

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**204977Orig1s000**

**PHARMACOLOGY REVIEW(S)**

Signed off in DARRTS on 8/5/2013

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 204977  
Supporting document/s: 000, and 0001  
Applicant's letter date: 1/31/2013, 3/6/2013  
CDER stamp date: 1/31/2013  
Product: AKR-963 soft gelatin capsules, liquid filled, 0.9 g.  
Indication: As an adjunct to diet to reduce triglyceride (TG)  
levels in adult patients with severe ( $\geq 500$  mg/dL)  
Hypertriglyceridemia  
Applicant: TRYGG Pharma Inc., Arlington, VA  
Review Division: DMEP  
Reviewer: Indra Antonipillai  
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## Executive Summary

1.1 **Introduction:** AKR-963 capsules (Proposed Trade Name: OMTRYGTM), a formulation of omega-3-acid ethyl esters sourced from fish oils, are indicated for the treatment of hypertriglyceridemia (triglycerides >500 mg/dl), which is the same indication as the approved reference drug, Lovaza (NDA 21-654). Similar to Lovaza, AKR-963 contains at least 900 mg omega-3 fatty acid ethyl esters per capsule. These are predominantly a combination of ethyl esters of eicosapentaenoic acid (EPAee, approximately 465 mg/capsule) and docosahexaenoic acid (DHAee, approximately 375 mg/capsule). The proposed daily dosage of AKR-963 is 4 capsules per day or 60 mg/kg/day.

1.1.2 **Additional Non Clinical Recommendations:**

No additional pre-clinical studies are required for this drug product.

1.1.3 **Labeling:** See the recommended labeling changes on page 95.

## 1.2 Brief Discussion of Nonclinical Findings

Sponsor conducted one 28-day comparative bridging toxicity study of AKR-963 with Lovaza in rats (main group= 10M + 10F rats) using doses of 200 and 4000 mg/kg/day with both AKR-963 and Lovaza. This study did not include control groups and only evaluated the high dose treated groups (AKR-963 and Lovaza) for histopathology. There's no evidence of pharmacologic activity in this study, as no effects were noted on triglycerides and total cholesterol in either treatment group compared to historical controls. The historical control ranges provided by the CRO are inadequate based on the age of the animals and the data years of collection to supplant concurrent controls, which may explain the apparent lack of pharmacodynamic or PD effects. In the 28-day toxicity study, AUC exposures of DHA and EPA were generally comparable with both drugs, and exposures were higher on day 28 than on day 1 (with both AKR-963 and Lovaza). At a HD, AUC exposures of DHA on day 28 were 1992 / 790 mcg.h/ml in male/females respectively (vs 1945/ 963 mcg.hr/ml in males/females with Lovaza). EPA exposures on day 28 were 2594/ 2201 mcg.h/ml (vs 2636/1636 mcg.h/ml in males/females respectively with Lovaza). Note that male rats had higher exposures of DHA, than female rats and drug accumulates over time. One male rat dosed with AKR-963 at 4000 mg/kg/day was found dead on Day 14 from a TK group (animal # 032 died, from 6M+6F/group), which was not noted with Lovaza. The cause of death is unknown but marked congestion in the liver and lungs was observed. Sponsor states that pale appearance of the heart and pulmonary congestion without any other obvious signs of toxicity was highly suggestive of an acute cardiac failure in this animal. Although the cause of a sudden death was undetermined, this condition was not considered treatment-related due to the lack of any other toxicity in this rat, and in the remainder of the rats from the same group. Additionally, sponsor outlined literature that shows a single death in a 28-day rat study, as well as shortened ventricular action potential in pigs, along with in vitro hERG channel effects, suggesting an occasional pro-arrhythmic effects of fish oil, despite a weight of evidence of anti-arrhythmogenic effects. Limited histopathology data were provided in this toxicity study. In general similar histopathology findings were noted with both drug products. Target organs of toxicity with both drugs were liver (inflammation in 3/20 rats vs 3/20 rats with Lovaza), kidney (inflammation in 3/20 rats vs 3/20 with lovaza) and eyes (focal retinal degeneration of mild severity in 1/10 female rats with both AKR-963 and Lovaza

respectively, but at the end of drug free recovery period it was only noted with AKR-963 in 1/5 males vs 0/5 with Lovaza). Note that findings in the urinary bladder were noted with AKR-963, not noted with Lovaza (in females subchronic and diffuse mucosal inflammation in 2/10 vs 0/10 with Lovaza). Additionally skin findings (hypotrichosis, of moderate severity) were noted in 1/5 female rats at the end of drug free recovery period with AKR-963, but not with Lovaza.

There are several fatty acid ethyl ester components in the AKR-963 drug product that exceed the levels found in Lovaza by >20X [REDACTED] (b) (4) [REDACTED], and some are present in clinical lots at higher concentrations than those tested in the lot used for toxicity testing. The sponsor has used literature NOAELs from repeat dose toxicity studies to establish safety of these 'related components'.

Note that there are limitations in the above pivotal toxicity study, these include 1) no concurrent controls were included in the bridging toxicity study; 2) we can-not tell if there is efficacy in animals, as no changes in triglycerides or total cholesterol were noted with either drug, 3) we cannot set a NOAEL, since the sponsor only provided histopathology at a HD of 4 g/kg/day with AKR-963 and Lovaza (no histopathology was conducted on the lower dose groups), 4) the toxicity noted in the above study does not appear to be the toxicity seen previously with the approved Lovaza (at 4X lower doses). It is possible that the relative absence of established target organ toxicity reflects the short dosing duration of 4 week study conducted here, and we do not have a 4-week rat toxicity study with the original Lovaza NDA, to compare these results to lovaza or AKR-963.

However the target organs of toxicity in this study with both drugs were similar, and appear to be liver, kidney, bladder, retina, and heart. Other than the bladder mucosal inflammation showing a higher incidence than Lovaza, and one male rat that died in the toxicokinetic group with acute cardiac failure with AKR-963, no other differences were noted in toxicity with both drugs (lovaza or AKR-963). The AUC exposures of EPA and DHA (the main ingredients of total omega-3-acid ethyl esters) were also in general similar with both drugs.

The NOAEL or tolerated doses of AKR-963 in a 4-week oral toxicity study in rats is considered 200 mg/kg/day (1200 mg/m<sup>2</sup>/day) which provides the safety margin of approximately 0.5X in humans at a recommended dose of 3600 mg/day (60 mg/kg/day or 2220 mg/m<sup>2</sup>/day, assuming 60 kg subject), based on body surface area. However the HD, that produced mortality (1/6 TK animal died) and histopathology findings in the liver, kidney and eyes (in both sexes), and in the urinary bladder (in females), provides safety margin of approximately 10X in humans at a recommended dose of 3600 mg/day (60 mg/kg/day or 2220 mg/m<sup>2</sup>/day, assuming 60 kg subject), based on body surface area. Thus, nonclinical data support approval of NDA 204977

1.2 **Recommendations:** The nonclinical data support approval of NDA 204977

## 2 Drug Information

### 2.1 Drug

Omega-3 polyunsaturated fatty acid ethyl esters or Omega-3 fatty acid ethyl esters

**Generic Name**

Omega-3 polyunsaturated fatty acid ethyl esters

**Code Name:** N/A

Proposed Trade name: OMTRYGTM

**Chemical Name:** of EPAee is

5,8,11,14,17-eicosapentaenoic acid, ethyl ester, (all-Z)-

Ethyl (5Z,8Z,11Z,14Z,17Z)-eicosa-5,8,11,14,17-pentaenoate

Chemical Name of DHAee is

4,7,10,13,16,19-docosahexaenoic acid, ethyl ester, (all-Z)-

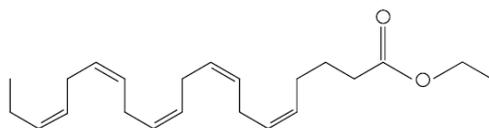
Ethyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate

**Molecular Formula/Molecular Weight:** EPAee  $C_{22}H_{34}O_2$  / 330.51

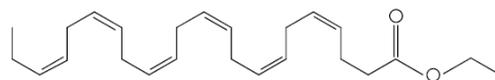
DHAee  $C_{24}H_{36}O_2$  / 356.55

**Structure:** The structures of the main components of the drug i.e EPAee and DHAee are shown below.

EPAee



DHAee

**Nonproprietary Name of Drug Substance**

Omega-3-acid ethyl esters

**Pharmacologic Class:** Fish oil supplement,  $\omega$ -3 poly-unsaturated fatty acids esters of eicosapentanoic (EPA) and docosahexaenoic acids (DHA). Both are essential fatty acids.

**Relevant INDs, NDAs, BLAs and DMFs:** NDA 21-654 (reference drug Lovaza, approved on 11/10/04), IND 107,259 (AKR-963, TRYGG Pharma). DMF (b) (4) (CMC information on the current drug), NDA 202-057 (Vascepa)

**Manufacturer for drug substance:** (b) (4)

(b) (4)

The drug product is manufactured at (b) (4)

**Drug Formulation:** The drug product (AKR-963 capsules, 1.16 g) is an immediate release capsule, which is comprised of at least 900 mg of total omega-3-acid ethyl esters that is (b) (4) with 4 mg/g  $\alpha$ -tocopherol. The drug product contains the following inactive ingredients, which are components of the capsule shell: gelatin, glycerin, and purified water.

Component	Function	Quality Standard	Amount per Capsule (g)
AKR-963 Drug Substance <sup>a</sup>		Trygg Pharma, Inc	1.16 (b) (4)
Total omega-3-acid ethyl esters	Active ingredient		
<i>d</i> $\alpha$ -Tocopherol	Antioxidant		
Capsule Shell	(b) (4)		
Gelatin		NF, Ph Eur	0.34
Glycerin		USP, Ph Eur	0.12
Purified water		USP, Ph Eur	<sup>b</sup>
White Ink	(b) (4)		Trace
		USP	
		NF	
		USP	<sup>c</sup>
		USP	<sup>c</sup>
		NF	<sup>c</sup>
		USP	<sup>c</sup>
		NF	
		USP	

<sup>a</sup> = Each capsule contains 1.16 g of AKR-963 drug substance, which is composed of at least 900 mg of total omega-3-acid ethyl esters, approximately 465-mg EPA<sup>ee</sup>, approximately 375-mg DHA<sup>ee</sup>, and 4.6-mg *d* $\alpha$ -tocopherol (b) (4)

**Excipients**

AKR-963 drug substance is filled into soft gelatin capsules without addition of other formulation components. The components of the soft gelatin capsule shells and printing ink are shown in Table 2.3.P-2. The component amounts in the drug product are all lower than the amounts found for oral dosage forms in the FDA Inactive Ingredients in Approved Drug Products database. All excipients comply with the USP/NF monographs.

Excipient	Function	Standard	Amount/Capsule,
Gelatin (b) (4)	(b) (4)	USP, Ph Eur	(b) (4)
Glycerin		USP, Ph Eur	(b) (4)
Purified water (b) (4)		USP, Ph Eur	
		USP	
		NF	
		USP	
		USP	
		NF	
		USP	
		NF	
	USP		

2.4 **Novel Excipients:** Sponsor states that the drug product does not contain novel excipients, or excipients of human or animal origin. Gelatin (pharmaceutical grade, manufactured by (b) (4) of AKR-963 capsules.

2.5 **Impurities/Degradants of Concern:**

Impurities present in the drug substance that are not degradation products are not monitored in the drug product. The only change in impurities observed in the drug product during the manufacturing process, or during storage, is an increase in the level of (b) (4) values.

(b) (4)

Thus, sponsor states that in the drug substance, an increase in the level of (b) (4) (b) (4) is observed. Note that (b) (4) is considered toxic with the (b) (4) judged to be the most toxic, and as a probable IARC human carcinogen. However, CMC clarified to us that (b) (4) is used to measure (b) (4) formation in the oil. It is not present in the fish oil, only used in the method. So, "anisidine level" refers to (b) (4) levels measured using (b) (4) (which forms a (b) (4) base adduct that is measurable by UV), and is not a concern.

## 2.6 Proposed Clinical Population and Dosing Regimen

Adult patients with severe ( $\geq 500$  mg/dL) hypertriglyceridemia. Dosing regimen is 4-Capsules once Daily, or 2- capsules twice Daily

## 2.7 Regulatory Background

The sponsor is submitting this application as 505(b)(2), based on prior approval of the drug product (Lovaza, NDA 21-654).

## 3 Studies Submitted

The sponsor has submitted a comparative bridging 28-day toxicity study in rats with AKR-963 and lovaza, and two geno-toxicity studies.

### 3.1 Studies Reviewed

The above 3 toxicity studies are reviewed here.

3.2 **Studies Not Reviewed.** All submitted new pre-clinical studies are reviewed here.

3.3 **Previous Reviews Referenced:** Most toxicity studies have been reviewed under NDA 21-654 (Lovaza)

## 4 PHARMACOLOGY

### INTRODUCTION AND DRUG HISTORY

Trygg Pharma, Inc. is seeking US marketing approval for AKR-963 capsules (Proposed Trade Name: OMTRYGTM), a formulation of omega-3-acid ethyl esters sourced from fish oils, for the treatment of hypertriglyceridemia (HTG), which is the same indication as the approved reference listed drug, Lovaza (NDA 21-654). Similar to Lovaza, AKR-963 contains at least 900 mg omega-3 fatty acid ethyl esters sourced from fish oils per capsule. These are predominantly a combination of ethyl esters of eicosapentaenoic acid (EPAee, approximately 465 mg/capsule) and docosahexaenoic acid (DHAee, approximately 375 mg/capsule). The proposed daily dosage is 4 capsules per day (3600 mg/day or 60 mg/kg/day).

The primary dietary source of omega-3 fatty acids is fish (and fish oils) from fatty, cold-water species. Fish oil contains approximately 30% EPA and DHA in a triacylglycerol form. Although omega-6 fatty acids are abundant in Western diets (particularly in vegetable oils rich in linoleic acid), humans cannot convert omega-6 fatty acids to omega-3-fatty acids and, hence, the latter must be obtained from separate dietary sources. The manufacturing process converts the EPA and DHA (b) (4)

from the concentrations found naturally. Both EPAee and DHAee are sparingly soluble in water at pH values ranging from 3 to 7.

The sponsor is submitting this application as 505(b)(2), based on prior approval of the drug product (Lovaza, NDA 21-654), and the published literature on fish oils to describe the toxicology of AKR-963

**Description of Manufacturing Process**

The starting material

(b) (4)

[Redacted text block]

(b) (4)

[Redacted text block]

**Comparison to Reference Listed Drug (and related components such as non-omega 3 fatty acids)**

The reference listed drug (RLD) for this NDA is Lovaza®(omega-3-acid ethyl esters) capsules. A comparison of the RLD and the proposed drug product, AKR-963 capsules, is given in [Table 2.3-1](#); all Lovaza information listed in the table are based on the approved labeling.

	Lovaza	AKR-963 Capsules
Route of administration	Oral	
Active Ingredient	Omega-3-acid ethyl esters	
Product source	Fish oil	
Expressed Strength (g)	1 <sup>b</sup>	0.9
Total omega-3-acid ethyl esters (mg/capsule) <sup>a</sup>	At least 900	
EPAee (mg/capsule)	Approximately 465	
DHAee (mg/capsule)	Approximately 375	
Minor omega-3-acid ethyl esters	Not specified in RLD label	(b) (4)
Dosage form	Capsule	
Inactive ingredients	Gelatin, glycerol, water, α-tocopherol (in a carrier of soybean oil)	Gelatin, glycerol, water, α-tocopherol (in a carrier of sunflower oil)
Capsule fill weight (g)	1	1.16
<sup>a</sup> =	(b) (4)	
<sup>b</sup> =	The RLD label states the description as each 1-gram capsule of Lovaza contains at least 900 mg of ethyl esters of omega-3 fatty acid sourced from fish oil. These are predominately a combination of ethyl esters of EPA - approximately 465 mg and DHA – approximately 375 mg.	

Note that both AKR-963 and Lovaza contain at least 900 mg total omega-3-acid ethyl esters, which include approximately 465 mg EPAee and approximately 375 mg DHAee, and thus both products have the same strength. Although the expression of strength listed in [Table 2.3-1](#) is 0.9 g and 1 g, respectively, the proposed strength for AKR-963 is 0.9 g to be consistent with the unit (g) for the strength as specified in the RLD label and to reflect that each capsule contains at least 900 mg of total omega-3-acid ethyl esters.

The concentration of the omega-3-acid ethyl esters in the drug substance for the proposed drug product (b) (4). Therefore, in order to deliver the same amount of omega-3-acid ethyl esters (b) (4).

Additionally they state that the fatty acid profiles for AKR-963 and Lovaza are marginally different. A comparison of the fatty acid profiles for the primary stability/registration lots and nonclinical and clinical drug substance batches of AKR 963 oil and Lovaza oil is presented in [Table 2.4-2](#). Variability in the fatty acid profiles is expected based on inherent variability in the starting material, (b) (4) and does not impact demonstration of comparable safety of AKR-963 with Lovaza (see [Section 2.6.6.8.1](#)).

Table 2-4.2. Fatty acid ethyl ester comparison of AKR and Lovaza

Table 2.4-2. Fatty Acid Ethyl Ester Comparison of AKR-963 and Lovaza						
Fatty Acid Ethyl Ester	Area %					
	Lovaza Oil (Batch 963134W)	AKR-963 Drug Substance Batch Lot Numbers				Primary Stability Lots <sup>c</sup>
		Nonclinical/Clinical Lots				
		M100086 <sup>a</sup>	M100021 <sup>b</sup>	M100022 <sup>c</sup>	M110013 <sup>d</sup>	(b) (4)
C14:0	Myristic					
C16:0	Hexadecanoic					
C16:1	Hexdecenoic					
C16:2	Hexdecadienoic					
C16:3	Hexdecatrienoic					
C17:1	Heptadecenoic					
C16:4	Hexadecatetraenoic					
C18:0	Stearic					
C18:1 n-9	Oleic					
C18:1 n-7	Cis-vaccenic					
C18:2 n-6	Linoleic					
C18:3 n-6	γ-linolenic					
C18:3 n-3	α-linolenic					
C18:4 n-3	Octadecatetraenoic					
C20:0	Eicosanoic					
C20:1	Eicosenoic					
C20:2 n-9	Eicosadienoic					
C20:2 n-6	Eicosadienoic					
C20:3 n-6	Eicosatrienoic					
C20:4 n-6	Arachadonic					
C20:3 n-3	Eicosatrienoic					
C20:4 n-3	Eicosatetraenoic					
C20:5 n-3	Eicosapentaenoic (EPA)					

Table 2-4.2. Fatty acid ethyl ester comparison of AKR and Lovaza (continued)

Table 2.4-2. Fatty Acid Ethyl Ester Comparison of AKR-963 and Lovaza							
Fatty Acid Ethyl Ester		Area %					
		Lovaza Oil (Batch 963134W)	AKR-963 Drug Substance Batch Lot Numbers				Primary Stability Lots <sup>e</sup>
			Nonclinical/Clinical Lots				
			M100086 <sup>a</sup>	M100021 <sup>b</sup>	M100022 <sup>c</sup>	M110013 <sup>d</sup>	
C22:0	Docosanoic						
C22:1 n-11	Cetoleic						
C22:1 n-9	Erucic						
C21:5 n-3	Heneicosapentaenoic						
C22:4 n-6	Docosatetraenoic						
C22:5 n-6	Docosapentaenoic						
C22:5 n-3	Docosapentaenoic						
C22:6 n-3	Docosahexaenoic (DHA)						
C24:1	Tetracosenoic						
Minor components							
Total omega-3							
Non-omega-3							
Sum for each lot							

(b) (4)

<sup>a</sup> = Used for drug product Lot 0003550700 and all toxicology studies and clinical studies TRGG-963-002 and TRGG-963-003.  
<sup>b</sup> = Used for drug product Lot 0003170601 and clinical studies TRGG-963-002.  
<sup>c</sup> = Used for drug product Lot 0003170602 and clinical studies TRGG-963-002.  
<sup>d</sup> = Used for drug product Lot 0003559500 and clinical studies TRGG-963-002, TRGG-963-004, TRGG-963-005, and TRGG-963-006.  
<sup>e</sup> = Range of values from 3 registration lots, batches M110015, M110017, and M110018. Where no range is provided, the values were the same across the 3 lots.

Sponsor has also provided a comparison of the results for the proposed product and the reference listed drug, Lovaza®, in Table 3.2.P.5.4-3, which lists the omega-3-acid ethyl ester content (in mg/capsule and mg/g of oil) and the fatty acid ethyl ester profiles (in area %). This comparison showed that the amount of the total omega-3-acid ethyl ester active ingredient on a per capsule basis is equivalent, although there are differences in the amounts of the individual fatty acid ethyl esters.

**Related components, such as non-omega 3 ethyl ester fatty acids.**

Table 2.4-2 shows that non-omega-3 ethyl ester fatty acids are present in higher amounts in AKR-963 than in lovaza (15-16% vs 6.5% in lovaza). Note that several fatty acid ethyl esters related components are present in higher amounts in the current drug product vs Lovaza.

These include

(b) (4)

Thus, as stated above, there are several non-omega-3 ethyl ester fatty acids components in AKR-963 that exceed the levels in Lovaza (b)

(b) (4)

(such as (b) (4)) see Table 2.6.6-8 below. Among these (such as (b) (4)) are present in clinical lots at higher concentrations than those tested in the lot used for toxicity testing. However, sponsor has provided literature NOAELs from repeat dose toxicity studies to establish safety of these 'related components'. See sponsor's description below.

*Potential daily exposure to individual non-omega-3 fatty acids and the omega-3 fatty acid 20:3 n-3 (eicosatrienoic acid) in AKR-963 that are not present in or that exceed the levels in Lovaza is low (Table 2.6.6-8). A summary of the available safety information for these fatty acids as a component of GRAS and dietary oils or from published nonclinical studies, in which oils containing these fatty acids were evaluated, (see Section 2.6.6.3.2) is provided below. For many of the published studies, it was possible to estimate exposure to an individual fatty acid based on the percentage in the diet. The exposure at the highest dose or NOAEL was used to calculate a safety margin as a multiple of the possible clinical exposure based on body surface area conversion (Table 2.6.6-8).*

**Table 2.6.6-8. Assessment of Fatty Acids in AKR 963 Not Present in or Exceeding Levels in Lovaza**

Lipid Number	Common Name Fatty Acid Ethyl Ester	Lovaza Area %	AKR-963 Area % <sup>a</sup>	Estimated Human Exposure from AKR-963, mg/kg <sup>b</sup>	NOAEL in Rats, mg/kg	Human Equivalent Dose, mg/kg <sup>c</sup>	Safety Margin <sup>d</sup> (b) (4)
C14:0	Myristic						
C16:0	Hexadecanoic (palmitic acid)						
C16:1	Hexadecenoic (palmitoleic acid)						
C16:2	Hexadecadienoic acid						
C16:3	Hexadecatrienoic acid						
C17:1	Heptadecenoic acid						
C18:0	Stearic acid						
C18:1 n-9	Oleic acid						
C18:1 n-7	Cis-vaccenic acid						
C18:2, n-6	Linoleic acid						
C18:3 n-6	γ-Linolenic acid						
C18:3 n-3	α-Linolenic						
C18:4 n-3	Octadecatetraenoic						
C20:0	Eicosanoic acid (eicosanoate)						
C20:1	Eicosenoic (eicosanoate)						
C20:2 n-6	Eicosadienoic acid (11,14-eicosadienoic acid)						
C20:3 n-6	Eicosatrienoic (homogamma-linolenic acid)						
C20:3 n-3,	Eicosatrienoic						
C20:4 n-3	Eicosatrienoic						
C22:0	Docosanoic						
C22:1 n-11	Cetoleic acid						

**Table 2.6.6-8. Assessment of Fatty Acids in AKR 963 Not Present in or Exceeding Levels in Lovaza**

Lipid Number	Common Name Fatty Acid Ethyl Ester	Lovaza Area %	AKR-963 Area % <sup>a</sup>	Estimated Human Exposure from AKR-963, mg/kg <sup>b</sup>	NOAEL in Rats, mg/kg	Human Equivalent Dose, mg/kg <sup>c</sup>	Safety Margin <sup>d</sup> (b) (4)
C22:1 n-9	Erucic acid						
C21:5 n-3	Heneicosapentaenoic						
C22:5 n-3	Docosapentaenoic						
C24:1	Tetracosenoic						

<sup>a</sup> = Value is the highest reported value for AKR-963 stability registration lots listed in *Table 2.6.1-1*.  
<sup>b</sup> = Obtained by multiplying area % in AKR-963 × 1160 mg oil/capsule × 4 capsules/60 kg man.  
<sup>c</sup> = Based on body surface area.  
<sup>d</sup> = Calculated using estimated exposure.  
<sup>e</sup> = Hammond, 2001a.  
<sup>f</sup> = Pillaia, 2011.  
<sup>g</sup> = MacKenzie, 2010.  
<sup>h</sup> = Rabbani, 1999 and 2001.  
<sup>i</sup> = Hammond 2008  
<sup>j</sup> = Blum, 2007a, 2007b.  
NA = No data available to calculate.  
Note: Excluding omega-3 fatty acids included in total omega-3 specifications.

Thus sponsor has qualified the levels of not only the omega-3-acid ethyl ester content, but also the non-omega-3-ethyl ester components in AKR-963 in the current submission, based on the 28-day toxicity study in rats (reviewed in this application), and by providing NOAEL's from the repeat dose toxicity studies (from literature references) to establish the safety of these related components.

Note that lot number used in all toxicity studies was 0003550700 (with drug substance lot # M100086), and for two clinical studies (TRGG-963-002, TRGG- 963-003), see the Table below.

Lot Number	0003910400	0003910500	0003910600	0003170601	0003170602	0003172900	0003550700	0003559500
Batch size (capsules)	(b) (4)							
Date of manufacturing	(b) (4)							
Release date	(b) (4)							
Drug substance lot number	M110015	M110017	M110018	M100021	M100022	M100002	M100086/S	M110013
Batch use	Registration/primary stability	Registration/primary stability	Registration/primary stability	Clinical supplies, supporting stability	Clinical supplies, supporting stability	Supporting stability	Nonclinical, clinical supplies, supporting stability	Clinical supplies, supporting stability
Fill weight (g)	(b) (4)							
Appearance <sup>a</sup>	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
EPAee ID	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
DHAee ID	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
EPAee (mg/g) <sup>a</sup>	(b) (4)							
DHAee (mg/g) <sup>a</sup>	(b) (4)							
EPAee + DHAee (mg/g) <sup>a</sup>	(b) (4)							
Total omega-3-acid ethyl esters (mg/g) <sup>a</sup>	(b) (4)							
Uniformity of dosage units (weight variation, total omega-3-acid ethyl ester, mg/sgc) <sup>b</sup>	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies

### Impurities

The potential impurities include environmental pollutants, residual solvents, and organic impurities from oxidation and thermal degradation. The quality of proposed GMP lots of drug substance was compared to the impurity profile of lots used in preclinical studies to assure that any impurities present above ICH qualification levels are fully qualified. The comparison of impurity profile of GMP lots of drug substance and lots used in nonclinical and clinical studies is provided in [Table 2.6.1-2](#). Additional information on the impurity profile of AKR-963 batches is found in [Section 3.2.S.3.2](#).

The impurity profile of the drug substance primary stability lots was compared to the impurity profile of lots used in preclinical and clinical studies to assure that any impurities present above ICH qualification levels are fully qualified (Table 2.6.1-2).

Impurity	AKR-963 Drug Substance Lot Numbers				Primary Stability Lots <sup>e</sup>
	M100086 <sup>a</sup>	M100021 <sup>b</sup>	M100022 <sup>c</sup>	M110013 <sup>d</sup>	
(b) (4) EPA (%)					(b) (4)
DHA (%)					
<sup>a</sup>	= Used for drug product Lot 0003550700 and all toxicology studies and clinical studies TRGG-963-002 and TRGG-963-003.				
<sup>b</sup>	= Used for drug product Lot 0003170601 and clinical studies TRGG-963-002.				
<sup>c</sup>	= Used for drug product Lot 0003170602 and clinical studies TRGG-963-002.				
<sup>d</sup>	= Used for drug product Lot 0003559500 and clinical studies TRGG-963-002, TRGG-963-004, TRGG-963-005, and TRGG-963-006.				
<sup>e</sup>	= Range of values from 3 registration lots, batches M110015, M110017, and M110018.				

Note that in all the toxicity studies, lot number 0003550700 (or M100086) has been used as stated above.

### Primary Pharmacology

AKR-963 is a mixture of omega-3 fatty acids isolated from the fish oil. It is composed primarily of the unsaturated omega-3 fatty acid esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

The mechanism of action of omega-3-acid ethyl esters is not completely understood. Potential mechanisms of action include inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase, increased mitochondrial and peroxisomal  $\beta$ -oxidation in the liver, decreased lipogenesis in the liver, and increased plasma lipoprotein lipase activity. Omega-3-acid ethyl esters may reduce the synthesis of triglycerides (TG) in the liver because EPA and DHA are poor substrates for the enzymes responsible for triglycerides synthesis, and EPA and DHA inhibit esterification of other fatty acids.

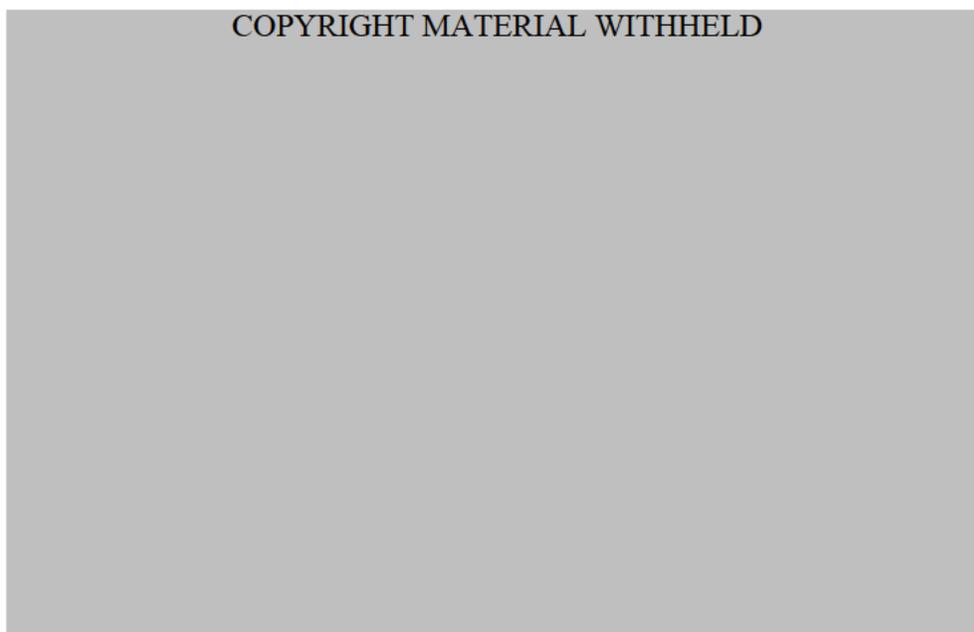
Sponsor states that there are several mechanisms of action of omega 3-acid ethyl esters and provides the description of these, see below:

From the published literature, several mechanisms of action for omega-3-acid ethyl esters are postulated (*Harris, 2006; GSK, 2010; Calder, 2012; Hofacer, 2012; Figure 2.4-3*):

1. Activation of peroxisome proliferator-activator receptor (PPAR) $\alpha$  by noncovalent binding of omega-3 acids to the receptor. This in turn leads to both fatty acid oxidation and reduced triglyceride and very low density lipoprotein (VLDL) secretion.

In a review of the literature on the potential mechanisms by which omega-3 acids reduce serum triglyceride levels, the majority of investigations were conducted in rats (*Harris, 2006*). While human kinetic studies have shown that fish oils rich in omega-3 acids reduce triglyceride levels by a decrease in very low-density triglyceride secretion rates, the most consistent effect of high omega-3-acid diet studies in rats is to reduce lipogenesis. Increased mitochondrial or peroxisomal  $\beta$ -oxidation was frequently reported and inhibition of triglyceride-synthesizing enzymes was only occasionally reported. However, the literature in rats is considered tentative due to the unphysiologically high doses of omega-3 acids used in the majority of studies.

Figure. See the potential mechanism described below (from Harris et al., 2006



**Figure 2.6.2-1. Potential Mechanism by Which Omega-3 Fatty Acids Influence Hepatic Triglyceride Metabolism (from Harris, 2006)**

DAG = diacylglycerol; DAGT = diacylglycerol acyl transferase; FA = fatty acids; NEFA = nonesterified fatty acids; PA = phosphatidic acid

Proposed genetic mechanisms of fish oils are described below:

Several hypotheses have been proposed regarding the mechanism of action of the beneficial effects of fish oil on fat metabolism, including down-regulation of genes involved in lipid biosynthesis such as SREBP-1c and SREBP-2 mRNA, and suppressed transcription of genes targeted by SREBP (including fatty acid synthase, SCD1, and acetyl-coenzyme A carboxylase- $\alpha$ ) (*Hirako, 2010; Yang, 2011; Hofacer, 2012*). In addition, oral administration of fish oil has been associated with up-regulation of genes involved in fatty acid oxidation such as PPAR $\alpha$  and AOX (a target gene of PPAR $\alpha$ ), which in turn leads to both fatty acid oxidation and reduced triglyceride and VLDL secretion (*Hirako, 2010; Hirako, 2011; Calder, 2012*).

EPA and DHA are hydrolyzed after oral administration, absorbed via the intestinal lymphatic system, and incorporated into various lipids, such as triglycerides in chylomicrons in the lymph, triglycerides in phospholipids in tissues, and triglycerides and phospholipids in lipoproteins in plasma (FAO, 2010; Yang, 1990; Ishiguro, 1988). EPA and DHA are incorporated in tissues; phospholipids can be metabolized by tissue cyclooxygenase to prostaglandins, prostacyclins, thromboxanes, and resolvins, and by lipoxygenases to leukotrienes, resolvins, and protectins (FAO, 2010).

### **Non-clinical studies**

Since most studies have been conducted with the reference drug lovaza, only a 28-day bridging toxicity / toxicokinetics study, and two geno-toxicity studies have been conducted with the present drug AKR-963.

## **5 Pharmacokinetics / ADME / Toxicokinetics**

### **5.2 Toxicokinetics in rats**

At a high dose of 4000 mg/kg/day, the AUC exposures with both drugs in general were similar. Plasma exposures of both DHA and EPA were higher in males than in females (both on day 1 and on day 28) following dosing with AKR-963 or Lovaza. Accumulation of the drug was generally noted on day 28 vs day 1.

On day 28, AUC exposures of EPA were slightly higher in females at a high dose of AKR-963 vs Lovaza (2201 vs 1636 mcg.hr/ml with Lovaza on day 28). Also, the AUC exposures of DHA were significantly higher in females at a low dose of AKR-963 vs Lovaza (536 vs 135 mcg.hr/ml with Lovaza).

Table. AUC exposure comparisons of DHA and EPA following administration of AKR-963 and Lovaza in a 28-day bridging toxicity study in rats.

	DHA	EPA
	AUC <sub>0-24 hrs</sub> mcg.hr/ml	AUC <sub>0-24 hrs</sub> mcg.hr/ml
	Day 1 / Day 28	Day 1 / Day 28
Dose: mg/kg/day		
<b>With AKR-963</b>		
<b>Males</b>		
200	492 / 1185	482 / 891
4000	1248 / 1992	2333 / 2594
<b>Females</b>		
200	119 / 536	398 / 576
4000	435 / 790	1597 / 2201
<b>With Lovaza</b>		
<b>Males</b>		
200	664 / 921	755 / 615
4000	1204 / 1945	2566 / 2636
<b>Females</b>		
200	258 / 135	344 / 408
4000	701 / 963	1592 / 1636

Table. Sponsor's summary of TK data from a 28-day toxicity study in rats is shown below:

<b>Table 2.6.5-4. Pharmacokinetics: Absorption after Repeated Doses</b>									
Report Number: 241342		Test Article: AKR-963 and Lovaza							
Species:		Sprague Dawley Rat							
Gender (M/F)/Number of Animals:		6M <sup>a</sup> /6F							
Feeding Condition:		Fed							
Vehicle/Formulation:		None							
Method of Administration:		Oral gavage							
Sample (eg, Whole Blood, Plasma, Serum):		Plasma							
Assay:		LC/MS/MS							
Dose (mg/kg):		200 (AKR-963) <sup>b</sup>		200 (Lovaza)		4000 (AKR-963) <sup>b</sup>		4000 (Lovaza)	
Analyte: <sup>c</sup>		DHA	EPA	DHA	EPA	DHA	EPA	DHA	EPA
PK Parameters: <sup>d</sup>		Gender							
C <sub>max</sub> (µg/mL) – day 1	Male	56 ± 8	85 ± 11	86 ± 11	126 ± 13	128 ± 15	247 ± 25	128 ± 23	264 ± 43
	Female	35 ± 7	62 ± 10*	39 ± 11*	47 ± 16*	54 ± 14*	130 ± 25*	48 ± 5*	106 ± 6*
C <sub>max</sub> (µg/mL) – day 28	Male	102 ± 9	127 ± 12	86 ± 12	98 ± 12	175 ± 15	371 ± 50	160 ± 20	381 ± 64
	Female	54 ± 9*	65 ± 7	43 ± 9	59 ± 12	116 ± 19*	231 ± 24	111 ± 18*	211 ± 40
t <sub>max</sub> (hours) – day 1	Male	2.3 ± 1.1	1.2 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3
	Female	1.7 ± 0.5	1.8 ± 0.5	6.7 ± 3.6	4.0 ± 1.3	7.2 ± 3.5	8.3 ± 3.3	12.7 ± 3.6*	8.0 ± 3.3
t <sub>max</sub> (hours) – day 28	Male	3.2 ± 1.0	1.3 ± 0.2	2.0 ± 0.4	1.3 ± 0.2	2.2 ± 0.5	2.0 ± 0.0	1.8 ± 0.2	1.8 ± 0.2
	Female	3.0 ± 1.1	2.0 ± 0.4	1.7 ± 0.2	1.5 ± 0.2	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	1.8 ± 0.2
AUC <sub>0 to 24</sub> (µg*h/mL) – day 1	Male	492 ± 176	482 ± 71	664 ± 146	755 ± 117	1248 ± 178	2333 ± 234	1204 ± 226	2566 ± 222
	Female	119 ± 89	398 ± 50	258 ± 144	344 ± 34	435 ± 105*	1597 ± 189*	701 ± 92*	1592 ± 132*
AUC <sub>0 to 24</sub> (µg*h/mL) – day 28	Male	1185 ± 144**	891 ± 79**	921 ± 189	615 ± 98	1992 ± 236	2594 ± 231	1945 ± 504	2636 ± 265
	Female	536 ± 167	576 ± 53	135 ± 128	408 ± 112	790 ± 174*	2201 ± 184	963 ± 273*	1636 ± 358
Mean residence time – day 1	Male	(19.2)	8.1	11.4	7.8	(19.6)	12.7	(14.5)	12.0
	Female	4.7	5.4	(29.5)	10.2	(75.8)	(42.5)	(66.6)	(27.6)

Table. Sponsor's summary of TK data from a 28-day toxicity study in rats (continued).

Table 2.6.5-4. Pharmacokinetics: Absorption after Repeated Doses									
Report Number: 241342		Test Article: AKR-963 and Lovaza							
Species:		Sprague Dawley Rat							
Gender (M/F)/Number of Animals:		6M <sup>a</sup> /6F							
Feeding Condition:		Fed							
Vehicle/Formulation:		None							
Method of Administration:		Oral gavage							
Sample (eg, Whole Blood, Plasma, Serum):		Plasma							
Assay:		LC/MS/MS							
Dose (mg/kg):		200 (AKR-963) <sup>b</sup>		200 (Lovaza)		4000 (AKR-963) <sup>b</sup>		4000 (Lovaza)	
Analyte: <sup>c</sup>		DHA	EPA	DHA	EPA	DHA	EPA	DHA	EPA
PK Parameters <sup>d</sup>		Gender							
Mean Residence Time – day 28	Male	11.9	8.5	(23.5)	8.4	(19.6)	10.3	11.4	8.8
	Female	10.5	8.5	(4.5)	10.5	10.9	10.0	13.7	11.2
t <sub>1/2(e)</sub> – day 1	Male	(14.7)	7.0	8.4	6.5	(14.0)	9.3	(10.6)	8.4
	Female	3.1	3.5	(20.9)	7.4	(51.4)	(29.5)	(45.4)	(18.8)
t <sub>1/2(e)</sub> – day 28	Male	8.7	6.9	(16.2)	7.0	(13.8)	8.0	8.3	7.1
	Female	7.7	6.3	(2.9)	8.2	8.5	7.2	10.1	8.5
CL/F (L/h/kg) – day 1	Male	(0.3)	0.4	0.3	0.3	(2.2)	1.4	(2.6)	1.3
	Female	1.4	0.6	(0.4)	0.5	(2.4)	(1.1)	(1.7)	(1.4)
CL/F (L/h/kg) – day 28	Male	0.1	0.2	(0.1)	0.3	(1.4)	1.4	6.9	1.4
	Female	0.3	0.3	(1.1)	0.4	4.3	1.6	3.3	1.8

Table. Sponsor's summary of TK data from a 28-day toxicity study in rats (continued).

Table 2.6.5-4. Pharmacokinetics: Absorption after Repeated Doses										
Report Number: 241342		Test Article: AKR-963 and Lovaza								
Species:		Sprague Dawley Rat								
Gender (M/F)/Number of Animals:		6M <sup>a</sup> /6F								
Feeding Condition:		Fed								
Vehicle/Formulation:		None								
Method of Administration:		Oral gavage								
Sample (eg, Whole Blood, Plasma, Serum):		Plasma								
Assay:		LC/MS/MS								
Dose (mg/kg):		200 (AKR-963) <sup>b</sup>		200 (Lovaza)		4000 (AKR-963) <sup>b</sup>		4000 (Lovaza)		
Analyte: <sup>c</sup>		DHA	EPA	DHA	EPA	DHA	EPA	DHA	EPA	
PK Parameters <sup>d</sup>		Gender								
Vz/F (L/kg) – day 1		Male	(6.1)	3.8	3.1	2.3	(45.1)	19.2	(40.3)	16.1
		Female	6.3	3.1	(13.0)	5.3	(175.4)	(45.4)	(109.5)	(37.8)
Vz/F (L/kg) – day 28		Male	1.7	2.0	(3.2)	3.0	(27.6)	15.7	22.3	14.2
		Female	3.2	2.8	(4.7)	4.9	53.0	16.8	48.7	22.3
<b>Additional Information:</b> Note: Values in parentheses are estimated TK parameters (AUC <sub>0-∞</sub> , t <sub>1/2(e)</sub> , CL/F, Vz/F, and MRT) for DHA and EPA that were considered unreliable due to the difficulty resolving all of the plasma concentration versus time data for each animal, especially at lower doses, where extrapolation of the AUC was > 20%.										
<sup>a</sup> = Only 5 males in the 4000 mg/kg/day group on day 28 due to incidental death of one rat.										
<sup>b</sup> = The reported dose levels were based on a minimum specification of 777 mg omega-3-acid ethyl esters per gram of oil (777 mg/g); however, the measured content of the tested lot was 851 mg/g. Therefore, actual dose levels of AKR-963 administered were 219 and 4381 mg/kg/day.										
<sup>c</sup> = Predose levels of endogenous DHA and EPA present in the plasma of individual rats on day 1 were subtracted from the plasma levels measured at the various blood collection times on day 1 and day 28 to obtain the net plasma levels of DHA and EPA. The TK analysis was performed using the net plasma levels per time point per group of rats. DHA and EPA (as free fatty acids) were measured from total lipids.										
<sup>d</sup> = All values are mean ± standard error about the mean.										
* = Females significantly different from males, $p < 0.05$ , One-Way ANOVA, SNK post-hoc analysis.										
** = Day 28 significantly different from day 1, $p < 0.05$ , One-Way ANOVA, SNK post-hoc analysis.										
ANOVA = Analysis of variance.										
LC/MS/MS = Liquid chromatography – tandem mass spectrometry.										
SNK = Student Newman–Keuls.										

Thus in general in rats, systemic exposure to AKR-963 and lovaza was similar, it was higher in males than in females, generally did not increase in a dose proportional manner from 200 to 4000 mg/kg in both sexes and exposures (more of DHA than EPA) increased over time suggesting accumulation over the 4-week period.

### Pharmacokinetic in humans

Sponsor has conducted a total of 5 clinical trials: these include four bioequivalence studies in healthy subjects (Studies: TRGG-963-003, TRGG- 963-004, TRGG-963-005; TRGG-963-006), and one Phase 3 study in patients with high triglycerides levels (TRGG-963-002, with fasting triglycerides levels of 500 mg/dL-1500 mg/dL).

The bioavailability studies were conducted under a variety of fed and fasted conditions to evaluate the comparative bioavailability of AKR-963 versus Lovaza. This series of studies used the same comparative dose of AKR-963 and Lovaza (3600 mg of omega-3-acid ethyl esters [4 capsules] as a single dose). Studies examined from testing total EPA and DHA lipid plasma concentrations after a moderate-fat meal (34% of total kcal) over a 24 hours period, to a replicate study after 1) either a high fat meal (50% of total kcal) or 2) in the fasting state, over a 72-hour period. The sponsor measured total EPA and DHA lipids (as well as EPA and DHA fatty acids) and also the EPA and DHA ethyl esters.

#### See sponsor's description below:

Following oral administrations, EPA increases in a dose-dependent manner, but DHA increases are less than dose-proportional. Table 7.0-1 presents in-house data for the range of mean values for total EPA and DHA pharmacokinetic data following a single, oral, 4000 mg dose under fed conditions.

**Table 7.0-1 Pharmacokinetic Estimations of Total EPA and Total DHA**

		Tmax (h)	Cmax (µg/mL)	AUC (µg•h/mL)
Total lipids	EPA	6.0–8.0	56.0–75.4	959.7–1700.2
	DHA	5.5–6.0	34.2–44.9	290.3–661.7

These data indicate that absorption of EPA and DHA is minimal and highly variable under fasting conditions.<sup>3</sup> The baseline-adjusted Cmax values from total lipids is between 19 and 26% for EPA and between 12 and 13% for DHA of the total measured concentration under fasting conditions.

In comparison, under fed conditions, the baseline-adjusted Cmax values from total lipids is between 73 and 82% for EPA and 40 and 43% for DHA of the total measured concentration.<sup>4,5</sup>

Initially, sponsor conducted two PK studies in humans (TRGG-963-003, TRGG- 963-004), see the Table below. Based on the above studies, definite bioequivalence studies were conducted in subjects after a high fat meal or in a fasting state (TRGG-963-005; TRGG-963-006) to show that the AKR-963 was bioequivalent to Lovaza

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) Dosage Regimen Route of Administration	Number of Subjects Enrolled (Planned/ Actual)	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status Type of Report
BE (fed)	<a href="#">TRGG-963-003</a>	Section 5.3.1.2	To evaluate the BE of AKR-963 relative to Lovaza by assessing plasma EPA + DHA concentrations following a single dose of 4 capsules	Randomized, double-blind, 2-period, 2-treatment, 2-sequence crossover design of single doses of AKR-963 and Lovaza immediately following a standardized moderate-fat (34% of kcal) meal	AKR-963 (3600 mg of omega-3 ethyl esters [4 capsules] single dose)  Lovaza (3600 mg of omega-3 ethyl esters [4 capsules] single dose) oral	72 subjects randomized  65 subjects included in PK population	Healthy men and women	2 single-dose treatment periods	Completed  Abbreviated report
BE (fasted vs fed)	<a href="#">TRGG-963-004</a>	Section 5.3.1.2	<b>Primary:</b> To evaluate the comparative BA of AKR-963 and Lovaza after a single dose in healthy subjects under fasting and fed conditions  <b>Secondary:</b> To evaluate the effect of food on AKR-963 and Lovaza after a single dose in healthy subjects under fasting and fed conditions	Open-label, single-dose, randomized, 4-period, 4-sequence, 4-treatment, crossover, comparative BA study under fasting and fed (FDA high-fat [50% of kcal], high-caloric breakfast) conditions	AKR-963 (3600 mg of omega-3 ethyl esters [4 capsules] single dose)  Lovaza (3600 mg of omega-3 ethyl esters [4 capsules] single dose)  Oral	16 subjects randomized  16 subjects included in PK population, 14 subjects in statistical analysis population	Healthy men and women	4 single-dose treatment periods	Completed  Full report

### **Bioequivalence of AKR-963 (studies TRGG-963-005 and TRGG-963-006)**

Trygg determined that the most appropriate bioequivalence approach was a full replicate, single-dose study (Study TRGG-963-005) to evaluate the comparative bioavailability and bioequivalence of single doses of AKR-963 and Lovaza following a standardized high-fat (50% of kcal), high-caloric breakfast. In addition, Trygg conducted a full replicate, single-dose study to evaluate the comparative bioavailability and bioequivalence of single doses of AKR-963 and Lovaza in the fasting state (study TRGG-963-006). Note that all subjects in above study were healthy, and no low fat diets were tested in these studies.

#### **Methods**

**Treatment A:** AKR-963 (omega-3-acid ethyl esters) capsules (Trygg Pharma Inc., USA) Lot No.: 0003559500 [4 capsules administered after a high fat, high calorie breakfast], total dose of 3600 mg

**Treatment B:** Lovaza® capsules (GlaxoSmithKline, USA) Lot No.: 1ZP2915 [4 capsules administered after a high fat, high calorie breakfast], total dose of 3600 mg.

Thus subjects were given AKR-963 soft gelatin capsule containing a mixture of at least 900-mg omega-3-acid ethyl esters, including approximately 465 mg of EPAee and 375 mg of DHAee.

Study design is described below:

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) Dosage Regimen Route of Administration	Number of Subjects Enrolled (Planned/ Actual)	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status Type of Report
BE (fed)	TRGG-963-003	Section 5.3.1.2	To evaluate the comparative BA of AKR-963 and Lovaza after a single dose in healthy subjects under fed conditions	Open-label, single-dose, randomized, 4-period, 2-sequence, 2-treatment, replicate, crossover, comparative BA study under fed (FDA high-fat [50% of kcal], high-caloric breakfast) conditions	AKR-963 (3600 mg of omega-3 ethyl esters [4 capsules] single dose)  Lovaza (3600 mg of omega-3 ethyl esters [4 capsules] single dose)  Oral	44 Subjects randomized  39 Subjects included in PK and statistical analysis population	Healthy men and women	4 single-dose treatment periods	Completed  Full report
BE (fasted)	TRGG-963-006	Section 5.3.1.2	To evaluate the comparative BA of AKR-963 and Lovaza after a single dose in healthy subjects under fasted conditions	Open-label, single-dose, randomized, 4-period, 2-sequence, 2-treatment, replicate, crossover, comparative BA study under fasted conditions	AKR-963 (3600 mg of omega-3 ethyl esters [4 capsules] single dose)  Lovaza (3600 mg of omega-3 ethyl esters [4 capsules] single dose)  Oral	50 Subjects randomized  49 Subjects included in PK and statistical data set	Healthy men and women	4 single-dose treatment periods	Completed;  Full report

BA = Bioavailability.  
BE = Bioequivalence.  
DHA = Docosahexaenoic acid.  
EPA = Eicosapentaenoic acid.  
HOMA-IR = Homeostatic Model Assessment for Insulin Resistance.  
PK = Pharmacokinetic.  
TG = Triglyceride.

## Results

Pharmacokinetic data showed that AKR-963 was bioequivalent to Lovaza under fed and fasted conditions in healthy subjects. As seen in Tables below, after taking the single dose of 4 g/day dose of AKR-963 under high-fat diet, the mean total EPA C<sub>max</sub> and AUC<sub>0-72h</sub> values were ~61 mcg/mL and ~2243 mcg.h/mL respectively. The total C<sub>max</sub> and AUC<sub>0-72h</sub> values of DHA values were 79 mcg/mL and 4252 mcg.h/mL respectively.

However, after taking a single 4g dose of AKR under fasted state, mean total EPA C<sub>max</sub> was ~18 mcg/mL and AUC<sub>0-72h</sub> was ~1003 mcg.h/mL; and for total DHA the C<sub>max</sub> of ~54 mcg/mL and AUC<sub>0-72h</sub> of ~3462 mcg.h/ml.

In general similar C<sub>max</sub> and AUC values were noted with Lovaza, see the Tables below

Table. Mean total EPA C<sub>max</sub> and AUC 0-72 hrs values in healthy subjects under fasted and under high fat diet with **AKR-963** single dose of 4 g/day.

	EPA		DHA	
	C <sub>max</sub> (mcg/ml)	AUC <sub>0-72 hrs</sub> (mcg.hr/ml)	C <sub>max</sub> (mcg/ml)	AUC <sub>0-72 hrs</sub> (mcg.hr/ml)
Fasted state	18	1003	54	3462
High fat diet	61	2243	79	4252

Table. Mean total EPA C<sub>max</sub> and AUC 0-72 hrs values in healthy subjects under fasted and under high fat diet with **Lovaza** single dose of 4 g/day.

	EPA		DHA	
	C <sub>max</sub> (mcg/ml)	AUC <sub>0-72 hrs</sub> (mcg.hr/ml)	C <sub>max</sub> (mcg/ml)	AUC <sub>0-72 hrs</sub> (mcg.hr/ml)
Fasted state	19	1053	53	3435
High fat diet	66 mcg/mL	2326	81	4299

Sponsor states that the results of both studies (TRGG-963-005 and TRGG-963-006) demonstrate that AKR-963 is bioequivalent to Lovaza with respect to EPA and DHA, as well as for the parent compounds EPA ethyl ester and DHA ethyl ester (the latter relevant to Study TRGG-963-005 only). Thus for total EPA lipids and total DHA lipids, the 90% confidence intervals of the test to reference ratio were entirely contained within the 80%-125% bioequivalence range for both C<sub>max</sub> and AUC<sub>0-72 hr</sub> using the unscaled approach.

The Tables below provide the sponsor's summary data on the pharmacokinetic and statistical analyses for the EPA and DHA on: 1) Total Lipids of Plasma, 2) Ethyl-esters 3) Free Fatty Acids of Plasma.

## Results from study TRGG-963-005

Table. EPA data in study TRGG-963-005.

Table 11.4.1-1 Summary of Study Results Based on Plasma Total EPA Levels

Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion
		Arithmetic	CV%	Geometric			Lower	Upper			
<i>Based on Baseline-Adjusted Total EPA Data</i>											
AUC <sub>0-72</sub> ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	A <sub>1</sub>	1218.16	41	1144.40	A vs. B	91.22	84.48 - 98.49	A: 36	N/A	N/A	
	A <sub>2</sub>	1259.32	31								
	B <sub>1</sub>	1345.60	33	1254.56							
	B <sub>2</sub>	1325.03	34								
C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	A <sub>1</sub>	44.79	33	44.97	A vs. B	89.78	85.35 - 94.43	A: 21	N/A	N/A	
	A <sub>2</sub>	49.50	36								
	B <sub>1</sub>	53.37	34	50.09							
	B <sub>2</sub>	51.41	30								
<i>Based on Measured Total EPA Data</i>											
AUC <sub>0-72</sub> ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	A <sub>1</sub>	2223.60	33	2161.23	A vs. B	96.68	93.96 - 99.48	A: 8	N/A	N/A	
	A <sub>2</sub>	2264.66	32								
	B <sub>1</sub>	2316.12	30	2235.40							
	B <sub>2</sub>	2335.85	31								
C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	A <sub>1</sub>	59.31	31	59.13	A vs. B	92.92	89.34 - 96.64	A: 15	N/A	N/A	
	A <sub>2</sub>	63.79	33								
	B <sub>1</sub>	66.94	31	63.64							
	B <sub>2</sub>	65.65	30								

Table. DHA data in study TRGG-963-005.

**Table 11.4.1-2 Summary of Study Results Based on Plasma Total DHA Levels**

Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion
		Arithmetic	CV%	Geometric			Lower	Upper			
<i>Based on Baseline-Adjusted Total DHA Data</i>											
<b>AUC<sub>0-72</sub></b> ( $\mu\text{g}^*\text{h/mL}$ )	A <sub>1</sub>	664.62	34	667.74	A vs. B	103.88	94.40 - 114.32	A: 24	N/A	N/A	
	A <sub>2</sub>	754.79	35								
	B <sub>1</sub>	696.49	39	642.79				B: 24	0.228		
	B <sub>2</sub>	728.11	41								
<b>C<sub>max</sub></b> ( $\mu\text{g/mL}$ )	A <sub>1</sub>	25.95	43	26.40	A vs. B	91.01	84.58 - 97.94	A: 29	N/A	N/A	
	A <sub>2</sub>	32.49	49								
	B <sub>1</sub>	31.06	47	29.01				B: 27	0.261		
	B <sub>2</sub>	31.97	36								
<i>Based on Measured Total DHA Data</i>											
<b>AUC<sub>0-72</sub></b> ( $\mu\text{g}^*\text{h/mL}$ )	A <sub>1</sub>	4142.03	26	4138.54	A vs. B	99.16	97.66 - 100.68	A: 6	N/A	N/A	
	A <sub>2</sub>	4362.48	28								
	B <sub>1</sub>	4189.26	25	4173.73				B: 5	0.056		
	B <sub>2</sub>	4409.73	26								
<b>C<sub>max</sub></b> ( $\mu\text{g/mL}$ )	A <sub>1</sub>	74.33	27	75.95	A vs. B	96.10	93.29 - 98.99	A: 13	N/A	N/A	
	A <sub>2</sub>	82.68	30								
	B <sub>1</sub>	79.72	26	79.03				B: 9	0.084		
	B <sub>2</sub>	83.19	27								

Table. Total EPA and DHA ethyl esters from study TRGG-963-005.

<i>Name of Sponsor: Trygg Pharma Inc.</i>													
<i>Name of Finished Product: AKR-963 (omega-3-acid ethyl esters) capsules</i>													
<i>Name of Active Ingredient: omega-3 fatty acid ethyl esters, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)</i>													
For both EPA Ethyl-esters and DHA Ethyl-esters, the ratio of the geometric means of the test to reference product for AUC <sub>t</sub> , AUC <sub>inf</sub> , and C <sub>max</sub> were all within the 80.00-125.00% range and the upper 95% bound of the confidence interval of the RSABE criterion is negative.													
<i>Ethyl-esters</i>													
Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion		
		Arithmetic	CV%	Geometric			Lower	Upper					
<i>Based on Measured/Baseline-Adjusted EPA Ethyl-ester Data</i>													
AUC <sub>0-t</sub> (ng*h/mL)	A <sub>1</sub>	381.378	46	N/A	A vs. B	88.73	N/A	-	N/A	N/A	N/A		
	A <sub>2</sub>	421.040	52										
	B <sub>1</sub>	482.751	49	N/A								0.401	-0.060215
	B <sub>2</sub>	429.334	49										
AUC <sub>0-inf</sub> (ng*h/mL)	A <sub>1</sub>	427.012	39	N/A	A vs. B	96.24	N/A	-	N/A	N/A	N/A		
	A <sub>2</sub>	414.545	51										
	B <sub>1</sub>	502.955	50	N/A								0.319	-0.012791
	B <sub>2</sub>	453.773	48										
C <sub>max</sub> (ng/mL)	A <sub>1</sub>	183.500	64	N/A	A vs. B	83.42	N/A	-	N/A	N/A	N/A		
	A <sub>2</sub>	192.450	68										
	B <sub>1</sub>	237.067	67	N/A								0.563	-0.122776
	B <sub>2</sub>	201.410	58										
<i>Ethyl-esters</i>													
Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion		
		Arithmetic	CV %	Geometric			Lower	Upper					
<i>Based on Measured/Baseline-Adjusted DHA Ethyl-ester Data</i>													
AUC <sub>0-t</sub> (ng*h/mL)	A <sub>1</sub>	1699.232	43	N/A	A vs. B	92.20	N/A	-	N/A	N/A	N/A		
	A <sub>2</sub>	1868.335	48										
	B <sub>1</sub>	2026.347	40	N/A								0.314	-0.039126
	B <sub>2</sub>	1866.066	46										
AUC <sub>0-inf</sub> (ng*h/mL)	A <sub>1</sub>	1762.930	39	1733.262	A vs. B	92.04	83.70	-	101.21	A: 25	N/A		
	A <sub>2</sub>	2096.299	45							B: 26	0.283	N/A	
	B <sub>1</sub>	2052.842	43	1883.138									
	B <sub>2</sub>	2106.363	39										
C <sub>max</sub> (ng/mL)	A <sub>1</sub>	683.003	56	N/A	A vs. B	86.47	N/A	-	N/A	N/A	N/A		
	A <sub>2</sub>	769.526	62										
	B <sub>1</sub>	859.846	56	N/A								0.459	-0.079450
	B <sub>2</sub>	767.172	54										

Table. Free EPA data from study TRGG-963-005.

**Table 11.4.1-5 Summary of Study Results Based on Plasma Free EPA Levels**

Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion
		Arithmetic	CV%	Geometric			Lower	Upper			
<i>Based on Baseline-Adjusted Free EPA Data</i>											
AUC <sub>0-72</sub> (ng*h/mL)	A <sub>1</sub>	7465.2	37	7678.6	A vs. B	92.18	87.26 - 97.38	A: 25	B: 15	0.153	N/A
	A <sub>2</sub>	8915.7	33								
	B <sub>1</sub>	8414.2	31	8330.2							
	B <sub>2</sub>	9146.5	36								
Cmax (ng/mL)	A <sub>1</sub>	698.0	51	N/A	A vs. B	90.86	N/A - N/A	N/A	N/A	0.314	-0.030003
	A <sub>2</sub>	773.4	43								
	B <sub>1</sub>	793.6	56	N/A							
	B <sub>2</sub>	883.2	62	N/A							
<i>Based on Measured Free EPA Data</i>											
AUC <sub>0-72</sub> (ng*h/mL)	A <sub>1</sub>	13338.2	42	13008.2	A vs. B	96.84	93.68 - 100.11	A: 11	B: 10	0.105	N/A
	A <sub>2</sub>	14616.2	43								
	B <sub>1</sub>	13701.2	35	13432.4							
	B <sub>2</sub>	14950.8	43								
Cmax (ng/mL)	A <sub>1</sub>	782.9	46	753.3	A vs. B	92.48	84.04 - 101.76	A: 30	B: 28	0.280	N/A
	A <sub>2</sub>	854.9	40								
	B <sub>1</sub>	868.6	51	814.5							
	B <sub>2</sub>	965.8	57								

Table. Free DHA data from study TRGG-963-005

**Table 11.4.1-6 Summary of Study Results Based on Plasma Free DHA Levels**

Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion
		Arithmetic	CV%	Geometric			Lower	Upper			
<i>Based on Baseline-Adjusted Free DHA Data</i>											
AUC <sub>0-72</sub> (ng*h/mL)	A <sub>1</sub>	19996.0	50	19833.7	A vs. B	88.99	81.66 - 96.96	A: 39	B: 21	0.214	N/A
	A <sub>2</sub>	25222.4	41								
	B <sub>1</sub>	22408.8	37	22288.5							
	B <sub>2</sub>	25423.9	41								
Cmax (ng/mL)	A <sub>1</sub>	1958.6	48	N/A	A vs. B	94.97	N/A - N/A	N/A	N/A	0.358	-0.064739
	A <sub>2</sub>	2321.6	43								
	B <sub>1</sub>	2145.4	59	N/A							
	B <sub>2</sub>	2487.3	57	N/A							
<i>Based on Measured Free DHA Data</i>											
AUC <sub>0-72</sub> (ng*h/mL)	A <sub>1</sub>	52432.6	41	51569.1	A vs. B	97.61	94.72 - 100.58	A: 12	B: 9	0.093	N/A
	A <sub>2</sub>	58080.2	38								
	B <sub>1</sub>	53140.2	33	52833.1							
	B <sub>2</sub>	58734.3	36								
Cmax (ng/mL)	A <sub>1</sub>	2457.5	39	2474.1	A vs. B	97.11	89.70 - 105.15	A: 27	B: 29	0.283	N/A
	A <sub>2</sub>	2823.0	36								
	B <sub>1</sub>	2612.3	48	2547.6							
	B <sub>2</sub>	2995.4	49								

**Results from study TRGG-963-006**

Table. EPA data from study TRGG-963-006.

<i>EPA Total Lipids of Plasma</i>														
Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion			
		Arithmetic	CV%	Geometric			Lower	Upper						
<i>Based on Baseline-Adjusted Total EPA Data</i>														
<b>AUC<sub>0-72</sub></b> ( $\mu\text{g}^*\text{h}/\text{mL}$ )	A <sub>1</sub>	81.29	205	N/A	A vs B	87.78	N/A -	N/A	N/A	N/A	-0.468892			
	A <sub>2</sub>	52.44	83											
	B <sub>1</sub>	90.21	157	N/A									N/A	0.956
	B <sub>2</sub>	65.58	84											
<b>C<sub>max</sub></b> ( $\mu\text{g}/\text{mL}$ )	A <sub>1</sub>	4.37	164	N/A	A vs B	81.94	N/A -	N/A	N/A	N/A	-0.048073			
	A <sub>2</sub>	3.09	56											
	B <sub>1</sub>	4.14	102	N/A									N/A	0.466
	B <sub>2</sub>	4.16	60											
<i>Based on Measured Total EPA Data</i>														
<b>AUC<sub>0-72</sub></b> ( $\mu\text{g}^*\text{h}/\text{mL}$ )	A <sub>1</sub>	1027.17	43	929.54	A vs B	95.43	92.74 -	98.19	A: 11	N/A	N/A			
	A <sub>2</sub>	979.26	47											
	B <sub>1</sub>	1097.59	44	974.06									B: 13	0.129
	B <sub>2</sub>	1009.58	45											
<b>C<sub>max</sub></b> ( $\mu\text{g}/\text{mL}$ )	A <sub>1</sub>	17.88	54	15.76	A vs B	92.98	89.12 -	97.01	A: 17	N/A	N/A			
	A <sub>2</sub>	16.79	50											
	B <sub>1</sub>	19.15	48	16.95									B: 17	0.166
	B <sub>2</sub>	17.83	44											

Table. DHA data from study TRGG-963-006.

<i>DHA Total Lipids of Plasma</i>												
Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion	
		Arithmetic	CV%	Geometric			Lower	Upper				
<i>Based on Baseline-Adjusted Total DHA Data</i>												
<i>AUC<sub>0-72</sub></i> ( <i>µg*h/mL</i> )	A <sub>1</sub>	83.85	66	N/A	A vs B	101.08	N/A -	N/A	N/A	N/A		
	A <sub>2</sub>	73.46	85	N/A								
	B <sub>1</sub>	90.92	71	N/A								
	B <sub>2</sub>	98.15	72	N/A								
<i>C<sub>max</sub></i> ( <i>µg/mL</i> )	A <sub>1</sub>	6.09	83	N/A	A vs B	103.51	N/A -	N/A	N/A	-0.310813		
	A <sub>2</sub>	5.20	49	N/A								
	B <sub>1</sub>	5.48	50	N/A								
	B <sub>2</sub>	5.65	32	N/A								
<i>Based on Measured Total DHA Data</i>												
<i>AUC<sub>0-72</sub></i> ( <i>µg*h/mL</i> )	A <sub>1</sub>	3452.06	31	3321.34	A vs B	100.89	99.24 -	102.57	A: 7	N/A		
	A <sub>2</sub>	3472.59	33									
	B <sub>1</sub>	3441.36	31	3292.02					B: 7	0.076		
	B <sub>2</sub>	3430.52	32									
<i>C<sub>max</sub></i> ( <i>µg/mL</i> )	A <sub>1</sub>	54.04	34	51.63	A vs B	101.57	99.59 -	103.58	A: 8	N/A		
	A <sub>2</sub>	53.92	33									
	B <sub>1</sub>	53.05	31	50.83					B: 8	0.089		
	B <sub>2</sub>	53.08	31									
Due to large variability ( <i>sWR</i> > 0.294) the reference-scaled approach was used for the baseline-adjusted <i>AUC<sub>72</sub></i> and <i>C<sub>max</sub></i> of both Total EPA and Total DHA. The results meet bioequivalence criteria: test/reference ratios are within the 80% to 125% range and the upper 95% bound of the confidence interval of the RSABE criterion is negative.												
Measured Total EPA and Total DHA were assessed using the standard average bioequivalence method for both <i>AUC<sub>72</sub></i> and <i>C<sub>max</sub></i> . The 90% confidence intervals for all these parameters are within the standard 80% to 125% bioequivalence range.												
<i>EPA Free Fatty Acids of Plasma</i>												
Parameter	TRT	Means			Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD (sWR)	95% Upper Bound for RSABE Criterion	
		Arithmetic	CV%	Geometric			Lower	Upper				
<i>Based on Baseline-Adjusted Free EPA Data</i>												
<i>AUC<sub>0-72</sub></i> ( <i>µg*h/mL</i> )	A <sub>1</sub>	2332.5	85	N/A	A vs B	94.86	N/A -	N/A	N/A	N/A		
	A <sub>2</sub>	2483.1	87	N/A								
	B <sub>1</sub>	2211.6	64	N/A								
	B <sub>2</sub>	2527.2	55	N/A								
<i>C<sub>max</sub></i> ( <i>µg/mL</i> )	A <sub>1</sub>	134.9	59	114.0	A vs B	101.63	95.29 -	108.40	A: 23	N/A		
	A <sub>2</sub>	131.2	73									
	B <sub>1</sub>	126.7	61	112.2					B: 23	0.229		
	B <sub>2</sub>	133.0	51									
<i>Based on Measured Free EPA Data</i>												
<i>AUC<sub>0-72</sub></i> ( <i>µg*h/mL</i> )	A <sub>1</sub>	7601.6	63	6680.3	A vs B	96.96	93.47 -	100.57	A: 15	N/A		
	A <sub>2</sub>	7538.7	65									
	B <sub>1</sub>	7616.7	50	6890.0					B: 15	0.153		
	B <sub>2</sub>	7660.5	53									

Sponsor: Trygg Pharma, Inc.		Individual Study Table Referring to Part of the Dossier									
Name of Finished Product: AKR-963		Module 5.3.1									
Name of Active Ingredient: Omega-3-acid ethyl esters											
$C_{max}$ ( $\mu\text{g}/\text{mL}$ )	A <sub>1</sub>	223.0	59	191.3	A vs B	99.74	95.83 - 103.82	A: 16	N/A	N/A	0.172
	A <sub>2</sub>	213.3	66								
	B <sub>1</sub>	217.2	56	191.8							
	B <sub>2</sub>	216.0	54								
<i>DHA Free Fatty Acids of Plasma</i>											
Parameter	TRT	Means		Contrast	Ratio	90% CI		Intra-Sub CV(%)	Intra-Sub Within Ref SD ( $\pm$ WR)	95% Upper Bound for RSABE Criterion	
		Arithmetic	CV%			Geometric	Lower				Upper
<i>Based on Baseline-Adjusted Free DHA Data</i>											
$AUC_{0-72}$ ( $\mu\text{g}^*\text{h}/\text{mL}$ )	A <sub>1</sub>	11716.7	62	N/A	A vs B	93.82	N/A - N/A	N/A	N/A	-0.111234	
	A <sub>2</sub>	12842.5	65								
	B <sub>1</sub>	12443.9	61	N/A							
	B <sub>2</sub>	13958.6	54								
$C_{max}$ ( $\mu\text{g}/\text{mL}$ )	A <sub>1</sub>	611.7	45	562.1	A vs B	100.10	94.90 - 105.59	A: 23	N/A	N/A	
	A <sub>2</sub>	622.5	52								
	B <sub>1</sub>	611.6	52	561.5							
	B <sub>2</sub>	638.7	45								
<i>Based on Measured Free DHA Data</i>											
$AUC_{0-72}$ ( $\mu\text{g}^*\text{h}/\text{mL}$ )	A <sub>1</sub>	42385.4	47	39512.1	A vs B	98.69	95.70 - 101.77	A: 12	N/A	N/A	
	A <sub>2</sub>	43912.4	46								
	B <sub>1</sub>	42796.6	43	40038.2							
	B <sub>2</sub>	44222.3	44								
$C_{max}$ ( $\mu\text{g}/\text{mL}$ )	A <sub>1</sub>	1127.3	45	1043.3	A vs B	100.43	97.31 - 103.65	A: 13	N/A	N/A	
	A <sub>2</sub>	1138.0	47								
	B <sub>1</sub>	1130.4	46	1038.8							
	B <sub>2</sub>	1127.3	45								

Due to large variability ( $s_{WR} > 0.294$ ) the reference-scaled approach was used for the baseline-adjusted  $AUC_{72}$  of both EPA and DHA from free fatty acids. The results meet bioequivalence criteria: test/reference ratios are within the 80% to 125% range and the upper 95% bound of the confidence interval of the RSABE criterion is negative. The standard average bioequivalence method was used for baseline-adjusted  $C_{max}$  and for measured  $AUC_{72}$  and  $C_{max}$  of both EPA and DHA from free fatty acids. The 90% confidence intervals for all these parameters are within the standard 80% to 125% bioequivalence range.

As stated before after a 4g single dose under fasted state, mean total EPA  $C_{max}$  was ~17 mcg/mL and  $AUC_{0-72h}$  was ~1000 mcg.h/mL; and for total DHA the  $C_{max}$  of ~53 mcg/mL and  $AUC_{0-72h}$  of ~3400 mcg.h/mL. Under high-fat diet these values were ~60 mcg/mL and ~2200 mcg.h/mL for total EPA  $C_{max}$  and  $AUC_{0-72h}$  respectively; and for total DHA was 80 mcg/mL and 4000 mcg.h/mL

**Efficacy study (TRGG-963-002)**

The results of Phase 3 study in patients with high triglycerides (TRGG-963-002) showed that the reduction in median fasting TG compared to placebo was 14.0% ( $p = 0.0234$ ) for Lovaza, and 12.2% ( $p = 0.0412$ ) for AKR-963, demonstrating the assay sensitivity for evaluating non-inferiority of the 2 active treatments.

As far as the efficacy is concerned, after 12 week of therapy, the non-inferiority of AKR-963 to Lovaza for reduction in TG was supported clinically and statistically by a demonstrated lack of difference between each treatment on the secondary efficacy parameters of non-HDL-C, VLDL-C, LDL-C, and HDL-C. In the above study, there were no clinically significant, treatment

*emergent changes in clinical laboratory parameters, vital signs, or other physical examination findings for any subject in the trial. The study drugs were both well tolerated by all subjects in the study.*

**Sponsor's Overall Conclusion:**

Sponsor states that the test product AKR-963 (omega-3-acid ethyl esters capsules from Trygg Pharma Inc., USA) is bioequivalent to the reference product (Lovaza® capsules from GlaxoSmithKline, USA) in healthy subjects after a single, oral dose, under fasted and fed conditions.

In conclusion as far as both Plasma total EPA and plasma total DHA are concerned, AKR-963 appears to be bioequivalent to LOVAZA under fasted state (Trial TRGG-963-006) as well as under fed high-fat diet state (Trial TRGG-963-005).

**Comparison of PK between rat and human studies**

In summary, the pharmacokinetic data between rat and human studies are hard to compare. In rats, the AUC exposures were measured for up to 24 hours, while in humans these were measured for up to 72 hrs. The pharmacokinetic data in rats after 28-day treatment were more meaningful, while data after a single dose in rats had more variability. The drug was given to humans only as a single dose for pharmacokinetic studies. Thus safety factor in rats (at the high dose of 4000 mg/kg/day) to humans (at therapeutic dose of 3600 mg/day or 60 mg/kg/day) was approximately 10X, based on the body surface area, see the integrated summary and evaluations.

**6 General Toxicology**

Following toxicity studies have been conducted by the current sponsor:

Table 2.6.7-1. Toxicology: Overview							Test Article: AKR-963
Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses <sup>a</sup> (mg/kg/day)	GLP Status	Testing Facility	Study Number
Single-dose toxicity	No studies						
Repeat-dose toxicity	Rat, Sprague Dawley	Oral gavage	28 days	200, 4000 (AKR-963 <sup>b</sup> and Lovaza)	GLP	(b) (4)	241342
Genotoxicity	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, <i>Escherichia coli</i> WP2 <i>uvrA</i>	In vitro (plate incorporation and preincubation assays)	Plated incubated for 48 to 72 hours	0.31-5 mg/plate (AKR-963 <sup>c</sup> )	GLP		241656
	Human peripheral blood lymphocytes	In vitro (cell culture)	3 hours exposure with metabolic activation; 3 and 20 hours exposure without metabolic activation	8.2-100 µg/mL (AKR-963)	GLP		241657
Carcinogenicity	No studies						
Reproductive and developmental toxicity	No studies						
Local tolerance	No studies						
Other toxicity studies	No studies						
<sup>a</sup> = For repeat-dose toxicity, the highest no observed adverse effect level (NOAEL) is underlined. <sup>b</sup> = The reported dose levels were based on a minimum specification of 777 mg omega-3-acid ethyl esters per gram of oil (777 mg/g); however, the measured content of the tested lot was 851 mg/g. Therefore, dose levels actually administered were 219 and 4381 mg/kg/day. <sup>c</sup> = The reported concentrations were based on a minimum specification of 777 mg omega-3-acid ethyl esters per gram of oil (777 mg/g); however, the actual purity of the tested lot was 851 mg/g. Therefore, concentration range actually tested was 0.34 to 5.5 mg/plate. GLP = Good Laboratory Practice.							

Table 2.6.7-2. Toxicokinetics: Overview of Toxicokinetics Studies						Test Article: AKR-963 and Lovaza
Type of Study	Test System	Method of Administration	Doses (mg/kg)	GLP Compliance	Report Number	
Repeat-dose toxicity	Rat, Sprague Dawley	Oral, gavage	200, 4000 AKR-963 <sup>a</sup> ; 200, 4000 Lovaza	GLP	241342	
<sup>a</sup> = The reported dose levels of 200 and 4000 mg/kg/day in the study report were based on a minimum specification of 777 mg omega-3-acid ethyl esters per gram of oil (777 mg/g); however, the measured content of the tested lot was 851 mg/g. Therefore, actual dose levels of AKR-963 tested were 219 and 4381 mg/kg/day.						

## 6.2 Repeat-Dose Toxicity

### **A comparative 28-day oral bridging toxicity study of AKR-963 and the marketed product (Lovaza®) in rats, followed by the 14-day drug-free recovery period**

**Key Study Findings:** This is a bridging toxicity study to determine if AKR-963 is similar to Lovaza. However, Sponsor did not include control groups and only evaluated the HD treated groups (at 4000 mg/kg/day of AKR-963 and Lovaza) for histopathology. There's no evidence of pharmacologic activity (no effect on triglycerides and total cholesterol) in this study in either treatment group compared to historical controls. The control ranges provided by the Contract Research Organization or CRO are inadequate, based on the age of the animals and the data years of collection. This may explain the apparent lack of PD. The AUC exposures of DHA and EPA were generally comparable with both drug products. At a HD, AUC exposures of DHA on day 28 were 1992 / 790 mcg.h/m in male/females respectively (vs 1945/ 963 mcg.hr/ml in males/females with Lovaza). EPA exposures on day 28 were 2594/ 2201 mcg.h/ml (vs 2636/1636 mcg.h/ml in males/females respectively with Lovaza). Based on histopathology of the HD groups, except the bladder mucosal inflammation in 2/10 females (even though the female rats have lower drug exposures than male rats) and a single dead male on day 14 due to apparent acute cardiac failure, the toxicity with two drugs in general was similar. Target organs of toxicity with both drugs were liver (inflammation in 3/20 rats vs 3/20 rats with Lovaza), kidney (inflammation in 3/20 rats vs 3/20 with lovaza), and eyes (focal retinal degeneration of mild severity in 1/10 female rats with both AKR-963 and Lovaza respectively, but at the end of drug free recovery period it was only noted with AKR-963 in 1/5 males vs 0/5 with Lovaza). The toxicity noted in the above study does not appear to be the toxicity seen previously with the approved Lovaza (at 4X lower doses). It is possible that the relative absence of established target organ toxicity reflects the short dosing duration of 4 weeks that animals were exposed to here, and we do not have a 4-week rat toxicity study with the original Lovaza NDA, to compare these results to lovaza or AKR-963. The high dose of 4000 mg/kg/day provides safety margin of approximately 10X in humans at a recommended dose of 3600 mg/day (60 mg/kg/day or 2220 mg/m<sup>2</sup>/day, assuming 60 kg subject), based on body surface area.

#### **Study title: Comparative 28-day oral toxicity study in rats with AKR-963 and with marketed product (lovaza®), followed by a 14-day recovery period**

Study no.:	241342 (TRGG-963-103)
Study report location:	Electronic submission
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	6/22/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A08552-001L04, Purity was not provided. Note that actual drug lot # is 0003550700 (see COA).

**Date of study initiation:** 6/22/2011

The details on the drug are provided below:

Identity: AKR-963  
Chemical Name/Category: Fish oil  
Formula: Principle components:  
Docosahexaenoic acid ethyl ester, C<sub>24</sub>H<sub>36</sub>O<sub>2</sub>  
Eicosapentaenoic acid ethyl ester, C<sub>22</sub>H<sub>34</sub>O<sub>2</sub>  
Molecular Weight: Docosahexaenoic acid ethyl ester: 356.5  
Eicosapentaenoic acid ethyl ester: 330.5  
Correction Factor: 0.777 (based on a minimum specification of  
777 mg omega-3-acid ethyl esters per gram of oil)  
Colour/Form: Light yellow viscous oil in capsules  
Lot No.: A08552-001L04

Certificate of analysis (COA) for AKR-963 is shown below:

(b) (4)

### Certificate of Analysis

NND11001

Product Name: AKR-963 Capsules  
Description: (b) (4) omega-3 EPA and DHA ethyl ester capsules  
Product Number: EE104  
Lot Number Finished Product<sup>2)</sup>: 0003550700  
Manufacturing Date Bulk Capsules: (b) (4)  
Date of Packaging: (b) (4)

Tests	Specification <sup>3)</sup>	Results <sup>4)</sup>
EPA Ethyl Ester Identity	Retention time in conformance with external EPA Ethyl Ester reference standard	Complies <sup>2)</sup>
DHA Ethyl Ester Identity	Retention time in conformance with external DHA Ethyl Ester reference standard	Complies <sup>2)</sup>
Appearance	Transparent oblong soft gelatin capsules filled with clear, faint yellow liquid	Complies <sup>2)</sup>
Fill mass	(b) (4)	(b) (4)
Uniformity of mass	(b) (4)	(b) (4)
Disintegration time	(b) (4)	(b) (4)
EPA Ethyl Ester	(b) (4)	(b) (4)
DHA Ethyl Ester	(b) (4)	(b) (4)
EPA +DHA Ethyl Ester	(b) (4)	(b) (4)
Total Omega 3 Acid Ethyl Ester	(b) (4)	(b) (4)
Tocopherol	(b) (4)	(b) (4)
Total aerobic microbial count	(b) (4)	(b) (4)
Total yeasts and moulds count	(b) (4)	(b) (4)
E. coli	Absent /g	Not detected /g <sup>2)</sup>
Staphylococcus aureus	Absent /g	Not detected /g <sup>2)</sup>
Salmonella	Absent /10g	Not detected /10g <sup>2)</sup>

(b) (4)

(b) (4)

This GMP lot was manufactured in accordance with the general principles as outlined in ICH Q6B Specification: "Test procedures and acceptance criteria for biotechnical/biological products".

(b) (4)

Page 1 of 1

**The comparator drug: Lovaza.** The details on the comparator are provided below:

Comparator

Identity:	Lovaza®
Chemical Name/Category:	Fish oil, mixture of the omega-3-acid ethyl esters of docosahexaenoic and eicosapentaenoic acids
Formula:	Principle components: Docosahexaenoic acid ethyl ester, C <sub>24</sub> H <sub>36</sub> O <sub>2</sub> Eicosapentaenoic acid ethyl ester, C <sub>22</sub> H <sub>34</sub> O <sub>2</sub>
Molecular Weight:	Docosahexaenoic acid ethyl ester: 356.5 Eicosapentaenoic acid ethyl ester: 330.5
Correction Factor:	0.900 (900 mg omega-3-acid ethyl esters per gram of oil)
Colour/Form:	Light yellow oil in capsules
Lot No.:	A08552-001L02

**Formulation/vehicle:** 0.4% w/v aqueous methylcellulose. Dosing volume was 5 ml/kg.

**Doses in administered units:** 0.2 and 4 g/kg/day. Note that this toxicity study is deficient, as no control group was included in the study, see below why the control group was not included:

A control group was not included as the objective of the study was to compare the safety and toxicokinetic profile of AKR-963 directly to that of Lovaza. As such, a control group was considered to be of limited value and an unnecessary use of animals. Historical control data for rats of similar age were used in lieu of a control group.

**Route, form, volume, and infusion rate:** Oral, gavage, at dose volumes of 5 ml/kg given once daily for 4 consecutive weeks, followed by a 14-day drug free recovery period.

**Methods (unique aspects):** Methods are described below.

The objective, study duration and parameters measured are described below:

This study examined the potential systemic toxicity and target organs for toxicity of fish oil product AKR-963 in comparison to Lovaza®, the marketed product, following a 28-day repeated oral dose administration in Sprague-Dawley rats.

**TEST SYSTEM**

Species:	<i>Rattus norvegicus</i>
Strain:	CD® [CrI:CD®(SD)BR] (Sprague-Dawley)
Source:	(b) (4)
Total No. of Animals on Study:	148 (74 males, 74 females); (91 males, 91 females prestudy)
No. of Study Groups:	4 (2 treated with Low or High Dose Test Article and 2 treated with Low or High Dose Comparator)
No. of Animals / Group:	Test Article - Low Dose: 16 males, 16 females - High Dose: 21 males, 21 females  Comparator - Low Dose: 16 males, 16 females - High Dose: 21 males, 21 females
Body Weight:	Males: Approx. 274-360 g at start of dosing Females: Approx. 181-238 g at start of dosing
Age at Start of Study:	7 - 8 weeks at start of dosing
Acclimatization Period:	22 days

The rats were offered the Teklad Certified Rodent Diet (#8728C) and municipal water ad libitum throughout the acclimatization and study periods. Main Study and Recovery animals were deprived of food (but not water) overnight (approximately 12 to 18 hours), prior to bleeding and during urine collection.

Four groups were used in the study (2 Low dose, and 2 High dose, test or comparator). Each Main Study group consisted of 10 male and 10 female rats [strain: CrI:CD®(SD)BR-Sprague-Dawley; (b) (4)]. Five (5) additional rats per sex were assigned for a 14-day recovery period in the high dose groups (4 g/kg) only. An additional 6 rats per sex were allocated to all groups for toxicokinetics evaluation of EPA and DHA on Days 1 and 28.

The animals were dosed at 0.2 or 4 g/kg with either AKR-963 or Lovaza®. The doses represent the total dose of omega-3-acid ethyl esters, docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid ethyl (EPA) ester. No control group was included in the study.

The dose volumes were based on the dose concentrations and a specific gravity of fish oil (0.928 g/mL at 15°C); the concentration of total omega-3-acid ethyl ester in AKR-963 was 0.777 g/g oil (based on the minimum specification) and in Lovaza® it was 0.90 g/g oil. Based on the concentration and specific gravity, the animals in the Low dose (0.2 g/kg) groups were dosed at 0.28 and 0.24 mL/kg, for AKR-963 and Lovaza®, respectively. For High dose (4 g/kg) groups, the dose volumes were 5.5 mL/kg and 4.8 mL/kg, respectively.

Table. Study design is shown below:

**Table 1: Animal Assignment per Group**

Group	Animal Identification (Tail Tattoos, Cage Cards)					
	Main Study		Recovery		Toxicokinetics	
	Males	Females	Males	Females	Males	Females
1	001 - 010	075 - 084	-	-	011 - 016	085 - 090
2	017 - 026	091 - 100	027 - 031	101 - 105	032 - 037	106 - 111
3	038 - 047	112 - 121	-	-	048 - 053	122 - 127
4	054 - 063	128 - 137	064 - 068	138 - 142	069 - 074	143 - 148

Groups, dose levels, and the dosing schedules for the study are presented in [Table 2](#).

**Table 2: Summary of Treatment Groups**

Groups	Dose Level (g/kg)	Dose Concentration (g/mL) <sup>a</sup>	Dose Volume (mL/kg)	Dose Route, Frequency	Number and Sex of Animals		
					Main Study	Recovery	TK Arm
1. Low Dose (AKR-963)	0.2	0.721	0.28	Oral, daily for 28 days	10M / 10F	—	6M / 6F
2. High Dose (AKR-963)	4	0.721	5.5	Oral, daily for 28 days	10M / 10F	5M / 5F	6M / 6F
3. Low Dose (Lovaza <sup>®</sup> )	0.2	0.835	0.24	Oral, daily for 28 days	10M / 10F	—	6M / 6F
4. High Dose (Lovaza <sup>®</sup> )	4	0.835	4.8	Oral, daily for 28 days	10M / 10F	5M / 5F	6M / 6F

<sup>a</sup> Dose concentration based on a specific gravity of fish oil of 0.928 g/mL at 15°C, concentration of active in AKR-963 of 0.777 g/g oil (minimum specification) and concentration of active in Lovaza<sup>®</sup> of 0.900 g/g oil.

M = male; F = female; TK = toxicokinetics

Rats were dosed either with test article or comparator for twenty-eight (28) consecutive days (Study Days 1 to 28). On Study Day 29, Main Study animals underwent necropsy. Recovery group animals underwent necropsy on Study Day 43.

### **Justification of the Dose Levels**

Doses were selected by the Sponsor based on the dose levels used in rat studies conducted with Lovaza<sup>®</sup> as well as in literature with other EPA- and DHA-rich fish oils. Doses of 0, 0.2, 1, and 4 g/kg/day were used in a 13-week study with Lovaza<sup>®</sup> resulting in severe clinical signs by Week 8-9, among other findings, at the High dose. Toxicology studies with other fish oils have shown that very high doses (up to 9 g/kg body weight/day, (1) are well tolerated in 28-day studies (2, 3). The Low dose of 0.2 g/kg body weight/day in this 28-day study with AKR-963 and Lovaza<sup>®</sup> was not expected to result in any significant changes while the High dose of 4 g/kg body weight/day was anticipated to produce effects associated with the high fish oil intake. The High dose also provided a margin of 67-fold over the proposed therapeutic dose of AKR-963 based on body weight and a 10-fold margin based on body surface area (proposed therapeutic dose is 3.6 g/day equivalent to 60 mg/kg body weight/day for a 60 kg individual).

#### **Following parameters were monitored:**

Clinical reactions to treatment were monitored as well as body weight assessment, food consumption, clinical pathology, organ weights, and gross pathology.

At the end of the treatment period, all Main Study animals were sacrificed and submitted for gross necropsy. The recovery animals were sacrificed at the end of the 14-day recovery period.

**Gross pathology:** At sacrifice.

**Organs weighed:** The list of organs weighed is provided below.

Adrenals	Pituitary Gland
Brain	Prostate
Heart	Spleen
Kidneys	Thymus
Liver	Testes
Lungs	Uterus
Ovaries	

**Histopathology:** Histopathology evaluations were performed on a comprehensive range of organs from all high dose main and recovery Study animals. With the exception of one TK animal that died during the study and which was subjected to gross necropsy and histopathology, all other TK animals were euthanized at the end of the study without further evaluations.

The following list was provided. The tissues listed in the Table below were examined from all high Dose Main Study and Recovery animals and one TK animal that died during the study were prepared for microscopic examination.

Abnormal Tissues	Liver (sample of central & left lobes)	Skin (inguinal)
Adrenals	Lungs (left & right diaphragmatic lobes) <sup>@</sup>	Spinal Cord (cervical, mid-thoracic, lumbar)
Animal Identification	Lymph Node (Mandibular)	Spleen
Aorta (Thoracic)	Lymph Node (Mesenteric)	Sternum & Marrow
Brain	Mammary Gland (inguinal)	Stomach
Cecum	Nasal Turbinates	Testes**
Colon	Optic Nerves**+	Thymus
Duodenum	Ovaries	Thyroid/Parathyroids +
Epididymides	Pancreas	Tongue
Esophagus	Pituitary	Trachea
Eyes**	Prostate	Urinary Bladder
Heart***	Rectum	Uterus (horns, cervix, body)
Ileum	Salivary Glands (Mandibular)	Vagina
Jejunum	Seminal Vesicles	
Kidneys	Sciatic Nerve	
Lacrimal glands	Skeletal Muscle (quadriceps)	

\*\* Fixed in Alcoholic Formalin (euthanized animals only)

\*\*\* Sections of left and right ventricles and atria, septum with papillary muscle

+ Parathyroids & optic nerves examined only if present in routine sections

@ Lungs of euthanized animals infused with formalin

Three femoral bone marrow smears were prepared from each sacrificed animal but were not evaluated and were retained for possible future examination

**Toxicokinetics:** TK were examined in the week-4 study as stated below:

Blood samples were collected on 2 occasions (Study Days 1 and 28) from Toxicokinetic Study rats only. Blood was collected at the following time points: pre-dose, and then 1, 2, 4, 8, and 24 hours post-dose. Toxicokinetics (AUC, T<sub>max</sub>, C<sub>max</sub>, t<sub>1/2</sub>(e), etc.) of DHA and EPA were calculated following the final blood collection, each Toxicokinetic Study rat was euthanized (by CO<sub>2</sub>/O<sub>2</sub> overdose) and was discarded without necropsy or further examination.

Plasma sample analysis (for DHA and EPA from AKR-963 and Lovaza) was conducted by  <sup>(b) (4)</sup> Laboratory by a validated LC/MS/MS Method (B-05-048, Validation 241236) under GLP conditions. Non-compartmental toxicokinetic profile analysis and TK parameters (T<sub>max</sub>, C<sub>max</sub>, AUC, kel, t<sub>1/2</sub>(e), CL/F, Vz/F, MRT etc.) determinations were conducted using validated computer software.

**Results**

**Mortality:** One male TK rat dosed with AKR-963 at 4 g/kg was found dead on Day 14 (032). This death was classified as incidental and not related to the treatment

Here is the sponsor description of these mortalities in the main group animals

Estimated time of death was sometime after 10-11 pm on the preceding day. The only gross finding was well demarcated discoloration (paleness) of the apex of the heart, and histologically there was moderate lung and liver congestion. Pale appearance of the heart and pulmonary congestion without any other obvious signs of toxicity was highly suggestive of an acute cardiac failure. Although the cause of a sudden death was undetermined, this condition was not considered treatment-related due to the lack of any other toxicity in this rat, and in the remainder of the rats from the same group. Sudden deaths are known to occasionally occur in the laboratory rats, and it is usually associated with stress (single housing, bleeding, manipulations, gavaging, etc.), vagal stimulation and/or diabetes/hypertension.

Under clinical signs on this animal (in individual animal), sponsor states the following: Retrospective analysis of rodent toxicity and carcinogenicity studies revealed that approximately 20% of deaths of rats in these studies were classified as undetermined (8). Historically at (b) (4), 1-2 sudden deaths per year occur (in different studies ranging from acute to 6-month repeated dose studies).

The following histopathology was provided on 3/6/13 (after request was made to the sponsor to submit this information). One male was found dead on day 14 at a HD of 4 g/kg/day.

**Table 1.11.2-1. Summary of All Individual Animal Histopathology Findings With Score > 0 in 28-Day Repeat Administration Study (Report 241342)**

<b>Animal Number (sex)</b>	<b>Dose Group</b>	<b>Sacrifice Day</b>	<b>Tissue (Score<sup>a</sup>)</b>	<b>Description of Observation</b>
32 (M) <sup>b</sup>	4 g/kg AKR-963	Found dead	Lungs (3)	Congested
			Liver (3)	Congested
			Prostate (2)	Focal subchronic inflammation

Further explanation on this animal's death is provided below:

In this study, one rat (no. 032, male) from a TK-subgroup dosed at 4 g/kg with AKR-963 (out of a total of 42 animals evaluated in the toxicology and TK portions of the study receiving 4 g/kg) was found dead on Day 14 of the study. Consistent with (b) (4) SOPs (e.g., the individual SOPs on clinical observations, necropsy, histopathology, etc.), it is our longstanding practice, during the course of any toxicology study in which an unscheduled death occurs, including this study that all reasonable efforts are made to determine the cause of death. This is an integrated process and includes (any) clinical signs of toxicity prior to death, PK-parameters ( $T_{max}/C_{max}$  in relation to time of death), clinical pathology data (if available), gross pathology and histopathology findings.

The Study Director, in consultation with the clinical veterinarian, and taking into account the suddenness of death, pale appearance of the apex of the heart, pulmonary congestion, and the complete lack of any other clinical or pathological signs of toxicity, offered as one plausible but theoretical explanation that this was suggestive of an acute cardiac arrest. However, like the pathologist, the Study Director also further concluded that the cause of death was undetermined. The statement in the study report that the death was suggestive of an acute cardiac arrest was not, and was not meant to be a diagnosis, but rather was a best guess, about the cause of death, and it did not and was not meant to change the conclusion of the pathologist, who did not review or sign off on this statement in the study report.

Further, in the opinion of this Study Director, the death of this rat was not considered to be treatment-related for the following reasons:

- The lack of any observable clinical toxicity in this rat or any other rat in this group.
- This was a TK rat, which endured much more stress than the rats from the main study groups (sudden deaths do occur more frequently in rats exposed to stress).
- PK data indicated that the  $T_{max}$  was approximately 2 – 3 hours, and the estimated time of death indicated that this rat died approximately 10 – 12 hours after the  $T_{max}/C_{max}$ , further suggesting that the death was not related to the peak concentration of AKR-963 in plasma.
- There were no gross or histopathological findings in any other rat in this group that would indicate any underlying pathological processes.
- Clinical pathology data did not indicate any abnormalities in this group of rats.

In conclusion, this rat died of undetermined cause(s). If an undetermined death occurs only in an individual animal and there is no apparent pattern of occurrence, this should not be a limiting factor in the interpretation of the toxicological effects of the test article in this study.

(b) (4)



**Clinical signs:** Sporadic soft feces were noted in 2/20 and 6/30 rats 0.2 and 4 g/kg/day of AKR-963 respectively; these were not noted with Lovaza. Alopecia was noted in 3/25 rats with AKR-963 vs 2/25 rats with Lovaza (these findings were with combined doses of 0.2 and 4 g/kg).

No summary Table on clinical signs were provided and only descriptions were provided as stated below.

Soft feces was observed in 2 out of 20 rats dosed with AKR-963 at 0.2 g/kg and in 6 out of 30 rats (gender combined) dosed at 4 g/kg. This observation was only sporadically noted and did not last for more than a day. In addition, alopecia was observed in 3 of 25 rats (Low and High dose combined) dosed with AKR-963 and in 2 of 25 rats (Low and High dose combined) dosed with Lovaza®. This finding was considered to be incidental and not related to the treatments.

**Body weights:** No significant differences in Mean body weight were noted. Mean body weights on day 28 in males were 437, 442, at 0.2, 4 g/kg of AKR-963 respectively; these with Lovaza were 444, and 460 g respectively. In females these values were 63, 66 g respectively (vs 67, 61 g respectively with Lovaza). The weight gains were similarly not significantly different between AKR-963 and Lovaza (males 132, 133 g with AKR-963 vs 134, 146 g respectively with Lovaza; females 63, 66 g vs 67, 61 g respectively with Lovaza).

**Food consumption:** No drug related effects on Food consumption (FC) were observed.

**Table 3: Summary of Body Weights, Body Weight Changes and Food Consumption<sup>1</sup>**

Group	Sex	Mean Body Weights $\pm$ S.D. (g)						Mean <sup>2</sup> Body Weight Changes	Mean Total Food Consumption (g)
		Day 1	Day 8	Day 15	Day 22	Day 28			
1. Low Dose (AKR-963)	M	304.6 $\pm$ 13.5	354.3 $\pm$ 18.8	393.7 $\pm$ 24.8	425.2 $\pm$ 27.3	436.7 $\pm$ 28.5	+ 132 g (+ 43%)	837 $\pm$ 51	
	F	209.9 $\pm$ 10.5	230.8 $\pm$ 11.8	246.4 $\pm$ 14.5	265.7 $\pm$ 14.7	272.6 $\pm$ 16.4	+ 63 g (+ 30%)	595 $\pm$ 32	
2. High Dose (AKR-963)	M	309.3 $\pm$ 18.1	358.8 $\pm$ 21.6	397.0 $\pm$ 24.0	430.7 $\pm$ 28.2	442.3 $\pm$ 26.3	+ 133 g (+ 43%)	815 $\pm$ 50	
	F	203.3 $\pm$ 9.5	227.4 $\pm$ 13.1	244.8 $\pm$ 16.5	262.9 $\pm$ 18.5	269.1 $\pm$ 16.6	+ 66 g (+ 32%)	586 $\pm$ 63	
3. Low Dose (Lovaza <sup>®</sup> )	M	310.2 $\pm$ 17.9	358.0 $\pm$ 21.1	399.4 $\pm$ 26.7	433.9 $\pm$ 31.3	444.1 $\pm$ 31.3	+ 134 g (+ 43%)	859 $\pm$ 67	
	F	208.5 $\pm$ 8.8	231.7 $\pm$ 13.3	249.6 $\pm$ 15.3	266.7 $\pm$ 19.3	275.1 $\pm$ 19.1	+ 67 g (+ 32%)	616 $\pm$ 57	
4. High Dose (Lovaza <sup>®</sup> )	M	313.3 $\pm$ 24.4	359.4 $\pm$ 19.8	403.4 $\pm$ 25.6	443.7 $\pm$ 32.9	459.6 $\pm$ 39.3	+ 146 g (+ 47%)	843 $\pm$ 87	
	F	210.4 $\pm$ 16.7	230.8 $\pm$ 17.7	248.4 $\pm$ 18.1	261.9 $\pm$ 20.0	271.4 $\pm$ 19.2	+ 61 g (+ 29%)	563 $\pm$ 33	

n = 10 (Groups 1 and 3); n = 15 (Groups 2 and 4)

M = Male

F = Female

1 = Body weights during acclimation were recorded, but were not reported. Body weights and food consumption of recovery animals are in Tables 8, 9, 16 and 17.

2 = Body weight changes over the 28-day period

**Ophthalmoscopy:** With both drug ophthalmological findings were noted, these included cataracts only in male rats (2/10 and 2/10 at 0.2 and 4.0 g/kg of AKR-963 respectively vs 3/10 and 0/10 with Lovaza), hyper-reflective lesions in the posterior segment (in both sexes combined in 1/20 and 3/20 rats with AKR-963 respectively vs 1/20 and 2/20 rats with Lovaza), and crystalline corneal deposits (in 2/20 and 1/20 rats, respectively vs in 5/20 and 1/20 rats with Lovaza).

Here is sponsor's description of these findings:

At the end of the treatment period, crystalline corneal deposits were observed in 2 and 1 rats in groups dosed with AKR-963 at 0.2 and 4.0 g/kg, respectively, and 5 and 1 rats dosed with Lovaza® at 0.2 and 4.0 g/kg, respectively, which were not present pre-study.

Cataracts were observed at the end of the treatment period in 2 and 2 rats in groups dosed with AKR-963 at 0.2 and 4.0 g/kg, respectively, and 3 and 0 rats dosed with Lovaza® at 0.2 and 4.0 g/kg, respectively, which were not present pre-study. Hyper-reflective lesions (Posterior Segment) were also observed in 1 and 3 rats in groups dosed with AKR-963 at 0.2 and 4.0 g/kg, respectively, and 1 and 2 rats dosed with Lovaza® at 0.2 and 4.0 g/kg, respectively, which were not present pre-study.

None of these were considered significant by the sponsor, see the following explanation:

Corneal deposits (crystalline) are the most frequently diagnosed spontaneous occurring lesions in Sprague-Dawley rats (11-46%) with a background incidence of cataracts also observed frequently up to 9.8% (9, 10). The corneal crystalline deposits, cataracts, and hyperreflective lesions noted in this study were spread throughout all groups (at the end of the treatment period). There was no apparent dose-dependency noted, thus these findings were not considered to be treatment-related.

Note that increased incidences of crystalline corneal deposits/cataracts/ hyper-reflective lesions were noted with Lovaza as well as with AKR-963.

**Hematology:** No effects on Platelet counts, WBC counts and differential counts were noted by both drugs. However, during the drug free recovery period in male rats at a HD, Hct was significantly lower with Lovaza (45 vs 43\* % with Lovaza), while reticulocytes were significantly higher with lovaza (178 vs 204\*  $10^9$  /L with Lovaza), but these were not toxicologically relevant as the differences were small, and no histological correlates were observed.

Sponsor describes above findings here "only statistically significant difference was noted at the end of the recovery period for Hct and reticulocytes between the AKR-963 and Lovaza® groups treated at 4 g/kg. Hct was lower and reticulocytes were higher in the Lovaza® group although all values remained within the historical ranges. These differences were not biologically significant".

Table. Hematology findings in **males** at the end of the treatment period with AKR-963 and the comparator Lovaza.

**Table 18: Haematology Group Summaries – Males – End of Treatment**

Parameters	Unit	Group Means $\pm$ S.D. (n = 10)				Normal Ranges
		Group 1 AKR-963 (0.2 g/kg)	Group 2 AKR-963 (4 g/kg)	Group 3 Lovaza (0.2 g/kg)	Group 4 Lovaza (4 g/kg)	
RBC	$\times 10^{12}$ / L	8.22 $\pm$ 0.21	8.01 $\pm$ 0.23	8.29 $\pm$ 0.25	8.11 $\pm$ 0.31	6.06 - 9.46
Hb	g / L	156 $\pm$ 4	153 $\pm$ 5	158 $\pm$ 6	157 $\pm$ 4	120 - 181
Hct	%	46.4 $\pm$ 1.1	45.5 $\pm$ 1.5	46.7 $\pm$ 1.8	46.4 $\pm$ 1.8	37.3 - 50.2
MCV	fL	56.5 $\pm$ 1.5	56.8 $\pm$ 1.4	56.3 $\pm$ 1.6	57.2 $\pm$ 1.3	47.5 - 66.1
MCH	pg	19.1 $\pm$ 0.4	19.1 $\pm$ 0.6	19.0 $\pm$ 0.5	19.4 $\pm$ 0.5	15.8 - 23.1
MCHC	g / L	337 $\pm$ 3	337 $\pm$ 7	338 $\pm$ 4	339 $\pm$ 8	287 - 401
Platelets	$\times 10^9$ / L	1021 $\pm$ 90	958 $\pm$ 65	1074 $\pm$ 80	1006 $\pm$ 67	579 - 1641
WBC	$\times 10^9$ / L	11.13 $\pm$ 1.2	11.51 $\pm$ 2.14	10.54 $\pm$ 2.57	10.36 $\pm$ 2.37	5.00 - 15.28
Neutrophils	$\times 10^9$ / L	1.16 $\pm$ 0.32	1.30 $\pm$ 0.35	1.23 $\pm$ 0.59	1.17 $\pm$ 0.23	0.05 - 2.37
Lymphocytes	$\times 10^9$ / L	9.56 $\pm$ 1.25	9.85 $\pm$ 2.19	8.94 $\pm$ 2.03	8.83 $\pm$ 2.29	1.67 - 14.00
Monocytes	$\times 10^9$ / L	0.19 $\pm$ 0.03	0.14 $\pm$ 0.05	0.16 $\pm$ 0.05	0.16 $\pm$ 0.07	0 - 0.46
Eosinophils	$\times 10^9$ / L	0.13 $\pm$ 0.05	0.13 $\pm$ 0.04	0.12 $\pm$ 0.09	0.12 $\pm$ 0.05	0 - 0.21
Basophils	$\times 10^9$ / L	0.04 $\pm$ 0.01	0.04 $\pm$ 0.02	0.03 $\pm$ 0.02	0.04 $\pm$ 0.02	0 - 0.06
LUC	$\times 10^9$ / L	0.06 $\pm$ 0.03	0.05 $\pm$ 0.02	0.06 $\pm$ 0.03	0.05 $\pm$ 0.02	0 - 0.14
Reticulocytes	$\times 10^9$ / L	186.6 $\pm$ 37.2	164.6 $\pm$ 21.6	181.5 $\pm$ 23.8	165.1 $\pm$ 24.7	100 - 400

Table. Hematology findings in **females** at the end of the **treatment period** with AKR-963 and the comparator Lovaza.

**Table 19: Haematology Group Summaries – Females – End of Treatment**

Parameters	Unit	Group Means $\pm$ S.D. (n = 10)				Normal Ranges
		Group 1 AKR-963 (0.2 g/kg)	Group 2 AKR-963 (4 g/kg)	Group 3 Lovaza (0.2 g/kg)	Group 4 Lovaza (4 g/kg)	
RBC	$\times 10^{12} / L$	8.00 $\pm$ 0.22	7.92 $\pm$ 0.31	7.83 $\pm$ 0.28	7.66 $\pm$ 0.33	6.16 - 9.09
Hb	g / L	154 $\pm$ 3	151 $\pm$ 5	151 $\pm$ 5	148 $\pm$ 5	127 - 172
Hct	%	44.8 $\pm$ 0.7	43.9 $\pm$ 1.6	44.0 $\pm$ 1.5	43.4 $\pm$ 1.7	35.3 - 47.5
MCV	fL	56.0 $\pm$ 1.6	55.4 $\pm$ 1.2	56.2 $\pm$ 1.3	56.7 $\pm$ 1.3	47.5 - 64.0
MCH	pg	19.3 $\pm$ 0.5	19.1 $\pm$ 0.6	19.2 $\pm$ 0.4	19.3 $\pm$ 0.6	17.9 - 21.6
MCHC	g / L	344 $\pm$ 5	345 $\pm$ 5	342 $\pm$ 4	341 $\pm$ 5	325 - 385
Platelets	$\times 10^9 / L$	1067 $\pm$ 234	966 $\pm$ 351	1025 $\pm$ 209	1054 $\pm$ 92	526 - 1648
WBC	$\times 10^9 / L$	8.04 $\pm$ 1.97	7.82 $\pm$ 3.07	7.24 $\pm$ 1.58	7.92 $\pm$ 3.15	4.30 - 13.00
Neutrophils	$\times 10^9 / L$	0.87 $\pm$ 0.38	0.97 $\pm$ 0.53	1.00 $\pm$ 0.47	0.75 $\pm$ 0.27	0.10 - 2.67
Lymphocytes	$\times 10^9 / L$	6.82 $\pm$ 1.63	6.53 $\pm$ 2.53	5.88 $\pm$ 1.24	6.90 $\pm$ 2.90	0.33 - 11.60
Monocytes	$\times 10^9 / L$	0.18 $\pm$ 0.09	0.14 $\pm$ 0.08	0.19 $\pm$ 0.08	0.13 $\pm$ 0.06	0 - 0.30
Eosinophils	$\times 10^9 / L$	0.08 $\pm$ 0.03	0.11 $\pm$ 0.07	0.08 $\pm$ 0.03	0.07 $\pm$ 0.04	0 - 0.20
Basophils	$\times 10^9 / L$	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0 - 0.04
LUC	$\times 10^9 / L$	0.07 $\pm$ 0.05	0.04 $\pm$ 0.02	0.07 $\pm$ 0.05	0.05 $\pm$ 0.03	0 - 0.11
Reticulocytes	$\times 10^9 / L$	190.8 $\pm$ 39.3	147.9 $\pm$ 28.9	158.3 $\pm$ 26.7	165.0 $\pm$ 32.5	100 - 400

Table. Hematology findings in males at the end of the of **recovery period** with AKR-963 and the comparator lovaza.

**Table 20: Haematology Group Summaries – Males – Recovery**

Parameters	Unit	Group Means ± S.D. (n = 5)		
		Group 2 AKR-963 (4 g/kg)	Group 4 Lovaza (4 g/kg)	Normal Ranges
RBC	$\times 10^{12} / \text{L}$	8.18 ± 0.30	8.05 ± 0.35	6.06 - 9.46
Hb	g / L	151 ± 4	146 ± 6	120 - 181
Hct	%	45.1 ± 0.7	<b>43.2 ± 1.3 *</b>	37.3 - 50.2
MCV	fL	55.1 ± 1.4	53.8 ± 1.5	47.5 - 66.1
MCH	pg	18.4 ± 0.4	18.2 ± 0.3	15.8 - 23.1
MCHC	g / L	334 ± 4	338 ± 5	287 - 401
Platelets	$\times 10^9 / \text{L}$	921 ± 41	942 ± 62	579 - 1641
WBC	$\times 10^9 / \text{L}$	7.80 ± 2.99	7.80 ± 1.57	5.00 - 15.28
Neutrophils	$\times 10^9 / \text{L}$	1.13 ± 0.48	1.35 ± 0.40	0.05 - 2.37
Lymphocytes	$\times 10^9 / \text{L}$	6.36 ± 2.50	6.07 ± 1.20	1.67 - 14.00
Monocytes	$\times 10^9 / \text{L}$	0.18 ± 0.07	0.22 ± 0.08	0 - 0.46
Eosinophils	$\times 10^9 / \text{L}$	0.08 ± 0.01	0.09 ± 0.04	0 - 0.21
Basophils	$\times 10^9 / \text{L}$	0.02 ± 0.01	0.02 ± 0.01	0 - 0.06
LUC	$\times 10^9 / \text{L}$	0.02 ± 0.01	0.06 ± 0.04	0 - 0.14
Reticulocytes	$\times 10^9 / \text{L}$	178.0 ± 16.7	<b>203.7 ± 13.5*</b>	100 - 400

\* Statistically significant difference from Group 2 ( $p < 0.05$ ).

Table. Hematology findings in females at the end of the **recovery period** with AKR-963 and the comparator lovaza.

**Table 21: Haematology Group Summaries – Females – Recovery**

Parameters	Unit	Group Means ± S.D. (n = 5)		
		Group 2 AKR-963 (4 g/kg)	Group 4 Lovaza (4 g/kg)	Normal Ranges
RBC	x10 <sup>12</sup> / L	7.97 ± 0.26	7.84 ± 0.16	6.16 - 9.09
Hb	g / L	151 ± 4	150 ± 5	127 - 172
Hct	%	43.7 ± 1.1	43.4 ± 1.9	35.3 - 47.5
MCV	fL	54.8 ± 1.3	55.3 ± 1.8	47.5 - 64.0
MCH	pg	19.0 ± 0.6	19.1 ± 0.6	17.9 - 21.6
MCHC	g / L	346 ± 4	346 ± 6	325 - 385
Platelets	x10 <sup>9</sup> / L	802 ± 430	1065 ± 121	526 - 1648
WBC	x10 <sup>9</sup> / L	6.78 ± 2.18	5.03 ± 0.25	4.30 - 13.00
Neutrophils	x10 <sup>9</sup> / L	0.87 ± 0.30	0.80 ± 0.24	0.10 - 2.67
Lymphocytes	x10 <sup>9</sup> / L	5.59 ± 2.05	3.95 ± 0.36	0.33 - 11.60
Monocytes	x10 <sup>9</sup> / L	0.17 ± 0.05	0.17 ± 0.05	0 - 0.30
Eosinophils	x10 <sup>9</sup> / L	0.09 ± 0.03	0.09 ± 0.02	0 - 0.20
Basophils	x10 <sup>9</sup> / L	0.01 ± 0.01	0.01 ± 0.00	0 - 0.04
LUC	x10 <sup>9</sup> / L	0.05 ± 0.03	0.02 ± 0.01	0 - 0.11
Reticulocytes	x10 <sup>9</sup> / L	184.4 ± 34.3	167.4 ± 15.0	100 - 400

**Coagulation:** During the drug free recovery period in male rats at a HD, Fibrinogen levels were higher with Lovaza (304\* vs 261 % with Lovaza). See sponsor's description of these findings below.

Activated partial thromboplastin time (APTT) and prothombin time (PT) were within the normal ranges in all test groups of rats at the end of study and recovery periods. At the end of recovery, fibrinogen in Lovaza® treated male rats was significantly (p<0.05) higher than fibrinogen in the AKR-963-treated group, but this difference was not biologically relevant and was well within the historical range.

**Table 22: Coagulation Group Summaries – End of Treatment**

Group		Parameter – Mean ± S.D. (n=10)		
		Prothrombin Time (sec.)	APTT (sec.)	Fibrinogen (mg/dL)
Male	Group 1 – AKR-963(0.2 g/kg)	18.0 ± 0.7	24.2 ± 3.2	227 ± 28
	Group 2 – AKR-963(4 g/kg)	17.6 ± 1.0	21.9 ± 3.4	250 ± 24
	Group 3 – Lovaza(0.2 g/kg)	18.1 ± 1.3	24.4 ± 5.6	238 ± 35
	Group 4 – Lovaza(4 g/kg)	18.2 ± 1.1	24.8 ± 2.1	239 ± 24
Female	Group 1 – AKR-963(0.2 g/kg)	16.2 ± 1.0	20.1 ± 3.1	222 ± 55
	Group 2 – AKR-963(4 g/kg)	15.7 ± 0.9	19.0 ± 2.9	233 ± 36
	Group 3 – Lovaza(0.2 g/kg)	16.2 ± 0.5	19.8 ± 2.9	213 ± 46
	Group 4 – Lovaza(4 g/kg)	16.3 ± 0.6	19.8 ± 2.3	251 ± 47
Normal Ranges		11.6 - 23.3	4.7 - 37.4	155 - 338

**Table 23: Coagulation Group Summaries – Recovery**

Group		Parameter – Mean ± S.D. (n=5)		
		Prothrombin Time (sec.)	APTT (sec.)	Fibrinogen (mg/dL)
Male	Group 2 – AKR-963(4 g/kg)	16.3 ± 0.8	19.8 ± 3.6	261 ± 6
	Group 4 – Lovaza(4 g/kg)	17.1 ± 0.4	18.7 ± 1.5	304 ± 33 *
Female	Group 2 – AKR-963(4 g/kg)	17.5 ± 3.1	20.8 ± 6.9	190 ± 74
	Group 4 – Lovaza(4 g/kg)	16.0 ± 0.8	17.2 ± 2.1	221 ± 18
Normal Ranges		11.6 - 23.3	4.7 - 37.4	155 - 338

\* Statistically significant difference from Group 2 ( $p < 0.05$ ).

**Clinical chemistry:** At a HD, no effects of AKR-963 or Lovaza on triglycerides or cholesterol levels were noted in both male or female rats, when compared to the historical controls, suggesting no evidence of pharmacologic activity in this study in either treatment group when compared to historical controls.

In male rats (at the end of treatment), ALT levels were increased at a HD with both drugs (73 vs 74 u/L with lovaza, low dose values were 59 u/L with both drugs); these in the historical control

group were 26-71 u/L, however these were reversible at the end of the drug free recovery period (50 u/L with both drugs). At the end of the recovery period at a HD with AKR-963 in male rats, creatinine (43 vs 38\*  $\mu\text{mol/L}$  with Lovaza), and in the female rats globulin levels (30 vs 28\* g/L with Lovaza) were significantly increased with AKR-963 vs lovaza. but these were not toxicologically relevant as the differences were small, and no histological correlates were observed. Also in female rats at a HD at the end of recovery period, LDH was increased with both drugs (5263  $\mu\text{L}$  by 22% with AKR-963 vs 6767  $\mu\text{L}$  by 57% with Lovaza). See sponsor's description of these findings.

Table. Clinical chemistry findings in males at the end of the treatment period with AKR-963 and the comparator Lovaza.

**Table 24: Serum Chemistry Group Summaries – Males – End of Treatment**

Parameters	Unit	Group Means $\pm$ S.D. (n = 10)				Normal Ranges
		Group 1 AKR-963 (0.2 g/kg)	Group 2 AKR-963 (4 g/kg)	Group 3 Lovaza (0.2 g/kg)	Group 4 Lovaza (4 g/kg)	
A/G	-	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	0.7 - 1.6
ALB	g / L	28 $\pm$ 1	28 $\pm$ 2	28 $\pm$ 2	29 $\pm$ 1	23 - 43
GLOB	g / L	27 $\pm$ 1	27 $\pm$ 2	27 $\pm$ 1	27 $\pm$ 2	22 - 36
ALP	u / L	175 $\pm$ 50	297 $\pm$ 80	157 $\pm$ 24	295 $\pm$ 135	47 - 426
Bil(T)	$\mu\text{mol} / \text{L}$	6.1 $\pm$ 1.6	3.8 $\pm$ 1.0	<b>4.1 <math>\pm</math> 1.4<sup>§</sup></b>	3.8 $\pm$ 1.3	1.7 - 5.7
BUN	mmol / L	6.4 $\pm$ 1.0	6.2 $\pm$ 0.6	6.8 $\pm$ 0.7	5.9 $\pm$ 0.6	3.0 - 8.4
Ca	mmol / L	2.35 $\pm$ 0.07	2.38 $\pm$ 0.06	2.39 $\pm$ 0.07	2.37 $\pm$ 0.08	2.24 - 3.00
Cl	mmol / L	101 $\pm$ 1	102 $\pm$ 2	102 $\pm$ 1	101 $\pm$ 1	90 - 116
Creatinine	$\mu\text{mol} / \text{L}$	41 $\pm$ 2	43 $\pm$ 4	41 $\pm$ 4	43 $\pm$ 4	24 - 66
Glucose	mmol / L	9.9 $\pm$ 2.0	8.5 $\pm$ 1.6	9.0 $\pm$ 1.7	8.4 $\pm$ 1.9	0.8 - 11.2
LDH	u / L	5358 $\pm$ 2026	3910 $\pm$ 2075	5974 $\pm$ 2861	3533 $\pm$ 1408	1050 - 6401
P	mmol / L	2.59 $\pm$ 0.13	2.50 $\pm$ 0.13	2.56 $\pm$ 0.18	2.49 $\pm$ 0.14	1.83 - 3.94
K	mmol / L	4.9 $\pm$ 0.3	4.8 $\pm$ 0.3	5.0 $\pm$ 0.3	4.8 $\pm$ 0.3	3.7 - 7.0
Protein (T)	g / L	55 $\pm$ 2	55 $\pm$ 3	55 $\pm$ 3	56 $\pm$ 3	47 - 75
AST	u / L	113 $\pm$ 25	105 $\pm$ 29	111 $\pm$ 23	100 $\pm$ 16	42 - 149
ALT	u / L	59 $\pm$ 7	73 $\pm$ 13	59 $\pm$ 7	74 $\pm$ 18	26 - 71
Na	mmol / L	143 $\pm$ 1	144 $\pm$ 1	143 $\pm$ 2	144 $\pm$ 1	136 - 152
Triglycerides	mmol / L	0.43 $\pm$ 0.13	0.40 $\pm$ 0.12	0.44 $\pm$ 0.12	0.38 $\pm$ 0.10	0.10 - 1.55
CK	u / L	499 $\pm$ 230	431 $\pm$ 218	521 $\pm$ 182	364 $\pm$ 125	228 - 529
Cholesterol	mmol / L	1.28 $\pm$ 0.24	1.24 $\pm$ 0.14	1.50 $\pm$ 0.32	1.23 $\pm$ 0.19	1.00 - 3.00
GGT	u / L	< 5 $\pm$ 0	< 5 $\pm$ 0	< 5 $\pm$ 0	< 5 $\pm$ 0	4 - 6

<sup>§</sup> Statistically significant difference from Group 1 (p < 0.05).

Table. Clinical chemistry findings in females at the end of the treatment period with AKR-963 and the comparator lovaza.

**Table 25: Serum Chemistry Group Summaries – Females – End of Treatment**

Parameters	Unit	Group Means $\pm$ S.D. (n = 10)				Normal Ranges
		Group 1 AKR-963 (0.2 g/kg)	Group 2 AKR-963 (4 g/kg)	Group 3 Lovaza (0.2 g/kg)	Group 4 Lovaza (4 g/kg)	
A/G	-	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	0.8 - 1.8
ALB	g / L	32 $\pm$ 2	31 $\pm$ 2	33 $\pm$ 2	32 $\pm$ 1	25 - 49
GLOB	g / L	28 $\pm$ 1	28 $\pm$ 1	28 $\pm$ 1	27 $\pm$ 1	22 - 34
ALP	u / L	116 $\pm$ 35	148 $\pm$ 28	105 $\pm$ 28	128 $\pm$ 40	29 - 309
Bil(T)	$\mu$ mol / L	4.7 $\pm$ 1.5	5.7 $\pm$ 2.6	5.2 $\pm$ 1.2	4.3 $\pm$ 0.8	1.7 - 5.9
BUN	mmol / L	5.2 $\pm$ 1.0	4.9 $\pm$ 0.8	5.6 $\pm$ 1.1	5.2 $\pm$ 1.1	3.2 - 8.0
Ca	mmol / L	2.41 $\pm$ 0.05	2.45 $\pm$ 0.09	<b>2.49 <math>\pm</math> 0.08*</b>	2.53 $\pm$ 0.07	2.31 - 3.03
Cl	mmol / L	103 $\pm$ 3	104 $\pm$ 2	103 $\pm$ 3	104 $\pm$ 2	93 - 117
Creatinine	$\mu$ mol / L	38 $\pm$ 4	37 $\pm$ 6	39 $\pm$ 4	39 $\pm$ 5	23 - 66
Glucose	mmol / L	8.0 $\pm$ 2.7	6.2 $\pm$ 1.0	8.2 $\pm$ 1.7	8.2 $\pm$ 2.3	1.2 - 11.4
LDH	u / L	5752 $\pm$ 1728	3992 $\pm$ 1121	4375 $\pm$ 2289	2533 $\pm$ 1314	1455 - 4306
P	mmol / L	2.24 $\pm$ 0.35	2.17 $\pm$ 0.23	2.38 $\pm$ 0.24	2.48 $\pm$ 0.26	1.50 - 3.47
K	mmol / L	4.9 $\pm$ 0.3	4.8 $\pm$ 0.3	5.0 $\pm$ 0.2	4.9 $\pm$ 0.2	3.6 - 6.5
Protein (T)	g / L	60 $\pm$ 3	58 $\pm$ 3	60 $\pm$ 3	59 $\pm$ 2	50 - 79
AST	u / L	115 $\pm$ 27	107 $\pm$ 24	115 $\pm$ 26	81 $\pm$ 15	48 - 134
ALT	u / L	45 $\pm$ 4	52 $\pm$ 9	46 $\pm$ 6	49 $\pm$ 6	22 - 66
Na	mmol / L	143 $\pm$ 2	143 $\pm$ 2	144 $\pm$ 2	145 $\pm$ 1	138 - 181
Triglycerides	mmol / L	0.46 $\pm$ 0.10	0.34 $\pm$ 0.02	0.42 $\pm$ 0.10	0.40 $\pm$ 0.11	0.10 - 1.25
CK	u / L	622 $\pm$ 201	426 $\pm$ 139	557 $\pm$ 194	287 $\pm$ 92	158 - 556
Cholesterol	mmol / L	1.54 $\pm$ 0.51	1.29 $\pm$ 0.18	1.69 $\pm$ 0.36	1.40 $\pm$ 0.21	0.94 - 3.26
GGT	u / L	< 5 $\pm$ 0	< 5 $\pm$ 0	< 5 $\pm$ 0	< 5 $\pm$ 0	3 - 8

\* Statistically significant difference from Group 1 ( $p < 0.05$ ).

Table. Clinical chemistry findings in males at the **end of the recovery period** with AKR-963 and the comparator lovaza.

**Table 26: Serum Chemistry Group Summaries – Males – Recovery**

Parameters	Unit	Group Means $\pm$ S.D. (n = 5)		
		Group 2 AKR-963 (4 g/kg)	Group 4 Lovaza (4 g/kg)	Normal Ranges
A/G	-	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	0.7 - 1.6
ALB	g / L	27 $\pm$ 2	30 $\pm$ 2	23 - 43
GLOB	g / L	28 $\pm$ 1	28 $\pm$ 1	22 - 36
ALP	u / L	142 $\pm$ 29	135 $\pm$ 34	47 - 426
Bil(T)	$\mu$ mol / L	4.0 $\pm$ 1.2	5.0 $\pm$ 1.9	1.7 - 5.7
BUN	mmol / L	5.6 $\pm$ 0.6	5.5 $\pm$ 0.8	3.0 - 8.4
Ca	mmol / L	2.39 $\pm$ 0.08	2.37 $\pm$ 0.06	2.24 - 3.00
Cl	mmol / L	102 $\pm$ 2	102 $\pm$ 2	90 - 116
Creatinine	$\mu$ mol / L	43 $\pm$ 3	38 $\pm$ 3 *	24 - 66
Glucose	mmol / L	8.9 $\pm$ 2.6	8.3 $\pm$ 1.4	0.8 - 11.2
LDH	u / L	4397 $\pm$ 1596	4777 $\pm$ 1418	1050 - 6401
P	mmol / L	2.43 $\pm$ 0.14	2.37 $\pm$ 0.08	1.83 - 3.94
K	mmol / L	4.7 $\pm$ 0.2	4.9 $\pm$ 0.4	3.7 - 7.0
Protein (T)	g / L	55 $\pm$ 2	58 $\pm$ 4	47 - 75
AST	u / L	109 $\pm$ 20	116 $\pm$ 12	42 - 149
ALT	u / L	50 $\pm$ 4	50 $\pm$ 4	26 - 71
Na	mmol / L	141 $\pm$ 1	142 $\pm$ 1	136 - 152
Triglycerides	mmol / L	0.56 $\pm$ 0.20	0.76 $\pm$ 0.32	0.10 - 1.55
CK	u / L	393 $\pm$ 159	440 $\pm$ 123	228 - 529
Cholesterol	mmol / L	1.37 $\pm$ 0.21	1.62 $\pm$ 0.53	1.00 - 3.00
GGT	u / L	< 5 $\pm$ 0	< 5 $\pm$ 0	4 - 6

\* Statistically significant difference from Group 2 ( $p < 0.05$ ).

Table. Clinical chemistry findings in females at the **end of the recovery period** with AKR-963 and the comparator lovaza.

**Table 27: Serum Chemistry Group Summaries – Females – Recovery**

Parameters	Unit	Group Means ± S.D. (n = 5)		
		Group 2 AKR-963 (4 g/kg)	Group 4 Lovaza (4 g/kg)	Normal Ranges
A/G	-	1.1 ± 0.1	1.2 ± 0.1	0.8 - 1.8
ALB	g / L	33 ± 3	32 ± 2	25 - 49
GLOB	g / L	30 ± 1	28 ± 1 *	22 - 34
ALP	u / L	87 ± 8	87 ± 28	29 - 309
Bil(T)	µmol / L	4.4 ± 0.4	4.5 ± 0.6	1.7 - 5.9
BUN	mmol / L	5.0 ± 0.2	5.0 ± 0.5	3.2 - 8.0
Ca	mmol / L	2.47 ± 0.03	2.43 ± 0.07	2.31 - 3.03
Cl	mmol / L	102 ± 1	103 ± 2	93 - 117
Creatinine	µmol / L	37 ± 6	35 ± 3	23 - 66
Glucose	mmol / L	8.0 ± 0.5	7.5 ± 0.7	1.2 - 11.4
LDH	u / L	5263 ± 3498	6767 ± 1730	1455 - 4306
P	mmol / L	2.37 ± 0.14	2.25 ± 0.19	1.50 - 3.47
K	mmol / L	4.4 ± 0.3	4.5 ± 0.2	3.6 - 6.5
Protein (T)	g / L	63 ± 4	60 ± 3	50 - 79
AST	u / L	108 ± 39	119 ± 15	48 - 134
ALT	u / L	40 ± 8	43 ± 5	22 - 66
Na	mmol / L	140 ± 1	141 ± 1	138 - 181
Triglycerides	mmol / L	0.41 ± 0.06	0.42 ± 0.07	0.10 - 1.25
CK	u / L	440 ± 239	601 ± 194	158 - 556
Cholesterol	mmol / L	1.57 ± 0.32	1.28 ± 0.20	0.94 - 3.26
GGT	u / L	< 5 ± 0	< 5 ± 0	3 - 8

\* Statistically significant difference from Group 2 ( $p < 0.05$ ).

Sponsor describes these findings below:

In females dosed with AKR-963 at 0.2 g/kg, both LDH and CK slightly exceeded the upper limits of the normal ranges at the end of the study (34% increase for LDH, and 12% increase for CK). In the groups dosed with AKR-963 or Lovaza® at 4 g/kg, LDH and CK levels were within the normal ranges. A similar trend was noted in males, where LDH and CK were higher in the 0.2 g/kg AKR-963 and Lovaza® groups compared to the 4 g/kg groups (although all values remained within historical control ranges for males). LDH levels were also increased in females dosed with either AKR-963 or Lovaza® at 4 g/kg at the end of the recovery period (22% and 57% increase for AKR-963 and Lovaza®, respectively). CK was also increased in

Lovaza® treated recovery females (8% increase). Overall, the trends for LDH and CK were similar for AKR-963 and Lovaza®-treated groups. None of the clinical chemistry parameters were considered to be significant.

These differences are known variabilities with these enzymes and are related to their release during regular handling of rats, grasping, dosing, etc. (12), blood collection procedures (13), or their release from cellular elements during clotting (14). There are also known issues with tissue non-specificity of LDH and CK isoenzymes (15).

Thus, the differences and variabilities were not considered to be treatment-related.

**Urinalysis:** All urinalysis were considered unremarkable. No summary data were provided.

**Organ weights:** No effects on changes in organ weights were noted, see below.

Since no control animals were used in this study, the difference in organ weights was considered only if the organ weights were outside the normal historical ranges for the age and sex of the animals and if the difference in the organ weights was more than 10% (Low dose compared to Low dose, and High dose compared to High dose).

Based on these criteria there was no apparent difference in the organ weights (gender matched) for spleen, liver, adrenals, testes, kidneys, prostate, lungs, heart, thymus, brain, pituitary, ovaries, and uterus.

The summary weights in males and females were provided here, see below:

Table. Organ weights in male rats with AKR-963 and Lovaza.

STUDY: Rat Oral Toxicity  
STUDY NO: 241342  
SEX: MALE

	ALL FATES	ALL DAYS	ALL BALANCES	
GROUP:	1-M	2-M	3-M	4-M
BODY WEIGHT (G) (ABSOLUTE)				
MEAN	405.9	419.8	416.2	421.5
SD	27.39	23.46	28.57	25.56
N	10	10	10	10
SPLEEN (G) (ABSOLUTE)				
MEAN	0.7536	0.7777	0.8416	0.8064
SD	0.14249	0.11413	0.10554	0.09148
N	10	10	10	10
LIVER (G) (ABSOLUTE)				
MEAN	11.54	12.90	12.25	12.84
SD	1.038	1.088	1.075	1.139
N	10	10	10	10
ADRENAL GLANDS (G) (ABSOLUTE)				
MEAN	0.0827	0.0809	0.0796	0.0900
SD	0.01852	0.01613	0.01410	0.01735
N	10	10	10	10
TESTES (G) (ABSOLUTE)				
MEAN	3.43	3.66	3.63	3.50
SD	0.161	0.196	0.265	0.266
N	10	10	10	10
KIDNEYS (G) (ABSOLUTE)				
MEAN	2.97	3.10	3.09	3.28
SD	0.223	0.201	0.223	0.245
N	10	10	10	10
PROSTATE (G) (ABSOLUTE)				
MEAN	1.0441	1.0836	1.0408	0.9044
SD	0.31386	0.27314	0.22629	0.30536
N	10	10	10	10
LUNGS AND TRACHEA (G) (ABSOLUTE)				
MEAN	1.75	1.75	1.77	1.80
SD	0.152	0.122	0.205	0.209
N	10	10	10	10

Table. Organ weights in male rats with AKR-963 and Lovaza (continued).

STUDY: Rat Oral Toxicity		ALL FATES		ALL DAYS		ALL BALANCES	
STUDY NO: 241342							
SEX: MALE							
GROUP:		1-M	2-M	3-M	4-M		
HEART (G) (ABSOLUTE)							
MEAN		1.29	1.31	1.28	1.27		
SD		0.079	0.137	0.128	0.078		
N		10	10	10	10		
THYMUS (G) (ABSOLUTE)							
MEAN		0.5449	0.5801	0.5742	0.5534		
SD		0.07948	0.09157	0.13982	0.11604		
N		10	10	10	10		
BRAIN (G) (ABSOLUTE)							
MEAN		2.06	2.10	2.12	2.09		
SD		0.053	0.076	0.057	0.080		
N		10	10	10	10		
PITUITARY GLAND (G) (ABSOLUTE)							
MEAN		0.0111	0.0113	0.0110	0.0116		
SD		0.00136	0.00105	0.00162	0.00250		
N		10	10	10	10		

Table. Organ weights in female rats with AKR-963 and lovaza.

STUDY: Rat Oral Toxicity  
STUDY NO: 241342  
SEX: MALE

	ALL FATES	ALL DAYS	ALL BALANCES	
GROUP:	1-M	2-M	3-M	4-M
SPLIEN (% BODY WEIGHT)				
MEAN	0.1864	0.1857	0.2028	0.1920
SD	0.03613	0.02837	0.02664	0.02567
N	10	10	10	10
LIVER (% BODY WEIGHT)				
MEAN	2.8407	3.0708	2.9439	3.0477
SD	0.12471	0.14664	0.19374	0.22613
N	10	10	10	10
ADRENAL GLANDS (% BODY WEIGHT)				
MEAN	0.0205	0.0193	0.0193	0.0213
SD	0.00479	0.00403	0.00412	0.00371
N	10	10	10	10
TESTES (% BODY WEIGHT)				
MEAN	0.8484	0.8729	0.8751	0.8343
SD	0.05707	0.06225	0.07868	0.09048
N	10	10	10	10
KIDNEYS (% BODY WEIGHT)				
MEAN	0.7338	0.7403	0.7449	0.7784
SD	0.06438	0.04607	0.05601	0.04402
N	10	10	10	10
PROSTATE (% BODY WEIGHT)				
MEAN	0.2565	0.2600	0.2501	0.2138
SD	0.07203	0.07579	0.05296	0.07041
N	10	10	10	10
LUNGS AND TRACHEA (% BODY WEIGHT)				
MEAN	0.4322	0.4178	0.4239	0.4275
SD	0.03624	0.03849	0.03214	0.05061
N	10	10	10	10
HEART (% BODY WEIGHT)				
MEAN	0.3190	0.3120	0.3075	0.3029
SD	0.02781	0.02966	0.01730	0.02496
N	10	10	10	10

Table. Organ weights in female rats with AKR-963 and Lovaza (continued).

STUDY: Rat Oral Toxicity						
STUDY NO: 241342						
SEX: MALE		ALL FATES	ALL DAYS	ALL BALANCES		
GROUP:		1-M	2-M	3-M	4-M	
THYMUS (% BODY WEIGHT)						
MEAN		0.1344	0.1379	0.1374	0.1312	
SD		0.01817	0.01820	0.02937	0.02523	
N		10	10	10	10	
BRAIN (% BODY WEIGHT)						
MEAN		0.5083	0.5025	0.5116	0.4971	
SD		0.03288	0.04259	0.03809	0.02270	
N		10	10	10	10	
PITUITARY GLAND (% BODY WEIGHT)						
MEAN		0.0027	0.0027	0.0027	0.0028	
SD		0.00030	0.00025	0.00038	0.00057	
N		10	10	10	10	

**Gross pathology:** At a HD with AKR-963, findings were noted in the sternum (thickening in 1/10 male rats), and uterus (fluid-filled in 1/10 rats both at 0.2 and 4 g/kg of AKR-963). During the drug free recovery period at a HD, these were observed in 1/5 female rats (alopecia) and 1/5 male rats in the right cerebrum (clear fluid-filled cavity, 3-4 mm size).

No summary Table was provided. Sponsor states that there were occasional findings in some animals that were considered to be incidental:

- sternum thickening: 1 male rat at 4 g/kg AKR-963;
- alopecia: 1 female at 0.2 g/kg AKR-963, 2 females at 0.2 g/kg Lovaza® and 1 recovery female at 4 g/kg AKR-963;
- fluid-filled uterus: 1 rat from each of 0.2 and 4 g/kg AKR-963;
- clear fluid-filled cavity (3-4 mm size) in the right cerebrum in 1 recovery male at 4 g/kg AKR-963; and
- adrenals small in size in 1 male recovery rat at 4 g/kg Lovaza®

None of these were considered significant.

It is not clear if the drug itself had any effects on these parameters because the control group was not included.

**Histopathology:** Initially, no summary Table was provided. This information was requested on 2/6/13 and sponsor provided it on 3/6/13, however the histopathology data provided was not very rigorous one, as not all tissues were included in the histopathology Table, see below. Earlier only individual report on each animal was provided and it was hard to make any sense of the individual animal data, and no individual histopathology report on the animal (male # 32) that died was provided.

Note that similar histopathology findings were noted with both drugs (AKR-963 and Lovaza). Target organs of toxicity with both drugs were liver (inflammation in 3/20 rats vs 3/20 rats with Lovaza) and kidney (inflammation in 3/20 rats vs 3/20 with lovaza), as seen in the Table below. However, histopathology findings were noted in the urinary bladder with AKR-963 (in females subchronic and diffuse mucosal inflammation in 2/10 vs 0/10 with Lovaza), these were not noted with Lovaza. Similarly in eyes, focal retinal degeneration of mild severity was noted during treatment in 1/10 female rats with both AKR-963 and Lovaza respectively, but at the end of drug free recovery period it was only noted with AKR-963 in 1/5 male rats (1/5 vs 0/5 with Lovaza). Additionally skin findings (hypotrichosis, of moderate severity) were noted in 1/5 female rats at the end of drug free recovery period with AKR-963, but not with Lovaza, see the Table below.

Table. Histopathology findings with AKR-963 and Lovaza are summarized below. Animals that had findings in the recovery group, are shown in the parenthesis.

Histopathology findings	AKR-963 (R=animals in the recovery group)		Lovaza (R=animals in the recovery group)	
	Males	Females	Males	Females
Liver-inflammation	0/10	3/10	2/10	1/10
Kidney -inflammation	0/10 (1/5 R)	2/10	1/10	2/10 (1/5 R)
Urinary bladder- mucosal inflammation	0/10	2/10	0/10	0/10
Eyes-focal retinal degeneration	0/10 (1/5 R)	1/10	0/10	1/10
Heart-Focal subchronic inflammation	0/10	0/10	0/10	1/10
Skin-Hypotrichosis	0/10	0/10 (1/5 R)	0/10	0/10

R= recovery animas

Thus the target organs of toxicity in this study appear to be liver, kidney, urinary bladder, retina, and heart (1/10 female rats with Lovaza). Other than the bladder mucosal inflammation showing a higher incidence than Lovaza, and one male rat that died in the toxicokinetic group with acute cardiac failure with AKR-963, it appears that the toxicity with both drugs (AKR-963 and lovaza) in general is similar with regard to incidence and severity in the current study.

However, previously, more toxicity was noted with lovaza in several organs at lower doses, not noted with either lovaza or AKR-963 (using 4-fold higher doses) in the current study.. Additionally because there are no control data in the current toxicity study, we can-not tell if

there is efficacy in animals. We cannot set a NOAEL, since the sponsor only provided histopathology at a HD of 4 g/kg/day with AKR-963 and Lovaza, and no histopathology was conducted on the lower dose groups. Thus there is no evidence of efficacy in animals, and the toxicity studies do not have concurrent controls.

See the sponsor’s description of these findings

Full histopathology was conducted at the end of dosing (EoT) on day 29 and at the end of the recovery period on day 43 (EoR) for animals in the high-dose groups. The majority of results were “normal” with a grade = 0 and a description of “no significant findings.” The individual animal histopathology findings in which the observation score for any tissue was > 0 are summarized in [Table 1.11.2-1](#). Abnormal findings in the low-dose group that necessitated histopathology were limited to hypotrichosis (grade 3) in a single animal in the AKR-963 and Lovaza groups (female animal numbers 83 and 117, respectively; data not included in [Table 1.11.2-1](#)). A tabulated summary of the data presented in [Table 1.11.2-1](#) is provided by group in [Table 1.11.2-2](#).

Following is sponsor’s submitted histopathology summary Table (corrected was provided on 7/12/13)

Tissue Grade: Description of Observation	AKR-963				Lovaza			
	End of Treatment		Recovery		End of Treatment		Recovery	
	M n(N)	F n(N)	M n(N)	F n(N)	M n(N)	F n(N)	M n(N)	F n(N)
Liver	0(10)	3(10)	0(5)	0(5)	2(10)	1(10)	0(5)	0(5)
Grade 1: Focal chronic inflammation	—	1(10)	—	—	1(10)	—	—	—
Grade 1: Focal subchronic inflammation	—	—	—	—	1(10)	—	—	—
Grade 2: Focal chronic inflammation	—	1(10)	—	—	—	—	—	—
Grade 2: Periportal lipidosis	—	—	—	—	—	1(10)	—	—
Grade 1: Disseminated focal chronic inflammation	—	1(10)	—	—	—	—	—	—
Kidney	0(10)	2(10)	1(5)	0(5)	1(10)	2(10)	0(5)	1(5)
Grade 2: Subchronic pelvic inflammation	—	1(10)	—	—	—	—	—	—
Grade 2: Subacute pelvic inflammation	—	1(10)	—	—	—	—	—	—
Grade 2: Focal subchronic inflammation	—	—	—	—	1(10)	1(10)	—	1(5)
Grade 2: Focal subchronic pelvic inflammation	—	—	—	—	—	1(10)	—	—
Grade 3: Focal chronic inflammation	—	—	1(5)	—	—	—	—	—
Urinary bladder	0(10)	2(10)	0(5)	0(5)	0(10)	0(10)	0(5)	0(5)
Grade 2: Subchronic mucosal inflammation	—	1(10)	—	—	—	—	—	—
Grade 2: Diffuse chronic mucosal inflammation	—	1(10)	—	—	—	—	—	—
Eyes	0(10)	1(10)	1(5)	0(5)	0(10)	1(10)	0(5)	0(5)
Grade 1: Focal retinal degeneration	—	—	—	—	—	1(10)	—	—
Grade 2: Focal retinal degeneration	—	1(10)	1(5)	—	—	—	—	—
Heart	0(10)	0(10)	0(5)	0(5)	0(10)	1(10)	0(5)	0(5)
Grade 2: Focal subchronic inflammation	—	—	—	—	—	1(10)	—	—
Skin	0(10)	0(10)	0(5)	1(5)	0(10)	0(10)	0(5)	0(5)
Grade 3: Hypotrichosis	—	—	—	1(5)	—	—	—	—

F = Female.  
M = Male.  
n = Number of animals with findings.  
N = Total number of animals evaluated.

Data Source: [Histopathology report](#), Appendix IX in Report 241342: Table 1, Table 2, and individual histopathology findings.

.Values are scored according to grades of responses: none = 0; minimal = 1; mild = 2; moderate = 3; marked = 4; severe = 5.  
Empty values indicate not examined.

These tables are a summary of the changes observed in each animal for each tissue evaluated as graded on a scale of: None = 0; Minimal = 1; Mild = 2; Moderate = 3; Marked = 4; Severe = 5.

See the sponsor's result description on histopathology below:

**Results:**

A list of grades for all tissues examined with means are provided in Table 1.

*Conditions of toxicological significance:*

There were no conditions of toxicological significance.

*Conditions of possible toxicological significance:*

There were no conditions of possible toxicological significance.

*Conditions lacking toxicological significance:*

A small number of animals had incidental lesions of inflammation or degeneration in isolated tissues. Such findings are not uncommon in laboratory rats and had no relationship to the test articles.

Rat # 032 of Group 2 was found dead on Day 14. Findings associated with death were lung and liver congestion. Other tissues were normal or had findings unrelated to death. The cause of death was undetermined.

**Conclusions:**

The daily oral administration of 0.2 g/kg or 4.0 g/kg AKR 963 or 0.2 g/kg or 4.0 g/kg Lovaza to Sprague-Dawley rats for 28 days caused no histopathological lesions of toxicological significance.

(b) (4)

25 October 2011  
Date

**Conclusions:**

The daily oral administration of 0.2 g/kg or 4.0 g/kg AKR 963 or 0.2 g/kg or 4.0 g/kg Lovaza to Sprague-Dawley rats for 28 days caused no histopathological lesions of toxicological significance.

The results of the 28-day repeat-dose toxicology study did not reveal any differences between AKR-963 and Lovaza at doses of 0.2 and 4 g/kg/day. One death occurred on day 14 of a male toxicokinetic animal dosed with AKR-963 at 4 g/kg/day. This death was not considered treatment-related due to the lack of any other toxicity in this animal or in any other animals in the study. There were no treatment-related adverse effects on body weight, body weight gain, food consumption, clinical signs, ophthalmology, clinical pathology, organ weights, macroscopic, or microscopic pathology. The toxicokinetic evaluation demonstrated dose-related (but less than proportional) increases in DHA and EPA and similar toxicokinetic profiles for AKR-963 and Lovaza with higher exposure in males than females and accumulation after 28 days of dosing. The NOAEL was 4 g/kg/day, a dose level which is 10-fold higher than the proposed therapeutic dose (60 mg/kg/day), based on comparison of doses by body surface.

### Information on Historical controls

The information on historical control data was provided by the sponsor on 3/6/13, when requested by this reviewer. Note that the laboratory data on historical control animals are from rats who are 9-13 weeks old, while the rats used in the present toxicity study were 7-8 weeks of age at the start of dosing. Also Laboratory data are from a large number of studies i.e. 18 to 43 studies (with n=140 to 385) from years 2002 to 2007, and do not represent the single study conducted here (with n=10 to 20). Thus this pivotal toxicity study lacks a vehicle control; and historical / background data submitted by the sponsor are not representative of a single study; and do not provide the complete assessment and differences between the two drugs and controls.

Table. Sponsor's historical control data

The historical data for Sprague-Dawley rats used instead of a control study are provided in [Table 1.11.2-3](#); data for years of data collection, number of rats, and number of studies are provided. The data are based only on the control rats 9 to 13 weeks old (toxicity studies) from Good Laboratory Practice (GLP) and quality control-checked non-GLP projects.

Parameter	Years of Data Collection	Number of Sprague-Dawley Rats <sup>a</sup>		Number of Studies <sup>c</sup>
		Male	Female	
Hematology	2002 – 2007 <sup>b</sup>	140	157	25
Coagulation	2002 – 2007 <sup>b</sup>	132	132	25
Serum chemistry	2002 – 2007 <sup>b</sup>	345	343	25
Organ weights	2000 – 2012	372	352	43
Body weights	2001 – 2012	372	385	32
Food consumption	2001 – 2012	345	385	32
Ophthalmology	2009- 2011	3007 (readings)		18
Histopathology	N/A <sup>d</sup>	180 <sup>e</sup>	158 <sup>e</sup>	14 <sup>e</sup>

<sup>a</sup> = CRL: CD® (SD) BR – Sprague-Dawley from (b) (4)

<sup>b</sup> = Clinical pathology data over the years were monitored and there was no significant shift in the values over the years, thus no current studies are included in these data.

<sup>c</sup> = Number of studies does not represent all studies (GLP or non GLP) conducted at (b) (4) over this time period.

<sup>d</sup> = (b) (4) does not collate normal ranges for histopathological data from short-term toxicology studies.

<sup>e</sup> = Values represent the available data that could be drawn upon related to specific histopathology findings for a period of 5 years (2007-2012) preceding the AKR-963 study.

GLP = Good Laboratory Practice.

**Toxicokinetics:**

With both AKR-963 or Lovaza at a high dose, plasma exposures of both DHA and EPA in general were similar; exposures were higher in males than in females, both on day 1 and on day 28. Accumulation of the drug was noted on day 28, as the average plasma exposure of both DHA and EPA were generally higher (1.9- and 1.2-fold, respectively) than on day 1 with both drugs.

Sponsor states that the increases of plasma exposure were only statistically significantly greater for AKR-963 low-dose males (day 28 versus day 1), but not for Lovaza low-dose males.

AUC exposures of EPA were 33% higher in females at a high dose of AKR-963 vs Lovaza (2201 vs 1636 mcg.hr/ml with Lovaza on day 28). Also, the AUC exposures of DHA were significantly higher in females at a low dose of AKR-963 vs Lovaza (536 vs 135 mcg.hr/ml with Lovaza).

Table. AUC exposure comparisons of DHA and EPA following administration of AKR-963 and Lovaza in a 28-day bridging toxicity study in rats.

	DHA	EPA
	AUC <sub>0-24 hrs</sub> mcg.hr/ml	AUC <sub>0-24 hrs</sub> mcg.hr/ml
	Day 1 / Day 28	Day 1 / Day 28
Dose: mg/kg/day		
<b>With AKR-963</b>		
<b>Males</b>		
200	492 / 1185	482 / 891
4000	1248 / 1992	2333 / 2594
<b>Females</b>		
200	119 / 536	398 / 576
4000	435 / 790	1597 / 2201
<b>With Lovaza</b>		
<b>Males</b>		
200	664 / 921	755 / 615
4000	1204 / 1945	2566 / 2636
<b>Females</b>		
200	258 / 135	344 / 408
4000	701 / 963	1592 / 1636

Thus systemic exposure of AKR-963 and lovaza at a HD in general were similar, the exposures were higher in males than in females, generally did not increase in a dose proportional manner from 200 to 4000 mg/kg in both sexes, and exposures increased over time suggesting accumulation over the 4-week period.

Sponsor's explanation of TK is provided below:

Toxicokinetic evaluations of DHA and EPA revealed that on Day 1, oral administration of

AKR-963 and Lovaza® resulted in elevation of DHA from endogenous levels by 2.1-fold for AKR-963 and 2.3-fold for Lovaza and elevation of EPA from endogenous levels by 31.3-fold for AKR-963 and 35.2-fold for Lovaza. Plasma exposure to DHA and EPA on Day 1 following oral administration of Low and High dose AKR-963 and Lovaza® was not proportional to dose, possibly due to saturation of omega-3 fatty acid transport in the gastrointestinal tract.

C<sub>max</sub> was higher in males compared to females (e.g., Day 1; DHA C<sub>max</sub> males 2.2 - 3.1-fold vs. females 1.6 - 1.8-fold; EPA C<sub>max</sub> males 15.5-36.0 fold vs. females 8.5 - 16.2-fold, respectively) both on Day 1 and on Day 28 following repeated dosing. These gender-specific observations may possibly be related to differences in metabolism and distribution of omega-3 fatty acids in males and females. Following repeated dosing and in general on Day 28, the average plasma exposure increased to both DHA and EPA on Day 28 (1.9- and 1.2-fold, respectively) compared to Day 1 and dose accumulation was observed; the increases of plasma exposure were slightly greater for AKR-963 compared to Lovaza®. In addition to dose accumulation, changes in the metabolism and distribution of omega-3 fatty acids may have contributed to the increase of plasma concentrations on Day 28.

Overall, there were no statistically significant differences in toxicokinetic parameters between AKR-963 and Lovaza groups, when inter-group statistical comparisons were made, and the products were found to produce similar toxicokinetic profiles for DHA and EPA with similar gender-specific differences and accumulation upon repeated dosing.

**Table 4: TK Parameters<sup>1</sup> for DHA and EPA in Male and Female Rats on Day 1**

Dose	Gender	C <sub>max</sub> (µg/mL)	T <sub>max</sub> (hr)	AUC <sub>0-24 hr</sub> (µg*hr/mL)	AUC <sub>0-∞</sub> (µg*hr/mL)	MRT (hr)
<b>0.2 g/kg</b>						
<b>DHA</b>						
AKR-963	M	56 ± 8	2.3 ± 1.1	492 ± 176	(691)	(19.2)
Lovaza	M	86 ± 11	1.5 ± 0.2	664 ± 146	774	11.4
AKR-963	F	35 ± 7	1.7 ± 0.5	119 ± 89	143	4.7
Lovaza	F	<b>39 ± 11*</b>	6.7 ± 3.6	258 ± 144	(464)	(29.5)
<b>EPA</b>						
AKR-963	M	85 ± 11	1.2 ± 0.2	482 ± 71	524	8.1
Lovaza	M	126 ± 13	1.3 ± 0.2	755 ± 117	809	7.8
AKR-963	F	<b>62 ± 10*</b>	1.8 ± 0.5	398 ± 50	322	5.4
Lovaza,	F	<b>47 ± 16*</b>	4.0 ± 1.3	344 ± 34	404	10.2
<b>4.0 g/kg</b>						
<b>DHA</b>						
AKR-963	M	128 ± 15	2.3 ± 0.3	1248 ± 178	(1792)	(19.6)
Lovaza	M	128 ± 23	2.3 ± 0.3	1204 ± 226	(1514)	(14.5)
AKR-963	F	<b>54 ± 14*</b>	7.2 ± 3.5	<b>435 ± 105*</b>	(1690)	(75.8)
Lovaza	F	<b>48 ± 5*</b>	<b>12.7 ± 3.6*</b>	<b>701 ± 92*</b>	(2390)	(66.6)
<b>EPA</b>						
AKR-963	M	247 ± 25	2.3 ± 0.3	2333 ± 234	2800	12.7
Lovaza	M	264 ± 43	2.3 ± 0.3	2566 ± 222	3016	12.0
AKR-963	F	<b>130 ± 25*</b>	8.3 ± 3.3	<b>1597 ± 189*</b>	(3757)	(42.5)
Lovaza	F	<b>106 ± 6*</b>	8.0 ± 3.3	<b>1592 ± 132*</b>	(2871)	(27.6)

<sup>1</sup> Based on mean net changes in plasma levels of DHA and EPA, values are ± S.E.; M - male; F - female.

\* Bolded values are significantly different from males, p < 0.05, One-Way ANOVA.

Values in parenthesis were determined based on an extrapolation of the AUC > 20% and are considered unreliable.

**Table 5: TK Parameters<sup>1</sup> for DHA and EPA in Male and Female Rats on Day 28**

Dose	Gender	C <sub>max</sub> (µg/mL)	T <sub>max</sub> (hr)	AUC <sub>0-24 hr</sub> (µg*hr/mL)	AUC <sub>0-∞</sub> (µg*hr/mL)	MRT (hr)
<b>0.2 g/kg</b>						
<b>DHA</b>						
AKR-963	M	102 ± 9	3.2 ± 1.0	<b>1185 ± 144**</b>	1464	11.9
Lovaza	M	86 ± 12	2.0 ± 0.4	921 ± 189	(1473)	(23.5)
AKR-963	F	<b>54 ± 9*</b>	3.0 ± 1.1	536 ± 167	697	10.5
Lovaza	F	43 ± 9	1.7 ± 0.2	135 ± 128	(179)	(4.5)
<b>EPA</b>						
AKR-963	M	127 ± 12	1.3 ± 0.2	<b>891 ± 79**</b>	1009	8.5
Lovaza	M	98 ± 12	1.3 ± 0.2	615 ± 98	673	8.4
AKR-963	F	65 ± 7	2.0 ± 0.4	576 ± 53	644	8.5
Lovaza,	F	59 ± 12	1.5 ± 0.2	408 ± 112	489	10.5
<b>4.0 g/kg</b>						
<b>DHA</b>						
AKR-963	M <sup>2</sup>	175 ± 15	2.2 ± 0.5	1992 ± 236	(2876)	(19.6)
Lovaza	M	160 ± 20	1.8 ± 0.2	1945 ± 504	2150	11.4
AKR-963	F	<b>116 ± 19*</b>	2.3 ± 0.3	<b>790 ± 174*</b>	922	10.9
Lovaza	F	<b>111 ± 18*</b>	2.3 ± 0.3	<b>963 ± 273*</b>	1199	13.7
<b>EPA</b>						
AKR-963	M <sup>2</sup>	371 ± 50	2.0 ± 0.0	2594 ± 231	2956	10.3
Lovaza	M	381 ± 64	1.8 ± 0.2	2636 ± 265	2878	8.8
AKR-963	F	231 ± 24	2.3 ± 0.3	2201 ± 184	2460	10.0
Lovaza	F	211 ± 40	1.8 ± 0.2	1636 ± 358	2202	11.2

<sup>1</sup> Based on mean net changes in plasma levels of DHA and EPA, values are ± S.E.; M - male; F - female;  
<sup>2</sup> n=5

Bolded values are: \* Significantly different from males;

\*\* Significantly different from Lovaza, p < 0.05, One-Way ANOVA.

Values in parentheses were determined based on an extrapolation of the AUC > 20% and are considered unreliable.

Overall, oral dosing of rats with the fish oil products AKR-963 and Lovaza resulted in plasma exposures of DHA and EPA that displayed similar gender specific differences and dose accumulation upon repeated dosing.

Sponsor's Tabulated summary findings are described below:

Table 2.6.7-7. Repeat-Dose Toxicity: Pivotal Studies									
Report Title: Comparative 28 Day Repeated Oral Dose Toxicity Study in Sprague Dawley Rats of Fish Oil Product (AKR-963) with Marketed Product (Lovaza®) as a Comparator Followed by a 14 Day Recovery Period					Test Article: AKR-963 and Lovaza				
Species/Strain: Rat/Sprague Dawley			Duration of Dosing: 28 days			Study: 241342			
Initial Age: 7 to 8 weeks			Duration of Postdose: 14 days						
Date of First Dose: June 15, 2011			Method of Administration: Oral, gavage						
			Vehicle/Formulation: None			GLP Compliance: Yes			
Special Features: Intergroup differences (low dose versus high dose and AKR-963 versus Lovaza) were evaluated statistically.									
No Observed Adverse Effect Level: > 4381 mg/kg/day (AKR-963) and > 4000 mg/kg/day (Lovaza)									
Repeat-Dose Toxicity: Study 241342, Rat, 28-Day									
Daily Dose (mg/kg/day)		200 (AKR-963) <sup>a</sup>		200 (Lovaza)		4000 (AKR-963) <sup>a</sup>		4000 (Lovaza)	
Sex		M	F	M	F	M	F	M	F
<sup>a</sup>		= The reported dose levels of 200 and 4000 mg/kg/day in the study report were based on a minimum specification of 777 mg omega-3-acid ethyl esters per gram of oil (777 mg/g); however, the measured content of the tested lot was 851 mg/g. Therefore, the actual dose levels of AKR-963 administered were 219 and 4381 mg/kg/day, respectively.							
<sup>b</sup>		= Data from day 28. Predose levels of endogenous DHA and EPA present in the plasma of individual rats on day 1 were subtracted from the plasma levels measured at the various blood collection times on day 1 and day 28 to obtain the net plasma levels of DHA and EPA. The toxicokinetic analysis was performed using the net plasma levels per time point per group of rats.							
<sup>c</sup>		= On day 14, one male toxicokinetic rat (032) dosed with AKR-963 at 4 g/kg was found dead during the morning check. This animal did not display any signs of ill health in the days prior to death. The only gross finding was well-demarcated discoloration (paleness) of the apex of the heart. Histological examination revealed moderate lung and liver congestion. Pale appearance of the apex of the heart and pulmonary congestion, without any other obvious signs of toxicity, was highly suggestive of an acute cardiac arrest. Although the cause of death of this rat was undetermined, this condition was not considered treatment-related, due to the lack of any other toxicity in this rat, and in the rest of the rats from the same group.							
<sup>d</sup>		= Data presented as number of animals with lesions at end of treatment which were not present pre-study. Findings were not considered treatment-related.							
<sup>e</sup>		= Values marginally above normal range. No statistical differences between groups.							
ALT		= Alanine aminotransferase.							
CK		= Creatinine kinase.							
DHA		= Docosahexaenoic acid.							
EPA		= Eicosapentaenoic acid.							
F		= Female.							
GLP		= Good Laboratory Practice.							
LDH		= Lactate dehydrogenase.							
M		= Male.							
NA		= Not applicable.							
SD		= Standard deviation.							
-		= No noteworthy findings.							

Table 2.6.7-7. Repeat-Dose Toxicity: Pivotal Studies								
Report Title: Comparative 28 Day Repeated Oral Dose Toxicity Study in Sprague Dawley Rats of Fish Oil Product (AKR-963) with Marketed Product (Lovaza <sup>®</sup> ) as a Comparator Followed by a 14 Day Recovery Period					Test Article: AKR-963 and Lovaza			
Species/Strain: Rat/Sprague Dawley			Duration of Dosing: 28 days			Study: 241342		
Initial Age: 7 to 8 weeks			Duration of Postdose: 14 days					
Date of First Dose: June 15, 2011			Method of Administration: Oral, gavage					
			Vehicle/Formulation: None			GLP Compliance: Yes		
Special Features: Intergroup differences (low dose versus high dose and AKR-963 versus Lovaza) were evaluated statistically.								
No Observed Adverse Effect Level: > 4381 mg/kg/day (AKR-963) and > 4000 mg/kg/day (Lovaza)								
Repeat-Dose Toxicity: Study 241342, Rat, 28-Day								
Daily Dose (mg/kg/day)	200 (AKR-963) <sup>d</sup>		200 (Lovaza)		4000 (AKR-963) <sup>d</sup>		4000 (Lovaza)	
Sex	M	F	M	F	M	F	M	F
Number of toxicokinetic animals	6	6	6	6	6	6	6	6
Toxicokinetics <sup>b</sup> : AUC <sub>0 to 24 hour</sub> (µg*h/mL) – DHA	1185	536	921	135	1992	790	1945	963
Toxicokinetics <sup>b</sup> : AUC <sub>0 to 24 hour</sub> (µg*h/mL) – EPA	891	576	615	408	2594	2201	2636	1636
Toxicokinetics <sup>b</sup> : C <sub>max</sub> (µg/mL) – DHA	102	54	86	43	175	116	160	111
Toxicokinetics <sup>b</sup> : C <sub>max</sub> (µg/mL) – EPA	127	65	98	59	371	231	381	211
Number of main study animals	10	10	10	10	10	10	10	10
Noteworthy findings:								
Died or sacrificed moribund	0	0	0	0	0 <sup>e</sup>	0	0	0
Body weight (%)	-	-	-	-	-	-	-	-
Food consumption (%)	-	-	-	-	-	-	-	-
Clinical observations	-	-	-	-	-	-	-	-
Ophthalmoscopy: <sup>d</sup>								
Crystalline corneal deposits	0	2	3	2	1	0	1	0
Cataracts	2	0	3	0	2	0	0	0
Hyper-reflective lesions	1	0	0	1	1	2	1	1
Hematology	-	-	-	-	-	-	-	-

Table 2.6.7-7. Repeat-Dose Toxicity: Pivotal Studies								
Report Title: Comparative 28 Day Repeated Oral Dose Toxicity Study in Sprague Dawley Rats of Fish Oil Product (AKR-963) with Marketed Product (Lovaza <sup>®</sup> ) as a Comparator Followed by a 14 Day Recovery Period					Test Article: AKR-963 and Lovaza			
Species/Strain: Rat/Sprague Dawley			Duration of Dosing: 28 days			Study: 241342		
Initial Age: 7 to 8 weeks			Duration of Postdose: 14 days					
Date of First Dose: June 15, 2011			Method of Administration: Oral, gavage					
			Vehicle/Formulation: None			GLP Compliance: Yes		
Special Features: Intergroup differences (low dose versus high dose and AKR-963 versus Lovaza) were evaluated statistically.								
No Observed Adverse Effect Level: > 4381 mg/kg/day (AKR-963) and > 4000 mg/kg/day (Lovaza)								
Repeat-Dose Toxicity: Study 241342, Rat, 28-Day								
Daily Dose (mg/kg/day)	200 (AKR-963) <sup>d</sup>		200 (Lovaza)		4000 (AKR-963) <sup>d</sup>		4000 (Lovaza)	
Sex	M	F	M	F	M	F	M	F
Serum chemistry (Values ± SD):								
ALT (u/L)	59 ± 7	45 ± 4	59 ± 7	46 ± 6	73 ± 13 <sup>e</sup>	52 ± 9	74 ± 18 <sup>e</sup>	49 ± 6
Normal range	26-71	22-66	26-71	22-66	26-71	22-66	26-71	22-66
LDH (u/L)	5358 ± 2026	5752 ± 1728 <sup>e</sup>	5974 ± 2861	4375 ± 2289 <sup>e</sup>	3910 ± 2075	3992 ± 1121	3533 ± 1408	2533 ± 1314
Normal range	1050-6401	1455-4306	1050-6401	1455-4306	1050-6401	1455-4306	1050-6401	1455-4306
CK (u/L)	499 ± 230	622 ± 201 <sup>e</sup>	521 ± 182	557 ± 194 <sup>e</sup>	431 ± 218	426 ± 139	364 ± 125	287 ± 92
Normal range	228-529	158-556	228-529	158-556	228-529	158-556	228-529	158-556
Urinalysis	-	-	-	-	-	-	-	-
Organ weights (%)	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-
Postdose evaluation: number evaluated	0	0	0	0	5	5	5	5

Sponsor's overall summary of this study is provided below:

This study examined the potential systemic toxicity and target organs for toxicity of fish oil product AKR-963 in comparison to Lovaza®, the marketed product, following a 28-day repeated oral dose administration in Sprague-Dawley rats. The animals were dosed at 0.2 or 4 g/kg with either AKR-963 or Lovaza®. The doses represent the total dose of omega-3-acid ethyl esters, docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid ethyl (EPA) ester.

Four groups were used in the study (2 Low dose, and 2 High dose, test or comparator). Each Main Study group consisted of 10 male and 10 female rats [strain: CrI:CD®(SD)BR-Sprague-Dawley; (b) (4)]. Five (5) additional rats per sex were assigned for a 14-day recovery period in the High dose groups (4 g/kg) only. An additional 6 rats per sex were allocated to all groups for toxicokinetics evaluation of EPA and DHA on Days 1 and 28. The dose volumes were based on the dose concentrations and a specific gravity of fish oil (0.928 g/mL at 15°C); the concentration of total omega-3-acid ethyl ester in AKR-963 was 0.777 g/g oil (based on the minimum specification) and in Lovaza® it was 0.90 g/g oil. Based on the concentration and specific gravity, the animals in the Low dose (0.2 g/kg) groups were dosed at 0.28 and 0.24 mL/kg, for AKR-963 and Lovaza®, respectively. For High dose (4 g/kg) groups, the dose volumes were 5.5 mL/kg and 4.8 mL/kg, respectively.

At the end of the treatment period, all Main Study animals were sacrificed and submitted for gross necropsy. The recovery animals were sacrificed at the end of the 14-day recovery period. Clinical reactions to treatment were monitored as well as body weight assessment, food consumption, clinical pathology, organ weights, and gross pathology. Histopathology evaluations were performed on a comprehensive range of organs from all High dose Main and Recovery Study animals. With the exception of one TK animal that died during the study and which was subjected to gross necropsy and histopathology, all other TK animals were euthanized at the end of the study without further evaluations.

One male TK rat dosed with AKR-963 at 4 g/kg was found dead on Day 14. Estimated time of death was sometime after 10-11 pm on the preceding day. The only gross finding was well demarcated discoloration (paleness) of the apex of the heart, and histologically there was moderate lung and liver congestion. Pale appearance of the heart and pulmonary congestion without any other obvious signs of toxicity was highly suggestive of an acute cardiac failure.

Although the cause of a sudden death was undetermined, this condition was not considered treatment-related due to the lack of any other toxicity in this rat, and in the remainder of the rats from the same group. Sudden deaths are known to occasionally occur in the laboratory rats, and it is usually associated with stress (single housing, bleeding, manipulations, gavaging, etc.), vagal stimulation and/or diabetes/hypertension. Daily cage side observations and detailed weekly physical examinations showed no treatment-related systemic toxicity in animals of both genders in all groups. Soft feces was observed in 2 out of 20 rats dosed with AKR-963 at 0.2 g/kg and in 6 out of 30 rats (gender combined) dosed at 4 g/kg. This observation was only sporadically noted and did not last for more than a day.

There were no treatment-related ophthalmological findings, and there was no apparent difference in body weights, body weight gains, and food consumption (gender matched) between groups. There were no clinical pathology (hematology, serum chemistry and urinalysis) parameter findings that were considered to be treatment-related, with the exception of marginally increased ALT in both groups of male rats dosed at 4 g/kg with either AKR-963 or

Lovaza®. ALT levels were  $73 \pm 13$  u/L and  $74 \pm 18$  u/L, for AKR-963 and Lovaza®-treated groups, respectively (normal ranges: 26-71 u/L). This may be the result of hepatocellular leakage due to higher lipid turnaround in the liver, although histopathological evaluation did not indicate hepatic lipidosis in either group dosed at 4 g/kg. At the end of the recovery period, ALT levels were normal in both groups of male rats. Therefore, since these increases were marginal, reversible, and without histopathological correlation, they were not considered clinically relevant. Gross necropsy and histopathology findings revealed no observations of toxicological significance.

**In summary in a 28-day comparative oral (gavage) toxicity study of AKR-963 with Lovaza in rats** (7-8-weeks old, n=10/sex/group), doses of 200, and 4000 mg/kg/day were used. The control group was not included in the study. The doses represent the total dose of omega-3-acid ethyl esters, i.e. mostly docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid ethyl (EPA) ester.

In general the toxicity of two drugs (AKR-963 and Lovaza) was similar. At a high dose, the AUC exposures of DHA and EPA were higher on day-28 vs day-1 with both drugs. AUC exposures of DHA in males/females were 1992/790 mcg.hr/ml (vs 1945/963 mcg.hr/ml with Lovaza). Same was generally true for EPA exposures on day 28 for males (2594 vs 2636 mcg.h/ml with Lovaza), but in females these were slightly higher with AKR-963 (2201 vs 1636 mcg.h/ml with Lovaza). At low doses, the exposures of DHA were different with AKR-963 vs lovaza in females (AUC values on days 1/28 were 119/536 vs 258/135 mcg.hr/ml with Lovaza)

In a TK group, 1/6 male rat dosed with AKR-963 at 4 g/kg was found dead on Day 14 (rat # 032), which was not noted with Lovaza. The cause of death is unknown but marked congestion in the liver and lungs was observed. Sponsor states that pale appearance of the heart and pulmonary congestion without any other obvious signs of toxicity was highly suggestive of an acute cardiac failure in this animal. Although the cause of a sudden death was undetermined, this condition was not considered treatment-related due to the lack of any other toxicity in this rat, and in the remainder of the rats from the same group. **Clinical signs** such as soft feces were noted in 2/20 and 6/30 rats at 0.2 and 4 g/kg/day of AKR-963 respectively; these clinical signs were not noted with Lovaza. No effects on body weights, weight changes or food consumption were noted with either drug. Similar ophthalmologic findings were noted with both drugs at a HD, including cataracts (in males only 2/10 and 2/10 rats at 0.2 and 4.0 g/kg/day respectively vs 3/100 and 0/10 with Lovaza), hyper-reflective lesions in the posterior segment (in both sexes combined in 1/20 and 3/20 rats with AKR-963 respectively vs 1/20 and 2/20 rats with Lovaza), and crystalline corneal deposits (in 2/20 and 1/20 rats respectively vs in 5/20 and 1/20 rats with Lovaza). Hematological findings (mostly with Lovaza) were noted during the drug free recovery period in male rats at a HD, these included significantly lower hematocrit (45 vs 43\* % with Lovaza), and significantly higher reticulocyte counts ( $178$  vs  $204^* 10^9$  /L with Lovaza).

**Clinical chemistry** showed no evidence of pharmacologic activity, as no effects on triglycerides or cholesterol were noted in this study in either treatment group compared to historical controls. The control ranges provided by the contract Research Organization or CRO are inadequate, based on the age of the animals and the data years of collection to supplant concurrent controls. This may explain the apparent lack of pharmacodynamic or PD effects. Other clinical chemistry findings included higher LDH levels in females with AKR-963 at both doses (5752 & 3992 vs 4375 & 2533  $\mu$ /L with lovaza at 200 and 4000 mg/kg/day respectively). At the end of the drug free recovery period, these LDH levels were above the historical control range with both drugs (5263 vs 6767 with lovaza), historical controls ranges were 1455-4306 u/L.

ALT levels were increased at a HD with both drugs (73 vs 74 u/L with lovaza, low dose values were 59 u/L with both drugs); these in the historical control group were 26-71 u/L, however these were reversible at the end of the drug free recovery period (50 u/L with both drugs). Also at the end of the recovery period at a HD increases in globulin levels in female rats were noted with AKR-963 (30 vs 28\* g/L with Lovaza), again these were not toxicologically relevant as the differences were small. No changes in organ weights were noted. **Gross pathologic findings** at a HD with AKR-963 were noted in the sternum (thickening in 1/10 male rats), uterus (fluid filled in 1/10 female rats at 0.2 and 4 g/kg of AKR-963), alopecia (in 1/5 female rats in a recovery group at 4 g/kg AKR-963), and in the right cerebrum (clear fluid-filled cavity, 3-4 mm size) in 1/5 recovery male at 4 g/kg AKR-963.

Sponsor states that no **histopathology** changes were observed in any tissue, however after the tabulated summary of histopathology data was provided by the sponsor, in general similar histopathology findings were noted with both drug products. Target organs of toxicity with both drugs were liver (inflammation in 3/20 rats vs 3/20 rats with Lovaza) and kidney (inflammation in 3/20 rats vs 3/20 with lovaza). However, findings in the urinary bladder were noted with AKR-963, not noted with Lovaza (in females subchronic and diffuse mucosal inflammation in 2/10 vs 0/10 with Lovaza). Similarly in eyes, focal retinal degeneration of mild severity was noted during treatment with both drugs (in 1/10 female rats with both AKR-963 and Lovaza respectively), but at the end of drug free recovery period it was only noted with AKR-963 (in 1/5 males vs 0/5 with Lovaza). Additionally skin findings (hypotrichosis, of moderate severity) were noted in 1/5 female rats at the end of drug free recovery period with AKR-963, but not with Lovaza.

Thus, in general findings with two drugs were similar (target organs of toxicity with both drugs were liver, kidney and eyes) and AUC exposures on day 28 at a HD were similar. However subtle findings were noted with AKR-963, not noted with Lovaza. These include AUC drug exposures of EPA, which were higher in females at a high dose of AKR-963 (i.e. 2201 vs 1636 mcg.hr/ml with Lovaza), and mortality in 1/16 male rats at a HD (10 main study rats+ 6 TK group rats, the cause of the death is not known), sponsor explains that this animal had marked congestion in liver and lungs and died of acute cardiac failure. Other differences noted with AKR-963 were findings in the urinary bladder in females (subchronic mucosal inflammation in 2/10 vs 0/10 with Lovaza), eyes (focal retinal degeneration during treatment in 1/10 and 1/10 females with AKR-963 and Lovaza respectively, but during the drug free recovery period with AKR-963 only in 1/5 males vs 0/5 with Lovaza), and skin (hypotrichosis, grade 3 severity) was noted in 1/5 females during the drug free recovery period with AKR-963, but not with Lovaza. Note that sponsor does not consider any of these histopathology findings significant.

Sponsor initially did not provide the laboratory data on historical control animals, but after requesting this information, data were provided and show that historical controls are from rats of 9-13 weeks age vs in the present toxicity study of 7-8 weeks of age. Also historical control data are from a large number of studies (i.e. 18 to 43 studies vs a single study here). These studies had higher numbers of animals per group (with n=140 to 385 vs n=10 in the current study). Thus in this pivotal toxicity study, historical / background data from years 2000-2007 submitted by the sponsor are not representative of a single study conducted here; and do not provide the complete assessment and differences between the drug groups and controls

The total omega-3-acid ethyl esters present in the current drug product are not only EPA and DHA, (b) (4)

(b) (4) Most of the above are present in higher amounts in AKR-963 than in Lovaza, and include (b) (4)

Thus several non-omega-3 fatty acid components in AKR-963 exceed the levels that are present in Lovaza ( (b) (4)

. Among these components such as (b) (4) are present in clinical lots at higher concentrations than those tested in the lot used for toxicity testing. However, sponsor has used literature NOAELs from repeat dose toxicity studies to establish safety of these 'related components' and has qualified these non-omega-3 fatty acid components in AKR-963.

Note that there are limitations in the above pivotal toxicity study for this 505(b)(2) application, such as 1) no concurrent controls were included in the study; 2) we can-not tell if there is efficacy in animals, as no changes in triglycerides or total cholesterol were noted with either drug, 3) we cannot set a NOAEL, since the sponsor only provided histopathology at a HD of 4 g/kg/day with AKR-963 and Lovaza (no histopathology was conducted on the lower dose groups), 4) the toxicity noted in the above study, does not appear to be the toxicity seen previously with the approved Lovaza (using 4X lower doses). May be the relative absence of established target organ toxicity reflects the short dosing duration of 4 weeks here, and we do not have a 4-week rat toxicity study with the original Lovaza NDA to compare these results to lovaza or AKR-963.

In summary, the target organs of toxicity in this study with both drugs appear to be liver, kidney, bladder, retina, and heart. Other than the bladder mucosal inflammation showing a higher incidence than Lovaza, and one male rat that died in the toxicokinetic group with acute cardiac failure with AKR-963, it appears that there is limited toxicity with either drug (lovaza or AKR-963) at 4000 mg/kg/day in the current study. AUC exposures with both drugs in general were also similar.

The NOAEL or tolerated doses of AKR-963 in a 4-week oral toxicity study in rats is 200 mg/kg/day (1200 mg/m<sup>2</sup>/day) which provides the safety margin of approximately 0.5X in humans at a recommended dose of 3600 mg/day (60 mg/kg /day or 2220 mg/m<sup>2</sup>/day, assuming 60 kg subject), based on body surface area. However, toxicity was noted at a HD in rats (mortality 1/6 TK animals, and histopathology findings in the liver & kidney in both sexes, and urinary bladder in female rats, as well in eyes and skin in rats). This HD of 4000 mg/kg or 24000 mg/kg/day, or 2220 mg/m<sup>2</sup>/day would provide the safety margin of approximately 10X in humans at a recommended dose of 3600 mg/day, based on body surface area.

Table 1. Safety margins in humans at NOAEL dose (of 200 mg/kg/day), and at a high dose of 4000 mg/kg (a toxic dose) in rats, both based on body surface area (mg/m<sup>2</sup>).

Species	NOAEL (m/k/d)	mg/m <sup>2</sup>	Safety margins in humans at 3600 mg/day dose
Rat	200	1200	0.5X
Rat	4000	24000	11X
Human dose			
3600 mg/day or 60 mg/kg/day (or 2220 mg/m <sup>2</sup> /day)		2220	

Conclusion: As noted earlier, the toxicity of two products in general is similar, with similar exposures (at a high dose on day 28, the AUC exposures of DHA in males/females were 1992/790 mcg.hr/ml (vs 1945/963 mcg.hr/ml with Lovaza, EPA exposures were 2594/2201 vs 2636/1636 mcg.h/ml with Lovaza), and although safety margin in rats to humans at the NOAEL dose of 200 mg/kg is low (< 1 fold, based on body surface area), at a high dose of 4000 mg/kg/day of AKR-963 (where toxicities were noted in the liver, kidney, bladder, retina, and heart), safety margins were 10-fold in rats to humans. Thus there is sufficient safety margin in this study in rats to humans.

## 7 Genetic Toxicology

Following gene-toxicity studies have been conducted with AKR-963

### 1. In vitro reverse mutation assay in bacterial cells (Ames assay)

#### Study title:

Study no.:	241656 ((TRGG-963-101)
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/14/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALMAC Lot No. A08552-001L04

Certificate of analysis indicates the lot number of the finished product is 0003550700, as shown below

Following certificate of analysis was provided on the Ames assay

(b) (4)

### Certificate of Analysis

NND11001

Product Name: AKR-963 Capsules  
 Description: (b) (4) omega-3 EPA and DHA ethyl ester capsules  
 Product Number: EE104  
 Lot Number Finished Product<sup>2)</sup>: 0003550700  
 Manufacturing Date Bulk Capsules: (b) (4)  
 Date of Packaging:

Tests	Specification <sup>5)</sup>	Results <sup>6)</sup>
EPA Ethyl Ester Identity	Retention time in conformance with external EPA Ethyl Ester reference standard	Complies <sup>3)</sup>
DHA Ethyl Ester Identity	Retention time in conformance with external DHA Ethyl Ester reference standard	Complies <sup>3)</sup>
Appearance	Transparent oblong soft gelatin capsules filled with clear, faint yellow liquid	Complies <sup>3)</sup>
Fill mass		(b) (4)
Uniformity of mass		(b) (4)
Disintegration time		(b) (4)
EPA Ethyl Ester		(b) (4)
DHA Ethyl Ester		(b) (4)
EPA +DHA Ethyl Ester		(b) (4)
Total Omega 3 Acid Ethyl Ester		(b) (4)
Tocopherol		(b) (4)
Total aerobic microbial count		(b) (4)
Total yeasts and moulds count		(b) (4)
E. coli	Absent /g	Not detected /g <sup>2)</sup>
Staphylococcus aureus	Absent /g	Not detected /g <sup>2)</sup>
Salmonella	Absent /10g	Not detected /10g <sup>2)</sup>

This GMP lot was manufactured in accordance with the general principles as outlined in ICH Q6B Specification: "Test procedures and acceptance criteria for biotechnical/biological products".

(b) (4)

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## Key Study Findings

AKR-963 was tested in duplicates using four strains of Salmonella typhimurium, TA98, TA1537, TA100, TA1535 and also the E. coli strain, WP2 uvrA, in the absence and in the presence of rat S9-mix. AKR-963 was not mutagenic in any of the above tested strains in the absence and in the presence of S9-mix. In conclusion, AKR-963 was not mutagenic in the Ames assay.

## Methods

Strains:	S. typhimurium strains, TA98, TA100, TA1535, TA1537, and the E. coli strain, WP2 uvrA,
Concentrations in definitive study:	0, 0.31, 0.63, 1.3, 2.5, and 5.0 mg per plate.
Basis of concentration selection:	No toxicity was observed at the highest dose
Negative control:	10% (v/v) Tween 80 in DMSO
Positive control:	<b>Without S9</b> sodium azide (NaAz), 2-nitrofluorene (2-NF), methyl methanesulfonate (MMS), 9-aminoacridine (9-AA). <b>With S9</b> Benzo[ $\alpha$ ]pyrene (B[ $\alpha$ ]P) 2- aminoanthracene (2-AMA), Cyclop-hosphamide monohydrate (CP).
Formulation/Vehicle:	10% (v/v) Tween 80 in DMSO

**Methods:** AKR-963 drug product was dissolved in 10% (v/v) Tween 80 in dimethyl sulfoxide (DMSO) for the experiments. The concentrations of AKR-963 investigated for both the plate incorporation and preincubation tests with or without metabolic activation were 0, 0.31, 0.63, 1.3, 2.5, and 5.0 mg per plate. Once plated, a white emulsion was observed only at the highest concentration of 5.0 mg per plate, both in the presence and absence of S9. No white emulsion, nor the small droplets of oil were visible at the lower concentrations. There was no indication of toxicity at any concentration as evidenced by a normal background lawn and colony counts similar to the negative control.

**Incubation and sampling times:** Both plate incorporation and pre-incubation methods were used. In the plate incorporation test, 0.1 mL of a test article, 0.5 mL of phosphate buffer (0.1 M), 0.5 mL of S9 mix and 0.1 mL of an overnight bacterial culture and 2 mL of appropriate molten top agar were combined. The contents were mixed and layered onto the appropriate bottom agar. In the pre-incubation test, these test components were preincubated at 37°C for 20 minutes prior to adding the top agar and plating. Negative control plates were similarly treated. Positive controls plates contained 0.1 mL of a mutagenic agents. Triplicate plates were used for each dose level. The plates were incubated at 37°C for 48 to 72 hrs and revertant colonies were counted.

**Study Validity:** The validity of the study was examined by use of several positive controls and a negative control (vehicle). The tester strain characteristics were checked for genotype (requirement of amino acid, ability of DNA repair and ampicillin resistance). The results were considered negative based on the results of statistics (Dunnetts test significance of  $p < 0.05$ ), and

the absence of a clear dose response, and the low magnitude of the response. The negative controls for each tester strain were within the historical negative control data. All concurrent positive controls induced at least 3-fold increase in colony counts per plate when compared to the corresponding negative controls

### Results:

For the plate incorporation test, with or without metabolic activation, AKR-963 did not produce any statistically significant increases in revertants over the concurrent negative controls. The pre-incubation test confirmed the negative results. The negative controls for each tester strain were within the historical negative control (DMSO) data. All concurrent positive controls induced significant increases ( $p < 0.001$ ) of at least 3-fold in colony counts per plate when compared to the corresponding negative controls.

Thus, it was concluded that AKR-963 was not mutagenic to *S. typhimurium* strains and *E. coli* strain (WP2 *uvrA*) in the plate incorporation test, with or without metabolic activation. The preincubation test confirmed the above negative results.

Summary of these findings are also described below

Table. Assay 1-Plate incorporation method (without and with metabolic activation

Table 2.6.7-8 (A). Genotoxicity: In Vitro - Report Number 241656							
Report Title: Bacterial Reverse Mutation Assay of Fish Oil Product (AKR-963)						Test Article: AKR-963	
Test for Induction of: Mutations			No. of Independent Assays: 2		Study No.: 241656		
Strains: <i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, <i>Escherichia coli</i> WP2 <i>uvrA</i>			No. of Replicate Cultures: 3		GLP Compliance: Yes		
Vehicles for Test Article: 10%(v/v) Tween 80 in DMSO				Vehicles for Positive Controls: Water or DMSO			
Treatment: Plate incorporation and pre-incubation assays			Metabolizing System: Aroclor 1254-induced rat liver S9		Date of Treatment: July 26 and 27, 2011		
Cytotoxic Effects: None							
Genotoxic Effects: None							
Metabolic Activation	Test Article	Concentration (mg/plate)	TA98 (Mean ± SD)	TA100 (Mean ± SD)	TA1535 (Mean ± SD)	TA1537 (Mean ± SD)	WP2 <i>uvrA</i> (Mean ± SD)
Assay 1: Plate Incorporation Method							
Without activation	10% (v/v) Tween 80 in DMSO	0	25 ± 4	135 ± 23	17 ± 1	9 ± 2	17 ± 8
	AKR-963 <sup>a</sup>	0.34	17 ± 5	123 ± 3	18 ± 3	10 ± 1	22 ± 4
		0.69	23 ± 3	112 ± 13	20 ± 3	10 ± 2	22 ± 7
		1.4	19 ± 2	123 ± 10	20 ± 6	8 ± 2	17 ± 7
		2.7	21 ± 4	131 ± 7	16 ± 1	12 ± 4	19 ± 3
		5.5 <sup>b</sup>	19 ± 6	130 ± 13	19 ± 2	13 ± 3	21 ± 2
	Positive control <sup>f</sup>	-	3161 ± 232***	1971 ± 20***	1593 ± 240***	1515 ± 275***	416 ± 7***
With activation	10% (v/v) Tween 80 in DMSO	0	30 ± 4	128 ± 20	16 ± 2	15 ± 2	35 ± 4
	AKR-963 <sup>a</sup>	0.34	28 ± 1	124 ± 10	18 ± 3	14 ± 4	36 ± 4
		0.69	31 ± 2	123 ± 15	10 ± 3	16 ± 4	42 ± 6
		1.4	36 ± 3	122 ± 6	14 ± 2	15 ± 4	40 ± 12
		2.7	35 ± 7	126 ± 3	19 ± 4	20 ± 4	46 ± 4
		5.5 <sup>b</sup>	31 ± 4	138 ± 7	13 ± 2	11 ± 2	47 ± 3
	Positive control <sup>d</sup>	-	368 ± 35***	1000 ± 85***	179 ± 14***	223 ± 19***	149 ± 13***

Table. Assay 2, pre-incubation method-**without** metabolic activation

Table 2.6.7-8 (A). Genotoxicity: In Vitro - Report Number 241656							
Report Title: Bacterial Reverse Mutation Assay of Fish Oil Product (AKR-963)						Test Article: AKR-963	
Test for Induction of: Mutations			No. of Independent Assays: 2			Study No.: 241656	
Strains: <i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, <i>Escherichia coli</i> WP2 <i>uvrA</i>			No. of Replicate Cultures: 3			GLP Compliance: Yes	
Vehicles for Test Article: 10%(v/v) Tween 80 in DMSO				Vehicles for Positive Controls: Water or DMSO			
Treatment: Plate incorporation and pre-incubation assays			Metabolizing System: Aroclor 1254-induced rat liver S9			Date of Treatment: July 26 and 27, 2011	
Cytotoxic Effects: None							
Genotoxic Effects: None							
Metabolic Activation	Test Article	Concentration (mg/plate)	TA98 (Mean ± SD)	TA100 (Mean ± SD)	TA1535 (Mean ± SD)	TA1537 (Mean ± SD)	WP2 <i>uvrA</i> (Mean ± SD)
Assay 2: Pre-Incubation Method							
Without activation	10% (v/v) Tween 80 in DMSO	0	24 ± 1	124 ± 12	17 ± 4	11 ± 2	23 ± 6
	AKR-963 <sup>a</sup>	0.34	19 ± 5	110 ± 4	21 ± 2	12 ± 2	26 ± 3
		0.69	21 ± 2	123 ± 5	20 ± 1	13 ± 4	24 ± 2
		1.4	21 ± 9	121 ± 14	19 ± 1	14 ± 1	19 ± 5
		2.7	21 ± 2	148 ± 9	18 ± 6	12 ± 2	24 ± 8
		5.5 <sup>b</sup>	20 ± 4	138 ± 15	12 ± 2	12 ± 3	28 ± 4
	Positive control <sup>c</sup>	-	2962 ± 401***	1916 ± 31***	1678 ± 28***	781 ± 72***	980 ± 16***

Table. Assay 2, pre-incubation method-**with** metabolic activation

Table 2.6.7-8 (A). Genotoxicity: In Vitro - Report Number 241656							
Report Title: Bacterial Reverse Mutation Assay of Fish Oil Product (AKR-963)						Test Article: AKR-963	
Test for Induction of: Mutations			No. of Independent Assays: 2			Study No.: 241656	
Strains: <i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, <i>Escherichia coli</i> WP2 <i>uvrA</i>			No. of Replicate Cultures: 3			GLP Compliance: Yes	
Vehicles for Test Article: 10%(v/v) Tween 80 in DMSO				Vehicles for Positive Controls: Water or DMSO			
Treatment: Plate incorporation and pre-incubation assays			Metabolizing System: Aroclor 1254-induced rat liver S9			Date of Treatment: July 26 and 27, 2011	
Cytotoxic Effects: None							
Genotoxic Effects: None							
Metabolic Activation	Test Article	Concentration (mg/plate)	TA98 (Mean ± SD)	TA100 (Mean ± SD)	TA1535 (Mean ± SD)	TA1537 (Mean ± SD)	WP2 <i>uvrA</i> (Mean ± SD)
Assay 2: Pre-Incubation Method							
With activation	10% (v/v) Tween 80 in DMSO	0	36 ± 10	122 ± 4	15 ± 3	16 ± 5	35 ± 5
	AKR-963 <sup>a</sup>	0.34	37 ± 7	116 ± 6	15 ± 2	16 ± 3	38 ± 1
		0.69	34 ± 8	116 ± 12	15 ± 6	17 ± 3	34 ± 5
		1.4	42 ± 4	120 ± 12	15 ± 1	19 ± 2	29 ± 4
		2.7	34 ± 4	121 ± 8	13 ± 2	14 ± 5	33 ± 5
		5.5 <sup>b</sup>	32 ± 5	130 ± 9	11 ± 4	12 ± 4	40 ± 6
	Positive control <sup>d</sup>	-	444 ± 58***	920 ± 46***	436 ± 33***	180 ± 22***	112 ± 12***

<sup>a</sup> = The intended concentrations were based on a minimum specification of 777 mg omega-3-acid ethyl esters per gram of oil (777 mg/g); however, the measured content of the tested lot was 851 mg/g and, therefore, corrected concentrations were 0.34, 0.69, 1.4, 2.7, and 5.5 mg/plate.  
<sup>b</sup> = Once plated, a white emulsion was observed only at the highest concentration of 5.0 mg per plate, both in the presence and absence of S9. With the aid of a microscope, this emulsion was observed as small droplets of oil.  
<sup>c</sup> = Positive controls: 2-nitrofluorene at 5 µg/plate (TA98); sodium azide at 5 µg/plate (TA100 & TA1535); 9-aminoacridine at 100 µg/plate (TA1537); and methyl methanesulfonate at 1 µL/plate (WP2*uvrA*).  
<sup>d</sup> = Positive controls: benzo[*a*]pyrene at 5 µg/plate (TA98, TA100, TA1537); cyclophosphamide monohydrate at 100 µg/plate (TA1535); and 2-aminoanthracene at 100 µg/plate (WP2*uvrA*).  
DMSO = Dimethylsulfoxide.  
GLP = Good Laboratory Practices.  
SD = Standard deviation.  
\*\*\* = *p* < 0.001 Dunnett's test.

**Summary**

In summary, it was concluded that AKR-963 was not mutagenic to S.typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli strain, WP2 uvrA, under the test conditions.

**2 In vitro chromosome aberration test of AKR-963 in cultured human lymphocytes, study # 241657 (TRGG-963-102)**

**Study title:**

Study no.:	<b>241657 (TRGG-963-102)</b>
Study report location:	Electronic submission
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	6/14/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALMAC Lot No. A08552-001L04

Certificate of analysis indicates the lot number of the finished product is 0003550700, as shown below:

(b) (4)

### Certificate of Analysis

NND11001

Product Name: AKR-963 Capsules  
 Description: (b) (4) omega-3 EPA and DHA ethyl ester capsules  
 Product Number: EE104  
 Lot Number Finished Product<sup>2)</sup>: 0003550700  
 Manufacturing Date Bulk Capsules: (b) (4)  
 Date of Packaging: (b) (4)

Tests	Specification <sup>5)</sup>	Results <sup>6)</sup>
EPA Ethyl Ester Identity	Retention time in conformance with external EPA Ethyl Ester reference standard	Complies <sup>1)</sup>
DHA Ethyl Ester Identity	Retention time in conformance with external DHA Ethyl Ester reference standard	Complies <sup>1)</sup>
Appearance	Transparent oblong soft gelatin capsules filled with clear, faint yellow liquid	Complies <sup>3)</sup>
Fill mass	(b) (4)	
Uniformity of mass		
Disintegration time		
EPA Ethyl Ester		
DHA Ethyl Ester		
EPA +DHA Ethyl Ester		
Total Omega 3 Acid Ethyl Ester		
(b) (4)		
Tocopherol		
Total aerobic microbial count		
Total yeasts and moulds count		
E. coli	Absent /g	Not detected /g <sup>2)</sup>
Staphylococcus aureus	Absent /g	Not detected /g <sup>2)</sup>
Salmonella	Absent /10g	Not detected /10g <sup>2)</sup>
(b) (4)		
(b) (4)		

This GMP lot was manufactured in accordance with the general principles as outlined in ICH Q6B Specification: "Test procedures and acceptance criteria for biotechnical/biological products".

(b) (4)

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**Key Study Findings:** AKR-963 was not clastogenic in cultured human lymphocytes under the conditions of the test.

## Methods

- Cell line: Primary human lymphocyte
- Concentrations in definitive study: Following doses of AKR-963 were used in the experiment:
- For 3 hours treatment: in the absence of S9 mix doses were: 0, 25, 50, 100 and 200 mcg/mL.
- For 20 hours treatment in the absence of S9 mix, the doses were: 0, 20, 40, 80, and 160 mcg/mL.
- For 3 hours in the presence of S9 mix, the used doses were: 0, 8.2, 21, 51, 130, 320, 800, 2000 and 5000 mcg/mL
- Basis of concentration selection: No toxicity was observed at the highest concentration.
- Negative control: DMSO or DMSO/Tween 80 were used to dissolve the test article and were used as the negative control.
- Positive control: Mitomycin C (MMC, at 0.1 and 0.5 mcg/ml) for cells without S9, and cyclophosphamide (CP-529414, at 10 and 20 µg/mL) with S9.
- Formulation/Vehicle: DMSO with S9, or in 10% (v/v) Tween 80 in DMSO without S9
- Incubation & sampling time: The AKR-963 was used in the experiment with 3 hours treatment in the absence of S9 mix (0, 25, 50, 100 and 200 mcg/mL). Cells were incubated for 20 hours in the absence of S9 Mix (at 0, 20, 40, 80 and 160 mcg/mL). For experiments in the presence of S9, 3 hr incubation was carried out (at 0, 8.2, 21, 51, 130, 320, 800, 2000 and 5000 mcg/mL).

**Methods**

Cultures of human lymphocytes were exposed to varying concentrations of AKR-963 under three different conditions: 1) a 3-hour exposure in the absence of S9 metabolic activation; 2) a 3-hour exposure in the presence of S9, and 3) a 20-hour exposure in absence of S9 activation. Cells were harvested at approximately 20 hours, or approximately 1.5 x the normal cell cycle length. None of the concentrations tested generated precipitates in the treatment medium, and the pH and osmolality of all treated cultures were well within the normal physiological ranges. The health status of the cultures was observed under a microscope. The cultures were free of Mycoplasma by direct culture test as indicated on the Certificates of Analysis supplied by vendor.

**Study Validity:** The dose design was appropriate, using concentrations that covered a range of no toxicity to severe toxicity in accordance with the current OECD guideline (OECD, 1997). Treatment with AKR-963 did not change the pH and osmolality of the treatment system. The amount of DMSO in the test system increased the osmolality as expected and was not a factor

that interfered with the testing as the osmolality levels were all within the physiological ranges of the cultures. The negative and positive controls presented levels of chromosome aberrations similar to the historical data described for human lymphocytes from this Laboratory. Most of the aberrations observed were chromatid breaks (tb). Moreover, there was no dose-related increase in the percent of cells with structural aberrations for all three conditions of treatment indicating that AKR-963 was not clastogenic, or an inducer of structural chromosome aberrations. No increase in the number of polyploidy or endo-reduplicated cells was noted for any of the treatments.

**Results:**

Three hours exposure without S9 to 25, 50, 100, and 200 µg/mL AKR-963, yielded relative cell growth (RCG) of 104, 84, 79, and 35% and relative mitotic indices (RMI) of 89, 109, 91, and 16%, respectively, Tables 1 and 2. Because of the increase in the length of treatment, cells exposed to 20 hours without S9, were exposed to a slightly lower concentration range of 20, 40, 80 and 160 mcg/mL AKR-963. This treatment resulted in RCG of 92, 94, 64, and 23%, respectively. The RMI were 89, 74, and 45%, while the highest concentration was not scored due to extreme toxicity.

Cell cultures were exposed to AKR-963 for 3 hours with S9 at doses of 8.2, 21, 51, 130, 320, 800, 2000, and 5000 mcg/mL. These treatments resulted in RCG of 109, 97, 89, 54, 54, 55, 53, and 6%, respectively. The RMI were 95, 74, 60, and 19%, while the four highest concentrations (320, 800, 2000, and 5000 mcg/mL) were not scored due to an extreme reduction in RMI (see Tables 1 and 2).

Under these test conditions, no structural or numerical chromosome aberrations were observed in the AKR-963-treated cultures beyond those seen in concurrent solvent controls, see Tables below.

Table 1. Relative cell growth after 3 and 20 hour treatment( without S-9 metabolic activation), and 3 hours after treatment (with S-9 metabolic activation, middle panel)

Table 1. Relative Cell Growth

AKR-963 (µg/ml)	-S9, 3 hr		AKR-963 (µg/ml)	+S9, 3 hr		AKR-963 (µg/ml)	-S9, 20 hr	
	Cells (x 10 <sup>5</sup> )	RCG %		Cells (x 10 <sup>5</sup> )	RCG %		Cells (x 10 <sup>5</sup> )	RCG %
0	9.20 9.70	100	0	11.60 12.00	100	0	11.80 11.20	100
25	10.00 9.60	104	8.2	13.10 12.70	109	20	10.00 11.20	92
50	8.90 6.90	84	21	12.90 9.90	97	40	10.70 11.00	94
100	6.30 8.60	79	51	10.00 10.90	89	80	7.80 6.90	64
200	3.90 2.70	35	130	5.90 6.90	54	160	2.80 2.60	23
-	- -	-	320	7.00 5.70	54	-	- -	-
-	- -	-	800	5.80 7.10	55	-	- -	-
-	- -	-	2000	6.40 6.20	53	-	- -	-
-	- -	-	5000	0.80 0.50	6	-	- -	-

RCG = Relative Cell Growth

-- Concentration not tested

Table 2. Relative mitotic index after (3 and 20 hour treatment without S-9 metabolic activation), and 3 hours after treatment (with S-9 metabolic activation, middle panel)

Table 2. Relative Mitotic Index

AKR-963 (µg/ml)	-S9, 3 hr			AKR-963 (µg/ml)	+S9, 3 hr			AKR-963 (µg/ml)	-S9, 20 hr		
	M/500 cells	MI	RMI		M/500 cells	MI	RMI		M/500 cells	MI	RMI
	M	%	%		M	%	%		M	%	%
0	25 20	4.5	100	0	28 34	6.2	100	0	35 27	6.2	100
25	21 19	4.0	89	8.2	32 27	5.9	95	20	27 28	5.5	89
50	26 23	4.9	109	21	25 21	4.6	74	40	21 25	4.6	74
100	25 16	4.1	91	51	16 21	3.7	60	80	13 15	2.8	45
200	5 2	0.7	16	130	5 7	1.2	19	160	NC NC	NC	NC

M = Metaphases  
 MI = Mitotic Index  
 RMI = Relative Mitotic Index  
 "NC" not counted due to high toxicity

Table 3.1. Chromosome aberrations without S-9 (after 3 hour treatment)

Table 3-1. Summary of Chromosome Aberrations, - S9, 3 hour exposure

AKR-963 (µg/mL)	Cells	Numerical Aberrations		Structural Aberrations											Other (sd)	Aberrations /100 Cells	Aberrant Cells (%)	
		e*	pp*	Chromatid Type						Chromosome Type								
				Simple			Complex			Simple		Complex						
				tg*	Tb	isb	tr	qr	cr	id	ci	sg*	sb	d				r
0	100			2	2												2	2
	100																0	0
	%			1	1												1	1
25	100			1	2												2	2
	100			2	1												1	1
	%			1.5	1.5												1.5	1.5
50	100			2	1								1				2	2
	100			2													0	0
	%			2	0.5												1	1
100	100				1												1	1
	101	1		3													0	0
	%	0.5		1.5	0.5												0.5	0.5
MMC 0.5	50			2	3	1	1			1	1	1		1			16	14
	50	1		2	4		1			2		2					18	16
	%	1		4	7	1	2			3	1	3		1			17	15

\* aberrations not included in calculations of Aberrations/100 Cells and Aberrant Cells (%)

Table 3.2. Chromosome aberrations with S-9 (after 3 hour treatment)

Table 3-2. Summary of Chromosome Aberrations, + S9, 3 hour exposure

AKR-963 (µg/mL)	Cells	Numerical Aberrations		Structural Aberrations													Other	Abberations /100 Cells	Aberrant Cells (%)			
		e*	pp*	Chromatid Type						Chromosome Type												
				Simple			Complex			Simple			Complex									
				tg*	tb	izb	tr	qr	cr	id	ci	zg*	zb	d	r	dm						
0	100			1	1								1	2							3	3
	100			2	4								1								4	4
	%			1.5	2.5								1	1							3.5	3.5
8.2	100			3	2	1							1	1							4	4
	100			2	2								1								2	2
	%			1.5	2	0.5							1	0.5							3	3
21	100	2		3	2								1	1							4	4
	101	1		2	4																4	4
	%	1.5		2.5	3								0.5	0.5	0.5						4	4
51	100	1		2	3								1	1							5	5
	100	1		4	2	1								1							4	4
	%	1		3	2.5	0.5							0.5	1							4.5	4.5
CP 10	50			5	4	1	3	1					1	3							26	20
	50			2	9	1	1						1	2							28	22
	%			7	13	2	4	1					2	5							27	21

\* Aberrations were not included in calculations of Aberrations/100 Cells and Aberrant Cells (%)

Table 3.3. Chromosome aberrations without S-9 (after 20 hour treatment)

Table 3-3. Summary of Chromosome Aberrations, - S9, 20 hour exposure

AKR-963 (µg/mL)	Cells	Numerical Aberrations		Structural Aberrations													Other (sd)	Abberations /100 Cells	Aberrant Cells (%)			
		e*	pp*	Chromatid Type						Chromosome Type												
				Simple			Complex			Simple			Complex									
				tg*	tb	izb	tr	qr	cr	id	ci	zg*	zb	d	r	dm						
0	101	1		3	2																2	2
	100			2	1								1								2	2
	%	0.5		2.5	1.5								0.5								2	2
20	100			1	1								1								2	2
	100			1	1																1	1
	%			1	1								0.5								1.5	1.5
40	100			4	3																3	3
	100	1		3	2									1							3	3
	%	0.5		3.5	2.5									0.5							3	3
80	100			2	1								1	1							2	2
	100			3	1									1							2	2
	%			2.5	1								0.5	0.5	0.5						2	2
MMC 0.1	50	1		5			1						1	2	1						20	18
	50			4	5		1						1	1							16	14
	%	1		4	10		2						2	3	1						18	16

\* Aberrations were not included in calculations of Aberrations/100 Cells and Aberrant Cells (%)

A low level of chromosome aberrations were observed at all concentrations, including solvent control, as tabulated below.

AKR-963 µg/mL	% Cells Aberrant 3 hrs -S9	AKR-963 µg/mL	% Cells Aberrant 3 hrs +S9	AKR-963 µg/mL	% Cells Aberrant 20 hrs -S9
0	1	0	3.5	0	2
25	1.5	8.2	3	20	1.5
50	1	21	4	40	3
100	0.5	51	4.5	80	2
Positive	15	Positive	21	Positive	16

The assay details and summary of these findings is also provided below:

Table 2.6.7-8 (B). Genotoxicity: In Vitro - Report Number 241657							
Report Title: Chromosome Aberration Test of Fish Oil Product (AKR-963) in Cultured Human Lymphocytes					Test Article: AKR-963		
Test for Induction of: Chromosome aberrations		No. of Independent Assays: 3		Study No.: 241657			
Strains: Human peripheral blood lymphocytes		No. of Replicate Cultures: 2		GLP Compliance: Yes			
Metabolizing System: Rat liver S9		No. of Cells Analyzed/Culture: 100					
Vehicles for Test Article: DMSO for experiment with S9; 10% (v/v) Tween 80 in DMSO for experiments without S9		Vehicles for Positive Controls: Water					
Treatment: Continuous treatment for 20 hours without S9; treatment for 3 hours with and without S9. All cultures harvested at 20 hours.					Date of Treatment: June 22, 2011		
Cytotoxic Effects: 3 hours -S9: RCG was 35% and RMI was 16% at 200 µg/mL; 3 hours +S9: RCG was ≤55% at concentrations ≥130 µg/mL and RMI was 19% at 130 µg/mL; 20 hours -S9: RCG was 23% and RMI was not counted due to high toxicity at 160 µg/mL.							
Genotoxic Effects: None							
Metabolic Activation	Treatment Time/Harvest Time (hours)	Test Article	Concentration (µg/mL)	Cytotoxicity (RCG %)	Cytotoxicity (RMI %)	Aberrations/100 Cells (%)	Aberrant Cells (Mean %)
Assay 1							
Without activation	3/20	10% (v/v) Tween 80 in DMSO	0	100	100	1	1
		AKR-963	25	104	89	1.5	1.5
			50	84	109	1	1
			100	79	91	0.5	0.5
		MMC	0.5	Not tested	Not tested	17	15****
Assay 2							
With activation	3/20	DMSO	0	100	100	3.5	3.5
		AKR-963	8.2	109	95	3	3
			21	97	74	4	4
			51	89	60	4.5	4.5
			10	Not tested	Not tested	27	21****
		CP	10	Not tested	Not tested	27	21****

Table 2.6.7-8 (B). Genotoxicity: In Vitro - Report Number 241657							
Report Title: Chromosome Aberration Test of Fish Oil Product (AKR-963) in Cultured Human Lymphocytes						Test Article: AKR-963	
Test for Induction of: Chromosome aberrations			No. of Independent Assays: 3		Study No.: 241657		
Strains: Human peripheral blood lymphocytes			No. of Replicate Cultures: 2		GLP Compliance: Yes		
Metabolizing System: Rat liver S9			No. of Cells Analyzed/Culture: 100				
Vehicles for Test Article: DMSO for experiment with S9; 10% (v/v) Tween 80 in DMSO for experiments without S9			Vehicles for Positive Controls: Water				
Treatment: Continuous treatment for 20 hours without S9; treatment for 3 hours with and without S9. All cultures harvested at 20 hours.						Date of Treatment: June 22, 2011	
Cytotoxic Effects: 3 hours -S9: RCG was 35% and RMI was 16% at 200 µg/mL; 3 hours +S9: RCG was ≤55% at concentrations ≥130 µg/mL and RMI was 19% at 130 µg/mL; 20 hours -S9: RCG was 23% and RMI was not counted due to high toxicity at 160 µg/mL.							
Genotoxic Effects: None							
Metabolic Activation	Treatment Time/Harvest Time (hours)	Test Article	Concentration (µg/mL)	Cytotoxicity (RCG %)	Cytotoxicity (RMI %)	Aberrations/100 Cells (%)	Aberrant Cells (Mean %)
Assay 3							
Without activation	20/20	10% (v/v) Tween 80 in DMSO	0	100	100	2	2
		AKR-963	20	92	89	1.5	1.5
			40	94	74	3	3
			80	64	45	2	2
		MMC	0.1	Not tested	Not tested	18	16***

CP = Cyclophosphamide.  
 DMSO = Dimethylsulfoxide.  
 GLP = Good Laboratory Practices.  
 MMC = Mitomycin C.  
 RCG = Relative cell growth.  
 RMI = Relative mitotic index.  
 \*\*\* =  $p < 0.001$  Chi-square test.

## Conclusions

It was concluded that exposure to AKR-963 did not induce chromosome aberrations in cultured human lymphocytes under the conditions of the test.

## Sponsor's overall conclusion are stated below (section 2.4.6)

Trygg is relying on FDA's finding of safety for the RLD, Lovaza. In support of the scientific validity of this extrapolation, a 28-day repeat administration study comparing AKR-963 and Lovaza was conducted. In this study, no toxicity was observed with either oil, and the toxicokinetics of AKR-963 and Lovaza were found to be similar. The NOAEL from the GLP 28-day repeat-dose toxicity study with AKR-963 in rats was 4000 mg/kg/day (based on nominal concentration of DHAee and EPAee in the oil). When compared with the proposed clinical dose of 60 mg/kg/day AKR-963 capsules (3.6 g/day in 4 capsules) on the basis of body surface area, the margin of safety was 10.7. Additionally, the safety of DHAee- and EPAee-rich oils is supported by reports from the published literature for GLP and non-GLP nonclinical studies. The difference in fatty acid profile for AKR-963 and Lovaza was further qualified in 2 genotoxicity studies in which AKR-963 was without genotoxic effect. The differences in fatty acid profile between AKR-963 and Lovaza are not expected to have any impact on the safety of the product, based on qualification in the 28-day repeat administration study in rats comparing AKR-963 and Lovaza, and genotoxicity studies with AKR-963, the exposure estimated for the clinical dose, and adequate safety profile for individual fatty acids, based on calculated safety margins or comparison with levels in GRAS oils and dietary sources.

## 11 Integrated Summary and Safety Evaluation

Trygg is seeking an NDA approval of an omega-3-acid ethyl esters drug product, i.e. AKR-963 capsules under 505(b)(2) application. They are relying primarily on FDA's finding of safety and efficacy for Lovaza (NDA 21-654) and the published literature on fish oils to describe the toxicology of AKR-963. The drug product (AKR-963) is a mixture of omega-3 fatty acids isolated from fish oil, approximately 80% are esters of two unsaturated FA, eicosapentaenoic acid (50%) and docosahexaenoic acid (30%). EPA and DHA are long chained polyunsaturated fatty acids. The chemical names of these fatty acids identify the number of carbon atoms, the number of double bonds, and the position of the first double bond. For example, eicosapentaenoic acid ethyl ester (EPAee) has (b) (4)

Each capsule of AKR-963 has EPA ethyl ester-465 mg, DHA ethyl ester-375 mg & (b) (4) 4 IU as (b) (4)

Omega-3 ethyl esters have been shown to decrease triglycerides (TG) levels in patients with TG levels > 500 mg/dL. The mechanism of action of omega-3 ethyl esters is not completely understood. Potential mechanisms of action include inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase, increased mitochondrial and peroxisomal  $\beta$ -oxidation in the liver, decreased lipogenesis in the liver, and increased plasma lipoprotein lipase activity. Omega-3 ethyl esters may reduce the synthesis of TG in the liver because EPA and DHA are poor substrates for the enzymes responsible for TG synthesis, and EPA and DHA inhibit esterification of other fatty acids.

The drug is indicated for the reduction of elevated TG levels in adult patients with hyperlipidemia (>500 mg/dL). AKR-963 (0.9 gram dose) is supplied as a transparent soft-gelatin filled capsule, it consists of a concentrated mixture of omega-3 fatty acid ethyl esters, where EPAee and DHAee are the main ingredients. The daily dose of AKR-963 is 4 capsules per day taken as a single dose, or as two-capsules given twice daily in adults.

Since most pharmacology and toxicology studies have been conducted with the reference drug lovaza, only a 28-day bridging toxicity / toxicokinetics study, and two geno-toxicity studies have been conducted with the current drug AKR-963

### Toxicity Studies:

**In a 28-day comparative oral (gavage) toxicity study of AKR-963 with Lovaza in rats (7-8-weeks old, n=10/sex/group), doses of 200, and 4000 mg/kg/day were used.** The control group was not included in the study. The doses represent the total dose of omega-3-acid ethyl esters, i.e. mostly docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid ethyl (EPA) ester. The AUC exposures of DHA and EPA were generally comparable with both drug products, and higher on day 28 than day 1 (with both AKR-963 and Lovaza). At a HD, AUC exposures of DHA on day 28 in males were 1992 mcg.h/ml; females 790 mcg.h/ml (vs 1945/963 mcg.hr/ml in males/females with Lovaza). EPA exposures on day 28 in males were 2594; females 2201 mcg.h/ml (vs 2636/1636 mcg.h/ml in males/females respectively with Lovaza); note that females had slightly higher EPA exposures with AKR-963 (AUC 2201 vs 1636 mcg.h/ml with Lovaza). Additionally at low doses, the exposures of DHA were 4-fold higher on day 28 with AKR-963 in females (days 1/28 exposures were 119/536 with AKR-963 vs 258/135 mcg.hr/ml with lovaza)

In a TK group (6M+6F/group), one male rat dosed with AKR-963 at 4000 mg/kg/day was found dead on Day 14 (animal # 032), which was not noted with Lovaza. The cause of death is unknown but marked congestion in the liver and lungs was observed. Sponsor states that pale appearance of the heart and pulmonary congestion without any other obvious signs of toxicity was highly suggestive of an acute cardiac failure in this animal. Although the cause of a sudden death was undetermined, this condition was not considered treatment-related due to the lack of any other toxicity in this rat, and in the remainder of the rats from the same group. . Additionally, sponsor outlined literature that shows a single death in a 28-day rat study, as well as shortened ventricular action potential in pigs, along with in vitro hERG channel effects (Verkerk, 2006, Guizy, 2005), suggesting an occasional pro-arrhythmic effects of fish oil, despite a weight of evidence of anti-arrhythmic effects.

**Clinical signs** such as soft feces (sporadically) were noted in 2/20 and 6/30 rats at 200 and 4000 mg/kg/day of AKR-963 respectively; these clinical signs were not noted with Lovaza. No effects on body weights, weight changes or food consumption were observed with either drug. Ophthalmologic findings were similar with both drugs at a HD; these included cataracts (in males only 2/10 and 2/10 rats at 0.2 and 4.0 g/kg/day respectively vs 3/100 and 0/10 with Lovaza), hyper-reflective lesions in the posterior segment (in both sexes combined in 1/20 and 3/20 rats with AKR-963 respectively vs 1/20 and 2/20 rats with Lovaza), and crystalline corneal deposits (in 2/20 and 1/20 rats respectively vs in 5/20 and 1/20 rats with Lovaza). Small differences in hematological findings were noted during the drug free recovery period in male rats at a HD with Lovaza, but were not toxicologically relevant; these included lower Hct (45 vs 43\* % with Lovaza), and higher reticulocyte counts (178 vs 204\*  $10^9$  /L with Lovaza).

Clinical chemistry findings showed no evidence of pharmacologic activity, as triglycerides or total cholesterol were not different in this study in either treatment group compared to historical controls. The control ranges provided by the contract Research Organization or CRO are inadequate, based on the age of the animals and the data years of collection to substitute concurrent controls. This may explain the apparent lack of pharmacodynamic or PD effects. Note that ALT levels were increased at a HD with both drugs in males (73 vs 74 u/L with lovaza, low dose values were 59 u/L with both drugs); these in the historical control group were 26-71 u/L), however these were reversible at the end of the drug free recovery period (50 u/L with both drugs). Other clinical chemistry findings included higher LDH levels in females with AKR-963 (5752 & 3992 u/L at 200 and 4000 mg/kg/day respectively vs 4375 & 2533  $\mu$ /L with lovaza). At the end of the drug free recovery period these LDH levels were above the historical control range with both drugs in females (5263 vs 6767 with lovaza), historical controls ranges were 1455-4306 u/L. Also at the end of the recovery period at a HD, increases in globulin levels in female rats were noted with AKR-963 (30 vs 28\* g/L with Lovaza). No changes in organ weights were noted. AKR-963 produced gross pathologic findings at a HD in the sternum (thickening in 1/10 male rats), in uterus (fluid filled in 1/10 female rats at 0.2 and 4 g/kg of AKR-963), in the right cerebrum (clear fluid-filled cavity, 3-4 mm size in 1/5 recovery male at 4 g/kg AKR-963) and alopecia (in 1/5 female rats in a recovery group at 4 g/kg AKR-963).

Sponsor did not provide the histopathology summary Table, and stated that no changes were observed in any tissue, however after the tabulated summary of histopathology data was requested, limited histopathology data were provided. In general similar histopathology findings were noted with both drug products. Target organs of toxicity with both drugs were liver (inflammation in 3/20 rats vs 3/20 rats with Lovaza), kidney (inflammation in 3/20 rats vs 3/20 with lovaza) and eyes, as focal retinal degeneration of mild severity was noted during treatment with both drugs (in 1/10 female rats with both AKR-963 and Lovaza respectively), but at the end of drug free recovery period it was only noted with AKR-963 (in 1/5 males vs 0/5 with Lovaza).. Additionally, findings in the urinary bladder were noted with AKR-963, not noted with Lovaza (in females subchronic and diffuse mucosal inflammation in 2/10 vs 0/10 with Lovaza). Also skin findings (hypotrichosis, of moderate severity) were noted in 1/5 female rats at the end of drug free recovery period with AKR-963, but not with Lovaza.

Thus, in general histopathology findings with both drugs are similar (target organs of toxicity with both drugs were liver and kidney) and AUC exposures on day 28 at a HD were similar with both drugs. However subtle findings were noted with AKR-963, not noted with Lovaza. These include AUC drug exposures of EPA were higher in females at a high dose of AKR-963 (2201 vs 1636 mcg.hr/ml with Lovaza), and mortality in 1/16 male rats at a HD (10 main study rats+ 6 TK group rats, the cause of the death is not known), this animal had marked congestion in liver and lungs and supposedly died of acute cardiac failure. Other differences noted with AKR-963 were findings in the urinary bladder in females (subchronic mucosal inflammation in 2/10 vs 0/10 with Lovaza), eyes (focal retinal degeneration during treatment in 1/10 and 1/10 females with AKR-963 and Lovaza respectively, but during the drug free recovery period with AKR-963 only in 1/5 males vs 0/5 with Lovaza), and skin (hypotrichosis, grade 3 severity) was noted in 1/5 females during the drug free recovery period with AKR-963, but not with Lovaza. Note that sponsor does not consider any of these histopathology findings significant.

As stated earlier, that the above pivotal toxicity study for the 505(b)(2) application lacks a vehicle control; sponsor provided the laboratory data and details on historical control animals after requesting this information, but these data are from rats who are 9-13 weeks old (vs rats were 7-8 weeks of age in the present study). Also Laboratory data are from a large number of studies (i.e. 18 to 43 studies vs a single study here) and they have higher numbers of animals/group (with n=140 to 385 vs n=10 in the current study). Thus historical / background data from years 2000-2007 submitted by the sponsor are not representative of a single study conducted here; and do not provide the complete assessment and differences between the drug products and controls.

Additionally, sponsor states that the active ingredient of this drug product is not only total omega-3-acid ethyl esters, which is defined as the ethyl esters of not only eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), but (b) (4)

Trygg Pharma acknowledges that AKR-963 capsules have a different overall formulation as that of Lovaza capsules, but that AKR-963 still delivers the same amount of "active ingredient" in the same dosage form and route as that of Lovaza. It is possible that the subtle differences in toxicity may be due to differences in other fatty acids present in the current drug product.

There are also non-omega-3 fatty acid components in AKR-963, which are present in higher amounts in AKR-963 than in Lovaza; these include (b) (4)

Thus there are several non-omega-3 fatty acid components in AKR-963, that exceed the levels in Lovaza (by (b) (4)

Among these, such as palmitoleic and stearic are present in clinical lots at higher concentrations than those tested in the lot used for toxicity testing. However, sponsor has used literature NOAELs from repeat dose toxicity studies to establish safety of these 'related components'

There are limitations in the above pivotal toxicity study for this 505(b)(2) application, these include 1) no concurrent controls in the bridging toxicity study; 2) we can-not tell if there is efficacy in animals, as no changes in triglycerides or total cholesterol were noted with either drug, 3) we cannot set a NOAEL, since the sponsor only provided histopathology at a HD of 4 g/kg/day with AKR-963 and Lovaza (no histopathology was conducted on the lower dose groups), 4) the toxicity noted in the above study, does not appear to be the toxicity seen previously with the approved Lovaza (using 4X lower doses). It is possible that the relative absence of established target organ toxicity reflects the short dosing duration of 4 weeks here, and we do not have a 4-week rat toxicity study with the original Lovaza NDA, to compare these results to lovaza and/or AKR-963 in the current application.

However, target organs of toxicity in this study with both drugs appear to be liver, kidney, bladder, retina, and heart. Other than the bladder mucosal inflammation showing a higher incidence than Lovaza, and one male rat that died in the toxicokinetic group with acute cardiac failure with AKR-963, it appears that there is limited toxicity with either drug (lovaza or AKR-963) in the current study.

Table. Safety margins in rats to humans with AKR-963 at NOAEL dose (of 200 mg/kg/day) and at a high dose (4000 mg/kg/day), based on the body surface area.

Species	NOAEL (mg/kg/ day)	NOAEL mg/m <sup>2</sup> /day	Safety Margin Based on body surface area (mg/m <sup>2</sup> /day)
Rat-28-day toxicity study	200	1200 mg/m <sup>2</sup> /day	0.5X
	4000	24000 mg/m <sup>2</sup> /day	11X
Human dose is 3600 mg/day (or 2220 mg/m <sup>2</sup> /day)			

**Mutagenicity:** The mutagenic/cytogenic potential of AKR-963 was examined in 2 tests. AKR-963 was negative in both the Ames test, and in vitro chromosomal aberration assay in cultured human lymphocytes.

**Conclusions:** The NOAEL or tolerated doses of AKR-963 in the 4-week oral toxicity study in rats is 200 mg/kg/day (1200 mg/m<sup>2</sup>/day) which provides the safety margin of approximately 0.5X

in humans at a recommended dose of 3600 mg/day (60 mg/kg/day or 2220 mg/m<sup>2</sup>/day, assuming 60 kg subject), based on body surface area. However, the HD that produced mortality (1/16 TK animal died) and histopathology findings (in the liver, kidney and eyes in both sexes, and in the urinary bladder in the females) provides safety margin of approximately 10X in humans at a recommended dose of 3600 mg/day (60 mg/kg/day or 2220 mg/m<sup>2</sup>/day, assuming 60 kg subject), based on body surface area. Thus, nonclinical data support approval of NDA 204977

**Labeling Review:** The pharmacology toxicology labeling in general is similar to the GlaxoSmithKline's label of Lovaza (NDA 21-654, approved on 11/10/04). In the current application, the submitted PLR label in pharmacology/toxicology sections, i.e. under "Pregnancy" and "Carcinogenesis, mutagenesis and impairment of fertility" are basically identical to the approved Lovaza label (NDA 21-654). The oral dose of 4 grams/day in the Lovaza label is substituted by 4 capsules/day in the AKR-963 label. However changes to the "Carcinogenesis, Mutagenesis, Impairment of Fertility" in the label are recommended, see the reviewer's recommendations below.

**Labeling Review:** Following is sponsor's proposed label from 1/31/2013 submission.

### 13 NONCLINICAL TOXICOLOGY

#### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a rat carcinogenicity study with oral gavage doses of 100, 600 , and 2,000 mg/kg/day, males were treated with omega-3-acid ethyl esters for 101 weeks and females for 89 weeks without an increased incidence of tumors (up to 5 times human systemic exposures following an oral dose of 4 capsules/day based on a body surface area comparison). Standard lifetime carcinogenicity bioassays were not conducted in mice.

Omega-3-acid ethyl esters were not mutagenic or clastogenic with or without metabolic activation in the bacterial mutagenesis (Ames) test with Salmonella typhimurium and Escherichia coli or in the chromosomal aberration assay in Chinese hamster V79 lung cells or human lymphocytes. Omega-3-acid ethyl esters were negative in the in vivo mouse micronucleus assay.

(b) (4)

In a rat fertility study with oral gavage doses of 100, 600, and 2,000 mg/kg/day, males were treated for 10 weeks prior to mating and females were treated for 2 weeks prior to and throughout mating, gestation, and lactation. No adverse effect on fertility was observed at 2,000 mg/kg/day (5 times human systemic exposure following an oral dose of 4 capsules/day based on a body surface area comparison).

**Reviewer's recommended changes (see the changes in bold letters):**

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a rat carcinogenicity study with oral gavage doses of 100, 600 , and 2,000 mg/kg/day, males were treated with omega-3-acid ethyl esters for 101 weeks and females for 89 weeks without an increased incidence of tumors (up to 5 times human systemic exposures following an oral dose of 4 capsules/day based on a body surface area comparison). Standard lifetime carcinogenicity bioassays were not conducted in mice.

Omega-3-acid ethyl esters were not mutagenic or clastogenic with or without metabolic activation in the bacterial mutagenesis (Ames) test with Salmonella typhimurium and Escherichia coli, in the chromosomal aberration assay in Chinese hamster V79 lung cells, **or in vitro in human peripheral lymphocytes**. Omega-3-acid ethyl esters were negative in the in vivo mouse micronucleus assay.

(b) (4)

In a rat fertility study with oral gavage doses of 100, 600, and 2,000 mg/kg/day, males were treated for 10 weeks prior to mating and females were treated for 2 weeks prior to and throughout mating, gestation, and lactation. No adverse effect on fertility was observed at 2,000 mg/kg/day (5 times human systemic exposure following an oral dose of 4 capsules/day based on a body surface area comparison).

#### Justification for the changes:

The sponsor already states that the Omega-3-acid ethyl esters were not mutagenic or clastogenic in the second sentence of "Carcinogenesis, Mutagenesis, Impairment of Fertility". Further repeating this in the third sentence with their drug does not add any extra value to the label. Therefore, the third sentence is deleted.

**Recommendations:** From the Pharmacology/toxicology point of view, this application is recommended for approval, pending labeling changes.

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_

Concurrence Yes \_\_\_ No \_\_\_

cc: IND Arch  
 HFD-510  
 HFD-510/davisbruno/antonipillai/chowdhury/haber,M/coleman,E/johnson  
 Review code: AP  
 File name: nda204977 (AKR-963, fish oil, RLD Lovaza)

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/s/  
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INDRA ANTONIPILLAI

08/05/2013

From the Pharmacology/toxicology point of view, this application is recommended for approval, pending labeling changes.

KAREN L DAVIS BRUNO

08/05/2013

Signed off in DARRTS on 3/13/13

45 Day Meeting Checklist  
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA 21-654: This NDA is a 505(b)(2) application.

Submission date: 1/31/13

Sponsor: Trygg Pharma Inc., Arlington, VA.

Drug: AKR-963 soft gelatin capsules. The proposed dose of AKR-963 is 4 capsules/day.

Introduction: AKR-963 soft gelatin capsule (liquid filled) drug product contains at least 900 mg of omega-3-acid ethyl esters, for oral administration. The major components are eicosapentaenoic acid ethyl ester (EPAee, approximately 465-mg) and docosahexaenoic acid ethyl ester (DHAee, approximately 375 mg), and (b) (4) (4.6-mg as (b) (4)).

AKR-963 lowers triglyceride (TG) by increasing mitochondrial and proximal beta oxidation of fatty acid. This is a 505(b)(2) application. The reference drug is Lovaza (NDA 21-654). Lovaza has been approved in USA and in several European countries for treatment of hypertriglyceridemia (at doses of 2-4 g/day) and myocardial infarction. Both drugs (Lovaza and AKR-963) contain ≥ 900 mg of omega-3-acid ethyl esters/capsule.

TEM: NDA 21-350	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	Yes		We recommended that the sponsor conduct a comparative bridging toxicology study (1-month, one species) with AKR-963 and Lovaza, and <i>in vitro</i> genotoxicity testing as per ICH Q3A and ICH Q3B). The sponsor has conducted a 28 day bridging toxicity study with AKR-963 and Lovaza, but did not include the control group. Additionally, they did not provide the tabulated summary of histopathology data during the treatment and recovery periods. Also tabulated summaries of gross pathology, and urine-analysis are missing in the above submission. The histopathology summary Tables were requested on 2/14/13, this information was provided by the sponsor on 3/6/13.
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	Yes		

<p>3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?</p>	<p>Yes</p>	<p>Sponsor has conducted the recommended toxicology studies, but <i>have not provided</i> the summary Tables of gross pathology, and urine analysis (at the end of treatment and recovery period),</p> <p>The tabulated summary of histopathology data was provided by the sponsor on 3/6/13.</p>
<p>4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)</p>	<p>Yes</p>	<p>Have electronic files of the carcinogenicity studies been submitted for statistical review?</p> <p>The carcinogenicity and other pre-clinical studies have previously been reviewed on the reference drug Lovaza, so carcinogenicity study is not required. Most of the non-clinical studies were conducted under reference drug Lovaza, previously submitted under NDA 21-654.</p> <p>We had recommended a comparative bridging toxicology study (1-month, one species) with AKR-963 and Lovaza and <i>in vitro genotoxicity testing (as per ICH Q3A and ICH Q3B)</i>, which would likely provide the necessary information as well as provide qualification of any differences in impurity/ degradant profiles between the products. As stated earlier, sponsor has conducted the above toxicology studies.</p>

TEM	YES	NO	COMMENT
<p>5) Were the studies adequately designed (i.e., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?</p>	<p>Yes</p>		<p>In general the studies are adequate, however, the control group was not included in the 28-day bridging toxicity study in rats, which is a review issue. Other non-clinical studies have been reviewed under the reference drug NDA 21-654.</p>

<p>6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (i.e., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?</p> <p>The major components are eicosapentaenoic acid ethyl ester (EPAee, approximately 465-mg) and docosahexaenoic acid ethyl ester (DHAee, , approximately 375 mg), and <math>\alpha</math>-tocopherol (4.6-mg)</p>	<p>Yes</p>	<p>One gram capsule of AKR-963 drug product consists of EPA ethyl ester (465 mg), DHA ethyl ester (375mg), &amp; (b) (4) (4.6 mg as (b) (4)).</p> <p>One gram capsule of the reference drug Lovaza consists also of EPA ethyl ester (465 mg), DHA ethyl ester (375mg), &amp; (b) (4) (4 mg as (b) (4)). Both also contain glycerol, gelatin and purified water.</p> <p>AKR-963 is obtained by esterification of the body oil of fish species primarily from the families (b) (4)</p> <p>The remaining components of the drug substance are ethyl esters (b) (4)</p>
<p>7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?</p>	<p>Yes</p>	<p>The route of administration is oral in toxicity studies, as was for the reference drug Lovaza (ND 21-654), which is the intended route in humans.</p>
<p>8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m<sup>2</sup> or comparative serum/plasma AUC levels?</p>	<p>Yes</p>	<p>Yes, the draft labeling submitted in general is in accordance with 21 CFR label, and data express human dose multiples in mg/m<sup>2</sup> (body surface area).</p>

TEM	YES	NO	COMMENT
<p>9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.</p>	<p>Yes</p>		<p>From the Pharmacology /toxicology point of view, the application is fileable. However note that the above pivotal toxicity study for the 505(b)(2) submission lacks a vehicle control; and there are differences between the products, which may be attributed to the finding of the drug (AKR-963), since there is no control included in the study.</p> <p>However, we will determine if there are clinical comparative PK data that would allow the sponsor to refer to the finding of safety and effectiveness of their product to Lovaza, such that the animal head-to-head comparison is not needed. Also there may be CMC issues in terms of impurities/ degradents / excipients of a concern, which will be considered.</p> <p>Additionally, we will evaluate if the tabulated histopathology and other data submitted by the sponsor are adequate based on the historical / background data submitted (instead of a control group in the bridging toxicity study).</p>

Reasons for refusal to file: N/A

Reviewing Pharmacologist: Indra Antonipillai, HFD-510

Supervisory Pharmacologist: Karen Davis-Bruno  
File name: 204977-filing.

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/s/  
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INDRA ANTONIPILLAI

03/13/2013

From the Pharmacology /Toxicology point of view, this application is fileable.

KAREN L DAVIS BRUNO

03/13/2013