CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-654

Pharmacology Review(s)

MEMORANDUM
Nov. 2, 2004

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-654

I have read the pharmacology/toxicology reviews of Omacor and concur that the product may be approved. The label, as amended at the request of the review division, is acceptable. Consideration should be given to the fact that omega-3 fatty acids are normally secreted in milk. Thus, the statement under the heading "Nursing Mothers" might be changed to include this information.

/S/

Kenneth L. Hastings, Dr.P.H.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluation II

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

/s/

Kenneth Hastings
11/2/04 03:22:39 PM
PHARMACOLOGIST

9/15/04

NDA 21-654

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-654
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	1/12/04
PRODUCT:	Omacor
INTENDED CLINICAL POPULATION:	Hypertriglyceridemic patients
SPONSOR:	Ross Products Division, Abbott Laboratories
DOCUMENTS REVIEWED:	Volume 1 and volumes 4-23
REVIEW DIVISION:	Division of Metabolic and Endocrine Drug Products (HFD-510)
PHARM/TOX REVIEWER:	Indra Antonipillai
PHARM/TOX SUPERVISOR:	Karen Davis Bruno
DIVISION DIRECTOR:	David Orloff
PROJECT MANAGER:	Valerie Jimenez

Date of review submission to Division File System (DFS): 9/15/2004

TABLE OF CONTENTS

Executive Summary

1. Recommendations

A. **Recommendation on approvability**
Pharmacology recommends approval of this drug for proposed indication.

B. **Recommendation for Nonclinical Studies**:
The preclinical studies are generally adequate to support the recommended doses up to 4 g/day, except the 2-year mouse carcinogenicity study, where the dose selection and study duration was inadequate (see Executive CAC recommendations). However, no further pre-clinical studies are required.

C. **Recommendation on Labeling**: Following labeling changes are recommended:

Under the heading: **Carcinogenesis, Mutagenesis, Impairment of fertility:**

1a. [redacted]

1b. [redacted]

1c. [redacted]

Under the heading: 'Pregnancy Category

The change in pregnancy category form to C is recommended, as follows:

2a. **Pregnancy Category C.** There are no adequate and well controlled studies in pregnant women.

[redacted]

2b. All HMG-CoA reductase inhibitors are contraindicated in pregnant and nursing women. When Omacor is administered with an HMG-CoA reductase inhibitor in a

[ ]

Under the heading: 'Nursing Mothers'

3. It is not known whether — is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Omacor is administered to — woman.

II. Summary of Nonclinical Findings:

A. Brief Review of Nonclinical studies

Omacor was [ ] However, since extensive nonclinical studies have been conducted with this drug under previous NDA, no additional toxicity studies were required.

In chronic one year toxicity studies in rats and dogs, the drug produced clinical signs such as fur staining in both rats and dogs, while tail lesions, paddling and high stepping gait was observed only in rats. Omacor increased adrenal and kidney weights at a HD in male rats (0, 100, 600, 2000 mg/kg/day), but the main target organ of toxicity in rats was liver (mottled liver in 19/40 vs 5/40 in controls, and increased liver vacuolation in 23/40 vs 8/40 in controls) and lungs (increased foamy histiocytes in 17/40 vs 13/40 in controls), additionally HD males had increased biliary proliferation (8/20 vs 3/20 in controls), and adrenal vacuolation (6/20 vs 1/20 in controls). In dogs, the target organs of toxicity were adrenals (at a HD vacuolation in zonal fasciculate & zona reticularis in ¾ females, accessory nodules at a MD & HD in ¼ + ¼ males respectively vs 0/4 controls), kidneys (basophilic tubules 0/4, 0/4, ¼, ¼ males at 0, 50, 300, 2000 mg/kg/day respectively), and testes (atrophy 0/4, 0/4, 2/4, 2/4 respectively). The NOAEL in rats was ≤600 mg/kg/day in both sexes (or 2 fold the human dose of 4 g/day, based on body surface area). The NOAEL in male dogs was 50 mg/kg/day and in female dogs was 300 mg/kg/day (or 0.4 fold and 2 fold respectively the human dose of 4 g/day, based on body surface area).

The dermal toxicity was observed in almost all pre-clinical toxicity studies (in rats, mice, rabbits and dogs) and was seen mainly and more severely in males for some unknown reason. This was of a concern in a 13–week study in rats where a HD of 4000 mg/kg/day not only produced fur staining and desquamation of tail and feet in all 40/40 male + female rats (with gross foot/leg lesions in 29/40 rats vs 0/40 controls) but lead to human sacrifice of all 20/20 male rats (due to tail lesions). This was not observed in a subsequent 1-year study in rats where a HD of 2000 mg/kg/day produced tail lesions only in male rats, but no mortalities. Similarly skin sores (sometimes in urogenital area), fur loss or staining, and skin dermatitis was observed in all tox studies of mice, dogs and rabbits (at usually high doses of 1000-4000 mg/kg/day). No dermal toxicity was observed in dogs at 300 mg/kg/day, and in rats at 600-1000 mg/kg/day in 3-12 month tox studies (which provides safety margin 2X the human dose based on body surface area). Skin rash has been reported as one of the adverse event with this drug in human studies. Sponsor attributes the skin lesions to the local irritation of the test

article metabolite through skin or urine. The dermal reactions are supposedly due to a deficiency in essential fatty acid(s). However, the deficiency of which essential fatty acid(s) contributes to these dermal reactions and humane sacrifice of animals (rats) is unknown.

Omacor tested negative in the Ames test, in Chinese hamster lung cells, in a clastogenic activity assay in human lymphocytes and in a mouse micronucleus test, suggesting it does not have mutagenic/genotoxic potential.

In 2-year carcinogenic studies in rats and mice, no significant neoplastic or non-neoplastic tumor findings were observed at doses up to 2000 mg/kg/day in rats, but at above doses in mice increased incidences of combined uterine smooth muscle tumors were observed (leiomyoma and leiomyosarcoma combined tumors 9/51 vs 8/102 in controls, p=0.019). These were not considered significant because these values did not reach the p of ≤ 0.01 for a common neoplasm. However, the high dose of 2000 mg/kg/day used in the mouse carcinogenicity study was less than 50% of the maximally tolerated dose (MTD) and the study was not carried out to the optimal 2-year duration. The 2000 mg/kg/day dose in rats and mice provides safety margin of 5X and 10X respectively the human dose of 4 g/day, based on the body surface area. However, ECAC considered the mouse study an inadequate assessment of carcinogenicity.

Omacor did not have any significant effects at doses up to 2000 mg/kg/day in segment I (fertility), and up to 6000 mg/kg/day in segment II (teratology) studies in rats (or 5X and 15X the maximal recommended human dose based on body surface area). In a segment II teratology study in rabbits (0, 375, 750, 1500 mg/kg/day), the HD of 1500 mg/kg/day (a maternally toxic dose) produced increases in post-implantation losses (18% vs 9% in controls). These doses in the rabbit provided safety margin of 2, 4, and 8 fold the human dose. The drug had no significant effects in a segment III (peri/postnatal) study in rats at doses up to 2000 mg/kg/day (or 5-fold the maximal recommended human dose based on body surface area).

B. Pharmacologic activity

Omacor 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). It lowers triglycerides by increased mitochondrial and peroxisomal beta oxidation in the liver. The omega-3 fatty acids replace arachidonic acid (AA) in phospholipids with EPA and DHA. EPA and DHA when released inhibit cyclo-oxygenase and lipoxygenase and reduce eicosanoid synthesis, particularly thrombosane (TXA2) and leukotriene B4 by platelets and macrophages. This leads to depression of the vasoactive anti-aggregatory prostacyclin (PGI_2) and the generation of the anti-aggregatory prostacyclin I_3 (PGI_3) from EPA.

C. Nonclinical safety issues relevant to clinical use

The target organs of toxicity in pre-clinical studies are liver, lungs and adrenal in rats, and adrenal, kidneys, testis in dogs. Dermal toxicity was observed in all species. Skin lesions were attributed to the local irritation of the test article

metabolite through skin or urine, but which metabolite(s) is responsible is unknown. The dermal reactions are supposedly due to a deficiency in essential fatty acids which led to euthanizing of all HD (≥ 2000 mg/kg/day) male rats. The sponsor suggests that due to excessive grooming behavior, exposures may have been recurrent to metabolite and/or drug. With Omacor, EPA and DHA levels are increased while linoleic acid (also an essential fatty acid) is decreased. In both rats and dogs, there is substantial dose-linked increase in EPA & DHA, followed by reduction in AA, palmatic, and linoleic acid. It is known that rats deficient in linoleic acid develop scaly, necrotic skin and necrotic tails. However, deficiency of which essential fatty acid(s) is responsible for tail lesions and humane sacrifice of rats is unknown. It is unclear how administration of omega-3-essential fatty acid (EFA) can result in signs of EFA deficiency in these rodents. However, these signs are present at doses ≥ 2000 mg/kg/day which exceed the therapeutic dose. These target organ toxicities were observed in animals at ≤2X human doses, suggesting low or insufficient safety margin in humans. This drug is approved and marketed in several countries. The mechanistic studies may answer some of theses deficiencies

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 21-654
Review Number: 1

Sequence number/date/type of submission: January 9, 2004 (original application). It is a 505(b)(1) application. The NDA

Information to sponsor: Yes ( ) No (X)

Sponsor: Ross Products Division, Abbott Laboratories, Columbus, OH.

Manufacturer for drug substance: The manufacturer of the drug substance (K85EE) is Pronova Biocare, Sandefjord, Norway. The drug product is manufactured at Cardinal Health, St. Petersburg, Florida.

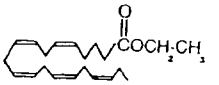
Reviewer name: Indra Antonipillai, Ph.D. Pharmacology Reviewer.
Division: Division of Metabolic and Endocrine Drug products, **HFD #:** 510
Review completion date: 8/31/2004

Drug:

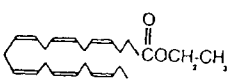
Trade name: Omacor.
Generic name (list alphabetically): Omega-3-acid ethyl ester
Code name: Ethyl ester K85 (or K85EE)
Chemical Name: The drug is mainly a mixture of two unsaturated fatty acids:
All cis 5,8,11,14,17-eicosapentaenoic acid ethyl ester (EPA ethyl ester)
All cis 4,7,10,13,16,19-docosahexaenoic acid ethyl ester (DHA ethyl ester)
CAS Registry Number: EPA ethyl ester: 86227-47-6
DHA ethyl ester: 81926-94-5
Mole file number: N/A
Molecular Formula/ Molecular Weight:
EPA ethyl ester: $C_{22}H_{34}O_2$/330.51
DHA ethyl ester: $C_{24}H_{36}O_2$/356.55

Structures: Structures of EPA and DHA are shown below

STRUCTURES
EPA- EICOSAPENTAENOIC ACID (ethyl ester) 20:5 -C_{20}, 5 double bonds
Five double bonds (@ carbons 5, 8, 11, 14, 17, all-cis)



DHA- DOCOSAHEXAENOIC ACID (ethyl ester) 22:6-C_{22}, 6 double bonds
Six double bonds (@ carbons 4, 7, 10, 13, 16, 19, all-cis)



Relevant INDs/NDAs/DMFs: [redacted] IND 45,998 (omacor). DMF number [redacted] (for omacor drug product from Cardinal health, FL)

Drug class: Fish oil supplement, ω-3 poly-unsaturated fatty acids esters of eicosapentaneoic (EPA) and docosahexaenoic acids (DHA). Both are essential fatty acids.

Intended clinical population: Hypertriglyceridemic patients. The drug will be used as an adjunct to diet to reduce triglyceride (TG) levels [redacted]

Clinical formulation: The drug is available in 1 gm capsule containing at least 900 mg omega-3-acid ethyl ester. These contain the active drug and inactive ingredients, see Table on page 8. The recommended dose is 4 g/day (single or divided daily dosing)

Route of administration: Oral.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise

Studies reviewed within this submission: Studies were previously reviewed under IND 45,998, [redacted], and are summarized here. Carcinogenicity studies were reviewed by ECAC on 8/10/04.

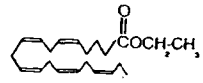
Studies not reviewed within this submission: None

2.62 PHARMACOLOGY

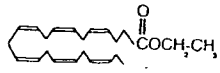
Omacor is a mixture of omega-3 fatty acids (FA) isolated from fish oil. The drug is a mixture of two predominant unsaturated fatty acids, eicosapentaenoic acid (—) and docosahexaenoic acid (—). The ethyl esters of EPA and DHA comprise at least — of the total drug substance. The fatty acids are similar in structure to prostaglandins and can serve as sources for prostaglandin formation both in vivo and in vitro. The remaining components of the drug substance are ethyl esters of alpha-linoleic acid, moroctic acid, eicosatetraenoic acid, heneicodapentaenoic acid and clupanodonic acid.

STRUCTURES

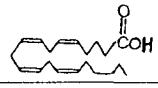
EPA- EICOSAPENTAENOIC ACID (ethyl ester) 20:5 ωC_{22}, 5 double bonds
Five double bonds (@ carbons 5, 8, 11, 14, 17, all-cis)



DHA- DOCOSAHEXAENOIC ACID (ethyl ester) 22:6-C_{22}, 6 double bonds
Six double bonds (@ carbons 4, 7, 10, 13, 16, 19, all-cis)



ARACHIDONIC ACID (20:4)
Four double bonds (@ carbons 5, 8, 11, 14, all-cis)



The Clinical formulation contains the active drug and following inactive ingredients:

Table. Composition of Omacor (K85):

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The following table provides the qualitative composition of the product.

Ingredients	Grade	Purpose
Active Ingredients		
Omega-3-acid ethyl esters	Ph. Eur.	Active Ingredient
Inactive Ingredients		
α-Tocopherol		
Partially hydrogenated vegetable oils including soybean oil		
Capsule Shell		
Gelatin	NF	
Glycerin, Natural	USP	
	EP	
	NF	
	USP	
Purified Water*	USP	
	NF	

* Processing aid. Not part of the final composition.

The sponsor's description on the drug substance is provided below

The drug substance of this application is a mixture of omega-3-acid ethyl esters derived from fish oil by trans-esterification of the purified triglyceride esters of the enriched omega-3-acids. The two predominant components of the drug substance are the ethyl esters of eicosapentaenoic acid and docosahexaenoic acid. For convenience, the acronyms, EPA and DHA, respectively, are used. The ethyl esters of EPA and DHA comprise at least — of the total drug substance. The primary remaining components of the drug substance are ethyl esters of alpha-linoleic acid,

The drug substance, K85EE, consists of the sum of EPA and DHA ethyl esters, approximately of which EPA ethyl ester amounts to approximately 465 mg/g (Ph. Eur.: Not less than 400 mg/g) and DHA ethyl ester amounts to approximately 375 mg/g (Ph. Eur.: Not less than 340 mg/g).

The total amount of omega-3-acid ethyl esters is not less than —

Each one gram capsule contains 465 mg of EPA ethyl ester, 375 mg of DHA ethyl ester & 4 IU (or 4 mg/g) of vitamin E as an antioxidant. are reduced in the drug to be not more than ug/g respectively. Thus in a 4 g of the drug, the will amount to approximately — which sponsor states is a fraction of the recommended daily allowance for men and women (the recommended dose is 700-900 µg). comprise the remainder of the formulation. are also present in the drug product but are supposedly lower than in unrefined fish oil, and are generally below the level of detection.

The drug is insoluble in water and very soluble in acetone, ethanol, heptane and methanol.

Omacor (which is a mixture of esters of two essential free fatty acids, concentrated from raw fish oil) has been marketed for hypertriglyceridemia in multiple European countries since 1994 for hyper-triglyceridemia and for post-myocardial infarction. This drug is indicated for the reduction of elevated TG levels in patients with hyperlipidemia as monotherapy [redacted] at initial recommended doses of 4 g/day (as a single dose or two 2 g divided doses) which would provide 28 mg/Kg of EPA, and 23 mg/Kg of DHA daily to a patient weighing 60 Kg. Thus in the present submission dosage and duration are comparable to that for the foreign-approved triglyceridemia indication.

Note that for toxicology/carcinogenicity studies corn oil is the vehicle.

2.6.2.2 Primary Pharmacodynamics

Omacor lowers triglyceride (TG) by increasing mitochondrial and peroxisomal beta oxidation of fatty acids (FA). The following study shows the hypolipidemic effect of omacor in Wistar rats (see the Figure and Table below). When rats were fed the drug (0, 0.6, 1.3, 2.5, 5 g/100 g of diet) for 21 days, the drug decreased plasma lipids significantly (3.1, 2.6, 1.5, 1.2 & 0.74 mmol/l respectively) as shown below. TG was also reduced by up to 24% of control with the 5 g/kg/day dose (0.74 vs 3.11 mM in controls).

Figure 3
Plasma lipid responses to increasing doses of E 85.
TG=triacylglycerol, PL=phospholipid
TC=total cholesterol, UC=unesterified cholesterol
CE=cholesteryl ester.
Mean values ± SEM, *p<0.05 vs.control group (dose 0).

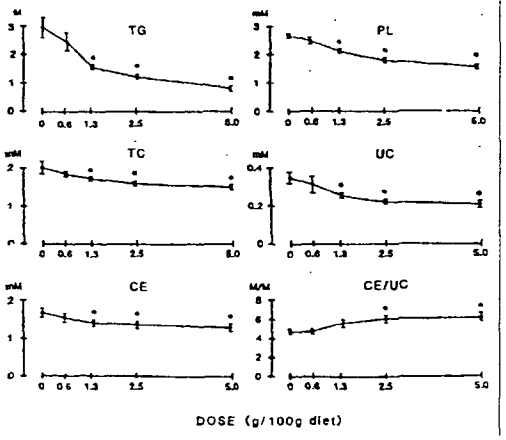

Table 1. Effect of K85 Administration on Plasma Lipid Levels in Male Rats

Start Dose (g/kg/day) [a]	0.0	0.6	1.3	2.5	5.0
End Dose (g/kg/day) [a]	0.0	0.4	0.9	1.7	3.3
	N =12	N =12	N =12	N =12	N =12
Triglycerides, mM	3.11	2.55	1.50*	1.20*	0.74*
(% of control)	(100)	(82)	(48)	(39)	(24)
Phospholipids, mM	2.71	2.56	2.11*	1.86 *	1.56*
(% of control)	(100)	(94)	(78)	(69)	(58)
Total Cholesterol, mM	2.05	1.92	1.72*	1.61*	1.49*
(% of control)	(100)	(94)	(84)	(79)	(73)
Unesterified cholesterol, mM	0.35	0.33	0.26*	0.23*	0.21*
(% of control)	(100)	(94)	(74)	(66)	(60)
Cholesterol esters, mM	1.70	1.59	1.46*	1.38*	1.28*
(% of control)	(100)	(94)	(86)	(81)	(75)

Note: Data are presented as mean plasma concentration (mM) and as the percentage of control value at the end of the 21-day dose period.

[a] In previous experiments it was shown that a rat eats about 20 g per day and during a 3-week period, its weight increases from about 200 g to 300 g. The approximate doses of K85 (g/kg/day) are presented for both the start and the end of the experiment.

* Statistically significant difference from control group, $P<0.05$ (student's t-test)

In a domestic swine model (Groot et al, Artherosclerosis: 77, 1: 1989), when animals were fed a high fat diet containing 21% mackerel oil (which contained 17 mol% EPA and 9 mol% DHA) or the lard fat (which had no EPA or DHA), after 8 weeks of treatment plasma TG and cholesterol were decreased by 62% and 55% respectively compared to controls. The fractionation studies showed that the hypo-triglyceridemic and hypocholesterolemic effects of mackerel on TG were exclusively due to the reduction of VLDL-C fraction, whereas cholesterol was reduced in all lipoprotein fractions (i.e in VLDL-C, IDL-C, LDL-C and HDL-C).

Several pharmacology studies have shown that omega- 3 fatty acids reduce TG, platelet aggregation and affect prostaglandin and leukotriene metabolisim. The studies with EPA & DHA suggest that a reduction in BP with these fatty acids is probably related to formation of the 3-series prostaglandins (PGS) from EPA with different biological activity than 2-series PGS formed from arachidonic acid (AA).

The poly unsaturated omega-3 fatty acids (n-3 PUFAS) depress lipids (essentially TGs), by inhibiting hepatic TGs and possibly apoprotein synthesis. They replace AA in phospholipids (from n-6 PUFAS) with EPA and DHA as shown below. EPA and DHA when released inhibit cyclo-oxygenase and lipoxygenase and reduce eicosanoid synthesis, particularly thromboxane (TXA2) and leukotriene B4 by platelets and macrophages. These lead to depression of the vasoactive anti-aggregatory prostacyclin (PGI_2) and the generation of the anti-aggregatory prostacyclin I_3 (PGI3) from the EPA. Thus, n-3 PUFAS reduce blood pressure and blood viscosity and modulate membrane fluidity and associated enzyme and receptor functions. However, the optimum intake of n-6 and n-3 PUFAS may be important in this regulation, since n-6 PUFAS can be immunosuppressive (Kinsella JE et al Am. J. Clin. Nutr. 52 (1):1, 1990).

Figure. The formation of 2-series and 3-series PGs is shown below:

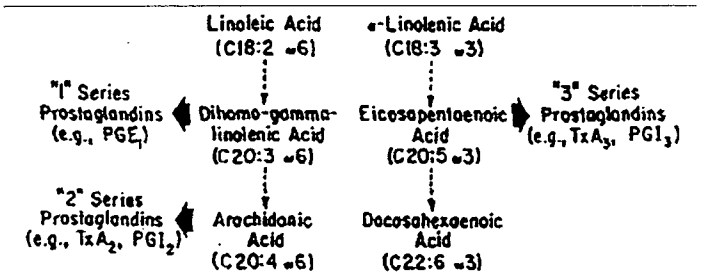

Figure 5. Prostaglandin synthesis from polyunsaturated fatty acids. Conversion of ω-6 and ω-3 fatty acids to various prostaglandins. The dotted lines indicate that the depicted elongation and desaturation steps occur in some but not all tissues.

Figure. The formation of docosapentaenoic acid (DPA from linoleic acid, n-6 series) & DHA (from linolenic acid, n-3 series) are shown below:

Linoleate series		Linolenate series	
C18:2w6	Linoleic acid	C18:3w3	Alpha-linolenic acid
	Δ^6 desaturase		Δ^6 desaturase
C18:3w6	Gamma-linolenic acid	C18:4w3	
C20:3w6	Dihomo-gamma-Linolenic Acid	C20:4w3	
	Δ^5 desaturase		Δ^5 desaturase
C20:4w6	Arachidonic acid	C20:5w3	Eicosapentaenoic acid
C22:4w6		C22:5w3	Docosapentaenoic acid
	Δ^4 desaturase		Δ^4 desaturase
C22:5w6	Docosapentaenoic acid	C22:6w3	Docosahexaenoic acid

FIG 3. Essential fatty acid metabolism desaturation and elongation of ω6 and ω3.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The active ingredients of omacor (EPA, DHA) are esters of the human essential fatty acids eicosapentaenoic and docosahexaenoic acid. The esters are hydrolyzed after administration, absorbed via the intestinal lymphatic system and incorporated into various lipids (e.g. in lymph mainly triglycerides in chylomicrons; in tissues to triglycerides in phospholipids and in plasma to lipoproteins). In the rat, chylomicrons are cleared by incorporation into serum LDL and HDL within 4 hours. EPA and DHA are metabolized by tissue lipoxygenases and cyclooxygenase to prostaglandins, thromboxane and leukotrienes in addition to mitochondrial β-oxidation. Seven days after

oral administration of labeled EPA and DHA to the rat <10% of the administered radiolabel was excreted in feces.

Table 29. Elimination of Ingested ^{14}C-EPA

Test Substance	Body Weight (g) (Mean ± SEM)	Radioactivity in Plasma, Day 7 (cpm/mL ± SEM)	Total Excretion on Days 1–7 (% ± SEM)
^{14}C-EPA ethyl ester	340 ± 13	129 ± 11	5.29 ± 0.40
^{14}C-EPA free acid	353 ± 5	163[a] ± 4	0.49[a] ± 0.25
^{14}C-EPA triglyceride	333[b] ± 6	104[b] ± 4	10.59[a,b] ± 0.18
^{14}C-DHA ethyl ester	386[a,b,c] ±11	140[c] ±10	7.87[a,b,c] ± 0.70

SEM = Standard error from the mean
[a]p<0.05 vs. EPA ethyl ester
[b]p<0.05 vs. EPA free acid
[c]p<0.05 vs. EPA triglyceride

The highest daily excretion took place on Day 2. During the week after intake of ^{14}C-fatty acid, 3.7% to 10.9% of the ingested dose was excreted in feces. The percentage excreted was highest with the EPA triglyceride, lowest with the EPA free acid, and at an intermediate level with EPA ethyl esters and DHA ethyl esters.

Radiolabel distribution studies indicate essentially the same profile after feeding EPA as an ethyl ester or a free fatty acid with 86% distribution to the liver, 8% plasma and 4% to the kidney after 24 hours in the rat.

Rat: Omacor increased the percent of EPA & DHA levels, while AA levels were decreased (in week 52) in a 1-year tox study in rats (at doses of 0, 100, 600, 2000 mg/kg/day), however the levels of DHA were not increased in a dose proportional manner, and levels of arachidonic acid (AA) were decreased, these are shown below:

Table 1. Plasma levels of EPA, DHA and AA in a 52-week tox study in rats:

Blood samples were obtained from 20 of the 40 rats in each group at Week 52. Additionally, blood samples were obtained from 20 health screen rats. The results are summarized in the following table. The values given are mean area percentage (of total fatty acids) ± standard deviation.

Fatty acid	Health Screen	Group 1 (0)[a]	Group 2 (100)	Group 3 (600)	Group 4 (2000)
EPA	0.41 ± 0.16	0.049 ± 0.043	0.34 ± 0.14	1.46 ± 0.44	4.71 ± 1.29
DHA	1.89 ± 0.48	1.32 ± 0.33	2.83 ± 0.75	3.84 ± 1.16	6.01 ± 1.06
AA	9.38 ± 2.11	18.21 ± 3.28	17.10 ± 2.41	11.42 ± 2.24	8.93 ± 1.52

[a] dosage (mg/kg/day)

The analytical results obtained in this study show a clear dose-dependent increase in rat serum EPA and DHA after receiving K85 for 1-year. These results confirmed that the test substance is readily taken up by the rat and demonstrated EPA and DHA absorption in this species. Since the animals in this study had been dosed with K85 for a period of one year, the values for EPA and DHA obtained were considered to indicate equilibrium levels.

Dog: The percent of EPA, DHA and AA levels formed on day 90 in a 13-week tox study in dogs (at doses of 0, 100, 600, 2000 mg/kg/day) are shown below. Both EPA and

DHA were increased with the dose, but DHA was not increased in a dose proportional manner again.

Table 2. Plasma levels of EPA and DHA in a 13-week tox study in dogs:

Group	Dose (mg/kg/day)	Fatty Acid	Pre-dose (%)	Week 13 (%)
1	0	EPA DHA	0.40 ± 0.15 1.50 ± 0.30	0.36 ± 0.16 0.55 ± 0.16
2	50	EPA DHA	0.41 ± 0.08 1.79 ± 0.51	0.72 ± 0.13 1.85 ± 0.31
3	300	EPA DHA	0.32 ± 0.07 1.30 ± 0.22	3.85 ± 1.05 3.85 ± 0.60
4	1000	EPA DHA	0.35 ± 0.05 1.57 ± 0.22	9.07 ± 4.84 5.59 ± 1.46

The results show a clear dose-dependent increase in serum EPA and DHA in dogs after 90 days of K85 administration. The serum levels of EPA and DHA obtained in the high-dose group are above physiological levels and are a result of the high doses given to this group.

Rabbit: The percent of EPA, docosapentaenoic acid (DPA) and DHA were meausred on day 18 of gestation in a dose range finding study in pregnant rabbits (at doses of 0, 750, 1500, 3000 mg/kg/day) as shown below (vol 1, page 40).

Table 3. Plasma levels of EPA, DPA and DHA in a dose range finding study in pregnant rabbits:

Serum samples from a dose range-finding study with K85 in pregnant rabbits[15,43] were analyzed for EPA, docosapentaenoic acid (DPA), and DHA. Serum samples were taken on Day 18 of gestation, prior to administration of the last dose.

Table 26. Bioavailability of EPA, DPA, and DHA in Pregnant Rabbits after Administration of K85

Dose (mg/kg/day) Fatty acid	0 N = 5	750 N = 5	1500 N = 5	3000 N = 5
EPA	0.75 ± 0.69	3.51 ± 1.09	5.94 ± 1.73	11.03 ± 5.74
DPA	0.55 ± 0.28	1.80 ± 0.70	2.15 ± 0.43	2.42 ± 0.75
DHA	0	4.23 ± 1.22	5.29 ± 0.87	8.68 ± 1.10

Note: Data are presented as mean area percentage of total fatty acids with standard deviation.

The study demonstrated absorption of EPA, and DHA from K85 in the rabbit. Levels of EPA, DPA, and DHA increased in serum with increasing doses of K85.

Human: The percent of EPA, DHA and AA levels at the end of 2-weeks in healthy males (at omacor doses of 0, 4, 8, 14 g/day) are shown below. Again, in both studies EPA somewhat was increased with the dose, but DHA did not increase in a dose proportional manner.

Table 4. Plasma levels of EPA and DHA in a 2-week study in healthy male subjects:

Studies **CK85-001** (Volume 24) **and CK85-002** (Volume 25) were open-label, multiple-dose studies in healthy volunteers with identical trial protocols conducted in the United Kingdom and Norway, respectively. In the first study (**CK85-001**), 24 healthy male volunteers, 18 to 53 years of age, were divided into 3 groups and given K85 4, 8, or 14 daily for 2 weeks. In the second study (**CK85-002**), 16 healthy volunteers were given a triglyceride preparation (Active-EPA) containing 30% omega-3 fatty acids, 12 or 24 g daily for 2 weeks. The increase of EPA and DHA in serum phospholipids is provided in the following table. Data shown are percentages of EPA or DHA in total phospholipid.

Table 5 EPA and DHA Incorporation Data (CK85-001 and CK85-002)

	EPA		DHA	
Treatment, Daily Dose	**Baseline**	**Day 15**	**Baseline**	**Day 15**
K85, 4 g	1.3 ± 0.2	6.2 ± 1.0***	5.1 ± 1.0	9.2 ± 0.7***
K85, 8 g	1.3 ± 0.6	10.3 ± 1.3***	5.5 ± 1.0	10.3 ± 1.1***
K85, 14 g	1.2 ± 0.7	11.0 ± 2.7***	4.4 ± 0.7	8.5 ± 1.0***
Active-EPA, 12 g	1.5 ± 2.1	7.0 ± 1.4***	6.5 ± 2.1	8.7 ± 1.8*
Active-EPA, 24 g	1.7 ± 1.4	9.2 ± 0.6***	6.8 ± 1.5	9.5 ± 1.2***

* $P<0.05$, ** $P<0.01$, *** $P<0.0001$

Thus EPA values at 2000 mg/kg/day in rats were 4.7% (1-year rat study), in dogs at 1000 mg/kg/day were 9.1% (3-month study), and in humans at 4, 8, 14 g/day (or 67, 133, 233 mg/kg/day assuming 60 kg weight) were 6.2, 10.3, 11% respectively (2-weeks study). While DHA values at 1000 or 2000 mg/kg/day in rats & dogs (in 3-12 month studies) were 6.0%, and in humans at 4, 8, 14 g/day (or 67, 133, 233 mg/kg/day assuming 60 kg weight) were 9.2, 10.3, 8.5% respectively (2-weeks study).

The EPA values at 2000 mg/kg/day in rats and mice were 10 and 29 mg/ml in a 2-year rat/mouse cac study, DHA levels were 29 &160 mg/ml in these rat & mice studies respectively

2.6.6 TOXICOLOGY STUDIES

2.6.6.2 Single-dose toxicity studies

No Acute toxicity studies have been conducted with this drug supposedly due to low toxicity. However, 4-week, 13-week and 52-week toxicity studies, repro tox studies, geno-tox studies and carcinogenicity studies have been conducted in rats and dogs under IND 45,998 .

2.6.6.3 Repeat-dose toxicity studies

Following repeat dose sub-chronic and chronic toxicity studies are summarized from IND 45,998

In a 13-week oral gavage toxicity study of omacor in rats (6-weeks old, n=20/sex/group), doses of 0, 200, 1000, 4000 mg/kg/day were used. The peroxidation number of the test substance to determine the quality of the drug and to see if it had been oxidized was provided (see Table 3), the peroxide levels in the drug in groups 1-3 were between 1-4 mmole/kg oil, but in group 4 (at a HD of 4000 mg/kg/day) these increased from 12 mmole/kg to 16 mmol/kg oil, but appear to be acceptable. Due to severe clinical signs at a HD in week 9, all 20/20 males were sacrificed during week 9

because of the severity of the desquamation in tails and feet (shedding of epithelial elements) and necrosis in tails. Also, 2 females (1/20 at MD and 1/20 HD) were sacrificed in week 8 due to panophthalmitis (severe protruding eye). HD also increased other clinical signs (rough hair coat in 20/20 M + 5/20 F, fur staining 40/40 in M + F, desquamation in tail and feet in 40/40 in M+F, necrotic tail in 10-20% in M+F). At a HD in males, body weight was decreased by 12%, and males had clinical chemistry parameters altered (increased AST/ALT by 2-fold, increased ALP/BUN by 20% and 50% respectively). The drug decreased cholesterol, total lipids, & phospholipids (by 30-70%) in both sexes. No effect on organ weights was noted but the drug produced gross pathological findings at a HD in the male liver (mottled in 12/20, pale in 6/20 vs 0/20 in controls), tail lesions in both sexes (40/40 vs 1/40 in controls), foot/leg lesions (29/40 vs 0/40 controls). Histopathology findings were observed at a HD in the spleen (hematopoiesis in 7/40 vs 3/40 controls), forestomach (gastritis/hyperkeratosis in females 5/20 vs 0/20 controls), tail dermatitis/foliculitis (35/4 0 vs 9/40 controls), foot leg dermatitis (13/40 vs 0/40 controls). All HD males had severe tail lesions, these consisted of hyperkeratosis/acanthosis, folliculitis, and in some cases necrosis of the tip, HD females had also developed similar but less extensive lesions. The foot lesions were relatively minor and included hyperkeratosis/acanthosis and in some cases with dermatitis. Additionally, mammary carcinomas were seen in two treated female rats (at 200 and 4000 mg/kg/day) in 13-week study, these tumors were very large: 35x25x20 (LD) and 20x10x5 (HD).

Table. Histopathology data from a 13-week oral toxicity study in rats

TABLE INCLUDES: SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL DEATH=ALL;FIND=ALL;SUBSET=ALL		--- NUMBER-OF-ANIMALS -							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	20	20	20	20	20	20	20	20
SPINAL CORD (SC)	NUMBER EXAMINED:	20	0	0	20	20	0	1	20
TAIL (TA)	NUMBER EXAMINED:	19	4	2	20	20	2	2	20
--DERMATITIS		0	1	1	9	0	1	1	6
--FOLLICULITIS		3	2	1	18	6	1	0	17
--HYPERKERATOSIS/ACANTHOSIS		0	1	0	20	1	1	0	20
--NECROTIC TIP		0	0	0	6	0	0	0	1
--BONE/JOINT LESION		0	0	0	0	1	0	0	0
FOOT/LEG (FO)	NUMBER EXAMINED:	0	1	1	13	0	0	0	16
--DERMATITIS		0	1	1	6	0	0	0	7
--HYPERKERATOSIS/ACANTHOSIS		0	0	0	13	0	0	0	14
ABDOMINAL CAVITY (AB)	NUMBER EXAMINED:	1	0	0	0	0	0	0	0
--PERITONITIS		1	0	0	0	0	0	0	0
ANIMAL (AA)	NUMBER EXAMINED:	0	9	12	0	0	14	14	0
--NOT EXAMINED HISTOLOGICALLY		0	9	12	0	0	14	14	0
CAUSE OF DEMISE (ZZ)	NUMBER EXAMINED:	0	0	0	20	0	0	1	1
--TAIL LESION		0	0	0	20	0	0	0	0
--EYE LESION		0	0	0	0	0	0	1	0
--MULTIFACTORIAL		0	0	0	0	0	0	0	1
## END OF LIST ##									

In a 90-day oral gavage toxicity study of omacor in dogs (n=4/sex/group), doses of 0, 50, 300, 1000 mg/kg/day were used. The peroxidation number of the test substance to determine the quality of the drug and to see if it had been oxidized was not provided. The drug increased clinical signs at a HD in 4/4 M +2/4 F dogs (fur staining of legs and abdomen was observed from week 4-5 onwards in both sexes, sponsor has not provided Table of clinical signs, just the above text), possibly due to excretion of metabolites through skin or urine. The drug increased body weight gains by 21-31% at 300 mg/kg/day in females and in both sexes at 1000 mg/kg/day. Omacor decreased plasma lipid concentration (cholesterol, TG levels and phospholipids). No effects on organ

weights, gross pathology or histopathology were observed. The NOAEL in this 90-day dog study was 1000 mg/kg/day (or 8-fold the human dose of 4 g/day based on body surface area).

In a one year oral gavage toxicity study of omacor in rats (6-weeks old, n=20/sex/group), doses of 0, 100, 600, 2000 mg/kg/day were used. The per-oxidation number of the test substance to determine the quality of the drug and to see if it had been oxidized was provided. At a HD of 2000 mg/kg/day, it was 52, 27, 9, 27, 38 meq/kg in weeks 1, 13, 26, 39 and 52 respectively, at a MD it was between 4-15 meq/kg. However nominal concentrations of the drug had been achieved. The FA concentration of the drug did not increase in a dose proportional manner, EPA values were 0.05, 0.34, 1.5, 4.7% of total fatty acids at 0, 100, 600, 2000 mg/kg/day respectively; DHA levels were 1.3, 2.8, 3.8, 6.0 % respectively and AA concentrations actually decreased with the dose (18.2, 17.1, 11.4, 8.9% respectively), see Table 1. The drug increased clinical signs at a MD and/or HD in rats (tail lesions at HD in males, fur staining at a HD in both sexes, paddling and high stepping gait at MD/HD in both sexes). Sponsor attributes the skin lesions to the local irritation of the test article metabolite through skin or urine. Excessive grooming was noted resulting in potential re-exposure. No effects on BW were observed at any dose. At a HD, the drug decreased total cholesterol, total lipids, TG, and phospholipids, but increased plasma urea at this dose (actual values were not provided). It produced increased adrenal weights in males at MD/HD, increased kidney weights at a HD in males. The target organ of toxicity at a HD was liver, as gross pathology showed increased incidences of mottled liver at all doses but higher incidences at HD (in 5/40, 12/40, 12/40 & 19/40 rats at 0, 100, 600, 2000 mg/kg/day respectively), along with increased microscopic liver vacuolation (slight to moderate severity in 23/40 vs 10/40 controls), and increased fat in the liver (slight to moderate severity in 23/40 at a HD vs 12/40 controls), as well as increased foamy histiocytes in the lungs (13/40, ¾, 2/3, 17/40 respectively). Also in HD males, there was increased biliary proliferation (3/20, 2/10, 1/11, 8/20 respectively), and adrenal vacuolation (1/20, 0/0, 0/1, 6/20 respectively). Histopathology was conducted only in control and HD animals (see Table below) and in animals with gross lesions and tissue masses. Since histopathology changes were not examined at low-mid doses in rats, it is unknown if there was a dose related trend in any of these findings in the liver. Since most of the histopathology changes were observed at a HD, the NOAEL may be ≤600 mg/kg/day in this 1-year study in rats which provides safety margin of <2 fold the human dose of 4 g/day based on body surface area.

Table 5. Summary of Important Findings: 52-Week Oral Toxicity in Rat

Dose (mg/kg/day)	Control		100		600		2000	
Sex	M	F	M	F	M	F	M	F
Number	N=20	N=20	N=20	N=20	N=20	N=20	N=20	N=20
Number of deaths	1[a]	1[a]	0	1[a]	1[a]	0	0	0
Clinical observations								
Fur staining	Perm +	Perm +	Perm +	Perm +	Perm +	Perm +	Perm ++	Perm ++
Tail lesions	Perm +	Perm +	Perm +	Perm +	Perm +	Perm +	Perm +	Perm +
Paddling and high stepping gait					Trans ++	Trans ++	Trans ++	Trans ++
Body weight gain					↑	↓*		↓*
Clinical chemistry (Week 52)								
Total cholesterol							↓***	↓***
Total lipids							↓*	↓***
Triglycerides							↓*	↓
Phospholipids							↓***	↓***
Plasma urea							↑***	↑*
Organ weights								
Adrenals					↑*		↑*	
Kidneys							↑*	
Microscopic findings – liver								
Vacuolation Absent	2	3	NE	NE	NE	NE	0	0
Minimal	10	15					6	11
Slight	5	2					12	8
Moderate	3	0					2	1
Fat in ORO Absent	0	2	NE	NE	NE	NE	0	0
Minimal	10	16					7	10
Slight	7	2					10	8
Moderate	3	0					3	2
No-toxic-effect dose level							Yes	Yes

Key: ↓ = decrease; ↑ = increase; NE = Not examined; ORO = oil-red-O stained tissue sections; Perm = permanent; Trans = transitory; + = mild; ++ = moderate; +++ = severe; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

a Two control rats died after accidents; a female given 100 mg/kg/day died due to pituitary tumor; and one cause of death was unknown.

In a one year oral gavage toxicity study of omacor in dogs (4-6 months old, n=4/sex/group), doses of 0, 50, 300, 1000 mg/kg/day were used. The peroxidation number of the test substance was not provided, however nominal concentrations had been achieved. The drug increased clinical signs at a HD in M+F dogs (fur staining). The drug increased ALT (at MD in 1/8 and at a HD in 1/8 dogs by 2-4 fold). The drug did not change total cholesterol, or TG levels. No effects on organ weights were observed. The target organs of toxicity were adrenals (at a HD vacuolation in zonal fasciculate & zona reticularis in ¾ females, accessory nodules at a MD & HD in ¼ + ¼ males respectively), kidney (basophilic tubules 0/4, 0/4, ¼, ¼ males at 0, 50, 300, 2000 mg/kg/day respectively), testes (atrophy 0/4, 0/4, 2/4, 2/4 respectively), epididymis/orchitis (0/4, 0/4, 0/4, ¼ respectively), also spleen pigmentation was observed in HD females, see Table below. The NOAEL in this one-year dog study is 50 mg/kg/day in males (or 0.4 fold the human dose of 4 g/day, based on body surface area) and 300 mg/kg/day in females (or 2 fold the human dose of 4 g/day, based on body surface area).

Table 8. Summary of Important Findings: 52-Week Oral Toxicity in Dog[25]

Dose (mg/kg/day)	50		300		1000	
Sex	M	F	M	F	M	F
Number	N=4	N=4	N=4	N=4	N=4	N=4
Number of deaths	0	0	0	0	0	0
Clinical observations						
Fur staining					Perm ++	Perm +
Clinical chemistry (Week 52)						
Free fatty acids						↑
Macroscopic/microscopic findings						
Adrenocortical vacuolation						↑+
Spleen pigmentation						↑+
No-toxic-effect dose level					Yes	Yes

Key: ↓ = decrease, ↑ = increase; Perm = permanent; + = mild; ++ = moderate; +++ = severe, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

The safety factor could not be determined based on exposures as EPA and DHA values (which increased) are provided as mean area percentages of total fatty acids. There were also other fatty acids like linoleic acid, which remained unchanged (see the IND 45,998 review). In contrast AA values although in percentages, decreased with increases in doses. Thus, exposures based on EPA and DHA percentages may not depict the actual drug exposures.

Calculations based on exposures of EPA+DHA (percentages of total fatty acids) in a 1-year rat and dog studies and 2-weeks human studies provide safety factor of 0.3 fold rat to human, and 0.5 fold dog to human, based on 4 g/day dose in humans.

2.6.6.4 Genetic toxicology

The following gene-toxicity studies are summarized from IND 45,998

Omacor was tested in a full battery of mutagenicity/clastogenicity assays. It was negative in the Ames test, in Chinese hamster V79 lung cells, and the in vivo micronucleus test in mouse bone marrow cells. There was a concern about a positive statistical significance in the clastogenic assay in human lymphocytes in the absence of metabolic activation at two high doses of 400-500 µg/ml. In a repeat assay this test was negative at doses up to 300 µg/ml. These assays were re-reviewed
The reason the first assay was positive in human lymphocytes was because of the excessive cytotoxicity and the poor quality of metaphases. In a second assay doses of 100-300 µg/ml were used, the drug was not clastogenic and the test was negative (see review in DFS).

2.6.6.5 Carcinogenicity

1. Study title: Oral gavage carcinogenicity study of omacor (ethyl ester K85) in mice

Key study findings: There was an increase in neoplastic uterine smooth muscle tumors, as increased incidences of leiomyoma and leiomyosarcoma were observed at a HD of 1860 mg/kg/day with omacor (combined tumors 9/51 vs 8/102 in controls, i.e 18% vs 8% in controls, p=0.019) in mice. The sponsor has not provided historical control data. However based on 12 Charles River laboratories Studies of 18 months duration the ranges are 0-4% for leiomyoma and 0-8% for leiomyosarcoma. In this study females were studies only up to week 88.

Adequacy of the carcinogenicity study and appropriateness of the test model:

The test model appears to be adequate, because mice are commonly used for carcinogenicity study in other lipid lowering drugs (statins). In a 13 week oral gavage dose range study in mice (0, 200, 1000, 4000 mg/kg/day, 12/sex/group), 4000 mg/kg/day produced no overt toxicity except an increase in liver weights by 4-7% at a HD and skin dermatitis at a HD in 2/2 males. Therefore in the current mouse assay, sponsor could have used doses of at least 4000 mg/kg/day. However, a high dose of

only 1860 mg/kg/day was used, which was way below MTD. In the current 80-88 week cac bioassay, no overt systemic toxicity was observed in mice, except accumulation of lipofuscin in mesenteric lymph nodes (males 6, 10, 4, 50, 86%; females 24,19; 13, 5, 76% at 0, 0, 200, 600, 2000 mg/kg/day respectively), at MD and/or HD, See Table on page 19. However, increased uterine smooth muscle tumors (leiomyoma and leiomyosarcoma) were observed at a HD (combined tumors 9/51 vs 8/102 in controls, p=0.019), but these were not considered significant. This is because these values did not reach p of ≤ 0.01, as confirmed by our FDA biometric reviewer. However, consideration should be given to the fact that the doses used in the mouse assay were below MTD and the assay was not carried out to the maximum 2-year time frame. Females were tested up to week 88 because of "enhanced survival" and males up to week 80. The sponsor suggests an anticipated decreased survival in the study based on high calorie/high fat consumption of the drug product. However, survival has not been effected in the study performed, nor is body weight significantly increased. The sponsor did not submit the dose selection/study design for ECAC review.

Evaluation of tumor findings: Increased incidence of neoplastic uterine smooth muscle tumors (leiomyoma and leiomyosarcoma) were observed at a HD when these two tumor types were combined (9/51 vs 8/102 in controls, p=0.019). These were not considered significant as these values did not reach p of ≤ 0.01. However, the mouse carci study does not provide adequate assessment of carcinogenicity because the dose selection and study duration were inadequate.

Study no.: 604/18-1050
Volume #, and page #: volume 14, page 001
Conducting laboratory and location: [redacted]
Date of study initiation: Study was initiated on 2/21/1992, In life ended 9/9/93, final report 8/94
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Batch number 9002M/P1210-3 was used with lot numbers 14, 15, 16, and 17. Purity data were in the certificate of analysis and the values were within the specification limits of the drug
CAC concurrence: Not obtained

Methods

Doses: 0, 115, 530, 1860 mg/kg/day (Nominal doses were suppose to be 100, 600, 2000 mg/kg/day but the dose formulations were approximately 115, 88 and 93% of nominal values)
Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: :CD(ICR)BR
Number/sex/group (main study): 51/sex/group
Route, formulation, volume: Orally by gavage in corn oil, 3 ml/kg. %-test article to vehicle (v/v) was 0, 3.3, 20.0, 66.7 at 0, 100, 600, 2000 mg/kg/day respectively.
Frequency of dosing: Once daily, for a minimum of 80 weeks in males, 88 weeks in females.
Satellite groups used for toxicokinetics or special groups: No

Age: Animals were obtained as weanlings, but age at the start of the study was not provided. Weights at initiation of the study in males was 23-41 g; females 19-28 g.
Animal housing: Three per polypropylene cage with stainless steel mesh floors
Restriction paradigm for dietary restriction studies: Animals had free access to mouse maintenance diet
Drug stability/homogeneity: The test article was prepared daily, solutions of the test article were stored at ambient temperature in a sealed container, and the air space above the solution was perged with nitrogen gas. Duplicate samples of the test formulations were taken in week 1, 13, 26, 39, 52, 65, 78, 89. No stability homogeneity data are provided
Dual controls employed: Yes
Interim sacrifices: No
Deviations from original study protocol: Study was designed for 80 weeks, but because of good survival in the females, the males were treated for a minimum of 80 weeks and females for a minimum of 88 weeks, but no data on why the survival rate would be lower was provided

Observation times

Analysis of formulations: The test article was prepared daily, solutions of the test article were stored at ambient temperature in a sealed container, and the air space above the solution was perged with [ ] Duplicate samples of the test formulations were taken in week 1, 13, 26, 39, 52, 65, 78, 89

Mortality: Twice daily

Clinical signs: Once daily, these were progressively reduced and discontinued in week 16, due to the absence of clinical signs
Body weights: At baseline, weekly to week 16, and monthly to week 80 (males), or week 88 (females) and at necropsy

Food consumption: weekly to week 16 and once a week every 4-weeks thereafter

Hematology: Blood samples were drawn in week 80 (males) or week 88 (females) for RBC & WBC.

Histopathology: Peer review: yes ( ), no (X). Histopathology was conducted only on control and high dosed mice at terminal necropsy, and in all tissues in mice that died, or if they had gross lesions or tissue masses.

Toxicokinetics: EPA, DHA , arachidonic acid and polyunsaturated n-6 fatty acid were measured in 0.5 ml blood samples from all mice that survived to terminal kill.

Results

Analysis of formulations: Nominal concentrations were suppose to be 3.3, 20 and 66.7% v/v for doses of 100, 600, 2000 mg/kg/day respectively. However, mean concentrations achieved were 3.8, 17.5, 61.8% v/v as shown below (Table 1).

Table 1: Concentration of the test article achieved vs the nominal doses in a mouse cac study

Week of study	CONCENTRATION (% v/v) OF KBS IN FORMULATION FOR GROUP NUMBER				
	1	2	3	4	5
Nominal	0	3.3	20.0	66.7	0
1	<0.1±0.0	2.7±0.0	17.1±0.4	63.2±0.6	<0.1±0.0
13	<0.1	2.89±0.05	17.40±0.20	61.58±0.40	<0.1
26	0	3.6	18.3	60.1	0
39	0	3.3	17.1	62.5	0
52	<0.1	6.3±0.2	17.9±0.1	62.7±0.3	<0.1
65	<0.1	6.1±0.1	17.5±0.1	59.5±0.2	<0.1
78	<0.1	2.6	17.3	62.3	<0.1
89	<0.1	2.7	17.1	62.9	<0.1
Mean	<0.1	3.8±1.5	17.5±0.4	61.8±1.4	<0.1

Additionally, the sponsor measured the peroxidation number of the test substance to determine the quality of the drug and if the drug had been oxidized. Peroxidation values in week 39 to week 89 were higher at a HD (values were in the range of [redacted] meq/kg), mean values for HD were 21 meq/kg and these were above the acceptance limit of 10 meq/kg according to the sponsor (see Table 2). However sponsor states that these are not of a concern as stated below in the text. It is possible that HD animals were not getting the active drug, or were only getting the partial dose of the active drug, as it was deteriorated.

Note that in a 13 week tox study in rats, in week 13 the peroxide levels in the drug in groups 1-3 were between 1-4 mmole/kg oil, but in group 4 (at a HD of 4000 mg/kg/day) were initially 12 mmole/kg and increased to 16 mmol/kg oil. This would suggest that in the present cac study the drug may be oxidized at certain doses and animals may not be getting the full amount of drug or were not continuously exposed to the drug.

Table 2: Peroxidation values of the test substance in a mouse cac study

Week of study	PEROXIDATION NUMBER (meq/kg) IN FORMULATION FOR GROUP NUMBER				
	1	2	3	4	5
Conc. (% v/v)	0	3.3	20.0	66.7	0
1	0.81±0.0	0.87±0.1	1.37±0.0	5.84±0.1	0.83±0.0
13	0.85±0.01	0.96±0.01	1.49±0.03	3.26±0.02	0.88±0.01
26	1.1±0.004	1.5±0.01	3.0±0.03	10.1±0.1	1.2±0.05
39	1.7±0.02	2.7±0.02	6.9±0.06	22.0±0.14	1.8±0.02
52	3.1±0.04	3.7±0.02	6.5±0.02	40.9±0.1	3.2±0.02
65	24.5±0.2	26.0±0.3	31.8±0.2	50.6±0.3	24.7±0.1
78	3.1	3.2	3.6	18.5	3.2
89	12.0	12.1	12.4	19.4	12.2
Mean	5.9±8.4	6.4±8.7	8.4±10.1	21.3±16.7	6.0±8.4

Background levels of lipid peroxidation are 6 meq/kg, at HD mean levels are increased by 3X.

Mortality: No effects on mortality were observed. Survival to terminal kill was not effected across all groups in both males and females (Table A). The Tables from the FDA biometric review show that in males in week 70, at least 68% survived in all groups, and in week 80 (at the termination of the study) more than 50% of animals survived (Table B). Similarly in females in week 80, at least 67% survived in all groups, which is why the sponsor extended the study to week 88 in females (Table C). The sponsor

indicates that the poor survival is likely a result of chronic administration of high caloric oils.

Table A. Mortality data in male and female mice from the sponsor (taken from NDA 21-654, volume 14, page 024

The survival to the terminal kill was as follows:

Group number	Group description	Dose level mg/kg/day	Survival (%) Males	Females
1	Control I	0	29(57)	28(55)
2	Low	100	26(51)	27(53)
3	Intermediate	600	31(61)	31(61)
4	High	2000	34(67)	25(49)
5	Control II	0	26(51)	30(59)

There was no significant difference ($p>0.05$) between the control groups or between the combined controls and the treated groups.

Table B. Mortality data in male mice from the FDA biometric review

Table 3 – Results of Statistical Analyses of Mortality Data for Male Mice

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	2000	0
Number of Deaths (* = Including 1 animal with accidental death)					
Weeks 0-50	4[**]	3	4[*]	6	7
Weeks 51-70	8	13[**]	6[*]	5	10[*]
Weeks 71-before term sac	10[*]	9[*]	10[*]	6	8[*]
Terminal Sacrifice Weeks	29	26	31	34	26
Unadjusted Mortality	19/51	22/51	17/51	17/51	23/51
Kaplan-Meier Estimate (Final)	0.388	0.448	0.349	0.333	0.458

Table C. Mortality data in female mice from the FDA biometric review

Table 4 – Results of Statistical Analyses of Mortality Data for Female Mice

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	2000	0
Number of Deaths (* = Including 1 animal with accidental death)					
Weeks 0-50	2	1	1	1	1
Weeks 51-75	7	10	13[**]	14[**]	6
Weeks 76-before term sac	14	13	6	11	14
Terminal Sacrifice Weeks	28	27	31	25	30
Unadjusted Mortality	23/51	24/51	17/51	23/51	21/51
Kaplan-Meier Estimate (Final)	0.451	0.471	0.353	0.475	0.412

Clinical signs: no treatment affects on fur staining, hair loss, rough coat, or sores/lesions.

Body weights: no treatment effects were observed

Food consumption: No treatment related food effects were observed.

Hematology: No treatment related effects were observed on RBC or WBC

Gross pathology: No drug related findings were observed.

Histopathology

Palpable Masses: No small or large moveable or stationary tissue masses were observed

NON-NEOPLASTIC:
Decedents: There were no significant differences in non-neoplastic lesions between controls and treated animals, except at a HD enhanced pigmentation (with slightly higher severity) was observed in the mesenteric lymph node (in both sexes), in adrenals in males and in ovaries in females.

Terminal: No significant differences in non-neoplastic lesions between controls and treated animals were observed. Mesenteric lymph node pigment was increased at a MD and/or HD and was observed in 6%, 10%, 4%, 50%, 86% at 0, 0, 115, 530, 1860 mg/kg/day in males. In females these values were 24, 19, 13, 5, 76% respectively. These values are provided in percentages above to better reflect the results, since the number examined varied in each group. Similarly enhanced pigmentation was observed at a HD with slightly higher degree of severity in the adrenal of males and in ovaries, see Table below. Selective staining indicated that the pigment contained lipofuscin (an end product of lipid peroxidation), but no gross or histopathological neoplastic lesions were observed.

Table below shows the histopathology findings at 0, 115, 530, 1860 and 0 mg/kg/day respectively in a cac study in mice

Text table 1: Incidence of selected non-neoplastic histopathology findings

Tissue and finding		Group and sex 1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Mesenteric lymph node	Number examined	48	24	22	50	50	49	24	21	51	47
Pigment	Grade -	45	23	11	7	45	37	21	20	13	38
	1	3	1	8	6	5	12	3	0	19	9
	2	0	0	3	27	0	0	0	1	16	0
	3	0	0	0	10	0	0	0	0	3	0
Adrenal gland	Number examined	51	25	20	51	51	51	25	20	51	51
Pigment	Grade -	44	22	15	31	45	30	13	16	26	29
	1	7	3	4	11	6	13	4	1	13	10
	2	0	0	1	6	0	6	5	2	6	6
	3	0	0	0	3	0	2	2	1	6	6
	4	0	0	0	0	0	0	1	0	0	0
Ovary	Number examined	0	0	0	0	0	51	46	46	51	51
Pigment	Grade -	0	0	0	0	0	41	37	37	32	47
	1	0	0	0	0	0	8	9	5	8	3
	2	0	0	0	0	0	2	0	4	9	0
	3	0	0	0	0	0	0	0	0	2	1

In the mesenteric node, the pigment was present within large cells, presumably macrophages, with a centrally placed nucleus and abundant pale brown/eosinophilic, finely granular cytoplasm. In the adrenal, the pigment was in cells at the cortico-medullary junction of the zona reticularis with abundant foamy, golden brown cytoplasm. The ovarian pigment was also present in cells with abundant foamy, golden brown cytoplasm. Staining of a small sample of mesenteric nodes, adrenals and ovaries from high dose animals indicated the pigment contained lipofuscin, an end product of lipid peroxidation. The increased levels of pigment in the high dose was possibly related to the altered lipid content of the test article compared with the control.

Neoplastic:
The drug increased uterine smooth muscle tumors (benign uterine leiomyoma and malignant uterine leiomyosarcoma) at a HD (combined tumors in drug treated mice were 9/51 vs 8/102 in controls, p=0.019), but these were not considered significant. This is because these values did not reach p of ≤ 0.01. These tumors were higher at 2000 mg/kg/day (8/102, 0/46, 1/37, 9/51 for two controls, LD, MD, HD respectively). Majority of uterine tumors encountered in control and treated cohorts were benign: a) leiomyoma incidence was 7/102, 0/46, 0/37, and 7/51 for combined controls, LD, MD, and HD respectively. b) While malignant leiomyosarcoma incidence was 1/102, 0/46, 1/37, and 2/51 for combined, LD, MD, and HD groups respectively. See Tables 1-6 (page 38)

Table. Uterine smooth muscle tumors (leiomyoma and leiomyosarcomas) incidences in decendents, at terminal sacrifice and in combined groups respectively.

	Doses: 0, 100, 600, 2000 mg/kg/day	
Incidences		
	Leiomyoma	Leiomyosarcomas
Decendents	0/44, 0/24, 0/20, 4/26	1/44, 0/24, 1/20, 0/26
Terminal sacrifice	7/58, 0/22, 0/17, 3/25	0/58, 0/22, 0/17, 2/25

Combined (decendents + terminal sacrifice)	7/102, 0/46, 0/37, 7/51	1/102, 0/46, 1/37, 2/51

Leiomyoma rate in combined controls, LD, MD, and HD groups in unscheduled decedents was 0/44, 0/24, 0/20, and 4/26, respectively, and for those sacrificed at the end of study was 7/58, 0/22, 0/17, and 3/25, respectively. Accordingly, since survival was not affected by treatment, the treatment-related doubling of the rate of leiomyoma was evidently in the context of an earlier tumor onset. Leiomyosarcoma rate in unscheduled decedents was 1/44, 0/24, 1/20, 0/26 respectively, and at terminal sacrifice was 0/58, 0/22, 0/17, 2/25 respectively. Thus two leiomyosarcomas were encountered at a HD at the terminal sacrifice. Sponsor's combined data in decendents + at terminal sacrifice are shown below.

Table. Mouse Tumor findings

TABLE 9.3
Group incidence: histopathology - neoplastic data - combined

Dose levels (mg/Kg/day): Gp 1 = 0, Gp 2 = 100, Gp 3 = 600 Gp 4 = 2000, Gp 5 = 0

PRINTED: 11-AUG-94
PAGE: 2

STUDY NUMBER: 60418

TABLE INCLUDES: SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL DEATH=ALL;FIND=B,M;SUBSET=ALL		--- NUMBER-OF-ANIMALS-AFFECTED ---									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	51	51	51	51	51	51	51	61	51	51
ADRENAL	NUMBER EXAMINED:	51	25	20	51	51	51	25	20	51	51
--B-SUBCAPSULAR CELL TUMOUR		0	0	0	0	0	1	0	0	0	0
--B-PHAEOCHROMOCYTOMA		1	0	0	0	0	0	1	0	0	0
--M-PHAEOCHROMOCYTOMA		0	1	0	0	0	1	0	0	0	0
KIDNEY	NUMBER EXAMINED:	51	26	21	51	51	51	28	24	51	51
--B-TUBULAR CELL ADENOMA		1	0	0	0	0	0	0	0	0	0
TESTIS	NUMBER EXAMINED:	51	26	21	51	51	0	0	0	0	0
--B-LEYDIG CELL TUMOUR		0	2	0	2	1	0	0	0	0	0
EPIDIDYMIS	NUMBER EXAMINED:	51	25	20	51	51	0	0	0	0	0
--B-BENIGN HISTIOCYTOMA		1	0	0	0	0	0	0	0	0	0
OVARY	NUMBER EXAMINED:	0	0	0	0	0	51	46	46	51	51
--B-LEIOMYOMA		0	0	0	0	0	1	0	0	0	0
--B-CYSTADENOMA		0	0	0	0	0	0	0	1	0	1
--B-LUTEOMA		0	0	0	0	0	0	2	0	0	1
--B-BENIGN GRANULOSA CELL TUMOUR		0	0	0	0	0	0	0	1	0	0
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	51	46	37	51	51
--B-HAEMANGIOMA		0	0	0	0	0	1	0	1	2	1
--B-POLYP		0	0	0	0	0	3	3	3	1	0
--B-LEIOMYOMA		0	0	0	0	0	5	0	0	7	2
--M-HISTIOCYTIC SARCOMA		0	0	0	0	0	0	2	3	2	3
--M-CARCINOMA		0	0	0	0	0	1	0	1	0	0
--M-LEIOMYOSARCOMA		0	0	0	0	0	0	0	1	2	1
--M-SARCOMA		0	0	0	0	0	1	1	0	0	0

Sponsor stated that uterine smooth muscle tumors are not uncommon in this strain and age of mouse, and indeed 8% of controls had this neoplasia in the current study submitted. There was essentially no excess leiomyoma or leiomyosarcoma in the smooth muscle of other tissues, nor was incidence of uterine carcinoma increased.

Historical control incidence: Sponsor did not provide historical range of spontaneous incidence of uterine smooth muscle tumors in the Crl:CD-1 BR mouse. Based on 12 studies, and 766 tissues examined, and a study duration of 18 months, it is 0-4% for leiomyoma, 0-2% for cervical leiomyoma, and 0-8% for leiomyosarcoma (Charles River Laboratories, "Spontaneous Neoplastic Lesions in the Crl:CD-1 BR mouse" March, 1995, compiled by Dr. P. Lang, consulting toxicologist: Table A2, p9). Accordingly, the combined incidence is evidently up to 14%, comparable to that seen at High Dose Omacor.

As indicated above these are common tumors, and their historical incidences range between 0-14% (see Dr. Defelice's review in DFS, appendix 2). In the current study these were present at 18% at a HD vs 8% in two controls combined, thus these appear to be at a higher rate than in the historical controls (0-14%). Additionally, consideration should also be given to the fact that the doses used in the mouse assay were below MTD and the assay was not carried out to the maximal 2-year time frame (as mice were sacrificed in weeks 80-88).

Since the concurrent combined control rates for the leiomyoma and consequently the combined leiomyoma and/or leiomyosarcoma were >1%, they were considered to be common tumor types. As a result, the increased incidences of those tumors in the high dose group over the combined control was not significant at p of 0.01.

Uterus	Combined Control	High Dose	Reviewer's p	Sponsor's p
Leiomyoma	7/102 (= 5/51 + 2/51)	7/51	0.0752	NA
Leiomyosarcoma	1/102 (= 0/51 + 1/51)	2/51	0.2139	NA
Combined tumors	8/102 (= 5/51 + 3/51)	9/51	0.0295	0.019

In conclusion: The mosue carci study does not provide adequate assessment of carcinogenicity because the dose selection and study duration were inadequate. In mice, uterine smooth muscle tumors (leiomyoma + leiomyosarcoma) were increased to 17% at a HD vs 8% in controls (p=0.019). These were not statistically significant.

Toxicokinetics: Plasma Levels in male/female mice at terminal sacrifice (mg/ml) in a cac study

Plasma levels of EPA, DHA, arachidonic acid (AA) and polyunsaturated n-6 fatty acid at the end of the study (in weeks 80-88) were measured in 0.5 ml blood samples from all mice which survived to terminal kill. Plasma concentrations of EPA levels increased with the dose (males 0.4-1.4, 2.6, 10.3, 29.2 mg/L at 0, 115, 530, 1860 mg/kg/day respectively, females 3-3.4, 2.3, 12.1, 41.3 mg/L respectively), but DHA values did not change (males 130-150, 166, 176, 179 mg/L; females 121, 126, 135, 141 mg/L respectively) see Table below. This would indicate that the treated mice did not receive significant levels of Omacor. However sponsor states that the dose related increases in both EPA and DHA levels were observed and that these results are similar to studies in other species. In human subjects, the plasma EPA levels increase by 5-8 fold with 4-8 g/day dose of omacor, and DHA levels by only 2 fold at these doses in a 2-week study, thus increases in DHA are not dose dependent.

This Table is taken from Dr. DeFelice's review of NDA 21-274.

Table 2: Plasma lipid data (Vol. 1.23, p 008)

GROUP: MALES	EPA(mg/ml)	DHA(mg/ml)	Arachidonic Acid	Poly unsat. N-6 fatty acids
Control 1	0.4±0.8	150±40	290±70	600±140
Control 2	1.4±0.8	130±40	250±70	550±130
Low Dose	2.6±1.2	170±32	210±70	510±110
Mid Dose	10.3±4.5	180±55	140±45	470±130
High Dose	29±15	180±55	80±25	380±110
GROUP: FEMALES	EPA(mg/ml)	DHA(mg/ml)	Arachidonic Acid	Poly unsat. N-6 fatty acids
Control 1	3.0±5.3	121±30	214±68	454±131
Control 2	3.4±5.1	121±30	213±62	449±110
Low Dose	2.3±0.7	126±48	166±65	391±161
Mid Dose	12.1±12.7	135±35	110±31	326±78
High Dose	41.3±30.9	141±21	72±19	275±45

In summary in a 80-88 week bioassay in mice, increased incidence of neoplastic uterine smooth muscle tumors (combined leiomyoma and leiomyosarcoma) were observed at a HD compared to controls (9/51 vs 8/102 in controls, I,e. 18% vs 8% in controls p=0.019). These were not considered significant as these values did not reach p of ≤ 0.01. However, doses used in the study were 'no effect doses' and were not based on MTD, the assay was not carried out to the optimal 2-year duration. Thus, this mouse carci study does not provide adequate assessment of carcinogenicity because the dose selection and study duration were inadequate.

2. Study title: Oral gavage carcinogenicity study of omacor (ethyl ester K85) in rats

Key study findings: No significant neoplastic or non-neoplastic findings were observed in rats compared to controls. However, the study duration for females was 89 weeks and for males was 101 weeks.

Adequacy of the carcinogenicity study and appropriateness of the test model:
The test model appears to be adequate, because rats are commonly used for carcinogenicity study in other lipid lowering drugs (statins). In a 13 week rat study 4000 mg/kg/day produced severe desquamation and tail necrosis at a HD, which led to their sacrifice in week 9, indicating that 4000 mg/kg/day is at or above MTD. In a 1-year study doses up to 2000 mg/kg/day were well tolerated except gross (mottled liver in 19/40 vs 5/40 in controls) and histopathology findings in liver (increased vacuolation in 23/40 vs 8/40 in controls, and increased fatty livers present in 23/40 vs 12/40 in controls) and lungs (increased foamy histiocytes in 17/40 vs 13/40 in controls) were observed at 2000 mg/kg/day, additionally HD males had increased biliary proliferation (8/20 vs 3/20 in controls), and adrenal vacuolation (6/20 vs 1/20 in controls). This suggests that MTD is between 2000 and 4000 mg/kg/day. However, a high dose of only 1800 mg/kg/day was selected in the current cac study. In the current 89-101 week cac bioassay, no overt systemic toxicity was observed except for the yellow/brown discoloration of the fur (at a HD in both sexes, 96-100% vs 8-48% in controls). No significant differences in body weights were noted. In gross pathology, increased incidences of mottled liver in

males (12/50, 12/50, 18/50, 28/50, 31/50 at 0, 0, 90, 540, 1800 mg/kg/day respectively) and pale pulmonary foci at MD & HD in both sexes (males 0/50, 3/50, 3/50, 4/50, 11/50; females 1/50, 0/50, 2/50, 1/50, 11/50 respectively) were observed. No significant histopathology findings, neoplastic or non-neoplastic lesions were observed in the drug treated rats vs controls. In summary, the high doses of 1800 mg/kg/day used here in the cac study are lower than MTD, however these did not produce significant toxicity/mortality or carcinogenicity in rats. This was also confirmed by our FDA stat reviewer.

The study was initially designed to be of 104 weeks duration, however males were tested up to week 101 and females up to week 89. The sponsor anticipated decreased survival in the study based on high calorie/high fat consumption of the drug product. In females (in the controls, LD and MD), survival rates were down to below 50% around the middle of the week 80, which is why sponsor terminated the study in week 89. In males in week 90, at least 50% survived in all groups, therefore in males the study was allowed to proceed till week 101. The sponsor notes that 30% of males and 85% females that were early decendents had mammary and pituitary tumors or both as a cause of death. The poor survival is attributed to chronic administration of high caloric lipids. However, survival was not effected in male rats, nor were the body weights significantly increased. The sponsor did not submit the dose selection/study design for ECAC review prior to actually conducting the cac study.

Evaluation of tumor findings: In both male and female rats, no significant increases in the incidences of any tumors (neoplastic or non-neoplastic) were observed in the high dose group when compared to two combined control groups

Study no.: 604/17-1050
Volume #, and page #: volume 18, page 246
Conducting laboratory and location: [ ]
Date of study initiation: In life began 12/90, necropsies finished 8/92, final report 1/94
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Following batch numbers were used. Purity data were in the certificate of analysis and the values were within the specification limits of the drug.

HE Lot number	Batch number	Quantity kg	Date of receipt at HE
11	1020		14 November 1990
12	8903WR/P1210-2		28 January 1991
13	8903WR/P1210-2		23 April 1991
14	9002M/P1210-3		25 November 1991
15	9002M P1210-3		26 May 1992
16	9002M P1210-3		29 May 1992

CAC concurrence: Not obtained

Methods

Doses: Nominal doses were suppose to be 100, 600, 2000 mg/kg/day, but achieved doses were 0, 90, 540, 1800 mg/kg/day and thus were approximately 10% lower.
Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: CD(SD)BR
Number/sex/group (main study): 50/sex/group
Route, formulation, volume: Orally by gavage in corn oil. All rats received 4 ml/kg. Controls got pure corn oil and treatment groups got the same total volume but Omacor comprising 2.5%, 15%, and 50% of the total. Thus, %-test article to vehicle (v/v) was 0, 2.5, 15, 50% at 0, 100, 600, 2000 mg/kg/day respectively.

Frequency of dosing: Once daily
Satellite groups used for toxicokinetics or special groups: No
Age: 6-weeks old
Animal housing: five/cage, in stainless steel mesh cages
Restriction paradigm for dietary restriction studies: Animals had free access to rat maintenance diet.
Drug stability/homogeneity: The test article was prepared daily, but no stability homogeneity data were provided
Dual controls employed: Yes
Interim sacrifices: No
Deviations from original study protocol: Study was designed for 104 weeks but because of lower expected survival, males were sacrificed in week 101 and females in week 89 after treatment, but no data why the survival was expected to be lower were provided

Observation times
Analysis of formulations: The test article was prepared daily, solutions of the test article were stored at ambient temperature in a sealed container, and the air space above the solution was perged with nitrogen gas. Duplicate samples of the test formulations were taken in weeks 1, 8, 13, 26, 39, 52, 65, 78, 91, 102. These were frozen and dispatched in [ ] to the sponsor for analysis.

Mortality: Twice daily

Clinical signs: Once daily
Body weights: At baseline, weekly to week 16, and monthly to week 100 (males), or week 88 (females)

Food consumption: weekly to week 16 and once a week every 4-weeks thereafter

Histopathology: Peer review: yes ( ), no (X). Histopathology was conducted only on control and high dosed rats at terminal necropsy plus in all tissues in rats that died, or if they had gross lesions or tissue masses. However, liver histopathology was done at low and mid doses due to a higher biliary hyperplasia seen at a HD.

Toxicokinetics: Blood samples were only collected from 10 males in each group at terminal necropsy for plasma analysis. Due to some error no blood samples were collected from females. The actual administered doses were 90, 540, 1800 mg/kg/day

respectively in three treated groups. Blood samples were also drawn in week 52 & 77 (males) and week 90 (females) and week 101 (males) for RBC & WBC

Results

Analysis of formulations: Nominal concentrations were suppose to be 2.5, 15 and 50% v/v for doses of 100, 600, 2000 mg/kg/day respectively. However mean achieved concentration were 2.5, 13.4 and 45.8% v/v as shown below (Table 1).

Table 1: Concentration of the test article achieved vs the nominal doses

Week of study	CONCENTRATION (% v/v) OF K85 IN FORMULATION FOR GROUP NUMBER				
	1	2	3	4	5
Nominal	0	2.5	15.0	50.0	0
1	0	4.29 ± 0.06	13.94 ± 0.04	46.38 ± 0.29	0
8	0	2.18 ± 0.23	13.17 ± 0.12	44.93 ± 0.61	0
13	0	2.19 ± 0.03	13.60 ± 0.18	48.27 ± 0.31	0
26	0	2.18 ± 0.03	13.13 ± 0.10	45.36 ± 0.42	0
39	0	2.32 ± 0.00	13.65 ± 0.23	44.19 ± 0.30	0
52	0	2.36 ± 0.02	13.68 ± 0.05	46.24 ± 0.13	0
65	< 0.1	2.5 ± 0.0	12.8 ± 0.2	45.8 ± 0.2	< 0.1
78	< 0.1	2.07 ± 0.07	13.25 ± 0.05	45.09 ± 0.42	< 0.1
91	0	2.5	13.7	46.0	0
102	0	2.3	13.4	45.5	0
Mean	0	2.49 ± 0.65	13.43 ± 0.34	45.78 ± 1.10	0

Additionally, the sponsor measured the peroxidation number of the test substance to determine the quality of the drug and if the drug had been oxidized. Peroxidation values in weeks 8-26 at a HD (in group 4) were higher (values were in the range of meq/kg) and these were above the acceptance limit of 10 meq/kg according to the sponsor (see Table 2). However sponsor states that these are not of a concern as stated below in the text. It is possible that HD animals were getting a lower dose of the active drug during weeks 8-26, as it was partially oxidized. This would suggest that in a current cac study animals were either not getting the full amount of drug, or getting a deteriorated drug. This level of peroxidation is not likely to be significant. The bile duct hyperplasia, foamy histiocytes (lungs), and cholesterol granuloma observed in the HD group are likely related to altered lipid metabolism suggesting that animals were receiving intact (non-degraded) fatty acids.

Table 2: Peroxidation values of the test substance

Week of study	PEROXIDATION NUMBER (meq/kg) IN FORMULATION FOR GROUP NUMBER				
	1	2	3	4	5
Conc. (% v/v)	0	2.5	15.0	50.0	0
1	1.73 ± 0.02	1.81 ± 0.01	2.08 ± 0.04	5.99 ± 0.13	1.75 ± 0.01
8	2.06 ± 0.03	2.93 ± 0.02	7.25 ± 0.00	21.17 ± 0.36	2.05 ± 0.04
13	2.1 ± 0.1	2.9 ± 0.1	7.0 ± 0.1	29.3 ± 0.1	2.1 ± 0.1
26	1.06 ± 0.01	1.72 ± 0.01	4.90 ± 0.02	13.73 ± 0.07	1.05 ± 0.01
39	1.40 ± 0.02	1.72 ± 0.02	3.29 ± 0.04	7.69 ± 0.05	1.45 ± 0.03
52	1.7 ± 0.0	2.0 ± 0.0	3.3 ± 0.1	6.9 ± 0.1	1.8 ± 0.0
65	0.66 ± 0.0	0.89 ± 0.1	2.09 ± 0.1	5.72 ± 0.1	0.60 ± 0.0
78	0.73 ± 0.01	1.03 ± 0.01	2.51 ± 0.02	6.68 ± 0.04	0.76 ± 0.01
91	1.1 ± 0.01	1.2 ± 0.02	1.7 ± 0.03	3.1 ± 0.02	1.2 ± 0.06
102	1.7 ± 0.01	2.1 ± 0.03	4.1 ± 0.05	10.0 ± 0.16	1.7 ± 0.01
Mean	1.42 ± 0.52	1.83 ± 0.70	3.82 ± 2.00	11.03 ± 8.22	1.45 ± 0.52

Sponsor's explanation on formulation analysis is provided below

4.1 Analysis of formulations (Appendix 4)
Analysed of formulations supplied by the study sponsor are included as appendix 4. Concentration analysis generally confirmed that formulations have been prepared correctly. Analysis of the formulation for group 2 at Week 1 showed a K85 concentration of 4.29± 0.06 % while the nominal concentration was 2.5 %. The measured mean concentration for groups 3 (13.43 %) and 4 (45.78 %) were somewhat lower than the nominal concentrations which were 15.0 and 50.0 %. These deviations are not considered to bias the study.

Analysis of peroxidation number measures the quality of the test substance and reflects to what extent the unsaturated material has been oxidized. As expected, the value increases with increasing content of K85. The specification of K85 states a maximum value of 10 mgeq/kg. Three of the formulation samples analyzed showed values above this (formulations for group 4 at Weeks 8, 13 and 26). The test substance might have been exposed to atmospheric oxygen during formulation, return transport to the sponsors laboratory and during analysis, therefore these values were not of concern since they did not necessarily reflect the quality of the formulation at dosing. No samples showed extremely high peroxidation number.

Mortality:
No effects on mortality were observed. Survival to terminal kill was not effected across all groups in rats (Table D). In males in week 90, at least 50% survived in all groups (Table E). In females in beginning of week 80, again at least 50% survived in all groups (Table F). However, the controls, LD and MD survival rates were down to below 50% around the middle of the week 80, which is why sponsor terminated the study in week 89. The sponsor notes that 30% of males and 85% females that were early decendents had mammary and pituitary tumors or both as a cause of death. The poor survival is attributed to chronic administration of high caloric lipids

Table D. Mortality data in male and female mice from the sponsor (taken from NDA 21-654, volume 18, page 270)

Group number	Group description	Dose level mg/kg/day	Group size	Survival (%) Male	Female
1	control	0	50	24(48)	22(44)
2	low	100	50	23(46)	24(48)
3	intermediate	600	50	22(44)	19(38)
4	high	2000	50	27(54)	28(56)
5	control II	0	50	25(50)	26(52)

Table E. Statistical analysis of mortality data for male rats

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	2000	0
Number of Deaths (* = Including 1 animal with accidental death)					
Weeks 0-52	1*	2*	4*	5**	2
Weeks 53-78	5	4	9*	6	6
Weeks 79-92	14	14*	10	8	9
Weeks 93-before term sac	7	8	7	6	11*
Terminal Sacrifice Weeks	23	22	20	25	22
Unadjusted Mortality	26/50	26/50	28/50	22/50	27/50
Kaplan-Meier Estimate (Final)	0.531	0.540	0.582	0.468	0.542

Table F. Statistical analysis of mortality data for female rats

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	20000	0
Number of Deaths (* = Including 1 animal with accidental death)					
Weeks 0-50	1*	2*	3	5*	4*
Weeks 51-75	13*	12	11*	5	10
Weeks 76-before term sac	14	14	18	12	11
Terminal Sacrifice Weeks	22	22	18	28	25
Unadjusted Mortality	26/50	27/50	31/50	21/50	24/50
Kaplan-Meier Estimate (Final)	0.542	0.551	0.632	0.428	0.489

Clinical signs: Fur staining: yellow/brown discoloration of the fur in week 89:
23, 28; 48, 39, 96% females respectively
8, 48; 52, 59 100% males respectively

Body weights: No significant effects were observed.
Food consumption: Higher in HD males 1st 13 weeks of study; no effects in females
Hematology: The WBC count was not altered at the end of the study

Gross pathology: At a HD, mottled liver was observed in males, and pale foci in the lungs of HD males+ females.

At 0, 0, 90, 540, 1800 mg/kg/day
MALES (no./50) FEMALES (no./50) LIVER:

Mottled:	12, 12, 18, 28, 31	17, 12; 13, 15, 17
Dark focus:	1, 1, 0, 5, 4	0, 0, 1, 0, 0

LUNG

Pale focus:	0, 3, 3, 4, 11	1, 0, 2, 1, 11
Pale area:	1, 0; 0, 0, 5	0, 0, 2, 3, 4

Thus, gross pathology findings in the liver were observed in males at MD & HD (mottled liver in 24/100, 18/50, 28/50, 31/50 at 0, LD, MD, HD respectively) and lungs at a HD in both sexes (pale foci in 4/200, 5/100, 5/100, 22/100 respectively).

Histopathology

Rat non-neoplastic: Only controls and HD rats were examined fully except for certain tissues (liver, kidney and lung). No significant histopath findings were observed in combined data of decedent animals and at terminal sacrifice, Table G. However, in decedent males, liver histopath showed a slightly higher incidence (53-64% vs 42% in controls) of slight to moderate biliary proliferation at MD/HD (males 23/55, 13/27, 16/30, 16/25 at 0, LD, MD, HD respectively). These were also increased in females at a HD and were 7/53, 7/28, 5/32, 6/22 respectively (or at a HD 27% vs 13% in controls). Adrenal vacuolation was similarly higher in decedent males (2/55, 3/28, 4/30, 6/25 respectively), but not effected in females.

Table G. Incidences of selected non-neoplastic histopathology findings in rats are shown below (the data are from decendents + terminal sacrifice).

Text table 1: Incidence of selected non-neoplastic histopathology findings

Tissue and finding		Group and sex 1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Liver	Number examined	50	49	50	50	50	50	50	50	50	50
Biliary proliferation	Grade -	22	26	19	10	23	39	36	37	29	35
	1	19	14	26	22	24	8	12	12	17	11
	2	9	9	5	14	3	3	2	1	4	3
	3	0	0	0	4	0	0	0	0	0	1
Kidney	Number examined	50	31	29	49	49	50	28	32	50	50
Increased pigment		0	0	1	5	0	2	0	3	15	3
Tubular vacuolation		16	13	13	20	18	13	6	7	19	13
Lung	Number examined	50	30	35	50	50	50	29	37	50	50
Foamy histiocytes	Grade -	30	19	14	11	26	37	16	21	15	40
	1	19	10	16	18	21	12	10	13	27	9
	2	1	1	4	14	2	1	3	3	5	1
	3	0	0	1	4	1	0	0	0	3	0
	4	0	0	0	3	0	0	0	0	0	0
Pigmented		3	3	2	11	4	3	1	3	8	0
Cholesterol granuloma		4	3	2	19	5	2	2	1	8	2

These findings are attributable to lipofuscin accumulation, an end organ product of lipid peroxidation. Vacuolation & biliary proliferation are result of lipid administration

Neoplastic: No drug related effects were observed. However, note that there was same problem as above in terms of thoroughness of exam. See Tables 7 to 16 (page 44).

Toxicokinetics: Plasma levels of EPA, DHA, AA and polyunsaturated n-6 fatty acid at the end of the study (in weeks 80-88 in males only) were measured in 0.5 ml blood samples from 10 males/group. At LD & MD, no difference between control and treated rats was observed in the two major components of Omacor (i.e. EPA and DHA). The plasma levels of EPA increased only at a HD by 3-folds (3.5, 2.9, 3.5, 10 mg/L at 0, 90, 540, 1800 mg/kg/day respectively). In contrast DHA levels were unchanged at all doses (21, 29, 32, 29 mg/L respectively), see Table below. Levels of arachidonic acid and polyunsaturated n-6 fatty acid were actually decreased

LEVELS IN MALE RATS AT TERMINAL SACRIFICE (mg/L)

GROUP	EPA# (mg/l)	DHA# (mg/l)	Arachidonic Acid	poly unsat. n-6 fatty acids
CONTROL I II	3.5±1.8* 0.9±0.2	20±10 21± 9	160±75 160±50	250±100 260± 70
90 mkd	2.9±2	29±16	110±60	190± 90
540 mkd	3.5±2	32±17	66±33	160±60
1800 mkd	10±11	29±21	45±24	120±50

Sponsor's also measured the levels of other fatty acids in the rat cac study at the terminal sacrifice, as shown below.

The resulting data from the fatty acid analysis are presented in the following table. The data are also grouped as saturated, monounsaturated, polyunsaturated omega-6 and polyunsaturated omega-3 fatty acids.

TABLE 32

Group/ Sex (dose level)[a]		Fatty Acid Concentration in Rat Serum (mg/L) at Terminal Euthanasia						
		EPA 20:5ω3	DHA 22:6ω3	AA 20:4ω6	Saturated	Monoun-saturated	Poly-unsatu-rated ω6	Poly-unsatu-rated ω3
1M (0)	Mean	3.5	20.3	163.0	335.7	36.4	254.9	29.8
	SD	1 8	10.2	75.0	102.9	15.6	103.2	13 3
2M (100)	Mean	2.9	29.4	105.2	273.3	27.8	190.0	35.7
	SD	1.6	15.5	58.8	102.3	12.2	89.9	18.5
3M (600)	Mean	3.5	32.2	66.2	265.5	26.8	158.4	41.6
	SD	1.6	17.4	32.5	61.9	7.5	54.7	20.1
4M (2000)	Mean	10 0	29.1	45.1	240.8	27.0	118.9	45.2
	SD	10.6	20.9	23.5	68.2	12.5	53.4	31.4
5M (0)	Mean	0.9	21 2	162.0	356.8	44.0	262.2	27.5
	SD	0 2	8.5	50.9	110.3	15.9	69.7	9.7

[a] Dose level = mg/kg/day
M = male
[b] SD = standard deviation

EPA analysis showed that the content of this fatty acid in serum increased with increased dosing of K85. EPA values were 0.9 and 3.5 mg/L in the control groups, while in the high-dose group it was 10.0 mg/L. DHA analysis showed much higher values for the control groups than those observed for EPA. The mean values for DHA in the control groups were 20.3 and 21.1 mg/L respectively, while in all dosed groups the levels were about 30 mg/L. The increases in EPA and DHA with increasing doses of K85 were accompanied by a marked decrease in arachidonic acid.

Grouped together, the omega-6 polyunsaturated fatty acids (18:2, 20:4) decreased with increasing K85 doses. The saturated fatty acids (14:0, 16:0, and 18:0) also decreased slightly with increasing K85 doses. The monounsaturated fatty acids (16:1, and 18:1) were constant, while the omega-3 polyunsaturated fatty acids (20:3, 20:5, 22:5, and 22:6) increased minimally with increasing dose.

In this study, reductions in arachidonic acid with increasing doses of K85 were marked, and decreases in arachidonic acid were larger than increases in EPA and DHA. This is in agreement with the results of similar studies with other species.[40,41,42] This is apparently due to some kind of competition between omega-3 and omega-6 fatty acids for incorporation into serum-phospholipids. However, this observation might also be due to the control substance, corn oil, which has a high content of linoleic acid (18:2ω6) that might be converted to arachidonic acid. Since corn oil was not only the control substance, but was also the vehicle, increasing doses of K85 were accompanied by decreasing doses of corn oil.

In summary, in both sexes in rats, no significant mortality or increases in tumors (neoplastic or non-neoplastic) were observed at doses up to 1800 mg/kg/day when compared to two combined controls. However, a high dose of 2000 mg/kg/day as selected (in this study) was appropriate, as 4000 mg/kg/day dose exceeded MTD. The study duration was short (101 weeks in males and 89 weeks in females) due to sponsor's decision to terminate the study at 50% survival. The sponsor attributes a proposed decreased survival effect (NS) to high caloric /lipid content of the test article or vehicle (corn oil). However, body weight is unaffected. Obesity is linked to hormone mediated neoplasia. The sponsor notes that 30% males and 85% females that were moribund had mammary, pituitary or both tumors identified as the cause of death.

Mouse CAC data:
Table 1, mouse data

Group incidence: histopathology - neoplastic data - combined

Dose levels (mg/Kg/day): Gp 1 = 0, Gp 2 = 100, Gp 3 = 600 Gp 4 = 2000, Gp 5 = 0

PRINTED: 11-AUG
PAGE: 1

STUDY NUMBER: 60418

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=ALL

		--- NUMBER-OF-ANIMALS-AFFECTED									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	51	51	51	51	51	51	51	51	51	51
** TOP OF LIST **											
SKIN SUBCUTIS	NUMBER EXAMINED:	51	30	23	51	51	51	25	22	51	51
--B-HISTIOCYTOMA		0	1	0	0	0	0	0	0	0	0
--M-FIBROSARCOMA		0	1	0	0	0	0	0	0	0	0
--M-SARCOMA		0	0	0	1	1	1	0	0	0	0
--M-HAEMANGIOSARCOMA		0	0	0	0	1	0	0	0	0	0
MAMMARY GLAND	NUMBER EXAMINED:	0	0	0	0	0	49	24	20	51	48
--M-CARCINOMA		0	0	0	0	0	2	1	3	1	1
FEMUR + MARROW	NUMBER EXAMINED:	51	25	21	51	51	51	24	20	51	51
--B-HAEMANGIOMA		1	0	0	1	0	0	0	0	0	0
--M-OSTEOSARCOMA		0	0	0	0	0	0	1	0	0	0
STERNUM + MARROW	NUMBER EXAMINED:	51	28	21	51	51	51	25	20	51	51
--B-HAEMANGIOMA		0	0	0	0	0	0	1	0	0	0
LIVER	NUMBER EXAMINED:	51	37	32	51	51	51	27	22	51	51
--B-HAEMANGIOMA		1	1	0	1	3	1	1	1	0	0
--B-ADENOMA		19	15	14	15	8	0	0	0	0	0
--M-CARCINOMA		3	0	3	0	1	0	1	0	0	0
SPLEEN	NUMBER EXAMINED:	51	29	20	51	51	51	29	24	51	50
--B-HAEMANGIOMA		1	0	0	0	0	1	0	0	0	0
MES.LYMPH NODE	NUMBER EXAMINED:	48	24	22	50	50	49	24	21	51	47
--B-HAEMANGIOMA		0	0	0	0	0	0	0	1	0	0
STOMACH	NUMBER EXAMINED:	47	29	23	50	48	50	29	23	50	48
--B-SQUAMOUS PAPILLOMA		0	0	0	1	0	0	0	0	0	0

Table 2, mouse data.

Group incidence: histopathology - neoplastic data - combined

Dose levels (mg/Kg/day): Gp 1 = 0, Gp 2 = 100, Gp 3 = 600 Gp 4 = 2000, Gp 5 = 0

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PAGE: 2

STUDY NUMBER: 60418

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED									
SEX:		----------MALE----------					----------FEMALE----------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		51	51	51	51	51	51	51	51	51	51
ADRENAL	NUMBER EXAMINED:	51	25	20	51	51	51	25	20	51	51
--B-SUBCAPSULAR CELL TUMOUR		0	0	0	0	0	1	0	0	0	0
--B-PHAEOCHROMOCYTOMA		1	0	0	0	0	0	1	0	0	0
--M-PHAEOCHROMOCYTOMA		0	1	0	0	0	1	0	0	0	0
KIDNEY	NUMBER EXAMINED:	51	26	21	51	51	51	28	24	51	51
--B-TUBULAR CELL ADENOMA		1	0	0	0	0	0	0	0	0	0
TESTIS	NUMBER EXAMINED:	51	26	21	51	51	0	0	0	0	0
--B-LEYDIG CELL TUMOUR		0	2	0	2	1	0	0	0	0	0
EPIDIDYMIS	NUMBER EXAMINED:	51	25	20	51	51	0	0	0	0	0
--B-BENIGN HISTIOCYTOMA		1	0	0	0	0	0	0	0	0	0
OVARY	NUMBER EXAMINED:	0	0	0	0	0	51	46	46	51	51
--B-LEIOMYOMA		0	0	0	0	0	1	0	0	0	0
--B-CYSTADENOMA		0	0	0	0	0	0	0	1	0	1
--B-LUTEOMA		0	0	0	0	0	0	2	0	0	1
--B-BENIGN GRANULOSA CELL TUMOUR		0	0	0	0	0	0	0	1	0	0
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	51	46	37	51	51
--B-HAEMANGIOMA		0	0	0	0	0	1	0	1	2	1
--B-POLYP		0	0	0	0	0	3	3	3	1	0
--B-LEIOMYOMA		0	0	0	0	0	5	0	0	7	2
--M-HISTIOCYTIC SARCOMA		0	0	0	0	0	0	2	3	2	3
--M-CARCINOMA		0	0	0	0	0	1	0	1	0	0
--M-LEIOMYOSARCOMA		0	0	0	0	0	0	0	1	2	1
--M-SARCOMA		0	0	0	0	0	1	1	0	0	0

Table 3, **mouse data**.

Group Incidence: histopathology - neoplastic data - combined

Dose levels (mg/Kg/day): Gp 1 = 0, Gp 2 = 100, Gp 3 = 600 Gp 4 = 2000, Gp 5 = 0

PRINTED: 1
PAGE: 3

STUDY NUMBER: 6

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=ALL

		--- NUMBER-OF-ANIMALS-AFFECT									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	51	51	51	51	51	51	51	51	51	51
VAGINA	NUMBER EXAMINED:	0	0	0	0	0	51	25	20	50	51
--B-LEIOMYOMA		0	0	0	0	0	0	0	0	1	0
--M-HAEMANGIOSARCOMA		0	0	0	0	0	0	0	0	0	1
LUNG	NUMBER EXAMINED:	51	31	25	51	51	51	31	28	51	51
--B-ADENOMA		5	9	6	10	10	7	8	3	2	4
--M-CARCINOMA		2	1	1	1	1	1	1	0	0	1
THYROID	NUMBER EXAMINED:	51	25	19	50	51	51	25	20	51	50
--B-FOLLICULAR ADENOMA		0	0	0	1	0	1	1	0	0	0
PITUITARY	NUMBER EXAMINED:	49	23	20	48	48	51	23	21	49	51
--B-ADENOMA		0	0	0	0	0	0	1	1	0	0
SPINAL CORD	NUMBER EXAMINED:	51	25	20	51	51	51	24	20	51	51
--M-HAEMANGIOSARCOMA		0	0	0	1	0	0	0	0	0	0
HAEM/LYMPH/RETIC	NUMBER EXAMINED:	51	25	20	51	51	51	26	22	51	51
--M-LYMPHOMA		0	0	1	0	0	0	0	1	0	1
--M-LYMPHOMA MIXED		0	2	1	0	0	1	4	2	3	3
--M-LYMPHOMA LYMPHOCYTIC		1	1	2	2	3	4	2	2	2	1
--M-LYMPHOMA HISTIOCYTIC		0	0	0	0	0	0	0	1	0	0
--M-LEUKAEMIA LYMPHOCYTIC		0	0	0	0	1	0	0	0	0	0
BONE	NUMBER EXAMINED:	1	1	0	0	0	3	0	0	1	0
--B-OSTEOFIBROMA		0	0	0	0	0	1	0	0	0	0
--M-OSTEOSARCOMA		0	1	0	0	0	0	0	0	1	0
PENIS	NUMBER EXAMINED:	2	1	1	2	0	0	0	0	0	0
--M-SQUAMOUS CELL CARCINOMA		0	0	0	1	0	0	0	0	0	0

Table 4, **mouse data**.

TABLE 9.1
Group incidence: histopathology - neoplastic data - combined

Dose levels (mg/kg/day): Gp 1 = 0, Gp 2 = 100, Gp 3 = 600 Gp 4 = 2000, Gp 5 = 0

PRINTED: 1
PAGE: 4

STUDY NUMBER: 6

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECT									
SEX:		----------MALE----------					----------FEMALE----------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		51	51	51	51	51	51	51	51	51	51
CONNECTIVE TISS	NUMBER EXAMINED:	1	0	0	3	0	0	0	0	0	1
--M-OSTEOSARCOMA		1	0	0	0	0	0	0	0	0	0
--M-HISTIOCYTIC SARCOMA		0	0	0	0	0	0	0	0	0	1
ABDOMINAL CAVITY	NUMBER EXAMINED:	0	1	2	2	1	4	4	1	3	5
--B-HAEMANGIOMA		0	0	0	0	0	0	0	1	0	0
CRANIAL CAVITY	NUMBER EXAMINED:	0	0	0	0	0	0	0	1	0	0
--B-OSSIFYING FIBROMA		0	0	0	0	0	0	0	1	0	0
HARDERIAN GLAND	NUMBER EXAMINED:	1	0	1	1	0	0	0	0	1	0
--B-ADENOMA		1	0	1	1	0	0	0	0	0	0
--M-CARCINOMA		0	0	0	0	0	0	0	0	1	0

** END OF LIST **

Table 5, mouse data.

Tumour incidence females - statistical analysis
Results of tests for a decreasing and increasing incidence

Tumour type	Number of tumour bearing animals	Gp 1	Gp 4	Gp 5	Included in analysis	p-values for increased tumour incidence Groups 1&5 vs Group 4	p-values for decreased tumour incidence Groups 1&5 vs Group 4	Method of analysis
Lung tumours	F	1	0	0	ALL	0.938	0.062	L
	NF	6	2	5				
	ALL	7	2	5				
Uterus ♀ tumours	F	1	0	0	ALL	0.864	0.479	P
	NF	3	1	0				
	ALL	4	1	0				
Uterus ♀ tumours	F	0	0	1	ALL	0.019*	0.981	L
	NF	5	9	2	NF	0.007**	0.993	L
	ALL	5	9	3				
Blood vessel tumours	F	0	1	1	ALL	0.600	0.730	P
	NF	2	1	1				
	ALL	2	2	2				
Histiocytic tumours	F	0	0	2	ALL	0.612	0.721	P
	NF	0	2	2				
	ALL	0	2	4				
Haemolymphoreticular tumours	F	4	2	2	ALL (U as F)	0.405	0.595	L
	NF	1	3	2	ALL (U as NF)	0.449	0.551	L
	U	0	0	1				
	ALL	5	5	5				
Mammary tumours	F	2	1	1	ALL	0.761	0.648	P
	NF	0	0	0				
	ALL	2	1	1				

KEY
F = Fatals
NF = Non-Fatals
U = Uncertain
L = Large sample tests
P = Permutation tests

* p<0.05
** p<0.01
*** p<0.001

Table 6, **mouse data**.

Tumour incidence rates - statistical analysis
Results of tests for a decreasing and increasing incidence

Tumour Type	Number of tumour bearing animals	Gp 1	Gp 4	Gp 5	Included in analysis	p-values for increased tumour incidence Groups 1&5 vs Group 4	p-values for decreased tumour incidence Groups 1&5 vs Group 4	Method of analysis
Lung tumours	F	0	1	0	ALL (U as F)	0.348	0.652	L
	NF	6	10	11	ALL (U as NF)	0.322	0.678	L
	U	1	0	0				
	ALL	7	11	11				
Blood vessel tumours	F	0	1	2	ALL	0.057	0.389	P
	NF	2	1	2				
	ALL	2	2	4				
Haemolymphoreticular tumours	F	1	1	4	ALL	0.756	0.547	P
	NF	0	1	0				
	ALL	1	2	4				
Testes tumours	NF	0	2	1	ALL	0.318	0.949	P
Liver tumours	F	1	0	1	ALL	0.523	0.477	L
	NF	19	15	8				
	ALL	20	15	9				

KEY
F = Fatals
NF = Non-Fatals
U = Uncertain
L = Large sample tests
P = Permutation tests

Table 7, rat data

RAT DATA

DOSE LEVELS (MG/KG/DAY): GROUP 1= 0, 2= 100, 3= 600, 4= 2000, 5= 0

PRINTED: 21-JAN
PAGE: 1

STUDY NUMBER: 60417

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=T

ORGAN AND FINDING DESCRIPTION		SEX:	---NUMBER-OF-ANIMALS-AFFECTED---									
			----------MALE----------					----------FEMALE----------				
		GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
		NUMBER:	50	50	50	50	50	50	50	50	50	50
** TOP OF LIST **												
EYE	NUMBER EXAMINED:		46	27	28	48	48	50	29	31	50	49
MUSCLE	NUMBER EXAMINED:		50	28	30	50	50	50	27	32	50	50
NERVE SCIATIC	NUMBER EXAMINED:		50	28	30	50	50	50	27	32	50	50
NERVE OPTIC	NUMBER EXAMINED:		49	28	30	50	50	50	28	32	49	50
SKIN SUBCUTIS	NUMBER EXAMINED:		50	37	40	50	50	50	40	39	50	50
--B-FIBROMA			4	7	4	10	6	3	1	0	0	1
--B-DERMAL FIBROMA			3	5	2	5	5	0	0	0	0	0
--B-KERATOACANTHOMA			8	4	4	4	8	0	0	0	0	1
--B-PAPILLOMA			1	3	0	1	0	0	0	1	0	0
--B-BASAL CELL TUMOUR			2	3	1	1	1	0	0	0	0	2
--B-SQUAMO-SEBACEOUS TUMOUR			1	0	0	0	0	0	0	0	0	0
--B-FIBROLIPOMA			0	0	0	0	2	0	0	0	0	0
--B-LIPOMA			2	2	1	1	1	1	2	0	0	0
--B-NEUROFIBROMA			0	0	0	0	1	0	0	0	0	0
--M-HISTIOCYTIC SARCOMA			0	1	0	1	1	0	1	1	0	0
--M-OSTEOSARCOMA			0	0	0	2	1	0	0	0	0	0
--M-SQUAMO-SEBACEOUS CARCINOMA			0	0	0	0	0	0	0	0	0	0
--M-SQUAMOUS CARCINOMA			0	0	0	0	1	0	0	0	0	0
--M-SARCOMA			2	0	2	2	1	0	1	0	0	0
--M-SCHWANN CELL TUMOUR			0	1	0	0	0	0	0	0	0	0
MAMMARY GLAND	NUMBER EXAMINED:		2	2	2	1	2	50	47	46	49	50
--B-FIBRO/ADENOMA			2	1	2	1	2	32	39	31	30	31
--M-CARCINOMA			0	0	0	0	0	9	5	7	4	9
--B-FIBRO/ADENOLIPOMA			0	0	0	0	0	1	1	0	0	0

Table 8, rat data

GROUP INCIDENCE: HISTOPATHOLOGY: NEOPLASTIC DATA: COMBINED

DOSE LEVELS (MG/KG/DAY): GROUP 1= 0, 2= 100, 3= 600, 4= 2000, 5= 0

1

PRINTED: 21-JAN
PAGE: 2

STUDY NUMBER: 60417

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=T

		--- NUMBER-OF-ANIMALS-AFFECTED									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	50	50	50	50	50	50	50	50	50	50
FEMUR + MARROW	NUMBER EXAMINED:	50	28	30	50	50	50	28	33	50	50
STERNUM + MARROW	NUMBER EXAMINED:	50	28	30	50	49	50	28	32	50	50
LIVER	NUMBER EXAMINED:	50	49	50	50	50	50	50	50	50	50
--B-ADENOMA		1	0	0	1	1	0	0	0	1	0
--M-HISTIOCYTIC SARCOMA		0	0	0	0	0	0	0	0	0	0
--M-CARCINOMA		0	0	1	0	0	0	0	0	0	0
SPLEEN	NUMBER EXAMINED:	49	29	30	49	50	50	28	33	50	50
PANCREAS	NUMBER EXAMINED:	50	29	30	50	49	50	29	33	50	50
--B-ACINAR CELL ADENOMA		4	3	4	2	5	0	0	1	0	1
--B-ISLET CELL ADENOMA		8	3	0	1	3	1	0	0	0	1
--M-ACINAR CELL CARCINOMA		0	2	0	1	0	0	1	0	0	0
--M-ISLET CELL CARCINOMA		0	0	0	0	1	0	0	0	0	0
MES.LYMPH NODE	NUMBER EXAMINED:	49	26	31	50	50	50	28	33	50	50
--B-HAEMANGIOMA		1	1	0	0	2	0	0	0	0	0
STOMACH	NUMBER EXAMINED:	49	32	34	50	50	50	31	32	50	50
--B-PAPILLOMA		0	1	0	0	0	0	0	1	0	0
DUODENUM	NUMBER EXAMINED:	44	27	28	49	45	50	27	30	47	50
JEJUNUM	NUMBER EXAMINED:	43	25	23	46	48	49	27	31	50	49
ILEUM	NUMBER EXAMINED:	41	23	24	48	46	49	27	30	47	48
CAECUM	NUMBER EXAMINED:	43	25	24	45	42	48	27	30	48	49

Table 9, rat data

GROUP INCIDENCE: HISTOPATHOLOGY: NEOPLASTIC DATA: COMBINED

DOSE LEVELS (MG/KG/DAY): GROUP 1= 0, 2= 100, 3= 600, 4= 2000, 5= 0

PRINTED: 21-JAN
PAGE: 3

STUDY NUMBER: 6G4L7

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	50	50	50	50	50	50	50	50	50	50
COLON	NUMBER EXAMINED:	45	28	26	49	48	49	27	31	49	50
RECTUM	NUMBER EXAMINED:	46	26	27	49	47	49	28	32	49	49
ADRENAL	NUMBER EXAMINED:	50	30	31	50	50	50	31	38	50	50
--B-PHAEOCHROMOCYTOMA		8	3	3	5	10	0	0	0	2	2
--B-ADENOMA		0	0	0	0	0	2	0	1	0	1
--M-PHAEOCHROMOCYTOMA		2	1	0	2	0	0	0	0	0	0
KIDNEY	NUMBER EXAMINED:	50	31	29	49	49	50	28	32	50	50
--M-LIPOSARCOMA		0	0	1	0	0	0	0	0	0	0
--M-HAEMANGIOSARCOMA		1	0	0	0	0	0	0	0	0	0
--M-TRANSITIONAL CELL CARCINOMA		1	0	0	0	0	0	0	0	0	0
--B-LIPOMA		0	1	0	0	0	0	0	0	0	0
TESTIS	NUMBER EXAMINED:	50	31	35	50	50	0	0	0	0	0
--B-LEYDIG CELL TUMOUR		3	2	2	2	1	0	0	0	0	0
EPIDIDYMIS	NUMBER EXAMINED:	50	29	30	50	50	0	0	0	0	0
SEMINAL VESICLE	NUMBER EXAMINED:	45	27	30	47	46	0	0	0	0	0
URINARY BLADDER	NUMBER EXAMINED:	47	28	30	50	49	50	27	32	49	49
--M-CARCINOMA		0	0	0	0	0	0	0	1	0	0
PROSTATE	NUMBER EXAMINED:	50	29	30	50	50	0	0	0	0	0
--M-CARCINOMA			0	0	1	0	0	0	0	0	0
SALIVARY GLAND	NUMBER EXAMINED:	50	28	30	50	50	50	28	32	50	50

Table 10, rat data

GROUP INCIDENCE: HISTOPATHOLOGY: NEOPLASTIC DATA: COMBINED

DOSE LEVELS (MG/KG/DAY): GROUP 1= 0, 2= 100, 3= 600, 4= 2000, 5= 0

PRINTED: 21-JAN-
PAGE: 4

STUDY NUMBER: 60417

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED -									
	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	50	50	50	50	50	50	50	50	50	50
MAND.LYMPH NODE	NUMBER EXAMINED:	50	28	29	50	49	50	28	32	49	50
THYMUS	NUMBER EXAMINED:	46	26	26	45	46	50	29	31	46	50
--B-THYMOMA		0	0	0	1	0	0	0	1	1	0
LUNG	NUMBER EXAMINED:	50	30	35	50	50	50	29	37	50	50
--B-ADENOMA		0	0	0	1	0	0	0	0	0	0
HEART	NUMBER EXAMINED:	50	28	30	50	50	50	28	32	50	50
--B-ENDOCARDIAL SCHWANN CELL TUMOUR		0	0	1	0	0	0	0	0	0	0
TRACHEA	NUMBER EXAMINED:	50	28	30	50	50	50	28	32	50	50
OESOPHAGUS	NUMBER EXAMINED:	50	28	30	50	50	50	28	32	50	50
THYROID	NUMBER EXAMINED:	46	25	29	49	49	50	27	33	50	50
--B-C-CELL ADENOMA		5	5	6	8	8	4	4	1	12	11
--B-FOLLICULAR ADENOMA		6	2	2	0	2	0	0	1	1	0
--M-C-CELL CARCINOMA		0	1	0	1	0	0	0	0	0	0
PARATHYROID	NUMBER EXAMINED:	46	23	25	50	48	47	25	31	47	48
--B-ADENOMA		0	0	0	0	0	1	0	0	0	0
PITUITARY	NUMBER EXAMINED:	50	31	32	50	50	50	43	42	50	50
--B-ADENOMA		34	17	19	25	28	35	35	32	38	34
--M-CARCINOMA		0	0	0	0	0	1	0	0	0	0
BRAIN	NUMBER EXAMINED:	50	28	30	50	50	50	28	32	50	50
--B-MENINGIOMA		0	0	1	1	3	0	0	0	0	1
--M-GLIOMA		1	1	0	1	2	1	0	0	0	0

Table 11, rat data

GROUP INCIDENCE: HISTOPATHOLOGY: NEOPLASTIC DATA: COMBINED

DOSE LEVELS (MG/KG/DAY): GROUP 1= 0, 2= 100, 3= 600, 4= 2000, 5= 0

PRINTED: 21-JAN-94
PAGE: 5

STUDY NUMBER: 60417

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:		----------MALE----------					----------FEMALE----------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		50	50	50	50	50	50	50	50	50	50
SPINAL CORD	NUMBER EXAMINED:	50	28	30	50	50	50	28	32	50	50
HAEM/LYMPH/RETIC	NUMBER EXAMINED:	50	28	30	50	50	50	28	33	50	50
--M-LEUKAEMIA GRANULOCYTIC		0	0	1	0	0	0	0	0	0	0
--M-LYMPHOMA		0	0	1	0	0	0	0	0	0	0
--M-LYMPHOMA MIXED		0	0	0	0	0	0	1	1	0	0
--M-LYMPHOMA LYMPHOCYTIC		0	0	0	2	0	0	2	2	0	0
--M-LEUKAEMIA LYMPHOCYTIC		1	0	0	0	0	0	0	0	0	0
--M-LEUKAEMIA HISTIOCYTIC SARCOMA		0	0	0	0	1	0	0	0	0	0
OVARY	NUMBER EXAMINED:	0	0	0	0	0	50	32	37	50	50
--B-GRANULOSA THECA TUMOUR		0	0	0	0	0	1	0	0	0	0
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	50	31	37	50	50
--B-STROMAL POLYP		0	0	0	0	0	2	4	2	2	1
--M-LEIOMYOSARCOMA		0	0	0	0	0	0	0	1	0	0
--M-STROMAL SARCOMA		0	0	0	0	0	0	1	0	2	0
--M-ENDOMETRIAL CARCINOMA		0	0	0	0	0	0	0	1	0	0
VAGINA	NUMBER EXAMINED:	0	0	0	0	0	49	28	32	50	48
--M-SARCOMA		0	0	0	0	0	0	0	0	0	0
--B-FIBROMA		0	0	0	0	0	1	0	0	0	0
THORACIC CAVITY	NUMBER EXAMINED:	2	2	0	1	1	3	1	1	0	1
BONE	NUMBER EXAMINED:	0	0	2	0	0	0	1	0	0	0
--M-OSTEOSARCOMA		0	0	1	0	0	0	0	0	0	0
ABDOMINAL CAVITY	NUMBER EXAMINED:	1	2	1	0	1	1	1	0	0	1
--B-LIPOMA		0	1	0	0	0	0	0	0	0	0

Table 12, rat data

GROUP INCIDENCE: HISTOPATHOLOGY: NEOPLASTIC DATA: COMBINED

DOSE LEVELS (MG/KG/DAY): GROUP 1= 0, 2= 100, 3= 600, 4= 2000, 5= 0

PRINTED: 21-JAN
PAGE: 6

STUDY NUMBER: 60417

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:		----------MALE----------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		50	50	50	50	50	50	50	50	50	50
TONGUE	NUMBER EXAMINED:	0	0	0	1	0	0	0	0	0	0
LACRIMAL GLAND	NUMBER EXAMINED:	15	9	15	11	11	0	1	0	0	2
--M-CARCINOMA		0	0	1	0	0	0	0	0	0	0
ORAL CAVITY	NUMBER EXAMINED:	3	2	4	3	5	8	5	3	5	4
--M-SARCOMA		0	1	0	0	0	0	0	0	0	0
ANIMAL	NUMBER EXAMINED:	0	0	0	0	0	0	0	0	0	0
TAIL	NUMBER EXAMINED:	9	12	14	11	11	5	10	4	8	5
--M-SCHWANN CELL TUMOUR		0	0	0	1	0	0	0	1	0	0
--B-KERATOACANTHOMA		1	0	1	0	0	0	0	0	0	0
--B-PAPILLOMA		0	1	0	1	1	0	0	0	0	0
EAR	NUMBER EXAMINED:	3	4	4	2	5	0	3	1	1	0
--M-SCHWANN CELL TUMOUR		0	1	0	0	0	0	0	0	0	0
FOOT/LEG	NUMBER EXAMINED:	8	6	9	7	5	3	3	3	2	1
--B-KERATOACANTHOMA		0	0	1	0	0	0	0	0	0	0
--B-PAPILLOMA		0	0	0	0	1	0	0	0	0	0
LARYNX	NUMBER EXAMINED:	0	0	1	0	0	0	0	0	0	0
CONNECTIVE TISS	NUMBER EXAMINED:	0	2	0	0	0	0	0	0	0	0
--M-HISTIOCYTIC SARCOMA		0	2	0	0	0	0	0	0	0	0
ZYMBAL GLAND	NUMBER EXAMINED:	0	0	0	1	0	0	0	0	0	0
--B-ADENOMA		0	0	0	1	0	0	0	0	0	0

Table 13, rat data

GROUP INCIDENCE: HISTOPATHOLOGY: NEOPLASTIC DATA: COMBINED

DOSE LEVELS (MG/KG/DAY): GROUP 1= 0, 2= 100, 3= 600, 4= 2000, 5= 0

PRINTED: 21-
PAGE: 7

STUDY NUMBER: 604

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M;SUBSET=T

		--- NUMBER-OF-ANIMALS-AFFECTE									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	50	50	50	50	50	50	50	50	50	50
LYMPH NODE	NUMBER EXAMINED:	0	0	0	0	0	1	1	0	0	0
CRANIAL CAVITY	NUMBER EXAMINED:	0	1	0	1	1	0	0	0	0	0
--M-SCHWANNOMA		0	0	0	1	0	0	0	0	0	0
--M-SARCOMA		0	0	0	0	1	0	0	0	0	0
DIAPHRAGM	NUMBER EXAMINED:	0	1	0	0	0	0	0	0	0	0
PENIS	NUMBER EXAMINED:	0	0	0	0	2	0	0	0	0	0
PREPUT/CLIT GL	NUMBER EXAMINED:	1	0	0	0	0	0	0	0	0	1
URETER	NUMBER EXAMINED:	0	0	0	1	0	0	0	0	0	0
NASAL CAVITY	NUMBER EXAMINED:	0	1	0	0	0	0	1	2	0	0

** END OF LIST **

Table 14, rat data

Tumour incidence males - statistical analysis
Results of tests for a decreasing and increasing incidence

Test article	Control I	Etylester K85			Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	100	600	2000	0

Tumour Type	Number of tumour bearing animals Gp 1	Gp 4	Gp 5	Included in analysis	p-values for increased tumour incidence Controls vs Group 4	p-values for decreased tumour incidence Controls vs Group 4	Method of analysis
Adrenal medulla	NF 9	7	10	ALL	0.722	0.278	L
Brain M-glioma	F 0	0	2	ALL (U as NF)	0.800	0.597	P
	NF 0	1	0	ALL (U as F)	0.810	0.582	P
	U 1	0	0				
	ALL 1	1	2				
Brain B-meningioma	NF 0	1	3	ALL	0.792	0.611	P
M/L/R tumours	F 1	0	1	ALL	0.400	0.894	P
	NF 0	2	0				
	ALL 1	2	1				
Liver adenoma and carcinoma	NF 1	1	1	ALL	0.712	0.736	P
Mammary tumours	F 2	0	2	ALL (U as NF)	0.846	0.503	P
	U 0	1	0	ALL (U as F)	0.850	0.499	P
	ALL 2	1	2				
Kidney M-haemangiosarcoma and Mes lymph node B-haemangioma	NF 2	0	2	ALL	1.000	0.197	P
Pancreas: endocrine	NF 8	1	4	ALL	0.977	0.023*	L
Pancreas: exocrine	NF 4	3	5	ALL	0.737	0.263	L

KEY
F = Fatals
NF = Non-fatals
U = Uncertain
L = Large sample tests
P = Permutation tests

* $p<0.05$
** $p<0.01$
*** $p<0.001$

Table 15, rat data

Tumour incidence males - statistical analysis
Results of tests for a decreasing and increasing incidence

Test article	Control I	Etylester KB5			Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	100	600	2000	0

Tumour type	Number of tumour bearing animals Gp 1 Gp 4 Gp 5				Included in analysis	p-values for increased tumour incidence Controls vs Group 4	p-values for decreased tumour incidence Controls vs Group 4	Method of analysis
Pituitary adenoma and carcinoma	F	7	7	9	ALL (U as NF)	0.793	0.207	L
	NF	25	16	19	ALL (U as F)	0.797	0.203	L
	U	2	2	0				
	ALL	34	25	28				
Skin 1	F	1	3	1	ALL	0.122	0.878	L
	NF	5	10	11				
	ALL	6	13	12				
Skin 2	F	1	2	2	ALL (U as NF)	0.421	0.833	P
	NF	0	1	0	ALL (U as F)	0.427	0.829	P
	U	1	0	0				
	ALL	2	3	2				
Skin 4	F	0	0	1	ALL	0.830	0.170	L
	NF	10	6	8				
	ALL	10	6	9				
Skin 8-basal cell tumour	NF	2	1	1	ALL	0.804	0.598	P
Skin 8-lipoma	F	0	0	1	ALL	0.786	0.618	P
	NF	2	1	0				
	ALL	2	1	1				
Testis 8-Leydig cell tumour	NF	3	2	1	ALL	0.625	0.709	P
Thyroid 8-follicular adenoma	NF	6	0	2	ALL	1.000	0.032*	P
Thyroid c-cell tumours	NF	5	9	8	ALL	0.186	0.814	L

KEY
F = Fatals
NF = Non-fatals
U = Uncertain
L = Large sample tests
P = Permutation tests

* p<0.05
** p<0.01
*** p<0.001

Table 16, rat data

Tumour incidence females - statistical analysis
Results of tests for a decreasing and increasing incidence

	Test article	Control I	Ethylester K85			Control II
	Group	1	2	3	4	5
	Level (mg/kg/day)	0	100	600	2000	0

Tumour Type		Number of tumour bearing animals Gp 1	Gp 4	Gp 5	Included in analysis	p-values for increased tumour incidence Controls vs Group 4	p-values for decreased tumour incidence Controls vs Group 4	Method of analysis
Adrenal B-adenoma	NF	2	0	1	ALL	1.000	0.438	P
Adrenal medulla	NF	0	2	2	ALL	0.394	0.903	P
Mammary tumours	F	15	7	13	ALL	0.868	0.132	L
	NF	20	24	21				
	ALL	35	31	34				
Pituitary adenoma and carcinoma	F	11	13	13	ALL	0.474	0.526	L
	NF	25	25	21				
	ALL	36	38	34				
Skin 1	F	1	0	0	ALL	1.000	0.203	P
	NF	2	0	1				
	ALL	3	0	1				
Thyroid c-cell tumours	NF	4	12	11	ALL	0.175	0.825	L
Uterus stromal polyp	NF	2	2	1	ALL	0.567	0.794	P

KEY
F = Fatals
NF = Non-fatals
L = Large sample tests
P = Permutation tests

Following are the Executive cac meeting minutes finalized and signed off on 8/13/04

Date of Meeting: August 10, 2004

Committee: David Jacobson-Kram, HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Chuck Resnick, Ph.D., HFD-110, Alternate Member
Indra Antonipillai, Ph.D., HFD-510, Presenting Reviewer

Author of Draft: Indra Antonipillai

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #: NDA 21-654
Drug Name: Omacor (Ethylester K85) soft gelatin capsules
Sponsor: Ross products Division, Abbott laboratories, Columbus, Ohio.

Background:

Omacor is isolated from fish oil. It is composed primarily of the unsaturated omega-3 fatty acid esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Its

mechanism of action is unknown but the poly-unsaturated omega-3 fatty acids depress other lipids (essentially triglycerides) by inhibiting hepatic triglycerides and possibly apoprotein synthesis. They replace arachidonic acid (AA) in phospholipids with EPA

and DHA. Omacor is indicated alone, or in combination with HMG-CoA reductase inhibitors in hypertriglyceridemic patients. Omacor does not have mutagenic/genotoxic potential.

Mouse carcinogenicity study

In a 2-year carcinogenicity study in mice CD-1(CR)BR, 51/sex/dose), doses of 100, 600, 2000 mg/kg/day were administered orally by gavage (in corn oil) for 80 weeks in males and for 88 weeks in females. The actual doses achieved in the carcinogenicity study were 90, 540, 1860 mg/kg/day.

The sponsor did not submit the dose selection/study design for ECAC review prior to conducting the carcinogenicity study. The highest dose selection (2000 mg/kg/day) for the mouse carcinogenicity study was based on a 13-week dose range study in mice where 4000 mg/kg/day produced no overt toxicity except increases in liver weights in the high dose group (4-7%) and skin dermatitis (in 2/2 males). However, a high dose of only 2000 mg/kg/day was chosen for the 2-year mouse assay, which was well below a MTD. The duration of the study was also shorter than the standard 2-year duration because of the sponsor's decision to terminate the study at 50% survival. However mortality in mice was not affected in the actual study. Therefore the study could have been continued for the full time period.

In the present carcinogenicity study, no AUC exposures to EPA and DHA were provided, but plasma levels of EPA increased with the dose (males 0.4-1.4, 2.6, 10.3, 29.2 mg/L at 0, 115, 530, 1860 mg/kg/day respectively, females 3-3.4, 2.3, 12.1, 41.3 mg/L respectively), while DHA values did not change (males 130-150, 166, 176, 179 mg/L; females 121, 126, 135, 141 mg/L respectively). This suggests that systemic levels of DHA in plasma from diet had not been exceeded.

Omacor had no effect on mortality or body weights in mice. No significant non-neoplastic lesions were observed in mice. Uterine smooth muscle tumors (benign uterine leiomyoma and malignant uterine leiomyosarcoma) were increased in the high dose group (combined tumors in drug treated mice were 9/51 vs 8/102 in controls, p=0.019), but these were not considered statistically significant for a common neoplasm.

In conclusion, the mouse carcinogenicity assay produced negative results. The study suffers from a number of deficiencies: the high dose used was below MTD, the assay was not carried out to the optimal 2-year duration (mice were sacrificed in weeks 80-88), the diet may have contained EPA, DHA and linolenic acid (a source of EPA, DHA), and the volume of vehicle (corn oil) varied with dose.

Rat carcinogenicity study

A 2-year carcinogenicity study in rats (Crl:CD (SD)BR, 50/sex/dose) was conducted, where doses of 100, 600, 2000 mg/kg/day were administered orally (by gavage) for 101 weeks in males and 89 weeks in females using a corn oil vehicle. AUC values were not provided, but plasma levels of EPA (available only in males) increased only at the high dose (3.5, 2.9, 3.5, 10 mg/L at 0, 90, 540, 1800 mg/kg/day respectively). In contrast, DHA levels were unchanged at all doses (21, 29, 32, 29 mg/L respectively),

As stated earlier, the sponsor did not submit the dose selection/study design for ECAC review prior to conducting the rat carcinogenicity study. The highest dose selected (2000 mg/kg/day) for rat CAC study was based on a 13-week dose range study, in which 4000 mg/kg/day produced clinical signs (severe desquamation, tail necrosis), gross foot/leg lesions (in 29/40 rats vs 0/40 controls), and humane sacrifice of 20/20 male rats due to the severe desquamation and tail necrosis indicating that 4000 mg/kg/day exceeds MTD. Therefore, a high dose of 2000 mg/kg/day as selected was appropriate. The duration of the study was shorter than the standard 2-year duration, again because of the sponsor's decision to terminate the study at 50% survival. The controls, low dose and mid dose survival rates were below 50% by the middle of week 80, which is why sponsor terminated the study in week 89 in females. However mortality in rats across the groups was not affected in this study and the study could have been continued for the full two-year period.

Omacor did not affect the mortality, or the body weights, and did not produce significant neoplastic or non-neoplastic lesions in this 2-year bioassay in rats.

In summary, although the duration of the study was shorter (89-101 weeks) than the standard 2-year optimal duration, the dose selection was adequate, and the drug at these doses (100, 600, 2000 mg/kg/day) did not produce significant toxicity/mortality or carcinogenicity in rats.

Executive CAC Recommendations and Conclusions:

Mouse

The Committee concurred that the mouse study did not meet the generally acceptable standards for adequate assessment of carcinogenicity. No drug-related tumors were observed in the mouse assay. The study suffers from a number of deficiencies: the high dose used was below MTD, the assay was not carried out to the optimal 2-year time duration (mice were sacrificed in weeks 80-88), the diet may have contained EPA, DHA and linolenic acid (a source of EPA, DHA), and the volume of vehicle (corn oil) was varied with dose. Despite these deficiencies the committee felt that the study need not be repeated.

Rat:

The committee noted that the doses used were adequate in rats, although there were a number of deficiencies: the study duration was less than the standard two year duration,

the diet may have contained EPA, DHA and linolenic acid (a source of EPA, DHA), and the volume of vehicle (corn oil) was varied with dose.

The rat study was negative for carcinogenicity.

In conclusion, although the study duration was less than optimal, the dose selection was adequate and ECAC accepts the study. The rat study was negative for carcinogenicity.

David Jacobson/Kram, Ph.D.
Chair, Executive CAC

cc:/

/Division File, HFD-510, NDA 21-654
/Team leader, HFD-510 Davis Bruno
/Reviewer, HFD-510 Antonipillai
/CSO, HFD-510, Jimenez
/HFD-024, ASeifried

2.6.6.6 Reproductive and developmental toxicology

Following repro and developmental toxicity studies are summarized from IND 45,998

Omacor was tested in a full battery of standard repro toxicity studies.

In a segment I fertility study in rats, (males 8 week old, females 12 weeks old) animals were given omacor orally (by gavage in corn oil) at doses of 0, 100, 600, 2000 mg/kg/day. Females were given the drug for 2-weeks prior to mating, throughout gestation and lactation. Males were given the drug for 70 days prior to mating. One half of females were sacrificed on day 20 of gestation and the other half allowed to litter and rear offspring (F1) to weaning. Males were dosed throughout mating period and the females throughout mating gestation and lactation. At a HD, clinical signs such as peri-anal staining (red scrotum in HD males begining week 6 and HD females during gestation) and fur staining (in anal area in HD males) were observed. Sponsor explains that the fur staining is due to excretion of one or more metabolites in the urine. Body weight gains were lower at a HD (during weeks 5-10) in males (mean body weight gains in week 10 were 557, 560, 551, 545 g at 0, 100, 600, 2000 mg/kg/day respectively), no effects on food consumption were observed. The HD produced decreases in the mean litter weight (58.3, 57.6, 58.7, 52.4 g at 0, 100, 600, 2000 mg/kg/day respectively). No effects on general maternal or paternal reproductive performance, or on the progression of pregnancy were observed in rats. The NOAEL doses in this fertility study in male and female rats were 2000 mg/kg/day (or 5 fold the human dose of 4 g/day or 67 mg/kg/day, based on body surface area).

In a segment II teratology study in rats, pregnant animals (8-10 weeks old, n=27/group) were given omacor by gavage (in corn oil) at doses of 0, 1000, 3000, 6000 mg/kg/day

from day 6 to day 15 of gestation. Females were sacrificed on day 20 PC and necropsied. The highest dose selection here was based on the dose range finding study in which 6000 mg/kg/day did not produce any toxicity in pregnant rats. In the actual teratology study except for an increase in the peri-anal and inguinal fur staining at a HD in late gestation, it had no effects in pregnant animals. Maternal and embryo-fetal NOAEL was 6000 mg/kg/day in segment II study in rats, as no maternal/embryo fetal or teratogenic effects were observed even at the highest doses of 6000 mg/kg/day (≈ 15 times the human dose of 4 g/day, based on body surface area).

In a segment II teratology study in rabbits, pregnant animals (16/group) were given omacor by gavage (as a pure test article, not diluted in the corn oil) at doses of 0, 375, 750, 1500 mg/kg/day from day 7 to day 19 of gestation. Females were sacrificed on day 29 PC and necropsied. The highest dose selection here was based on the toxicity in the dose range finding study in pregnant rabbits, where ¾ rabbits were euthanized at a HD of 6000 mg/kg/day before day 29 of gestation due to clinical signs (anorexia, liquid colon content, swelling or reddening of anogential orifices, enlarged anus and sores in anogenital region on hind legs and tail) and weight loss (% body weight change days 0-29 was 24.2, 19.1, 22.5, 9.5 respectively). In the current study one HD rabbit was killed following abortion on day 24. The clinical signs at MD/HD were observed in the mouth (desquamation, sores/lesions), and in the anus or vagina (red skin/sores/staining). At necropsy all animals had increased incidences of "minor lung changes". Sponsor states that small quantities of test article drained from the esophagus into lungs and caused local irritation. The drug produced maternal toxicity at MD/HD as there were significant decreases in body weights (% BW change days 0-29 was 27.5, 26.5, 21.2, 19.3 respectively, % BW change days 7-19 was 7.2, 5.7, 1.2, 1.2 respectively) and food consumption (mean intake days 0-29 was 157, 160, 129, 130 g/animal/day respectively). The HD of 1500 mg/kg/day (a maternally toxic dose) produced an increase in post-implantation loss (18% vs 9% in controls), and a corresponding reduction in mean number of fetuses per dam (7.6% vs 8.3% in controls), these losses were considered secondary to weight loss in mothers. This HD in rabbits also produced an increase in male fetuses (47, 54, 52, 59% at 0, 375, 750, 1500 mg/kg/day respectively), and decrease in mean fetal weights (42, 41, 39, 38 g respectively). The maternal NOAEL was 375 mg/kg/day and embryo-fetal NOAEL was 750 mg/kg/day in segment II study in rabbits. The embryo fetal toxicity seen at a HD was mainly due to the maternal toxicity. These doses of 375, 750, 1500 mg/kg/day in the rabbit provide safety margin of 2, 4, 8 fold the human dose of 4g/day (based on body surface area).

In the rat Segment III study, omacor was given to rats, orally by gavage (in corn oil) on gestation days 14 to lactation day 21 (0, 100, 600, 2000 mg/kg/day, n=24/group, 10-12 weeks old). Physical development in F1 was monitored up to day 21 postpartum. The doses up to 2000 mg/kg/day in this study did not produce any effects. However, in a dose range segment III study, where higher doses (0, 1000, 3000 and 6000 mg/kg/day) were used, the two high doses had effects on the mean number of pups born/female (14.5, 12.9, 12.1, 11.3 respectively), and mean number of fetuses alive on day 1 and 4 (9.5, 11.9, 5.9, 8.2 respectively). The body weights at 6000 mg/kg/day in the dose range study were decreased by 10% (on day 29 BW were 3.69, 3.55, 3.70, 3.33 kg respectively, the % body weight change on days 0-29 was 24.2, 19.1, 22.5, 9.5 respectively). The NOAEL doses in the current segment III study in rats were 2000 mg/kg/day (or 5 fold the human dose of 4 g/day or 67 mg/kg/day, based on body surface area). However in a dose range segment III study, 3000 mg/kg/day (the dose that was

not maternally toxic) was toxic for pups and fetuses, as this dose decreased the mean number of pups born/female and the mean number of fetuses alive on day 1 and 4.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Omacor is a mixture of omega-3 fatty acids (FA) isolated from fish oil, approximately — are esters of two unsaturated FA, eicosapentaenoic acid (—) and docosahexaenoic acid (—). EPA and DHA are long chained polyunsaturated fatty acids which can also be designated 20:5ω3 and 22:6ω3 according to the number of carbon atoms and number of unsaturated bonds. Each one gram capsule of Omacor has EPA ethyl ester- — mg, DHA ethyl ester- — mg & vitamin E-4 IU as antioxidant. Omacor lowers triglyceride (TG) by increasing mitochondrial and proxisomal beta oxidation of fatty acids.

The drug is indicated for the reduction of elevated TG levels in adult patients with hyperlipidemia. It is recommended for the use as a monotherapy [ ] at doses of 4 g/day (as a single dose or two 2 g divided doses) in adults. Omega–3 fatty acids lower triglyceride and cholesterol levels, promote the HDL/LDL- cholesterol ratio, and affect prostaglandin and leukotriene metabolism. Pharmacology studies show that Omacor (0, 0.6, 1.3, 2.5, 5 g/100 g of diet) administered to rats for 21 days decrease plasma lipids significantly (triglyceride levels were reduced by up to 24% of control & total cholesterol by up to 58% of control at 5 g/kg/day dose).

Toxicity Studies:
In one-year chronic toxicity studies in rats and dogs, high doses (rats 2000 mg/kg/day and dogs 1000 mg/kg/day) produced clinical signs in both rats (in males tail lesions, in both sexes fur staining, paddling and high stepping gait) and dogs (in both sexes fur staining). The drug increased adrenal and kidney weights at a HD in male rats, but the main target organ of toxicity in rats was liver (mottled liver in 19/40 vs 5/40 in controls, and increased liver vacuolation in 23/40 vs 8/40 in controls). The sponsor states that a slightly higher incidence of bile duct proliferation and adrenal vacuolation was observed in HD male rats but these were of minor nature, and probably relate to perturbations in lipid content due to test article. Additional evidence of perturbations in lipid metabolism were observed in the rat carcinogenicity where animals had lung histiocytes and cholesterol granuloma. No liver toxicity in dogs was observed. In contrast, the target organs of toxicity in dogs were adrenals (at a HD vacuolation in zonal fasciculate & zona reticularis in ¾ females, accessory nodules at a MD & HD in ¼ + ¼ males respectively vs 0/4 controls), kidneys (basophilic tubules 0/4, 0/4, ¼, ¼ males at 0, 50, 300, 2000 mg/kg/day respectively), and testes (atrophy 0/4, 0/4, 2/4, 2/4 respectively). Sponsor states that the testicular atrophy was of a low grade in 3 of 4 dogs which frequently occurs in beagles of this age. Note that the testicular atrophy was not observed in a 90-day study in dogs, and no effects of the drug were observed in the rat male fertility study. The NOAEL in a one-year rat study is ≤ 600 mg/kg/day in both sexes (<2 fold the human dose of 4 g/day or 67 mg/kg/day, based on body surface area). The NOAEL in a 1-year study in male dogs was 50 mg/kg/day (or 0.4 fold the human dose of 4 g/day, based on body surface area) and in female dogs was 300 mg/kg/day (or 2 fold the human dose of 4 g/day, based on body surface area).

The dermal toxicity was consistently observed in all studies and was seen mainly and more severely in males for some unknown reason and concerned skin lesions. **In rats**, fur staining was observed in a 28-day tox study (at a HD of 4000 mg/kg/day in all 20/20 rats), and in a 90-day tox study (fur staining and desquamation of tail and feet in all 40/40 rats, with gross foot/leg lesions in 29/40 rats vs 0/40 controls, and deaths in all 20/20 male rats were due to tail lesions). Sponsor attributes this local irritation effect to excretion of one or more metabolites of the drug in urine or skin that were spread by grooming and at high doses these were locally irritating. In a 1-year rat tox study, tail lesions were observed mainly at a HD of 2000 mg/kg/day in males only, while fur staining was observed in both sexes at a HD. **In mice** in a 13-week study, skin sores or fur loss was noted in a gross pathology at a HD of 4000 mg/kg/day in 2/12 males and in histopathology evaluation, 2/2 males examined had skin dermatitis. **In dogs**, fur staining was observed in a 28 day tox study (fur staining of the chest or entire body at a HD of 1000 mg/kg/day and sores in a urogenital area of 1M+1F), while in a 13-week and a 1-year dog study, a HD of 1000 mg/kg/day produced fur staining in both sexes (and occasionally in other dose groups in a one-year study). No dermal toxicity was observed at 300 mg/kg/day in dogs, and at 600-1000 mg/kg/day in rats and in 3-12 month tox studies (or 2X the human dose based on body surface area). Changes in the relative amounts of different fatty acids in the plasma of drug treated animals are proposed as a possible explanation for skin problems. With Omacor, EPA and DHA levels are increased while linoleic acid (also an essential fatty acid) is decreased. It is known that rats deficient in linoleic acid develop scaly, necrotic skin and necrotic tails. However similar plasma changes are observed in dogs, where there is substantial dose-linked increase in EPA, DHA, followed by reduction in AA, palmitic, and linoleic acid. Sponsor attributes the skin lesions to local irritation of the test article metabolite through skin or urine, while dermal reactions which led to euthanizing of all HD males may be due to a deficiency in essential fatty acid(s). It is not known deficiency of which essential fatty acid(s) led to the deaths in males in a 13 week study in rats.

Mutagenicity: The mutagenic/cytogenic potential was examined in 4 tests. Omacor was negative in the Ames test, in vitro mutagenicity assay in Chinese hamster V79 lung cells and in vivo micronucleus test in mice. There was a concern that in a clastogenic activity assay in human lymphocytes, the drug was positive in the absence of metabolic activation at two high doses of 400-500 µg/ml, this was suggested to be due to the use of a deteriorated drug in the assay and also due to the excessive cytotoxicity (mitotic index was depressed by 86% in that assay). In a repeat assay this test was negative (see genotox section in DFS)

Reproductive toxicity: Omacor did not have any significant effects on fertility at doses up to 2000 mg/kg/day in segment I study & on peri/postnatal parameters in a segment III study in rats at doses up to 2000 mg/kg/day (or 5-fold the maximal recommended human dose based on body surface area). Similarly in a segment II teratology studies in rats, it was not teratogenic at doses up to 6000 mg/kg/day (15 fold the maximal recommended human dose based on body surface area). However in a segment II teratology study in rabbits (0, 375, 750, 1500 mg/kg/day), a HD of 1500 mg/kg/day (a maternal toxic dose) produced an increase in post-implantation loss (18% vs 9% in controls), and a corresponding reduction in a mean number of fetuses per dam (7.6% vs 8.3% in controls). The maternal NOAEL was 375 mg/kg/day and embryo-fetal NOAEL was 750 mg/kg/day in this rabbit study and provided safety margin of 2 & 4 fold respectively the human dose, based on body surface area. No effects in segment III

study in rats were observed at doses up to 2000 mg/kg/day. However, in a dose range finding segment III study in rats, where higher doses (of 0, 1000, 3000 and 6000 mg/kg/day) were used, the two high doses decreased the mean number of pups born/female (14.5, 12.9, 12.1, 11.3 pups/female respectively), and mean number of fetuses alive on day 1 (13.8, 12.5, 10.1, 11.2 respectively) and day 4 (9.5, 11.9, 5.9, 8.2 respectively). The HD in this study produced maternal toxicity as the body weights were decreased by 10%. However, 3000 mg/kg/day dose (7X the human dose based on body surface area) which did not produce maternal toxicity, produced toxicity in fetuses and pups.

Carcinogenicity studies: The rat cac study (0, 100, 600, 2000 mg/kg/day) conducted for 89-101 weeks, did not produce increased mortality or a significant increase in tumors (neoplastic or non-neoplastic) compared to two combined controls. The mouse cac study (0, 115, 530, 1860, 0 mg/kg/day) conducted for 80-88 weeks also did not show increased mortality, but produced increased incidence of neoplastic uterine smooth muscle tumors (combined leiomyoma and leiomyosarcoma) at a HD vs two combined controls (9/51 vs 8/102 in controls, i.e 18% vs 8% in controls, p=0.019). These were not considered significant for a common neoplasm (as p values were not less than 0.01). Since the doses used in the mouse carcinogenicity study were below MTD, the study was shorter (80-88 weeks) and not carried out to the optimal 2-year time duration, it was considered deficient.

The ECAC was consulted on 8/10/04. They agreed that the 2-year carcinogenicity assay in mice was inadequate, but felt that the study need not be repeated. The dose selection for the rat 89-101 week carcinogenicity study was appropriate, although rat study duration was less than the standard two year duration, the study was acceptable and negative for carcinogenicity.

Labeling Review: The following changes in labeling are recommended:

Suggested labeling:

Sponsor's suggested labeling

Carcinogenesis, Mutagenesis, Impairment of fertilitv:

[ ]

Reviewer's recommended changes:

Carcinogenesis, Mutagenesis, Impairment of fertility:

[ ]

[ ]

Justification for the changes:

1. The carcinogenicity study in mice was inadequate. The combined uterine leiomyoma and leiomyosarcoma were not significant at a dose of 2000 mg/kg/day in a mouse carcinogenicity study because the dose selection and study duration were inadequate.
2. In the rat carcinogenicity study, the dose selection was adequate, and although the duration of the study was shorter than the standard 2 year duration, the assay was acceptable and considered negative.

Sponsor's suggested labeling on Genotox:

[ ]

Reviewer's recommended changes:

[ ]

Justification for the changes:

1) The in vitro human lymphocyte chromosomal assay is considered negative at doses up to 300 ug/ml in the absence of metabolic activation. 2) The data on fertility study are included under the heading Carcinogenesis, Mutagenesis, Impairment of fertility

Sponsor's suggested label on pregnancy category

[ ]

Reviewer's recommended changes:

Pregnancy Category C

[

]

Justification for the changes:

1) The pregnancy category is changed from — to C.
2) The doses of Omacor used in segment II studies in rats and rabbits are specified.
3) Omacor produced effects in pups and fetuses in a segment III study in rats
4) Since Omacor will be used in the [

]

Sponsor's suggested label on Nursing Mothers

[

]

Reviewer's recommended changes:

It is not known whether — is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Omacor is administered to — woman.

Recommendations

From Pharm/tox point of view this application is recommended for approval pending labeling changes

Signatures (optional): /S/

Reviewer Signature _____

/S/

Supervisor Signature_____
Concurrence Yes ___ No ___

cc: IND Arch
HFD-510
HFD-510/davisbruno/antonipillai/parks/jimenez
Review code: AP
File name: nda21654 (Omacor/fish oil)

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

/s/

Indra Antonipillai
9/15/04 11:02:58 AM
PHARMACOLOGIST
From the pahrm/tox point of view, this application is recommended for approval pending labeling changes
This application is recommended for approval pending labeling changes

Karen Davis-Bruno
9/15/04 11:11:16 AM
PHARMACOLOGIST
Label Re: TL Memo 8/26

6/29/04

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

STATISTICAL REVIEW AND EVALUATION

CARCINOGENICITY STUDIES

NDA/Serial Number:	21-654/N-000
Drug Name:	Omacor® (omega-3-acid ethyl esters)
Indication(s):	Hypertriglyceridemia
Applicant:	Ross Products Division, Abbott Laboratories
Date(s):	Received 01/09/04; user fee (10 months) 11/09/04
Review Priority:	Standard
Biometrics Division:	Division of Biometrics II (HFD-715)
Statistical Reviewer:	Cynthia Liu, MA
Concurring Reviewer(s):	Karl K. Lin, Ph.D., Expert Mathematical Statistician (Applications in Pharmacology and Toxicology)
Medical Division:	Div. of Metabolic and Endocrine Drug Products (HFD-510)
Pharmacology Team:	Indra Antonipillai, Ph.D., Pharmacological Reviewer
	Karen Davis-Bruno, Ph.D., Team Leader
Project Manager:	Valerie Jimenez
Keywords:	NDA review, carcinogenicity studies, survival, neoplastic lesions

TABLE OF CONTENTS

Summary of Statistical Review

- Documents of two carcinogenicity studies (rat and mouse) with two sexes each, submitted by the sponsor along with electronic data sets, were reviewed.

- Dose levels were 0, 100, 600, and 2000 mg/kg/day for both species. There were 2 identical controls in those studies. Route of administration was oral gavage.

- The rat and mouse studies were designed to be of 104 and 80 weeks, respectively. According to the sponsor, because of lower than expected survival, the male and female rats were killed after 101 and 89 weeks of treatment, respectively. However, due to good survival, the female mouse study was extended to the 50% survival point and terminated at Week 88.

- The number of animals with adequate treatment exposure was generally sufficient with respect to the duration of each study.

- In both the rat and mouse studies, there were no significant positive trends or group comparisons in mortality in either sex, nor were there significant increases in the high dose tumor incidences when compared to the combined control.

- There were no analyses of combining tumors, tissues, and/or related hyperplastic lesions requested by the reviewing pharmacologist.

- This reviewer's findings of the survival and tumor analyses for both the rat and mouse studies agree with the sponsor's.

Introduction

The sponsor has submitted two carcinogenicity studies (rat and mouse) with two sexes each, for the new drug application (NDA 21-654) for Omacor® (omega-3-acid ethyl esters). The purpose of these studies was to evaluate the effect of the test article, Etylester K85, on the incidence and morphology of tumors following oral (gavage) administration once daily to the rats and mice for at least 104 and 80 weeks, respectively.

[ ]: conducted the rat study during Years 1990-1992 and the mouse study during Years 1992-1993. According to the review report (dated 11/1/2000) of Dr. Albert DeFelice (previous reviewing pharmacologist), the data had been submitted to the agency before, but the formats were not in compliance. Therefore, no analysis or verification on the data or results was conducted by the agency at that time.

The sponsor finally reformatted the tumorigenicity data and this reviewer was able to perform her own independent statistical analyses on survival and neoplastic lesions, using the electronic data sets submitted by the sponsor on 1/9/2004. The data files are located in \\Cdsesub1\n21654\N_000\2004-01-09\Tox. However, no electronic study reports were submitted. The paper volumes this reviewer used as references are Vols. 14 (mouse) and 18-19 (rat).

Study Design

The group designation, dose level, and number of animals per group for the rat and mouse studies are provided below. The strains of rats and mice were ....CD(SD)BR and CD-1(ICR)BR, respectively. Note that the two controls were identical.

Group Number	Group Description	Rat			Mouse		
		Dose Level mg/kg/day	Animals/group		Dose Level mg/kg/day	Animals/group	
			Male	Female		Male	Female
1	Control I	0	50	50	0	51	51
2	Low	100	50	50	100	51	51
3	Intermediate	600	50	50	600	51	51
4	High	2000	50	50	2000	51	51
5	Control II	0	50	50	0	51	51

According to the sponsor, because of lower than expected survival, the male and female rats were killed after 101 and 89 weeks of treatment, respectively. However, due to good survival, the female mouse study was extended to the 50% survival point. In other words, for the rat study, the males were treated for a minimum of 101 weeks and the females for a

minimum of 89 weeks; for the mouse study, the males were treated for a minimum of 80 weeks and the females for a minimum of 88 weeks.

Reviewer's Analysis Methods

Survival. Evaluations of dose-response trend in mortality and group comparisons were conducted using Cox-Tarone binary regression (parametric) and Gehan-Breslow (nonparametric) tests. The former method is weighted more heavily toward late incidences and the latter method is weighted more heavily toward early incidences due to treatment. As a result, both are valuable tools for incidence data with onset times. Kaplan-Meier product limit survival curves were a supplementary tool to examine the survival distribution patterns among the study groups. Two-sided tail probabilities for trend and group comparisons are evaluated at the 5% significance level.

Neoplastic Lesions. The occult tumors (incidental and/or fatal) were analyzed by interval-based exact permutation test incorporating cause of death information. The cut-off points used for the intervals were Weeks 0-52, 53-78, 79-92, 93-before terminal sacrifice (T_{sac}), and T_{sac} for the male rats, which are based on the suggestions from National Toxicology Program (NTP). Since the durations of the other studies were shorter than 104 weeks, this reviewer used Weeks 0-50, 51-75, 76-before T_{sac}, and T_{sac} for the 89-week female rat and 88-week female mouse studies, and Weeks 0-50, 51-70, 71-before T_{sac}, and T_{sac} for the 80-week male mouse study. The palpable (superficial) tumors were also analyzed by interval-based exact permutation test as in the case of fatal tumors, using the first palpation time (provided in the sponsor's electronic data files) as the tumor onset time. SAS PROC MULTTEST (1999) was used to implement the interval-based exact permutation test. Since the low and intermediate dose groups did not have all the animals examined microscopically for the protocolled tissues (unless noted otherwise), they were excluded from the statistical analyses.

The benign and malignant neoplastic lesions were evaluated individually as well as combined. In the cases of multiple-organ findings (e.g., hemangioma and hemangiosarcoma), the incidences were counted and evaluated by animal as well as by tissue type. The statistical results for these cases may be biased because not all the animals were examined for every tissue. This reviewer has selected combined tumor types and/or combined organ types, where appropriate, for the analyses based on the work of McConnell et al. (1986) and her past experience. There were no combining cases requested by the reviewing pharmacologist.

Since whether tumor incidence rates increase as doses increase is the main concern of the FDA/CDER pre-clinical review team regardless of the real direction indicated by the data,

upper-tailed probabilities (p-values) were, therefore, always computed in testing for positive trend and group comparisons in tumor incidences. The following table provides the criterion for determining the statistical significance according to the FDA's Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May 2001).

	Test for Positive Trend	Control-High Pairwise Comparisons
Standard 2-Year Studies with 2 Species and 2 Sexes	Common and rare tumors are tested at 0.005 and 0.025 significance levels, respectively.	Common and rare tumors are tested at 0.01 and 0.05 significance levels, respectively.

Common tumor is defined as a tumor type with background (control) rate >1% and rare tumor with background (control) rate ≤1%. The concurrent controls and historical control (where applicable) were taken into consideration in determining commonality of a tumor.

Based on this reviewer's initial analyses at 0.05 significance level (2-sided), there were no striking differences between the two controls in the mortality and tumor incidence rates. Therefore, this reviewer used combined control (Groups 1+5) in all the statistical analyses.

There are some minor differences between the sponsor and reviewer's analysis methods. For example, the cut-off points used by the sponsor were Weeks 1-50, 51-80, 81-before terminal sacrifice, and terminal sacrifice, which are based on the suggestions from FDA. Whether the onset-rate method or onset times were used in the sponsor's analyses for palpable tumors is unknown. Interval-based methods were applied to both incidental and fatal tumors in this reviewer's analyses, while only incidental tumors were analyzed by the interval-based method in the sponsor's analyses.

Results and Discussion

In Tables 1-4, p-value under Group 1 is for trend analysis and p-values under Groups 2, 3, and 4 are for group comparisons.

The Rat Study

Survival. As shown in Tables 1 (male) and 2 (female), there were no significant positive trends or group comparisons in mortality in either of the two sexes in the rat study. The non-monotonic dose-response curve in each sex was mainly caused by the high dose group (2000 mg/kg/day), where the mortality was smaller than that of the combined control. In fact,

based on the Kaplan-Meier estimates, a monotonic positive trend in mortality was observed up to the intermediate dose group (600 mg/kg/day) in both sexes.

In the males, there were at least 50% of the animals in each group still surviving at the beginning of Week 90, indicating that a sufficient number of the male rats were exposed to the treatment adequately, even though the study was terminated at Week 101. In the females, there were at least 50% of the animals in each group still surviving at the beginning of Week 80. However, the Control-I, low-dose, and intermediate-dose groups survival rates were down to below 50% around the middle of Week 80, which was probably the reason for the sponsor to terminate the study at Week 89. The Kaplan-Meier product limit survival curves for the males and females are presented in Figures 1 and 2, respectively.

Neoplastic Lesions. In either sex of the rat study, there were no significant increases in the incidences of any tumors in the high-dose group when compared to the combined control. The summary incidences can be found in Volume 19, Table 8.6 of Reference 44.

The Mouse Study

Survival. As shown in Tables 3 (male) and 4 (female), there were no significant positive trends or group comparisons in mortality in either of the two sexes in the mouse study. In the males, there were at least 68% of the animals in each group still surviving at the beginning of Week 70 and more than 50% of animals were alive when the study was terminated at Week 80. In the females, by Week 80 (the original planned study termination week), there were at least 67% of the animals in each group still surviving, which was probably the reason for the sponsor to extend the study to Week 88. With respect to the duration of the mouse study, there were a sufficient number of animals exposed to the treatment adequately.

Neoplastic Lesions. The sponsor noted a statistically significant increase in uterine smooth muscle tumors (leiomyoma and leiomyosarcoma) in the high-dose group compared with controls. Since the concurrent combined control rates for the leiomyoma and consequently the combined leiomyoma and/or leiomyosarcoma were >1%, they were considered to be common tumor types. As a result, both the sponsor and this reviewer's analyses showed that the increased incidences of those tumors in the high dose group over the combined control were judged not to be significant at 0.01 significance level according to the FDA's guidance.

Uterus	Combined Control	High Dose	Reviewer's p	Sponsor's p
Leiomyoma	7/102 (= 5/51 + 2/51)	7/51	0.0752	NA
Leiomyosarcoma	1/102 (= 0/51 + 1/51)	2/51	0.2139	NA
Combined tumors	8/102 (= 5/51 + 3/51)	9/51	0.0295	0.019

In summary, there were no significant positive tumor findings in either sex of the mouse study. The summary incidences can be found in Volume 14, Table 9.3 of Reference 39.

Conclusion

In both the rat and mouse studies, no significant positive findings in mortality or tumor incidence rates were observed in either sex. Based on examination of the validity of the study designs, the majority of the rats and mice were exposed to treatment adequately.

In general, this reviewer's conclusions for survival and tumor analyses for the rat and mouse studies agree with the sponsor's.

Labeling Comments

The sponsor might be interested in adding [ ]' to the text where it says [

]

Prepared by: Cynthia Liu, MA, Statistical Reviewer

Concurred by: Karl K. Lin, Ph.D., Expert Mathematical Statistician (Applications in Pharmacology and Toxicology)

CC: HFD-510/VJimenez, KDavisbruno, IAntonipillai
HFD-715/ENevius, KLin, TSahlroot, CLiu
HFD-700/CAnello

Table 1 – Results of Statistical Analyses of Mortality Data for Male Rats

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	2000	0
Number of Deaths ([a] = Including 1 animal with accidental death)					
Weeks 0-52	1[a]	2[a]	4[a]	5[aaa]	2
Weeks 53-78	5	4	9[a]	6	6
Weeks 79-92	14	14[a]	10	8	9
Weeks 93-before term sac	7	8	7	6	11[a]
Terminal Sacrifice Weeks	23	22	20	25	22
Unadjusted Mortality	26/50	26/50	28/50	22/50	27/50
Kaplan-Meier Estimate (Final)	0.531	0.540	0.582	0.468	0.542
Cox-Tarone Test (two-sided p)	0.6025 –	0.9680 –	0.4453 +	0.6583 –	
Gehan-Breslow Test (two-sided p)	0.8143 –	0.8996 –	0.2164 +	0.7426 –	

Figure 1 – Kaplan-Meier Product Limit Survival Curves for Male Rats

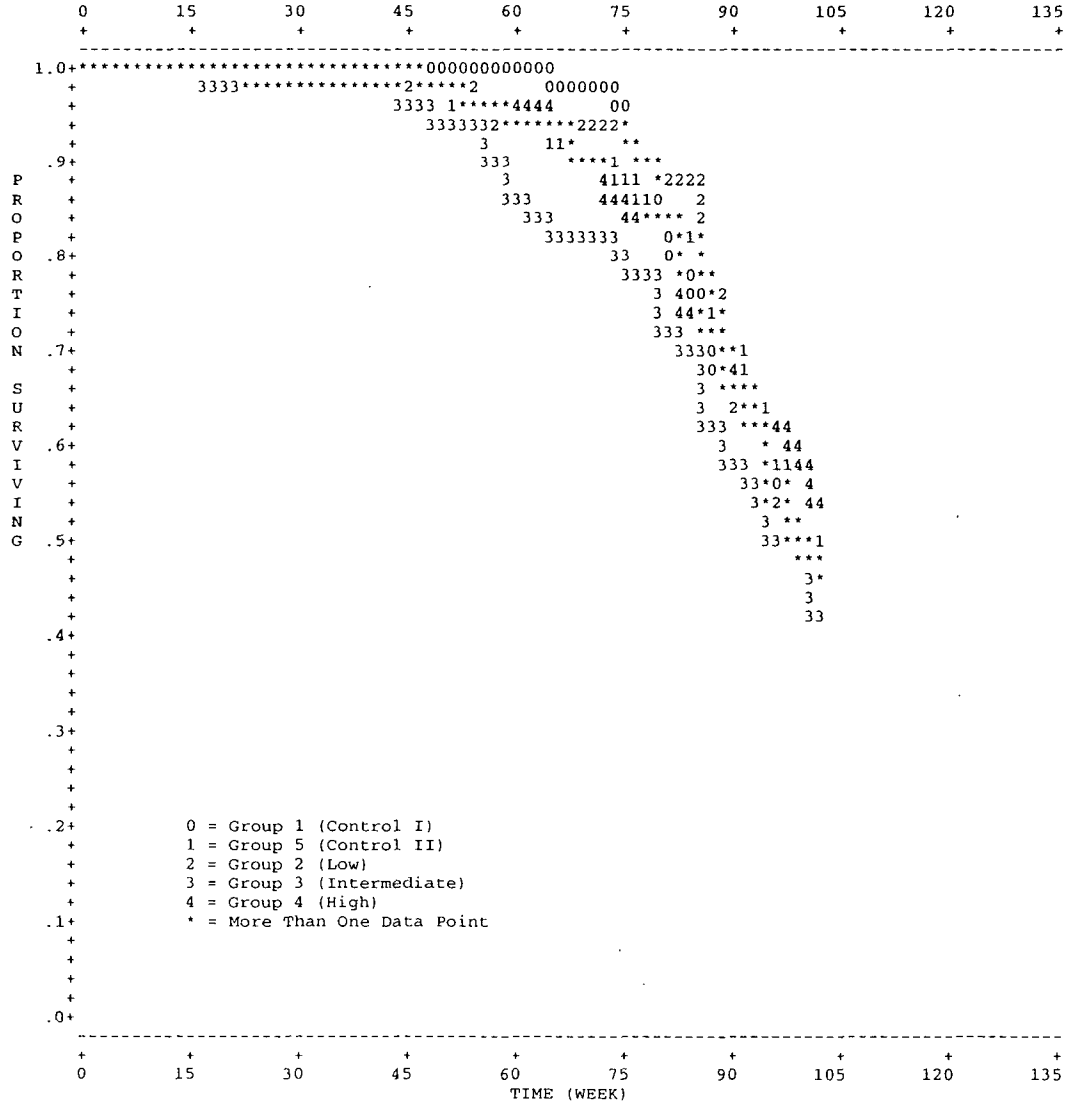


Table 2 – Results of Statistical Analyses of Mortality Data for Female Rats

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	20000	0
Number of Deaths ([a] = Including 1 animal with accidental death)					
Weeks 0-50	1[a]	2[a]	3	5[a]	4[a]
Weeks 51-75	13[a]	12	11[a]	5	10
Weeks 76-before term sac	14	14	18	12	11
Terminal Sacrifice Weeks	22	22	18	28	25
Unadjusted Mortality	26/50	27/50	31/50	21/50	24/50
Kaplan-Meier Estimate (Final)	0.542	0.551	0.632	0.428	0.489
Cox-Tarone Test (two-sided p)	0.2689 –	0.8691 +	0.3400 +	0.3806 –	
Gehan-Breslow Test (two-sided p)	0.2869 –	0.9001 +	0.4305 +	0.3289 –	

Figure 2 – Kaplan-Meier Product Limit Survival Curves for Female Rats

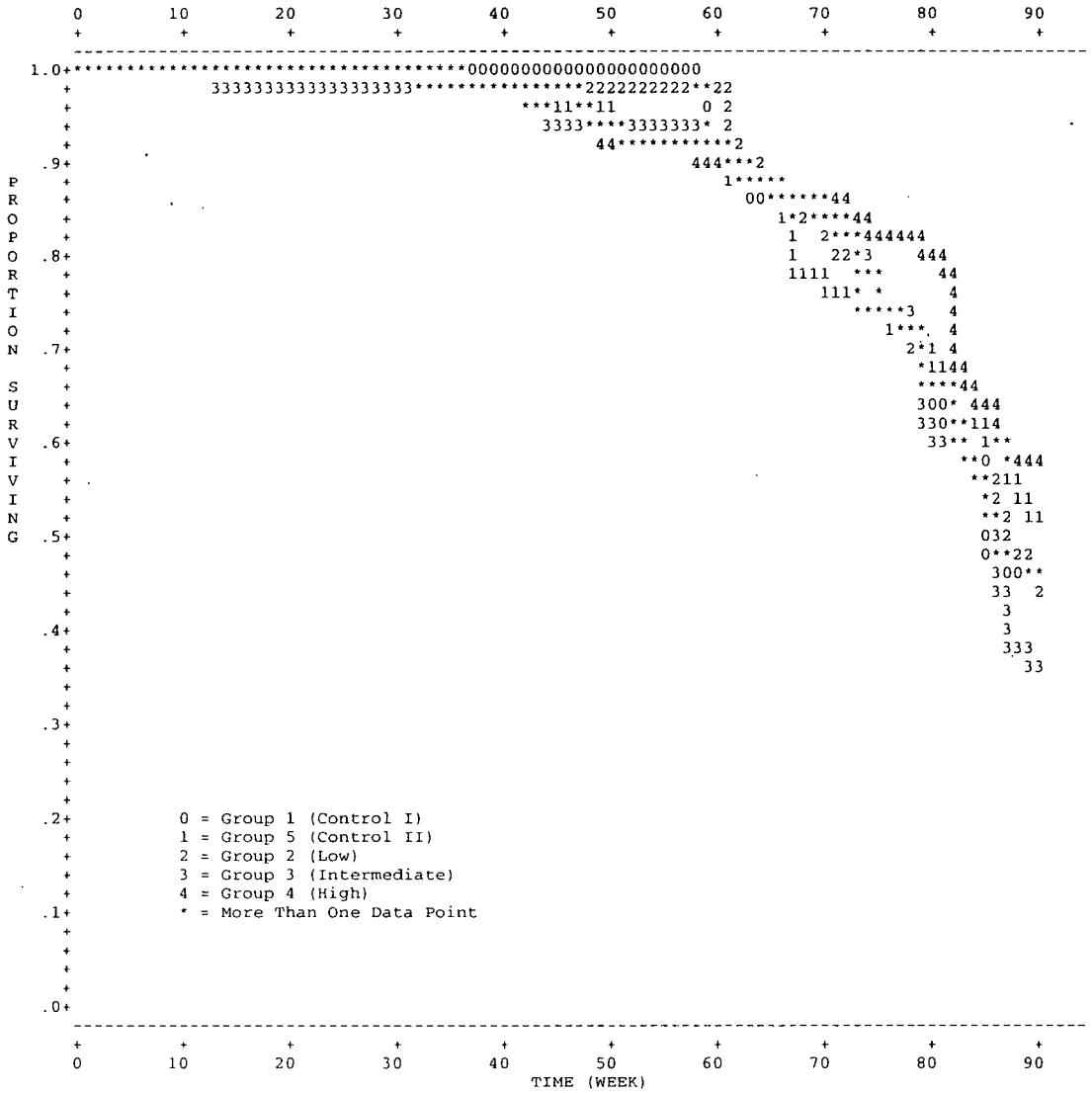


Table 3 – Results of Statistical Analyses of Mortality Data for Male Mice

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	2000	0
Number of Deaths ([a] = Including 1 animal with accidental death)					
Weeks 0-50	4[aa]	3	4[a]	6	7
Weeks 51-70	8	13[aa]	6[a]	5	10[a]
Weeks 71-before term sac	10[a]	9[a]	10[a]	6	8[a]
Terminal Sacrifice Weeks	29	26	31	34	26
Unadjusted Mortality	19/51	22/51	17/51	17/51	23/51
Kaplan-Meier Estimate (Final)	0.388	0.448	0.349	0.333	0.458
Cox-Tarone Test (two-sided p)	0.2717 –	0.9398 +	0.3753 –	0.4359 –	
Gehan-Breslow Test (two-sided p)	0.3598 –	0.8944 +	0.2531 –	0.4581 –	

Figure 3 – Kaplan-Meier Product Limit Survival Curves for Male Mice

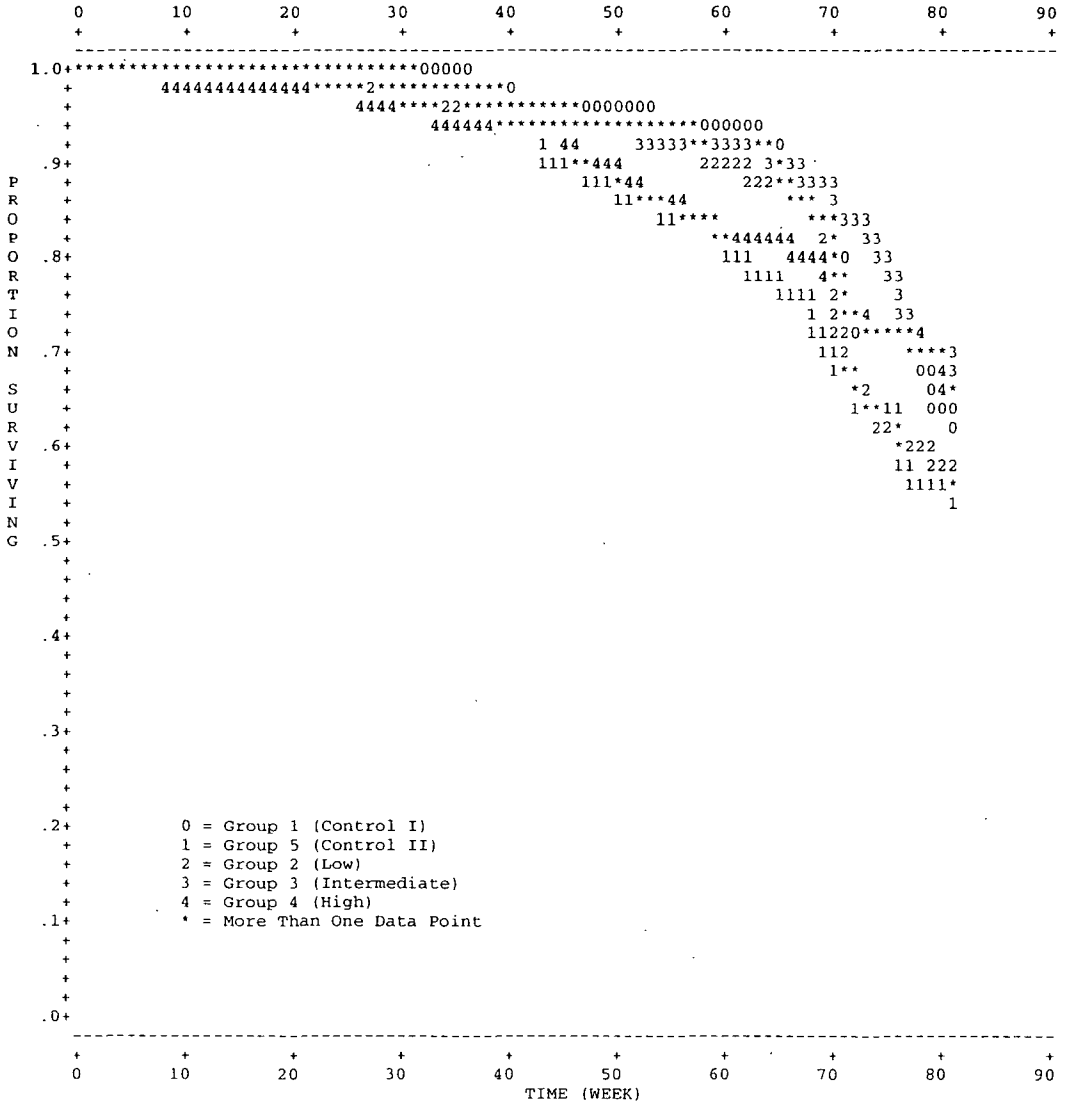
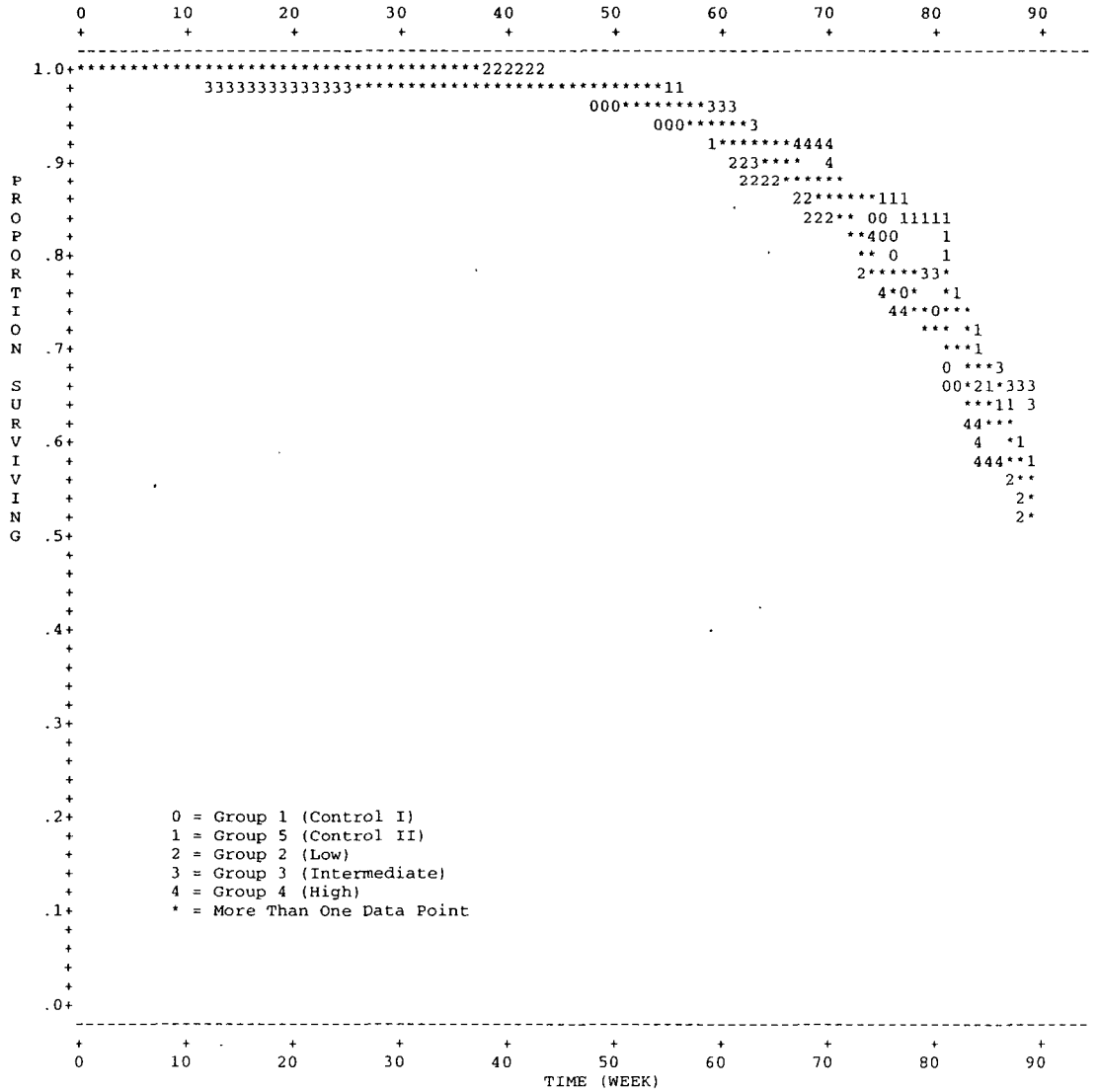


Table 4 – Results of Statistical Analyses of Mortality Data for Female Mice

Group	1	2	3	4	5
Dose (mg/kg/day)	0	100	600	2000	0
Number of Deaths ([a] = Including 1 animal with accidental death)					
Weeks 0-50	2	1	1	1	1
Weeks 51-75	7	10	13[aaa]	14[aaa]	6
Weeks 76-before term sac	14	13	6	11	14
Terminal Sacrifice Weeks	28	27	31	25	30
Unadjusted Mortality	23/51	24/51	17/51	23/51	21/51
Kaplan-Meier Estimate (Final)	0.451	0.471	0.353	0.475	0.412
Cox-Tarone Test (two-sided p)	0.7110 +	0.7302 +	0.5608 –	0.6702 +	
Gehan-Breslow Test (two-sided p)	0.7104 +	0.6377 +	0.5889 –	0.5700 +	

Figure 4 – Kaplan-Meier Product Limit Survival Curves for Female Mice



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/s/

Cynthia Liu
6/29/04 11:18:13 AM
BIOMETRICS

Karl Lin
6/29/04 11:22:51 AM
BIOMETRICS
Concur with review