oxidation and expired breath. EPA is rapidly taken up into phospholipids with a slower incorporation into cell membranes; however EPA also slowly declines from lipid membranes if administration is stopped.

Plasma protein binding was assessed from rat, dog and human samples. EPA (free) distributed predominantly to the plasma fraction where it was highly bound to plasma proteins in the rat, dog and human. There was a low association of EPA to red blood cells; however this non-dose dependent interaction was highest in human samples. EPA was found to be a weak inhibitor of all CYPs tested such as CYP2C19, 2C9, 2C8, and 2B6 in human liver microsomes. The lowest IC_{50} for EPA from *in vitro* human liver

microsome studies was 8.4 µM for CYP2C19 (followed by 12.8 µM (CYP2C8), and 14.0 µM (CYP2C9 and 2B6)). For reference, the calculated C_{max} plasma levels of un-esterified EPA in humans (28 day study with 4g/day) was 5.04 µM, making it theoretically possible that EPA may cause DDIs. In cultured human hepatocytes, there was no CYP induction of 2C9, 3A4 or 1A2 at a concentration up to 100 µM. Studies with drug transporters (P-gp, OAT1, OAT3, BCRP, OCT2, OATP1B1 and OATP1B3) showed that 1 µM EPA has the potential to inhibit OAT3 by 24.2% in the Caco-2 cell line. An additional study of 50 mg ethyl-EPA orally administered to P-gp knockout mice showed that there was decreased brain and liver corticosterone levels following ethyl-EPA administration but was not P-gp dependent (*i.e.* ethyl-EPA can affect the HPA-axis in mice).

Figure 4: Metabolism of Ethyl-EPA



[Figure reproduced from literature reference: J. Ishiguro et al. *Chem. Pharm. Bull.* 36(6), 1988: 2158-2167]

Table 1: Summary of Pharmacokinetic Parameters for Unesterified EPA in Plasma Across Species

Species, Sex	Time Point	AMR101 Dose	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)	AUC_{0-24h} (ng.h/mL)
Mouse, Male	Day 28	2 g/kg/day*	9910	1.00	81020	-
Mouse, Female	Day 28	2 g/kg/day*	4540	8.00	73720	-
Rat, Male	Day 28	2 g/kg/day*	16400	4.00	196000	-
Rat, Female	Day 28	2 g/kg/day*	17300	4.00	140200	-
Dog, Male	Week 39	1 g/kg/day*	15600	0.50	190800	-
		2 g/kg/day	42200	0.25	471500	-
Dog, Female	Week 39	1 g/kg/day*	17900	1.00	132000	-
		2 g/kg/day	29800	0.00	324300	-
Human	Day 28	2 g/day	770	5	-	8790
		4 g/day	1520	5	-	20300

- Denotes not applicable
* denotes NOAEL dose
LC-MS/MS assay for unesterified EPA was used
Median for T_{max}; mean for all other PK parameters.
Mouse data are from (b) (4) Study 459549(Amendment 1); rat data are from (b) (4) Study Z0C0001 (Amendment 1); dog data are from (b) (4) Study 515194 (Amendment 1); human data are from healthy volunteer study AMR-01-01-0018

[Table reproduced from applicant's NDA 202057, Module 2.6.4]

AMR-NC-11-01: Inhibition of Human Cytochromes P450 by Eicosapentaenoic Acid

The purpose of this study was to evaluate the potential of EPA (the active metabolite of ethyl-EPA) to inhibit CYP enzymes in human liver microsomes (*in vitro*). Results showed that EPA inhibited all CYP isoforms tested (IC_{50} ranged from 8.4 µM - 250 µM) with significant CYP2E1 inhibition increased by pre-incubation. The IC_{50} with pre-incubation with CYP2D6 could not be calculated but a leftward shift in the curve was not evident. A slight increase in inhibition with CYP3A4 pre-incubation was observed.

The CYP2C family was most sensitive to inhibition by EPA with the lowest IC_{50} value of 8.4 µM achieved with CYP2C19. For reference, in the 28 day human study with healthy volunteers (Study AMR101-01-01-0018), C_{max} plasma levels were 2.55 µM and 5.04 µM after dosing with 2 g and 4 g AMR101, respectively.

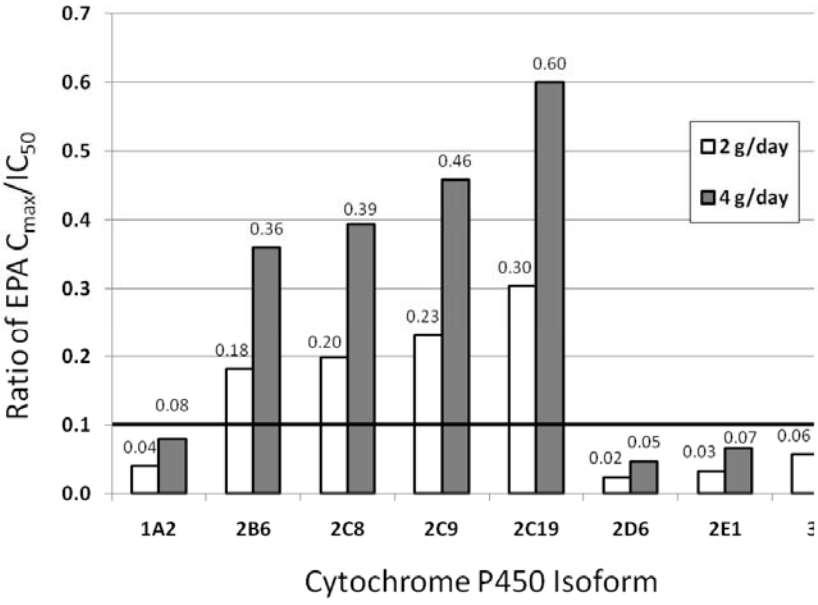
Based on FDA guidance, the following CYP isoforms indicated a possible DDI: CYP2C19, CYP2C9, CYP2C8 and CYP2B6. Additionally, CYP3A at a dose of 4 g/ day AMR101 also indicated a possible DDI with a ratio of 0.11.

Table 2: Summary of EPA Inhibition of CYP Isoforms

CYP isoform	EPA IC_{50} (µM)	
	Without preincubation	With preincubation
1A2	63.2	68.3
2B6	14.0	35.1
2C8	12.8	18.4
2C9	14.0	11.0
2C19	8.4	19.0
2D6	105	-
2E1	>250	76.5
3A4	67.1	44.7

[Table reproduced from NDA Submission 202057]

Figure 5: Ratio of C_{max}/IC_{50} for EPA for Different CYP Isoforms Based on the Plasma (Unesterified) EPA C_{max} After Dosing with 2 and 4 g/day AMR101 for 28 Days in Healthy Volunteers



The horizontal line at a Cmax/IC_{50} ratio of 0.1 represents the cut-off according to the FDA guidance12, above which a clinical DDI is "possible".

[Figure reproduced as is from NDA Submission 202057]

AMR-NC-11-02: Induction of Human Cytochromes P450 by Eicosapentaenoic Acid

The purpose of this study was to evaluate the potential of EPA to induce CYP3A4, CYP2C9 and CYP1A2 in human cultured hepatocytes. EPA did not significantly induce any of the tested CYP enzymes at the concentrations tested.

Table 3: Metabolic Activity of CYP Isoforms Following Incubation with EPA

CYP isoform	Metabolic activity following incubation with EPA					
	Percent of negative control			Percent of positive control		
	1 µM	10 µM	100 µM	1 µM	10 µM	100 µM
3A	75 (±4)	73 (±3)	76 (±4)	-5 (±3)	-5 (±2)	-4 (±1)
2C9	103 (±8)	78 (±13)	82 (±6)	1 (±9)	-24 (±14)	-22 (±6)
1A2	79 (±5)	119 (±45)	121 (±43)	-7 (±3)	5 (±15)	6 (±14)

[Table reproduced from NDA Submission 202057]

Study 789867: Determination of the In Vitro Binding of EPA (Free Acid) to the Plasma Proteins and Partitioning into the Blood Cells of Rat, Dog and Human

Study conducted at (b) (4)

The purpose of this study was to assess the potential for EPA (free acid, Sigma-Aldrich) to bind to plasma proteins and blood cells in various species. Blood was collected from three male CD(Crl:CD(SD)) Sprague Dawley rats, from three male Beagle dogs, and from three male human volunteers. Concentrations of EPA tested included 750, 1500, 7500 and 15,000 ng/mL and endogenous EPA concentrations were subtracted from EPA measured in the ultrafiltrate. EPA (free acid) was highly bound to plasma proteins where dog (100%) > human > rat and increased slightly with concentration in human and rat. EPA had a low association with blood cells; however the highest binding was to human cells (non-dose dependent). Results indicated that EPA (free acid) was predominantly distributed to the plasma fraction.

Table 4: Percent EPA Binding to Plasma Protein and Blood Cells

Target Test Item Concentration (ng/mL)	Plasma Protein Binding (%)		
	Rat	Dog	Human
750	97.3 ± 0.9	100 ± 0.0	98.8 ± 2.4
1500	98.1 ± 0.7	100 ± 0.0	99.3 ± 0.5
7500	99.7 ± 0.1	100 ± 0.0	99.5 ± 0.3
15000	99.9 ± 0.1	100 ± 0.0	99.8 ± 0.1

Target Test Item Concentration (ng/mL)	Blood Cell Association (%)		
	Rat	Dog	Human
750	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1500	0.0 ± 0.0	0.0 ± 0.0	11.1 ± 9.7
7500	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 4.7
15000	0.0 ± 0.0	4.4 ± 7.7	1.4 ± 2.4

[Tables reproduced from NDA Submission 202057]

Study OPT-2010-157 and OPT-2010-158: Assessment of EPA as a potential inhibitor of human P-gp, BCRP, OAT1, OAT3, OCT2, OATP1B1 and OATP1B3-mediated transport, and AMR101 as a potential inhibitor of human P-gp

Study conducted [redacted] (b) (4)

The purpose of this study was to determine if EPA can inhibit the OAT1, OAT3, OCT2, OATP1B1 and OATP1B3 transport proteins expressed on MDCK-II or the BCRP transporter in Caco-2 cells. Additionally, AMR101 and EPA were tested to determine if they would inhibit the transport of substrate by P-glycoprotein (P-gp) in MDCK-MDR1 cells. A vehicle control of 0.5% DMSO was used for comparison of substrate uptake.

At a concentration of 1 µM EPA, there was no inhibitory effect on P-gp, BCRP, OCT2, OAT1, OATP1B1 or OATP1B3. OAT3 was significantly inhibited by 1 µM EPA by 24.2%. At a 15µM EPA or AMR101 concentration, there was no effect on P-gp inhibition of substrate transport (maximum of approximately 5% inhibition).

Figure 6: Inhibition of Probe Substrate Transport by 1 µM EPA

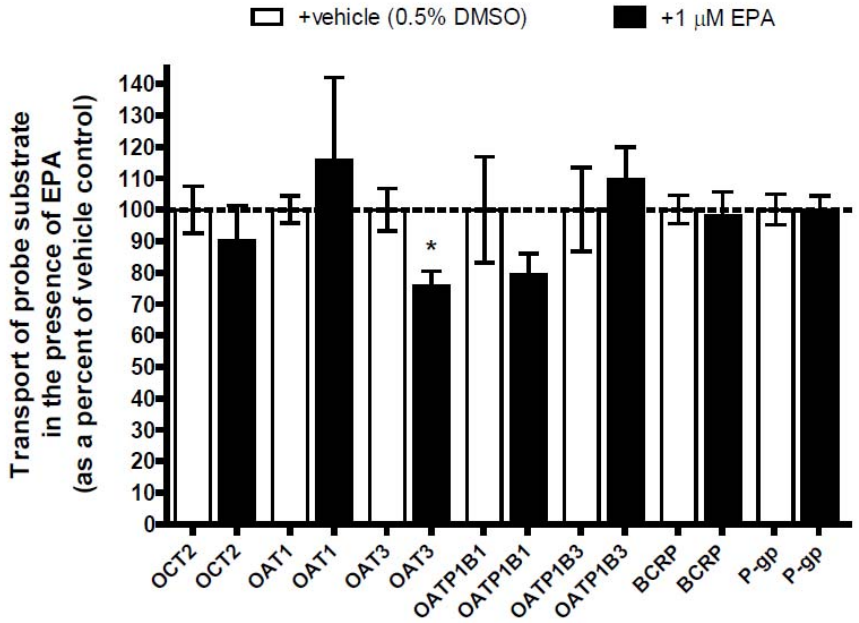
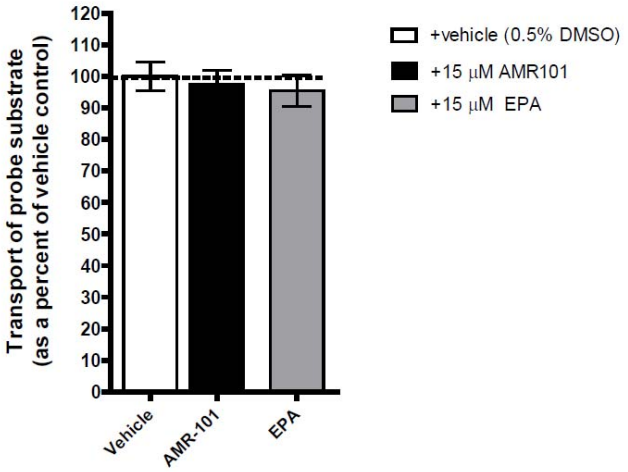


Figure 7: Inhibition of P-gp Mediated Probe Substrate Transport by 15 µM EPA and 15 µM AMR101



[Above figures reproduced from NDA Submission 202057]

The Effects of Ethyl-EPA in P-gp Knockout Mice

It was stated that this study was performed for Amarin Neuroscience Limited (b) (4) . The purpose was to determine the effect of ethyl-EPA on the hypothalamus-pituitary-adrenocortical (HPA) axis in male wild-type (FVB/N) and p-glycoprotein knockout mice (P-gp; disruption of both *ABCB1a* and *ABCB1b* genes), as compared to placebo. 50 mg ethyl-EPA was mixed into 950 mg chocolate nut cream versus 1000 mg chocolate nut cream given as placebo, and administered once every 2 days for 50 days followed by every day from Day 51 to Day 65. The average weight of wild type mice administered ethyl-EPA was 34.06 g (estimated 1468 mg/kg ethyl-EPA) and for P-gp KO mice was 33.86 g (estimated 1477 mg/kg ethyl-EPA). The following steroid hormones were measured in the brain, serum, kidney, lung, spleen, liver, testis, intestine and adipose tissue: corticosterone, progesterone and testosterone. Ethyl-EPA, EPA and DHA levels were also measured in the brain and other tissues.

Results showed that when compared to placebo (in both animal models, wild type and P-gp knockout); ethyl-EPA decreased brain concentrations of corticosterone and may influence the HPA-axis, but was not dependent upon P-gp. The concentration of corticosterone in the liver was decreased in the presence of ethyl-EPA, also in both animal models.

Table 5: Plasma and Tissue Concentrations of Corticosterone

Treatment Group	Tissue	unit	Wild type control mice*		P-gp knockout mice*	
			Mean	SEM	Mean	SEM
Placebo	Serum	ng/ml	73,16	12,34	103,58	8,59
	Cerebrum	ng/g	9,08	1,54	14,75	3,66
	Intestine	ng/g	4,28	1,04	6,97	1,13
	Liver	ng/g	20,64	4,52	31,97	5,52
	Kidney	ng/g	5,34	0,70	9,44	1,05
	Spleen	ng/g	6,49	1,43	12,17	2,29
	Lung	ng/g	10,05	1,98	14,58	1,59
	Testis	ng/g	3,90	0,57	6,73	0,97
	Adipose	ng/g	5,11	0,86	6,49	0,87
Ethyl-EPA	Serum	ng/ml	79,49	11,34	70,53	15,69
	Cerebrum	ng/g	5,69	1,40	6,11	1,66
	Intestine	ng/g	4,79	0,75	4,57	1,17
	Liver	ng/g	14,42	2,93	15,51	3,35
	Kidney	ng/g	7,15	1,01	6,38	1,18
	Spleen	ng/g	7,95	2,00	8,21	1,75
	Lung	ng/g	10,05	1,85	10,65	2,42
	Testis	ng/g	4,89	0,88	4,81	0,91
	Adipose	ng/g	4,23	1,10	4,37	0,93

* n = 9

[Table reproduced from NDA Submission 202057]

Testosterone levels were higher in the testis in both mouse models; however there was a large standard error and no statistical significance.

Table 6: Plasma and Tissue Concentrations of Testosterone

Treatment Group	Tissue	unit	Wild type control mice*		P-gp knockout mice*	
			Mean	SEM	Mean	SEM
Placebo	Serum	ng/ml	7,39	2,54	7,52	2,52
	Cerebrum	ng/g	4,08	1,75	3,16	1,17
	Intestine	ng/g	1,40	0,38	0,90	0,27
	Liver	ng/g	0,18	0,02	0,17	0,01
	Kidney	ng/g	1,30	0,26	1,13	0,21
	Spleen	ng/g	1,67	0,44	1,28	0,44
	Lung	ng/g	2,17	0,66	1,96	0,53
	Testis	ng/g	222,46	78,85	203,50	73,90
	Adipose	ng/g	16,13	4,21	6,10	2,46
Ethyl-EPA	Serum	ng/ml	9,50	3,70	11,65	3,13
	Cerebrum	ng/g	2,96	1,24	3,61	1,10
	Intestine	ng/g	0,97	0,31	1,51	0,45
	Liver	ng/g	0,16	0,02	0,17	0,03
	Kidney	ng/g	1,30	0,22	1,40	0,29
	Spleen	ng/g	1,65	0,59	2,27	0,63
	Lung	ng/g	1,95	0,59	3,38	0,99
	Testis	ng/g	373,93	120,34	359,85	124,20
	Adipose	ng/g	4,66	1,08	10,49	2,51

* n = 9

[Table reproduced from NDA Submission 202057]

Progesterone was detected in the serum, testis and adipose with a small non-significant decrease in progesterone in the testis with ethyl-EPA in both mouse models. Significant increases in ethyl-EPA and EPA were observed in the serum as well as in tissues, with the largest increases versus placebo observed in the adipose, spleen, intestine, liver, lung, cerebrum and kidney (both animal models). Ethyl-DHA was increased but DHA was decreased in P-gp mice versus wild type mice administered ethyl-EPA.

Study No. 309365: Validation of an Analytical Method for the Determination of AMR101 and EPA in Dog, Rat, and Mouse Plasma by LC-MS/MS

Test Facility: (b) (4)

Analytical methods were validated to detect AMR101 (ethyl eicosapentaenoate) as well as EPA (eicosapentaenoic acid) from dog, rat and mouse plasma over a concentration range of 20.0 – 2000 ng/mL AMR101 and 50.0 – 5000 ng/mL EPA. The assay was performed following protein precipitation and solid phase extraction and quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples of control matrix (5% Fatty Acid Free Bovine Serum Albumin prepared in phosphate buffered saline) were also spiked with AMR101 and EPA.

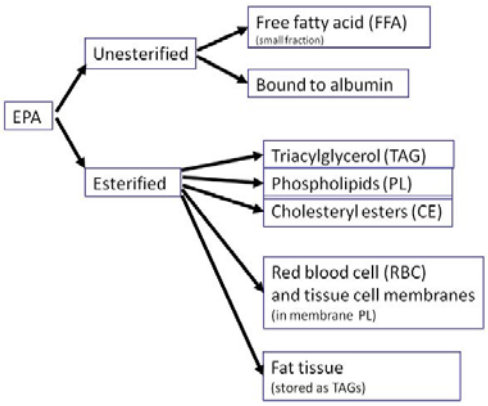
The assay was found to be specific for differential determination between EPA and ethyl-EPA in rat, dog and mouse plasma. AMR101 was stable in dog, rat and mouse plasma after 3 freeze/thaw cycles, and EPA was found to be stable in dog, mouse and rat plasma after 4, 3 and 2 freeze/thaw cycles, respectively. AMR101 was stable for 4 hours at room temperature in rat and dog plasma without the addition of any inhibitors of degradation. EPA was stable for 2 hours in dog plasma, and for 4 hours in rat plasma at room temperature without any inhibitors of degradation. Both AMR101 and EPA were stable in mouse plasma for 4 hours on ice with the addition of inhibitors of degradation.

Study No. 314194: Validation of an Analytical Method for the Determination of Total Eicosapentaenoic Acid (EPA) in Rat Plasma by LC-MS/MS

Test Facility: (b) (4)

Analytical methods were validated to detect total EPA, eicosapentaenoic acid, in rat plasma which includes un-esterified EPA as well as EPA liberated from phospholipids, triglycerides, and cholesteryl esters.

Figure 8: EPA distribution



[Figure reproduced from Applicant's NDA 202057 submission]

The method was validated for use to detect total EPA in rat plasma over the concentration range of 1 – 1000 µg/mL in a volume of 10 µL. Stability of EPA in rat plasma was determined to be at least 24 hours at room temperature, through 4 freeze/thaw cycles and for at least 3 weeks at -80°C, and for at least 208.5 hours at 4°C.

5.2 Toxicokinetics

A summary of TK data for Un-esterified EPA is presented below:

Table 7: TK Data for Un-esterified EPA

Study: 459549	**4-Week Oral (Gavage) Toxicity Study in Wild Type CByB6F1 (rasH2) Mice**					
Dose AMR101:	**0.3 g/kg**		**1.0 g/kg**		**2.0 g/kg**	
Day 28 EPA TK	**M**	**F**	**M**	**F**	**M**	**F**
$AUC_{0\text{-}t}$ (ng•h/mL)	6007	6527	46010	43280	81020	73720
C_{max} (ng/mL)	509	534	5820	3910	9910	4540
T_{max} (h)	1.00	1.00	2.00	4.00	1.00	8.00
Study: 8222196	**26-Week Oral Gavage Oncogenicity Study with AMR101 in 001178-T (Hemizygous) Mice**					
Dose AMR101:	**0.5 g/kg**		**2.0 g/kg**		**4.6 g/kg**	
Day 28 EPA TK	**M**	**F**	**M**	**F**	**M**	**F**
$AUC_{0\text{-}t}$ (ng•h/mL)	11340	15120	40790	61690	71190	77820
C_{max} (ng/mL)	1400	1580	2470	4280	7450	5720
T_{max} (h)	2.00	2.00	2.00	1.00	2.00	2.00
Study: ZOC0001	**4 Week Oral (Gavage) Toxicity Study in the Rat**					
Dose AMR101:	**0.3 g/kg**		**1.0 g/kg**		**2.0 g/kg**	
Day 28 EPA TK	**M**	**F**	**M**	**F**	**M**	**F**
$AUC_{0\text{-}t}$ (ng•h/mL)	60310	54970	138200	151600	196000	140200
C_{max} (ng/mL)	7340	6490	19600	13100	16400	17300
T_{max} (h)	2.00	2.00	2.00	2.00	4.00	4.00
Study: 522093	**A 4 Week Study of AMR101 and Epadel by Oral Gavage Administration in Rats**					
Dose (g/kg):	**AMR101 (1 g/kg)**		**AMR101 (2 g/kg)**		**Epadel (2 g/kg)**	
Day 28 TK	**M**	**F**	**M**	**F**	**M**	**F**
$AUC_{0\text{-}t}$ (ng•h/mL)	212800	163900	265500	201600	290500	296500
C_{max} (ng/mL)	25000	12700	28400	19400	21400	25400
T_{max} (h)	2.0	2.0	2.0	1.17	2.67	2.67
Study: 515194	**AMR101: 39 Week Toxicity Study in Dogs by Oral (Gavage) Administration with an Interim Kill and 4 Week Recovery After 13 Weeks**					
Dose AMR101:	**0.3 g/kg**		**1.0 g/kg**		**2.0 g/kg**	
Week 13 EPA TK	**M**	**F**	**M**	**F**	**M**	**F**
$AUC_{0\text{-}t}$ (ng•h/mL)	30220	27340	206800	201300	365600	355900
C_{max} (ng/mL)	3140	3280	22600	20000	49000	47500
T_{max} (h)	0.50	1.00	0.50	0.00	0.75	0.50
Week 39 EPA TK	**M**	**F**	**M**	**F**	**M**	**F**
$AUC_{0\text{-}t}$ (ng•h/mL)	33280	29830	190800	132000	471500	324300
C_{max} (ng/mL)	2750	2970	15600	17900	42200	29800
T_{max} (h)	2.00	1.50	0.50	1.00	0.25	0.00

6 General Toxicology

6.1 Single-Dose Toxicity

There were no Amarin sponsored single dose toxicology studies conducted with AMR101 (ethyl-EPA). There was a single literature reference to assess the acute toxicity of ethyl-EPA (EPA-E) and its metabolites in mice and rats (Shibutani *et al.* Iyakuhin Kenkyu. 1989; 20: 801-7; Amarin provided a certified translation), which was sponsored by Mochida Pharmaceutical. Ethyl-EPA was administered as a single dose (20 g/kg) by oral, intraperitoneal and subcutaneous methods to Slc:ICR mice and Slc:Wistar rats. In addition, oral administration of the following ethyl-EPA metabolites and impurities ((b) (4) were also given to assess toxicity:

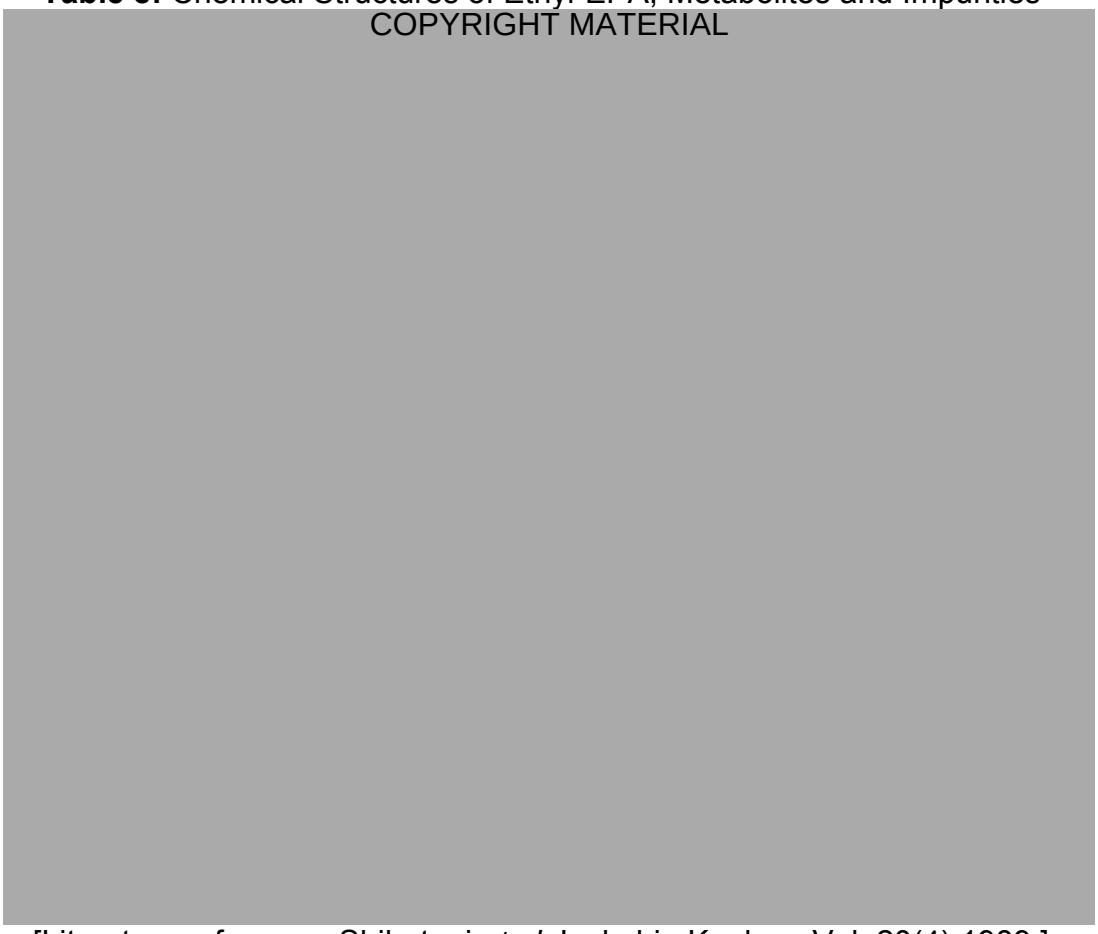
- (b) (4)
- 5,8,11,14,17-eicosapentaenoic acid (EPA), a metabolite
- 7,10,13,16,19-docosapentaenoic acid (DPA), a metabolite
- 4,7,10,13,16,19-docosahexaenoic acid (DHA), a metabolite

(b) (4)

For the parent compound, ethyl-EPA, the LD_{50} for oral and subcutaneous routes of administration in both species and sexes was >20 g/kg. Following intraperitoneal injection of ethyl-EPA in female rats, the LD_{50} was 15-20 g/kg, and was >20 g/kg for mice (both sexes) and for male rats. For DPA, (b) (4) oral administration resulted in an LD_{50} of >20 g/kg in both species and sexes. For the metabolites EPA and DHA, the LD_{50} in mice (both sexes) was >20 g/kg, but for rats (both sexes) was 10-20 g/kg.

Toxicities presenting from oral administration of ethyl-EPA and its metabolites included oily leakage from the anus in mice and rats. Intraperitoneal injection of EPA-E as well as some metabolites/ impurities in mice and rats led to weight loss for several days after dosing but eventually recovered. In surviving animals, no gross pathology abnormalities were noted upon necropsy.

Table 8: Chemical Structures of Ethyl-EPA, Metabolites and Impurities



[Literature reference: Shibutani *et al.* Iyakuhin Kenkyu, Vol. 20(4) 1989.]

6.2 Repeat-Dose Toxicity

Repeat-Dose toxicology studies in the rat and mouse up to 4 weeks and in the dog up to 39 weeks were sponsored by Amarin Pharma. Mochida sponsored repeat dose toxicology studies performed in support of Epadel (ethyl-EPA) marketing included 3-month and 12-month rat studies. In general, the main target organs of toxicity included the GI tract, the hematopoietic system including WBCs, RBCs and clotting parameters, the liver, the skin and the adrenal gland (dog). High doses of ethyl-EPA in the rat and dog frequently led to GI tract related findings that may have been due to excess oil as an oily discharge was observed from the anus. Two dogs (one high dose (HD) and one low dose (LD)) were moribund sacrificed due to intussusception of the ileocecocolic junction in the HD male and an infarcted jejunum followed by a blockage between the large and small intestines in the LD male. The skin was frequently irritated either through direct interaction with oil, such as near the anus or tail and presented as red, flaky/ dry skin and hair loss or may have been due to systemic depletion of omega-6 fatty acids also leading to hair loss, scabbing, and fibrosis/ epidermal hyperplasia. WBC increases were observed which may have been due to skin irritation and inflammation

although RBC and clotting parameters were also affected. Liver enzymes such as ALT and ALP were increased in both the rat and dog. In the dog, ethyl-EPA also showed an effect on the adrenal gland which included vacuolar degeneration of the inner cortex and decreased cortisol production. The effect on the adrenal gland is noted as published literature has shown that EPA accumulates in the adrenal gland in the rat (Becker and Bruce 1985); however cortisol levels were not tested in the rat.

Mochida sponsored studies published in the scientific literature were performed in the Wistar rat for 3 or 12 months at oral doses of 100, 300, 1000 and 3000 mg/kg/day (plus an additional 6000 mg/kg/day group in the 3 month study). Following 3 months of ethyl-EPA repeat oral dosing, clinical signs included an oily substance leaking from the anus, scabbing and hair loss associated with skin irritation (fibrosis, inflammation, granuloma and hyperkeratosis) at 3000 and 6000 mg/kg. Increased WBCs and eosinophils may reflect the inflammation observed in the skin. At the highest dose tested, 6000 mg/kg, both sexes presented with increased ALP which frequently in the rat is indicative of intestinal toxicity and may reflect the inability to absorb excess oil. Also at this high dose, increased urine protein and increases in organ weights such as the heart, thyroid, testes, adrenals, and kidney were observed that persisted through recovery. The pharmacodynamic (PD) effect of ethyl-EPA was observed as cholesterol, triglycerides (TG) and HDL levels all declined. The NOAEL in the 3-month rat study using ethyl-EPA (Epadel) was determined to be 3000 mg/kg (HED = 484 mg/kg with a body surface area exposure margin of 7X based on a clinical dose of 4g/day icosapent ethyl).

The longest toxicology study sponsored by Mochida was a 12 month rat study with daily oral doses of ethyl-EPA up to 3000 mg/kg. Similar findings were reported in the 3-month rat study such as an oily substance observed leaking from the anus; nodulation of the tail (hyperkeratosis, fibrosis, and abscess around tail) increased ALP, and increased leukocytes predominantly at 3000 mg/kg/day. Other findings included enhanced liver toxicity as observed by increased ALT at 3000 mg/kg and an increase in the severity of bile duct hyperplasia and liver weight. Heart weight increased at 3000 mg/kg with an increase in the severity of fibrosis and bronchopneumonia (lung) was dose dependently increased. Retinal hemorrhage was only observed transiently at week 26 in males treated with 3000 mg/kg (3/20), but was not observed by ophthalmoscopy after 50 weeks. A decrease in hemoglobin and hematocrit were also observed at 3000 mg/kg. In summary, following 12 month administration of ethyl-EPA to rats, the skin was affected and presented with inflammation and nodulation at the site of oil leakage from the anus, liver enzymes and liver histopathology indicated toxicity, and changes in RBC parameters were observed with transient retinal hemorrhage at the HD of 3000 mg/kg. Although increased bronchopneumonia may have been attributed to inadvertent inhalation of oil, a possible suppression of WBC function cannot be dismissed due to the known immune modulating mechanism of omega-3 fatty acids. The NOAEL following 12 months of repeat oral dosing of ethyl-EPA to the rat was 1000 mg/kg/day (HED = 161 mg/kg with a body surface area exposure margin of 2.4X based on a clinical dose of 4 g/day icosapent ethyl).

Amarin Pharma sponsored five repeat dose nonclinical toxicology studies in three species including: a 4-week GLP study in the rat, a 4-week GLP bridging toxicology and PK study in the rat comparing AMR101 to Epadel, a 4-week oral gavage dose-ranging toxicity study in wild-type CByB6F1 (rasH2) mice, a 14-day non-GLP dose range finding study in the Beagle dog and a 39-week GLP study in the Beagle dog with an interim kill and recovery at 13-weeks. In the 4-week AMR101 toxicology study in the rat, excessive salivation and hair loss was observed in both sexes and mean prothrombin times were slightly increased up to 10% at the HD of 2000 mg/kg. The PD effect of ethyl-EPA was noted with dose-dependent decreasing cholesterol, HDL and TG. The bridging study also completed in the rat, used oral repeat daily doses of 1000 and 2000 mg/kg AMR101, as well as 2000 mg/kg of Epadel for comparison. Again there was mild toxicity observed with scabs, fur staining and hyperkeratosis of the skin in both AMR101 and Epadel treated groups at 2000 mg/kg. A mild increase in RBCs and decreases in reticulocytes and RDW were also noted at 2000 mg/kg in both AMR101 and Epadel groups. A PD effect was noted as cholesterol, and TG decreased in all ethyl-EPA treated groups (AMR101 and Epadel). The NOAEL for both of these 4-week studies in the rat based on toxicology findings was 2000 mg/kg/day (HED = 323 mg/kg with a body surface area exposure margin of 5X based on a clinical dose of 4g/day icosapent ethyl).

The AMR101 and Epadel 4-week bridging study in the rat was to support the use of published literature conducted for the marketing of Epadel by Mochida Pharmaceutical, in the Applicant's 505(b)(2) NDA submission. The toxicology profiles of these two ethyl-EPA compounds in this study were similar with no obvious differences at the same dose of 2000 mg/kg. When the PK profile was examined, mild differences in C_{max} or AUC usually less than 2-fold were observed in a single sex or following Day 1 administration. Both un-esterified EPA (free EPA) as well as total EPA (un-esterified EPA and EPA liberated from phospholipids, triglycerides and cholesteryl esters) were quantitated from rat plasma on Day 1 and Day 28. Overall, the PK profiles were similar between AMR101 and Epadel with accumulation of total EPA and un-esterified EPA observed between day 1 and day 28 of dosing. T_{max} was approximately at 2 hours in both AMR101 and Epadel treated rats on day 28. Epadel had mild increases in exposure (AUC and C_{max}) primarily in females over AMR101 but it is unlikely that these differences are toxicologically relevant as no difference in the toxicology profile was noted in the bridging study conducted for 28 days. Furthermore, when assessing the general variability of un-esterified EPA exposure, a comparison can be made between the 2000 mg/kg AMR101 dose administered in the rat 4-week bridging study and the 4-week AMR101 toxicology study in the rat (#ZOC0001). Again although these were two separate studies, there was a higher exposure of un-esterified EPA in the bridging study versus the original rat toxicology study using the same test compound, AMR101. Comparison of toxicology results between these two 28-day studies also did not show any significant differences. It is therefore likely that at least in the rat, where there is a less than dose proportional increase in EPA exposure from AMR101 doses higher than 1000 mg/kg, that a certain variability or range in exposure levels will result in similar toxicity profiles. If the rate of absorption has been exceeded however, certainly additional toxicities such as that related to the GI tract and excess oil leakage from the rectum could be expected at least in the rat.

Table 9: Comparison of **Un-Esterified** EPA Plasma Levels Across Rat 4-Week Toxicology Studies at a Dose of 2000 mg/kg/day (Day 28)

	Male			Female		
	Bridging Study		4-Wk Tox	Bridging Study		4-Wk Tox
	Epadel	AMR101	AMR101	Epadel	AMR101	AMR101
AUC (µg•hr/mL)	290.5	265.5	196.0	296.5	201.6	140.2
C_{max} (µg/mL)	21.4	28.4	16.4	25.4	19.4	17.3

Table 10: Combined (♂/♀) Average Exposure of **Un-Esterified** EPA Plasma Levels in the Rat in 4-week Toxicology Studies at a dose of 2000 mg/kg (Day 28)

	Bridging Study		4-Wk Tox
	Epadel	AMR101	AMR101
AUC (µg•hr/mL)	293.5	233.55	168.1
C_{max} (µg/mL)	23.4	23.9	16.9

The 4-week mouse study was conducted as a dose range finding study to set dosing for the 6-month transgenic mouse (rasH2) carcinogenicity study. AMR101 was administered orally to wild type CByB6F1 (rasH2) mice at doses of 0.3, 1.0 and 2.0 g/kg/day. Decreased white blood cells including neutrophils, lymphocytes, monocytes and large unclassified cells were observed at a dose of 2000 mg/kg in both sexes. Liver weights decreased at all doses and in both sexes while adrenal gland weight increased in males at all doses. Glucose decreased in females at ≥1000 mg/kg. A PD effect was observed at doses ≥1000 mg/kg where cholesterol and triglyceride levels were declining.

The dog was the third nonclinical species used to assess AMR101 toxicity. In a 14-Day dose ranging study in the dog, a dose of 2000 mg/kg AMR101 resulted in red/flaky skin on the abdomen and groin, increased WBC and neutrophils that may have been attributed to the skin irritation, increased ALP and ALT and a PD effect of decreased cholesterol, HDL/LDL and triglycerides. The 39-week dog toxicology study used AMR101 dosing of 300, 1000 and 2000 mg/kg/day and had an interim kill at 13- weeks with a 4-week recovery period. There were two dogs in the study that were sacrificed moribund: one male administered the HD of 2000 mg/kg AMR101 was sacrificed on Day 45 due to intussusception of the ileocecocolic junction (blockage at the junction of the ileum and cecum, or between the large and small intestine) and one male administered the LD of 300 mg/kg AMR101 was sacrificed on Day 154 with an infarcted jejunum followed by a blockage between the small and large intestines. Both of these incidents while at different doses of AMR101 involved a blockage of the GI tract. The skin was a site of toxicity as red/flaky/dry skin with hair loss was observed around the anogenital region with histopathology at 39 weeks showing increased severity of epidermal hyperplasia at ≥1000 mg/kg (HED ≥556 mg/kg; 8X exposure margin to a clinical dose of 4g/day). The adrenal gland in the dog was a site of toxicity at doses of ≥1000 mg/kg in both sexes. Beginning at 13 weeks but also observed at 39 weeks,

vacuolar degeneration of the inner adrenal cortex was observed (persisted throughout recovery following 13 weeks of AMR101 administration) and cortisol levels at 39 weeks were decreased at ≥1000 mg/kg in females and at 2000 mg/kg in males. There was an increase in ALP levels that could theoretically be attributed to a dog specific corticosteroid-ALP (C-ALP); however it is not known which ALP isotype was elevated in this study. EPA can localize to the adrenal cortex and poly-unsaturated fatty acids such as omega-3s have been shown to have corticosteroid-like activity and affect the hypothalamus-pituitary-adrenal axis; thereby decreasing the need for and production of cortisol by the adrenal glands. Therefore, in the dog, there is evidence that ethyl-EPA can alter cortisol production and affect adrenal function. Additionally, there was an increase in the incidence (3/4 animals) of anterior lobe pituitary cysts in both sexes at the HD of 2000 mg/kg following 39 weeks of ethyl-EPA administration (HED = 1111 mg/kg; 17X exposure margin to a clinical dose of 4g/day).

Table 11: Comparison of toxicities in Repeat Dose ethyl-EPA Studies

Study Type Study No. GLP Status	Species/ strain Number/ group	Dose Levels	Study Findings
Mochida Sponsored Studies			
Literature Ref. Shibutani 1989 *Iyakuhin Kenkyu* (20:808-25) GLP? – Not Stated	Wistar Rat 10/ sex/ group	0, 100, 300, 1000, 3000, 6000 mg/kg **90 Days** **NOAEL: 3000 mg/kg (HED = 484 mg/kg; Exp 7X)**	• Oily substance leaking from anus, scabbing, hair loss, fibrosis, hyperkeratosis, and inflammation of the skin at 3000 and 6000 mg/kg • ↑ leukocytes and eosinophils (+88%) at 6000 mg/kg • ↑ ALP at 6000 mg/kg (♀/♂), 3000 mg/kg (♀) • ↓ Cholesterol, TG, HDL • Urine: ↓ electrolytes, ↑ protein 6000 mg/kg • ↓BW, ↑ organ weights (multiple incl. thyroid, heart, liver, kidney, spleen, adrenal and testes), persisting through recovery at 6000 mg/kg
Literature Ref. Shibutani 1989 *Iyakuhin Kenkyu* (20:826-44) GLP? – Not Stated	Wistar Rat 20/ sex/ group	0, 100, 300, 1000, 3000 mg/kg/day **12 months** **NOAEL: 1000 mg/kg (HED = 161 mg/kg; Exp 2X)**	• Oily substance from anus 3000 mg/kg • ↓ BW gain at 3000 mg/kg • Retinal hemorrhage observed at 3000 mg/kg (♂) at week 26 only • ↑ ALP at ≥ 1000 mg/kg (♂),↑ ALT 3000 mg/kg (♀) • ↓ Hb and HT, ↑PMN and WBCs at 3000 mg/kg • Urine: At 3000 mg/kg ↓volume and electrolytes (♂); ↓ calcium (♀) • Lung: bronchopneumonia (dose dependent- possibly related to inhalation of oil) • Heart: ↑ relative weight (♂) at 3000 mg/kg with increased severity of fibrosis and scarring • Liver: (♂) ↑ severity of bile duct hyperplasia at all doses; (♀) ↑ relative weight at 3000 mg/kg • Kidney: (♂) ↑ relative weight at 3000 mg/kg and ↑cell infiltration (all doses) • Uterus: at ≥ 1000 mg/kg uterine cyst and endometrial stromal polyp
Amarin Sponsored Studies			

#ZOC0001 GLP? – YES	Wistar Rat 10/ sex/ group	0 (mineral oil), 300, 1000, 2000 mg/kg/day **4 Weeks** **NOAEL: 2000 mg/kg (HED = 323 mg/kg; Exp 5X)**	• Excessive salivation, hair loss (2000 mg/kg) • ↓ eosinophils ~40% at 2000 mg/kg (♀/♂) • ↓ Cholesterol, TG, HDL • Mean PT slight increase ~10% at 2000 mg/kg
#522093 GLP? -YES	SD Rat 10/sex/ group (3/sex/ group for TK)	0 (water), 1000 or 2000 mg/kg AMR101; 2000 mg/kg Epadel **4 Weeks** **NOAEL: 2000 mg/kg (HED = 323 mg/kg; Exp 5X) for both AMR101 and Epadel**	• Scabs, fur staining, sparse hair with hyperkeratosis of skin at 2000 mg/kg • ↑ RBC, ↓ RDW and reticulocytes (♂) at 2000 mg/kg • ↓ cholesterol, TG ≥ 1000 mg/kg • Mild ↑ ALP at 2000 mg/kg
#515147 GLP? - NO	Beagle Dog (non-naïve) 2/sex/group	0 (mineral oil), 2000 mg/kg/day **14 Days**	• Skin reddening/ flaky skin on abdomen and groin (♀/♂) • ↑ PMN and WBC (♀/♂) • ↑ spleen weight (♀/♂) • ↓ cholesterol, TG, LDL/HDL • ↑ ALP (♀/♂) and ALT (♀)
#515194 GLP? - YES	Beagle Dog 4/sex/group for 39 weeks; 3/sex/group for 13 weeks	0 (2000 mg/kg mineral oil), 300, 1000, 2000 mg/kg/day **39 Weeks** (with a 13 week interim kill)	**<u>13 Weeks:</u>** • 2000 mg/kg: premature sacrifice of one male with intussusception of the ileocecocolic junction • ≥ 1000 mg/kg: red, flaky dry skin with hair loss in the anogenital region • ≥ 1000 mg/kg : ↑ WBC and PMN (possibly skin related) • ≥ 1000 mg/kg: ↑ ALP 3-9X • Adrenal: ≥ 300 mg/kg vacuolar

		NOAEL: 300 mg/kg (HED = 167 mg/kg; Exp 2.5X)	degeneration of inner cortex • Heart: ≥ 1000 mg/kg papillary mineralization • Skin: ≥ 300 mg/kg epidermal hyperplasia • Lung: ≥ 1000 mg/kg (♀) alveolar foamy macrophages **<u>39 Weeks:</u>** • One male in the 300 mg/kg dose group was sacrificed early on Day 154 due to deterioration in general condition. It was found that he had an infarcted jejunum followed by a blockage between the small and large intestines. • ≥ 1000 mg/kg: red, flaky dry skin with hair loss in the anogenital and abdominal region; corresponding histopath showed increased severity of epidermal hyperplasia • (♀): slight decrease in Hb and Hct at 2000 mg/kg • ↑ALP at 2000 mg/kg (♀/♂) • ↓Cortisol at 2000 mg/kg (♂) and ≥ 1000 mg/kg (♀) • Urine: intermittent increases in urine protein at 1000 and 2000 mg/kg (♀/♂) • Spleen: ↑ absolute and relative weight (♀/♂) at 1000 and 2000 mg/kg • Heart: ↑ absolute weight (♀) at 1000 and 2000 mg/kg; histopath (♀/♂) showed valvular endocarditis and inflammatory cells at 1000 and 2000 mg/kg (not dose dependent) • Pituitary: ↑ incidence of anterior lobe cysts at 2000 mg/kg • Adrenal: Increased severity of vacuolar degeneration of inner cortex at 1000 and 2000 mg/kg (♀/♂)

HED = Human Equivalent Dose
Exp = Exposure margin based on body surface area using a 60 kg human and a clinical dose of 4.0 g/day ethyl-EPA (icosapent ethyl)

Study title: A 4 Week Study of AMR101 and Epadel by Oral Gavage Administration in Rats

Study no.:	522093
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	January 26, 2012
GLP compliance:	Yes, page 7
QA statement:	Yes, page 8
Drug, lot #, and % purity:	AMR101 (icosapent ethyl; ethyl-EPA) from Nisshin Pharma Inc.: Lot # 264818A 1/3, 97.9% purity Epadel (ethyl EPA) from Mochida Pharmaceutical (gelatin capsules with 300 mg ethyl-EPA): Lot # 073, 96.1% purity

Key Study Findings

- The purpose of this study was to provide a bridge from AMR101 (ethyl-EPA, icosapent ethyl) to the Japanese marketed ethyl-EPA product, Epadel. Toxicity and TK was compared between AMR101 and Epadel after 4 weeks oral administration to SD rats.
- There was one unscheduled death of a 2000 mg/kg AMR101 treated female on Day 24; however there was evidence that this death was due to a gavage-related injury.
- Clinical signs for both AMR101 and Epadel included scabs, fur staining and sparse hair. Skin discoloration at necropsy was noted at low incidence at 2000 mg/kg AMR101 or Epadel.
- Females treated with 2000 mg/kg AMR101 or Epadel had an increase in BW gain over control that reached statistical significance with AMR101.
- In males administered 2000 mg/kg AMR101 or Epadel, there were minor increases in RBC count and minor decreases in RDW and reticulocytes. There were individual animals in both AMR101 and Epadel groups that had elevated LDH levels (above that observed in control animals) and may be indicative of hemolysis. One additional finding was that two females (one administered 2000 mg/kg AMR101 and one administered 2000 mg/kg Epadel) had increased urine urobilinogen as compared to the control range for this urinary parameter, and also may indicate the potential for hemolysis.
- Cholesterol and triglyceride levels decreased in both AMR101 and Epadel treated groups from control, and were an expected pharmacologic effect.
- ALP had only a minor increase in 2000 mg/kg AMR101 or Epadel treated groups, but is noted as this change in a clinical chemistry parameter was observed in several other repeat dose toxicology studies with ethyl-EPA. “Prominent lobular

architecture" of the liver was noted upon necropsy in all AMR101 and Epadel treated groups (both sexes) but was most prevalent at a 2000 mg/kg dose. No corresponding histopathology or organ weight change was reported.

- There was a low incidence of histopathology findings without dose dependence or difference between AMR101 or Epadel treatment. This included cardiomyopathy in 1000 mg/kg AMR101 treated males, lung infiltration and histiocytosis, and skin hyperkeratosis.
- Pharmacokinetic assessment between 2000 mg/kg AMR101 and 2000 mg/kg Epadel was completed. Total EPA as well as un-esterified (free) EPA was analyzed from rat plasma. **Total EPA:** Initial exposure on Day 1 in males was 1.6 – 2.1X greater for Epadel than AMR101. By Day 28 there was evidence of some accumulation for both substances and exposures were roughly equivalent between AMR101 and Epadel for both sexes. One difference of note was that females had a 2.2X higher C_{max} when administered Epadel by Day 28. T_{max} at Day 28 for all groups and both sexes was approximately 2 hours. **Un-esterified EPA:** The un-esterified fraction was only 3-5% of total EPA following repeat dosing. Similar to total EPA, the un-esterified EPA fraction also showed some accumulation in all dose groups and both sexes. Systemic exposure again was roughly equivalent between AMR101 and Epadel in both sexes, with a slightly higher AUC in Epadel treated females. T_{max} was approximately at 2 hours. In sum, while there were minor differences in C_{max} values between AMR101 and Epadel in females and between male and females treated with AMR101 in general, the overall exposure profile between an equivalent dose of Epadel and AMR101 are relatively comparable. There is normal variability in total EPA incorporation into phospholipids, triglycerides and cholesteryl esters as well as differences observed in exposures of EPA in AMR101 treated animals at identical dose in other toxicology studies (*i.e.* ZOC0001). The exposure differences observed between Epadel and AMR101 are not likely toxicologically meaningful as no differences in toxicity were observed under the design of this study.

Methods

Doses:

Group No.	No. of Animals				Test Item	Dose Level (mg/kg/day)	Dose Volume (mL/kg)
	Main Study Animals		Toxicokinetics Animals				
	Male	Female	Male	Female			
1	10	10	3	3	Water	0	2.20
2	10	10	3	3	AMR101	1000	1.10
3	10	10	3	3	AMR101	2000	2.20
4	10	10	3	3	Epadel	2000	2.20

[Reproduced from Sponsor's Study No. 522093]

Frequency of dosing: Once daily for 28 or 29 days
Route of administration: Oral gavage
Dose volume: 1.10 mL/kg for 1000 mg/kg AMR101; 2.20 mL/kg for all other dose groups
Formulation/Vehicle: Neat test-article, no vehicle
Species/Strain: Crl:CD(SD) Sprague-Dawley Rat
Number/Sex/Group: 10/sex/group
Age: 8-9 weeks at commencement of dosing
Weight: Males: 300 - 405 g
Females: 208 – 270 g
Diet: PMI Nutrition International Certified Rodent Diet No. 5CR4 (14% protein)
Satellite groups: 3/sex/group for TK analysis
Unique study design: None
Deviation from study protocol: On Day 18, animal 75F was observed to reflux some Epadel immediately after dosing and did not receive the full dose on this day.

Observations and Results

Mortality

Animals were checked for mortality in the early morning and as late as possible on each study day. One female animal was found dead from the 2000 mg/kg AMR101 treated group (No. 66F) on Day 24 and was attributed to a gavage related injury. Gross pathology correlates included a rupture of the esophagus, white accumulation on the heart, red discoloration of lungs and an enlarged bronchial lymph node.

Clinical Signs

Detailed clinical observations were recorded once each week beginning in pretrial period. Examination included appearance, movement, behavior, skin and hair condition, eyes and mucous membranes, respiration and excreta. Additionally, treatment related clinical observations were recorded from pre-dose through 1 hour post-dose (for days 1-2 only, observations were made through 4 hours post-dose).

Fur staining on the dorsal neck was observed in animals treated with AMR101 or Epadel; with slightly higher prevalence in AMR101 treated females. Scabbing and sparse hair was also noted in a couple of animals in both AMR101 and Epadel treated animals. One female (No. 75F) in the 2000 mg/kg Epadel dose group on Day 17 presented with ploughing (lowering of head to below surface of cage shavings and walking forward) and excess salivation immediately after dosing.

Table 12: Clinical Observations between AMR101 and Epadel Treated Rats

	Male				Female			
	0 mg/kg (water)	1000 mg/kg AMR101	2000 mg/kg AMR101	2000 mg/kg **Epadel**	0 mg/kg (water)	1000 mg/kg AMR101	2000 mg/kg AMR101	2000 mg/kg **Epadel**
Scab(s)	-	2	-	2	-	-	-	-
Fur stain (neck)	-	-	-	1	-	2	3	1
Sparse hair	-	-	-	1	-	1	-	1
Ploughing	-	-	-	-	-	-	-	1
Excess salivation	-	-	-	-	-	-	-	1

Body Weights

During the treatment period, body weights were recorded daily. Females receiving 2000 mg/kg/day AMR101 had an increase in body weight gain over controls, which reached statistical significance, and males had a slightly higher increase in body weight gain but this was not statistically significant. These changes were not thought to be toxicologically relevant.

Table 13: Mean Body Weight Gain Comparing AMR101 and Epadel Treated Rats

Weight (g)	Male				Female			
	0 mg/kg (water)	1000 mg/kg AMR101	2000 mg/kg AMR101	2000 mg/kg **Epadel**	0 mg/kg (water)	1000 mg/kg AMR101	2000 mg/kg AMR101	2000 mg/kg **Epadel**
Ending Weight (Day 28)	480	473	489	461	293	282	294	290
Weight Change 0-28 days	123	117	136	119	45	42	62*	55

P<0.01 as compared to control

Feed Consumption

Food consumption was recorded twice weekly beginning at one week prior to dosing commencement. Water consumption was monitored weekly by visual inspection of water bottles.

There were no significant changes in food consumption between control, AMR101 or Epadel treated groups in either sex. No observable changes in water consumption were noted.

Table 14: Mean Food Consumption (g/animal/day) Between AMR101 and Epadel Treated Rats

	Male				Female			
	0 mg/kg (water)	1000 mg/kg AMR101	2000 mg/kg AMR101	2000 mg/kg **Epadel**	0 mg/kg (water)	1000 mg/kg AMR101	2000 mg/kg AMR101	2000 mg/kg **Epadel**
Day 0	26.0	26.7	26.6	25.5	18.9	18.3	17.0	18.5
Day 14	27.9	27.9	29.4	26.7	20.3	20.0	20.6	20.1
Day 28	27.2	28.1	27.1	24.7	17.9	20.8	17.7	18.8

Ophthalmoscopy

An ophthalmoscopic examination was conducted at pre-trial and also at week 4 during the treatment period. Anterior, lenticular and fundic areas were evaluated by an indirect ophthalmoscope.

There were no dose dependent changes in ophthalmoscopy findings that were attributed to either AMR101 or Epadel. Most frequently, lens opacities were observed but were usually detected at both pretrial as well as at week 4.

ECG

Not performed.

Hematology

Blood samples were taken via capillary tube from the orbital sinus under isoflurane anesthesia on the morning of necropsy (week 5). Parameters measured included red blood cell count, hemoglobin concentration, hematocrit, mean cell volume, mean cell hemoglobin concentration, mean cell hemoglobin, reticulocytes, reticulocyte count (absolute), red blood cell distribution width, blood smear (prepared but not evaluated), white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells, platelets, activated partial thromboplastin time, fibrinogen and prothrombin time.

Statistically significant changes in hematology were limited to male rats and included mild decreases in RDW and percent reticulocytes in both 2000 mg/kg AMR101 and 2000 mg/kg Epadel. Administration of 2000 mg/kg Epadel additionally had statistically

significant increases in RBC counts and reticulocyte counts with similar but non-statistically significant changes in the 2000 mg/kg AMR101 dose group.

Table 15: Mean Hematology Parameter Values Following 28 Days Ethyl-EPA Dosing

	Male				Female			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
n =	**9**	**10**	**10**	**8**	**10**	**8**	**9**	**9**
RBC (10^{12}/L)	8.05	8.17	8.30	**8.54[b]**	7.88	7.81	7.76	7.70
RDW (%)	13.0	12.7	**12.1[b]**	**12.2[a]**	11.4	11.2	11.2	11.2
Reti (%)	2.7	2.8	**2.4[a]**	**2.2[b]**	2.3	2.1	2.2	2.3
Ret (10^9/L)	219	225	196	**184[a]**	185	162	167	179

Group 1 = water control
Group 2 = 1000 mg/kg AMR101
Group 3 = 2000 mg/kg AMR101
Group 4 = 2000 mg/kg Epadel
(a): $p<0.05$ vs. Group 1 Control
(b): $p<0.01$ vs. Group 1 Control

Clinical Chemistry

Blood samples were taken via capillary tube from the orbital sinus under isoflurane anesthesia on the morning of necropsy (week 5). Parameters measured included urea, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, sodium, potassium, chloride, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, creatinine, total bilirubin, calcium, inorganic phosphate, and triglycerides.

It was noted that increases in lactate dehydrogenase (LDH) were observed in individual animals as follows, that were outside the range observed in control animals (116 – 226 U/L in males and 108 – 230 U/L in females:
No. 17M (1000 mg/kg AMR101): 507 U/L
No. 19M (1000 mg/kg AMR101): 302 U/L
No. 20M (1000 mg/kg AMR101): 318 U/L
No. 36M (2000 mg/kg Epadel): 378 U/L
No. 59F (1000 mg/kg AMR101): 274 U/L
No. 79F (2000 mg/kg Epadel): 344 U/L

The individual animal increases in LDH in both AMR101 and Epadel treated groups may be indicative of hemolysis and anemia as red blood cells are abundant in LDH. Decreases in cholesterol and triglycerides were observed in both AMR101 and Epadel treated rats and is a pharmacologic effect of ethyl-EPA.

Table 16: Mean Clinical Chemistry Values Following 28 Days Ethyl-EPA Dosing

	Male				Female			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
n =	**9**	**10**	**9**	**10**	**10**	**10**	**9**	**10**
ALP (U/L)	178	197	210	202	109	110	**137[a]**	128
Chol (mmol/L)	2.1	**1.7[b]**	**1.5[c]**	**1.5[c]**	2.3	**1.9[b]**	**1.6[c]**	**1.7[c]**
Trig (mmol/L)	2.48	**1.26[c]**	**1.41[c]**	**1.38[c]**	1.41	1.48	1.24	1.05
Alb (g/L)	40	**42[a]**	**42[a]**	41	47	48	48	48
LDH (U/L)	176	231	166	227	162	208	192	201

Group 1 = water control
Group 2 = 1000 mg/kg AMR101
Group 3 = 2000 mg/kg AMR101
Group 4 = 2000 mg/kg Epadel
(a): $p<0.05$ vs. Group 1 Control
(b): $p<0.01$ vs. Group 1 Control
(c): $p<0.001$ vs. Group 1 Control

Urinalysis

Urine was collected individually from animals in metabolism cages over a four hour period during week 4 of study treatment. Urinalysis parameters assayed included microscopic evaluation of spun deposit, color, turbidity, specific gravity, volume, pH, protein, glucose, bilirubin, ketones, leukocytes, blood pigments, and urobilinogen.

It was noted that urobilinogen was increased in female No. 52 (1000 mg/kg AMR101) to 4.0 mg/dL (control values were 0.2 – 1.0 mg/dL) along with increased ketones (20 mg/dL). Female 74 (2000 mg/kg Epadel) also had an elevation in urobilinogen of 3.0 mg/dL. These elevations in urobilinogen could indicate hemolysis and anemia and may correspond with increased LDH levels observed in clinical chemistry results, but were not from the same rats. There were also sporadic elevations in urinary leukocytes in all AMR101 and Epadel groups in females and in 1000 mg/kg AMR101 and 2000 mg/kg Epadel groups in males.

Gross Pathology

All main study animals were necropsied on Day 29 of the study which included a full examination of the musculoskeletal system, external surfaces and orifices, cranial cavity and external surfaces of brain, thoracic, abdominal and pelvic cavities.

There was an increased incidence of abnormal liver (prominent lobular architecture, all lobes) in both AMR101 groups as well as in the 2000 mg/kg Epadel group. Skin discolorations were noted in both 2000 mg/kg AMR101 and Epadel groups.

Table 17: Necropsy Findings Following 28 Days Ethyl-EPA Dosing

	Male				Female			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
n =	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Liver, Prominent lobular architecture	0	1	5	4	0	1	2	3
Lung, Foci, Dark	1	5	0	3	1	2	2	3
Skin/Subcutis, discoloration	0	0	0	1	0	0	2	1

Group 1 = water control
Group 2 = 1000 mg/kg AMR101
Group 3 = 2000 mg/kg AMR101
Group 4 = 2000 mg/kg Epadel

Organ Weights

The following organs were weighed at necropsy with the exception of animal No. 66F (found dead): brain, epididymis (paired weight), adrenal gland (paired weight), pituitary gland, prostate, thyroid, heart, kidney (paired weight), liver, lung, ovary (paired weight), spleen, testis (paired weight), thymus (paired lobe weight), and uterus.

There was a mild increase in the weight of the thymus in males and females in both 2000 mg/kg dose groups of AMR101 and Epadel; however neither group reached statistical significance from control.

Table 18: Mean Organ weights Following 28 Days Ethyl-EPA Dosing

	Male				Female			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
n =	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**10**
BW (g)	**471**	**477**	**484**	**456**	**289**	**289**	**290**	**289**
Thymus (g)	0.381	0.390	0.419	0.417	0.364	0.405	0.460	0.391
% BW	0.0806	0.0828	0.0858	0.0916	0.1257	0.1403	0.1601	0.1350

Group 1 = water control
Group 2 = 1000 mg/kg AMR101
Group 3 = 2000 mg/kg AMR101
Group 4 = 2000 mg/kg Epadel

Histopathology

Adequate Battery: Yes

Tissues were collected from main study animals and preserved in 10% neutral buffered formalin, except for the optic nerve, eye, harderian gland (Davidson's fixative) and the testis (Modified Davidson's fixative). Bone marrow smears were prepared and stained but not evaluated. The following tissues were examined:

Table 19: Histopathology Tissues Examined in the Rat

Animal identification[a]	Liver
Artery, aorta	Lung
Bone marrow, femur	Lymph node, mandibular
Bone marrow, sternum	Lymph node, mesenteric
Bone, femur	Muscle, skeletal
Bone, sternum	Nasal cavity[a]
Brain	Nerve, optic[b]
Cervix	Nerve, sciatic
Epididymis	Oesophagus
Eye[b]	Ovary
Gland, adrenal	Oviduct
Gland, harderian[b]	Pancreas
Gland, lacrimal	Skin
Gland, mammary	Small intestine, duodenum
Gland, parathyroid	Small intestine, ileum
Gland, pituitary	Small intestine, jejunum
Gland, prostate	Spinal cord
Gland, salivary	Spleen
Gland, seminal vesicle	Stomach
Gland, thyroid	Testis[c]
Gross lesions/masses	Thymus
Gut-associated lymphoid tissue	Tongue
Heart	Trachea
Kidney	Ureter
Large intestine, caecum	Urinary bladder
Large intestine, colon	Uterus
Large intestine, rectum	Vagina

[a] Not examined.
[b] Preserved in Davidson's fixative.
[c] Preserved in Modified Davidson's fixative.

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Peer Review:Yes, with at least 20% of the animals conducted by (b) (4) [redacted]

Histological Findings:

In general, there was a low incidence of histopathology findings in rats treated with AMR101 or Epadel for 28 days. There was a slight increase in lymphocytic infiltration of the Harderian gland in Epadel treated rats, although there is no human clinical correlate to this finding. Three males and one female administered 1000 mg/kg AMR101 (but not at higher dose) had focal cardiomyopathy; however since this was not present at 2000 mg/kg the finding is equivocal. Hyperkeratosis of the skin (focal or multifocal) was observed at all doses of AMR101 or Epadel. There were other sporadic histopathology findings in one or both sexes, but were not dose dependent and no clear differences in AMR101 versus Epadel histopathology were observed in this study.

Table 20: Histopathology Findings Following 28 Days Ethyl-EPA Dosing

	Male				Female			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
n =	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**10**
Eye								
dysplasia, retinal, unilat, focal	0	0	0	0	0	0	1	0
dysplasia, retinal, unilat, multifo.	0	0	0	1	0	0	0	0
Harderian gland								
infiltration, lymphocytic focal	0	0	0	1	0	0	1	2
infiltration, lymphocytic multifocal	0	1	0	1	0	0	0	2
Heart								
inflamm. mixed, epicardium	0	0	0	0	0	0	1	1
cardiomyopathy, focal	0	3	0	0	0	1	0	0
cardiomyopathy, multifocal	1	0	0	0	0	0	0	0
Kidney								
cyst, multifocal	0	0	2	0	0	0	0	0
Lung								
infiltration, focal	0	2	1	0	0	1	2	0
infiltration, multifocal	1	2	1	0	0	0	0	1
histiocytosis, alveolar, focal	0	0	0	0	0	1	0	2
histiocytosis, alveolar, multifocal	0	1	1	1	0	2	0	1
Lymph Node, Mesenteric								
hemorrhage	0	0	1	0	1	0	0	0
Nerve, Sciatic								
degeneration, axonal	0	0	1	1	0	0	0	0
Skin								
hyperkeratosis, focal	0	1	1	0	1	2	2	0
hyperkeratosis, multifocal	0	1	1	0	0	0	1	2

Group 1 = water control
Group 2 = 1000 mg/kg AMR101
Group 3 = 2000 mg/kg AMR101
Group 4 = 2000 mg/kg Epadel

For female 66F (2000 mg/kg AMR101, found dead on Day 24), pathology and histopathology findings included: ruptured esophagus near lung with inflammation; heart epicardium inflammation; aorta inflammation of the fat; lung discoloration with congestion, fibrosis and inflammation; bronchial lymph node enlargement with hemorrhage and fibrosis; spleen and lymphoid depletion. These findings are consistent with esophageal rupture due to gavage error.

Special Evaluation

None performed.

Toxicokinetics

Blood samples were collected at 0 (pre-dosing), 0.5, 1, 2, 4, 8 and 24 hours post-dose on study days 1 and 28 from the tail vein. TK was determined for both total EPA (including un-esterified EPA and EPA liberated from phospholipids, triglycerides and cholesteryl esters) and un-esterified EPA alone.

The results of TK analysis were as follows:

- Control animals had a low level of EPA as this is an endogenous substance.
- On Day 1, there was a less than dose proportional increase in systemic exposure of both total EPA and un-esterified EPA between 1000 and 2000 mg/kg/day of AMR101, indicating that in the rat, absorption may be limited above 1000 mg/kg/day.
- Total EPA on Day 1 for Epadel was 1.6 – 2.1 fold greater in males than observed with AMR101.
- On Day 28 of oral administration, total EPA exposure was similar to Day 1 exposures in both AMR101 and Epadel treated groups; however there was some accumulation of total EPA in females administered 1000 mg/kg AMR101. In general, C_{max} was 1.5 – 1.9 fold higher on Day 28 for AMR101 (except for 2000 mg/kg AMR101 females) and increased 2.4 fold in females administered 2000 mg/kg Epadel (slight decrease in males).
- Between Days 1 and 28, un-esterified EPA in all dose groups showed some accumulation in both sexes.
- Comparing AMR101 to Epadel on Day 28, systemic exposure of Total EPA was roughly equivalent in both males and females; however C_{max} was 2.2 fold greater in Epadel treated females versus AMR101 (2000 mg/kg).
- Comparing AMR101 to Epadel on Day 28, systemic exposure of Unesterified-EPA was roughly comparable in both sexes with slightly higher AUC in females administered 2000 mg/kg Epadel.
- Following 28 days of Dosing, C_{max} of total and un-esterified EPA in males was generally higher than females in AMR101 groups (range 1.5 – 2.6 fold).
- T_{max} on Day 28 was approximately at 2 hours post-dose for total EPA and un-esterifed EPA for all AMR101 and Epadel dose groups. $T_{1/2}$ was roughly equivalent for all dose groups on Day 28 with 10.25 hours for 1000 mg/kg AMR101, 9.21 hr for 2000 mg/kg AMR101 and an average of 8.86 hr (male and female average) for 2000 mg/kg Epadel. It is noted however that in many profiles the terminal elimination phase could not be calculated due to insufficient data or continuing increases in total or un-esterified EPA concentrations.
- Un-esterified EPA was a small fraction (3-5% on average) of total EPA levels in rat plasma following repeat dosing in all groups.

Table 21: Mean Values of Total EPA in Rat Plasma (Day 28)

	AUC_{0-T} (µg•hr/mL)		**C_{max} (µg/mL)**	
	Male	**Female**	**Male**	**Female**
AMR101 (2000 mg/kg)	6026	4598	988	384
Epadel (2000 mg/kg)	5513	5509	799	857

Table 22: Mean Values of **Un-Esterified** EPA in Rat Plasma (Day 28)

	AUC_{0-T} (µg•hr/mL)		C_{max} (µg/mL)	
	Male	**Female**	**Male**	**Female**
AMR101 (2000 mg/kg)	265.5	201.6	28.4	19.4
Epadel (2000 mg/kg)	290.5	296.5	21.4	25.4

Table 23: Mean T_{max} Values (hours) on Day 28

	Total EPA		Unesterified EPA	
	Male	**Female**	**Male**	**Female**
AMR101 (2000 mg/kg)	2.0	2.67	2.0	1.17
Epadel (2000 mg/kg)	2.0	2.0	2.67	2.67

The mean $T_{1/2}$ was only reported for Total EPA and with the exception of Epadel, only for males. The $T_{1/2}$ of 2000 mg/kg AMR101 was 9.21 hours (male) and for 2000 mg/kg Epadel was 8.86 hours (average of male and female).

In summary, there were small differences in total and un-esterified EPA exposure (AUC and C_{max}) between AMR101 and Epadel but was usually limited to one sex. Males administered AMR101 had slightly higher C_{max} and AUC concentrations of total and un-esterified EPA than females; which were not observed between sexes with Epadel administration. In summary, the exposure profile including AUC, C_{max} and T_{max} between 2000 mg/kg AMR101 and Epadel were generally comparable; however exposures were slightly higher in females administered 2000 mg/kg Epadel versus 2000 mg/kg AMR101 following 28 days of dosing.

A method for determining total EPA in rat plasma by liquid chromatography-tandem mass spectrometry was validated under Study No. 314194 and un-esterified EPA methodology was validated under Study No. 309365.

Table 24: Mean Values of Toxicokinetic Parameters of Total EPA in Rat Plasma

Sex	Day	Dose Level (g/kg/day)		Cmax (µg/mL)	Cmax/D (µg/mL)/ (mg/kg/day)	Tmax (h)	AUC(0-t) (µg.h/mL)	AUC(0-t)/D (µg.h/mL)/ (mg/kg/day)
Male	1	AMR101 (1 g)	Mean	498	0.498	2.00	3850	3.850
			CV%*	63.4	63.4	0	29.2	29.2
		AMR101 (2 g)	Mean	607	0.303	2.00	4717	2.358
			CV%*	43.8	43.8	0.0	42.3	42.3
		Epadel (2 g)	Mean	1300	0.649	2.00	7617	3.809
			CV%*	48.8	48.8	0.0	24.9	24.9
Female		AMR101 (1 g)	Mean	237	0.237	1.59	2590	2.590
			CV%*	48.1	48.1	41.7	19.6	19.6
		AMR101 (2 g)	Mean	491	0.245	2.00	3442	1.721
			CV%*	45.0	45.0	0.0	29.3	29.3
		Epadel (2 g)	Mean	355	0.177	2.00	4277	2.139
			CV%*	41.0	41.0	0.0	47.4	47.4
Male	28	AMR101 (1 g)	Mean	768	0.768	2.00	4149	4.149
			CV%*	52.0	52.0	0.0	31.4	31.4
		AMR101 (2 g)	Mean	988	0.494	2.00	6026	3.013
			CV%*	89.4	89.4	0.0	58.6	58.6
		Epadel (2 g)	Mean	799	0.399	2.00	5513	2.756
			CV%*	63.7	63.7	0.0	44.3	44.3
Female		AMR101 (1 g)	Mean	457	0.457	1.59	4519	4.519
			CV%*	58.3	58.3	41.7	52.3	52.3
		AMR101 (2 g)	Mean	384	0.192	2.52	4598	2.299
			CV%*	36.0	36.0	41.7	6.7	6.7
		Epadel (2 g)	Mean	857	0.429	2.00	5509	2.754
			CV%*	13.5	13.5	0.0	9.4	9.4

*Calculated from the Geometric mean

Table 25: Mean Values of Toxicokinetic Parameters of **Un-Esterified** EPA in Rat Plasma

Sex	Day	Dose Level (g/kg/day)		Cmax (µg/mL)	Cmax/D (µg/mL)/ (mg/kg/day)	Tmax (h)	AUC(0-t) (µg.h/mL)	AUC(0-t)/D (µg.h/mL)/ (mg/kg/day)
Male	1	AMR101 (1 g)	Mean	5.87	0.00587	2.00	56.59	0.05659
			CV%*	30.4	30.4	0.0	21.8	21.8
		AMR101 (2 g)	Mean	10.8	0.00541	2.52	64.03	0.03201
			CV%*	13.2	13.2	41.7	37.1	37.1
		Epadel (2 g)	Mean	8.95	0.00447	2.52	87.66	0.04383
			CV%*	7.6	7.6	41.7	9.0	9.0
Female		AMR101 (1 g)	Mean	5.02	0.00502	2.00	39.17	0.03917
			CV%*	24.7	24.7	0.0	6.8	6.8
		AMR101 (2 g)	Mean	9.18	0.00459	2.00	70.78	0.03539
			CV%*	25.5	25.5	0.0	47.3	47.3
		Epadel (2 g)	Mean	7.31	0.00365	5.77	70.94	0.03547
			CV%*	55.9	55.9	204.4	70.8	70.8
Male	28	AMR101 (1 g)	Mean	25.0	0.0250	2.00	212.8	0.2128
			CV%*	53.6	53.6	0.0	37.3	37.3
		AMR101 (2 g)	Mean	28.4	0.0142	2.00	265.5	0.1327
			CV%*	41.6	41.6	0.0	62.9	62.9
		Epadel (2 g)	Mean	21.4	0.0107	2.52	290.5	0.1452
			CV%*	19.8	19.8	41.7	5.2	5.2
Female		AMR101 (1 g)	Mean	12.7	0.0127	2.00	163.9	0.1639
			CV%*	38.0	38.0	0.0	19.0	19.0
		AMR101 (2 g)	Mean	19.4	0.00971	1.00	201.6	0.1008
			CV%*	10.3	10.3	78.5	8.0	8.0
		Epadel (2 g)	Mean	25.4	0.0127	2.52	296.5	0.1482
			CV%*	25.1	25.1	41.7	16.3	16.3

*Calculated from the Geometric mean

[Tables reproduced from Applicant's NDA submission; Study report 522093]

Dosing Solution Analysis

The stability of AMR101 and extracted Epadel were confirmed. No formulations were prepared for this study as Epadel (ethyl-EPA oil) was extracted from capsules and AMR101 was also administered neat.

7 Genetic Toxicology

Vascepa (AMR101) was tested in an Ames mutagenesis assay, in two chromosomal aberration assays in Chinese Hamster Ovary (CHO) cells (plus one clastogenicity assay with EPA), and in an *in vivo* mouse micronucleus study. Results of the Ames test and the *in vivo* micronucleus assay were negative; however ethyl-EPA was determined to be positive for clastogenicity. In multiple assays, both ethyl-EPA and EPA (with or without toxicity) were positive for clastogenicity in the presence and absence of S9 metabolic activation. As reference compounds, linoleic (omega-6) and oleic (omega-9) acids were also tested and gave variable results for both clastogenicity (±S9) and toxicity. The following table is a summary of genetic toxicology studies submitted under IND 102,457:

Table 26: Genetic Toxicology Studies Conducted with Ethyl-EPA

Study	Study Design	Compound Tested
Ames	One dose was tested (25 µl/plate = 2.275 µg/plate = 1:10 dilution of test article into ethanol) based on cytotoxicity, plate incorporation ± S9	Ethyl-EPA + 0.2% DL-alpha tocopherol
Chromosomal Aberration	0.5, 1, 2, 5 µl/mL CHO cells ± S9	Ethyl-EPA + 0.2% DL-alpha tocopherol
Chromosomal Aberration	+S9: 20, 25, 30, 35, 40 $\mu g.mL^{-1}$ -S9: 20, 40, 75, 100, 150 $\mu g.mL^{-1}$ CHO cells	Ethyl-EPA + 0.2% DL-alpha tocopherol
Chromosomal Aberration	+S9: 10, 20, 25, 30, 35, 40 $\mu g.mL^{-1}$ -S9: 50, 60, 70, 80, 90, 100 $\mu g.mL^{-1}$ CHO cells	EPA
Toxicity Test	+S9: 20 – 5000 $\mu g.mL^{-1}$ -S9: 20 – 5000 $\mu g.mL^{-1}$ CHO cells	Ethyl-EPA
Toxicity Test	+S9: 0.16 – 40 $\mu g.mL^{-1}$ -S9: 0.63 – 150 $\mu g.mL^{-1}$ CHO cells	EPA
In Vivo Mouse Bone Marrow Micronucleus	*i.p.* injection of 50 mL/kg	Ethyl-EPA + 0.2% DL-alpha tocopherol

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

The Ames assay was negative in the presence and absence of S9 metabolic activation when used at a maximum concentration of 25 µL/plate (neat oil at a density of 0.910 mg/µL) in the bacterial strains TA98, TA100, TA1535, TA1537 and WP2. Although the assay was considered negative, it was noted that in one tester strain TA100, there was a non-statistically significant increase (1.2 fold) in revertant colonies in the presence of metabolic activation that was reproducible in the confirmatory assay. As this did not meet the positive criteria of at least a 2-fold increase for this bacterial strain, Ethyl-EPA was considered to be non-mutagenic in the Ames assay.

7.2 *In Vitro* Assays in Mammalian Cells

Several clastogenicity studies were completed as ethyl-EPA induced chromosome aberrations in CHO cells, and were therefore considered clastogenic. In the first study, four concentrations were used (0.5, 1, 2, and 5 µl/mL) in the presence and absence of S9 metabolic activation. There was an apparent dose dependent increase in the number of chromosome aberrations (-S9) up to 2 µl/mL, where 5 µl/mL resulted in significant cellular toxicity and an insufficient number of analyzable cells. In the presence of S9 (+S9) there was an increase in the number of cells with aberrations at a dose of ≥ 1 µl/mL. There was an apparent dose-dependent increase in the number of chromosome aberrations ± S9 and therefore ethyl-EPA was considered clastogenic under the conditions of this study.

In the second ethyl-EPA clastogenicity assay in CHO cells ethyl-EPA, ethyl-oleate (omega-9 fatty acid) and ethyl linoleate (omega-6 fatty acid) were tested. The top dose of ethyl-EPA (+S9) was 40 $\mu g.ml^{-1}$ and (-S9) was 150 $\mu g.ml^{-1}$. Ethyl-EPA was again considered clastogenic -S9 at 150 $\mu g.ml^{-1}$ and there were indications of clastogenicity (including and excluding gaps) +S9 at 25-30 $\mu g.ml^{-1}$ (30-40 $\mu g.ml^{-1}$ was toxic in cell culture). The omega-9 and omega-6 fatty acids, ethyl-oleate and ethyl-linoleate, respectively, were not considered clastogenic ±S9 metabolic activation.

As ethyl-EPA is rapidly converted to EPA in all species, this omega-3 fatty acid was tested along with oleic (omega-9) and linoleic acids (omega-6) for clastogenicity. In this third assay in CHO cells, the highest doses of EPA were 40 $\mu g.ml^{-1}$ (+S9) and 100 $\mu g.ml^{-1}$ (-S9). EPA was clastogenic in the presence and absence of S9 and was also considered toxic to cells. EPA was positive at 25 $\mu g.ml^{-1}$ (+S9) and 80 $\mu g.ml^{-1}$ (-S9). Oleic acid was toxic and clastogenic at 20 $\mu g.ml^{-1}$ (+S9); however was negative (-S9) up to a top concentration of 70 $\mu g.ml^{-1}$. Linoleic acid was toxic and clastogenic at a concentration of 20 $\mu g.ml^{-1}$ (+S9) and had mixed findings (one positive/ one negative) for clastogenicity (-S9) at a concentration of 70 $\mu g.ml^{-1}$.

The high concentration of fatty acids could have been excessively toxic leading to cell rupture (detergent effect) as ethyl-EPA or EPA was directly added to cells in culture; however positive results were observed even in the absence of toxicity. Three separate chromosomal aberration assays were performed ± S9 in CHO cells. Results from these assays showed that ethyl-EPA was positive (using gap exclusion, the most relevant

analysis to potential genotoxicity) ± S9 in the absence and presence of cellular toxicity. EPA had a positive response +S9 with moderate toxicity (40% viable) while -S9 cell viability was <10% with a positive finding. The omega-9 monounsaturated and omega-6 polyunsaturated fatty acids had variable toxicities and clastogenicity results ±S9. An argument could be made that when delivered *in vivo* (physiologically relevant), ethyl-EPA is rapidly converted into EPA and then incorporated into triglycerides and chylomicrons with only a small portion remaining as free fatty acid. In addition, the mouse micronucleus assay (an *in vivo* test system) was negative.

Table 27: Clastogenic Effect – Replicate Cultures (% Aberrations without Gaps)

Fatty Acid	+S9	Toxic	-S9	Toxic
Ethyl-EPA				
Study 01-0680-G2	**+**	**-**	**+**	**-**
Study 21549	**±/±** [25 µg.mL^{-1}]	**-**	**+/+**	**+** (25%)
Study 21549	**±/+** [30 µg.mL^{-1}]	**+** (90%)		
Ethyl-Oleate				
Study 21549	**-/-**	**+** (50-60%)	**-/-**	**-**
Ethyl-Linoleate				
Study 21549	**-/-**	**+** (60%)	**-/-**	**-**
EPA				
Study 21883	**+/+**	**+** (40%)	**+/+**	**+** (<10%)
Oleic Acid				
Study 21883	**+/-**	**+** (20%)	**-/-**	**+** (75%)
Linoleic Acid				
Study 21883	**-/+**	**+** (40%)	**-/+**	**+** (70%)

"Toxic" refers to whether there was observed cytotoxicity at the clastogenic dose.
(%) in Toxic columns refers to the % viable cells relative to control
(±) indicates equivocal results (one negative and one positive replicate)
Note: Lot # EE171GQ Ethyl-EPA (Study #01-0680-G2) was also used in the mouse micronucleus assay with negative results

Additionally, when examining the calculated concentrations of Ethyl-EPA and EPA based on NOAEL C_{max} values in the 4 week rat and 9 month dog studies versus the concentrations shown to the clastogenic *in vitro*, there are large safety margins in the dog for ethyl-EPA and EPA. In the rat, Ethyl-EPA TK values were below the limit of detection but EPA levels were high. For reference, human C_{max} exposure (free-EPA) following 28 days of 4g/day dosing = 5.04 µM or 1.52 µg/mL. This would give a relative exposure margin for humans to the positive EPA clastogenicity result of 16X (+S9) and 53X (-S9) as observed in CHO cells.

Table 28: Comparison of *in vitro* concentrations positive for clastogenicity to C_{max} values in nonclinical toxicology studies

		4 Week Rat NOAEL = 2000 mg/kg (♂/♀)	9 Month Dog NOAEL = 300 mg/kg (♀)
Ethyl-EPA Cmax (µg/mL) / (µM)		BLQ	0.0738/ 0.22
EPA Cmax (µg/mL) / (µM)		16.85/ 55.71	2.97/ 9.82
Lowest dose (+) for clastogenicity in *in vitro* CHO cells (tested ethyl-EPA and EPA)	Ethyl-EPA (+S9) (µg/mL) / (µM)		25/ 75.6
	Safety Margin	ND	344X
	Ethyl-EPA (-S9) (µg/mL) / (µM)		150/ 453.8
	Safety Margin	ND	2063X
	EPA (+S9) (µg/mL) / (µM)	25/ 82.7	25/ 82.7
	Safety Margin	1.5X	8X
	EPA (-S9) (µg/mL) / (µM)	80/ 264.5	80/ 264.5
	Safety Margin	5X	27X

BLQ = Below the Limit of Quantitation; ND = No Data
Ethyl-EPA FW = 330.51 g/mole; EPA FW = 302.451 g/mole

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

Swiss Albino mice were used for the *in vivo* micronucleus assay, where five males and five females were given a single intraperitoneal injection of 50 mL/kg of ethyl-EPA with sampling times of 24 and 48 hours post-injection. Given the volume administered was 1.2 – 1.8 mL depending on animal weight, the average dose for a 30 g mouse was 1.5 mL (496 mg ethyl-EPA = HED 1344 mg/kg), which was a 20-fold higher exposure than the proposed human MRHD of 4 g/day (67 mg/kg/day), therefore this dose was sufficiently qualified. A total of at least 2000 polychromatic erythrocytes per animal were scored for the presence of micronuclei. All test groups (positive control, negative control and test article) gained weight and no toxicity as assessed by clinical observations was observed at study termination. There was no statistically significant increase in the number of micronucleated cells in the test article groups at either 24 or 48 hours using a dose of 50 mL/kg ethyl-EPA + 0.2% DLαTocopherol; and therefore was considered non-mutagenic.

7.4 Other Genetic Toxicity Studies

No additional studies were conducted.

8 Carcinogenicity

Two carcinogenicity studies were completed: 1. A 2-year rat carcinogenicity study which did not receive ECAC concurrence, and 2. A 6-month HRAS transgenic mouse carcinogenicity study that received ECAC concurrence. Both carcinogenicity studies were positive for tumors. In the rat 2-year carcinogenicity study, the following tumors were statistically significant by trend analysis when compared to corn oil or undosed control: hemangiomas at the mesenteric lymph node in both sexes, hemangiomas (all sites) in males, combined hemangiomas and hemangiosarcomas of the mesenteric lymph node in females. Additionally, pairwise statistical significance was also achieved for combined hemangiomas and hemangiosarcomas at the mesenteric lymph node in HD females. In the 6-month transgenic mouse, there was a positive finding for squamous cell papillomas of the proximal tail in male mice; however these neoplasms are attributed to fecal excretion of excess oil and dermal irritation. Due to the hemangioma findings of the mesenteric lymph node in the 2-year rat, it is of note that the 6-month mouse had a non-neoplastic histopathology finding of increased thrombosis of the mesenteric vein (ileum) and the perimesenteric vein (mesenteric lymph node), indicating this site is vulnerable to ethyl-EPA mediated toxicity due to its route of absorption.

Based on body surface area, there were low safety margins to the HD in both rat (2.2X) and mouse (5.5X). Both of these high doses had increased incidence of tumor findings including mesenteric lymph node hemangiomas (both sexes) and combined skin fibromas, fibrosarcomas and NOS sarcomas (males) that were predominantly associated with the abdomen in rats, and skin papillomas of the proximal tail in male mice. An increase in mesenteric lymph node hemangiomas was also identified in female rats at the MD with a safety margin of <1X.

There were also low safety margins based on EPA plasma exposure (derived from a 28-day human PK study) as compared to the MRHD of 4 g/day Vascepa. Ethyl-EPA (Vascepa) is rapidly converted to EPA by pancreatic lipase; therefore free (unesterified) EPA was measured in the plasma. As omega-3 fatty acids are incorporated into triacylglycerol (TAG) and chylomicrons it should be noted that exposure levels are only based on one fraction of the EPA pool (*i.e.* free fatty acid). In the rat, the safety margin is 7X to the HD rat where mesenteric lymph node hemangiomas were present in both sexes and 3.7X to mouse papillomas. Given the method of absorption of omega-3 fatty acids from enterocytes of the small intestine into the lymph, the site of these hemangiomas would indicate that they are related to ethyl-EPA and would be relevant to humans. Additionally, in the 6-month transgenic mouse study, although no hemangiomas formed, there was a significant increase in the incidence of thrombosis of the mesenteric vein (ileum) and perimesenteric vein (mesenteric lymph node) predominantly at the HD in both sexes.

Amarin conducted one 28-dat rat repeat dose toxicology study and one 9-month dog repeat dose toxicology study, although neither had histopathology findings of

hemangiomas. The 9-month dog study did have two events of premature sacrifice related to the GI tract where at week 7, a HD dog (2000 mg/kg = HED 1111 mg/kg) was sacrificed due to intussusception of ileocecocolic junction, and at week 22 a LD dog (300 mg/kg = HED 167 mg/kg) was sacrificed due to an infarcted jejunum and blockage between the small and large intestine. The only pathology reported at the mesenteric lymph node at 3 months (interim sacrifice) and 9 months was a low incidence of erythrophagocytosis at all doses. The combined toxicologically relevant events at the mesenteric lymph nodes in multiple species indicate that this site is sensitive to EPA, given its known absorption route from the small intestine into the lymph before systemic absorption. The 6-month transgenic HRAS mouse had increased mesenteric lymph node thrombosis/ inflammation (perimesenteric vein) in both sexes at terminal sacrifice and the 9-month dog had increased erythrophagocytosis at the mesenteric lymph node that was assessed by histopathology due to a necropsy finding (reddened mesenteric lymph node). These findings in two species may indicate red blood cell or endothelial cell damage at this site that could be explained by a localized high EPA fatty acid concentration. Continual absorption at this site could alter membrane fluidity and cellular signaling due to omega-3 FA incorporation into the endothelium, which may then affect RBC or platelet interaction leading to further cellular damage/ clumping and inflammation. One hypothesis may be that the rat hemangioma findings at the mesenteric lymph following 2-year administration by oral gavage began with continual damage at this site and led to a regenerative angiogenic response and ultimately hemangioma formation. Amarin did not conduct a rat toxicology study longer than 1 month so it is difficult to know if there were histopathology findings in this species with intermediate dosing duration. However, the Applicant relies on literature references conducted on ethyl-EPA for marketing of Epadel where a 3-month and a 1-year rat toxicology study were conducted. In these studies, the mesenteric lymph nodes were not assessed by histopathology, so it is not known if physiological changes were occurring earlier than 2 years in this species.

In all studies (conducted by Amarin or from literature references for Epadel), animals from a 9 month dog, 1 month rat, 3 month rat and 12 month rat all had some type of skin finding and severity increased with dose and length of treatment. In the 9 month dog study, reddened, flaky and dry skin was noted with hair loss around the anogenital region and abdomen and was associated with epidermal hyperplasia histologically at doses ≥300 mg/kg/day. A one month rat study (Amarin) showed hair loss from the back at most doses but increased at the HD of 2000 mg/kg/ day. The 3 month rat study (literature reference) had findings of yellow coat discoloration, hair loss, edema, scabbing and nodulation of the tail near the base at ≥3000 mg/kg/day (HED = 484 mg/kg) with histopathology correlates of inflammation, fibrosis and granuloma. The 12 month rat study (literature reference) had fur discoloration and nodulation at the tail (3000 mg/kg = HED 484 mg/kg) which correlated to hyperkeratosis, fibrosis, abscess and acanthosis. In the 6-month transgenic mouse carcinogenicity study, there was a prominent finding of nodule(s) at the proximal tail in both sexes at

the HD, acanthosis and hyperkeratosis, with only males having papilloma formation following 6 months of dosing. It is plausible that the papillomas may have been due to irritation and a hyperplastic response to oxidation of fatty acids on the skin.

In summary, it is likely that the findings of mesenteric lymph node hemangiomas are related to the long term administration of Vascepa (ethyl-EPA) to rats over the duration of 2 years, as the results were statistically significant by trend analysis even if considered a common tumor in both sexes, are relevant to the route of absorption of omega-3 fatty acids, and occur at a low margin of safety as compared to the proposed MRHD of 4 g/day in humans. The findings of papillomas (proximal tail) in mice are noted as there is a signal for dermal toxicity in all previous toxicology studies conducted. The vascular and skin tumors in two rodent species may translate to human risk as omega-3 fatty acids have the potential to cause cutaneous reactions and hemorrhage as treatment-emergent adverse events.

The following were the Executive CAC Recommendations and Conclusions for the two Carcinogenicity Studies conducted for ethyl-EPA (Vascepa, AMR101):

Tg.rasH2 Mouse:

- The Committee agreed that the study was adequate.
- The Committee concurred that there were no drug-related neoplasms in females and that the skin/subcutis papillomas in the tail of males were drug-related but not relevant to humans.

Rat:

- The Committee agreed that the study was adequate.
- The Committee concurred that there were no neoplasms clearly drug-related in male rats. Mesenteric lymph node hemangiomas/ hemangiosarcomas appeared to be drug related in female rats. However the incidences of hemangiomas/ hemangiosarcomas at all sites, combined, were not statistically significantly increased. The Committee noted that the increased incidence of mesenteric lymph node thrombosis of the perimesenteric vein as well as ileum mesenteric vein thrombosis and inflammation, both seen in the TgRasH2 mice and the high drug exposure at the mesenteric lymph nodes in the rats suggest that the mesenteric lymph node hemangiomas/ hemangiosarcomas in rats are drug-related.

Study title: EPA-E (5, 8, 11, 14, 17-Eicosapentaenoic Acid Ethyl Ester) 104 Week Carcinogenicity Study in Rats with Administration by Gavage

Study no.:	456223
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	21 February 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	EPA-E (5, 8, 11, 14, 17-eicosapentaenoic acid ethyl ester) obtained from Nisshin Pharma Inc., Japan, Batch No. EE141IS (97.3% purity), Batch No. EE030HU (99.1% purity)
CAC concurrence:	No

Key Study Findings

- There was a statistically significant increase in the number of HD female decedents and this dose group was sacrificed early at week 98 due to declining survival.
- Although there was no overall common cause of death in the female decedents, there was a slight increase in the incidence of pituitary adenomas as the cause of death in HD females.
- There was an increase in fibromas and fibrosarcomas in males as the cause of death.
- Body weights trended to be higher in HD males and females throughout the majority of the study. After week 87, HD females began to lose weight which correlated with decreased food consumption, declining clinical condition and decreased survival.
- Hematology parameters were assessed and female platelet counts significantly decreased at all doses (non-dose dependent).
- Prostate weight significantly decreased in MD and HD males.
- Non neoplastic findings included: 1. an increased incidence of thyroid C-cell hyperplasia in male decedents at all doses versus corn oil control; 2. hyperplasia of the non-glandular stomach mucosa in MD and HD decedents of both sexes; 3. an increase in the total incidence of pancreas islet cell hyperplasia in HD males.
- Neoplastic findings included an increased incidence of hemangiomas predominantly localized to the mesenteric lymph node in both sexes at HD and additionally at MD in females. The combined incidence of hemangiomas and hemangiosarcomas at this same site also reached statistical significance only in females. Males additionally had significant increases in brain/ spinal cord astrocytomas (only benign; but not with the combination with malignant astrocytomas) as well as the combined incidence of fibromas/fibrosarcomas/ and sarcoma (not otherwise specified, NOS) by trend analysis. These were not significant by pairwise statistical analyses.

Adequacy of Carcinogenicity Study

This study was valid as rats developed tumors in response to EPA-E and declining female survival before planned study termination also indicated that an MTD had been reached.

Appropriateness of Test Models

The 2 year rat carcinogenicity study was an appropriate model however it is noted that although the diet did not contain any fish meal, there was 0.06% C18:3ω3 linolenic acid which can be metabolically converted to EPA and DHA. Neither control (undosed or corn oil) was appropriate for this model.

Evaluation of Tumor Findings

A complete tumor evaluation was conducted by CDER statistical review. Additionally, (b) (4) (a contracted company by the applicant) evaluated the hemangioma/ hemangiosarcoma tumor findings as well as the decrease in mortality of females in the HD group. CDER analysis found an increased incidence of hemangiomas in both sexes by trend analysis, as well as a combined increase in hemangiomas and hemangiosarcomas in females. As this site (mesenteric lymph node) is relevant to the absorption of omega-3 fatty acids from the small intestine, the formation of this tumor is physiologically relevant. Although the (b) (4) analysis confirmed that the incidence of hemangiomas was higher in high-dose animals than in control it was within levels reported in background historical control data and not likely biologically relevant to humans. The applicant also originally conducted their own statistical analysis (Peto analysis) but did not report any statistically significant differences in tumor incidence.

Methods	
Doses:	0.1, 0.3 and 1.0 mL/kg/day (~91, 273 and 911 mg/kg/day)
Frequency of dosing:	Once daily
Dose volume:	Variable (0.1, 0.3 or 1.0 mL/kg)
Route of administration:	Oral gavage
Formulation/Vehicle:	No vehicle used, doses were adjusted by changing the dosing volume of the neat test article
Basis of dose selection:	At the time of the carcinogenicity study initiation, dose level limitations were based on the percent caloric intake (Kcal intake/day) from the test article relative to that of the diet to no more than approximately 5% (7% and 5% at HD for males and females, respectively).
Species/Strain:	Rat/ Wistar (Crl:WI)BR)
Number/Sex/Group:	50/sex/group
Age:	~7 weeks at dosing initiation
Animal housing:	Animals were housed in suspended polypropylene cages with stainless steel grid tops and solid bottoms. Sterilized white wood shavings were used as bedding, which was changed up to 3 times per week.
Paradigm for dietary restriction:	None. Rat and mouse No. 1 Expanded SQC Diet, supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK. *Note: the mouse feed contained no fish meal, and therefore no marine-derived omega-3 fatty acids.*
Dual control employed:	Yes Control 1: corn oil + 0.2% α-tocopherol (1.0 mL/kg/day) Control 2: undosed
Interim sacrifice:	No
Satellite groups:	No (no TK analysis)
Deviation from study protocol:	On 29 November 2003 (Week 61), all Group 3 females (351-400) were dosed with the incorrect volume of test item formulation (received 1.0 mL/kg/day instead of 0.1 mL/kg/day) in error. There were also sporadic dates when animals in all dose groups were not dosed due to condition or behavior.

Observations and Results

Table 29: Groups in the 2 Year Rat Carcinogenicity Study

	Males					Females				
Group Number	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
EPA-E mg/kg/day	**0**	**0**	**91**	**273**	**911**	**0**	**0**	**91**	**273**	**911**
EPA-E mL/kg/day	**0**	**0**	**0.1**	**0.3**	**1.0**	**0**	**0**	**0.1**	**0.3**	**1.0**

Group 1 was administered 1.0 mL/kg/day corn oil; Group 2 was undosed

Mortality

Mortality was assessed twice daily. There were 259 premature decedents as follows:

Table 30: Mortality of Rats in 2 Year Carcinogenicity Study

Sex	Dose Level (mL/kg/day)				
	(0)	(undosed)	(0.1)	(0.3)	(1.0)
Males	31	25	34	32	27
Females	20	17	22	19	32

[Table reproduced from NDA 202057 submission; Study No. 456223]

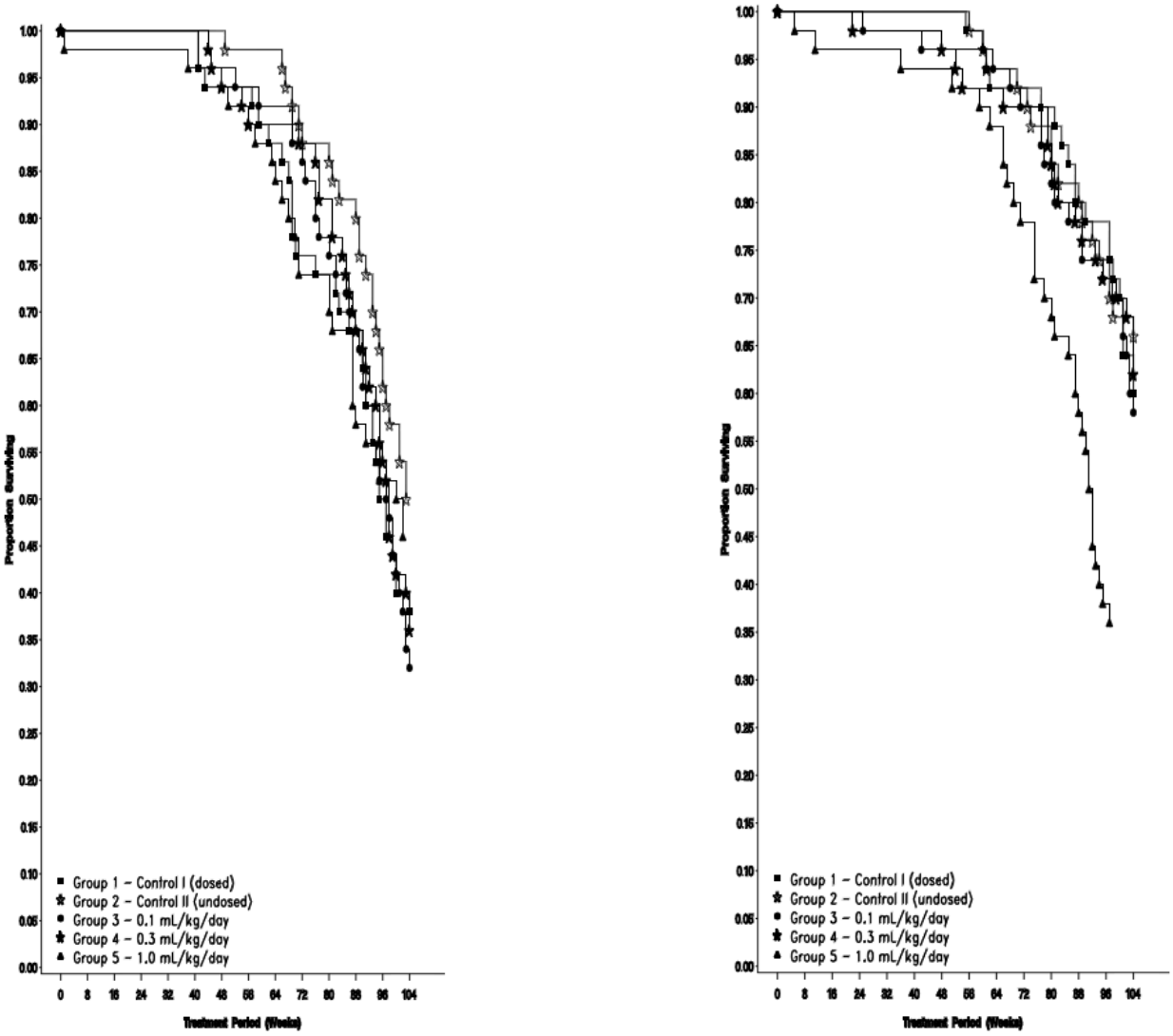
Table 31: Rat Mortality in Males and Females

	Males					Females				
Group Number	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
EPA-E mg/kg/day	**CO**	**0**	**91**	**273**	**911**	**CO**	**0**	**91**	**273**	**911**
Number/group	50	50	50	50	50	50	50	50	50	50
Terminal Kill	19	25	16	18	23	30	33	28	31	18
Killed Prematurely	22	15	26	20	18	16	15	18	16	28
Found Dead	9	10	8	12	9	4	2	4	3	4

CO = Corn Oil

Figure 9: Mortality (Kaplan-Meier Plots) in 2-year Male and Female Rats

Kaplan-Meier Plot: MALES **Kaplan-Meier Plot: FEMALES**



[Figures reproduced from NDA 202057 submission; Study No. 456223]

Female Mortality

There was a statistically significant increase in the number of HD female decedents versus the corn oil control ($p<0.001$). Due to the deteriorating condition of the HD females, this group was terminated early after 98 weeks of treatment.

Anterior lobe pituitary adenoma as a cause of death was slightly increased in incidence in treated females at 1.0 mL/kg/day compared to Group 1 females as follows:

Table 32: Neoplasm Cause of Death in Female Rats

	EPA-E Dose Group (mg/kg/day)				
	0 (corn oil)	**0 (undosed)**	**91**	**273**	**911**
Decedents	**20**	**17**	**22**	**19**	**32**
Pituitary Adenoma	7	9	8	3	14

Table 33: Statistical Analysis of Rat Mortality in the 2 year carcinogenicity study

Detailed below are the results of the Wilcoxon test comparing survival times for the Control group 1 and all other test groups.

Males	Test Statistic	P-Value
Control I vs Control II	2.795	0.095
Combined Control vs 0.1 mL/kg/day	0.010	0.92
Combined Control vs 0.3 mL/kg/day	0.146	0.70
Combined Control vs 1.0 mL/kg/day	0.045	0.83

Females	Test Statistic	P-Value
Control I vs Control II	0.084	0.77
Combined Control vs 0.1 mL/kg/day	0.182	0.67
Combined Control vs 0.3 mL/kg/day	0.001	0.97
Combined Control vs 1.0 mL/kg/day	12.456	<0.001

[Table reproduced from NDA 202057 submission; Study No. 456223]

Male Mortality

There were no statistically significant differences in mortality between Control I and any of the treated male groups.

There was an increased incidence of fibroma and fibrosarcoma in males as the cause of death.

Table 34: Neoplasm Cause of Death in Male Rats

	EPA-E Dose Group (mg/kg/day)				
	0 (corn oil)	**0 (undosed)**	**91**	**273**	**911**
Decedents	**31**	**25**	**34**	**32**	**27**
Fibrosarcoma (M)	0	0	2	3	4
Fibrosarcoma (M), renal	0	0	0	0	1
Fibroma	1	1	2	2	4

(M) = malignant tumor

Clinical Signs

Clinical signs were recorded daily, prior to treatment and at suitable intervals after dosing.

Dermal and subcutaneous masses were detected but did not increase with treatment of EPA-E. In HD females, there was an increase in respiratory abnormalities and the observation of "leaning to one side" which reflects the deteriorating clinical condition of this group.

Table 35: Clinical Signs of 2-year Ethyl-EPA treated Rats

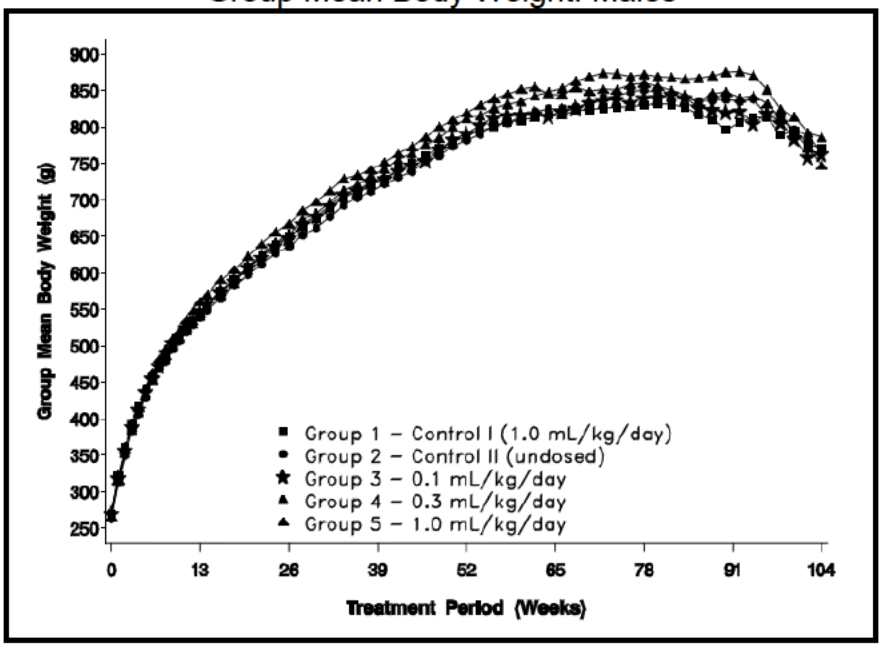
	Males					Females				
EPA-E mg/kg/day	**CO**	**0**	**91**	**273**	**911**	**CO**	**0**	**91**	**273**	**911**
Number/group	50	50	50	50	50	50	50	50	50	50
Subcutaneous masses	10	20	11	14	20	19	20	21	16	18
Dermal masses	7	11	8	4	6	4	2	2	2	0
Excessive salivation	0	0	0	1	1	0	0	0	1	1
Respiration abnormal.	17	10	14	18	10	9	8	13	14	22
Leaning to one side	1	2	1	1	2	0	2	3	2	10

Body Weights

Body weights were recorded daily for the first 13 weeks, then weekly thereafter.

In male HD rats (1.0 mL/kg/day), there was a small increase in mean body weight throughout the majority of the study as compared to control. Female HD animals had an increase in body weight over control from approximately weeks 26 – 65 before stabilizing. In female HD rats from week 87, there was a reduction in body weight and a loss in mean body weight gain that was associated with the decreasing survival of this group.

Figure 10: 104 Week Carcinogenicity Study in Rats with Administration by Gavage - Group Mean Body Weight: Males

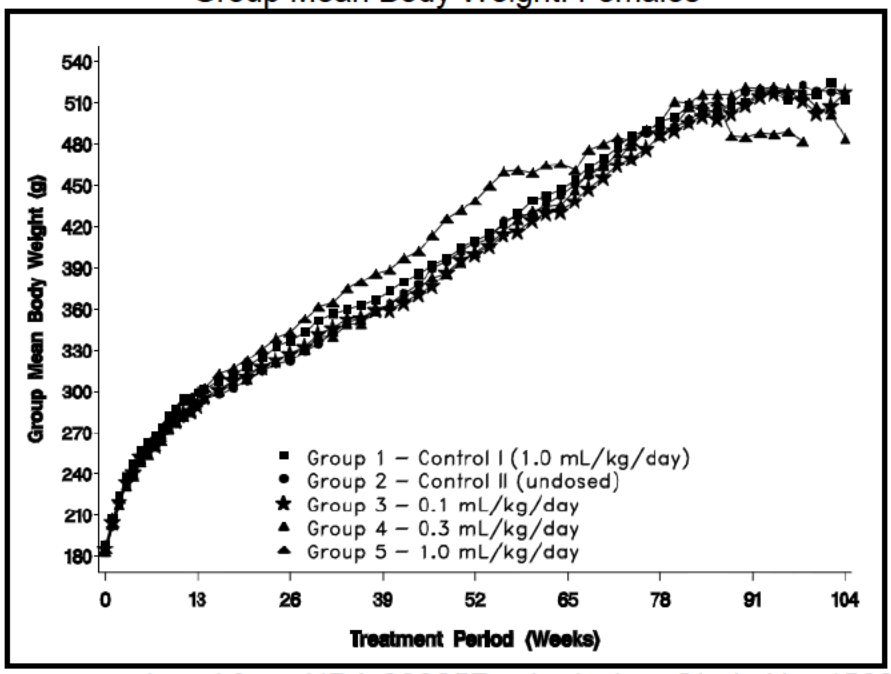


[Figure reproduced from NDA 202057 submission; Study No. 456223]

Table 36: Weight Change in Male Rats

	MALE - Change in weight from Predose (%)							
Week	**13**	**26**	**39**	**52**	**65**	**78**	**91**	**104**
Group 1: corn oil	102	141	168	192	203	207	199	186
Group 2: undosed	105	142	173	197	213	224	219	191
Group 3: 91 mg/kg	102	140	170	193	202	211	205	183
Group 4: 273 mg/kg	104	144	175	203	216	225	226	193
Group 5: 911 mg/kg	115	152	182	209	220	225	217	182

Figure 11: 104 Week Carcinogenicity Study in Rats with Administration by Gavage - Group Mean Body Weight: Females



[Figure reproduced from NDA 202057 submission; Study No. 456223]

Table 37: Weight Change in Female Rats

	FEMALE - Change in weight from Predose (%)							
Week	**13**	**26**	**39**	**52**	**65**	**78**	**91**	**104**
Group 1: corn oil	59	79	97	118	138	164	173	172
Group 2: undosed	58	76	95	123	144	168	183	181
Group 3: 91 mg/kg	56	76	94	115	132	162	176	178
Group 4: 273 mg/kg	60	79	97	119	140	171	186	164
Group 5: 911 mg/kg	63	85	110	137	149	163	158	--

Feed Consumption

The amount of food consumed/week/cage (5 rats/cage) was recorded for the first 13 weeks, then for 1 week out of every 4 weeks until study end.

There was a statistically significant decrease in food consumption in HD females (1.0 mL/kg/day) during weeks 88-92 and correlated with a reduction in body weight.

Table 38: Mean Food Consumption in Male Rats

	MALE – Mean Food Consumption (g/animal/day)							
Week	**-1**	**12**	**24**	**36**	**48**	**60**	**72**	**104**
Group 1: corn oil	28.4	27.3	27.6	27.0	27.0	26.6	26.5	24.1
Group 2: undosed	28.4	29.0**	29.2*	29.3**	29.0**	29.8***	29.2**	25.1
Group 3: 91 mg/kg	28.8	28.8**	29.5**	29.3**	29.3**	28.6**	28.7*	24.0
Group 4: 273 mg/kg	27.9	28.0	28.2	28.2	28.3	28.5**	28.7*	22.5
Group 5: 911 mg/kg	28.5	28.6*	28.7	28.6*	28.6*	28.4*	27.5	22.4

* = P<0.05 ; ** = P<0.01 ; *** P<0.001 from corn oil control

Table 39: Mean Food Consumption in Female Rats

	FEMALE – Mean Food Consumption (g/animal/day)							
Week	**-1**	**12**	**24**	**36**	**48**	**60**	**72**	**104**
Group 1: corn oil	19.8	21.2	21.6	21.6	21.5	21.2	22.7	19.2
Group 2: undosed	19.6	22.3	22.2	22.6	22.5	22.0	23.1	20.6
Group 3: 91 mg/kg	18.5	21.7	22.0	22.4	22.3	21.8	23.0	21.4
Group 4: 273 mg/kg	19.4	21.5	21.7	22.0	21.4	22.5	23.0	17.6
Group 5: 911 mg/kg	19.8	21.5	21.8	22.6	23.1	21.6	23.2	--

Ophthalmoscopy

The eyes were examined using an indirect ophthalmoscope after the application of 1% tropicamide. Anterior, lenticular and fundic areas were evaluated. An ophthalmoscopic examination was completed on all animals during Pretrial, and during Weeks 52 and 104. Surviving High dose females were examined prior to terminal sacrifice during Week 99.

There were no ophthalmologic changes that were attributed to EPA-E in any dose group.

Hematology

Blood samples for hematology were taken from all surviving animals during Weeks 103 or 104 and high dose females had a terminal blood sample taken prior to premature sacrifice at Week 99 of treatment. The parameters evaluated included: Hemoglobin, Reticulocytes, Red Blood Cell Count, Hematocrit, Mean Cell Hemoglobin, Mean Cell Volume, Mean Cell Hemoglobin Concentration, White Blood Cell Count, Platelets, Differential White Blood Cell Count including Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, and Large Unclassified Cells.

A statistically significant decrease (~20%) in group mean platelet count was observed for females treated with EPA-E at all doses (non-dose dependent).

Table 40: Hematology (Mean ± SD)

	Males					Females				
EPA-E mg/kg/day	**CO**	**0**	**91**	**273**	**911**	**CO**	**0**	**91**	**273**	**911**
Number/group	19	24	15	20	22	29	31	29	27	18
Platelets	872 ± 148	957 ± 221	853 ± 215	889 ± 198	794 ± 209	832 ± 309	810 ± 411	629 ± 207**	644 ± 219*	639 ± 206*

*P<0.05, **P<0.01

Gross Pathology

All surviving animals and premature decedents were subject to necropsy and gross pathology. Organ weights were recorded from 10/sex/group at terminal sacrifice.

The most prominent findings included reddened mesenteric lymph nodes (HD) and skin scabs (MD and HD) in animals of both sexes treated with AMR101 when compared to control. Females also had an increase in the incidence of a dark pituitary gland at the HD. Selected findings are presented below:

Table 41: Males – Gross Pathology

Dose (mg/kg)	Corn Oil Control		Untreated Control		91		273		911	
Survival Status	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number examined	31	19	25	25	34	16	32	18	27	23
Liver, prominent lobulation	10	8	7	7	10	6	10	7	12	14
Total	18		14		16		17		26	
Liver, Pale Focus	0	1	0	1	1	3	1	2	3	0
Total	1		1		4		3		3	
Lung, pale focus	13	11	11	12	21	13	15	14	15	15
Total	24		23		34		29		30	
Mesenteric lymph nodes, reddened	7	1	2	2	3	0	3	4	8	5
Total	8		4		3		7		13	
Skin, scabs	1	2	0	0	1	0	3	3	7	2
Total	3		0		1		6		9	
Skin, staining	20	15	13	14	17	7	20	11	14	14
Total	35		27		24		31		28	

DOS = died or euthanized on study; SNC = scheduled necropsy

Table 42: Females – Gross Pathology

Dose (mg/kg)	Corn Oil Control		Untreated Control		91		273		911	
Survival Status	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number examined	20	30	17	33	22	28	19	31	32	18
Liver, prominent lobulation	3	9	4	11	5	6	6	9	14	7
Total	12		15		11		15		21	
Lung, pale focus	5	6	6	10	11	15	9	12	24	8
Total	11		16		26		21		32	
Mesenteric lymph nodes, reddened	1	6	1	2	2	3	5	2	6	5
Total	7		3		5		7		11	
Ovary, reddened, both	2	0	0	1	1	3	3	1	3	2
Total	2		1		4		4		5	
Pituitary Gland,	0	0	1	0	0	0	1	0	5	0

dark										
Total	**0**		**1**		**0**		**1**		**5**	
Pituitary Gland, enlarged	8	12	9	9	9	6	5	10	15	4
Total	**20**		**18**		**15**		**15**		**19**	
Skin, scabs	1	0	1	0	0	1	2	1	3	0
Total	**1**		**1**		**1**		**3**		**3**	
Skin, staining	11	9	11	8	10	10	6	13	19	9
Total	**20**		**19**		**20**		**19**		**28**	

DOS = died or euthanized on study; SNC = scheduled necropsy

Organ Weights

A statistically significant reduction in absolute prostate gland weight was observed at 0.3 and 1.0 mL/kg/day in males.

Table 43: Absolute Organ Weights in Males and Females (Mean ± SD)

	Males					**Females**				
EPA-E mg/kg/day	**CO**	**0**	**91**	**273**	**911**	**CO**	**0**	**91**	**273**	**911**
Number	10	10	10	10	10	10	10	10	10	10
Ave. Body Weight (g)	767	732	765	741	803	505	517	514	508	466
Pituitary Gland (g)	0.047 ± 0.062	0.037 ± 0.038	0.026 ± 0.019	0.027 ± 0.027	0.028 ± 0.022	0.045 ± 0.056	0.034 ± 0.032	0.019 ± 0.045	0.045 ± 0.053	0.067 ± 0.107
Prostate (g)	0.705 ± 0.194	0.686 ± 0.125	0.594 ± 0.259	0.461 ± 0.151 **	0.528 ± 0.181 *					
Thyroid (g)	0.057 ± 0.034	0.047 ± 0.011	0.040 ± 0.012	0.041 ± 0.010	0.039 ± 0.013	0.030 ± 0.005	0.031 ± 0.011	0.032 ± 0.011	0.032 ± 0.011	0.029 ± 0.009

*P<0.05, **P<0.01

Histopathology

Peer Review: Yes (internal peer review)

Table 44: Tissues Analyzed for Histopathology

Tissues Collected	Weigh	Examine	Comments
Abnormal Tissue	-	X	Included local lymph nodes to masses.
Adrenal x 2	X	X	-
Aortic Arch	-	X	-
Blood Smear	-	-	Taken from animals killed prematurely
Brain	X	X	Forebrain, midbrain and cerebellum.
Epididymis x 2	X	X	-
Exorbital Lacrimal Glands	-	-	-
Eye x 2	-	X	Fixed in Davidson's fluid. One eye was examined.
Femur Bone (including Stifle Joint)	-	X	-
Gastro-intestinal Tract:			Opened at necropsy and mucosa examined. Peyers patch examined from small intestine.
Stomach	-	X	
Duodenum	-	X	
Jejunum	-	X	
Ileum	-	X	
Caecum	-	X	
Colon	-	X	
Rectum	-	X	

Harderian Glands	-	-	Fixed in Davidson's fluid. Only one examined.
Heart	X	X	-
Implant(s)	-	-	For identification purposes.
Interscapular Brown Fat Pads	-	X	-
Kidney and Ureter x 2	X	X	-
Larynx	-	X	-
Liver	X	X	Three lobes fixed, two examined.
Lung	X	X	Inflated with fixative after weighing, all lobes examined.
Marrow Smear	-	-	Smear air-dried and fixed in methanol.
Mesenteric Lymph Node	-	X	-
Nasal Cavity	-	-	-
Oesophagus	-	X	-
Optic Nerve x 2	-	X	Fixed in Davidson's fluid. Only one was examined.
Ovary x 2	X	X	-
Pancreas	-	X	-
Parotid Salivary Gland	-	-	-
Pituitary	X	X	-
Preputial / Clitorial Gland	-	-	-
Prostate	X	X	-
Rib	-	X	Including costochondral junction.
Sciatic Nerve	-	X	-
Seminal Vesicles	-	X	-
Skin + Mammary Gland	-	X	-
Spinal Cord	-	X	Sections from cervical, midthoracic and lumbar regions.
Spleen	X	X	-
Sternum	-	X	Including bone marrow.
Submandibular Lymph Node	-	X	-
Submaxillary and Sublingual Salivary Gland	-	X	-
Testis x2	X	X	-
Thigh Muscle	-	X	-
Thymus	X	X	-
Thyroid with Parathyroid x 2	X	X	Weighed after fixation
Tongue	-	X	-
Trachea	-	X	-
Urinary Bladder	-	X	Contracted bladders distended with fixative; epithelial surface examined after fixation.
Uterus with Cervix + Oviduct	X	X	-
Vagina	-	X	-
Zymbals Glands	-	-	-

[Table reproduced from NDA 202057 submission; Study No. 456223]

Neoplastic

Major neoplastic findings include the following:

1. In males and females there was an increased incidence of hemangiomas primarily of the mesenteric lymph nodes. As this is the site where ethyl-EPA is absorbed from the gut into the lymph, it has physiological relevance. Combined hemangiomas at all sites in both sexes were also highly statistically significant. There are differing results (depending on the study or database) as to whether hemangiomas and hemangiosarcomas (all or mesenteric lymph node) are common or uncommon in the Wistar rat; however the incidence of hemangiomas in the MLN in both sexes were significant even if considered a common tumor. Combined hemangioma + hemangiosarcomas of the MLN were significant in females if common but were not significant in males.
2. The combined incidence of skin/ subcutis fibromas, fibrosarcomas and sarcomas (not otherwise specified) was statistically significant in males when considered a common tumor by trend analysis.
3. Although the number of benign astrocytomas (brain alone or brain + spinal cord combined) was low, it did reach statistical significance in males when considered an uncommon tumor by trend analysis. If one spinal cord malignant astrocytoma was included in the analysis, statistical significance was lost.

Table 45: Summary of Tumor Incidence in Males

Dose (mg/kg)	Corn Oil Control		Untreated Control		91		273		911	
Survival Status	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number examined	31	19	25	25	34	16	32	18	27	23
Total Number	50		50		50		50		50	
Exposure Margin[1]	-		-		<1X		<1X		2X	
Est. Exposure Margin (AUC)[2]	-		-		-		3X		7X	
Thyroid, C-cell Adenoma	2	3	2	2	0	3	1	1	4	3
Total	**5**		**4**		**3**		**2**		**7**	
Fibrosarcoma (skin)	1	2	0	4	2	2	4	3	5	3
Total	**3**		**4**		**4**		**7**		**8**	
Fibrosarcoma (kidney)	0	0	0	0	0	0	0	0	1	0
Total	**0**		**0**		**0**		**0**		**1**	
Hemangioma (all)	1	0	1	1	0	0	0	0	3	3
Total	**1**		**2**		**0**		**0**		**6**	
Hemangiosarcoma (all)	6	2	3	5	2	4	3	3	5	3
Total	**8**		**8**		**6**		**6**		**8**	
HA + HS (all) combined	7	2	4	6	2	4	3	3	8	6
Total	**9**		**10**		**6**		**6**		**14**	
Hemangioma (MLN)	0	0	0	1	0	0	0	0	3	2
Total	**0**		**1**		**0**		**0**		**5**	
Hemangiosarcoma (MLN)	5	2	0	4	1	3	2	3	5	2
Total	**7**		**4**		**4**		**5**		**7**	
HA + HS (MLN) combined	5	2	0	5	1	3	2	3	8	4
Total	**7**		**5**		**4**		**5**		**12**	
Astrocytoma (Brain)	0	0	1	0	0	0	0	0	1	2
Total	0		1		0		0		3	
Astrocytoma (Brain + Spinal Cord) (B)	0	0	1	0	0	0	1	0	1	2
Total	0		1		0		1		3	

(1) exposure Margin is based on the proposed MRHD of 4 g/day = 67 mg/kg for a 60 kg human (HED conversion), Body Surface Area
(2) estimated exposure margins are based on the 28-day rat study (ZOC0001, Amendment) where week 4 male rats had an exposure of $AUC_{0\text{-}t}$ = 60,310 ng.hr/mL for 300 mg/kg/day and $AUC_{0\text{-}t}$ = 138,200 ng.hr/mL for 1000 mg/kg/day EPA-E (AMR101). Human exposure (Study AMR-01-01-0018, 28 days healthy volunteers) at the MRHD of 4g/day AMR101 was $AUC_{0\text{-}24hr}$ = 20,305 ng.hr/mL (all values reported are for unesterified EPA)
MLN = Mesenteric Lymph Node
DOS = died or euthanized on study; SNC = scheduled necropsy
(B) = Benign

Table 46: Summary of Tumor Incidence in Females

Dose (mg/kg)	Corn Oil Control		Untreated Control		91		273		911	
Survival Status	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number examined	20	30	17	33	22	28	19	31	32	18
Total Number	50		50		50		50		50	
Exposure Margin[1]	-		-		<1X		<1X		2X	
Est. Exposure Margin (AUC)[2]	-		-		-		3X		7X	
Thyroid, C-cell Adenoma	1	1	1	2	0	4	2	3	3	2
Total	2		3		4		5		5	
Fibrosarcoma (skin)	0	0	1	1	0	0	3	0	0	0
Total	0		2		0		3		0	
Hemangioma (all)	0	0	0	1	0	0	2	2	2	2
Total	0		1		0		4		4	
Hemangiosarcoma (all)	1	2	2	1	0	2	1	2	1	1
Total	3		3		2		3		2	
HA + HS (all) combined	1	2	2	2	0	2	3	4	3	3
Total	3		4		2		7		6	
Hemangioma (MLN)	0	0	0	0	0	0	1	2	2	2
Total	0		0		0		3		4	
Hemangiosarcoma (MLN)	0	1	0	0	0	0	1	1	1	1
Total	1		0		0		2		2	
HA + HS (MLN) combined	0	1	0	0	0	0	2	3	3	3
Total	1		0		0		5		6	

(1) exposure Margin is based on the proposed MRHD of 4 g/day = 67 mg/kg for a 60 kg human (HED conversion), Body Surface Area
(2) estimated exposure margins are based on the 28-day rat study (ZOC0001, Amendment) where week 4 female rats had an exposure of AUC_{0-t} = 54,970 ng.hr/mL for 300 mg/kg/day and AUC_{0-t} = 151,600 ng.hr/mL for 1000 mg/kg/day EPA-E (AMR101). Human exposure (Study AMR-01-01-0018, 28 days healthy volunteers) at the MRHD of 4g/day AMR101 was AUC_{0-24hr} = 20,305 ng.hr/mL (all values reported are for unesterified EPA)
MLN = Mesenteric Lymph Node
DOS = died or euthanized on study; SNC = scheduled necropsy
HA = Hemangioma
HS = Hemangiosarcoma

Table 47: Statistical Analysis to Group 1 Corn Oil Control - Rat

Gender	Tissue	Tumor	Common/ Uncommon	Pairwise Analysis P - value	Pairwise Analysis Sig?	Trend Analysis P - value	Trend Analysis Sig?
Female	Mesenteric LN	Hemangioma	Common	0.0266	No	0.0042	YES
Male	Mesenteric LN	Hemangioma	Common	0.0289	No	0.0009	YES

Female	Mesenteric LN	Hemangiomas and Hemangiosarcomas	Common	0.0206	No	0.0024	YES
Male	Mesenteric LN	Hemangiomas and Hemangiosarcomas	Common	0.1954	No	0.0313	No
Female	All	Hemangiomas	Common	0.0266	No	0.0069	No
Male	All	Hemangiomas	Common	0.0530	No	0.0009	YES
Female	All	Hemangiomas and Hemangiosarcomas	Common	0.1140	No	0.0360	No
Male	All	Hemangiomas and Hemangiosarcomas	Common	0.1776	No	0.0288	No
Male	Brain	Astrocytoma (B)	Uncommon	0.1249	No	0.0156	YES
Male	All (brain + spinal cord)	Astrocytoma (B)	Uncommon	0.1196	No	0.0176	YES
Male	All (brain + spinal cord)	Astrocytoma (B and M)	Uncommon	0.3069	No	0.0593	No
Male	Skin/Subcutis	Fibroma	Common	0.0516	No	0.0110	No
Male	Skin/Subcutis	Fibromas, Fibrosarcomas, and sarcomas (NOS)	Common	0.0101	No	0.0030	YES

Pairwise analysis is to the HD of 911 mg/kg

Table 48: Statistical Analysis to Group 2 Undosed Control - Rat

				Pairwise Analysis		Trend Analysis	
Gender	Tissue	Tumor	Common/ Uncommon	P - value	Sig?	P - value	Sig?
Female	Mesenteric LN	Hemangioma	Common	0.0282	No	0.0044	YES
Male	Mesenteric LN	Hemangioma	Common	0.0771	No	0.0036	YES
Female	Mesenteric LN	Hemangiomas and Hemangiosarcomas	Common	0.0047	YES	0.0009	YES
Male	Mesenteric LN	Hemangiomas and Hemangiosarcomas	Common	0.0429	No	0.0078	No
Female	All	Hemangiomas	Common	0.0924	No	0.0161	No
Male	All	Hemangiomas	Common	0.0924	No	0.0024	YES
Male	All	Hemangiomas and Hemangiosarcomas	Common	0.1013	No	0.0187	No
Male	Brain	Astrocytoma (B)	Uncommon	0.2585	No	0.0455	No
Male	Skin/Subcutis	Fibromas, Fibrosarcomas, and sarcomas (NOS)	Common	0.1191	No	0.0247	No

Pairwise analysis is to the HD of 911 mg/kg

Table 49: Wistar Rat Carcinogenicity Historical Controls for Hemangiomas and Hemangiosarcomas

Reference	Tumor Type	Tissue Site	Male	Female
(b) (4)	Hemangioma	Mesenteric Lymph Nodes	Common	Uncommon
	Hemangiosarcoma	Mesenteric Lymph Nodes	Common	Uncommon
	Hemangioma	All Lymph Nodes	Common	Common
	Hemangiosarcoma	All Lymph Nodes	Common	Uncommon
	Hemangioma + hemangiosarcoma (no distinction)	All Sites	Common	Common
	Hemangioma	All sites	Common	Common
	Hemangiosarcoma	All sites	Common	Uncommon
	Hemangioma	Mesenteric Lymph Nodes	Common	Common
	Hemangioma	Mesenteric Lymph Nodes	Common	Common
	Hemangiosarcoma	Mesenteric Lymph Nodes	Common	Uncommon
	Hemangioma	Mesenteric Lymph Nodes	Uncommon	Uncommon
	Hemangiosarcoma	Mesenteric Lymph Nodes	Uncommon	Uncommon
	Hemangioma	All sites	Uncommon	Uncommon
	Hemangiosarcoma	All sites	Uncommon	Uncommon
	Hemangioma	Mesenteric Lymph Nodes	Common	Common
	Hemangiosarcoma	Mesenteric Lymph Nodes	Common	Uncommon
Current Study Controls[1]	Hemangioma	Mesenteric Lymph Nodes	Uncommon	Uncommon
	Hemangiosarcoma	Mesenteric Lymph Nodes	Common	Common
	Hemangioma	All sites	Common	Uncommon
	Hemangiosarcoma	All sites	Common	Common
Current Study Controls[2]	Hemangioma	Mesenteric Lymph Nodes	Common	Uncommon
	Hemangiosarcoma	Mesenteric Lymph Nodes	Common	Uncommon
	Hemangioma	All sites	Common	Common
	Hemangiosarcoma	All sites	Common	Common

[1] Group 1 (Corn Oil) control; [2] Group 2 untreated control

Table 50: Combined Hemangiomas/ Hemangiosarcomas By Site

	Males					Females				
EPA-E mg/kg/day	**CO**	**0**	**91**	**273**	**911**	**CO**	**0**	**91**	**273**	**911**
Number/group	50	50	50	50	50	50	50	50	50	50
Hemangiosarcoma (M), skin	1	3	2	1	0	1	0	1	0	0
Hemangioma (B), skin	0	1	0	0	0	0	0	0	0	0
	1	4	2	1	0	1	0	1	0	0
Hemangiosarcoma (M), LN mesent.	7	4	4	5	7	1	0	0	2	2
Hemangioma (B), LN mesenteric	0	1	0	0	5	0	0	0	3	4
	7	5	4	5	12	1	0	0	5	6
Hemangiosarcoma (M), LN axillary	0	0	0	0	0	0	1	0	0	0
Hemangioma (B), LN axillary	0	0	0	0	1	0	0	0	0	0
	0	0	0	0	1	0	1	0	0	0
Hemangiosarcoma (M), Abdom cav	0	0	0	0	0	0	0	1	1	0
Hemangioma (B), Abdominal cavity	1	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	1	1	0

Table 51: ALL NEOPLASMS

	Males					Females				
EPA-E mg/kg/day	**CO**	**0**	**91**	**273**	**911**	**CO**	**0**	**91**	**273**	**911**
Number/group	50	50	50	50	50	50	50	50	50	50
Larynx										
Hemangioma (B)	0	0	0	0	0	0	0	0	1	0
Lung										
Bronchiolo-Alveolar Carcinoma (M)	0	0	0	0	0	0	0	0	0	1
Bronciolo-Alveolar Adenoma (B)	0	1	0	0	1	0	0	0	0	0
Squamous Cell Carcinoma (M)	0	0	0	0	0	1	0	0	0	0
Hemopoietic System										
Leukemia, granulo (M)	0	1	0	0	0	0	0	0	0	0
Histiocytic Sarcoma (M)	0	2	1	2	2	0	1	0	0	0
Lymphoma, Lympho (M)	2	1	1	0	0	1	0	0	1	1
Lymphoma, Foll Ctr (M)	0	0	0	0	0	0	1	0	0	0
Lymph Node (Mesenteric)										
Sarcoma (M)	0	0	0	0	0	1	0	0	0	0
Hemangiosarcoma (M)	7	4	4	5	7	1	0	0	2	2
Hemangioma (B)	0	1	0	0	5	0	0	0	3	4
Lymph Node (Axillary)										
Hemangiosarcoma (M)	0	0	0	0	0	0	1	0	0	0
Hemangioma (B)	0	0	0	0	1	0	0	0	0	0
Spleen										
Hemangiosarcoma (M)	0	0	0	0	0	0	1	0	0	0
Thymus										
Malignant Thymoma (M)	1	0	0	0	0	0	1	0	0	0
Thymoma (B)	0	0	0	0	0	3	2	2	1	1
Thymoma (M) metast.	0	0	0	0	1	0	0	0	1	0
Heart										
Schwannoma (B)	0	0	0	0	1	0	0	0	0	1
Thyroid Gland										
Follicular Cell Carcinoma (M)	1	0	0	0	0	0	0	0	0	0
Cystadenocarcinoma (M)	0	0	0	1	0	0	0	0	0	0
Cystadenoma (B)	1	0	0	0	0	0	0	0	0	0
C-Cell Adenoma (B)	5	4	3	2	7	2	3	4	5	5
Follicular Cell Adenoma (B)	3	3	0	0	3	2	3	2	1	1
Parathyroid Gland										
Adenoma (B)	1	2	0	1	0	2	0	0	1	0
Adrenal Gland										
Cortical Carcinoma (M)	0	0	0	1	0	0	0	0	0	0
Pheochromocytoma (M)	1	1	1	1	0	0	0	0	0	0
Cortical Adenoma (B)	0	0	1	0	0	1	1	1	1	0
Pheochromocytoma (B)	0	9	4	0	1	2	2	2	1	3
Pituitary Gland										
Carcinoma (M), ant. lobe	0	0	0	1	0	1	0	1	1	1
Mixed Glioma (M)	0	0	0	0	0	0	0	0	1	0
Adenoma (B) ant. Lobe	18	17	23	25	12	25	28	15	19	23
Adenoma (B) int. lobe	2	2	1	0	2	1	0	1	0	0
Pancreas (Endocrine)										
Islet Cell Carcinoma (M)	0	0	1	0	0	1	0	0	0	0
Islet Cell Adenoma (B)	4	5	5	5	2	0	0	0	1	0
Testis										
Mesothelioma (M)	1	1	0	0	0					

Interstitial Cell Adenoma (B)	5	5	1	1	3					
Epididymis										
Mesothelioma (M)	0	0	0	1	0					
Preputial Gland										
Cystadenoma (B)	0	-	0	-	0	0	0	1	0	-
Ovary										
Granulosa Cell Tumor (B)						0	0	0	1	0
Uterus										
Adenocarcinoma (M)						1	0	0	0	0
Malignant Schwannoma (M)						0	1	0	0	0
Stromal Sarcoma (M)						0	1	0	0	0
Leiomyosarcoma (M)						2	0	0	0	0
Carcinoma (M)						0	0	1	0	0
Fibroma (B)						0	1	0	0	0
Stromal Polyp (B)						6	7	3	11	4
Cervix										
Stromal Sarcoma (M)						0	0	0	1	0
Leiomyosarcoma (M)						0	1	0	0	0
Vagina										
Stromal Sarcoma (M)						0	0	1	1	1
Granular Cell Tumor (B)						0	0	0	0	1
Fibroma (B)						0	0	0	1	0
Kidney										
Fibrosarcoma (M)	0	0	0	0	1	0	0	0	0	0
Liposarcoma (M)	0	3	0	0	1	0	0	0	0	0
Renal Mesenchymal Tumor (M)	0	0	0	0	0	0	0	0	1	0
Lipoma (B)	0	1	0	0	0	0	0	0	0	0
Urinary Bladder										
Mast Cell Tumor (B)	0	0	1	0	0	0	0	0	0	0
Tongue										
Squamous Cell Carcinoma (M)	0	0	0	0	0	0	0	0	1	1
Stomach										
Mesothelioma (M)	0	0	0	0	0	1	0	0	0	0
Duodenum										
Adenocarcinoma (M)	0	1	0	0	0	0	0	0	0	0
Carcinoma (M)	0	0	1	0	0	0	0	0	0	0
Fibrosarcoma (M)	0	0	0	0	0	0	0	0	0	1
Leiomyoma (B)	0	0	0	0	0	1	0	0	0	0
Jejunum										
Adenocarcinoma (M)	0	0	0	0	0	1	0	0	0	0
Liver										
Hemangiosarcoma (M)	0	0	0	0	0	1	0	0	0	0
Cholangioma (B)	0	0	0	0	0	1	0	0	0	0
Hepatocellular Adenoma (B)	0	2	0	0	1	1	0	0	1	0
Salivary Gland (Parotid)										
Fibroma (B)	-	-	-	-	1	-	-	-	-	-
Pancreas (Exocrine)										
Acinar Cell Carcinoma (M)	1	0	0	0	0	0	0	0	0	0
Acinar Cell Adenoma (B)	6	1	1	2	4	0	1	0	0	0
Abdominal Cavity										
Hemangiosarcoma (M)	0	0	0	0	0	0	0	1	1	0
Carcinoma (M)	0	0	0	0	0	1	0	0	0	0
Hemangioma (B)	1	0	0	0	0	0	0	0	0	0
Thoracic Cavity										
Liposarcoma (M)	0	-	-	-	0	1	-	-	-	-
Head										

Osteosarcoma (M)	-	-	-	-	-	-	-	-	1	-
Skin and Subcutis										
Fibrosarcoma (M)	3	4	4	7	8	0	2	0	3	0
Hemangiosarcoma (M)	1	3	2	1	0	1	0	1	0	0
Squamous Cell Carcinoma (M)	0	0	2	1	0	0	0	0	0	0
Osteosarcoma (M)	0	0	0	0	1	0	0	0	0	0
Liposarcoma (M)	0	2	0	0	0	0	0	0	0	0
Sarcoma (M)	0	0	0	1	0	0	1	0	0	0
Basal Cell Carcinoma (M)	0	0	1	1	1	0	0	0	0	0
Carcinoma (M)	0	0	0	0	0	0	0	0	0	1
Basal Cell Adenoma (B)	0	1	0	0	1	0	1	3	0	1
Trichoepithelioma (B)	0	0	0	0	0	1	0	0	0	0
Keratoacanthoma (B)	7	6	4	3	2	0	0	1	0	2
Fibroma (B)	2	7	3	2	8	3	2	2	2	1
Fibrolipoma (B)	0	0	0	0	0	0	0	0	1	0
Schwannoma (B)	0	1	0	0	0	0	0	0	0	0
Papilloma (B)	0	3	0	0	1	0	0	0	0	0
Dermal Fibroma (B)	0	1	0	0	0	0	0	0	0	0
Hemangioma (B)	0	1	0	0	0	0	0	0	0	0
Lipoma (B)	0	2	0	0	0	1	0	0	0	1
Hamartoma (B)	1	0	0	0	0	1	0	0	0	0
Mammary Gland										
Carcinoma (M)	0	0	0	0	0	1	2	2	6	3
Fibroadenoma (B)	0	0	0	0	0	19	17	14	12	13
Adenoma (B)	0	0	0	0	0	0	4	3	2	0
Cystadenoma (B)	0	0	0	0	0	0	1	0	0	0
Ear										
Fibroma (B)	-	-	0	0	0	0	0	-	1	0
Eye										
Carcinoma (M)	0	0	1	0	0	0	0	0	0	0
Harderian Gland										
Squamous Cell Carcinoma (M)	0	0	0	1	0	0	0	0	0	0
Brain										
Granular Cell Tumor (M)	0	1	0	0	0	0	0	0	0	0
Granular Cell Tumor (B)	0	0	0	1	0	0	0	1	0	0
Astrocytoma (B)	0	1	0	0	3	0	0	0	0	0
Spinal Cord										
Malignant Astrocytoma (M)	1	0	0	0	0	0	0	0	0	0
Astrocytoma (B)	0	0	0	1	0	0	0	0	0	0
Skeletal Muscle										
Hemangiosarcoma (M)	0	0	0	0	1	0	0	0	0	0
Foot/ Leg										
Hemangioma (B)	0	0	0	0	0	-	1	0	-	0
Bone										
Hemangiosarcoma (M)	-	0	-	0	-	0	1	-	0	-
Sarcoma (M)	-	0	-	1	-	0	0	-	0	-
Cranium										
Neuroblastoma (M)	-	-	-	1	-	-	-	-	-	-
Tail										
Squamous Cell Carcinoma (M)	0	0	0	0	1	-	0	-	-	0

CO = Corn oil; 0 = undosed; (-) lesion not identified (only select animals were examined)

Table 52: Combined Incidence for Statistical Analysis

	Males					Females				
EPA-E mg/kg/day	**CO**	**0**	**91**	**273**	**911**	**CO**	**0**	**91**	**273**	**911**
Number/group	50	50	50	50	50	50	50	50	50	50
Skin/ Subcutis										
Basal Cell Carcinoma (M)	0	0	1	1	1	0	0	0	0	0
Basal Cell Adenoma (B)	0	1	0	0	1	0	1	3	0	1
	0	1	1	1	2	0	1	3	0	1
Squamous Cell Carcinoma (M)	0	0	2	1	0	0	0	0	0	0
Keratoacanthoma (B)	7	6	4	3	2	0	0	1	0	2
	7	6	6	4	2	0	0	1	0	2
Fibrosarcoma (M)	3	4	4	7	8	0	2	0	3	0
Sarcoma (M)	0	0	0	1	0	0	1	0	0	0
Fibroma (B)	2	7	3	2	8	3	2	2	2	1
	5	11	7	10	16	3	5	2	5	1
Lipoma (B)	0	2	0	0	0	1	0	0	0	1
Liposarcoma (M)	0	2	0	0	0	0	0	0	0	0
	0	4	0	0	0	1	0	0	0	1
Mammary Gland										
Fibroadenoma (B)	0	0	0	0	0	19	17	14	12	13
Adenoma (B)	0	0	0	0	0	0	4	3	2	0
Cystadenoma (B)	0	0	0	0	0	0	1	0	0	0
	0	0	0	0	0	19	22	17	14	13
Lung										
Bronchiolo-Alveolar Carcinoma (M)	0	0	0	0	0	0	0	0	0	1
Bronciolo-Alveolar Adenoma (B)	0	1	0	0	1	0	0	0	0	0
	0	1	0	0	1	0	0	0	0	1
Hematopoietic System										
Lymphoma, Lympho (M)	2	1	1	0	0	1	0	0	1	1
Lymphoma, Foll Ctr (M)	0	0	0	0	0	0	1	0	0	0
	2	1	1	0	0	1	1	0	1	1
Pancreas (exocrine)										
Acinar Cell Carcinoma (M)	1	0	0	0	0	0	0	0	0	0
Acinar Cell Adinoma (B)	6	1	1	2	4	0	1	0	0	0
	7	1	1	2	4	0	1	0	0	0
Pancreas (endocrine)										
Islet Cell Carcinoma (M)	0	0	1	0	0	1	0	0	0	0
Islet Cell Adenoma (B)	4	5	5	5	2	0	0	0	1	0
	4	5	6	5	2	1	0	0	1	0
Pituitary Gland										
Carcinoma (M), ant. lobe	0	0	0	1	0	1	0	1	1	1
Adenoma (B) ant. Lobe	18	17	23	25	12	25	28	15	19	23
	18	17	23	26	12	26	28	16	20	24
Thyroid Gland										
Follicular Cell Carcinoma (M)	1	0	0	0	0	0	0	0	0	0
Follicular Cell Adenoma (B)	3	3	0	0	3	2	3	2	1	1
	4	3	0	0	3	2	3	2	1	1
Adrenal Gland										
Cortical Carcinoma (M)	0	0	0	1	0	0	0	0	0	0
Cortical Adenoma (B)	0	0	1	0	0	1	1	1	1	0
	0	0	1	1	0	1	1	1	1	0
Pheochromocytoma (M)	1	1	1	1	0	0	0	0	0	0
Pheochromocytoma (B)	0	9	4	0	1	2	2	2	1	3
	1	10	5	1	1	2	2	2	1	3

Uterus										
Stromal Sarcoma (M)						0	1	0	0	0
Stromal Polyp (B)						6	7	3	11	4
						6	8	3	11	4
Nervous Tissue										
Astrocytoma (B), brain	0	1	0	0	3	0	0	0	0	0
Malignant Astrocytoma (M), sp. cord	1	0	0	0	0	0	0	0	0	0
Astrocytoma (B), spinal cord	0	0	0	1	0	0	0	0	0	0
Mixed Glioma (M), pituitary gland	0	0	0	0	0	0	0	0	1	0
	1	1	0	1	3	0	0	0	1	0
Thymus										
Malignant Thymoma (M)	1	0	0	0	0	0	1	0	0	0
Thymoma (B)	0	0	0	0	0	3	2	2	1	1
Thymoma (M) metast.	0	0	0	0	1	0	0	0	1	0
	1	0	0	0	1	3	3	2	2	1
Kidney										
Liposarcoma (M)	0	3	0	0	1	0	0	0	0	0
Lipoma (B)	0	1	0	0	0	0	0	0	0	0
	0	4	0	0	1	0	0	0	0	0
All Sites										
Hemangiosarcoma (M), skin	1	3	2	1	0	1	0	1	0	0
Hemangioma (B), skin	0	1	0	0	0	0	0	0	0	0
Hemangiosarcoma (M), Sk. muscle	0	0	0	0	1	0	0	0	0	0
Hemangiosarcoma (M), Bone	-	0	-	0	-	0	1	-	0	-
Hemangioma (B), foot/leg	0	0	0	0	0	-	1	0	-	0
Hemangioma (B), larynx	0	0	0	0	0	0	0	0	1	0
Hemangiosarcoma (M), LN mesent.	7	4	4	5	7	1	0	0	2	2
Hemangioma (B), LN mesenteric	0	1	0	0	5	0	0	0	3	4
Hemangiosarcoma (M), LN axillary	0	0	0	0	0	0	1	0	0	0
Hemangioma (B), LN axillary	0	0	0	0	1	0	0	0	0	0
Hemangiosarcoma (M), spleen	0	0	0	0	0	0	1	0	0	0
Hemangiosarcoma (M), liver	0	0	0	0	0	1	0	0	0	0
Hemangiosarcoma (M), Abdom cav	0	0	0	0	0	0	0	1	1	0
Hemangioma (B), Abdominal cavity	1	0	0	0	0	0	0	0	0	0
(TOTAL ALL SITES)	**9**	**9**	**6**	**6**	**14**	**3**	**4**	**2**	**7**	**6**
(TOTAL Hemangiomas)	**1**	**2**	**0**	**0**	**6**	**0**	**1**	**0**	**4**	**4**
(TOTAL Hemangiosarcomas)	8	7	6	6	8	3	3	2	3	2
Mesothelioma (M), testis	1	1	0	0	0					
Mesothelioma (M), epididymis	0	0	0	1	0					
Mesothelioma (M), stomach	0	0	0	0	0	1	0	0	0	0
	1	1	0	1	0	1	0	0	0	0

Table 53: NEOPLASMS AS CAUSE OF DEATH

	Males					Females				
EPA-E mg/kg/day	**0[a]**	**0[b]**	**91**	**273**	**911**	**0[a]**	**0[b]**	**91**	**273**	**911**
Number	31	25	34	32	27	20	17	22	19	32
Fibrosarcoma	0	0	**2**	**3**	**4***	0	0	0	2	0
Fibrosarcoma, renal	0	0	0	0	1	0	0	0	0	0
Fibrosarcoma, duodenum	0	0	0	0	0	0	0	0	0	1
Fibroma	1	1	**2**	**2**	**4**	0	1	0	0	0
Hemangiosarcoma	1	1	2	1	0	0	0	0	0	0
Pituitary carcinoma, ant.	0	0	0	0	0	0	0	0	1	0
Pituitary adenoma	4	3	3	6	1	7	9	8	3	**14**
Mammary Fibroadenoma	0	0	0	0	0	0	1	1	0	2
Mammary Adenoma	0	0	0	0	0	0	0	0	0	1

(a) corn oil control (b) undosed; *P<0.05, (from corn oil control)

Non Neoplastic

EPA-E related non-neoplastic findings included the following:

1. There was an increased incidence of thyroid C-cell hyperplasia in male decedents at all doses versus corn oil control. Additionally, parathyroid hyperplasia was also increased in the male MD and HD decedent population.
2. Hyperplasia of the non-glandular stomach mucosa increased in MD and HD male and female decedents when compared to corn oil control. Stomach erosion/ ulcer in female decedents and scheduled sacrifice animals increased at HD.
3. Seventy-four percent of LD male decedents had adrenal cortical cell focal hyperplasia versus 19% in corn oil control.
4. Bile duct hyperplasia increased in male and female decedents over control at all doses as well as in total incidence in males only.
5. Larynx squamous epithelial cell hyperplasia and pituitary gland hypertrophy increased in male MD and HD decedents over control.
6. Pancreas islet cell hyperplasia increased in incidence in HD male decedents.
7. Chronic progressive nephropathy had a higher incidence in female HD decedents.

Table 54: Non-Neoplastic Findings in Males

EPA-E Dose (mg/kg)	Corn Oil Control		Untreated Control		91		273		911	
Survival Status	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**
Number/group	**31**	**19**	**25**	**25**	**34**	**16**	**32**	**18**	**27**	**23**
Total Number	**50**		**50**		**50**		**50**		**50**	
Thyroid, Diffuse C-cell hyperplasia	9 (29%)	18	9 (36%)	25	19* (56%)	16	19** (59%)	18	18*** (67%)	23
Total	**27**		**34**		**35**		**37****		**41*****	
Stomach, non-glandular mucosa hyperplasia	1 (3%)	2	1 (4%)	0	2 (6%)	2	7 (22%)	5	6* (22%)	2
Total	**3**		**1**		**4**		**12***		**8**	
Parathyroid, diffuse hyperplasia	0 (0)	6	1 (4%)	4	1 (3%)	8	5 (16%)	7	4* (15%)	7
Total	**6**		**5**		**9**		**12**		**11**	
Adrenal, focal cortical cell hyperplasia	6 (19%)	7	11* (44%)	12	25*** (74%)	11	5 (16%)	4	1 (4%)	12
Total	**13**		**23***		**36*****		**9**		**13**	
Adrenal, focal cortical cell hypertrophy	4	15	6	16	5	10	8	10	11*	15
Total	**19**		**22**		**15**		**18**		**26**	
Bile Duct hyperplasia	19 (61%)	19	18 (72%)	23	28* (82%)	16	30** (94%)	17	22 (81%)	23
Total	**38**		**41**		**44**		**47***		**45**	
Larynx, Squamous epithelial hyperplasia	1	6	0	4	0	7	13***	10	8**	6
Total	**7**		**4**		**7**		**23*****		**14**	
Pituitary Gland, ant. focal hypertrophy	0	5	0	8	0	4	7*	6	4*	8
Total	**5**		**8**		**4**		**13**		**12**	

Pancreas, islet cell hyperplasia	0	3	1	2	1	1	2	0	4*	3
Total	**3**		**3**		**2**		**2**		**7**	

DOS = died or euthanized on study; SNC = scheduled necropsy
*P<0.05, **P<0.01, ***P<0.001 (from corn oil control Group 1)

Table 55: Non-Neoplastic Findings in Females

Dose (mg/kg)	**Corn Oil Control**		**Untreated Control**		**91**		**273**		**911**	
Survival Status	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**	**DOS**	**SNC**
Number examined	**20**	**30**	**17**	**33**	**22**	**28**	**19**	**31**	**32**	**18**
Total Number	**50**		**50**		**50**		**50**		**50**	
Stomach, non-glandular mucosa hyperplasia	1 (5%)	2	4 (24%)	0	2 (9%)	0	4 (21%)	0	14** (44%)	0
Total	**3**		**4**		**2**		**4**		**14****	
Stomach, erosion/ ulcer	3 (15%)	0 (0)	3 (18%)	0 (0)	2 (9%)	0 (0)	3 (16%)	0 (0)	8 (25%)	4* (22%)
Total	**3**		**3**		**2**		**3**		**12***	
Liver, Bile Duct hyperplasia	10 (50%)	25	12 (71%)	27	17 (77%)	22	17* (89%)	28	22 (69%)	16
Total	**35**		**39**		**39**		**45***		**38**	
Lung, alveolar foamy macrophage accum.	12	16	7	15	14	18	13	21	24	9
Total	**28**		**22**		**32**		**34**		**33**	
Ovary, hyperplasia epithelial	1	20	8**	17	5	13	5	14	13**	6*
Total	**21**		**25**		**18**		**19**		**19**	
Kidney, chronic prog. nephropathy	9 (45%)	29	11 (65%)	31	19** (86%)	23	12 (63%)	28	28** (88%)	15
Total	**38**		**42**		**42**		**40**		**43**	

DOS = died or euthanized on study; SNC = scheduled necropsy
*P<0.05, **P<0.01, ***P<0.001 (from control Group 1)

Toxicokinetics

Toxicokinetics were not conducted in this study. The table below is from the 28-day repeat-dose toxicology study (#ZOC0001) in Wistar rats (once daily administration by oral gavage). This table is the Amended Version submitted to NDA 202057, as the original TK analysis did not subtract out EPA present in control matrix; and therefore all original reported EPA values were significantly higher than presented below. The following table can be found under Module 2.6.6 (toxicology written summary) under NDA 202057 but may also be found in Study ZOC0001 final report amendment.

Table 56: TK parameters for 28-Day Rat Toxicology Study ZOC0001

Table 2.6.6-5 Toxicokinetic Parameters for Plasma Concentrations of EPA in Rats (Amendment 1 Study ZOC0001)

Sex	Dose (g/kg/day)	Day	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	CL/F (mL/h/kg)	$T_{½el}$ (h)
Male	0.3	1	35990	4010	4.00	7798	5.79
		28	60310	7340	2.00	4974	11.47
	1.0	1	150000	21100	4.00	6461	4.38
		28	138200	19600	2.00	7235	13.24
	2.0	1	72470	9680	4.00	25350	6.14
		28	196000	16400	4.00	10200	6.21
Female	0.3	1	36020	3560	1.00	7345	7.84
		28	54970	6490	2.00	5457	17.14*
	1.0	1	75700	13300	4.00	11840*	6.69*
		28	151600	13100	2.00	6598	12.38
	2.0	1	114600	9230	4.00	13740	9.90
		28	140200	17300	4.00	14270	9.75*

* Unreliable estimate

[Table reproduced from NDA 202057 submission; Study No. 456223]

Dosing Solution Analysis

The study report stated, "Analysis of the formulations and analytical results subsequently generated are the responsibility of the Sponsor and are not considered to be part of the study and are therefore not in this report."

Table 57: Exposure Margins – 2yr. Rat

AMR101 Doses (mg/kg)	91 mg/kg	273 mg/kg	911 mg/kg
Rat mg/m^2	546	1638	5466
Human mg/m^2 (MRHD 4g/day)	2479	2479	2479
Safety Margin (body surface area)	<1X	<1X	2.2X
Rat AUC_{0-t} (ng.hr/mL) Day 28	ND	57,640	144,900
Human AUC_{0-24hr} (ng.hr/mL) Day 28	20,300	20,300	20,300
Safety Margin (4g AUC exposure)	ND	3X	7X

- MRHD of 4 g/day = 67 mg/kg for a 60 kg human adult
- **Rat AUC_{0-t} data is derived from the 28-day repeat dose toxicology** (Amarin sponsored) study # ZOC0001. Average of male and female from week 4. TK data from doses used in the 28 day rat study (#ZOC0001, amended) that approximated that used in the 2 yr. CARC study were used for comparison (*i.e.* 300 mg/kg dose group used for 273 mg/kg and 1000 mg/kg dose group used for 911 mg/kg)
- Human Exposure Data is from Study AMR-01-01-0018 CSR (28-Day PK study) where data was originally reported as AUC_{0-24hr} (µg.hr/mL) and is baseline-unadjusted for unesterified (free) plasma EPA (4g/day given 2g BID)

Study title: 26-Week Oral Gavage Oncogenicity Study with AMR101 in 001178-T (Hemizygous) Mice

Study no.:	8222196
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	April 7, 2010
GLP compliance:	Yes, page 2
QA statement:	Yes, page 3
Drug, lot #, and % purity:	AMR101 (ethyl-EPA), Lot # EE070IX, 98.2% Purity
CAC concurrence:	Yes; ECAC Meeting held March 2, 2010

Key Study Findings

- Mortalities (aside from accidental causes) included sacrifice of one HD male for skin ulceration/ inflammation of the proximal tail on day 88 and one HD male was found dead with a histopathology correlate of squamous cell carcinoma on day 122. One female in the 1 g/kg dose group was sacrificed with histopathology evidence of erythroleukemia. One HD female was found dead with an ovarian carcinoma and one HD female died from an undetermined cause.
- Clinical signs included nodules around the tail associated with swelling, scabs, sores and alopecia in both sexes predominantly at the 4.6 g/kg HD but also to a lesser extent at the Mid-High dose of 2.0 g/kg. Histopathology correlates of acanthosis/ hyperkeratosis, erosion/ ulceration and inflammation were observed at doses of ≥2.0 g/kg. In HD males, benign squamous cell papilloma was observed histologically.
- Although HD females tended to have a decrease in food consumption, their overall body weight gain was higher than control animals, which might be explained by the high caloric intake of AMR101. Males had an overall decrease in BW gain when compared to control and correlated with an intermittent decrease in food consumption.
- There was an increase in neutrophils at the HD in both sexes that may have been due to the skin irritation observed in these animals (nodules, swollen, alopecia, sores, scabs).
- There were minor organ weight changes including an increase in spleen weight in both sexes at HD (possibly attributed to inflammation of the skin at the site of the proximal tail), increased kidney weight and decreased seminal vesicle weight in HD males, and a decrease in uterine weight in HD females.
- Non-neoplastic lesions predominantly consisted of acanthosis/ hyperkeratosis, erosion/ ulceration and inflammation of the skin/ subcutis of the proximal tail as well as perirectal skin at doses ≥2.0 g/kg; mesenteric lymph node vein thrombosis and inflammation was observed in males at doses ≥2.0 g/kg and in females at 4.6 g/kg; ileum mesenteric vein thrombosis predominantly at HD in both sexes; bone marrow

(femur and sternum) myeloid hyperplasia in both sexes at HD; extramedullary hematopoiesis of the liver and spleen in both sexes at HD; increased gall bladder hyaline secretion at HD in both sexes; nonglandular stomach hyperplasia/ hyperkeratosis and inflammation predominantly at ≥2.0 g/kg in both sexes; and epididymis inflammation in HD males.

- A statistically significant increase in skin/ subcutis squamous cell papillomas of the proximal tail in AMR101 HD males was observed.
- TK analysis was conducted and determined that exposure at steady state was less than dose proportional in both sexes, increased from Day 1 to Day 28, and was slightly greater in females than males.

Adequacy of Carcinogenicity Study

The study design of this carcinogenicity 6 month transgenic mouse model was adequate. The positive control animals treated with 75 mg/kg *i.p.* MNU on Day 1 of the dosing phase resulted in 2 males and 4 females with increased leukocyte counts indicative of benign or neoplastic lymphocytosis. MNU increased the incidence of lymphosarcomas in males and females, increased nonglandular stomach squamous cell B-papillomas in males and females, increased nonglandular stomach squamous cell B-papilloma, M-carcinomas in males and increased B-papilloma of proximal tail or miscellaneous sites in females.

Appropriateness of Test Models

The transgenic HRAS mouse model was appropriate to assess carcinogenicity.

Evaluation of Tumor Findings

AMR101 was found to be positive for squamous cell papillomas associated with the proximal tail in male mice.

Table 58: Statistical Analysis – Tg.rasH2 Mouse

Gender	Tissue	Tumor	Common/ Uncommon	Pairwise Analysis		Trend Analysis	
				P - value	Sig?	P - value	Sig?
Male	Skin/ Subcutis	Papilloma, Squamous Cell (Proximal Tail), single	Common	0.0546	No	0.0014	YES
Male	Skin/ Subcutis	Papilloma, Squamous Cell (Proximal Tail), all	Common	0.0248	No*	0.0003	YES

Pairwise analysis is to the HD of 4.6 g/kg
(*): Not statistically significant when using the criteria of P<0.01 for 2 year studies. For transgenic mouse studies, due to the lower number of animals used, statistics review would consider a P value of P<0.05 statistically significant for a pairwise comparison.

Table 59: MALE - Significant Tumor Incidence in the 6 month Tg.rasH2 Mouse

AMR101 (mg/kg)	0	500	1000	2000	4600
Skin/Subcutis Squamous cell Papilloma (Prox. Tail)	0	0	0	1	4
Skin/Subcutis Papillomas (Prox. Tail)	0	0	0	1	5

Methods

Doses:	

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Group[a]	No. of Animals Male	No. of Animals Female	Dose Level (g/kg)	Dose Volume[b] (microliter/kg)
Main Study				
1 (Control - RODI water)	25	25	0	5000
2 (Low)	25	25	0.50	550
3 (Mid)	25	25	1.00	1100
4 (Mid-High)	25	25	2.00	2200
5 (High)	25	25	4.60	5000
6 (Positive Control)[c]	15	15	75 mg/kg[c]	10 mL/kg[c]
Toxicokinetic Study				
7 (Control - RODI water)	6	6	0	5000
8 (Low)	48	48	0.50	550
9 (Mid-High)	48	48	2.00	2200
10 (High)	48	48	4.60	5000

a Groups 1 and 7 received reverse osmosis/deionized (RODI) water only.
b The dose level was achieved by varying the volume of the test article formulation delivered. For Groups 1 through 5 and 7 through 10, glass microliter syringes were used for dosing. Dose volumes were calculated to take into account the density of AMR101 (0.911 g/mL).
c Group 6 animals were dosed with one intraperitoneal dose of N-methyl-N-nitrosourea (MNU) on Day 1 of the dosing phase at a dose level of 75 mg/kg/mouse and a dose volume of 10 mL/kg.

Frequency of dosing:	Once daily
Dose volume:	Dose volumes of 0.55, 1.1, 2.2, and 5.0 mL/kg/day (for 0.50, 1.00, 2.00, and 4.60 AMR101 g/kg/day, respectively)
Route of administration:	Oral gavage for AMR101 and intraperitoneal injection for positive control MNU (N-methyl-N-nitrosourea)
Formulation/Vehicle:	Test article delivered neat
Basis of dose selection:	A 4-week oral gavage toxicity study (Study No. 459549) with AMR101 using wild type CByB6F1 (rasH2) mice at doses up to 2.00 g/kg/day (2.2 mL/kg/day).
Species/Strain:	Male and female Model 001178-T (hemizygous), CByB6F1-Tg(HRAS)2Jic (main study) and 001178-W (wild), CByB6F1-Tg(HRAS)2Jic (toxicokinetic) mice (b) (4)
Number/Sex/Group:	25/sex/group for AMR101 main study; 15/sex for positive control
Age:	7-8 weeks old at initiation of dosing
Animal housing:	Individually housed in stainless steel cages except during acclimation (pair-housed by sex)
Paradigm for dietary restriction:	None. Certified Rodent Diet #2016C (b) (4) and water *ad libitum*. C18:3w3 linolenic fatty acid was present at 0.1% in the diet (can be converted to EPA and DHA). This diet did not contain fish meal.
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	TK: 48/sex for 0.5, 2 and 4.6 g/kg/day AMR101 dose groups; 6/sex for TK water control
Deviation from study protocol:	None that affected study integrity

Observations and Results

Mortality

Animals were checked twice daily for mortality, abnormalities and signs of pain or distress.

1. One male administered 4.6 g/kg AMR101 was sacrificed in moribund condition on Day 88 due to skin ulceration/inflammation of the proximal tail (No. A15900). One control male was found dead on Day 29 after struggling during dosing (No. A15798).
2. One male administered 4.6 g/kg AMR101 was found dead on Day 122. Squamous cell carcinoma of the non-glandular stomach was noted histologically (No. A15880).
3. One control female was found dead on Day 46 and was attributed to a cage injury (No. A16094).
4. One female administered 1.0 g/kg was sacrificed on Day 89 after presenting with few feces, irregular respiration, brown perineal haircoat, hypoactivity, and cold to the touch. Erythroleukemia was noted histologically (Animal No. A16121).
5. One female administered 1.0 g/kg was found dead on Day 123 (No. A16141). This death was determined at necropsy to be accidental.
6. Three females administered 4.60 g/kg were found dead:
   No. A16176 died on Day 39 as a result of a gavage error
   No. A16182 was found dead on Day 149, and an ovarian carcinoma was noted
   No. A16185 died on Day 148 and the cause was undetermined.
   Clinical signs for these females included nodules, sores or swelling on the proximal tail, rough haircoat, and/or hunched posture.
7. In the positive control group, ten males and four females were found dead or sacrificed early. Overall survival was 73% for females and 33% for males

Adjusted survival at week 27 was ≥92% for all groups given AMR101.

Table 60: Adjusted Survival at Week 27 in the Tg.rasH2 mouse study

Adjusted Survival* at Week 27

Dose Level(g/kg)	0	0.50	1.00	2.00	4.60
Males	24 (100%)	25 (100%)	25 (100%)	25 (100%)	23 (92%)
Females	24 (100%)	25 (100%)	23 (96%)	25 (100%)	22 (92%)

* = Adjusted survival reflects the original number of animals/sex/group (25) minus the number of accidental deaths/gavage errors/sex/group.

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Reviewer's Note: When calculating the absolute percent survival (not adjusted to accidental deaths), female survival at the HD of 4.6 g/kg was 88%.

Clinical Signs

Once daily (dosing phase), cageside observations were made for each animal and weekly detailed observations were also recorded.

The main clinical observations that were noted included nodules around the tail associated with swelling, scabs or sores and alopecia in both sexes predominantly at a dose of 4.6 g/kg AMR101 but also noted to a lesser extent at 2.0 g/kg. Rough haircoat was also noted at doses of ≥ 2.0 g/kg in both sexes. Masses were noted in 1 male/ 1 female in the 2.0 g/kg dose group and in 3 males/ 1 female in the 4.6 g/kg dose group.

Animal A15900 (4.6 g/kg male) that was sacrificed on Day 88 had multiple large nodules on the proximal tail, was hypoactive, cold to the touch, had irregular respiration and brown perineal haircoat.

Table 61: Clinical Signs in the Tg.rasH2 Mouse

	Males (n=25)					Females (n=25)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Hunched	-	1	-	1	-	-	-	-	1	2
Swollen, Proximal Tail	-	-	-	2	25	-	-	-	-	22
Swollen, Sacral	-	-	-	-	5	-	-	-	-	6
Thin	-	2	-	1	1	-	-	1	3	2
Small Nodule, Proximal Tail	-	-	-	-	6	-	-	-	-	12
Large Nodule, Proximal Tail	-	-	-	-	18	-	-	-	-	14
Large Nodule, Sacral	-	-	-	-	1	-	-	-	-	-
Multiple small nodules, proximal tail	-	-	-	1	4	-	-	-	-	4
Multiple large nodules, proximal tail	-	-	-	1	19	-	-	-	-	8
Hypoactive	-	-	-	-	4	-	-	1	-	-
Mass	-	-	-	1	3	-	-	-	1	1
Irregular Respiration	-	-	-	-	1	-	-	1	-	-
Alopecia, left dorsal abdomen	-	-	-	-	2	-	-	-	-	-
Alopecia, Proximal Tail	-	-	-	-	-	-	-	-	-	2
Alopecia, Sacral	-	-	-	-	12	-	-	-	-	7
Brown Haircoat, perineal area	-	-	-	-	1	-	-	1	-	-
Cold to Touch, entire body	-	-	-	-	1	-	-	1	-	-
Rough Haircoat	4	5	5	20	14	1	-	1	10	12
Sore/scab, Proximal Tail	-	-	-	-	20	-	-	-	-	7
Sore/scab, sacral	-	-	-	-	1	-	-	-	-	1

Body Weights

Body weights were recorded during the predose phase, before dosing on Day 1 and weekly during the dosing phase. There were body weight gain decreases (as compared to controls) observed beginning at week 9 for males and week 7 for females that correlated to reduced food consumption; however the overall weight gain during the dosing period for females was similar to control while the HD animals had a higher weight gain than control. Males had an overall decrease in weight gain in all dose groups as compared to control.

Table 62: Mean Body Weights in Tg.rasH2 Mice (g)

	Males (n=25)					Females (n=25)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Week 1 Dosing	22.7	24.1	23.2	22.8	23.6	18.7	18.4	18.5	18.8	18.5
Week 13 Dosing	26.0	26.5	26.2	25.4	26.1	21.5	21.3	21.2	21.3	21.9
Week 27 Dosing	27.8	28.8	27.3	26.8	27.7	22.4	22.5	22.3	22.6	23.4
Week 1 - 13 Weight Change	3.3	2.4	3.0	2.6	2.5	2.8	2.9	2.7	2.5	3.4
Week 13 - 27 Weight Change	1.8	2.3	1.1	1.4	1.6	0.9	1.2	1.1	1.3	1.5
Week 1 - 27 Weight Change	5.1	4.7	4.1	4.0	4.1	3.7	4.1	3.8	3.8	4.9

Table 63: Mean Body Weight Gain in Tg.rasH2 Mice

Mean Body Weight Gain Percent Difference from Controls - Males

Dose (g/kg)	0.50	1.00	2.00	4.60
Weeks 1-13	↓25.0*	↓9.4	↓18.8*	↓18.8
Weeks 1-27	↓7.8	↓21.6	↓21.6	↓17.6

↓= Decrease; * = Significant at $p \leq 0.05$.

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Feed Consumption

Food consumption was recorded weekly for each animal during the dosing phase.

Mean food consumption decreased for most weekly intervals for males at all AMR101 doses and for females at AMR101 doses of ≥1.0 g/kg. While HD (4.6 g/kg) females generally had a trend for decreased food consumption, these animals gained weight as compared to control. This may be due to the high caloric intake of the test article (omega-3 fatty acids).

Table 64: Mean Food Consumption in Tg.rasH2 Mice (g/animal/period)

	Males (n=25)					Females (n=25)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Week 1 Dosing	34.8	35.4	34.7	33.4	34.8	32.7	32.5	30.5	30.9	31.0
Week 7 Dosing	34.2	33.4	33.2	28.9*	31.5	33.5	32.5	30.3*	28.6*	30.2*
Week 14 Dosing	32.5	31.0	32.2	32.1	36.1*	30.5	29.6	31.4	30.4	33.3
Week 21 Dosing	35.1	34.5	32.2	32.0*	31.7	35.2	33.2	32.3*	29.0*	27.7*
Week 26 Dosing	32.0	32.2	32.3	30.6	31.7	30.1	31.3	30.3	29.9	28.6*

(*) $p \leq 0.05$

Hematology

Blood samples were collected at study termination from surviving non-fasted main study animals via cardiac puncture for analysis of the following hematology parameters: red blood cell count, hemoglobin, hematocrit, platelet count, white blood cell count, differential blood cell count, blood smear, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume and reticulocyte count.

There were mild increases in neutrophil counts predominantly at the HD in both sexes. This increase is likely related to the nodules/ skin irritation observed in this dose group.

Table 65: Hematology Values in Tg.rasH2 Mice

	Males (n=25)					Females (n=25)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
HCT (%)	60.0	58.5	57.9	58.1	57.4	53.0	57.4*	54.3	55.8*	54.6
NEUT (E3/ µL)	0.78	0.78	0.98	0.98	1.07	0.81	0.80	0.75	0.94	1.10

(*) p≤ 0.05

When compared to the range of values from control animals, the following mice had elevated neutrophil counts as follows:

Table 66: Neutrophil Counts in Individual Tg.rasH2 Mice

Dose Group	Sex	Animal No.	Neutrophil count (10^3/µL)
4	M	A15879	2.25
5	M	A15893	1.82
5	M	A15892	1.99
4	F	A16160	2.28
4	F	A16162	1.97
5	F	A16190	3.75
5	F	A16192	2.41

Total leukocyte count of > 10,000 cells/ µL (predominantly lymphocytes) suggestive of benign or neoplastic lymphocytosis occurred at the following incidence:

Table 67: Elevated Leukocyte Counts in Male and Female Tg.rasH2 Mice

Group	Male	Female
Control (water)	1	3
0.5 g/kg AMR101	1	1
1.0 g/kg AMR101	1	2
2.0 g/kg AMR101	1	-
4.6 g/kg AMR101	1	-
75 mg/kg MNU (+) Control	2	4*

(*) One additional female had increased total leukocyte counts, predominantly with neutrophils

Gross Pathology

Necropsy was performed on animals that died or were sacrificed before study termination. All surviving animals were weighed and necropsied. Specified organ weights (shown below) were recorded and bone marrow smears were prepared and stained (but not evaluated) for the following: adrenal gland (2), brain, epididymis (2), heart, kidney (2), liver with gall bladder (drained), lung, ovary (2), prostate, salivary gland (mandibular, 2), seminal vesicle, spleen, testis (2), thyroid (2 lobes) with parathyroid, uterus.

Nodules, abrasion and/or masses in the skin (proximal tail region) were observed that correlated to microscopic findings of benign squamous cell papilloma in males given 4.6

g/kg and acanthosis/ hyperkeratosis, erosion/ ulceration, and/ or inflammation in animals administered ≥2.0 g/kg.

Macroscopic Observations

Table 68: Summary of Macroscopic Observations - All Animals / (Unscheduled Deaths)

	Males (n=25)					Females (n=25)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Spleen, Large	-	-	-	-	1/(1)	1	-	-	-	3/(1)
Stomach GI, Raised Area	-	-	-	-	1	4	1	1	-	7
Skin, Abrasion	-	-	-	-	13/(1)	-	-	-	-	7/(1)
Skin, Nodule	-	-	-	1	25/(2)	-	-	-	-	20
Skin, Confirmed mass	-	-	-	1	3	-	-	-	-	-
Ovary, Cyst						-	-	-	1	1/(1)
Uterus, Distended						-	6	5	4	4
Tail, Mass	-	-	-	-	-	-	-	-	-	1/(1)
Tail, Confirmed mass	-	-	-	-	1	-	-	-	-	1
LN, Inguinal, Large	-	-	-	-	3/(1)	-	-	-	-	-

Organ Weights

Seminal vesicle and uterine weights decreased at the HD by all three parameters (absolute weight, % body weight and % brain weight). Although not significant, spleen weights increased in both sexes at HD which could be attributed to skin nodules and corresponding inflammation.

Table 69: Organ Weights in Tg.rasH2 Mice

	Males (n=25)					Females (n=25)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Kidney (g)	0.5669	0.5780	0.5451	0.5373	**0.5256***	0.3834	0.3896	0.3870	0.3766	0.3893
% BW	2.0786	2.0441	2.0278	2.0249	1.9876	1.7410	1.7556	1.7567	1.7128	1.7572
% Brain Wt.	113.41	113.26	109.18	107.35	**106.13***	73.06	74.48	74.88	73.33	75.98
Spleen (g)	0.0818	0.1039	0.0888	0.0884	0.1301	0.1113	0.1078	0.1199	0.1135	0.1484
% BW	0.2998	0.3681	0.3300	0.3326	0.4893	0.5056	0.4847	0.5420	0.5145	0.6700
% Brain Wt.	16.33	20.35	17.81	17.63	26.20	21.10	20.65	23.21	22.22	28.91
Liver/ Gall bladder (g)	1.4903	1.5644	1.4622	**1.4080***	1.5377	1.2220	**1.1848***	**1.1879***	**1.1342***	1.2322
% BW	5.4643	5.5301	5.4451	5.2996	5.8113	5.5516	5.3335	5.3891	**5.1415***	5.5537
% Brain Wt.	298.29	306.40	292.78	281.34	310.88	233.04	**226.69***	230.02	**220.92***	240.52
Seminal Vesicles (g)	0.4187	0.4358	0.3831	0.3701	**0.3088***					
% BW	1.5301	1.5440	1.4250	1.3936	**1.1668***					
% Brain Wt.	83.78	85.59	76.41	73.97	**62.20***					
Uterus (g)						0.3358	0.3604	0.3785	0.2810	**0.2591***
% BW						1.5178	1.6176	1.7140	1.2636	**1.1634***
% Brain Wt.						64.21	69.03	73.16	54.77	**50.58***

(*) p≤ 0.05

Histopathology

Peer Review: No

Tissues were preserved in 10% neutral-buffered formalin, with the exception of the eyes, optic nerves, Harderian glands, and testes, which were preserved in modified Davidson's fixative. All tails from scheduled sacrifices and any collected at unscheduled sacrifices were processed and examined from groups 1-5. Macroscopic lesions and

thymus from all animals in Group 6 (positive control) were processed and examined microscopically. The following tissues were preserved:

Table 70: Tissues Examined for Histopathology (Tg.rasH2 Mice)

adrenal (2)	lymph node (mesenteric)
aorta	mammary gland (females)
brain	optic nerve (2)[a]
cecum	ovary (2)
cervix	pancreas
colon	pituitary gland
duodenum	prostate
epididymis (2)	rectum
esophagus	salivary gland [mandibular (2)]
eye (2)[a]	seminal vesicle
femur with bone marrow (articular surface of the distal end to include femoro tibial joint)	skeletal muscle (thigh)
	skin/subcutis
	spinal cord (cervical, thoracic, and lumbar)
gallbladder	spleen
heart	sternum with bone marrow
ileum	femoro tibial joint
incisors (upper and lower right to include maxilla and mandible)	stomach
	testis (2)[a]
jejunum	thymus
kidney (2)	thyroid (2 lobes) with parathyroid
lacrimal gland	tongue
lesions	trachea
liver	urinary bladder
lung with large bronchi	uterus
lymph node (mandibular)	vagina

a Preserved in modified Davidson's fixative.

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Note: Protocol Amendment 1 removed the femoro tibial joint and added the sciatic nerve and harderian gland (Davidson's fixative). Protocol Amendment 6 stated that although incisors were collected they were not processed for histology as it was not specified in the study design.

Protocol Deviations:
The listed tissues for the following animals were recorded as missing and not examined microscopically. For tissues marked with an asterisk, only one of the pair was missing:

Table 71: Missing Tissues from Individual Animals (Tg.rasH2 Mice)

Tissue	Animal No.	Group/Sex
Eye	A15842*	3/M
	A16071*	1/F
Prostate	A15874	4/M
Epididymis	A15884*	5/M
Ovary	A16086*	1/F
	A16114*	2/F
	A16119*	2/F
	A16120*	3/F
	A16134*	3/F
	A16136*	3/F
	A16141*	3/F
	A16146*	4/F
	A16151*	4/F
	A16167*	4/F
	A16192*	5/F
Tail	A16121	3/F
	A16141	3/F
	A16146	4/F
	A16176	5/F
Adrenal, cortex	A15794*	1/M
	A15815*	2/M
	A15817*	2/M
	A15818*	2/M
	A16117*	2/F
	A16186*	5/F
	A16189*	5/F
Adrenal, medulla	A15794*	1/M
	A15798*	1/M
	A15815*	2/M
	A15817	2/M
	A15818*	2/M
	A15880*	5/M
	A15891*	5/M
	A16117*	2/F
	A16186*	5/F
	A16189*	5/F
Pituitary	A15798	1/M
	A16100	2/F
	A16179	5/F
Trachea	A15847	3/M
	A16176	5/F
Thyroid	A15784*	1/M
	A15785*	1/M
	A15788*	1/M
	A15866*	4/M
	A16086*	1/F
	A16090	1/F
	A16120	3/F
	A16137*	3/F
	A16139*	3/F
	A16163*	4/F
	A16166*	4/F
	A16176	5/F
	A16181*	5/F
	A16189*	5/F
Parathyroid	A16120	3/F
	A16176	5/F
Tongue	A15887	5/M
Gallbladder	A15853	3/M
	A16137	3/F
Thymus	A16180	5/F
Urinary bladder	A15859	4/M
Stomach, glandular	A15798	1/M
Stomach, nonglandular	A15798	1/M
Duodenum	A15798	1/M
Rectum	A15791	1/M
	A16120	3/F
Lymph node, mesenteric	A15780	1/M
	A15853	3/M
	A15898	5/M
	A16137	3/F
Lymph node, mandibular	A15785	1/M
	A15794	1/M
	A15798	1/M
	A15859	4/M
	A15861	4/M
	A16129	3/F
	A16134	3/F
	A16146	4/F
	A16150	4/F
Lacrimal gland	A15784	1/M
	A15793	1/M
	A15795	1/M
	A15851	3/M
	A15852	3/M
	A15860	4/M
	A15875	4/M
	A15893	5/M
	A16079	1/F
Optic nerve	A15783	1/M
	A15802	1/M
	A15807	2/M
	A15808*	2/M
	A15822	2/M
	A15825	2/M
	A15830	3/M
	A15839	3/M
	A15842*	3/M
	A15848	3/M
	A15850	3/M
	A15853	3/M
	A15855	4/M
	A15864	4/M
	A15867	4/M
	A15869	4/M
	A15874	4/M
	A15880	5/M
	A15894	5/M
	A16077	1/F
	A16095	2/F
	A16107	2/F
	A16131	3/F
	A16145	4/F
	A16154	4/F
	A16161*	4/F
	A16167	4/F
	A16168	4/F
	A16182	5/F
	A16183	5/F

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Neoplastic

There was a statistically significant increased incidence of benign squamous cell papilloma (5/25 animals) in the skin/ subcutis (proximal tail) of males given 4.60 g/kg

and correlated with the gross pathology finding of skin masses. Histologically these lesions were well differentiated and associated with proliferation and inflammation.

Table 72: Incidence of Neoplasms in Tg.rasH2 Mice (All)

	Males (All)					Females (All)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Liver										
B-Adenoma, Hepatocellular					1					
C-Hematopoietic neoplasm								1		
C-Vascular Neoplasm			1							
Spleen										
C-Vascular Neoplasm	1	1	2	1				2	1	
C-Hematopoietic neoplasm								1		
Stomach, Glandular										
I-Squamous cell carcinoma					1					
C-Vascular Neoplasm							1			
Stomach, Nonglandular										
B-Papilloma, Squamous cell	1				1					1
M-Carcinoma, Squamous cell					1					
C-Vascular Neoplasm							1			
Skin/Subcutis										
B-Papilloma, Sq. cell (Prox Tail)				1	4				1	
B-Papilloma, Sq. cell (Prox Tail, multi)					1					
Epididymis										
N-Squamous cell carcinoma, metastat.					1					
Ovary										
C-Vascular Neoplasm										1
M-Carcinoma										1
Body, Whole/Cav										
M-Hemangiosarcoma	1	1	3	1		1	1	2		
B-Benign Hemangioma									1	1
M-Leukemia, Erythrocytic								1		

B Primary, benign neoplasm **C** Multicentric neoplasm **I** Locally invasive neoplasm
M Primary, malignant neoplasm **N** Metastatic neoplasm

Table 73: Incidence of Neoplasms in Tg.rasH2 Mice (Unscheduled Deaths)

	Males (Unscheduled Deaths)					Females (Unscheduled Deaths)				
No. in group	**1**	**0**	**0**	**0**	**2**	**1**	**0**	**2**	**0**	**3**
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Liver										
C-Hematopoietic neoplasm								1		
Spleen										
C-Hematopoietic neoplasm								1		
Stomach, Glandular										
I-Squamous cell carcinoma					1					
Stomach, Nonglandular										
B-Papilloma, Squamous cell										1
M-Carcinoma, Squamous cell					1					
Pancreas										
I-Squamous cell carcinoma, invasive					1					
Skin/Subcutis										
B-Papilloma, Sq. cell (Prox Tail)					1					
Epididymis										
N-Squamous cell carcinoma, metastat.					1					
Ovary										
M-Carcinoma										1
Body, Whole/Cav										
M-Leukemia, Erythrocytic								1		

Positive Control MNU:
In positive control animals treated with 75 mg/kg *i.p.* MNU on Day 1 of the dosing phase, there were 2 males and 4 females with increased leukocyte counts indicative of benign or neoplastic lymphocytosis. Results of statistical analyses indicated that MNU increased the incidence of lymphosarcomas in males and females, increased nonglandular stomach squamous cell B-papillomas in males and females, increased nonglandular stomach squamous cell B-papilloma, M-carcinomas in males and increased B-papilloma of proximal tail or miscellaneous site in females.

CDER statistical analysis also confirmed statistically significant increases in lymphosarcomas (♀/♂), skin/subcutis papillomas (♀), nonglandular stomach squamous cell papillomas (♀/♂), and vagina squamous cell papillomas (♀) in MNU treated mice.

Non Neoplastic
There was a statistically significant increased incidence of:

- acanthosis/hyperkeratosis, erosion/ulceration, and/or inflammation involving the skin/subcutis (proximal tail) and perirectal skin of animals given ≥2.00 g/kg
- mesenteric vein thrombosis and inflammation in males given ≥2.00 g/kg and females given 4.60 g/kg
- increased pigment in the mesenteric lymph node at all dose levels
- hyperplasia/hyperkeratosis and inflammation in the nonglandular stomach of males given ≥1.00 g/kg and females given ≥2.00 g/kg
- increased hyaline secretion in the gallbladder mucosal epithelial cells in males and females given 4.60 g/kg
- increased inflammation of the epididymis of males given 4.60 g/kg

Table 74: Non-Neoplastic Histopathology Findings in Tg.rasH2 Mice (All)

	Males (All)					Females (All)				
AMR101 (g/kg)	**0**	**0.5**	**1**	**2**	**4.6**	**0**	**0.5**	**1**	**2**	**4.6**
Parathyroid, cyst	1	2	1		1				1	3
Heart, cardiomyopathy									1	1
Liver, hematopoiesis, extramed.	1				2	1				3
Gall Bladder, hyaline secretion, muc. epi.	1	2		1	7				1	8
Spleen, hematopoiesis, extramed.	1	4	3	4	13	12	13	10	11	19
Stomach, Nonglandular										
Hyperplasia/hyperkeratosis squam. cell		2	5	10	10	3	2	2	8	12
Inflammation			3	4	3				2	3
Ileum, mesen. vein, thrombosis/inflamm.	3	3	1	7	13		2		2	4
Rectum										
Perirectal skin, inflammation			1	13	15				16	15
Perirectal skin, acanthosis/hyperkeratosis				14	18				16	18
LN, Mesenteric										
Perimesenteric vein, thrombosis					7				1	3
Increased pigment	1	3	5	7	12	1	4	2	9	12
Skin/Subcutis										
Acanthosis/Hyperkeratosis, prox. tail				21	25				15	24
Erosion/ulcer, prox. tail					11					8
Inflammation, prox. tail				15	25			1	9	23
Epididymis, inflammation	1	2	2	3	9					
Marrow, Femur, Hyperplasia, myeloid					21			1	1	10
Marrow, Sternum, Hyperplasia, myeloid					21			1	1	10
LN, Inguinal, Hyperplasia, lymphocytes					3					

Table 75: Non-Neoplastic Histopathology Findings in Tg.rasH2 Mice (Unscheduled Sacrifice)

	Males (Unscheduled Sacrifice)					Females (Unscheduled Sacrifice)				
No. in group	1	0	0	0	2	1	0	2	0	3
AMR101 (g/kg)	0	0.5	1	2	4.6	0	0.5	1	2	4.6
Liver, hematopoiesis, extramed.					1					2
Gall Bladder, hyaline secretion, muc. epi.										1
Spleen, hematopoiesis, extramed.					2					3
Stomach, Nonglandular										
Hyperplasia/hyperkeratosis squam. cell										1
Inflammation										1
Ileum, mesen. vein, thrombosis/inflamm.					1					1
Rectum										
Perirectal skin, inflammation					1					2
Perirectal skin, acanthosis/hyperkeratosis					1					2
LN, Mesenteric										
Perimesenteric vein, thrombosis					1					
Increased pigment										2
Skin/Subcutis										
Acanthosis/Hyperkeratosis, prox. tail					2					2
Erosion/ulcer, prox. tail					1					1
Inflammation, prox. tail					2					2
Epididymis, inflammation					1					
Marrow, Femur, Hyperplasia, myeloid					2					2
Marrow, Sternum, Hyperplasia, myeloid					2					2
LN, Inguinal, Hyperplasia, lymphocytes					1					

Table 76: Incidence of Skin/ Subcutis Histopathology (Tg.rasH2 Mice)

Incidence of Test Article-Related Acanthosis/Hyperkeratosis, Erosion/Ulceration, Inflammation, and Squamous Cell Papilloma

	AMR101									
Sex	Males					Females				
Dose Level (g/kg)	0	0.50	1.00	2.00	4.60	0	0.50	1.00	2.00	4.60
Skin/Subcutis (Proximal Tail)										
Acanthosis/Hyperkeratosis	0	0	0	21	25	0	0	0	15	24
Erosion/Ulceration	0	0	0	0	11	0	0	0	0	8
Inflammation	0	0	0	15	25	0	0	1	9	23
Squamous Cell Papilloma[a]	0	0	0	1	5	0	0	0	1	0
Rectum (Perirectal Skin)										
Acanthosis/Hyperkeratosis	0	0	0	14	18	0	0	0	16	18
Inflammation	0	0	1	13	15	0	0	0	16	15
Erosion/Ulceration	0	0	0	0	2	0	0	0	0	0

a Includes multiple squamous cell papilloma in Animal No. A15892 (Group 5 male).

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Table 77: Incidence of Thrombosis and Inflammation in the Mesenteric Vein

Incidence of Thrombosis and Inflammation in the Mesenteric Vein

	AMR101									
Sex	Males					Females				
Dose Level (g/kg)	0	0.500	1.0	2.00	4.60	0	0.50	1.00	2.00	4.60
Ileum (mesenteric vein)										
Thrombosis/Inflammation	3	3	1	7	13	0	2	0	2	4
Mesenteric Lymph Node (perimesenteric vein)										
Thrombosis	0	0	0	0	7	0	0	0	1	3

Note: Mesenteric vein present in mesenteric fat attached to ileum and mesenteric lymph node.

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Reviewer's Note:

1. Statistical analysis for the mesenteric lymph node thrombosis: females p= 0.0070, males p<0.0001 (trend analysis); females p=0.0070 (pairwise), males p=0.0050 (pairwise)
2. Statistical analysis for the ileum mesenteric vein thrombosis: females p= 0.0170, males p<0.0001 (trend analysis); females p=0.0550 (pairwise), males p=0.0030 (pairwise)

Table 78: Incidence of Histopathology Findings in Tg.rasH2 Mice

Incidence of Test Article-Related Findings in Nonglandular Stomach, Gallbladder, Mesenteric Lymph Node, and Epididymis

	AMR101									
Sex	Males					Females				
Dose Level (g/kg)	0	0.50	1.00	2.00	4.60	0	0.50	1.00	2.00	4.60
Nonglandular Stomach										
Hyperplasia/Hyperkeratosis, Squamous Cells	0	2	5	10	10	3	2	2	8	12
Inflammation	0	0	3	4	3	0	0	0	2	3
Gallbladder										
Hyaline Secretion, Mucosal Epithelium	1	2	0	1	7	0	0	0	1	8
Mesenteric Lymph Node										
Pigment, Increased	1	3	5	7	12	1	4	2	9	12
Epididymis										
Inflammation	1	3	2	3	9	NA	NA	NA	NA	NA

NA = Not applicable.

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Toxicokinetics

Blood samples were collected via cardiac puncture from non-fasted TK animals on Days 1 and 28 of the dosing phase as follows:

- Group 7 (water control): 3/sex, approximately 2 hours post-dose
- Groups 8, 9, and 10 (AMR101 dose groups): 4/sex/group/time point approximately 0.5, 1, 2, 4, 8, and 24 hours post-dose

EPA (hydrolyzed from AMR101) was detected in all plasma samples from Groups 8, 9, and 10 (0.5, 2.0 and 4.6 mg/kg AMR101, respectively). The increase in EPA exposure was less than dose proportional between groups, there was an increase in exposure from Day 1 to Day 28 for both sexes and females had a slightly higher systemic exposure than males. EPA was detected in some samples from control animals but is expected as it is an endogenous fatty acid.

Table 79: TK Parameters

Toxicokinetic Parameters Indicative of Systemic Exposure to EPA: Effect of Repeat Dosing in Males and Females Following Once-Daily Oral Dosing with AMR101 for 28 Days

Sex	*Group*	*Dose (g/kg)*	*Day*	*AUC(0-∞) (ng.h/mL)*	*AUC(0-t) (ng.h/mL)*	*Cmax(obs) (ng/mL)*
Male	8	0.5	Day 1	21770	7775	759
			Day 28	15950*	11340	1400
	9	2.0	Day 1	22360	19140	1610
			Day 28	86190	40790	2470
	10	4.6	Day 1	40110	34460	3640
			Day 28	143600*	71190	7450
Female	8	0.5	Day 1	11240	9992	1570
			Day 28	20150*	15120	1580
	9	2.0	Day 1	43730	29500	2190
			Day 28	120600	61690	4280
	10	4.6	Day 1	NC	135100	11000
			Day 28	87430	77820	5720

NC Non Calculable
* Unreliable estimate

[Table reproduced from NDA 202057 submission; Study No. 8222196]

Dosing Solution Analysis

Analysis of the test article demonstrated that appearance, anisidine value, tocopherol assay, ethyl-EPA assay, ethyl-EPA-related substances, and microbiological testing results were all within specification.

The test article peroxide value result was higher than the acceptance criteria of 2 meq/kg. An out-of-specification investigation verified the original result, and the test material was deemed suitable for use.

Table 80: Exposure Margins – Tg.rasH2 Mouse

AMR101 Doses (mg/kg)	**500 mg/kg**	**2000 mg/kg**	**4600 mg/kg**
Mouse mg/m^2	1500	6000	13,800
Human mg/m^2 (MRHD 4g/day)	2479	2479	2479
Safety Margin (body surface area)	<1	2.4X	5.5X
Mouse $AUC_{0\text{-}t}$ (ng.hr/mL) Day 28	13,230	51,240	74,505
Human $AUC_{0\text{-}24hr}$ (ng.hr/mL) Day 28	20,300	20,300	20,300
Safety Margin (AUC exposure)	<1	2.5X	3.7X

- MRHD of 4 g/day = 67 mg/kg for a 60 kg human adult
- Mouse AUC is calculated from an average of male/ female exposure values on Day 28
- Human Exposure Data is from Study AMR-01-01-0018 CSR (28-Day PK study) where data is originally reported as $AUC_{0\text{-}24hr}$ (µg.hr/mL); is baseline-unadjusted for unesterified (free) plasma EPA; and was administered as 2g BID

9 Reproductive and Developmental Toxicology

The following reproductive and developmental toxicology studies are summarized in each section from IND 102,457, which include one Amarin sponsored developmental toxicology study in the SD rat and four referenced literature studies sponsored by Mochida Pharmaceuticals and published in support of Epadel, ethyl-EPA, marketing.

Table 81: Study Design Comparison of Reproductive Toxicology Studies

Study	**Amarin** Developmental	**Mochida Sponsored Literature References** Fertility	Teratogenicity	Peri-Post-Natal	Teratogenicity
Species/ Strain	Rat/ Crl:CD(SD) 9 wks	Rat/ Slc: Wistar ♂ 7 wks ♀ 10 wks	Rat/ Slc: Wistar 10 wks	Rat/ Slc: Wistar 13 wks	Rabbit/ New Zealand White
Dosing	2000 mg/kg mineral oil (control) 300, 1000, 2000 mg/kg/day E-EPA	0 (dH_2O) 300, 1000, 3000 mg/kg/day E-EPA	0 (dH_2O) 300, 1000, 3000 mg/kg/day E-EPA	0 (dH_2O) 300, 1000, 3000 mg/kg/day E-EPA	0 (dH_2O) 100, 300, 1000 mg/kg/day E-EPA
Duration	GD6 – GD16	♂: 63d pre-mating until successful copulation ♀: 14d pre-mating to GD7	GD7 – GD17	GD17 – PD20	GD6 – GD18
E-EPA purity	98% Batch# EE070IX	90.65% Lot#K113	90.65% Lot#K113	93.9% Lot#30415	93.9% Lot#30415
Diet	Rat & Mouse Breeder Diet No. 3 (Expanded) SQC (Special Diets)*	Irradiated solid feed FR-2 (Funahashi Nojo)	Irradiated solid feed FR-2 (Funahashi Nojo)	Irradiated solid feed FR-1 (Funahashi Nojo)	Solid Feed GB-1 (Funahashi Nojo)
TK Data	No	No	No	No	No

* included fish meal in ingredient list

In summary, assessment of fertility in the Wistar rat, demonstrated that copulation, fertility and the estrus cycle were not affected by ethyl-EPA doses up to 3000 mg/kg/day. Reproductive toxicity showed the potential for mild variations such as cervical rib or 13th rib (SD rat) and an equivocal developmental abnormality of missing optic nerve that was not dose dependent and could be due to genetic strain differences in the Wistar rat. It is noted that in ADME studies, it was shown that placental transfer in the rat was low; however accumulation of ^{14}C-EPA-E in fetal brain was higher than in maternal brain. ^{14}C-EPA-E was also detected in milk at a higher concentration than in

plasma (6-14 fold higher). The rabbit was a second species used to assess teratogenicity; however malformations were not observed. Body weight and food consumption decreases were observed in HD dams (1.0 g/kg) that were associated in one case with total litter loss. In the F_1 generation in rats, females had a non-statistically significant delay in the estrus cycle of 10 days that could be correlated with decreased copulation and decreased implantation at the HD of 3.0 g/kg. F_1 males also at the HD had a slight increase in the incidence of testis atrophy. While these results are equivocal, they are mentioned here as development and maintenance of the retina (optic nerve) and reproductive organs are very sensitive to levels of the omega-3 fatty acid DHA. While fatty acids compete for cell membrane incorporation and are not necessarily interchangeable in their biologic action, one could argue that the high levels of EPA outcompete for DHA; however EPA can be readily converted to DHA through chain elongation and desaturation reactions in the cell or in the liver.

A tabulated summary of referenced reproductive toxicology studies for ethyl-EPA (used in support of Epadel marketing) sponsored by Mochida as well as one Amarin sponsored rat developmental reprotox study is below:

Table 82: Reproductive Toxicology Studies (Ethyl-EPA) – Summary Table

Study Type Study No. GLP Status	Species/ strain Number/ group	Dose Levels	Study Findings
Literature Reference: Saito 1989 GLP? – Not stated	Wistar Rat Fertility	0, 300, 1000, 3000 mg/kg/day ♂: Treat 63 days before mating ♀: Treat 14 days before mating through GD7; sac GD21	• ↓ BW gain/ ↓ food consumption at 3000 mg/kg (♂) • No fertility or copulation effect (♂/♀) • ↑ placental weight • Skeletal abnormality: cervical rib 0-2-2-5 • 3000 mg/kg: Soiling of fur around anus; lower jaw rubbing and salivation • 1 HD ♂ (parent) had slight atrophy of the **optic nerve**
Literature Reference: Saito 1989 (20:853-66) GLP? – Not stated	Wistar Rat Teratogen-icity	0 (H_2O), 300, 1000, 3000 mg/kg GD7-17 Sac GD21	• Soiling of fur and slight hair loss around anus at 3000 mg/kg • Optic nerve missing in one pup each dose GD21 • One PD21 F_1 was missing optic nerve (3000 mg/kg); PD56 one LD and 1 HD also missing optic nerve.

		or F_1 to PD21/ PD56	• **Total animals missing optic nerve: 0-2-2-2** • **Micro/ano-phthalmia in one LD pup (300 mg/kg) – originates as retinal defect** • Unilateral testis atrophy (1 each in all dose groups) on PD56 • One infertile F_1 ♂, testis atrophy at 3000 mg/kg • Increase in % cervical rib in all doses • F_1: ↓ copulation rate 3000 mg/kg; ♀ delayed estrus cycle 10 days (3000 mg/kg) • Early eruption of lower incisors at 1000 and 3000 mg/kg • Water T-maze test: increase in time to goal for 300 and 1000 mg/kg on 2nd day only
Literature Reference: Shibutani 1989 (20:867-72) GLP? – Not stated	Rabbit (New Zealand white)	0 (H_2O), 100, 300, 1000 mg/kg GD6-18; Sac GD29	• Preliminary tests with 6000 mg/kg/day led to 3/6 ♀ deaths or moribund sacrifice (stomach erosion or ulceration) • One ♀ at 1000 mg/kg had an entire litter of absorbed fetuses with extreme ↓ in food consumption • ↓ BW and ↓ food consumption at 1000 mg/kg but recovered after dosing • ↑ cervical rib at 1000 mg/kg
Literature Reference: Shibutani 1989 (20:873-84) GLP? – Not stated	Wistar Rat	0 (H_2O), 300, 1000, 3000 mg/kg GD17-PD20 (perinatal and nursing) F_0, F_1, F_2 assess-ment	• F_0 ♀ at 3000 mg/kg: oily substance leakage from anus • F_1 ♀ at 1000 mg/kg autopsy: atrophy of cerebral cortex from right lobe to occipital lobe • F_2 fetus at 3000 mg/kg had cervical rib
Study #	Crl:WI(Han)	0 (2000	• Slight increase in staining of ear/

494981 GLP? - YES	Rat	mg/kg mineral oil), 300, 1000, 2000 mg/kg GD6-16; Sac GD20	muzzle/ head at 1000 and 2000 mg/kg (♀) • Minor Variants/Abnormalities: 13th Vestigial Ribs: 0-0-0-2 13th Reduced Ribs: 0-1-3-5

cervical rib = supernumerary rib arising from a cervical vertebra

9.1 Fertility and Early Embryonic Development

One fertility study (segment I) in the Wistar rat was referenced from the scientific literature (M. Saito et al. Iyakuhin Kenkyu, 1989; 20:845-852; certified translation provided) where ethyl-EPA was administered at doses of 0 (distilled water), 0.3, 1.0 and 3.0 g/kg/day by oral gavage. Ethyl-EPA was administered to male rats 63 days before mating until successful copulation and to female rats from 14 days before mating until gestation day 7. On gestation day 21, all dams were sacrificed and the fetuses were examined. Clinical signs of adult animals included soiling of fur at 3.0 g/kg in both sexes as well as lower jaw rubbing and salivation. Males administered 3.0 g/kg had a significant inhibition of weight gain and in females body weights initially decreased but quickly recovered to control levels. One adult male necropsied from the 3.0 g/kg had slight atrophy of the right optic nerve. There was no effect of ethyl-EPA on copulation rate (100%) or fertility (91.7% - 100%) in adult animals of both sexes. There was a minor but statistically significant increase in the placental weights at the 3.0 g/kg dose. There was a dose dependent increased incidence of cervical rib observed that reached statistical significance at the high dose (0-2-2-5 for 0, 0.3, 1.0 and 3.0 g/kg/day, respectively). The NOAEL for this fertility study in male and female rats was 3.0 g/kg (3.0 g/kg = HED 0.484 g/kg = 29 g/day for a 60 kg human = 7-fold higher than the MRHD of 4g/day).

9.2 Embryonic Fetal Development

In a segment II developmental toxicity study in SD rats sponsored by Amarin, twenty pregnant females per group were dosed once daily by oral gavage with control (2000 mg/kg mineral oil), 300, 1000 and 2000 mg/kg ethyl-EPA (AMR101), between gestation days (GD) 6 – 16. As a note, the breeding diet used contained fish meal and therefore was an additional source of omega-3 fatty acids. Fetuses were collected by cesarean section on GD 20 and dams were also necropsied. Mid-dose and high-dose dams had a slight increase in staining of the ears/muzzle/head/ and limbs; however no other effect on mortality, body weight or food consumption was observed. There was no effect on embryo survival, embryo weight or sex ratio. There was a minor variation in that there was an increased incidence of 13th reduced ribs in fetuses (0-1-3-5 for control, 0.3, 1.0 and 3.0 g/kg AMR101, respectively/ litter incidence was 0-1-2-2) from MD and HD animals, and no effect was observed for skeletal ossification. Also there was a slight

increase in 13th vestigial ribs at the 2.0 g/kg dose (0-0-0-2 for control, 0.3, 1.0 and 3.0 g/kg AMR101, respectively). TK studies were not performed to verify levels of systemic EPA exposure. The maternal NOAEL was 2000 mg/kg (2.0 g/kg = HED 0.323 g/kg =19 g/day for a 60 kg human = 5-fold higher than the MRHD of 4g/day) and the embryo-fetal NOAEL was 1000 mg/kg (1.0 g/kg = HED 0.16 g/kg = 9.6 g/day for a 60 kg human = 2.4-fold higher than the MRHD of 4g/day).

In a referenced literature study (segment II) in Wistar rats (M. Saito et al. Iyakuhin Kenkyu, 1989; 20:853-866; certified translation provided), animals were dosed with 0 (distilled water), 0.3, 1.0 and 3.0 g/kg/day ethyl-EPA. Thirty-nine rats per group were used and were administered ethyl-EPA by oral gavage from GD7-17. Fetuses were collected at GD21 from two-thirds of the dams and the remaining surviving offspring were observed following parturition (one-third of dams). Soiling of the fur and slight hair loss around the anus was observed in the HD treated dams and although food consumption decreased in MD and HD animals, there was no significant change in weight gain. In fetuses examined on Day 21, the optic nerve was missing from one animal in each of the ethyl-EPA dose groups (LD, MD and HD) and microphthalmia/ anophthalmia were observed in one LD fetus. F_1 offspring were assessed on postnatal days (PD) 21 and 56, where one HD animal on PD 21 had an absent optic nerve and one LD and one MD animal had an absent optic nerve identified on PD 56 (total incidence of absent optic nerve from GD21 – PD56 was 0-2-2-2 for 0, 0.3 ,1.0 and 3.0 g/kg ethyl-EPA, respectively). Unilateral testis atrophy was also observed in one animal in each of the EPA-E dose groups at day 56 and an infertile F_1 male was also found to have testicular atrophy at HD. Other findings included a non-statistically significant increase in the percent of cervical rib at all EPA-E doses (1.9%, 5.4%, 6.8%, 6.1% for 0, 0.3, 1.0 and 3.0 g/kg ethyl-EPA, respectively), a decrease in the number of rearings of both males and females (open field test) at HD and a non-dose dependent increase in time to goal for the T-Maze Test at LD and MD (in the first maze attempt, but no difference in second attempt). F_1 offspring were tested for fertility and there was a significant decrease in the copulation rate from the HD group in both the first and second mating attempts, and females were found to have a delayed estrus cycle of 10 days. The number of implantations at LD and HD was decreased with an increased corresponding fetal weight. Only one F_2 embryo had an external malformation of exencephaly at the LD. The NOAEL for reproductive toxicity for the dam was 3.0 g/kg (3.0 g/kg = HED 0.484 g/kg = 29 g/day for a 60 kg human = 7-fold higher than the MRHD of 4g/day) as the number of implantations, number of corpora lutea, the number of live fetuses and number of total fetuses was similar between dosed and control groups. The NOAEL for the developmental toxicity of the F_1 generation was 1.0 g/kg (1.0 g/kg = HED 0.161 g/kg = 10 g/day for a 60 kg human = 2.5-fold higher than the MRHD of 4 g/day) as HD animals had a higher combined incidence of retina/ optic nerve defects, testis atrophy, decreased copulation rate, delayed estrus cycle, and decreased number of implantations for the F_1 generation. As a note, The optic nerve finding may be related to the genetic strain of rat (Wistar) as a literature reference has reported unilateral optic nerve aplasia in young (6 wk) Slc:Wistar rats (K. Shibuya *et al.* Vet Pathol. 26; 1989); however optic nerve atrophy is usually observed in rats greater than 1 year of age.

In a referenced literature teratogenicity study (segment II) in New Zealand White rabbits (Y. Shibutani et al. Iyakuhin Kenkyu, 1989; 20:867-872; certified translation provided), 16-18 pregnant animals per group were administered 0 (distilled water), 0.1, 0.3 and 1.0 g/kg/day ethyl-EPA from GD 6-18. On GD 29, dams were sacrificed and the fetuses were examined. One low-dose (LD) dam died on GD 16 but was due to a liver parasite discovered upon necropsy. It is noted however that preliminary testing at 6.0 g/day ethyl-EPA resulted in 3 of 6 animal deaths or moribund sacrifice where pathology showed erosion or ulceration of the fundus gland of the stomach. While no overt clinical signs were observed up to a 1.0 g/kg dose to the dam, a preliminary study with 6 g/kg resulted in an oily substance leaking from the anus, soiling of the fur, decreased body weight and food consumption, blue-white discoloration of the eyes and ears, decreased motility, increased respiration rate and lowered body temperature. HD dams (1.0 g/kg) had a significant decrease in body weight and food consumption that likely led to the resorption of one whole litter in a HD female noted with an extreme decrease in food consumption. There were no treatment related malformations or skeletal anomalies. There was one discrepancy in the literature report as the text stated 2 cases of cervical rib from the HD group (not present in any other dose or control group), but the tabulated data reported only one (of 99 fetuses examined). The NOAEL for reproductive and fetal toxicity is 0.3 g/kg (0.3 g/kg = HED 0.1 g/kg = 6 g/day for a 60 kg human = 1.5-fold higher than the MRHD of 4 g/day) due to decreased body weight, decreased food consumption and loss of one litter at the HD.

9.3 Prenatal and Postnatal Development

In a referenced literature prenatal and postnatal development study (segment III) in Wistar rats (Y. Shibutani et al. Iyakuhin Kenkyu, 1989; 20:873-884; certified translation provided), 23 pregnant animals per group were administered 0 (distilled water), 0.3, 1.0 and 3.0 g/kg/day ethyl-EPA by oral gavage from GD 17-PD 20 (perinatal and nursing period). All dams were examined during the study and at necropsy, F_1 offspring were evaluated for physical development, behavioral differences and reproduction, and F_2 offspring were examined for abnormalities. HD dams had leakage of an oily substance from the anus during ethyl-EPA administration. All of the offspring of two dams in the LD group and one dam in the MD group died by PD 4. There were no physical or behavioral differences in F_1 offspring with the exception of one MD female that upon necropsy was noted with atrophy of the cerebral cortex from the right lobe to the occipital lobe. No differences in F_1 reproduction rate or fertility were noted and F_2 offspring had only one incidence of cervical rib from the HD of 3.0 g/kg. The NOAEL for the reproductive toxicity to F_0 dams and the developmental toxicity to F_1 and F_2 offspring was 3.0 g/kg (3.0 g/kg = HED 0.484 g/kg = 29 g/day for a 60 kg human = 7-fold higher than the MRHD of 4g/day) under the conditions of this study.

10 Special Toxicology Studies

No studies were conducted.

11 Integrated Summary and Safety Evaluation

Background
Under NDA 202057, Amarin Pharmaceuticals proposes the use of Vascepa (icosapent ethyl/ ethyl-EPA/ AMR101) for the treatment of severe hypertriglyceridemia (≥500 mg/dL) at a clinical dose of 4g/day. This 505(b)(2) application relies on scientific literature references that were published in support of Epadel marketing on Japan, an ethyl-EPA product used at a clinical dose of 1.8 g/day for the indications of hyperlipidemia, arteriosclerotic ulceration and alleviation of pain and feeling of cold. It is noted on the package insert for Epadel that when an excess of triglycerides are present, dosage may be increased to 900 mg, three times daily for a total dose of 2.7 g/day. Amarin Pharma relies predominantly on the reproductive toxicology literature for Epadel as well as supplemental studies for repeat dose toxicology (3- and 12- month rat), single dose toxicology in rodents and an extensive literature database on the pharmacology of ethyl-EPA, EPA, and omega-3 fatty acids in general.

There is a long history of human consumption of fish oil containing omega-3 fatty acids as these are available as dietary supplements in both the United States and the European Union. Additionally, menhaden oil (fish oil composed of multiple fatty acids including 18% EPA) at a dose of 3g/day (EPA + DHA) is designated as GRAS by the FDA. This level was set as there remains a concern that both EPA and DHA may increase bleeding times, increase LDL levels and have an effect on glycemic control in non-insulin dependent diabetics. It should be noted however that fish oil is a complex mixture of multiple fatty acids with varying degrees of unsaturation. Even among omega-3 fatty acids, they are not considered as biologically interchangeable as there are differential effects on cellular signaling, receptor targets and even on cell type specificity.

EPA is an essential omega-3 fatty acid (FA) available naturally in the diet predominantly through the consumption of fish, fish oil or at low level in some types of nuts or seeds. Ethyl-EPA is the ethyl-ester of EPA and therefore is a pro-drug. It is hydrolyzed very quickly to EPA by esterases such as pancreatic lipase in the intestine and is usually below the level of analytical detection in serum. As EPA is a long-chain fatty acid (≥20 carbons), it is absorbed into the villi of the small intestine, incorporated into chylomicrons and transported to the blood via the intestinal lymphatics and ultimately distributed to the systemic circulation. As FA compete with each other for membrane incorporation, increases in the available fraction of omega-3 FA such as EPA would result in decreases in other FA such as the omega-6 FA arachidonic acid or linoleic acid. At a cellular level, EPA as well as other omega-3 fatty acids can activate PPAR signaling, regulate leukotriene and prostaglandin production (anti-inflammatory), alter the synthesis of triglycerides and cholesterol production by the liver, affect the hypothalamus-pituitary-adrenal (HPA) axis and may also modulate thyroid hormone production.

Human experience with purified ethyl-EPA is limited to the product Epadel, which has been marketed in Japan since 1991. The package insert for Epadel lists the following

side effects that have been observed in incidence between 0.1 – 5%: hypersensitivity (rash, tingling), tendency to hemorrhage, anemia, diarrhea/ stomach pain/ heartburn/ nausea, liver function abnormalities (AST, ALT, LDH), and increased CK (CPK). Additionally, Mochida conducted the JELIS trial in Japan to evaluate the effect of 1.8 g/day Ethyl-EPA ± statin over a 5-year follow-up period. The results of this study were published (M. Yokoyama *et al.* Lancet 369: 2007) and the two adverse events that had the highest statistical significance were GI disturbance (nausea, diarrhea, epigastric discomfort) and skin abnormalities (eruption, itching, eczema, exanthema). Hemorrhage (cerebral, fundal, epistaxis, subcutaneous) was also highly statistically significant followed by an increase in GOT (glutamic oxaloacetic transaminase or AST) and pain (joint, lumbar, muscle).

The Applicant has conducted repeat dose toxicology studies in two species, a full panel of genotoxicity studies, limited pharmacology/ pharmacokinetics, one Seg II reproductive toxicology study, two carcinogenicity studies and a 28 - Day bridging study in the rat directly comparing the pharmacokinetics and toxicology profile of Epadel to AMR101. In addition to these Applicant sponsored studies, there is reliance on Seg I, II, and III reproductive toxicology studies, two additional repeat dose toxicology studies in the rat and several pharmacology and pharmacokinetics literature references conducted to support Epadel marketing. Amarin's product Vascepa (AMR101/ icosapent ethyl/ ethyl-EPA) is similar to Epadel (ethyl-EPA) in that both are derived from fish, specifications for purity of ethyl-EPA are identical, the use of (b) (4) tocopherol is identical, related substances/ impurity/ degradant profiles are similar but not identical, and the nonclinical toxicology profiles are similar in the rat. The combined scientific and toxicologic information provided in addition to a well established understanding of fatty acid absorption, biological activity and metabolism is sufficient to support this 505(b)(2) application.

Pharmacology

There is sufficient information in the published scientific literature pertaining to omega-3 fatty acids and their pharmacologic actions. In general, long chain polyunsaturated fatty acids (PUFAs) are absorbed from the small intestine and transported by the lymph before systemic distribution. Fatty acids can be incorporated into triglycerides, cholesteryl esters, phospholipids or may remain as unesterified (free) fatty acids. Fatty acids are incorporated into the cell and can affect the fluidity of the membranes, regulate cellular signaling and alter the production of prostaglandin and leukotriene to modulate the immune response. The omega-3 fatty acids EPA and DHA inhibit the conversion of arachidonic acid by the cyclooxygenase pathway and reduce the production of platelet activating factor (PAF). PUFAs have also been shown to be endogenous agonists for PPARs such as PPARα and GPCR receptors such as GPR119. In addition to PPARs and GPCRs, other nuclear receptors such as farnesol X receptor (FXR) and liver X receptor (LXR) can be modulated by nonesterified fatty acids. The downstream activation of these receptors includes the regulation of gene products that modulate lipid synthesis, glucose metabolism and fatty acid oxidation. PUFAs such as EPA are therefore poor substrates for triglyceride (TG) synthesis, promote fatty acid degradation (peroxisomal or mitochondrial β-oxidation), inhibit

lipogenesis in the liver and increase triglyceride clearance from the plasma. Therefore the net effect of omega-3 polyunsaturated fatty acids such as EPA (derived from hydrolysis of ethyl-EPA) is a metabolic shift from triglyceride production and storage to oxidation and elimination.

Other secondary pharmacologic effects of EPA as described in the literature include its ability to reduce platelet aggregation (due to membrane incorporation of this PUFA), support cardiovascular health by the reduction of thrombus formation and increased vasodilation, and reduce inflammation. Changes in cellular membrane lipid composition can, for example, alter the production of pro-inflammatory mediators such as prostaglandins, leukotrienes and clotting factors (PAF). Intracellular signaling is affected as increased PUFAs can increase the cell membrane fluidity and decrease the formation of lipid rafts such as required in T-cell activation. Once PUFAs such as EPA are incorporated into cell membranes such as in RBCs or WBCs, the half-life is long and can influence the cell's activity for an extended period of time.

Amarin Pharmaceuticals did not conduct any dedicated safety pharmacology studies; however repeat dose toxicology studies did not indicate a concern for cardiovascular, neurological or respiratory toxicities. Potential safety concerns have been identified such as the potential to increase bleeding time or increased risk of hemorrhage, increased liver function enzymes, skin hypersensitivity and gastrointestinal upset. Additionally, a large randomized trial was conducted in humans administered 1.8 g/day ethyl-EPA where results demonstrated a favorable-to-neutral effect on cardiac arrythmias and sudden death. Due to the extensive data available from human subjects, dedicated nonclinical safety pharmacology studies would not provide additional information required for a regulatory decision.

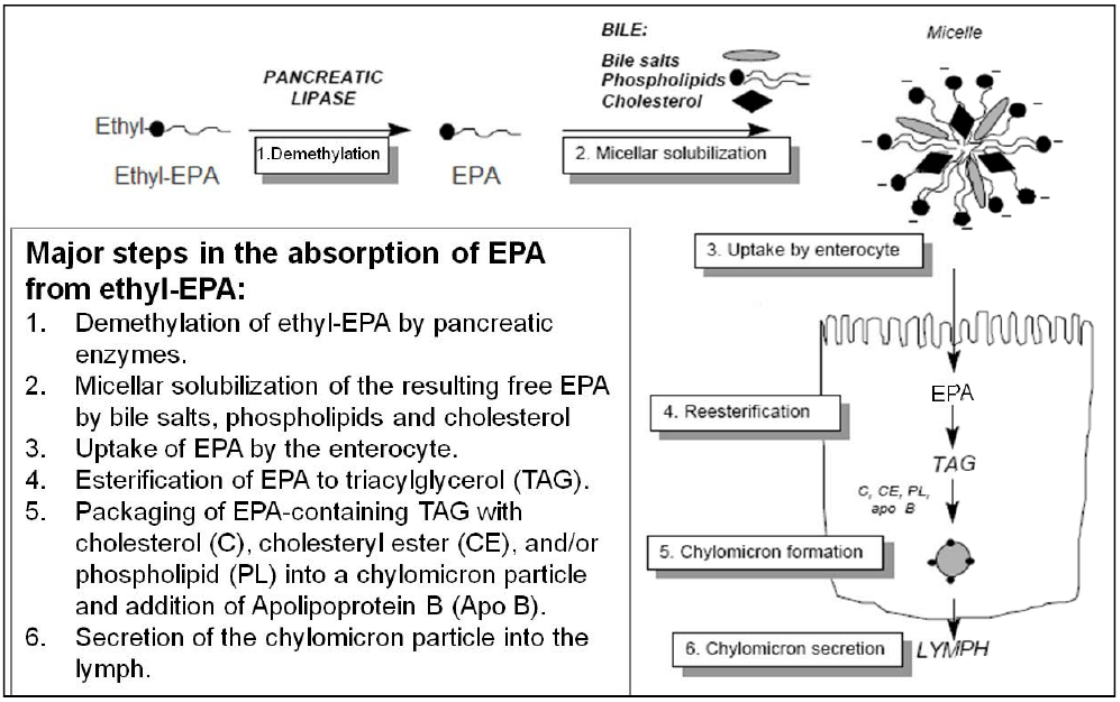
In summary, the pharmacology of ethyl-EPA and EPA has been well described in nonclinical and clinical studies either submitted under NDA 202057 or available within the scientific literature. For the proposed indication of severe hypertriglyceridemia, EPA has been shown to reduce triglyceride and cholesterol levels in multiple species. In addition to this intended pharmacologic effect, EPA can also decrease platelet aggregation, can have a potential beneficial effect on cardiovascular function and can reduce inflammation.

Pharmacokinetics

Amarin conducted limited ADME studies with the remainder of published literature studies on ethyl-EPA conducted by Mochida Pharmaceutical Company in support of the marketed product Epadel. Ethyl-EPA is rapidly converted to free (unesterified) EPA by esterases in the stomach and small intestine following oral administration, and absorption is rapid from the small intestine as triacylglycerol/ phospholipids/ cholesteryl esters as well as unesterified EPA. EPA is then absorbed into the lymphatic system before systemic distribution where plasma steady state levels are reached within 7-10 days in nonclinical species. As ethyl-EPA is rapidly converted to EPA, it is frequently below the LLOQ in PK studies.

Clearance of EPA from the serum can be through conversion to DHA, membrane incorporation, or beta-oxidation and elimination in expired air. Omega-3 FAs are extremely prone to peroxidation due to the high level of unsaturation; therefore the administration and storage of ethyl-EPA must be in the presence of [redacted] (b) (4) such as α-tocopherol.

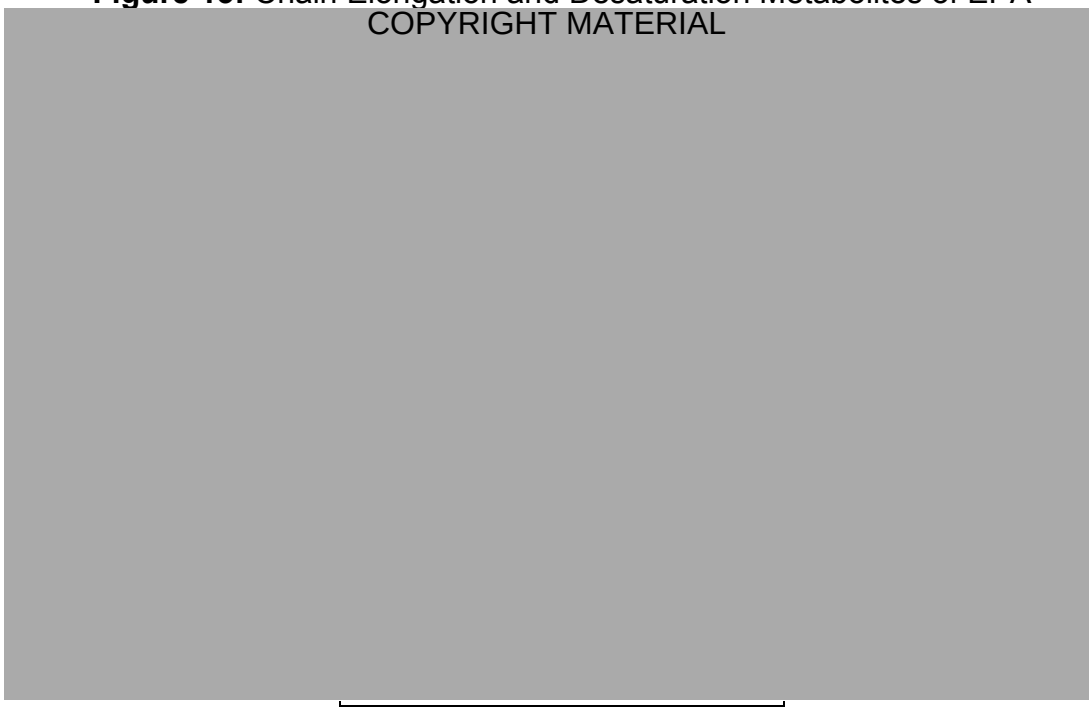
Figure 12: Conversion of Ethyl-EPA to EPA and Subsequent Intestinal Absorption



[Figure reproduced from Applicant's NDA 202057 submission]

Supplementation with EPA promoted the omega-3 fatty acid metabolic pathway and led to the displacement of omega-6 and omega-9 fatty acids in different lipid fractions. EPA is rapidly taken up into phospholipids with a slower incorporation into cell membranes; however EPA also slowly declines from lipid membranes if administration is stopped. When rats were given an oral administration of ^{14}C-EPA-E, the majority of absorption was found to be from the small intestine (95.4%) with only 3.7% absorption from the stomach and was found to be through a lymphatic route. While bile increased absorption, the purity of EPA-E had no effect on absorption. In rats, aside from the GI tract, the highest concentrations of radiolabeled EPA were found in the brain, liver, skin, fat and muscle although EPA had a wide distribution to most tissue types including the heart, arteries, adrenals, urinary bladder, ovaries, and bone marrow. All radioactivity present in the total lipids of lymph was de-ethylated EPA with no detection of ethyl-EPA or its metabolites. In plasma, only EPA was detected at 1 hour post dose, DPA (a chain elongated metabolite) was detected at 9 hours post-dose and DHA (chain elongated and desaturated) was detected after 24 hours. Clearance of EPA is through metabolism to DPA/DHA or by cell membrane incorporation, and elimination is primarily through beta-oxidation by the mitochondria or peroxisomes primarily in the liver but also in other tissues and released as CO_2 and H_2O in expired air.

Figure 13: Chain Elongation and Desaturation Metabolites of EPA



[Literature reference: CE Childs *et al.* Proc. Nutr. Soc. 2008 (67), p.19-27]

Toxicokinetic studies indicated that in rats, there was a less than dose proportional increase in exposure above a dose of 1.0 g/kg/day AMR101 while in dogs there was a greater than dose proportional increase in exposure. Rats also showed some evidence of accumulation with repeat exposure as did the male dog (between 3 and 9 months of dosing).

Unesterified EPA (free) distributed predominantly to the plasma fraction where it was highly bound to plasma proteins in the rat, dog and human and had low association with RBCs. EPA was found to be a weak inhibitor of all CYPs tested such as CYP2C19, 2C9, 2C8, and 2B6 in human liver microsomes where the lowest IC_{50} for EPA was 8.4 µM for CYP2C19. For reference, the calculated C_{max} plasma levels of un-esterified EPA in humans (28 day study with 4g/day) was 5.04 µM, making it theoretically possible that EPA may cause DDIs. In cultured human hepatocytes, there was no CYP induction of CYPs 2C9, 3A4 or 1A2 at a concentration up to 100 µM. Studies with drug transporters (P-gp, OAT1, OAT3, BCRP, OCT2, OATP1B1 and OATP1B3) showed that 1 µM EPA had the potential to inhibit OAT3 by 24.2% in the Caco-2 cell line. Taken together, EPA has the potential to cause DDI however considering the extensive clinical human data available for EPA, the risk may be low.

Toxicology

Single and Repeat Dose Toxicology

A single dose toxicology study was completed in the rat and mouse for ethyl-EPA, its metabolites and impurities to assess acute toxicity (Mochida sponsored literature reference). Routes of administration included oral, intraperitoneal and subcutaneous. Toxicities present from a single dose of ethyl-EPA (20g/kg) or one of its metabolites (10-

20 g/kg) resulted in oily leakage from the anus when delivered orally and led to recoverable weight loss when administered intraperitoneally.

Repeat dose toxicology studies were completed in the rat (a combination of cited literature references conducted by Mochida as well as Amarin studies), in the mouse (Amarin) and in the dog (Amarin). Combined, these studies included a 28-day dose range finding study in the mouse (Amarin); 28-day (including a bridging study with comparison to Epadel, Amarin), 90-day and 12-month rat studies (Mochida sponsored literature references); and a 14 day as well as a 39-week with 13-week interim kill dog study (Amarin sponsored). The pharmacologic effect of ethyl-EPA was noted across species as triglycerides and cholesterol levels decreased variably but reproducibly.

The toxicology profile of ethyl-EPA (AMR101) supports that this test article is relatively well tolerated. At high doses of this fatty acid, an oily anal discharge was noted that was associated with skin irritation and oily or discolored fur. In dogs, there was skin irritation and two dogs were euthanized due to GI tract related issues involving blockage between the small and large intestines (intussusception of the ileocecocolic junction and an infarcted jejunum at 2000 mg/kg and 300 mg/kg, respectively). The skin was frequently affected in all species with scabs, reddened areas, fur discoloration, fibrosis, hyperkeratosis and hyperplasia. These findings, localized near the anus, tail or abdomen, are likely related to the topical irritation of EPA (including metabolites or oxidation) as anal leakage of oil has been frequently noted. The skin findings cannot however be completely dismissed as depletion of omega-6 fatty acids from cell membranes (and replacement with omega-3 fatty acids) has been shown to induce scaly and necrotic skin in rats in scientific literature. Human correlates of skin hypersensitivity to ethyl-EPA have also been reported and therefore it is possible that systemic distribution of EPA can lead to adverse skin manifestations.

Additional toxicities observed include increased liver enzymes such as ALT or ALP (however increased ALP may have been due to a GI effect or even due to a corticosteroid effect in the dog). Altered liver morphology (prominent lobular architecture) has also been observed in nonclinical studies with AMR101 and may be related to the pharmacologic effect of decreased TG and cholesterol synthesis. Elevations in liver enzymes have been reported in human clinical studies with ethyl-EPA as adverse effects; therefore these nonclinical findings are relevant to humans. Multiple nonclinical studies had altered WBC counts that could translate into altered immune function that may increase the risk of infection if suppressed and may indicate skin hypersensitivity and inflammation if increased. The effect of omega-3 fatty acids such as EPA have been studied in the scientific literature with results including down-regulation of adhesion molecules on endothelial cells, decreased chemotaxis of neutrophils and suppression of viral clearance from the lung. The net effect may be an increased risk of infection or pathogen clearance; however this risk is theoretical based only on a plausible mechanism of action. The effect of EPA on clotting, RBC parameters, and the risk of increased hemorrhage has been repeatedly shown in nonclinical studies. Small increases in clotting times (PT), decreased RBC, RDW, Hb, Hct and reticulocytes were noted in multiple species, a low incidence of hemorrhage was noted in the rat,

increased erythrophagocytosis was reported in the dog, the Epadel bridging study had findings of individual animals treated with either AMR101 or Epadel having increased lactate dehydrogenase (high concentration present in RBCs), or increased urobilinogen in urine (possible hemolysis and anemia). It is also worth mentioning here that in the 6-month transgenic mouse carcinogenicity study that there was a statistically significant increase in the incidence of perimesenteric vein and mesenteric vein thrombosis in both sexes. Given the already known theoretical human risk of increased bleeding and tendency to hemorrhage with EPA or high dose omega-3 fatty acids, these nonclinical toxicology findings are relevant to humans.

In the dog, there was an increased incidence of adrenal vacuolar degeneration of the inner adrenal cortex in both sexes that was associated with a decrease in cortisol levels. In the rat, radioactively labeled EPA has been shown to not only distribute to the adrenal gland but remains at a high level at least 24 hours after administration while other tissue levels have subsided. EPA has been reported to have a corticosteroid like effect and in fact has been shown to permit a decrease in the dose of administered glucocorticoids in dogs. As EPA stimulates a corticosteroid like effect there is a negative feedback on the hypothalamus, pituitary gland and ultimately the adrenal gland to decrease cortisol production. As cortisol was not measured in any other species, it is unknown if EPA is affecting this pathway in rodents.

An important comparison between AMR101 and Epadel (both ethyl-EPA preparations) was completed in the 28-day bridging study in the rat where both PK and toxicology profiles were assessed. At an identical dose (2000 mg/kg/day) of AMR101 or Epadel, the toxicology profile was similar for both ethyl-EPA compounds with expected findings of skin/ fur clinical signs, RBC parameter decreases, increased ALP (possibly intestinal or liver) and decreased lipids. These were compared to a previous Amarin sponsored 28-day rat toxicology study which had slightly different toxicities but all within the expected scope of the overall EPA profile (*i.e.* clotting, WBC, RBC changes). The bridging study also demonstrated a reasonably similar PK profile between Epadel and AMR101 with a slightly higher mean exposure for Epadel over AMR101 but almost identical mean C_{max} values. Certain variability is to be expected as even exposures between studies using the same compound (*i.e.* AMR101) can vary even when the same dose, species and length of administration is used. The results from this bridging study together with comparable toxicity profiles in nonclinical studies conducted by Mochida in support of Epadel marketing, and a similar chemical profile of ethyl-EPA (use of tocopherol, high purity ethyl-EPA derived from fish oil, and similar degradants and impurities (b) (4)) are supportive of comparability between AMR101 and Epadel.

Table 83: Comparison of Toxicity and Un-esterified EPA Exposures at Study Termination (Day 28)

Most Common Tox Findings	1-month Rat Bridging Study #522093		28 Day Rat Tox #ZOC0001
	Epadel	AMR101	AMR101
	2000 mg/kg	2000 mg/kg	2000 mg/kg
Fur/Skin changes; excess salivation	✓	✓	✓
WBC change (any)			✓
↑ Prothrombin time			✓
↓ RBC/ Retic/ RDW	✓	✓	
↑ ALP	✓	✓	
↓ cholesterol/TG	✓	✓	✓
NOAEL	2000 mg/kg	2000 mg/kg	2000 mg/kg
AUC (µg•hr/mL)	293.5	233.6	168.1
C_{max} (µg/mL)	23.4	23.9	16.9
T_{max} (hr)	2.67	1.59	4.5

AUC, C_{max} and T_{max} are all reported as an average of male and female data.
Exposure data from Study ZOC0001 was obtained from the Amended (corrected) data.

Genotoxicity

Amarin Pharma conducted a full panel of genetic toxicology studies with AMR101. Study results indicated that ethyl-EPA (AMR101) was not mutagenic in the Ames assay, was negative in the *in vivo* mouse micronucleus assay; however was positive in three separate chromosomal aberration assays in *in vitro* CHO (Chinese Hamster Ovary) assays. The relevance of the clastogen risk to humans in an *in vivo* setting is partially countered by the 20+ years of human exposure to ethyl-EPA in Japan (in a background of a high fish and therefore high omega-3 FA diet) as no known carcinogenic risk of this fatty acid has been identified. One caveat to this however is that Epadel is approved at a maximum dose of 1.8 g/day (or up to 2.7 g/day when "an excess of triglycerides are presented"), where Amarin is seeking approval for their ethyl-EPA product AMR101 at a clinical dose of 4 g/day. Various scientific publications in the literature have also proposed that omega-3 fatty acids are anti-carcinogenic and may even blunt damage from known carcinogens.

An argument can be made that ethyl-EPA delivered *in vivo* would not be a clastogen as the *in vivo* mouse micronucleus study was negative and high concentrations of fatty acids delivered directly onto a cell monolayer can cause detergent like effects resulting in cellular toxicity (*i.e.* would not be physiologically relevant). However, due to the differences in the clastogenicity of other unsaturated fatty acids (±S9, different toxicities) as compared to ethyl-EPA or EPA, a clastogenic risk cannot be completely dismissed. As the ultimate concern of any positive genetic toxicology assay is the risk of a carcinogenic effect, the results of both carcinogenicity assays conducted in the rat and the mouse are discussed.

Carcinogenicity

Two carcinogenicity studies were conducted with AMR101: a 2-year rat study and a 6-month Tg.rasH2 mouse study. The 2-year rat study did not receive ECAC concurrence for protocol approval, and despite the absence of an appropriate control group (corn oil control and an undosed control were used), the study was considered adequate. The 6-month transgenic mouse study received ECAC concurrence for the protocol and was also adequate.

In the 6-month transgenic mouse study, there were no drug related neoplasms in females. There was a positive finding in males only for papilloma(s) of the skin/subcutis at the tail. The incidence was 0-0-0-1-5 for doses of 0, 0.5, 1, 2 and 4.6 g/kg/day. As multiple nonclinical studies have shown that with increasing dose of ethyl-EPA, there is an increased incidence of oil leakage from the anus and depositing on the skin or fur it is possible that these papillomas are due to direct interaction with metabolized or oxidized EPA. Other histopathology findings at the proximal tail included acanthosis/ hyperkeratosis, erosion/ ulceration and inflammation. All of these findings are consistent with a localized skin irritant effect of the oil that may have led to increased proliferation resulting in papilloma formation. If this was the mechanism of this tumor type in the mouse, then it is not likely relevant to humans. As EPA has been shown to cause skin hypersensitivity or deplete omega-6 fatty acids from epithelial cell membranes, it is also possible that systemic exposure could also lead to this skin neoplasm. Given the location of the papillomas at the proximal tail, the evidence would support that in this model the cause of these lesions is likely localized oil deposition on the skin leading to irritation/ inflammation and a corresponding proliferative response.

Non-Neoplastic histopathology findings in the transgenic mouse included a statistically significant increase in both sexes of thrombosis and inflammation at the ileum (mesenteric vein) and thrombosis at the mesenteric lymph node (perimesenteric vein) as well as increased pigment at the mesenteric lymph node (MLN) in both sexes. These findings were particularly of interest as the small intestine to the mesenteric lymph is the primary route of EPA absorption and is likely at the highest concentration before systemic distribution.

In the 2-year rat carcinogenicity study, there were no neoplasms clearly drug-related in male rats. In female rats, there was a statistically significant increase in the incidence of hemangiomas + hemangiosarcomas at the mesenteric lymph node. If all hemangiomas and hemangiosarcomas from all sites across the body were added together, there was no statistical significance; however it should be noted that the majority of these tumors were present at the mesenteric lymph node. Again, as in the mouse, the mesenteric lymph node was of particular interest as this is the primary site of absorption from the small intestine. From early decedents in the study, the earliest hemangiosarcoma was found in a 58 week old male rat, although MLN hemangiosarcoma was the cause of death only in one low-dose male (91 mg/kg) at week 77. The 12-month rat toxicology study conducted by Mochida in support of Epadel marketing did not report the mesenteric lymph nodes to be examined at necropsy or for histopathology; therefore no additional information from a chronic repeat dose study on this finding is available.

Table 84: FEMALE – Total Tumor Incidence at the MLN in the 2 yr. Wistar Rat

AMR101 (mg/kg)	0	91	273	911
MLN: Hemangioma	0	0	3	4
MLN: Hemangiosarcoma	0	0	2	2
MLN: Hemangioma + hemangiosarcoma	0	0	5	6

0 = Un-Dosed Control; MLN = Mesenteric Lymph Node

Table 85: MALE – Total Tumor Incidence at the MLN in the 2 yr. Wistar Rat

AMR101 (mg/kg)	0	91	273	911
MLN: Hemangioma	1	0	0	5
MLN: Hemangiosarcoma	4	4	5	7
MLN: Hemangioma + hemangiosarcoma	5	4	5	12

0 = Un-Dosed Control; MLN = Mesenteric Lymph Node

Table 86: Exposure Margins – 2yr. Wistar Rat

AMR101 Doses (mg/kg)	91 mg/kg	273 mg/kg	911 mg/kg
Rat mg/m^2	546	1638	5466
Human mg/m^2 (MRHD 4g/day)	2479	2479	2479
Safety Margin (body surface area)	**<1X**	**<1X**	**2.2X**
Rat $AUC_{0\text{-}t}$ (ng.hr/mL) Day 28	ND	57,640	144,900
Human $AUC_{0\text{-}24hr}$ (ng.hr/mL) Day 28	20,300	20,300	20,300
Safety Margin (4g AUC exposure)	**ND**	**3X**	**7X**

- MRHD of 4 g/day = 67 mg/kg for a 60 kg human adult
- **Rat $AUC_{0\text{-}t}$ data is derived from the 28-day repeat dose toxicology** (Amarin sponsored) study # ZOC0001. Average of male and female from week 4. TK data from doses used in the 28 day rat study (#ZOC0001, amended) that approximated that used in the 2 yr. CARC study were used for comparison (*i.e.* 300 mg/kg dose group used for 273 mg/kg and 1000 mg/kg dose group used for 911 mg/kg)
- Human Exposure Data is from Study AMR-01-01-0018 CSR (28-Day PK study) where data was originally reported as $AUC_{0\text{-}24hr}$ (µg.hr/mL) and is baseline-unadjusted for unesterified (free) plasma EPA (4g/day given 2g BID)

In the 9-month dog repeat dose toxicology study, there was a non-dose dependent finding of increased erythrophagocytosis at the mesenteric lymph node indicating RBC damage and clearance. This finding may also be similar to the 6-month transgenic

mouse where increased MLN pigment and thrombosis with inflammation was present. Given the high concentration of EPA transiting through this site and EPA's know risk of increasing bleeding and altering RBCs, it is likely that the MLN is a site of toxicity or at least of cellular stress to these species. In the dog, the erythrophagocytosis was observed at the 3-month interim kill as well as at the 9 month terminal sacrifice; however no progression of severity was noted. It is possible that continual absorption of EPA in the dog leads to an adaptive response where potential hemolysis does not progress into a proliferative response.

The question remains as to the relevance of the hemangioma/ hemangiosarcoma finding at the MLN in female rats, to humans. Certainly, the route of absorption and lipid distribution into the lymph would be similar across species including humans. As the mechanism of action of hemangioma formation in the rat is unknown it is difficult to predict if the same risk is present in humans. There has been active debate on whether or not hemangiomas(sarcomas) are relevant to humans when compared to rodents (SM Cohen et al. Tox Sci. 2009, 111(1), 4-18). The spontaneous incidence in mice is higher than in rats and humans; therefore less weight is given to this finding in this species. One theory of a potential mechanism of action for hemangioma formation in rodents involves hemolysis (and is certainly relevant to the toxicity profile of ethyl-EPA). Upon hemolysis, there is an increase in reactive oxygen species, recruitment of WBCs such as macrophages, increased cytokines and ultimately increased endothelial cell proliferation. If the site of the MLN at the mesenteric or perimesenteric vein has a high concentration of EPA as it distributes into the systemic circulation, then this site is predisposed to increased hemolysis. This may account for the multiple histopathology findings including increased pigment (*e.g.* heme), erythrophagocytosis, thrombosis and inflammation and in the rat hemangioma(sarcoma) formation. While this is certainly plausible, this hypothesis is unproven and does not take into account differences in human physiology that may or may not alleviate this concern.

For example, if antioxidants would dampen the initial ROS burst following hemolysis perhaps humans have higher circulating concentrations of antioxidants than rodents or dogs. Another factor to consider may be one of a genetic predisposition to dietary intake of fat and oil. The rat, whose optimized dietary intake of all fats combined is 5%, is much less than that of a healthy human diet of 20 – 35% total fat intake. Furthermore, the normal composition of any omega-3 fatty acid in the rat diet is less than 0.5%. The point being that species differences in dietary requirements may result in a difference in how fats are processed and adding additional fat into the human diet may be less of a "stress" on absorption than for a rodent. It is also not clear how normal proliferative rates of endothelial cells may compare across species and how that may influence hemangioma formation. Finally, it is noted that although a clinical dose of 4g/day is proposed for humans, this total daily dose is to be administered as 2g twice per day; therefore decreasing the amount of oil being absorbed at any one given time.

In summary, the finding of hemangiomas/hemangiosarcomas at the mesenteric lymph node in female rats is drug-dependent and it is important that this finding is included in labeling as it is mechanistically plausible that increased absorption of EPA at this site

can lead to hemolysis and potentially induce a proliferative endothelial cell repair response. While this is hypothetically possible, it should also be countered with the human experience that at doses of 1.8 -2.7 g/day of ethyl-EPA (Epadel) marketed since 1991, in a Japanese population whose diet consists of high omega-3 fatty acid intake (fish), there are no reports of an increased incidence of hemangiomas(sarcomas).

Reproductive Toxicology

Reproductive toxicology studies were primarily referenced from the literature from Mochida sponsored studies in support of Epadel (ethyl-EPA) marketing. These studies included fertility, embryo-fetal (teratogenicity) and peri-and post-natal developmental studies in the Wistar rat as well as an embryo-fetal study in the rabbit. Amarin supplemented these studies with one embryo-fetal study in the SD rat. Pharmacokinetic studies conducted in support of Epadel marketing, demonstrated that placental transfer of EPA in the rat was low; however accumulation of ^{14}C-EPA-E in fetal brain was higher than in maternal brain. ^{14}C-EPA-E was also detected in milk at a higher concentration than in plasma (6-14 fold higher).

There was no effect on fertility of adult rats when ethyl-EPA was administered for 9 weeks before mating to males and 14 days before mating to females. Reproductive toxicity showed the potential for mild skeletal variations such as cervical rib, vestigial rib or increased 13th rib and an equivocal developmental abnormality of missing optic nerve and micro/ano-phthalmia (originates as a retinal defect) that was not dose dependent and could be due to genetic strain differences in the Wistar rat. The overall incidence was 0-2-2-2 for 0, 300, 1000 and 3000 mg/kg ethyl-EPA administered to the dam from GD 7-17 (1.4 -1.7%). In the Wistar rat historical database (b) (4) there was no listing of missing optic nerve. In the Crl(CD) rat, retina agenesis (failure to develop) had an incidence of 0.006% (fetal) and 0.044% (litter). Upon aging, atrophy of the optic nerve in rats is observed but usually occurs after 1 year of age. One literature reference (K. Shibuya et al. Vet Path. 1989) states that unilateral optic nerve aplasia was observed in two young female Slc: Wistar rats at 6 weeks of age (from 200 male and 200 females = 0.5% incidence); however the incidence with ethyl-EPA although not dose dependent is at least 3-fold higher than any of the literature or historical control data.

In the F_1 generation, females had a non-statistically significant delay in the estrus cycle of 10 days that could be correlated with decreased copulation and decreased implantation at the HD of 3000 mg/kg. F_1 males also at the HD of 3000 mg/kg had a slight increase in the incidence of testis atrophy. While these results are equivocal, they are mentioned here as development and maintenance of the retina (optic nerve) and reproductive organs are very sensitive to levels of the omega-3 fatty acid DHA.

DHA is the most prominent fatty acid present in certain tissues such as the brain, retina and spermatozoa. It was therefore of interest that in the embryo-fetal toxicity study sponsored by Mochida in support of Epadel marketing, missing optic nerve and testis atrophy was found at low incidence. While one could argue that increased EPA levels would out-compete DHA for membrane incorporation and alter cellular function, EPA

can be readily converted to DHA by chain elongation and desaturase activity. Provided that these mechanisms in the cell (or via liver conversion) were functional, DHA levels should also increase in the presence of EPA administration; therefore a mechanistic theory of developmental abnormalities due to an out-competition of DHA with EPA is unclear.

In summary, reproductive toxicology studies conducted by Mochida in support of Epadel marketing in addition to one Seg II study in the rat conducted by Amarin Pharma indicated that skeletal variations and visceral abnormalities were present in multiple studies. Although the incidence of these findings was low, the potential risk to the developing fetus cannot be ruled out.

Potential Human Toxicities Based Upon Non-Clinical Studies Sponsored by Amarin or Mochida and Scientific Literature References

As an omega-3 fatty acid, ethyl-EPA (hydrolyzed to EPA) has multiple roles within the cell and can influence not only cellular function but organ function as well (*e.g.* brain/ retinal development). Polyunsaturated fatty acids (PUFA) such as EPA and DHA influence the fluidity of cell membranes and therefore can modulate receptor mediated signaling or cellular adhesion (*e.g.* influence lipid raft and caveolae formation); are endogenous ligands for PPARs (*e.g.* PPARα); can bind to orphan GPCRs that may mediate insulin secretion and glucose homeostasis (*e.g.* GPR119); and can influence prostaglandin and leukotriene synthesis or cytokine release (*e.g.* immune modulatory).

While PUFAs all share similar effects, this should not be taken as all PUFAs are interchangeable in their signaling mechanisms and biological properties; therefore products with mixtures of fatty acids or fish oil dietary supplements may have differing toxicologic and biologic properties from high dose ethyl-EPA alone. For example, published literature (Review: R. Gorjao *et al.* Pharmacology & Therapeutics 2009) has demonstrated that EPA and DHA have different effects on leukocyte function such as phagocytosis as well as signaling pathways involved in lymphocyte proliferation. In particular, high dose EPA (4g/day for 8 weeks) has been shown to decrease neutrophil chemotaxis by as much as 70% in humans (DG Payan *et al.* J. Clin. Immunol. 1986).

Based upon submitted non-clinical toxicology studies, there are several concerns for toxicity that are further reinforced by scientific MOA studies and/or correlative findings in human clinical trials. These include a possible effect on clotting time and hemorrhagic effects, alterations in WBCs and increased susceptibility to infection, GI tract disturbances, an HPA axis effect including decreased cortisol production, alteration in liver enzymes, and skin abnormalities or hypersensitivity.

Table 87: Safety Margins to Nonclinical Toxicology Studies

Species	Duration	NOAEL	EPA AUC_{0-t} (ng•h/mL)	Multiples of Human Dose Levels	
				Body Surface Area	AUC
Rat	4-weeks	2000 mg/kg	♂: 196,000 ♀: 140,200	5X	8X
Rat	4-Weeks (Bridge)	2000 mg/kg	♂: 265,500 ♀: 201,600	5X	12X
Rat	3-months	3000 mg/kg	ND	7X	--
Rat	12-months	1000 mg/kg	ND	2.4X	--
Mouse	4-weeks	2000 mg/kg	♂: 81,020 ♀: 73,720	2.4X	4X
Dog	9-months	♂: <300 mg/kg ♀: 300 mg/kg	♂: <33,280 ♀: 29,830	≤ 2.5X	1.5X

Human AUC_{0-24h} Day 28 = 20,300 ng•h/ml (4g/day): un-esterified EPA
ND = No Data
Proposed Clinical Dose = 4000 mg/d = 67 mg/kg/d = 2467 mg/m^2
3-month and 12-month rat studies were conducted with Epadel (ethyl-EPA)

12 Appendix/Attachments

The following are amended Toxicokinetic Parameter Tables from Studies No. 459549 (4-Week wild-type CByB6F1 rasH2 mice), No. ZOC0001 (28-Day rat) and No.515194 (39- week dog). These studies were amended as the original EPA results were incorrectly calculated for samples that were diluted with control matrix; therefore reported EPA values were higher than they actually were at the time of the initial review.

Table 88: Toxicokinetic Parameters for Plasma Concentrations of EPA in Mice (Amendment 1 Study No. 459549)

Sex	Dose (g/kg/day)	Day	$AUC_{0-\infty}$ (ng.h/mL)	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{½el}$ (h)	Kel (1/h)	CL/F (mL/h/kg)	Vd/F (mL/kg)
Male	0.3	1	25540	23530	3610	2.00	6.94	0.10	13610	136200
		28	10760	6007	509	1.00	20.40	0.03	49940	1470000
	1.0	1	75090	41220	3890	2.00	23.34	0.03	13320	448500
		28	52970	46010	5820	2.00	9.07	0.08	21730	284300
	2.0	1	108300*	66130	4000	2.00	20.52*	0.03*	18460*	546600*
		28	150900	81020	9910	1.00	23.08	0.03	24690	822000
Female	0.3	1	23360	18150	3010	1.00	13.24	0.05	18730	358000
		28	7318	6527	534	1.00	7.51	0.09	45960	498200
	1.0	1	45690	38150	3240	2.00	8.93	0.08	21880	281800
		28	48320	43280	3910	4.00	7.11	0.10	23110	237100
	2.0	1	47240	39330	4940	1.00	9.34	0.07	42340	570600
		28	84350	73720	4540	8.00	8.54	0.08	21730	334100

* Unreliable estimate

Table 89: Toxicokinetic Parameters for Plasma Concentrations of EPA in Rats (Amendment 1 Study No. ZOC0001)

Sex	Dose (g/kg/day)	Day	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	CL/F (mL/h/kg)	$T_{½el}$ (h)
Male	0.3	1	35990	4010	4.00	7798	5.79
		28	60310	7340	2.00	4974	11.47
	1.0	1	150000	21100	4.00	6461	4.38
		28	138200	19600	2.00	7235	13.24
	2.0	1	72470	9680	4.00	25350	6.14
		28	196000	16400	4.00	10200	6.21
Female	0.3	1	36020	3560	1.00	7345	7.84
		28	54970	6490	2.00	5457	17.14*
	1.0	1	75700	13300	4.00	11840*	6.69*
		28	151600	13100	2.00	6598	12.38
	2.0	1	114600	9230	4.00	13740	9.90
		28	140200	17300	4.00	14270	9.75*

* Unreliable estimate

Table 90: Toxicokinetic Parameters for Plasma Concentrations of AMR101 and EPA in Dogs (Amendment 1 Study No. 515194)

Group/ Sex	Dose Level (g/kg/day)	Time point	AMR101			EPA		
			C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)
2M	0.3	Day 1	NC	NC	NC	592	2.00	4541
		Week 13	22.5 n=1	1.00 n=1	5.625 n=1	3140	0.50	30220
		Week 39	50.5 n=2	2.00 n=2	25.25 n=2	2750 n=3	2.00 n=3	33280 n=3
3M	1.0	Day 1	148 n=5	8.00 n=5	157.8 n=5	3550	2.00	28410
		Week 13	239	8.00	584.4	22600	0.50	206800
		Week 39	378	8.00	808.6	15600	0.50	190800
4M	2.0	Day 1	248 n=7	8.00 n=7	438.9 n=7	8330	24.00	69890
		Week 13	431 n=7	8.00 n=7	1470 n=7	49000 n=8	0.75 n=8	365600 n=8
		Week 39	274	6.00	929.6	42200	0.25	471500
2F	0.3	Day 1	23.2 n=1	8.00 n=1	46.40 n=1	683	1.00	5783
		Week 13	22.2 n=1	4.00 n=1	54.60 n=1	3280	1.00	27340
		Week 39	73.8 n=1	2.00 n=1	36.90 n=1	2970	1.50	29830
3F	1.0	Day 1	198 n=6	8.00 n=6	449.2 n=6	3590	8.00	44250
		Week 13	150 n=6	8.00 n=6	262.7 n=6	20000	0.00	201300
		Week 39	192	4.50	319.1	17900	1.00	132000
4F	2.0	Day 1	418 n=8	8.00 n=8	1148 n=8	13000	8.00	158100
		Week 13	381	8.00	1120	47500	0.50	355900
		Week 39	286	1.50	299.1	29800	0.00	324300

NC=non calculable
N=7 in Groups 2 and 3 and N=9 in Group 4 on Day 1 and Week 13 and N=4 during Week 39 unless otherwise specified.

[The above tables were reproduced from the Applicant's NDA 202057 submission]

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/s/

STEPHANIE J QUINN
06/05/2012

KAREN L DAVIS BRUNO
06/05/2012
concur w/AP recommendation

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 202057	**Applicant: Amarin Pharmaceuticals**	**Stamp Date: Sept. 26, 2011**
Drug Name: Vascepa	**NDA/BLA Type: 505(b)(2)**	**Filing Date: Nov. 25, 2011**

Amarin Pharmaceuticals, Inc. has submitted NDA 202057 for Vascepa (AMR101, icosapent ethyl, ethyl-eicosapentaenoic acid, ethyl-EPA) as a 505(b)(2) application, and are relying on non-clinical literature references published by Mochida in support of the marketed Japanese product Epadel (ethyl-EPA). From a PharmTox perspective, bridging studies are needed to provide evidence that Vascepa and Epadel are sufficiently comparable to rely on the published nonclinical toxicology data for this NDA submission.

- Amarin did not conduct any toxicology or PK bridging studies with a direct side-by-side comparison of Vascepa to Epadel.
- There is no published PK data on ethyl-EPA (Epadel) that would directly compare to PK data from AMR101 (*i.e.* differences in methodology, radioactivity, and length of administration). *As a note, single dose PK data for **EPA** (following oral ethyl-EPA administration) was published with the most relevant comparison between the literature and AMR101 as follows:*

Source	Species	Dose	AUC (µg.hr/mL)	C_{max} (µg/mL)	T_{max} (hr)
Published (Ishiguro 1987b, p. 99-118)	Rat, Male Wistar (Single Dose)	1000 mg/kg (90.5% purity) ^{14}C-EPA-E	7629	204	6
Amarin Study ZOC0001 (28 Day repeat dose Tox)*	Rat, Male Crl:WI (Han) (Day 1 PK of 28 Day Tox Study)	1000 mg/kg (98% purity)	150	21	4

(*) Data derived from an amended study report submitted under NDA 202057 due to incorrect (higher) calculations of EPA submitted under IND 102,457

It is worth noting that this single dose comparison between the two products would not support similarity between Vascepa (AMR101) and Epadel due to the wide differences in exposure likely due to the experimental differences in EPA plasma quantitation methodology.

- In the absence of any nonclinical bridging study conducted with the comparator Epadel, the use of bridging via chemical analysis was considered. The purity of ethyl-EPA (icosapent ethyl) in both products was ≥96% and impurities consisted (b) (4) with similar (but not identical) concentration (See attached table). However, to consider a chemical bridge, CMC requires a more extensive analysis as a side-by-side characterization of the two products would have to be completed, using the same physicochemical, structural and functional test methods.
- The sponsor stated that their bridging studies consisted of one 28-day rat toxicology study and one embryonic development study in the rat using only their product (AMR101). They maintain that as the toxicology profiles of AMR101 in these studies are similar to those published for Epadel, they serve as an adequate bridge.

From a **scientific perspective**, it would be reasonable to conclude that Amarin's ethyl-EPA product AMR101 (Vascepa) is highly similar to Mochida's marketed ethyl-EPA product Epadel, for the following reasons:

1. The source of ethyl-EPA before further purification is derived from fish.

2. The specifications for purity of ethyl-EPA are identical (≥96%).
3. The specifications for (b) (4), tocopherol, are identical.
4. The (b) (4) impurities for each product are similar but not identical; however the concentration limits for these (b) (4) are generally low, they are intermediates in fatty acid synthesis/ metabolism and are part of the complex mixture of unsaturated fatty acids in fish.
5. The pharmacology of poly-unsaturated fatty acids is well understood including their metabolism, synthesis, distribution and excretion. As fatty acids compete with one another for phospholipid and membrane incorporation, a purity of ≥96% icosapent-ethyl will likely have a similar biological effect even in the presence of other (b) (4) impurities.
6. Nonclinical repeat dose toxicology study results were similar but not completely identical between both products for the rat (possibly due to acceptable experimental variability between studies). Similarities included for example, fur/skin changes with oily discharge, WBC changes, clotting parameter alterations, and liver enzyme increases.

From a **regulatory perspective**, it is not clear if Amarin has fulfilled the bridging study requirements for a 505(b)(2) application for the following reasons:

1. Although the sponsor was informed at the pre-IND meeting that a rat study including a comparator dose group with Epadel would be required to establish a scientific basis to rely on the published literature, they chose not to do this citing multiple reasons.
2. No comparison to PK data could be made, as PK studies with AMR101 and Epadel were not completed by Amarin and PK data was not published in the referenced literature for Epadel.
3. Although the identities of (b) (4) impurities present in Epadel and Vascepa (AMR101) are similar, there are differences in the specifications of these biologically active (b) (4)
4. The 505(b)(2) FDA Guidance states that a 505(b)(2) application should include, "A Bioavailability/Bioequivalence (BA/BE) study comparing the proposed product to the listed drug (if any)". It further states that, "Before submitting the application, the applicant should submit a plan to the appropriate new drug evaluation division identifying the types of bridging studies that should be conducted". As Amarin originally submitted their NDA application as a 505(b)(1) and changed this to a 505(b)(2) before the filing meeting, they did not adequately plan for this type of submission.

Based upon scientific evidence of similar toxicologic profiles, similar (not identical) chemical profiles of highly purified icosapent-ethyl, and the well understood pharmacology of polyunsaturated fatty acids, it would be reasonable to conclude that Epadel and Vascepa are similar products. However, from a regulatory perspective, Amarin Pharma has not conclusively demonstrated direct comparability between Epadel and Vascepa due to the absence of any direct bridging study conducted by Amarin Pharma or an acceptable PK comparison to the literature references. Unless we may defer to scientific rationale, the regulatory recommendation based on the lack of a traditional bridging study, is a RTF.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

TEST	Ethyl-EPA Used in Mochida Toxicology Studies (Nippon Suisan Kaisha Ltd.)				AMR101 (Ethyl-EPA) Used in Amarin Toxicology Studies		Amarin Ethyl-EPA API (Nisshin Pharma Inc.)	EPADEL Ethyl-EPA API (Nippon Suisan Kaisha Ltd.)
Studies Where Used	K850416: 12 mo. rat 840424: 3 mo. rat K113: Rat Seg 1, Seg 2 K30415: Rabbit Seg 2, Rat Seg 3				EE171GQ: Genotox (Ames, Chrom Ab, Micronuc) EE070IX: 4Wk rat, 4Wk mouse, 39 Wk dog, Rat Seg 2, 6 mo. CARCI mouse		2 yr. Rat CARCI (Also used in >900 patients in CNS program under IND (b) (4)	Used in human population
Batch Numbers	K850416	840424	K113	K30415	EE171GQ	EE070IX	EE141IS, EE030HU	--
Assay as EPA-E, %	91.2	90.5	90.5	93.9	97.4%	NLT 96.0	NLT 96.0	NLT 96.0
Tocopherol, % (b) (4)	(b) (4)							
Refractive Index (20°C)								
Specific Gravity (20°C) (b) (4)								
Related Substances (b) (4) **as ethyl esters, %**								

(b) (4)

n.d. = not determined; (--) = not stated; NMT = Not More Than; NLT = Not Less Than

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

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?	✓		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		
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)?		✓	**Lack of Adequate Bridging Study:** 1. There was no non-clinical bridging study submitted with a direct comparison between Vascepa and the referenced product Epadel (PK or toxicology). The sponsor was informed during the PIND meeting on July 14, 2008 that "…Before the initiation of Phase 3 clinical studies, at a minimum, a 3-month rat study including a comparator dose group with Epadel will be required to establish a scientific basis to rely on the published data…". The sponsor subsequently stated that this study was "impractical and unnecessary" given the quality of Epadel data that exist. 2. Referenced literature for Epadel submitted in tabulated form for the pre-NDA meeting did not have acceptable PK data for comparison to AMR101 PK parameters upon review (Toxicology or Pharmacokinetic Studies); therefore bridging to Epadel via PK parameters could not be used. 3. A chemical bridge was considered; however in the absence of a toxicology bridge reliance on a CMC bridge would require extensive side-by-side characterization of Vascepa to Epadel, which also has not been provided.
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 formulation to be marketed is the same as used in the toxicology studies.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
	the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			
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?	✓		Route of administration is via oral hard gelatin capsules. Nonclinical studies were performed by oral gavage.
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?	✓		Pivotal Amarin sponsored toxicology studies were performed under GLP regulations. Referenced literature on which they rely for their 505(b)(2) application does not have any GLP designation, but was used in support of marketing Epadel (ethyl-EPA) in Japan.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			N/A (See content parameter 4)
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	✓		The proposed nonclinical study related language is found under Sections 8.1 (Pregnancy), 12.1 (Mechanism of Action), and 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility)
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	✓		Impurities have been identified (b) (4) [redacted]
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___No_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Amarin Pharma submitted AMR101 under pre-IND 102,457 to DMEP for the treatment of hypertriglyceridemia on May 9, 2008 with a pre-IND meeting request. The pre-IND meeting was held on July 14, 2008. There were multiple nonclinical questions regarding the use of published literature from Mochida's marketed product Epadel (ethyl-EPA) in Japan as well as the reliance

on previous human use of ethyl-EPA. It was repeatedly conveyed to the sponsor that it was unclear how AMR101 compared with the referenced literature product (Ethyl-EPA/ Epadel) and scientific justification would need to be provided to allow for reliance on the published data. Furthermore, the following was also communicated to the sponsor, *"Before the initiation of Phase 3 clinical studies, at a minimum, a 3-month rat study including a comparator dose group with Epadel will be required to establish a scientific basis to rely on the published data and a 9-month toxicity study in a nonrodent species will be required because of the apparent lack of nonrodent toxicity data."* While the sponsor did not provide a 3 month rat study with a comparator Epadel group, they did complete a 28-day rat and 9-month dog (with 3 month interim results) study to address the toxicologic safety profile of AMR101, and clinical trials were permitted to proceed. Amarin subsequently stated that an Epadel comparator study was "impractical and unnecessary" given the quality of Epadel data that exist.

Preliminary review of submitted studies at pre-NDA appeared appropriate and studies reviewed at that time supported that a sufficient toxicologic profile to assess the safety of ethyl-EPA was scientifically established. Based upon submitted study titles of Mochida's published literature studies in the meeting package, it was reasonable to conclude that Amarin referenced PK studies that included sufficient data to compare (bridge) to AMR101. Upon complete review of all published studies conducted by Mochida in support of Epadel, there was no sufficient PK data to use as a bridge to AMR101 in support of Amarin's 505(b)(2) application. Epadel repeat dose toxicology studies as well as single and repeat dose PK studies either did not contain PK data or did not experimentally compare with the measurement of PK parameters in the Amarin sponsored 28-day rat toxicology study.

In the absence of a clinical bioequivalence bridging study with Epadel, a nonclinical bridging study is needed to demonstrate comparability between the referenced literature (Epadel) and AMR101. Given the lack of any non-clinical bridging study, such as a 3-month toxicology study with an Epadel comparator group recommended at the pre-IND meeting, there is no sufficient bridging study to adequately compare AMR101 (Vascepa) to Epadel. Additionally, while product specifications for the main active ingredient, ethyl-EPA (icosapent ethyl), are identical for the two products (≥96%) the concentration specifications of the biologically active (b) (4) impurities present in the two products are not identical.

Comments To The Applicant:
Not applicable as the NDA will be filed.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
1. There is not sufficient evidence of the comparability of Epadel to Vascepa due to a lack of adequate toxicologic or pharmacokinetic bridging data. While Epadel and AMR101 have specifications for high purity icosapent ethyl (ethyl-EPA), the impurities in these products are biologically active (b) (4) that are not identical in their concentrations. As you did not complete a repeat dose toxicology study with an Epadel comparator group, an acceptable bridge would have been to show comparability of PK parameters between Epadel and AMR101.

The referenced toxicology literature studies that Mochida conducted in support of Epadel do not contain PK parameter data. Furthermore, referenced pharmacokinetic studies also conducted by Mochida do not contain experimentally comparable PK data to that of the 28-Day rat toxicology study with AMR101. For example, ^{14}C-EPA-E PK parameter data from published studies cannot be directly compared to PK data from the AMR101 28-day rat toxicology study as the methodology for analysis and reported values are not similar.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

As there is no adequate bridging information to Epadel, please conduct a 28-Day repeat dose toxicology study in the rat with an Epadel comparator group including PK analysis to provide an adequate bridge to the published Epadel (ethyl-EPA) literature, so that you may rely on these studies for your 505(b)(2) application.

2. Please submit all chemical analyses completed for Epadel to NDA 202057.

Stephanie Leuenroth-Quinn, Ph.D.	11/18/2011
Reviewing Pharmacologist	Date
Karen Davis-Bruno, Ph.D.	
Team Leader/Supervisor	Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

STEPHANIE J QUINN
11/18/2011

KAREN L DAVIS BRUNO
11/18/2011