CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 207648
Supporting document/s:	000
Applicant's letter date:	September 25, 2014
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Product:	Smoflipid 20 % (lipid injectable emulsion)
Indication:	(b) (4)
Applicant:	Fresenius Kabi
Review Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
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1 Executive Summary

1.1 Introduction

Smoflipid 20 % is an intravenous (IV) lipid emulsion containing essential and nonessential fatty acids for use in total parenteral nutrition (TPN). It is a mixture of fatty acids derived from four different oils: soybean oil (6 %), medium chain triglycerides (6 %), olive oil (5 %), and fish oil (3 %). The Applicant is seeking an approval of Smoflipid as a supplement (b) (4) Smoflipid is supplied in a (b) (4) single chamber plastic container of 100 ml, 200 ml or 500 ml, and can be administered by IV infusion into a central or peripheral vein.

1.2 Brief Discussion of Nonclinical Findings

The applicant has provided adequate nonclinical pharmacology and toxicology information in support of the NDA application. Nonclinical studies submitted are with Smoflipid or its constituent lipids: long chain triglycerides (LCT), medium chain triglycerides (MCT) and omega 3 (n-3) fatty acids (Omegaven) and Intralipid (soybean oil emulsion). In nonclinical studies, Smoflipid or its constituent fatty acids were generally well-tolerated in a rodent (rats) and a non-rodent (dogs) species at the maximum doses administered, with slight or moderate, but reversible adverse effects. The safety of Smoflipid and/or the individual lipid constituent, soybean oil (Lipovenos 20 %, Intralipid 20 %), fish oil (Omegaven 10%) has been established in safety pharmacology, repeat dose IV toxicity and genotoxicity studies. There are no significant nonclinical safety concerns for Smoflipid 20 %.

In repeat-dose toxicity studies with Smoflipid 20 % in rats, it was tolerated for only up to 4 weeks. Administration of Smoflipid beyond 4 weeks at the highest dose of 18 g/kg/day (3.8 mL/kg/hr) was associated with mortalities and and a wide range of toxicities, related to the continuous 24 hour infusion of a large volume of the fat emulsion. In dogs, infusion of Smoflipid 20% (6 hr/day) for 13 weeks was well-tolerated with limited adverse effects, related to the nature of the formulation and administration of a large volume of the parenteral emulsion. There were no mortalities in dogs, and no changes in hemodynamic or ECG parameters were observed. Mild to moderate indurations observed at the infusion site, and slight discoloration and fatty changes in the liver were fully or partially reversible at the end of the 4-week recovery period. The other componets of SMOFlipid were similarly well-tolerated in rodents and non-rodets following repeated administration for up to 13 weeks.
Smoflipid and/or its constituents were not genotoxic in a standard battery of genotoxicity assays. Reproductive toxicology studies were not performed with Smoflipid. However, in a pre- and post-natal developmental study in rabbits with Intralipid, no teratogenic effects, or effects on fetal development were observed.
The applicant has performed a comprehensive safety assessment of the potential leachables and extractables from the one chamber (b) (4) container closure system.

The safety assessment of the potential leachables/extractables appears to be adequate and acceptable, and does not raise any safety concerns.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, the NDA application is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended.

1.3.3 Labeling

The proposed draft labeling generally conforms to the format specified under 21CFR 201.56(d) and 201.57 for the content and format of labeling for human prescription drugs. However, the following changes in the proposed labeling are recommended.

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Proposed Version:

(b) (4)

13 NONCLINICAL TOXICOLOGY

Proposed Version

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies with Smoflipid have not been performed to evaluate the carcinogenic potential, or effects on fertility. (b) (4)

No mutagenic effects were observed in the following *in vitro* studies (b) (4) [redacted]

In an *in vivo* bone marrow cytogenetic study in rats, no mutagenic effect was observed.

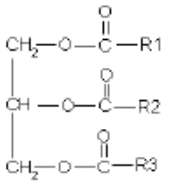
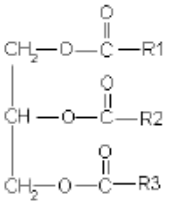
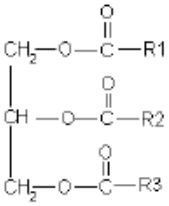
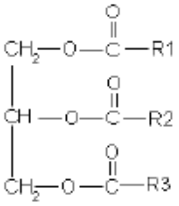
2 Drug Information

2.1 Drug

Smoflipid 20 % (lipid injectable emulsion)

CAS Registry Number 8016-13-5
Generic Name
N/A
Code Name
N/A
Chemical Name
SMOFlipid 20% is an intravenous lipid emulsion containing fatty acids derived from four different oils: soybean oil (6%), medium chain triglycerides (6%), olive oil (5%), and fish oil (3%).

Molecular Formula/Molecular Weight

API	Structural formula	Molecular formula
Soybean oil	 R = Fatty acid chains	Triacylglycerol with fatty acid chains mainly C16:0, C18:1, C18:2, C18:3
Medium chain triglycerides (MCT)	 R = Fatty acid chains	Triacylglycerol with fatty acid chains mainly C8:0, C10:0
Olive oil	 R = Fatty acid chains	Triacylglycerol with fatty acid chains mainly C16:0, C18:1, C18:2
Fish oil	 R = Fatty acid chains	Triacylglycerol with fatty acid chains mainly C20:5, C22:6

Pharmacologic Class

Lipid Injectable Emulsion

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 102137 (Smoflipid; Fresenius Kabi), NDA 200656 (Kabiven and Perikabiven; Fresenius Kabi), NDA 017643, NDA 018449, NDA 019942, NDA 020248 (Intralipid; Fresenius Kabi)

2.3 Drug Formulation

2.3.1 Drug formulation.
SMOFlipid 20% is an intravenous lipid emulsion containing fatty acids derived from four different oils: soybean oil (6%), medium chain triglycerides (6%), olive oil (5%), and fish oil (3%). Smoflipid has three packaging sizes, 100 ml, 250 ml and 500 ml in a 1 chamber bag.

Strength	**20%**		
Packaging Configuration (Bag size)	**100 mL**	**250 mL**	**500 mL**
Product Code	**830307310**	**830570310**	**830820310**
SMOFlipid	**Content (per 1000 mL)**	**Function**	**Quality of ingredient**
• **Active ingredients**			
Soybean oil	60 g	Active substance	USP
Triglycerides, medium-chain	60 g	Active substance	NF
Olive oil, refined	50 g	Active substance	NF
Fish oil, rich in omega-3 acids	30 g	Active substance	USP Dietary supplements
• **Other ingredients**			
all-rac-α-Tocopherol (Vitamin E)	163-225 mg	(b) (4)	USP
Purified egg phospholipids	12 g		NF
Glycerol (b) (4)	25 g		USP
Sodium oleate (b) (4)			In-house specification
Sodium hydroxide	to pH (b) (4)		NF
Water for injections	to 1000 ml		USP
(b) (4)			NF

2.4 Comments on Novel Excipients

There are no novel excipients in the Smoflipid drug product

2.5 Comments on Impurities/Degradants of Concern

Potential impurities from Smoflipid consist of process impurities, environmental impurities and degradation product impurities. The process impurities (b) (4) were tested by the Sponsor and meet the USP standards. Similarly, environmental impurity levels (b) (4) were tested and meet the USP standards. Degradation impurity levels (b) (4) were also analyzed and found to be within the specified USP limits. Thus, there are no safety concerns for the impurities/degradants present in the drug product.

2.6 Proposed Clinical Population and Dosing Regimen

Smoflipid 20 % is proposed to be used (b) (4)

2.7 Regulatory Background

At a Pre-IND Type B meeting (December 6, 2011) between FDA and the sponsor (Fresenius Kabi and APP Pharmaceuticals), there was an agreement that the sponsor will submit a 4-week rat and 3-month dog IV toxicity studies with Smoflipid in support of an NDA application. The sponsor was also asked to add testing for heavy metals to their drug product specification as well as to conduct a hemolysis potential study with the drug product.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology Studies

Primary pharmacodynamics:

1. Report on the infusion of fish oil supplemented emulsions in dogs

Safety pharmacology:

1. Investigation for hypotensive substances (European pharmacopoeia): Study on Lipovenos 20 % (Experiment code: **004-051**)
2. Action of Lipovenos 20 % (batch KE 112) on the hemodynamics, respiration and acid-base equilibrium of the anesthetized dog. Comparison with Intralipid 20 % (batch 71135-51), (Experiment code: **806-083**).
3. Cardiovascular investigation of fat emulsion 73403 (a mixture of long and medium chain fatty acids) in anesthetized cat (**Study No. 89-9)**)

Pharmacokinetic Studies (ADME)

1. Plasma elimination kinetics of Lipovenos 20 % in the dog; comparison with Intralipid 20 % (fat emulsion) (Experiment code **100-001**).
2. Estimation of the elimination rate of fat particles from the blood stream in rabbits after injection of intralipid 20 %
3. Half-lives of intralipid particles in mammals (Document No. **82 99 026**)
4. Elimination test from blood serum of intravenous infusion of 30 % fat emulsion (b) (4) (**Study No. 84-7**)
5. Elimination test from blood serum of intravenous infusion of fat emulsion 4501 20 % (a mixture of long and medium chain fatty acids) (**Study No. 86-6**)
6. Eliomination from the blood after intravenous infusion of fat emulsion 73403 20 % (**Study No. 89.5**)

Toxicology

Single Dose Toxicology Studies

1. Acute toxicity via the intravenous route in the male and female mouse: Determination of the LD_{50}; comparison between lipovenos 20 % - Intralipid 20 % (Experiment codes: **001-098 and 001-101**)
2. Estimation of acute toxicity (LD_{50}) of intralipid 20 %
3. Omegavenous: acute intravenous toxicity study in the mouse **(Report No. 5613-630/1)**
4. Acute toxicity in mice and rats after single intravenous administration of fat emulsion 73403 **(Study No. 89-17)**
5. Acute toxicity via the intravenous route in the male and female rat: Determination of the LD_{50}; study on Lipovenos 20 % (Experiment codes**: 013-018 and 013-019**)
6. Omegavenous: acute intravenous toxicity study in the rat **(Report No. 5614-630/2)**
7. Acute toxicity study of SMOF 20 % by intravenous infusion to Sprague-Dawley rats **(Report No. 9356/95)**

Repeat Dose Toxicology Studies

1. 13-Week subchronic toxicity study of SMOF 20 % by daily 6-Hour intravenous infusion to beagle dogs **(Study No. 10825/97)**
2. Plasma medium chain dicarboxylic acids and medium chain 3-hydroxy fatty acids in dogs administered fat emulsion 73403 intravenously – Preliminary study
3. A three months intravenous tolerance study in dogs of fat emulsion 4501 containing a (b) (4) mixture of soybean and (b) (4) oils. (**Study No. 87-2)**
4. Fat emulsion 73403: 13-Week intravenous toxicity study in the beagle dog followed by a four-week recovery period (**Study No. 90-25**)
5. Omegaven – 13-Week intravenous infusion (16 hours per day) toxicity study in the beagle dog followed by a 4-Week treatment-free period **(Study No. AA76192)**
6. Repeated dose toxicity of Lipovenos 20 % via the intravenous route in the Beagle dog (30 days) (**Study No. 003 – 062**)

7. Omegavenous: 4-Week intravenous administration sub-chronic toxicity study in the beagle dog **(Study No. 5820-630/5)**
8. A tolerance study in dogs of fat emulsion 73403 administered intravenously for one month (**Study No. 88-20**)
9. 4-Week subchronic toxicity study of SMOF 20 % by daily 6-Hour intravenous infusion to beagle dogs (Report No. **9358/1/95**)
10. Omegaven – 6-Week preliminary intravenous (16-Hour infusion) toxicity study in the beagle dog **(Study No. AA76191)**
11. Omegaven – 6-Week preliminary intravenous (16-Hour infusion) toxicity study in the beagle dog **(Study No. AA76191, Amendment No. 1)**
12. 28-Day toxicity of Lipovenos-infusion solution (20 %), Lot No. KI 145 and Intralipid 20 % vitrium (preparation for comparison), Lot No. 5489251 – called for short Lipovenos 20 % and Intralipid 20 % - by intravenous infusion to Beagle dogs.
13. A tolerance study in dogs of fat emulsion 4501, containing a (b) (4) mixture of soybean and (b) (4) oils, with intravenous administration for 28 days (**Study No. 86-2**)
14. A tolerance study in dogs of fat emulsion 73403 administered intravenously for eight days (**Study No. 90-6**)
15. Repeated dose toxicity of Lipovenos 20 % via the intravenous route in the rat (30 days): Comparison with Intralipid 20 % (**Study No. 002 – 007**)
16. Omegavenous: 4-Week intravenous administration subchronic toxicity study in the rat **(Study No. 5874-630/6)**
17. SMOFlipid 20 %: MTD/DRF study by continuous intravenous infusion in the Han Wistar rat **(Study No. AA78299)**
18. Smoflipid 20 %: Preliminary toxicity/long-term tolerance study by daily intravenous infusion to male Sprague-Dawley rats for 4-Weeks. **(Study No. HK0002)**
19. A tolerance study in rats of a new fat emulsion 4501 infused intravenously during 28 days (**Study No. 86-1)**
20. A two weeks intravenous tolerance study in the rat of fat emulsion 73403 (**Study No. 89-11**)
21. An intravenous tolerance study in rats of fat emulsion 73403 administered at a high dose during 27 days (**Study No. 90-12**)

Genetic Toxicology Studies

1. Mutagenicity study of SMOF 20 % in mammalian cells (V79) in the in vitro gene mutation assay (HPRT Test) (**Study No. 10554/97**)
2. In Vitro assessment of the clastogenic activity of SMOF 20 % in cultured human peripheral lymphocytes (**Study No. 10555/97**)
3. Mutagenicity study of Omegavenos in the *Salmonella Typhimurium* reverse mutation assay *in vitro* **(Study No. 8197/93)**
4. Mutagenicity study of Omegavenos in mammalian cells (V79) in the *in vitro* gene mutation assay HGPRT test **(Study No. 8198/93)**
5. *In vitro* assessment of the clastogenic activity of Omegavenos in cultured human peripheral lymphocytes **(Study No. 8199/93)**

6. Study to determine the ability of fat emulsion 73403 to induce mutation in four Histidine-requiring strains of *Salmonella Typhimurium* and two Tryptophan-requiring strains of *Eschericia Coli* **(Study No. 982/3)**
7. Study to evaluate the chromosome damaging potential of fat emulsion 73403 by its effects on cultured human lymphocytes using an in vitro cytogenetics assay **(Study No. KVS11/HLC)**
8. Study to determine the ability of fat emulsion 73403 to induce mutations at the Thymidine Kinase (tk) locus in mouse lymphoma L5178Y cells using a fluctuation assay **(Study No. KVS11/TK)**
9. Mutagenicity study of SMOF 20 % in the *Salmonella Typhimurium* reverse mutation assay *in vitro* (Report No. **9357/95**)
10. In Vivo Bone marrow cytogenetic test of SMOF 20 % by intravenous administration to Sprague-Dawley rats (chromosomal analysis) (**Report No. 10556/97**)
11. In vivo bone marrow cytogenetic test of Omegavenos by intravenous administration in Sprague-Dawley rats **(Study No. 8200/93)**
12. Study to evaluate the potential of fat emulsion 73403 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice **(Study No. KVS11/NMT)**

Reproductive and Developmental Toxicology Studies

1. Fat emulsion 73403: Teratology study in the rabbit **(Study No. 94/0212)**
2. Developmental toxicity study in rats and rabbits administered an emulsion containing medium chain triglycerides as an alternative caloric source
3. Study on the perinatal and postnatal toxicity in the rat of a mixture based on eicosapentaenoic acid and docosahexaenoic acid at 85 %

Local Tolerance Studies

1. Local tolerance test of Omegavenos (10 % fat emulsion) in rabbits after a single intravenous, intraarterial, paravenous, intramuscular and subcutaneous administration **(Study No. 8876/94)**
2. Local tolerance test of SMOF 20 % in rabbits after a single intravenous, intraarterial, paravenous, intramuscular and subcutaneous administration **(Study No. 9802/1/96)**
3. Local tolerance test of lipovenos 20 % in rabbits after a single intravenous, intra-arterial, paravenous, intramuscular and subcutaneous administration **(Study No. 9803/1/96)**

Special Toxicology Studies

1. Systemic antigenicity test of Omegavenos 10 % **(Study No. 90C-05014-00)**
2. Examination of SMOFLIPID for compatibility and hemolytic properties in EDTA-anticoagulated human blood in vitro **(Study No. 29103)**
3. Functional activity of the reticuloendothelial system of the rat after total parenteral nutrition with intralipid or fat emulsion 73403 **(Study No. 92 96 104)**
4. The effect of infusions of intralipid 20 % and fat emulsion 73403 on reticuloendothelial function in the rat **(Study No. 92 96 106)**

5. Toxicological evaluation of the [redacted] container packaging system
6. Toxicological evaluation of the [redacted] used to store the drug substance (Soybean oil, olive oil and fish oil components) of Smoflipid 20 %

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Report on the Infusion of Fish Oil Supplemented Emulsions in Dogs

The study objective of the study was to determine the effects of four different lipid emulsions:
1. A [redacted] long chain triglyceride (LCT) soy emulsion
2. A mixed [redacted] medium chain triglyceride (MCT) / [redacted] LCT emulsion
3. A [redacted] LCT soy emulsion supplemented with [redacted] fish oil triglycerides
4. A mixed [redacted] MCT / [redacted] LCT emulsion supplemented with [redacted] fish oil Triglycerides;

on the fatty acid (FA) composition of dog tissues, when infused daily over 15 days as a substantial component of a total parenteral nutrition (TPN).

Methodology:

Sixteen healthy Beagle dogs (mean body weight of 14.7 ± 1.1 kg) were randomly divided into 4 groups, with each group assigned to 1 of 4 different TPN lipid emulsion transfusion groups. Two weeks prior to the start of TPN infusion, the dogs were surgically implanted with a jugular vein catheter under anesthesia. During the anesthesia, the dogs underwent laparotomy in order to obtain large size tissue biopsies. During the 2-week recovery period, the dogs were fed normal chow diet (20 g/kg).

During 15 days, for 10 hours daily, starting from treatment day 1, all dogs received glucose at 1 g/kg/hr (Glucosteril 40 %), amino acid at 0.325 g/kg/hr (Aminosteril 10 %), vitamins, minerals and trace elements. In addition, the dogs were infused with 0.5 g/kg/hr of one of the 4 lipid admixtures. The 4 different admixtures are:

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

All the lipid preparations contained (b) (4) triglycerides and (b) (4) egg yolk phospholipids per liter of emulsion. The fish oil emulsion (Omegavenos) was supplemented with (b) (4) (b) (4) α –tocopherol All the TPN preparations were mixed in all-in-one 3 liter bags and infused via the implanted central catheter, with the infusion rates controlled by volumetric pumps.

Sampling:

Serum samples were obtained at 0 hr on days 1, 8, and 15, from which hepatic and renal function parameters were measured, and hematological status determined. Surgical biopsies from the liver, muscle (rectus abdominis), and adipose tissue (greater momentum) were obtained 2 weeks before starting the infusion. In the morning following the last infusion, a second biopsy was performed, in addition to removal of the spleen. Fatty acid pattern characterization was performed within 15 days.

Results:

There were no mortalities, and no clinical or laboratory test abnormalities were observed in any of the 16 dogs during the 15-day study.

A marked decrease (from 23.5 ± 0.2 % to 16.6 ± 4.1 %) in arachidonic acid level was observed after 2 weeks infusion with the LCT soy emulsion, in contrast to a much less pronounced effect in the other 3 groups. A significant rise of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in erythrocyte phospholipids (PL) was observed in the 2 groups supplemented with fish oil, as shown in the Sponsor's table below.

Fatty acid pattern of erythrocyte PL

fatty acid	day 1 (before TPN)				day 16 (after TPN)			
	LCT	LCT/MCT	LCT+FO	LCT/MCT+FO	LCT	LCT/MCT	LCT+FO	LCT/MCT+FO
C8:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C10:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C16:0	18.4±0.4	18.5±0.3	18.2±0.1	18.3±0.3	21.6±1.2	20.7±0.6	19.1±0.2	19.5±0.4
C18:0	25.6±0.1	24.8±0.3	25.9±0.4	25.5±0.4	27.3±2.4	25.7±0.3	24.6±0.2	24.5±0.3
C18:n-9	7.9±0.2	7.9±0.3	7.9±0.2	8.0±0.2	11.0±0.9	10.1±0.4	9.7±0.1	9.5±0.0
C18:2n-6	8.7±0.3	8.6±0.2	8.9±0.4	8.7±0.1	8.5±0.1	8.7±0.2	8.5±0.3	7.7±0.2
C18:3n-3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C20:4n-6	23.5±0.2	23.5±0.1	24.4±0.4	23.2±0.3	16.6±4.1	21.8±1.0	21.2±0.6	21.9±0.6
C20:5n-3	0.5±0.1	0.9±0.2	0.5±0.1	0.7±0.2	0.6±0.1	0.5±0.1	1.5±0.2*	1.4±0.2
C20:6n-3	0.6±0.0	0.7±0.1	0.7±0.0	0.6±0.1	0.7±0.2	1.0±0.1	1.5±0.0**	1.6±0.1**

Liver lipids

Liver triglycerides were low, but large individual variability was evident in the initial samples prior to TPN initiation, and became more homogenous in biopsies obtained from all groups after 2 weeks of TPN administration. Similarly, liver PL and total cholesterol (TC) did not increase in any group after TPN administration. The free cholesterol (FC) measured in initial samples became esterified by 25-40 % in all groups on day 16. Changes in liver triglyceride fatty acids (TGFA) pattern consists of increased linoleic (18:2n-6) in both LCT treated groups (1 and 3), which was compensated for by a relative decrease of 16:0 and 18:1n-9. Although linoleate was unchanged in both MCT/LCT groups 2 and 4, there was marked reduction in the 2 groups. In the fish oil supplemented groups 3 and 4, EPA (C20:5n-3), and to a lesser extent, DHA (C20:6n-3), were detected in liver triglycerides (TG) and accounted for 3 % and 1.2 %, respectively of TG fatty acids. In both fish oil supplemented groups increases of EPA (0.5 % to 2.5 %), and mainly of DHA (3.5 % to 10 %) were observed. This increase of n-3 FA in liver PL was associated to a significant decrease of arachidonic acid 20:4n-6, which was not observed in the non-supplemented groups.

Muscle lipids

There was no significant change in muscle TG, PL, TC and FC in any group after 2 weeks of TPN administration. LCT treatment induced changes of TGFA pattern in muscles, mainly consisting of an increase in 18:2n-6 (linoleic), which was, however, suppressed in the LCT/MCT groups. Similar to liver TG, but to a smaller extent, MCFA were also found in the muscle TGFA of dogs infused with LCT/MCT. In contrast, EPA and DHA were not detected in the muscle TG of fish-oil supplemented groups. After 2 weeks of TPN administration, 18:2n-6 was unchanged in muscle PL in all groups, but 20:4n-6 level decreased significantly, particularly in the LCT group. EPA and DHA content increased significantly in muscle PL, only in the fish oil treatment groups.

Spleen lipids

There were no differences observed in the TG, PL, TC and FC contents in the spleen of all the study animals, suggesting similar accumulation of infused lipids. Fatty acid (FA) pattern in the spleen TG was influenced by the composition of the infused emulsions: dogs infused with LCT showed higher concentration of 18:2n-6 than the corresponding LCT/MCT groups. Dogs receiving MCT showed substantial amounts of MCFA (5-13 %),

while n-3 LCPUFA was present in animals receiving fish oil supplementation. The only difference of FA pattern in spleen PL between the groups consisted of a marked rise of EPA and DHA content in the fish oil supplemented animals.

Adipose tissue triglycerides
Since the majority of lipids in adipose tissues are triglycerides (TG), the fatty acid (FA) pattern was measured in total lipids. The infusion of soy emulsion resulted in a marked increase of 18:2n-6 (linoleic acid) and a moderate increase of 18:3n-3 (alpha-linolenic acid), that was not observed in both MCT/LCT groups. Although EPA and DHA were not detected in adipose tissue, traces of 8-carbon fatty acid and substantial amounts of 10-carbon fatty acid were measured in the adipose tissue after 15 days of MCT/LCT infusion.

In summary, following a two week (15 day) total parenteral nutrition (TPN) in 4 groups of 4 dogs each infused with LCT, or LCT and (b) (4) fish oil, or LCT/MCT, or LCT/MCT and (b) (4) fish oil, there were no differences in safety or tolerance to the different TPN preparations. However, linoleate content of tissue triglycerides (TG) was markedly increased with the LCT emulsions, but reduced with LCT/MCT preparations.
Omega 3 fatty acids (n-3FA) were incorporated (slightly) into liver TG, but remained undetected in extra hepatic tissue TG, in contrast to medium chain fatty acid (FA), which was found in tissue TG after infusion of the mixed MCT/LCT emulsions.
Changes in tissue phospholipids (PL) involved increased linoleate in the liver PL, but not in other tissues with infusion of long chain triglyceride (LCT) soy emulsion. MCT/LCT infusion did not markedly affect phospholipids fatty acid (PLFA) pattern in any tissue, but supplementation with fish-oil was associated with the incorporation of n-3FA into tissue PL, especially in the liver.

4.3 Safety Pharmacology

Investigation for hypotensive substances (European pharmacopoeia): Study on Lipovenos 20 % (Experiment code: **004-051**)

Two healthy male cats were anesthetized and catheterized with a common carotid cannula through the trachea (for continuous monitoring of blood pressure). A second cannula was inserted into the jugular or femoral vein (for the injection of histamine or Lipovenos 20 % (a soybean oil emulsion). Histamine at a dose of 0.1 and 0.15 µg/kg (dissolved in 0.9 % (w/v) saline to a volume of 1 ml or 1.5 ml/kg) was injected IV at regular intervals, four times, prior to the injection of 5 ml/kg of Lipovenos 20 % for over 30 secconds. The histamine test injection was repeated 2 times in each animal, to elicit a drop in blood pressure (BP) with each injection, prior to the test substance injection.
Results: The injection of 5 ml/kg of Lipovenos 20 % for over 30 seconds did not induce a drop in arterial BP in the study animals. In contrast, histamine injection elicited a drop in arterial BP of -50 and -47 mm Hg in test animal #1, and -53 and -47 mm/Hg in test animal #2.

Action of Lipovenos 20 % (batch KE 112) on the hemodynamics, respiration and acid-base equilibrium of the anesthetized dog. Comparison with Intralipid 20 % (batch 71135-51), (Experiment code: **806-083**).

Six healthy female Beagle dogs (mean weight of 11 kg) assigned (3 animals/group) to 1 of 2 groups (Lipovenos 20 % or Intralipid 20 %) were fasted for 16 hours and anesthetized (with IV sodium pentobarbital, 30 mg/kg) prior to dosing with 20 ml/kg (4 g lipid/kg) of Lipovenos 20 % or Intralipid 20 % for 30 minutes. The animals were implanted with catheters for the recording of arterial blood pressure (systolic and diastolic), heart rate (HR), left intraventricular pressure (LIVP), isometric myocardial contractility (dp/dt), electrocardiogram, respiration tidal volume rate (TV), pH of arterial blood, and PO_2-PCO_2 of arterial blood. Other vascular parameters calculated from the study are, differential arterial pressure (DAP), mean arterial pressure (MAP), Ejection time (ET), tension-time index (ET•MAP•HR), total CO_2 (tCO_2), base excess (BE), oxygen saturation (O_2 sat), standard bicarbonate (HCO_3).

Results: At the end of the 30 min infusion of lipovenos 20 % or intralipid 20 %, cardiovascular parameters such as the HR, ET, and the electrocardiogram, and the acid-base equilibrium all remained unchanged. The moderate MAP elevation observed was ascribed to the rapid infusion of the emulsions and was not considered biologically significant.

In 2 of the 3 dogs infused with Lipovenos 20 %, there was elevation of the LIVP by 20 % and 24 % during the infusion, with a subsequent decrease by 9 % and 5 %, respectively, after the 30 minutes infusion period. Elevation of dp/dt occurred in the 3 animals receiving lipovenos 20 % by 40 %, 12 %, and 25 % during infusion, but decreased to values identical to the 2 groups of animals at the end of the 30 min infusion period.
The measured tension-time index showed an increase of 13 %, 22 %, and 10 % in the animals receiving lipovenos 20 % during the infusion period, but the changes were low and not considered pharmacologically significant.
A single animal developed irregular respiration during the study period, and was administered artificial respiration. Other animals with observed changes in respiration were considered affected by the degree of anesthesia, and not by the TPN infusion.

In summary, the infusion of 4 g/kg lipovenos 20 % and intralipid 20 % in female beagle dogs for 30 min did not induce any undesirable cardiovascular effect such as hypotension or change in acid-base equilibrium. Infusion of the lipid emulsions did not result in any undesirable hemodynamic effects, but rather increased myocardial performance on occasions in some animals administered lipovenos 20 %. The dose of infused lipids (4 g/kg body weight for 30 min) is higher than the recommended maximum clinical dose of 3 g/kg for 24 hours.

Cardiovascular investigation of fat emulsion 73403 (a mixture of long and medium chain fatty acid) in anesthetized cats (**Study No. 89-9)**)

Eight healthy 6-10 months old male cats (2.6 to 4 kg;4 animals/group) were anesthetized prior to dosing with 27 ml/kg (at a rate of 0.15 ml/kg/min for 180 minutes) of fat emulsion 73403 or Intralipid 20 % at 1.8 g TG/kg/hr or 5.4 g TG/kg/day through the cephalic vein. Cannulas were inserted into the trachea to monitor respiration and into the right femoral artery to monitor blood pressure. Heart rate was monitored through a tachygraph connected to an amplifier, and blood flow was measured electromagnetically through a flow probe inserted around the central mesenteric artery. ECG was recorded from electrodes attached to the extremities of the animals. Blood gas measurements were obtained through a pH/blood gas analyzer, and hemoglobin levels were determined with a spectrophotometer.

Blood samples were obtained prior to the start of infusion, 90 mins after the start of infusion, immediately after the end of infusion (180 min), and 60 min after the end of infusion (240 min).

Results: The increases in blood pressure, heart rate, blood flow, respiratory frequency and tidal volume were similar in the two dose groups during the infusion of fat emulsion 73403 or Intralipid 20 %. There was a slight increase in the S-T segment duration after the infusion of fat emulsion 73403 or Intralipid 20%.

For blood gases, PCO_2 decreased and PO_2 increased following infusion of fat emulsion 73403 as well as Intralipid 20 %. The changes were as a result of the increase in respiratory frequency.

The pre-dose hemoglobin (Hb) levels were similar for the two groups, and following lipid infusion, the Hb values increased to a similar extent for the two emulsions. However, the pH and ABE decreased (especially in cat #1 from group 1) during the infusion of fat emulsion 73403, in contrast to observed pH increase, and no change in ABE during Intralipid infusion in group 2 animals. The slight decrease in pH and ABE observed in group 1 animals is an indication of induction of metabolic acidosis by fat emulsion 73403, and falls within the normal range of evaluation, without any biological significance.

There were increases in lactate and β-hydroxybutyrate levels during the infusion of fat emulsion 73403 when compared to the infusion of Intralipid 20 %. The increases were more pronounced in cat #1 from group 1, and an indication of the slight metabolic acidosis by fat emulsion 73403.

There were slight increases in platelet count following the infusion of the two lipid emulsions, whereas, the hematocrit levels in all the animals remained unchanged throughout the study period.

Although the triglyceride levels increased in both animal groups for the study duration, the fat emulsion 73403 group values were higher, due to the higher moles/ml of lipid content in comparison to Intralipid 20 %. However, there were no changes in the cholesterol levels of the animals in both groups.

In conclusion, following 3 hr infusion of fat emulsion 73403 and Intralipid 20 % in groups of male cats, there were no adverse cardiovascular events observed in both treatment groups. However, the pH and ABE values were slightly lower, while the lactate and β-hydroxybutyrate levels were higher after Fat emulsion 73403 infusion as compared to Intralipid 20 % infusion. The differences in pH, ABE and β-hydroxybutyrate values were only observed in a single cat (#1), whereas, the difference in lactate levels were observed in 3 of 4 group 1 cats dosed with fat emulsion 73403.

5 Pharmacokinetics/ADME/Toxicokinetics

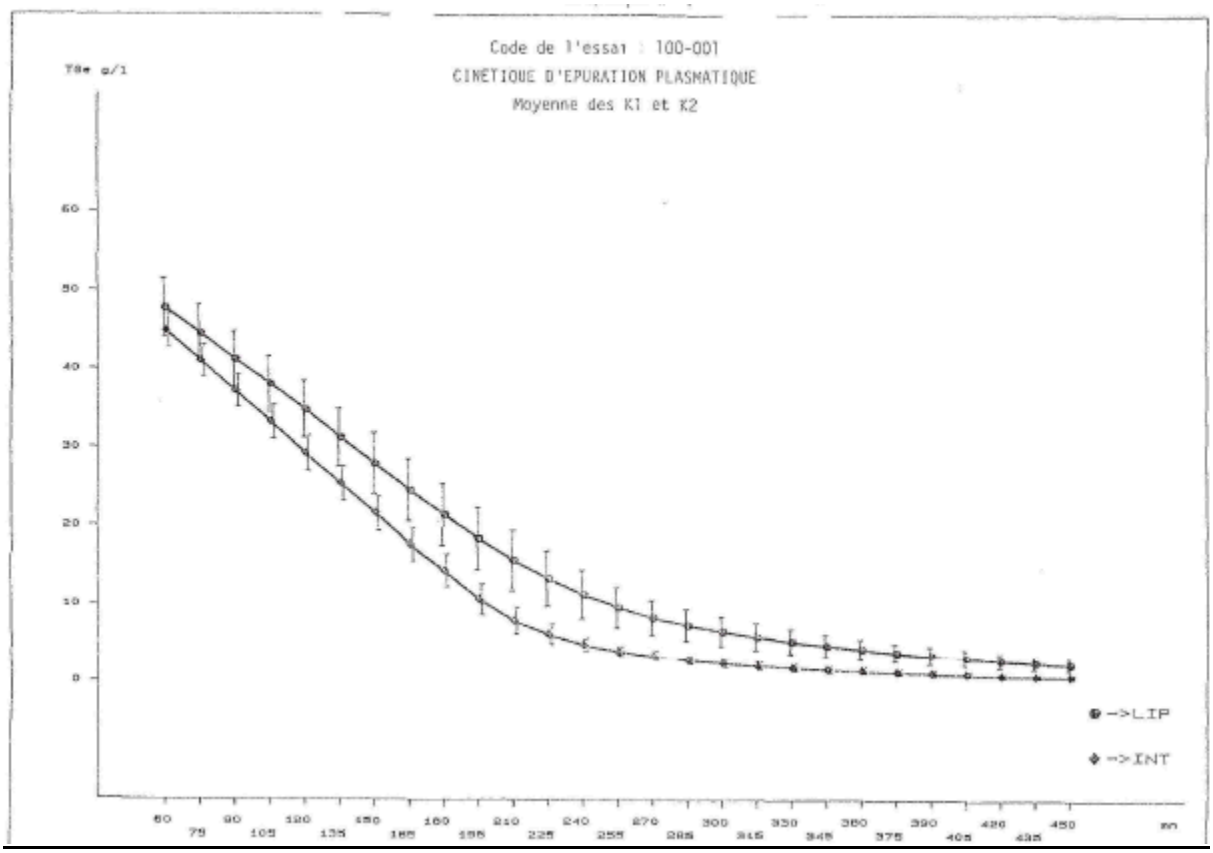
5.1 PK/ADME

Plasma elimination kinetics of Lipovenos 20 % in the dog; comparison with Intralipid 20 % (fat emulsion) **(Experiment code 100-001)**

Six healthy 1 year old male Beagle dogs (average weight 12 kg) were intravenously infused with 15 ml/kg (3 g lipid/kg) of lipovenos 20 % and Intralipid 20 % for 60 min., with one week interval between the administration of each lipid to each dog. Blood samples were taken prior to infusion and every 15 min. for 7 hours after the infusion. Samples were diluted in saline (0.9 % NaCl), erythrocytes separated by centrifugation, and triglyceride levels in the supernatant determined by nephelometry. The method allowed the calculation of maximum elimination capacity (K1), the fractional clearance rate (K2), and the critical concentration (C.C) of each lipid in the dog plasma.
Results: The plasma elimination curve of the two lipid emulsions was (a mixed linear-exponential model) biphasic with K1 (0.217 ± 0.017; 0.261 ± 0.006 g/L/min), K2 (0.885 ± 0.131, 1.132 ± 0.141g/L/min), C.C (26.47 ± 3.21, 24.76 ±3.03 g/l) for lipovenos 20 % and Intralipid 20 %, respectively; with the values not showing any statistically significant difference. The low exogenous plasma lipid concentration obtained at an infusion rate of 15 ml/kg in 1 hr (is 10X the rate used clinically) followed the zero order elimination kinetics; where the rate is constant, and independent of the lipid concentration.

At the lower lipid concentration, the elimination was a first order (exponential elimination) kinetics, where the elimination rate is proportional to the plasma lipid concentration.
The concentration of exogenous triglycerides at which the kinetics changed from zero order kinetics to first order kinetics, is termed the critical concentration. This value, expressed in g/l, is obtained using the formula (K1/K2) X 100. The elimination curve of Lipovenos 20 % and Intralipid 20 % in male Beagle dogs is shown in the sponsor's Figure below.

The Plasma Elimination of Lipovenos 20 % and Intralipid 20 % in Beagle Dogs



In conclusion, the plasma elimination kinetics of Liponenos 20 % and Intralipid 20 % administered in male Beagle dogs are biphasic and identical.

Estimation of the Elimination Rate of Fat Particles from the Blood stream in Rabbits after Injection of Intralipid 20 %

Nine white New Zealand rabbits (2.2 to 3.4 kg), fasted for 18 hrs, were infused with 100 mg fat/kg body weight of Intralipid 20 % via the marginal ear vein at a rate of 1.0 ml/min. Venous blood samples from the central ear vein of the opposite ear were drawn at 2, 4, 8, 16, and 64 min after infusion, and the light scattering indexes (LSI) analyzed by nephelometry. The elimination rate K_2 and the $t_{1/2}$ were calculated from the slope of the estimated regression line.

Results: The elimination rate K_2 was calculated to be 5.98 ± 0.38 and the half-life ($t_{1/2}$) was calculated to be 11.59 ± 0.74 min from the slope of the regression line.

Half-lifes of intralipid particles in mammals (Document No. **82 99 026**)

Adult animals (Beagle Dog, European Cat, and New Zealand White Rabbit, Sprague Dawley Rat, and NMRI mouse) were fasted for 16 hrs. prior to the IV administration of Intralipid 10 % at a dose of 0.1 g fat/kg body weight/min. Only the cat was anesthetized with chloralose, whereas other animals were dosed normally.

Venous blood samples were drawn before dosing at time 0, and at 2, 4, 8, 16, 32, and 64 min after injections. In the mouse, retroocular blood vessels were used for blood sampling, which limited the number of samples from each animal to 2. Each 0.1 ml blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and lipid levels in the supernatant were measured.
Results: The combined results of the calculated half-lives (obtained from the slopes of the regression lines) of Intralipid 10 % for all the animals, except the cat are shown in the table below. A comparison of all animal values was made to human half-life values for Intralipid 10 % (Rössner, S. Studies on an intravenous fat tolerance test. Acta Med. Scand., Suppl. 564, (1974).
Half-Lifes for Intralipid 10 % Fat Particles in Different mammalian Species

	$T_{1/2}$ ± S.E. min.	n
Dog	13.5 ± 0.83	4
Cat	10.2 ± 0.35	1
Rabbit	10.3 ± 0.92	8
Rat	9.7 ± 1.74	5
Mouse	11.3 ± 6.16	12
Man x		
male	11.4 ± 0.77	45
female	9.7 ± 0.45	43

x)Calculated from Rössner (5)

In conclusion, the fractional removal rate of fat particles of Intralipid 10 % from the blood of different mammalian animal species was similar. No differences exist between man and the commonly used laboratory animals. A mean half-life of 10 minutes was determined for all species.

Elimination test from blood serum of intravenous infusion of 30 % fat emulsion (b) (4) in Rabbits (**Study No. 84-7**)

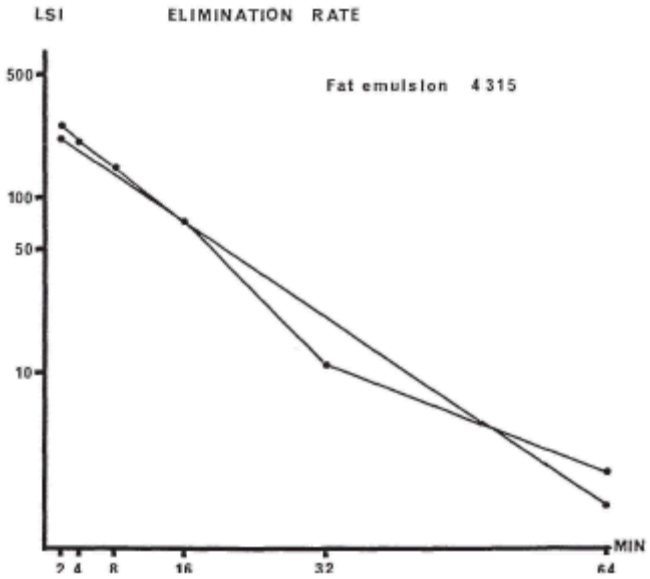
Four adult female rabbits (2.45 to 3 kg) were administered IV infusion of a 30 % fat emulsion (b) (4) at a dose of 100 mg fat/kg (0.33 ml/kg) at a rate of 1 ml/min. Blood samples of 0.1 ml were taken from a central ear artery of the animals before dosing (at time 0), and then at 2, 4, 8, 16, 32, and 64 min after the end of infusion. Each blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and triglyceride levels in the supernatant determined by nephelometry.
Results: The plasma lipid clearance rate expressed in light scattering index (LSI) following Fat emulsion (b) (4) infusion is shown in the Sponsor's table below. The

elimination curve of the lipids was estimated as a regression line in the graph below, with a half-life ($t_{1/2}$) of 9.00 ± 0.64 min and a fractional clearance rate (K_2) of 7.70 ± 0.55 %/min. For comparison, the $t_{1/2}$ of Intralipid 20 % in an earlier study was measured at 12.58 ± 0.88 min, and the K2 measured at 5.51 ± 0.39 %/min.

Nephelometric Values of Fat Emulsion (30 %) (b) (4) Elimination from Plasma of Rabbits

Min.after end of inf.	Rabbit No 1	Rabbit No 2	Rabbit No 3	Rabbit No 4
2	245	267	199	335
4	191	183	178	273
8	158	137	102	200
16	78	65	32	114
32	18	17	5	6
64	2	0	5	4

Mean Elimination Rate (LSI changes over time) of 30 % Fat Emulsion (b) (4) from the Plasma of Rabbits



Elimination test from blood serum of intravenous infusion of fat emulsion 4501 20 % (a mixture of long and medium chain fatty acids) (**Study No. 86-6**)

Four adult male rabbits (2.1 to 2.9 kg) were administered IV infusion of a 20 % fat emulsion 4501, at a dose of 100 mg fat/kg (0.50 ml/kg), with a total fat concentration of 241 ± 17 mg or Intralipid 20 % at a dose of 200 mg fat/ml, with a total fat concentration of 238 ± 16 mg at a rate of 1 ml/min, with one week interval between the administration of each lipid to each rabbit. Blood samples of 0.1 ml were collected from a central ear artery of each animal before dosing (at time 0), and then at 2, 4, 8, 16, 32, and 64 min after the end of infusion. Each blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and triglyceride levels in the supernatant determined.

Results: The plasma lipid clearance rate for fat emulsion 20 % and Intralipid 20 % are expressed in light scattering index (LSI) in the Sponsor's table below. The elimination curves of the lipids are estimated as regression lines below. The $t_{1/2}$ was found to be 6.56 ± 1.0 min and the K_2 was 10.57 ± 1.61 %/mins for fat emulsion 4501. Similarly, the $t_{1/2}$ was 7.66 ± 1.54 min while the K_2 was 9.05 ± 1.82 %/min for Intralipid 20 %. There was no statistical difference between the fat emulsion 20 % (test article) result and the Intralipid 20 % (reference article) result.

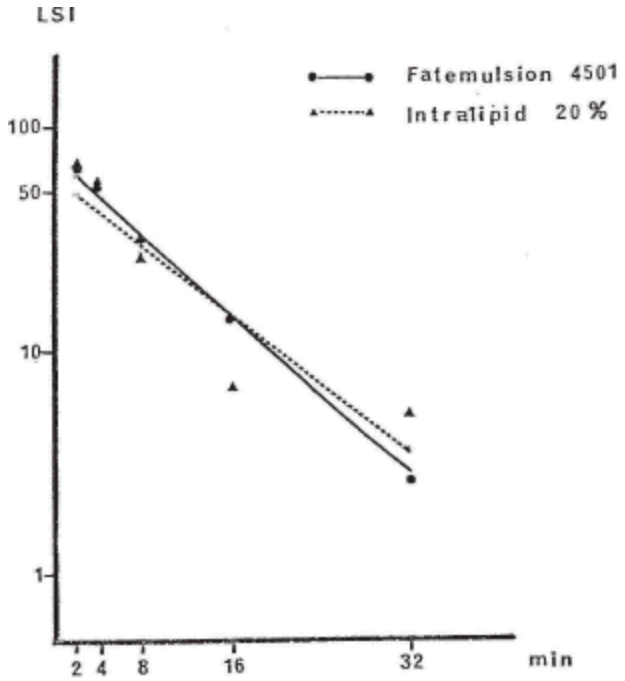
Nephelometric Values of 20 % Fat Emulsion 4501 Elimination from Plasma of Rabbits

Min.after end of inf.	Rabbit No 1	Rabbit No 2	Rabbit No 3	Rabbit No 4
2	78	63	43	87
4	73	46	31	72
8	49	29	12	51
16	21	15	3	33
32	0	3	1	18
64	0	2	0	7

Nephelometric Values of Intralipid 20 % Emulsion Elimination from Plasma of Rabbits

Min. after end of inf.	Rabbit No 1	Rabbit No 2	Rabbit No 3	Rabbit No 4
2	58	75	65	80
4	48	59	53	63
8	22	25	16	47
16	5	19	0	19
32	10	12	0	5
64	0	3	1	1

Mean Elimination Rate (LSI changes over time) of 20 % Fat Emulsion 4501 and Intralipid 20 % Emulsion from the Plasma of Rabbits



Elimination from blood after intravenous infusion of 20 % fat emulsion 73403 (**Study No. 89-5**)

Four (6-8 month old) New Zealand white male Rabbits (2.4 - 2.7 kg) were randomly infused each with a 20 % fat emulsion 73403 and Intralipid 20 %, at a dose of 100 mg fat/kg body weight (0.5 ml/kg) at a rate of 1 ml/min. The lipids were each administered to the animals following a 24-hr fasting period. Blood samples of 0.1 ml were taken from a central ear artery of each animal before dosing (at time 0), and at 2, 4, 8, 16, 32, and 64 min after the end of infusion. Each 0.1 ml blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and lipid levels in the supernatant were measured.

Results: The results show that the 20 % fat emulsion 73403 is eliminated faster than Intralipid 20 % at the administered doses.

Mean LSI Values in Serum of Rabbits after IV Infusion of 20 % Fat Emulsion 73403

Min after end of inj.	Rabbit no 1	Rabbit no 2	Rabbit no 3	Rabbit no 4	Mean value (n=4)
2	118	88	118	88	103
4	68	70	74	51	66
8	42	26	53	29	38
16	10	12	27	18	17
32	2	1	2	7	3
64	0	4	0	0	-

Mean LSI Values in Serum of Rabbits after IV Infusion of Intralipid 20 %

Min after end of inj.	Rabbit no 1	Rabbit no 2	Rabbit no 3	Rabbit no 4	Mean value (n=4)
2	125	123	112	116	119
4	119	80	84	102	96
8	71	56	56	66	62
16	44	42	22	27	34
32	10	9	11	20	13
64	1	6	2	1	3

Total Lipid Administered, Half-Life, and K2 Following IV Infusion in Male Rabbits

	Fat mg	$T_{\frac{1}{2}}$ min	K_2 %/min
Fat emulsion 73403	263±8	5.7±0.4*	12.2±0.9*
Intralipid 20 %	260±7	9.2±0.6	7.6±0.5

*Significantly different ($p<0.001$) from Intralipid 20 %

In conclusion, the 20 % Fat emulsion 73403 is eliminated faster than Intralipid 20 % when administered at a dose of 100 mg fat/kg (0.5 ml/kg) body weight.

6 General Toxicology

6.1 Single-Dose Toxicity

Acute toxicity via the intravenous route in the male and female mouse: Determination of the LD_{50}; comparison between lipovenos 20 % - Intralipid 20 % (Experiment codes: **001-098 and 001-101**)

Thirty male and 30 female CD-1 mice (22-23 g) were randomly assigned (10/sex/dose) to three groups and administered a single IV injection of Lipovenos 20 % or Intralipid 20 % at doses of 112.5, 125 or 137.5 ml/kg in male mice and at doses of 100, 112.5 or 125 ml/kg in female mice (1 ml/min). The animals were observed for signs of acute toxicity for 14 days. Animal mortality was recorded and a linear dose-response regression curve was used to calculate the LD50 dose of the lipid emulsion formulations.

Results: Mortality in male mice dosed with Lipovenos 20 % was, 1 mouse (112.5 ml/kg) on day 2; 4 mice (125 ml/kg) on day 2; 1 mouse (125 ml/kg) on day 5; and 9 mice (137.5 ml/kg) on day 2. The dosed animals were observed to be moribund, developed clonic convulsions, and had loss of righting reflex and irregular breathing at the 125 and 137.5 ml/kg doses. Macroscopic observations included mottled spleen, pale, swollen kidneys, with white and yellow liver spots. The LD_{50} for lipovenos 20 % in male mice was determined to be 129 ml/kg, with a range between 122 – 137 ml/kg.

Mortality in male mice dosed with 137.5 and 112.5 ml/kg intralipid 20 % occurred within a maximum period of 3 days following IV dosing, with total mortality at 3 mice and 7 mice in the 112.5 ml/kg and the 137 ml/kg dose groups, respectively. The dosed animals were observed to be moribund, and with diminished respiratory activity in all dose groups. In the 137.5 mL/kg (high) dose group, the male mice showed catatonia, loss of righting reflex, opisthotonos and tonic convulsion. Macroscopic observations include mottled spleen, clear swollen kidneys, and mottled and yellow spotted liver. The LD_{50} for intralipid 20 % in male mice could not be determined because the percentage mortality was not proportional to the administered dose.

Mortality in the female mice dosed with lipovenos 20 % occurred within a maximum period of 6 days following IV dosing, with total mortality at 4, 1, and 9 mice in the 100, 112.5, and 125 ml/kg dose groups, respectively. The only clinical observation of diminished activity was seen in the 125 ml/kg dose group; however, macroscopic observations of mottled spleen, swollen kidneys, and white/yellow liver spots were present in the animals. The LD_{50} for lipovenos 20 % in female mice could not be determined because the percentage mortality was not proportional to the administered dose.

Mortality in the female mice dosed with intralipid 20 % was observed to occur within a period of five days following IV dosing, with the exception of one animal dosed at 100 ml/kg which died on day 9. Total mortality in female mice dosed with intralipid 20 % were 1, 8, 8 mice in the 100, 112.5, and 125 ml/kg dose groups respectively. The animals in the 112.5 and 125 ml/kg dose groups were observed to be moribund, with irregular breathing, with loss of righting reflex and tonic convulsions. Macroscopic observations include mottled spleen, swollen kidneys, and yellow mottled liver. The LD_{50} for intralipid 20 % in female mice could not be determined because the percentage mortality was not proportional to the administered dose.

Estimation of Acute Toxicity (LD_{50}) of Intralipid 20 %

Intralipid 10 % at doses of 80, 100 and 125 ml/kg were administered IV (via the dorsal tail vein) in male NMRI mice (20/dose) at a rate of 0.25 ml/min (batch # 1900700) and to a second group of male NMRI mice (20/dose) at a rate of 0.50 ml/min (batch # 1900075). The mice were fasted for 16 hrs but allowed access to water prior to the start of the study. Mortality frequencies in the animals were observed at 1/20, 0/20 and 5/20 in mice dosed at 80, 100 and 125 ml/kg, respectively, a rate of 0.25 ml/min. In the animals injected at a rate of 0.50 ml/min, mortality frequencies were observed at 0/20, 2/20 and 5/20 at doses of 80, 100, 125 ml/kg, respectively. The low mortality frequencies did not allow the estimation of the LD_{50} values in the animals.

Omegavenous: Acute Intravenous Toxicity Study in the Mouse **(Report No. 5613-630/1)**

Twenty (6-10 weeks old) male and female (26-38 g) CD-1 mice were fasted for 3-4 hours prior to dosing. Ten of the animals were randomly assigned (1/sex/dose) to 5 screening study groups and administered a single IV injection (via a lateral caudal vein) of Omegavenous (10 % fish oil) at doses of 500 (0.5 ml/kg), 1,000 (1.0 ml/kg), 1,500 (1.5 ml/kg), 3,000 (3.0 ml/kg) and 5,000 (5.0 ml/kg) mg/kg. The animals were observed frequently for seven days for mortality only. For the limit test, 10 fasted animals 5/sex was administered a single IV dose of Omegavenous at a dose of 5,000 (5.0 ml/kg) mg/kg. The animals were observed for acute signs of toxicity and or mortality for 14 days. All the animals were sacrificed, necropsied, and acute median lethal dose of the test article estimated from the mortality results on day 15.
Results: There were no mortalities during the 14 day study period, and all the animals appeared normal on observations. All the animals showed weight gain at termination, with the exception of 1 male and 2 female animals. One female animal showed a loss of body weight, and two animals had distended uteri at necropsy.

Acute toxicity in Mice and Rats after single intravenous administration of fat emulsion 73403 **(Study No. 89-17)**

Fat emulsion 73403 was administered IV at 45 ml/kg to 6 male and 4 female (35-37 day old) NMRI mice, and at 60 ml/kg to 5 male and 5 female mice. Fat emulsion 73403 was also administered IV at 60 ml/kg to 45 day old SD rats (5/sex). The animals received the emulsion at an infusion rate of 0.5 ml/min (mice) or 2 ml/min (rats) through the tail vein. The animals were observed for 15 days for behavioral signs of toxicity or mortality. Each animal body weight was recorded prior to dosing and on study day 2, 7 and 14. All the surviving animals were necropsied on day 15, including an animal that was sacrificed on day 9 due to injury.
Results: One female mouse dosed at 60 ml/kg was found to be lethargic the second day after dosing, and died shortly thereafter. This animal was not necropsied. There were no treatment-related clinical signs observed in the rats dosed at 60 ml/kg or in the mice dosed at 45 ml/kg of the fat emulsion. There was slight piloerection observed in 2 male mice dosed at 60 ml/kg on study day 2. A male mouse dosed at 45 ml/kg was observed with a biting tail wound on study day 8 and was sacrificed on day 9 for animal welfare reasons.
In mice dosed at 60 ml/kg, reduced body weight gain or body weight loss was observed during the first week of treatment, but returned to normal body weight gain in the second week of treatment. Body weight gain was not adversely affected in treated rats although a somewhat reduced body weight gain was initially observed in the female rats.

Macroscopic examination showed that both kidneys of a male mouse C212 dosed at 60 ml/kg had a pale appearance. Histopathology examination of both kidneys showed regenerative and degenerative changes in the kidney cortex. The lesions are thought to

be treatment-related infarcts. No other changes were observed in any other mice or rats examined.

In conclusion, the administration of a single IV dose of Fat emulsion 73403 to male and female mice and rats at 45 and 60 ml/kg, respectively, resulted in a macroscopic observation of pale kidneys and treatment-related histopathologic lesions in the kidney cortex of a male mouse dosed with 60 ml/kg of the fat emulsion. There were no treatment-related mortalities.

Acute toxicity via the intravenous route in the male and female rat: Determination of the LD_{50}; study on Lipovenos 20 % (Experiment codes**: 013-018 and 013-019**)

Lipovenos 20 % at doses of 87.50, 100, 112.50 and 137.50 ml/kg were randomly administered IV to CD rats (in groups of 10/sex/dose) at 1ml/min, to determine the toxicity of the lipid emulsion. The animals were observed for signs of acute toxicity for 14 days prior to necropsy at the end of the study period. Mortality was also recorded, and a linear dose-response regression curve was used to calculate the LD_{50} dose of the lipid emulsion.
Results: Total mortality in male rats dosed with lipovenos 20 % were, 1, 7, and 9 animals in the 87.50, 100, and 112.50, ml/kg dose groups, respectively. The animals in the 100 and 112.50 ml/kg dose groups showed clinical signs of moribundity, whereas the 137.5 ml/kg dose groups showed paralysis, irregular breathing, tonic spasm, loss of righting reflex, and muscular tonus. Macroscopic observations seen in all dose groups include mottled spleen, white, greenish and swollen kidneys, and white/yellowish liver spots. The LD_{50} of IV Lipovenos 20 % in male rats was 98 ml/kg, and ranges between 93-103 ml/kg.

Mortality in the female rats dosed with Lipovenos 20 % was observed within a variable period following IV dosing, with total mortality of 2, 6, and 7 rats occurring at 100, 112.50, and 137.50 ml/kg dose groups, respectively. Only female animals in the 137.50 ml/kg dose groups showed clinical signs of moribundity, loss of righting reflex, muscular tone, irregular breathing and catatonia. Macroscopic observations seen in all the dose groups include mottled spleen, white, greenish and swollen kidneys, and white/yellowish liver spots. The LD_{50} of Lipovenos 20 % infused IV in female rats is 116 ml/kg, and ranges between 103-130 ml/kg.

Omegavenous: acute intravenous toxicity study in the rat **(Report No. 5614-630/2)**

Twenty (6-10 weeks old) male and female (200-332 g) SD rats were fasted overnight prior to dosing. Ten of the animals were randomly assigned (1/sex/dose) to 5 screening study groups and administered a single IV injection (via a lateral caudal vein) of Omegavenous (10 % fish oil) at doses of 250 (0.5 ml/kg), 500 (1.0 ml/kg), 1,000 (1.5 ml/kg), 3,000 (3.0 ml/kg) and 5,000 (5.0 ml/kg) mg/kg. The animals were observed frequently for seven days for mortality only. For the limit test, 10 fasted animals (5/sex)

was administered a single IV dose of Omegavenous at a dose of 5,000 (5.0 ml/kg) mg/kg. The animals were observed for acute signs of toxicity and or mortality daily for 14 days. Individual body weights were recorded prior to dosing, on dosing day 1, and day 7 and 14 after dosing. All the animals were sacrificed, necropsied, and acute lethal dose of the test article estimated from the mortality results on day 15.
Results: There were no mortalities during the 14 day study period. Loose feces were observed in one rat, 4 hours following dosing. Otherwise, the animal appeared normal afterwards. All other animals showed body weight gain at terminal necropsy. There were no toxicities observed at necropsy in any of the study animals. The acute IV median lethal dose of Omegavenous was determined to be in excess of 5,000 mg/kg since no animal died in the limit study.

In conclusion, the administration of Omegavenous (10 % fish oil) at a maximum dose of 5,000 mg/kg IV to male and female SD rats did not result in any mortality in the study animals. The acute lethal dose of Omegavenous was determined to be higher than 5,000 mg/kg.

Acute toxicity study of SMOF 20 % by intravenous infusion to Sprague-Dawley rats **(Report No. 9356/95)**

SMOF 20 % at doses of 45 (9 g TG/kg), 90 (18 g TG/kg) and 180 (36 g TG/kg) ml/kg were randomly administered by a single IV infusion to 46-56 day old male and female (184-216 g) SD rats (in groups of 5/sex/dose). The animals were observed daily for clinical signs of acute toxicity for 14 days prior to necropsy at the end of the study period. Mortality was also recorded, and a linear dose-response regression curve was used to calculate the LD_{50} dose of the lipid emulsion in the animals. Individual body weights of the animals were recorded prior to dosing and weekly thereafter, up to the end of the study and at necropsy. All the surviving animals were sacrificed, necropsied, and all the gross pathological changes recorded. Necropsy and gross pathological examination of the animals that died prematurely was also carried out immediately.
Results: All male and female rats dosed at 180 ml/kg (36 g TG/kg) with SMOF 20 % died within 18 hours of dosing on the first day, as shown in the Sponsor's table below. These animals showed toxic signs of reduced motility, ataxia, catalepsy, reduced muscular tonus and dyspnea prior to their expiration. Necropsy findings of pale liver and spleen were observed in the dead animals. The cause of death at the highest tested dose of 180 ml/kg was probably due to the infused volume and not the emulsion treatment. The NOEL dose in this study was 90 ml/kg (18 g TG/kg). The 14 day LD50 was calculated to be 127 ml/kg (25.4 g TG/kg) body weight.

In conclusion, the administration of SMOF 20 % at a maximum dose of 180 ml/kg IV to male and female SD rats for 6 hours resulted in the death of all animals on the first day of dosing. The lipid emulsion was well tolerated by the animals at the 45 and 90 ml/kg dose level.

6.2 Repeat-Dose Toxicity

Study title: A Two Week IV Tolerance Study in Rats of Fat Emulsion 73403

Study no.:	89-11
Study report location:	Project No. 734, Page 283 to 345
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 26, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat Emulsion 73403 20 %, lot # 3568-177; Intralipid 20 %, lot # 43365-51

Key Study Findings

Intravenous administration of Fat emulsion 73403 (a mixture of medium chain and long chain triglycerides) or Intralipid 20 % at 9 g TG/kg for 20 hrs/day to male rats for 2 weeks was well tolerated by the study animals. There were no significant differences in food consumption or body weight gain between the study groups, and there were no findings of biological significance in the the test and reference animals.

Methods

Doses:	45 ml/kg (9 g TG/kg/day
Frequency of dosing:	20 Hours infusion daily for 2 weeks
Route of administration:	IV via a central venous jugular catheter
Dose volume:	45 ml/kg/day
Formulation/Vehicle:	(b) (4) Triglyceride (fat emulsion 73403), (b) (4) soybean oil (intralipid 20 %) per liter.
Species/Strain:	CD: Sprague-Dawley Rats
Number/Sex/Group:	9 males/group
Age:	37 days old
Weight:	142.8-162.4 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minor protocol deviation reported as technical problems with catheter.

Key study findings: Intravenous administration of Fat emulsion 73403 or Intralipid 20 % at 9 g TG/kg 20 hrs/day to male rats for 2 weeks was well tolerated.
The platelet counts were similarly reduced in test and reference article group animals, and individual blood parameter values for some rats in both groups differed slightly, but were not of toxicological significance.

Inflammatory changes indicative of sepsis were seen in two animals, one each from the test and reference article groups, but there were no discernible macroscopic differences between the animals.

Microscopic changes observed included, deposition of fine droplets in a small number of thymic macrophages in two animals from the intralipid 20 % dose group; deposition of fat droplets in red pulp macrophages of the spleen in all study animals; fatty change that is characterized by vacuolation of cells with mononuclear necrotic or granulomatous infiltration of the liver in animals from both dose groups.

In conclusion, there were no differences in findings between the test and reference article group animals receiving fat emulsion 73403, 20 % or Intralipid 20 %. There was no control animal group in this study.

Study title: A Intravenous Tolerance Study in Rats of Fat Emulsion 73403 Administered at a High Dose During 27 Days

Study no.:	90-12
Study report location:	Project No. 1992-06-04, Page 387 to 467
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 19, 1990
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat Emulsion 73403 20 %, lot #356900-1; Intralipid 20 %, lot #50782-91; Sodium chloride, lot #84056-51

Key Study Findings

Intravenous infusion of 20 % Fat emulsion 73403 (18 g TG/kg/day) for 27 days in rats resulted mainly in a decrease in platelet count in treated rats when compared to animals infused with Intralipid 20 % (18 g TG/kg/day) or saline infused control animals for 27 days. Eleven animals (1 from the test article group, 4 from the reference article group and 7 from the control group) were excluded from the study due to microbial infection. The histopathological findings and fatty changes in the liver and spleen were comparable in the two lipid emulsion treated groups. The study demonstrated a good tolerance to the high dose Fat emulsion 73403 20 % for 27 days in the study animals.

Methods

Doses:	0 (control), 18 g TG/kg
Frequency of dosing:	20 hrs daily for 27 days
Route of administration:	Intravenous via a central venous catheter
Dose volume:	90 ml/kg
Formulation/Vehicle:	[redacted] (b) (4) (Fat emulsion 73403 20 % or Intralipid 20 %)/0.9 % NaCl (Saline)
Species/Strain:	Sprague-Dawley Rats (Male)
Number/Sex/Group:	10 males/group
Age:	40 days of age.
Weight:	158.1-182.6 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Deviation from study protocol was due to microbial infection of some animals and technical problems with infusion catheter

In summary, intravenous infusion of a high dose of 20 % Fat emulsion 73403 (18 g TG/kg) as the test article, or a similarly high dose Intralipid 20 % (18 g TG/kg) as the reference article, were similarly tolerated by Sprague-Dawley rats for 27 days. There were no significant differences observed between the test and reference article groups in body weight gain and organ weights, clinical chemical and morphological findings.
Eleven of the study animals (1 from the test group, 4 from the reference group, and 7 from the control group) were excluded from the study due to microbial infection. Most of the clinical, hematology, and blood chemistry changes were slight, similar in the two treatment groups, not considered to be of toxicological importance, and were found mainly in the animals excluded from the study.

Macroscopic examinations showed slight paleness of the liver in 4 of 10 rats in the test article group, and 3 of 10 rats in the reference article group; purulent abscesses adjacent to the catheter insertion site in 1 of 10 rats in the test article group, 4 of 10 rats in the reference article group, and 6 of 10 rats in the control group.

Microscopic changes observed included the presence of fine fatty droplets in pulmonary macrophages (in five rats), and thymic macrophages (in 3 rats) from the test article group. However, the occurrences of fatty changes in the spleen and liver (hepatocytes and kupffer cells) were scored at the same degree in all the rats treated with Fat emulsion 73403 or Intralipid 20 %.

Deposition of iron-containing pigment was observed in the liver and spleen of some animals in all the 3 study groups, coupled with focal mononuclear cell infiltrations, focal necrotic changes and slight focal myocardial degeneration in some animals in both the test and reference article groups.

The major difference observed between rats from the test and reference article treatment groups was a pronounced decrease in the number of thrombocytes in the fat emulsion 73403 treated animals versus the Intralipid 20 % treated animals. However, only one animal from the test article group and 2 animals from the reference article group had values below the normal range.

In conclusion, the administration of 20 % Fat emulsion 73403 or Intralipid 20 % at 18 g TG/kg/day did not result in any differences between the test or reference lipid emulsion groups regarding body weight gain, food consumption, organ weights, clinical and macroscopic changes. The minor pathological changes observed were considered to be spontaneous and seen in the control, test and reference article treated animals. The administration of Fat emulsion 73403 resulted in a higher reduction in platelet counts when compared to that observed in animals administered Intralipid 20 %.

Study title: A Tolerance Study in Rats of a New Fat Emulsion 4501 Infused Intravenously During 28 Days

Study no.:	86-1
Study report location:	Project No. 715, Page 232 to 282
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 7, 1986
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 4501 20 %, lot # 3575-004; Intralipid 20 %, lot # 55854-51

Key Study Findings

In the evaluation of tolerance to a dose of 18 g TG/kg of fat emulsion 4501 20 % (consisting of (b) (4) soybean oil and (b) (4) oil), or Intralipid 20 % in male rats, all study animals were in good clinical conditions without any significant differences in food consumption or weight gain between the test and reference article groups.
There were no changes in organ weights, clinical chemistry, and total fatty acid contents in the liver, and macro- or microscopic pathology between the test and reference article treated animals.
There were similarities in weight gain, food consumption, organ weights, clinical chemistry and histopathology between the animals infused with fat emulsion 4501 20 % and Intralipid 20 %. Thus, Lipid emulsion 4501 20 % was well tolerated for 28 days by male rats.

Methods

Doses:	18 g triglycerides (TG)/kg/day of fat emulsion 4501 (20 %) or Intralipid 20 %
Frequency of dosing:	20 hrs/day for 28 days
Route of administration:	Intravenous via central venous catheter
Dose volume:	100 ml total volume
Formulation/Vehicle:	18 g TG/kg
Species/Strain:	SPF Sprague-Dawley rats
Number/Sex/Group:	12 male rats (fat emulsion 4501 20 % group); 8 male rats (Intralipid 20 % reference group)
Age:	37 days old
Weight:	152-214 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minor deviations in daily infusion dose in some animals due to technical problems

Infusion of 18 g TG/kg/day fat emulsion 4501 20 % (test article) or Intralipid 20 % (reference article) was well tolerated by male Sprague-Dawley rats. There were macroscopic observations of patechial bleeding in the thymus, pulmonary, kidney, and one cranial lobe, in 4 fat emulsion 4501-treated animals; whereas, there were bilateral dilatations of the renal pelvis, and bleeding of a lobe of the lung in animals treated with Intralipid 20 %. The macroscopic observations did not show significant differences between the two groups.

Microscopic changes include slight focal inflammation of the lungs, vacuolation and yellowish pigment deposition in the spleen of most rats, focal hemorrhage and/or venous congestion in the lungs of some animals from both groups, vacuolated cells with mononuclear infiltration, and glycogen deposition in the liver of all rats; fat deposition in the hepatocytes and kupffer cells in all rats. The changes observed following the infusion of Fat emulsion 4501 20 % in male rats was not more severe than for Intralipid 20 % in the 28 day-treatment. There was no control group in this study.

In conclusion, the administration of 20 % Fat emulsion 4501 or Intralipid 20 % at 18 g TG/kg/day did not result in any differences between the tests. The tolerance of Fat emulsion 4501 was similar to that for Intralipid 20 % in the study animals.

Study title: Repeated Dose Toxicity of Lipovenos 20 % via the Intravenous Route in the Rat (30 Days): Comparison with Intralipid 20 %

Study no.:	85-8
Study report location:	Project 002-007, Page 1 to 164
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 6, 1985
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lipovenos 20 %, lot # KE 112; Intralipid 20 %, lot # 71133-51; Isotonic Saline, lot # 31001

Key Study Findings

In the administration of Lipovenos 20 % or Intralipid 20 % at 9 g TG/kg to rats for 30 days, there was decreased food intake in both lipid emulsion treated groups, compared to control, without changes in animal weight gain. Mottled liver was observed in 5 lipovenos and 6 Intralipid treated animals, with slight increases in liver and spleen weight.
Excessive lipid and pigment hemosiderosis, and hypertrophy of the reticular cells of the liver and spleen were observed in the treated animals.

Methods

Doses:	9 g TG/kg/day of lipovenos 20 % or Intralipid 20 %
Frequency of dosing:	Daily for 30 days
Route of administration:	Intravenous
Dose volume:	45 ml/kg/day
Formulation/Vehicle:	9 g TG/kg /Isotonic NaCl
Species/Strain:	Sprague-Dawley Rats
Number/Sex/Group:	4/sex/group
Age:	6 weeks old
Weight:	200-250 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No major deviations reported.

Observations and Results

Mortality

There was no animal mortality in this study.

Clinical Signs

Animals were observed daily from the start of infusion to the end at regular intervals.
Clinical observation in the animals included pink urine coloration in lipovenos 20 % and Intralipid 20 % treated animals, and on a single occasion in 3 control animals. The pink coloration, which is indicative of hemoglobinuria, disappeared after the end of the infusion.

Body Weights

There were no differences in body weight between the control and the treated animals.

Feed Consumption

Food consumption was recorded twice weekly for each animal.
The food intake in Lipovenos 20 % and Intralipid 20 % treated animals was reduced, when compared with the control animals. The decreased food intake was attributable to calories from the lipid emulsion. However, the changes were not statistically significant.

Ophthalmoscopy

No ophthalmological changes were observed.

Hematology

Blood samples were taken 18 hrs after the last infusion.
There were no observed differences in hematological parameters between the control and treated animals, with the exception of diminished hematocrit values in one animal in each of the 3 groups.

Clinical Chemistry

Blood samples were taken 18 hrs after the last infusion.
There were no changes attributable to the treatment with Lipovenos 20 % or Intralipid 20 %. All clinical chemistry results from all the study animals were comparable.

Urinalysis

Urine samples were taken on day 30, following diuresis on day 29 for each animal.
There were no observed differences in urine analysis between the control and treated animals.

Gross Pathology

Lipovenos 20 % treated animals had mottled liver (5 rats), and 1 animal each with spleen showing clear zone and heart with a zone of infarct. Intralipid treatment group also had 6 animals with mottled liver and 1 animal each with white inclusions in the spleen, clear zone in the spleen, and congested zone in the kidney. All other organs appeared normal.

Organ Weights

The weights of the following organs were recorded:

- brain
- heart
- liver
- adrenal glands
- lungs
- spleen
- kidneys

In comparison to control animals, the liver weight expressed as a percentage of body weight was slightly higher in animals treated with Lipovenos 20 % (by 1 %), and Intralipid 20 % (by 1-5 %).
Similarly, in comparison to control animals, the splenic weight expressed as a percentage of body weight was slightly higher in animals treated with Lipovenos 20 % or Intralipid 20 % (by 1 %).

Histopathology

The following organs were examined histopathologically:

- Adrenal glands
- Aorta (arch and abdominal section)
- Bladder
- Brain
- Colon
- Duodenum
- Eye and optic nerve
- Heart
- Hypophysis
- Ileum
- Jejunum
- Kidneys
- Liver
- Lung
- Oesophagus
- Pancreas
- Prostate or uterus
- Sciatic nerve
- Skeletal muscle
- Skin
- Spleen
- Sternum
- Stomach
- Testicles or ovaries
- Thyroids
- Tongue
- Trachea

Adequate Battery: Yes

Peer Review: No

Histological Findings
Histopathological observations in both Intralipid 20 % and Lipovenos 20% treated animals include an excess of lipids and pigment hemosiderosis, and hypertrophy of the reticular cells of the liver and spleen. There were no significant differences between the appearances of the organs in the two treatment groups.
The number of rats/sex/group was inadequate at 4/sex/group, instead of 10/sex/group.

In conclusion, the administration of Lipovenos 20 % or Intralipid 20 % at a dose of 9 g TG/kg body weight daily for 30 days in rats resulted in decreased food intake in both lipid emulsion treated groups, compared to control without changes in animal weight gain. Mottled liver was observed in 5 lipovenos and 6 Intralipid treated animals, with slight increases in liver and spleen weight of treated animals. Excessive lipid and pigment hemosiderosis, and hypertrophy of the reticular cells of the liver and spleen were observed in treated animals. There were no major toxicological changes observed in the treated animals when compared to control animals.

Study title: Omegavenous: 4-Week Intravenous Administration Subchronic Toxicity Study in the Rat

Study no.:	5874-630/6
Study report location:	Volume 1 + 2, Page 333 to 802
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 9, 1988
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Omegavenous, lot #1 (batch # MBA 01), 10 % fish oil; Lipovenos 10 %, lot #3 (batch #NC1040R6), lot #4 (batch #NA1077R10), lot #4 (batch #NC1101R9).

Key Study Findings

Intravenous administration of Omegavenous at doses of, 500, 1,000 and 5,000 mg/kg/day for four weeks did not result in any toxicity or an increase in localized reaction at the site of injection. The death of 4 males in the 5,000 mg/kg/day group, 2 males and 1 female in the 1,000 mg/kg/day group were considered to be related to the technical procedure in the study.

Methods

Doses:	0, (control), vehicle (control), 250, 1,000 and 5,000 mg/kg/day
Frequency of dosing:	Once, Daily (1 ml/min)
Route of administration:	Intravenous, via lateral caudal vein
Dose volume:	5 ml/kg
Formulation/Vehicle:	Lipovenos 10 %/Saline
Species/Strain:	Rat Crl: CD(SD) BR strain
Number/Sex/Group:	20/sex/group
Age:	6-8 weeks of age
Weight:	170 – 210 g (males), 130-165 g (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Several animals were above the specified weight range at study initiation (i.e. 224.4 g for males, and 181.6 g for females); On 3 occasions, the room temperature exceeded the range specified in the protocol, up to 27^0C; a number of blood samples collected at week 5 were clotted; measured blood urea nitrogen was presented as urea. These deviations were considered minor and did not affect the study outcome.

Observations and Results

Mortality

All animals were checked twice daily for mortalities or moribundity.

A total of 7 animal mortalities were recorded. The animal deaths were considered to be related to technical procedures and not treatment related. Four male animals from the high dose (5,000 mg/kg/day) group were sacrificed on day 24 because the tails of the animals were not patent for dosing. Two male animals in the mid-dose (1,000 mg/kg/day) group died on day 27 (from the restraint used) and on day 38 (after blood sample collection), respectively. One female animal from the mid-dose group was inadvertently left in the urine collection cage on day 32, where it was found dead.

Clinical Signs

All animals were examined once daily.

There were no observed clinical signs considered to be treatment-related.

Body Weights

Individual body weights were recorded before the first day of treatment, and at weekly intervals, thereafter.

The body weight gains observed in treated animals were similar to their respective controls.

Feed Consumption

Food consumption by each animal was recorded weekly

There were no significant differences observed in the food consumption of treated animals when compared to the respective control groups.

Ophthalmoscopy

Ophthalmic examination of all the animals were conducted pre-dose and during week 5

There were no treatment-related ophthalmic findings observed at the week 5 examinations of animals.

Hematology

Blood samples for hematology were collected from the orbital plexus of all surviving animals under light anesthesia, following an overnight fast, during weeks 5 and 6.

There were no treatment-related effects of Omeganenous administration on the hematological parameters measured in male and female rats.

Clinical Chemistry

Blood samples for clinical chemistry were collected from the orbital plexus of all surviving animals under light anesthesia, following an overnight fast, during weeks 5 and 6.

There were no effects of Omegavenous treatment observed on clinical chemistry parameters measured in male and female rats

Urinalysis

Urine samples were collected from animals during week 2 and from all surviving animals during week 5.

There were no treatment-related effects on urinalysis parameters measured in all the animal groups that was different from the respective control group.

Gross Pathology

All the surviving or moribund animals were examined, sacrificed and necropsied. Animals found dead were also examined and necropsied.

There were no treatment-related observations seen at the necropsy of study animals.

Organ Weights

The following animal organs were dissected and weighed prior to fixation:

adrenals	brain (including brain stem)
heart	kidneys
liver	ovaries
prostate	spleen
testes (with epididymides)	

Paired organs were weighed separately.

There were no significant differences observed in organ weights and organ/body weight ratios between the treated and control animal groups.

Histopathology

Histopathology samples were fixed in 10 % neutral buffered formalin, with the exception of the eyes, which were fixed in Davidson's fluid. The following organs were examined:

adrenals	brain (including brain stem)
caecum	colon
duodenum	epididymides
eyes (with optic nerves)	heart
ileum	injection site
jejunum	kidneys
liver	lungs (with mainstem bronchi)
lymph nodes (mandibular and mesenteric)	oesophagus
	ovaries
pancreas	prostate
pituitary	rectum
salivary glands (submaxillary and sublingual)	skin and mammary gland
	spinal cord (lumbar, cervical thoracic)
spleen	
sternum (with bone marrow)	stomach
testes	thymus
thyroids (with parathyroids)	trachea
urinary bladder	uterus
vagina	all gross lesions

Adequate Battery
Yes
Peer Review
Yes
Histological Findings

There was no treatment-related histopathological evidence of systemic tissue or target organ toxicity in any of the study animals. The injection site lesions observed were mainly vascular and perivascular reactions. There was no increase in the severity of the reactions when compared to the vehicle control group.

In conclusion, the intravenous administration of Omegavenous at doses of 500, 1,000 and 5,000 mg/kg/day for four weeks did not result in overt or systemic toxicity or any increase in localized reaction at the site of injection in male and female rats.

Study title: SMOFlipid 20 %: MTD/DRF Study by Continuous Intravenous Infusion in the Han Wistar Rat.

Study no.:	AA78299
Study report location:	SMOF-007-CNC, Page 1 to 610
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 28, 2008
GLP compliance:	The study was conducted according to "OECD Principles of Good Laboratory Practice".
QA statement:	Yes
Drug, lot #, and % purity:	SMOFlipid 20 %, batch #s F0361 (Phase I), F0363 (Phase II),

Key Study Findings

The administration of Smoflipid by IV infusion at doses of 9, 12 and 18 g/kg/day by 24 hours continuous daily infusion in male and female rats resulted in treatment- induced mortalities at all the dose levels, leading to the early termination of the study during 4th week of the study. The main histopathological findings consisted of vessel wall necrosis, inflammation and local abscess formation at the injection site, as well as pulmonary inflammation and lung abscesses in both moribund, early death and terminally sacrificed animals. Similar injection site findings with lesser severity were observed in control animals. Based on this finding, it is concluded that the rat continuous infusion model is not suitable for chronic administration of lipid emulsions.

Methods

Doses:	9 (1.9 ml/kg/hr), 12 (2.5 ml/kg/hr), 15 (3.1 ml/kg/hr) and 18 (3.8 ml/kg/hr) g/kg/day Phase I (dose selection) ; 0 (control, 3.8 ml/kg/hr), 12 (2.5 ml/kg/hr), 15 (3.1 ml/kg/hr) and 18 (3.8 ml/kg/hr) g/kg/day Phase II (main study)
Frequency of dosing:	Daily 24 hours continuous IV infusion.
Route of administration:	Intravenous (IV) via the left femoral vein.
Dose volume:	45.6 – 91 ml/kg/day
Formulation/Vehicle:	SMOFlipid 20 %/0.9 % saline
Species/Strain:	Wistar rats: Crl: Wi (Han)
Number/Sex/Group:	2/sex/group (Phase I), 3/sex/group (Phase II).
Age:	9 weeks of age.
Weight:	249 – 263 g males, 170 – 198 g females (Phase I), 247 – 282 g males, 151 – 168 g females (Phase II), 253 – 284 g males, 153 – 188 g females (Satellite animals)
Satellite groups:	2/sex/group was designated as Toxicokinetics (TK) animals.
Unique study design:	None
Deviation from study protocol:	There were minor study deviations that were not considered to affect the study outcome.

Observations and Results

Mortality

Morbidity/mortality checks in the study animals were performed at least twice daily

One female rat dosed at 18 g/kg/day (No. 16) was found dead on day 3. The animal did not show any clinical signs prior to death, and the cause of death could not be determined at necropsy.
Seven out of 10 rats in the low or high dose groups, and all the mid-dose animals (main and TK) were found dead or prematurely sacrificed at the end of dosing day 26. Most of the rats dosed with SMOFlipid 20 % died or were prematurely sacrificed between days 10 and 26 in all the treatment groups. There was no mortality in the control group. The treatment-related deaths were not dependent on the level of dosing in the animals.

Clinical Signs

Clinical observations, including a full clinical examination of animals, were performed daily. For phase I, full clinical examination of the animals was performed once daily, but for phase II, full clinical examinations were performed twice daily.

There were no treatment-related clinical signs observed among the surviving phase I animals. The dead phase I female rat did not show any clinical signs prior to death. Clinical signs noted in the treated phase II animals prior to death included irregular breathing, subdued behavior, soiled fur, piloerection, lacrimation and cold to touch. The

incidence and severity of the clinical signs were not dose-related. The surviving animals in the low and high dose groups did not show any of the clinical signs exhibited by the dead animals. Clinical signs caused by scabs and sores from the infusion jacket were comparable in control and treated animals.

Body Weights

All the animals were weighed prior to dosing and on days 0, 4, 7 during phase I and twice weekly during phase II.

There were no treatment-related effects on the body weight of phase I animals over the 4-day infusion period and the 4-day observation period. Although the high mortality rate in the treated animals in phase II did not allow reliable interpretation, there was a relative high body weight gain throughout the infusion period for the single surviving high dose male rat, when compared to control.

Feed Consumption

Food consumption was measured daily for phase I animals and twice weekly for phase II main group animals.

Food consumption in treated phase I male and female rats, at all the dose levels were dose-dependently reduced in comparison to that of control animals during the 4-day dosing period. However, food consumption in the treated animals returned to normal at the end of the 4-day observation period.
Food consumption in treated phase II male and female rats, at all the dose levels were reduced in comparison to the control group. The reduction was dose-dependent for the female rats but not for the males.

Hematology

Blood was collected for measurement of hematology parameters from phase I animals on day 4 at the end of infusion, and on days 1, 8 and 15 for phase II animals.

There were no treatment-related effects on the hematology parameters in phase I animals.

Phase II animals in both treatment and control groups occasionally showed a slight reduction in red blood cell count during the first 15 days of infusion. This tendency was slightly more marked in the treatment group than in the control group with no dose-response correlation.

Male and female animals in both treatment and control groups showed slightly reduced platelet count, which was more slightly marked in the treated groups. One high dose male had a low white blood cell (WBC) count on day 15, and a TK male rat had low platelet and WBC counts on treatment day 23.

Clinical Chemistry

Blood was collected for measurement of clinical chemistry parameters from phase I animals on day 4 at the end of infusion, and on days 1, 8 and 15 for phase II animals.

Dose-related increase in serum cholesterol concentration was observed in treated phase I group 4 male and female rats with marked increase in the 18 g/kg/day of the animals. The increase observed in group 4 was due to an increase in LDL cholesterol in males.
Increased triglyceride concentration was observed in the high dose (18 g/kg/day) male rats, but increased free fatty acid level was observed only in a single high dose male rat.

The serum total bilirubin level was high in the high dose male and female animals in comparison to the other dose groups. A dose-dependent increase in glucose concentration was observed in treated animals, particularly for the high dose males. A male rat in the control group was observed to have a slightly elevated serum LDH activity.

In the phase II animals, total LDL and HDL cholesterol concentrations in all the male and female treatment groups were higher than the respective control animals from treatment day 1 through the dosing period duration. The serum free fatty acid concentrations were also observed to be markedly increased in Smoflipid 20 % treated animals from dosing day 1, showing a 3 to 5-fold increase over the respective control animal values. The triglyceride concentrations were markedly increased in all treated animal groups from infusion day 1, with increases of up to 55-fold when compared to the respective control animal value. There were however, no dose-response correlations in any of the observed increase in lipid levels in treated animals. The serum samples in the treated animals had a milky appearance, with slight serum hemolysis for occasional rats from each of the treatment groups.

In the phase II animals, total serum bilirubin levels were higher in all the treated female animals than in the control animals on the first day of dosing, but there was no dose-response correlation. This tendency decreased with fewer rats affected, with subsequent sampling as the dosing progressed daily. One control male animal was noted with an elevated serum ASAT enzyme activity on treatment day 15.

Unscheduled blood samples taken from moribund phase II animals showed similar tendencies of increased cholesterol, free fatty acid and triglycerides as discussed above. Other changes in glucose, urea, creatinine and protein concentrations, including elevated serum enzyme activity reflects the poor clinical conditions of these animals.

Urinalysis

No urinalysis data was reported.

Gross Pathology

All the phase I surviving animals were sacrificed on day 8 (following a 4-day observation), and necropsied. All the phase I animals found dead were also necropsied. Phase II dead and surviving animals were necropsied. The surviving animals were sacrificed at the end of the infusion period on day 26 and necropsied.

There were no treatment-related gross lesions observed at necropsy of phase I animals. At the necropsy of phase II animals, the main macroscopic findings were adhesions, dark and pale areas and nodules of the lungs and induration at the injection sites. These changes were accompanied by enlarged spleen and dark or clear fluids in the thoracic or abdominal cavities in most of the 24 dead or unscheduled sacrifice animals. The causes of death and/or moribundity in the animals were attributed to changes in the respiratory tract.

In the terminally sacrificed animals, including control animals, induration (12 out of 16) and/or edematous aspects (2 out of 16) were observed at the injection site. Enlarged spleen and dark areas in the lung were detected in 9 out of 16 of treated and control animals. Single cases of nodules and pale raised areas were also noted in these animals. A summary of the macroscopic findings of Smoflipid 20 % administration in rats is shown in the Sponsor's table below.

Organ/finding	Premature decedents (24 treated rats out of 40 included in the study)		Surviving animals terminated on day 26			
			control animals (10)		treated animals (6)	
	Incidence		Incidence		Incidence	
	No. of rats	% decedent	No. of rats	% control	No. of rats	% treated
Lung						
- adhesion	8	33	-	-	-	-
- dark area	16	67	4	40	5	83
- nodule	5	21	-	-	1	17
- pale area	20	83	1	10	1	17
Injection site						
- oedematous	4	17	1	10	1	17
- induration	23	96	6	60	6	100

Organ Weights

The liver of dead and sacrificed phase 1 animals was weighed at necropsy. The following organs were weighed at scheduled necropsy for all surviving Phase II (main and TK) animals:

Heart
Kidneys
Liver
Lungs
Spleen

Organs from dead or moribund phase II animals were not weighed, unless the size was observed to be abnormal. Paired organs were weighed together and organ weights expressed as absolute or relative values.

There were no effects of Smoflipid 20 % administration on liver weight in phase I animals.

Body and organ weights of the surviving phase II animals show a dose-dependent increase in lung, liver and spleen weight. Due to the high mortality in phase II animals, data for the other organ weights were not reported.

Histopathology

For phase I animals, samples of the liver and any abnormal tissues were fixed and preserved in 10 % neutral formalin.
For phase II animals, the following organs/tissues were sampled for all (main and TK) animals:

Heart
Injection sites (a sample was taken from the injected area)
Kidneys and ureters
Liver
Lungs
Lymph node (mandibular)
Lymph node (mesenteric)
Spleen
All gross lesions

All organs/tissues sampled were fixed in 10 % formalin.
Sampling was performed on 8 male and female rats from the main and TK groups for bacteriological contamination analysis.

Adequate Battery
No
Peer Review
Yes
Histological Findings

Treatment-related changes observed in the lungs and injection sites (catheterized and non-catheterized vein) in the 24 dead or unscheduled sacrifice phase II animals consist of septic inflammatory changes at the injection sites, characterized by slight to marked vessel wall necrosis and perivascular active chronic inflammation, with the formation of abscess in the surrounding tissue in most of the animals. These were accompanied by minimal to slight multiple lipid droplets in a few of the animals. In several animals, these changes were associated with extensive inflammation in the organs of the abdominal cavity, including the adrenal glands, kidneys ureters and liver.

The lungs of the premature death animals consists of, slight to marked multiple abscesses characterized by necrosis and cell debris with the presence of bacterial colonies surrounded by vacuolated macrophages with eosinophilic content, alveolar

edema and the presence of frothy material (probably lipids) in the veins of these animals were observed.

In 4 high dose animals (nos. 213 to 216) sacrificed moribund on day 12, oil-red-O staining (positive reaction) revealed lipid surrounding abscess as well as in macrophages in some veins. These findings were accompanied by alveolar inflammatory changes (edema/hemorrhage), thrombosis, perivascular mixed cell infiltrate, and pleural fibrosis in several animals. This septic inflammatory process in the lungs is secondary to the events at the injection sites, and related to test-article treatment, including injection-site infection that may have enabled bacterial growth.

In the 16 animals terminally sacrificed on day 26, changes observed in the injection sites include, slight to marked vessel wall necrosis with minimal to moderate perivascular inflammatory changes with the formation of abscess in the surrounding tissue, and were the main findings observed in both treated and control animals. Despite the lower incidence, the severity of the microscopic changes observed in these animals was similar to the severity noted in the premature dead animals and unscheduled sacrifice animals. Other observed changes associated with the dosing procedure such as mural thrombus, endothelial inflammation and intimal thickening/endothelial hyperplasia were seen in both control and treated, terminally sacrificed animals.

Microscopic observations in the terminally sacrificed animals consists of septic pulmonary inflammatory changes, including slight to marked abscess, which were seen in control and treated animals. These findings were accompanied by alveolar inflammatory changes, hemorrhage, thrombosis, and perivascular mixed cell infiltrate. These changes are test article-related findings that are possibly exacerbated by the vascular dissemination from the injection site of a possible infusion-related infection.

Samples of lung tissue taken and examined for bacteriological examination showed that one control male rat and 1 treated female rat tested positive for *Pseudomonas Aeruginosa* infection. A summary of the microscopic findings of Smoflipid 20 % administration in rats is shown in the Sponsor's table below.

Organ/finding	Premature decedents (24 treated rats out of 40 included in the study)		Surviving animals sectioned at the end of the observation period on day 26 (16 out of 40 rats)			
			control animals (10)		treated animals (6)	
	Incidence		Incidence		Incidence	
	No. of rats	% decedent	No. of rats	% control	No. of rats	% treated
Lung						
-Alveolar oedema	23	96	-	-	-	-
-Perivascular infiltration	5	21	10	100	6	100
- Abscess	21	88	2	20	3	50
-Alveolar inflammation	8	33	2	20	4	67
-Alveolar haemorrhage	18	75	3	30	2	33
-Alveolar macrophages	14	58	2	20	1	17
Injection site						
-Vessel wall necrosis	23	96	3	30	5	83
-Thrombus	11	46	3	30	1	17
-Perivascular inflammation	22	92	6	60	6	100
-Lipid droplets	15	63	2	20	3	50
Non-catheterized vein						
-Vessel wall necrosis	21	88	3	30	3	50
-Thrombus	6	25	1	10	2	33
-Perivascular inflammation	23	96	2	20	4	67
-Lipid droplets	4	17	-	-	1	17

* including 2 micro abscesses; No.: number; -: not applicable

In conclusion, the administration of Smoflipid by IV infusion at doses of 9, 12 and 18 g/kg/day continuous daily infusion for 24 hours in male and female rats resulted in treatment-related mortalities at all dose levels, leading to the early termination of the study during the 4th week of the study.

Toxicokinetics

Blood samples for TK analysis were taken prior to dosing, and at the end of the 4-day infusion from all the phase I surviving animals. For the phase II animals, blood samples were taken at 6, 12 and 24 hours prior to dosing on day 0 and at 6, 12 and 24 hour on days 1, 12, 20 and 26. Control TK animals (group 1) and the surviving main group 2 (low dose), group 3 (mid-dose) and group 4 (high dose) animals were sampled on day 26 at the end of infusion.

No toxicokinetic data were provided in this report. Only descriptive results of fatty acids in the blood samples were provided.

Only the dose dependent relevant increases of fatty acid concentration in the study animals are described. The fatty acids detected in blood samples from rats are: Palmitic acid (16:00), Stearic acid (18:00), Oleic acid (18:01), Linoleic acid (18:02), Arachidonic acid (20:04), Eicosapentaenoic acid (20:05), Docosahexaenoic acid (20:06). There were no relevant differences detected between male and female rats.

In the phase 1 TK animals, fatty acids 8:0 – 14:0 were determined to be below or close to the detection limit for all groups, except for 8:0 and 10:0 fatty acids in group 4 (high dose) males.

In phase I TK animals, dose-dependent increases in fatty acids 16:00, 18:00, 18:01, 18:02, 20:04, 20:05 and 20:06 were observed in treated rats. The maximum plasma concentrations of fatty acids measured at the highest dose level (18 g/kg/day) were increased by a factor of 2 (16:0), 3 (18:0), 5 (18:01), 2 (18:02), 1 (20:04) or 5 (22:06), when compared with the TK control animals. However, for eicosapentaenoic acid (EPA, 18:5) which was detected at a maximum concentration of 1.98 mmol/L at the 18 g/kg/day dose, no plasma levels were detected in the control animals.

In phase II TK animals, fatty acids 12:0 and 14:0 concentrations were below the detection limit and fatty acids 8:0 and 10:0 were only detected at the higher dose levels.

On day 0 in each of the treated animal dose groups, a steady state plasma concentration was reached at the 6 hour time point, and remained constant up to the end of the infusion day at the 24 hour time point. For each examined time point, a dose-dependent increase in plasma levels of 16:0, 18:0, 18:01, 18:02 and 20:05 was seen at the 12 and 18 g/kg/day dose levels. The maximum plasma concentrations measured at the dose of 18 g/kg/day on day 1 at the 24 hour time point were increased by a factor of 4 (16:0), 4 (18:0), 10 (18:01), 3 (18:02), 1 (20:04) and 2 (22:06) in comparison to TK control animals. However, for EPA (20:05), which was detected at a maximum concentration of 1.20 mmol/L at the 18 g/kg/day dose, no plasma levels of this fatty acid was seen in the control animal group. Similar results were seen at the other time points observed in weeks 2, 3 and 4, without any further elevation of concentrations at these times. These results do not show any evidence of lipid accumulation.

Study title: Smoflipid 20 %: Preliminary Toxicity/Long-Term Tolerance Study by Daily Intravenous Infusion to Male Sprague-Dawley Rats for 4-Weeks.

Study no.:	HKQ0002
Study report location:	SMOF-010-C NC, page 1 to 411
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 15, 2009
GLP compliance:	Study was conducted under the OECD Principles of Good Laboratory Practice
QA statement:	Yes
Drug, lot #, and % purity:	Smoflipid 20 %, batch # F1042, 20 % lipid emulsion; Intralipid 20 %, batch # 10CB9842, 20 % lipid emulsion.

Key Study Findings

The continuous 24-hour infusion of Saline (control), Smoflipid 20 % at doses of 3,000, 6,000, 9,000 mg TG/kg/day, and Intralipid 20 % (reference control) at 9,000 mg TG/kg/day in rats for 4 weeks resulted in the death or premature sacrifice of 18 out of 42 animals, particularly in the and 9,000 mg TG/kg/day dose groups. The wide range of pathological changes observed were at the site of the catheter tip in the vena cava and consists of inflammation, necrosis, thrombosis, bacterial colonies, granulation tissue and thrombosis in the majority of animals treated with Smoflipid or Intralipid. Findings in other organs (lung, liver, kidney, heart) included inflammation, thrombi, emboli, infarction and lipid-induced (foreign body) granulomatous inflammatory response.

Methods

Doses:	0 (45 ml saline/kg/day,control, group 1), 3 (0.625 ml/kg/hr, group 3), 6 (1.25 ml/kg/hr, group 4), 9 (1.875 ml/kg/hr, group 5) and 9 (1.875 ml/kg/hr, reference control, group 2) g TG/kg/day
Frequency of dosing:	Daily 24 hours continuous IV infusion
Route of administration:	Intravenous via the femoral vein.
Dose volume:	15 – 45 ml/kg/day
Formulation/Vehicle:	Smoflipid 20%, Intralipid 20 % (reference), Saline (control)
Species/Strain:	Crl:CD(SD)IGS BR Rats
Number/Sex/Group:	6 male animals/group
Age:	7 to 8 weeks of age
Weight:	290 to 330 g
Satellite groups:	4 male animals from groups 1, 2 and 5 were designated as extended study animals
Unique study design:	None
Deviation from study protocol:	From dosing day 17, the dosing flow rate of animals in the reference control group 2 and high dose group 5 was reduced from 1.875 ml/kg/hour to 1.25 ml/kg/hour so that the dose level is reduced to 6 g TG/kg/day because of the mortality rate in the animals. The flow rate in the group 1 control animals was also reduced to 1.25 ml/kg/hour to provide concurrent control, on the same day. In error, the flow rates for group 1 saline control and group 2 reference control was reduced on dosing day 18 instead of day 17. The temporary protocol deviation was not considered to adversely affect the integrity of the study.

Observations and Results

Mortality

Animals were observed daily for moribundity and were sacrificed for welfare reasons or for irretrievable loss of cannula potency. Where possible, blood samples were taken ante mortem and analyzed. A complete necropsy was performed on all dead or sacrificed animals.

Eighteen animals; 1 (saline control), 5 (Intralipid 20 % control), 2 (low dose, 3,000 mg/kg/day group 3), 4 (mid dose 6000 mg/kg/day group 4) and 6 (high dose 9,000 mg/kg/day group 5) were either found dead or prematurely sacrificed during the treatment period.

Nine of these male rats consisting of:1 from the control group, 1 from the reference control group (given Intralipid 20 % at 9,000 mg/kg/day), I from the Smoflipid 20 % group (given 3,000 mg/kg/day), 2 from the Smoflipid 20 % group (given 6,000 mg/kg/day), and 4 from the Smoflipid 20 % group (given 9,000/6,000 mg/kg/day) were sacrificed due to cannula regression under the skin or because of irreparable cannula disconnection preventing the continuation of dosing. 2 moribund males from the Smoflipid 20 % group (given 6,000 mg/kg/day), 2 other male rats given the reference article (Intralipid 20 %) and 1 male rat given Smoflipid 20 % (9,000/6,000 mg/kg/day) were sacrificed because of large masses found at the femoral vein access site.

The 4 male rats found dead with cardiovascular trauma consists of 2 Intralipid 20 % dosed animal, 1 Smoflipid 20 % (3,000 mg/kg/day) and 1 Smoflipid 20 % (6,000 mg/kg/day) dosed animal.

Clinical Signs

Animals were inspected at least twice daily for adverse clinical signs or reaction to test article treatment. Detailed physical examination was also performed weekly on each animal.

There were no clinical signs considered related to treatment with Smoflipid 20 % or Intralipid 20 %.

Body Weights

The weight of each animal was recorded prior to dosing initiation, on the day of dosing, and twice weekly throughout the treatment period, and before necropsy.

Animals dosed with Smoflipid 20 % gained less weight during the treatment period, when compared to saline control animals, although the effect did not show any dose-response relationship. Rats dosed with Intralipid 20 % at the high (9,000 mg/kg/day) and reduced (6,000 mg/kg/day) dose gained more weight than rats on the same dose regimen of Smoflipid 20 %.

Feed Consumption

The mean daily food consumption by each animal was calculated.

Food consumption in Smoflipid 20 % treated animals was reduced (0.64 – 0.77 %) dose-dependently, when compared to the food consumption in control animals. A similar reduction in food consumption (0.72x of control) was observed in Intralipid 20 % treated animals, in a similar respect to the corresponding dose of Smoflipid 20 %.

Hematology

Blood samples (0.5 ml) for hematology were collected from surviving animals via the sublingual vein after an overnight fast, during weeks 2 and 4.

Changes observed include decreased hematocrit (0.90x of control) and hemoglobin concentration (0.91x of control) during week 2 of treatment in animals receiving 9,000 mg/kg/day of Smoflipid 20 %. During week 4 of treatment, there was a reduction in hematocrit (0.82 – 0.90x of control) and hemoglobin concentration (0.80 – 0.89x of control) in animals receiving Smoflipid 20 % at 3,000 or 6,000 mg/kg/day. The changes were not dose dependent. There were also decreased erythrocyte counts (0.82 – 0.93x of control), and increased reticulocyte counts (1.3 – 2.6x of control) for all the treated animals in comparison to control animals in the groups receiving Smoflipid 20 % at a dose of 9,000/6,000 mg/kg/day. A reduction in platelet count was also observed during week 2 of treatment in animals receiving Smoflipid 20 % (0.72x of control) or Intralipid 20 % (0.66x of control) at a dose 9,000 mg/kg/day. This change was still present in treated animals during week 4, with a significant difference in animals dosed with Smoflipid 20 % at 9,000/6,000 mg/kg/day.

Increased prothrombin times (PT) were slightly increased in Smoflipid 20 % (9,000 mg/kg/day) dosed animals during week 2 of treatment, but absent during treatment week 4. However, animals dosed with 9,000/6,000 mg/kg/day Intralipid 20 % showed a significant increase in PT during treatment week 4, when compared to the control animals. Activated partial thromboplastin time (APTT) were reduced (0.87 – 0.90x of control) in animals receiving 9,000/6,000 mg/kg/day Smoflipid 20 % during treatment week 4 in comparison to control animals. The observed fluctuation in white blood cell counts during the treatment period does not show a definite relationship to Smoflipid 20 % or Intralipid 20 % treatment.

Clinical Chemistry

Blood samples (0.7 ml) for clinical chemistry were collected from the surviving animals via the sublingual vein after an overnight fast, during weeks 2 and 4.

During treatment week 2, a dose-dependent increase in non-esterified fatty acid (NEFA) (1.7 – 2.9x) and triglyceride concentrations (2.4 – 6.3x) were observed in animals administered Smoflipid 20 %. An increase in NEFA of 3.3x of control animals and triglyceride concentrations of 10.1X of control animals observed in animals receiving 9,000 mg/kg/day of Intralipid 20 % were higher than that of animals receiving an equivalent dose of Smoflipid 20 %. During treatment week 4, NEFA and triglyceride levels remained increased, although at a lower level for treated animals, in comparison to control animals. Although NEFA concentrations were similar between the Smoflipid 20 % and Intralipid 20 5 treated animals, the triglyceride concentration was slightly higher in the Intralipid 20 % animal group.

Blood samples obtained from animals at necropsy, prior to the premature termination of the remaining animals (planned extended study) on day 30 showed that the NEFA and

triglyceride levels in the treated animals were significantly lower than that of the control animals. The lipid infusion had been stopped for 45 min to 2 hrs. prior to blood sampling.

During treatment week 2 urea and bilirubin concentrations were reduced in treated animals in comparison to control. Alanine aminotransferase (ALT) activity was comparably decreased in animals receiving Smoflipid 20 % or Intralipid 20 % at 6,000 or 9,000 mg/kg/day. In contrast, glucose levels were increased in animals dosed with Smoflipid or Intralipid 20 % when compared to levels in control animals.

During treatment week 4 urea concentrations were decreased in animals receiving Smoflipid or Intralipid 20 %, in comparison to that in control animals. Although cholesterol levels were decreased in Smoflipid 20 % treated animals at doses of 3,000 or 6,000 mg/kg/day, there was no similar effect seen in animals dosed with Intralipid 20 %. Glucose levels were increased in all the treated animals but without a dose-response correlation. The mean ALT activity was decreased in all the treated animals, when compared to the control animals, even in blood samples taken from animals on treatment day 30, prior to the termination of the planned treatment extended phase.

Urinalysis

No urinalysis data was reported in this study.

Gross Pathology

All animals sacrificed prior to study termination and those surviving till the end of the study were sacrificed and subjected to full necropsy. A full tissue macroscopic examination was performed on each animal.
There are no significant difference in the incidence and range of macroscopic findings observed in animals treated with the test article (Smoflipid 20 %), when compared to those treated with the reference article (Intralipid 20 %). In most of these animals the liver, spleen, adrenals and heart were enlarged with pale, dark areas; the kidneys also had pale areas, pelvic dilatation, depressions, masses (2 high dose animals) and a prominent vasculature in a high dose animal. The lungs appeared edematous, thick, with pale, dark areas. The vein access sites and vena cava also appeared edematous, with masses and dark thickened and pale areas. The abdomens of some animals were distended with fluids, and masses. The adipose tissue was edematous with dark thickened areas in one of the animals. There were no significant changes observed in the vena cava from control animals infused with saline.

Organ Weights

The following organs taken from each animal sacrificed after 4 weeks were dissected and weighed:

Adrenals	Prostate
Brain	Salivary glands≠ - submandibular - sublingual
Epididymides	Seminal vesicles
Heart	Spleen
Kidneys	Testes
Liver	Thymus
Lungs with mainstem bronchi	Thyroid with parathyroids*
Pituitary	

* Weighed after partial fixation
≠ Weighed together, reported as salivary glands

At the end of treatment week 4, the mean absolute and (bodyweight) adjusted liver and lung weights were increased in animals dosed with Smoflipid 20 % at 3,000 mg/kg/day. The lung weights were also increased in animals given Smoflipid 20 % or Intralipid 20 % at 9,000/6,000 mg/kg/day, when compared with control animals. The mean absolute spleen weights were increased in all animals dosed with Smoflipid 20 %, up to 4.5 times the control value, but not dose-responsively. The spleen weights were increased in all the animals given Intralipid 20 %, up to 2.2 times the control value.

Histopathology

The following organs/tissues taken from all animals were dissected and samples were preserved in 10 % neutral buffered formalin, except for the testes and the eyes that were fixed in Davidson's fluid:

Adrenals	Pancreas
Aorta - thoracic	Peyer's patches
Brain	Pituitary
Caecum	Prostate
Cannula exteriorisation site	Rectum
Colon	Salivary glands - submandibular
Duodenum	- parotid
Epididymides	- sublingual
Eyes	Sciatic nerves
Femoral vein access site	Seminal vesicles
Femurs+	Skeletal muscle
Harderian glands	Skin with mammary glands
Head#	Spinal cord
Heart	Spleen
Ileum	Sternum
Jejunum	Stomach
Kidneys	Testes
Lachrymal glands	Thymus
Larynx	Thyroid with parathyroids
Liver	Tongue
Lungs	Trachea
Lymph nodes - lumbar	Ureters
Oesophagus	Urinary bladder
Optic nerves	Vena cava

+ Both hindlimbs retained, one sectioned where appropriate
# Including nasal cavity, paranasal sinuses and nasopharynx

Tissue sections were processed, embedded in paraffin wax, and stained with hematoxylin and eosin for histopathology. In addition, Liver, lungs, spleen, kidneys and heart tissues were stained with Oil-Red-O for fat.

Adequate Battery
Yes
Peer Review
Yes
Macroscopic and Histopathological Findings

Many of the histopathological findings for the femoral vein access site were observed in saline control animals as well as the Smoflipid 20 % or Intralipid 20 % treated animals. Changes such as suture granuloma, perivascular and subcutaneous inflammation and necrosis are commonly seen at the catheter site in control animals undergoing continuous infusion studies. However, the severity of inflammation and necrosis tended to be greater in Smoflipid 20 % or Intralipid 20 % treated animals than in the control animals in this study.

There were severe macroscopic pathological findings observed in and around the vena cava, at or around the tip of the infusion cannula. In most of the animals treated with Smoflipid 20 % or Intralipid 20 %, significant levels of perivascular inflammation, bacterial colonies, necrosis and granulation tissue/fibrosis were observed. Thrombi formations were also present in the vena cava of animals treated with Smoflipid 20 % or Intralipid 20 %. There were no significant pathological changes or thrombi observed in the vena cava of control animals infused with saline. A summary of the findings is presented in the Sponsor's table below.

Summary of Microscopic findings in the Vena Cava (LS section of the cannula tip) for Rats found dead or Sacrificed before the Scheduled Termination Date

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Perivascular Inflammation						
	Minimal	0	0	0	0	1
	Slight	0	0	0	0	2
	Marked	0	1	2	1	2
	Severe	0	1	0	0	0
	Total	0	2	2	1	5
Bacterial Colonies						
	Slight	0	0	1	1	2
	Moderate	0	2	0	0	1
	Marked	0	0	0	1	0
	Total	0	2	1	2	3
Perivascular Necrosis						
	Moderate	0	0	1	0	1
	Marked	0	2	0	2	1
	Total	0	2	1	2	2
Thrombus						
	Slight	0	0	0	1	1
	Moderate	0	1	1	2	0
	Marked	0	0	0	0	1
	Severe	0	1	0	0	1
	Total	0	2	1	3	3
Perivascular Granulation Tissue and Fibrosis						
	Minimal	0	0	0	0	1
	Slight	0	0	0	1	1
	Moderate	0	0	0	1	0
	Marked	0	2	2	2	3
	Total	0	2	2	4	5
Perivascular Lipid Vacuoles						
	Slight	0	1	0	0	0
	Moderate	0	0	1	2	0
	Total	0	1	1	2	0
Myofibre Inflammation/Necrosis						
	Minimal	0	1	0	0	1
	Slight	0	0	0	1	1
	Moderate	0	0	2	0	0
	Marked	0	1	0	1	0
	Total	0	2	2	2	2
Number of Animals Examined		1	4	2	4	6

Summary of Microscopic findings in the Vena Cava (LS section of the cannula tip) for Rats killed after 4 Weeks of Treatment

Group/sex Total dose (mg triglycerides/kg/day)	1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Perivascular Inflammation					
Minimal	0	3	0	0	0
Slight	0	1	0	0	0
Marked	0	0	2	1	4
Severe	0	0	1	0	0
Total	0	4	3	1	4
Bacterial Colonies					
Minimal	0	1	0	0	0
Slight	0	0	1	1	1
Moderate	0	0	2	0	0
Total	0	1	3	1	1
Perivascular Necrosis					
Marked	0	0	2	0	2
Total	0	0	2	0	2
Thrombus					
Slight	0	2	0	0	2
Moderate	0	0	1	1	0
Marked	0	0	2	0	0
Severe	0	0	0	0	2
Total	0	2	3	1	4
Perivascular Granulation Tissue and Fibrosis					
Minimal	1	0	0	0	0
Slight	0	1	0	0	0
Marked	0	0	3	1	4
Severe	0	0	1	0	0
Total	1	1	4	1	4
Myofibre Inflammation/Necrosis					
Minimal	0	1	0	0	1
Slight	0	0	0	1	1
Moderate	0	0	2	0	0
Marked	0	1	0	1	0
Total	0	2	2	2	2
Perivascular Lipid Vacuoles					
Slight	0	0	2	1	0
Moderate	0	0	1	0	1
Total	0	0	3	1	1
Number of Animals Examined	9	5	4	2	4

The presence of minimal or slight levels of perivascular inflammatory cells and medial hypertrophy were observed in lung tissues from control, Smoflipid 20 % or Intralipid 20 % animals. However, there was an increase in the severity of these findings in the treated animals versus the control animals. Interstitial pneumonitis was observed in the Smoflipid 20 % and Intralipid 20 % animals, but not in the saline control animals. Lipid vacuoles (from Oil Red O staining) were observed in the lung parenchyma of some of the treated animals. The lipid vacuoles were associated with a granulomatous inflammatory response, indicating the presence of lipid stimulated foreign body-type inflammatory response. However, there was no clear association of incidence or severity of lesions with the lipid dose received. A summary of the findings is presented in the Sponsor's table below.

Microscopic Findings in the Lungs and Bronchi for Rats found Dead or Sacrificed before the Termination Date

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Lung Parenchyma Oil Red O Staining						
	Minimal	0	1	1	4	3
	Slight	0	2	0	0	1
	Total	0	3	1	4	4
Perivascular Inflammatory Cells						
	Minimal	0	1	1	1	1
	Slight	1	1	1	1	4
	Moderate	0	2	0	2	0
	Marked	0	1	0	0	1
	Total	1	5	2	4	6
Granulomatous Inflammation and Lipid Vacuoles						
	Minimal	0	0	0	0	1
	Slight	0	1	0	1	1
	Total	0	1	0	1	2
Interstitial Pneumonitis						
	Minimal	0	2	0	0	1
	Slight	0	0	0	1	3
	Moderate	0	1	1	0	1
	Marked	0	1	0	1	1
	Total	0	4	1	2	6
Aggregations of Alveolar Macrophages						
	Minimal	0	1	0	1	0
	Slight	0	1	0	0	0
	Moderate	0	0	0	1	1
	Marked	0	0	0	1	0
	Total	0	2	0	3	1
Alveolar Haemorrhage/Oedema						
	Minimal	0	1	0	1	2
	Slight	0	0	0	0	1
	Moderate	0	0	0	1	1
	Total	0	1	0	2	4
Number of animals examined		1	5	2	4	6

Microscopic findings in the Lungs and Bronchi for Rats killed after 4 Weeks of Treatment

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Lung Parenchyma Oil Red O Staining						
	Minimal	0	3	4	0	4
	Slight	0	1	0	1	0
	Total	0	4	4	1	4
Arterial Medial Hypertrophy						
	Minimal	2	2	1	2	1
	Slight	0	1	1	0	3
	Moderate	0	1	1	0	0
	Total	2	4	3	2	4
Interstitial Pneumonitis						
	Minimal	0	0	0	0	2
	Slight	0	1	1	0	0
	Moderate	0	2	3	1	1
	Marked	0	0	0	0	1
	Total	0	3	4	1	4
Granulomatous Inflammation and Lipid Vacuoles						
	Minimal	0	1	1	1	0
	Slight	0	2	2	0	1
	Total	0	3	3	1	1
Aggregations of Alveolar Macrophages						
	Minimal	0	1	1	0	2
	Slight	0	2	0	0	0
	Total	0	3	1	0	2
Alveolar Haemorrhage/Oedema						
	Minimal	0	0	1	0	1
	Slight	0	1	0	0	0
	Moderate	0	1	0	0	0
	Total	0	2	1	0	1
Number of animals examined		9	5	4	2	4

The kidneys of animals treated with Smoflipid 20 % or Intralipid 20 % showed increased severity of cortical tubular basophilia/interstitial inflammation and increased incidence and severity of cortical tubular necrosis, in comparison to control animals with significant but minimal severity of tubular basophilia/interstitial inflammation (which were within the historical control range). A minimal or slight positive staining with Oil Red O seen in the kidneys of these animals is an indication of some uptake of infused triglyceride into the kidney parenchyma cells. A summary of the findings is presented in the Sponsor's table below.

Microscopic Findings in the Kidneys for Rats found Dead or Sacrificed before the Termination Date

Group/sex Total dose (mg triglycerides/kg/day)	1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Cortical Tubular Basophilia/Interstitial Inflammation					
Minimal	0	1	0	1	2
Slight	0	0	0	0	2
Marked	0	2	0	3	1
Severe	0	0	1	0	0
Total	0	3	1	4	5
Oil Red O Staining of Renal Parenchyma					
Minimal	0	0	0	1	0
Slight	0	1	1	1	0
Total	0	1	1	2	0
Cortical Tubular Necrosis					
Moderate	0	0	0	0	1
Marked	0	1	1	1	0
Total	0	1	1	1	1
Septic Embolus					
Slight	0	1	1	0	0
Total	0	1	1	0	0
Bacterial Colonies					
Minimal	0	0	1	0	0
Slight	0	0	0	1	0
Moderate	0	1	0	1	0
Total	0	1	1	2	0
Pelvic Dilatation					
Minimal	0	0	0	1	0
Marked	0	1	1	1	0
Total	0	1	1	2	0
Papilla – Inflammation/Necrosis					
Moderate	0	1	1	1	1
Total	0	1	1	1	1
Hyperplasia – Papillary Epithelium					
Slight	0	0	0	0	1
Moderate	0	1	0	1	0
Total	0	1	0	1	1
Number of animals examined	1	5	2	4	6

Summary of Microscopic Findings in the Kidneys for Rats Killed after 4 Weeks of Treatment

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Cortical Tubular Basophilia/Interstitial Inflammation						
	Minimal	5	1	0	2	3
	Slight	0	1	1	0	0
	Moderate	0	1	2	0	0
	Marked	0	1	0	0	0
	Total	5	4	3	2	3
Papilla – Inflammation/Necrosis						
	Moderate	0	1	0	0	0
	Marked	0	1	0	0	0
	Total	0	2	0	0	0
Oil Red O Staining of Renal Parenchyma						
	Minimal	0	1	0	1	0
	Total	0	1	0	1	0
Cortical Tubular Necrosis						
	Marked	0	1	0	0	0
	Total	0	1	0	0	0
Number of animals examined		9	5	4	2	4

The liver of a few animals treated with Smoflipid 20 % or Intralipid 20 % showed minimal or slight portal inflammation and Kupffer cell proliferation, which is an indication of increased presence of lipid/foreign material within the liver parenchyma. Most of the liver sections stained positive for lipid with Oil Red O, indicating an uptake of triglycerides by hepatocytes. A summary of the findings is presented in the Sponsor's table below.

Microscopic Findings in the Liver for Rats found Dead or Sacrificed before the Termination Date

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Oil Red O Staining of Hepatocytes						
	Minimal	0	1	1	2	2
	Slight	0	0	0	0	2
	Moderate	0	1	0	1	0
	Total	0	2	1	3	4
Inflammation, Portal						
	Minimal	0	1	0	1	0
	Total	0	1	0	1	0
Kupffer Cell Proliferation						
	Minimal	0	0	1	0	1
	Slight	0	0	0	1	0
	Moderate	0	1	0	0	1
	Total	0	1	1	1	2
Number of animals examined		1	5	2	4	6

Microscopic Findings in the Liver for Rats Killed after 4 Weeks of Treatment

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Oil Red O Staining of Hepatocytes						
	Minimal	1	1	2	0	2
	Slight	0	1	0	2	0
	Moderate	0	2	0	0	0
	Total	1	4	2	2	2
Inflammation, Portal						
	Minimal	0	0	3	0	0
	Slight	0	1	0	0	0
	Total	0	1	3	0	0
Kupffer Cell Proliferation						
	Minimal	0	0	1	0	2
	Slight	0	0	1	0	0
	Total	0	0	2	0	2
Number of animals examined		9	5	4	2	4

Increased severity in myocardial inflammation/necrosis was observed in the heart of Smoflipid or Intralipid 20 % treated animals when compared to control animals. This observation was likely due to the presence of lipid and/or vascular damage in the tissue. Marked or severe thrombi were seen in the atria of three animals (Nos. 18, 22, 29) found dead. Although bacterial colonies were associated with the thrombi, there was no evidence of bacterial infection within the myocardial tissue. A summary of the findings is presented in the Sponsor's table below.

Microscopic Findings in the Heart for Rats found Dead or Sacrificed before the Termination Date

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Myocardial Inflammation/Necrosis						
	Minimal	1	2	1	1	2
	Moderate	0	0	0	0	1
	Marked	0	1	1	1	0
	Total	1	3	2	2	3
Myocardial Tissue Oil Red O Staining						
	Minimal	0	0	0	1	0
	Slight	0	1	0	0	0
	Total	0	1	0	1	0
Atrial Thrombus						
	Marked	0	1	1	0	0
	Severe	0	0	0	1	0
	Total	0	1	1	1	0
Bacterial Colonies						
	Moderate	0	1	1	0	0
	Marked	0	0	0	1	0
	Total	0	1	1	1	0
Number of animals examined		1	5	2	4	6

Microscopic Findings in the Heart for Rats Killed after 4 Weeks of Treatment

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Myocardial Inflammation/Necrosis						
	Minimal	3	0	2	1	1
	Moderate	0	1	0	0	0
	Marked	0	1	0	0	0
	Total	3	2	2	1	1
Myocardial Tissue Oil Red O Staining						
	Minimal	0	1	0	0	0
	Total	0	1	0	0	0
Number of animals examined		9	5	4	2	4

In the spleen of Smoflipid or Intralipid 20 % treated animals, significant increase in the incidence of extramedullary hematopoiesis and increased cellularity of the white pulp were observed, when compared to control animals. These findings are associated with systemic inflammatory response, and are accompanied by similar elevations of white blood cell parameters in the animals. A summary of the findings is presented in the Sponsor's table below.

Microscopic Findings in the Spleen for Rats found Dead or Sacrificed before the Termination Date

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Extramedullary Haemopoiesis						
	Minimal	0	0	0	0	1
	Slight	0	0	0	1	0
	Moderate	1	1	1	2	2
	Marked	0	1	0	0	1
	Severe	0	0	0	0	1
	Total	1	2	1	3	5
White Pulp – Increased Cellularity						
	Slight	0	0	0	0	1
	Moderate	0	0	1	0	0
	Marked	0	0	0	0	1
	Total	0	0	1	0	2
Number of animals examined		1	5	2	4	6

Microscopic Findings in the Spleen for Rats Killed after 4 Weeks of Treatment

Group/sex Total dose (mg triglycerides/kg/day)		1M 0	2M Reference 9000/6000	3M 3000	4M 6000	5M 9000/6000
Increased Germinal Centre Development						
	Slight	0	2	0	1	0
	Moderate	0	0	1	0	3
	Total	0	2	1	1	3
Extramedullary Haemopoiesis						
	Slight	0	0	1	0	0
	Moderate	0	2	3	0	4
	Marked	0	1	0	0	0
	Total	0	3	4	0	4
White Pulp – Increased Cellularity						
	Slight	0	2	0	0	0
	Moderate	0	0	1	0	0
	Total	0	2	1	0	0
Number of animals examined		9	5	4	2	4

The findings observed at the site of catheter placement and other organs appear to occur as a result of vascular disturbance and are more severe in the animals treated with Smoflipid 20 % or Intralipid 20 %, as compared to that in the control animals. There were no significant differences in the pathological findings observed in animals treated with either Smoflipid or Intralipid 20 %, suggesting that there is a lipid effect to the findings rather than an inherent effect of Smoflipid 20 %.

In conclusion, continuous IV infusion of Smoflipid 20 % to rats at doses of 3,000, 6,000 or 9,000 Triglycerides (TG) mg/kg/day, over 24-hour period for 4 weeks resulted in premature deaths in the high dose group. The wide range of pathological changes observed was believed to be due to vascular disturbance. The physical nature of the test material, the flow rate and the continuous 24 hour/day exposure were found not to be compatible with the intended duration of chronic infusion in rats. There were no significant differences in the pathological findings observed in animals treated with either Smoflipid or Intralipid 20 %.

Toxicokinetics

Blood samples for toxicokinetic (TK) analysis was obtained from surviving main study animals on dosing day 29 and from the extended study animals on dosing day 30 prior to the end of infusion. Plasma isolated from the blood samples were analyzed for the following fatty acids as shown in the sponsor's table below.

Shorthand designation	Trivial name	LOD (mmol/L)	LOQ (mmol/L)
8:0	Caprylic acid	0.02	0.06
10:0	Capric acid	0.01	0.05
12:0	Lauric acid	0.01	0.04
14:0	Myristic acid	0.01	0.04
16:0	Palmitic acid	0.01	0.03
16:1	Palmitoleic acid	0.01	0.03
18:0	Stearic acid	0.01	0.03
18:1	Oleic acid	0.01	0.03
18:2	Linoleic acid	0.01	0.03
18:3	Linolenic acid	0.01	0.03
20:1	Gadoleic acid	0.01	0.03
20:4	Arachidonic acid	0.01	0.03
20:5	Eicosapentaenoic acid	0.01	0.03
22:5	Docosapentaenoic acid	0.01	0.02
22:6	Docosahexaenoic acid	0.01	0.02

Plasma concentrations of fatty acids; caprylic acid, capric acid, lauric acid, gadoleic acid, eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) in samples taken on Day 29 or 30 from control animals and from animals receiving Intralipid 20 % (reference formulation) were generally below the limit of detection. Plasma concentrations of these fatty acids in samples taken on Day 29 or 30 from the animals receiving Smoflipid 20 % were also below the limit of detection, with the exception of EPA. However, there were insufficient data to calculate the mean concentrations at any dose level of Smoflipid 20 %. There was no apparent evidence of a relationship between plasma concentration of EPA and the administered dose level of Smoflipid.

Plasma concentrations of myristic acid, palmitoleic acid and linolenic acid were quantifiable in the samples taken from control animals, but not in the samples taken from Smoflipid 20 % or Intralipid 20 %.

Plasma concentrations of palmitic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid, and docosahexaenoic acid were generally quantifiable in the samples taken from all animals. For Palmitic acid, stearic acid, oleic acid and linoleic acid, there was no significant difference between the treated and control groups, and no evidence of a relationship between plasma concentration and dose level of Smoflipid 20 %.

Plasma concentrations of arachidonic acid were significantly lower in the treated animal groups than in the controls. The mean plasma concentrations of arachidonic acid were similar in all Smoflipid dose groups, and there does not seem to be any correlation between the plasma concentration of arachidonic acid and the dose of Smoflipid 20 %.

In contrast, plasma concentrations of docosahexaenoic acid in the Smoflipid 20 % dose groups were significantly higher than those of the control animals, with no correlation between plasma (docosahexaenoic acid) concentration and Smoflipid 20 % dosing.

In conclusion, there was no strong correlation between the administered dose level of Smoflipid 20 % and plasma concentration of any fatty acids analyzed.

Dosing Solution Analysis

The overall mean achieved doses for the dosing period (days 1 to 28) were 2,884, 5,690, 8,495 and 8,519 mg/kg/day for the treatment doses of 3,000 (group 3), 6,000 (group 4), 9,000 (reference group 2) and 9,000 mg TG/kg/day, respectively.

In conclusion, the continuous 24-hour infusion of Saline (control), Smoflipid 20 % at doses of 3,000, 6,000, 9,000 mg TG/kg/day, and Intralipid 20 % (reference control) at 9,000 mg TG/kg/day in rats for 4 weeks resulted in the death or premature sacrifice of 18 out of 42 animals, particularly in the high dose (9,000/6,000 mg TG/kg/day) groups. The pathological changes of inflammation, necrosis, bacterial colonies, granulation tissue and thrombosis in the majority of animals treated with Smoflipid or Intralipid, and the accompanying findings of inflammation, thrombi, emboli, infarction and lipid-induced granulomatous inflammatory response in the lungs, liver, kidneys, and heart appear to be due to vascular disturbance. The high mortality in lipid treatment groups under the conditions of the current study and the associated pathology is due to the physical nature of the test material and indicate that sub-chronic or chronic 24 hour a day continuous intravenous infusion of total parenteral nutrition products in the rat model is not feasible.

A Tolerance Study in Dogs of Fat Emulsion 73403 Administered Intravenously for Eight Days. (**Study No. 90-6**)

Methods: Fifteen male Beagle dogs aged 27-32 weeks and weighing 10.8 – 15.3 kg were randomly divided into five groups of 3 dogs each. The animals were intravenously administered fat emulsion 73403 (consisting of long and medium chain fatty acids, as well as medium and long chain triglycerides) at dose levels of 6,000, 9,000, or 12,000 mg TG/kg/day, or Vasolipid 20 % at 9,000 mg TG/kg/day, or Intralipid 20 % at 12,000 mg TG/kg/day for 8 days. The test and reference emulsions were infused for 6 – 9 hours/day. Clinical signs, body weight and food consumption were recorded daily, and electrocardiograms (ECG) were recorded from all dogs once during the pre-test period and at the end of the dosing period. Hematological and blood chemistry analyses were performed on blood samples taken from the animals once prior to the start of dosing and at the end of the last dosing day. Blood samples for the determination of blood glucose, blood gases, plasma beta-hydroxybutyrate, lactate, free fatty acids and triglycerides were also obtained from the animals before, and at 3 and 6 hours after the start of infusion on days 1 and 8. All the dogs were sacrificed, necropsied, selected organs were weighed, and principal organs examined histopathologically. Tissue samples from the liver and heart were taken for the analysis of fatty acid content.

Results: There were no animal deaths in the study. Clinical signs of vomiting were observed in one dog from the high dose (12,000 mg TG/kg/day) test article group, and frequent drowsiness and vomiting were seen in dogs from the vasolipid 20 % dose group at the end of infusion day 1. There were no clinical signs seen in dogs dosed with Intralipid 20%.

Food consumption decreased in a dose responsive manner in all treatment groups, with

a 50 % reduction in the high dose (12,000 mg/kg/day TG) fat emulsion 73403 and 9,000 mg vasolipid 20 % dose groups. A decrease in food consumption of 50 % was seen in the high dose (Fat emulsion 73403) and Vasolipid 20 % groups and a 25 % to 35 % reduction in food consumption was seen in the low and intermediate (6,000, 9,000 mg/kg/day TG) fat emulsion 73403 dose groups and the Intralipid 20% group.

Body weight gains were increased for most of the study dogs with no differences noted between the study groups.

The plasma urea concentrations were decreased in most of the dogs from all treatment groups, whereas the alkaline phosphatase (ALP) activities were increased in the high dose test emulsion (73403), Vasolipid 20 % or Intralipid 20 % groups.
The observed increase in serum cholesterol and phospholipid levels were dose related in the test groups, and the slightly elevated triglyceride values seen in the high dose test emulsion and Intralipid 20 % (reference) groups were similar, when compared with the test article.

The plasma concentrations of triglycerides (TG) and free fatty acids (FFA) increased during the infusion of the fat emulsions on days 1 and 8. The triglyceride levels on both days were higher after the infusion of the high dose fat emulsion 73043 than after the Intralipid 20 % infusion. The TG concentrations were lower after the infusion of low and mid dose fat emulsion 73043 or Vasolipid 20 %, with no apparent differences in TG levels between animals in the three groups. The differences in the increased FFA seen between the high and intermediate fat emulsion 73403 groups and the Vasolipid 20 % group were less obvious following repeated lipid infusion.

The daily Infusion of fat emulsion 73403 caused a transient increase in plasma beta-hydroxybutyrate, which resulted in a metabolic acidosis in the high dose group, but was comparable at the two lower dose levels. Infusion of vasolipid 20 % resulted in metabolic effects comparable to the high dose fat emulsion 73403. The decreased blood gases and increased metabolic acidosis observed in all the fat emulsion 73403 dose groups declined with repeated dosing. There were no significant changes in beta-hydroxybutyrate, lactate or blood gases during the infusion of Intralipid 20 % on days 1 and 8.

A slight reduction in blood glucose of the same magnitude was seen in all the study animals during the first 3-6 hours of infusion on days 1 and 8, but persisted throughout the infusion on day 1, and was of shorter duration on day 8 in the high dose fat emulsion 73403 and reference groups.

In the liver, fatty change in hepatocytes and Kupffer cells, deposition of fat pigment and granulomatous reactions of slight degree were seen in dogs from all treatment groups. The fatty change in the hepatocytes was slightly more pronounced in the Intralipid 20 % dosed dogs than in the fat emulsion 73403 or Vasolipid 20 % dose groups.

In the myocardium, fat droplets in interstitial macrophages were observed in the dogs receiving the high dose of Fat emulsion 73403, Vasolipid 20 % or Intralipid 20 %, and in one dog from each of the intermediate and low dose groups of emulsion 73404.

A reaction mainly characterized by hemorrhage and inflammatory changes was observed at the sites of the intravenous injections in dogs from all treatment groups.

In conclusion, the repeated infusion of Fat emulsion 73403 at doses of 6,000, 9,000 or 12,000 mg TG/kg/day was well tolerated in male dogs, resulting in clinical signs of vomiting in one dog from the high dose (12,000 mg TG/kg/day) test article group. There were no animal deaths, and the histopathological finding corresponded well with those previously reported in dogs after intravenous infusion of Intralipid 20 %.

Study title: A Tolerance Study in Dogs of Fat Emulsion 4501, containing a (b) (4) Mixture of Soybean and (b) (4) Oils, with Intravenous Administration for 28 Days.

Study no.:	86-2
Study report location:	Project 715, Page 1 to 110
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 8, 1986
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat Emulsion 4501, lot # 3575-003; Intralipid 20 %, lot # 56654; Ringer Acetate, lot # LG 46970

Key Study Findings

Daily intravenous infusion of Fat emulsion 4501 (as 9 g triglycerides (TG)/kg/day mixture with 18 mg L-carnitine) for 28 days in dogs, resulted in a lower lipid content of the liver, when compared with Intralipid 20 % (as 9 g TG/kg/day) infusion for 28 days. The clinical chemical and histopathological findings were comparable between the two treatment groups. There were no treatment related clinical signs after a 6-hr daily repeated infusion of Fat emulsion 4501 in dogs for 28 days. The total fatty acid content in the liver from fat emulsion 4501 treated animals were higher than the liver fatty acid contents of control dogs, but significantly lower than that of the Intralipid 20 % (reference article) treated dogs.
In the fat emulsion 4501 treated dogs, the plasma levels of gamma-linolenic acid and its chain elongation product homo-gamma-linolenic acid were significantly increased, as compared to the Intralipid 20% treated dogs, but there was no difference in the arachidonic acid content.

Methods

Doses:	9 g TG/kg/day and 18 mg L-carnitine/kg/day (Fat emulsion 4501); 9 g TG/kg/day (Intralipid 20 %)
Frequency of dosing:	6 hrs/day for 28 days
Route of administration:	Intravenous Infusion
Dose volume:	45 ml/kg/day
Formulation/Vehicle:	9 g TG and 18 mg L-carnitine, or 9 g TG/Ringer-acetate
Species/Strain:	Beagle Dogs
Number/Sex/Group:	2/sex/group
Age:	28-29 weeks
Weight:	7.4-9.1 kg (males), 6.4 8.8 kg (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None reported

In summary, IV infusion of 9 g TG/kg/day of fat emulsion 4501 (containing a (b) (4) mixture of (b) (4) soybean oil and (b) (4) oil) or 9 g TG/kg/day Intralipid 20 % for 28 days in dogs were well tolerated and did not result in any deaths or treatment related clinical signs. All the fat emulsion treated dogs showed increased body weight initially during the first two weeks of the test period, but later showed body weight gain similar to that of the pre-test period, in spite of reduced food intake. Slight anulocytosis and/or anisocytosis of no biological significance were present in blood smears from some female dogs treated with fat emulsion.
Clinical chemistry and histopathology findings from the two treatment groups were similar to that of control group, with the exception of significantly lower lipid contents in the liver of fat emulsion 4501 treated dogs in comparison to Intralipid 20 % treated dogs.

Study title: 28-Day Toxicity of Lipovenos-Infusion Solution 20 %, Lot No. KI 145 and Intralipid 20 % Vitrum (Preparation for Comparison), Lot No. 5489251 – called for short; Lipovenos 20 % and Intralipid 20 % - by Intravenous Infusion to Beagle Dogs.

Study no.:	Not provided
Study report location:	Pages 1 to 117
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 5, 1985
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lipovenos 20 %, lot # KI 145; Intralipid 20 %, lot # 5489251

Key Study Findings

In lipovenos 20 %-treated animals, increased serum alkaline phosphatase, total lipids, phospholipids, free cholesterol and esterified fatty acids were observed, with fatty deposits in the liver, lungs and spleen. Minor, but similar findings were observed with Lipovenos 20 % and Intralipid 20 % administration in Beagle dogs.

Methods

Doses:	45 ml/kg or 9 g/kg body weight, 4 hr/day
Frequency of dosing:	Daily for 28 days
Route of administration:	Intravenous
Dose volume:	45 ml/kg
Formulation/Vehicle:	9 g lipid/kg/ Ringer's solution
Species/Strain:	Beagle Dog
Number/Sex/Group:	3/sex/group
Age:	12 to 16 months
Weight:	8.0 to 8.7 kg (male); 7.0 to 9.2 kg (female)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No protocol deviations were reported.

Observations and Results

Mortality

No animal mortality was observed in this study

Clinical Signs

Behavior, examination of reflexes, and external appearance were observed daily. There were no observations of treatment-related changes in behavior or external appearance. A single vomiting episode in a male dog in the Intralipid 20 % group was recorded after the start of the 8th infusion, and was considered an incidental occurrence.

Body Weights

Body weight was determined once a week, at the same time.
Body weight gain was within normal range during the entire test period.

Feed Consumption

Food consumption was estimated daily.

Food consumption by the animals in the treatment groups was lower in comparison to the control animals for the entire test period. At the end of the infusion period, the food intake was reduced by 44 % in the male dogs, and by 50 % in the female dogs treated with Lipovenos 20 %. The food intakes were reduced by 53 % in the male dogs and by 37 % in the female dogs treated with Intralipid 20 %.

Ophthalmoscopy

Ophthalmological examinations were conducted after 28 test days in all animals.
No treatment-related effects on the eyes were observed in any of the study animals.

ECG

Electrocardiography was monitored on the first test day and in test week 4 in all animals. Examination was done before and directly after the 4 hr infusion period if necessary, until there are no changes observed. Blood pressure was also measured after 28 test days in all animals.

No effects of Lipovenos 20 % or Intralipid 20 % were observed on the electrocardiogram in any of the treatment animals. Minimal changes in the heart rate of control and treated animals are attributed to the lack of relaxation during the recording period.

There were no observed differences in peripheral blood pressure recordings among all the study animals, up to 20 hrs after the end of the study period.

Hematology

Venous blood was collected before the first infusion, 20 hrs after dosing on fasting animals, and in test week 4 in all animals.

In both lipid treatment groups, the leukocyte and segmented neutrophil granulocyte cell count were increased, while the lymphocytes count were significantly decreased in comparison to control animals during the treatment period. Furthermore, a slight anemia was observed in Intralipid 20 % treated animals, with decreases in erythrocyte count, hemoglobin and hematocrit values. Normal values were observed for all other blood parameters in all the animal groups.

Clinical Chemistry

Venous blood was collected before the first infusion, 20 hrs after dosing on fasting animals, and in test week 4 in all animals.

In both lipovenos 20 % and Intralipid 20 % treatment groups, the activity of liver alkaline phosphatase was significantly increased, and moderate to markedly significant increases were also observed for total lipids, phospholipids, total and free cholesterol, and esterified fatty acids in the treated animals in contrast to control animals. There were also increases in bile acids in Intralipid 20 % treated animals versus control animals.
However, the values of glucose, blood urea, total protein, bilirubin, ALT and AST activities, and plasma LDH activity remain unchanged in all study animals.

Urinalysis

Urine was collected from all animals before the first infusion and in test week 3 over 5 hrs.
There were no observed treatment related changes in urinalysis in any treatment group.

Gross Pathology

There were no observed differences from macroscopic examinations between control and treated animals at the end of the study period. Minimal bleeding occurred at the injection sites in both control and treated animals.

Organ Weights

After macroscopic inspection, the weights of the following organs were determined: Heart, Liver, Brain, Lungs, Kidneys, Adrenals, Gonads, Thyroids, Thymus, Spleen and Pituitary
There were no differences in organ weights between control and treated animals.

Histopathology

The following organs were fixed in 10 % neutral buffered formalin and stained with hematoxylin-eosin after preparation of paraffin sections, and examined histopathologicallly:

heart	prostate/uterus (incl. cervix)	bone
lungs	stomach	trachea
liver	duodenum	aorta
spleen	jejunum	oesophagus
kidneys	ileum	pancreas
adrenals	colon	lymph node ((mes.)
thymus	rectum	peripheral nerve
pituitary	salivary gland	skeletal muscle
gonads	eyes with optic nerve	skin
thyroids	urinary bladder	spinal cord
brain	bone marrow	gall bladder
		mammary gland
		infusion site

Adequate Battery
Yes
Peer Review
Yes
Histological Findings
In both Lipovenos 20 % and Intralipid 20 % treated animals, there were accumulation of fat in the liver and mononuclear cellular proliferation (partly with siderosis, as well as infiltration of mononuclear cells), including lesions of the spleen, (sinus histiocytosis), and lungs (slight to moderate bronchopneumonia). Other changes occurring in single animal organs are regarded as spontaneous occurrences.

In conclusion, 28-day Intravenous infusion at 45 ml/kg (9 g/kg) of Lipovenos 20 % or Intralipid 20 % for 4 hr/day to Beagle dogs resulted in reduced food consumption, but not weight changes in the treated animals. Increased leukocyte and decreased lymphocyte counts were observed along with elevations of total and free cholesterol and esterified lipids. The lipid accumulations were confirmed by histopathological findings of fatty deposits in hepatocytes, and mononuclear cellular proliferation, spleen histiocytosis, and accumulation of macrophages in the lungs. In addition to the findings mentioned, Intralipid 20 % treated animals also showed slight anemia and increased value for bile acids.

Study title: Repeated Dose of Lipovenos 20 % via the Intravenous Route in the Beagle Dog: Comparison with Intralipid 20 % (30 Days)

Study no.:	003-062
Study report location:	Pages 1 to 216 of the electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 1985
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lipovenos 20 %, lot # KE 112; Intralipid 20 %, lot # 71133-51

Key Study Findings

Lipovenos 20 % and Intralipid 20 % IV infusion in Beagle dogs, at a daily dose of 45 ml/kg (9 g/kg) for 30 days were well tolerated, without adverse changes. Mild, but similar changes observed for both Lipovenos 20 % and Intralipid 20 % include decreased levels of RBCs, hemoglobin, hematocrit, increase in reticulocytes, alkaline phosphatase, bilirubin, cholesterol and phospholipids.

Methods

Doses:	9 g/kg
Frequency of dosing:	150 ml/hr for 3 hrs daily for 30 days
Route of administration:	Peripheral Intravenous
Dose volume:	45 ml/kg/day
Formulation/Vehicle:	9 g lipids/kg; Isotonic NaCl solution
Species/Strain:	Beagle Dog
Number/Sex/Group:	3 groups of 2 dogs/sex/group
Age:	10 months
Weight:	11 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No deviation from study protocol reported.

Observations and Results

Mortality

No mortality was recorded in study animals.

Clinical Signs

Animals were observed daily from the start of infusion to its completion.
Sporadic vomiting observed in 2 female dogs dosed Lipovenos 20 % on days 24, 26, and 28

Body Weights

The animals were weighed twice weekly at regular intervals from study day 7.

Mean weight gain (+ 1.20 kg) for in animals dosed with Lipovenos 20 % (between day 1 and day 29) was higher than that for control animals (+ 0.18 kg), or animals dosed with Intralipid 20 % (+ 0.15 kg). The differences are not statistically significant.

Feed Consumption

Food consumption in animals was recorded daily.
Food intake for animals dosed with Intralipid 20 % was slightly less than that of control animals, but the difference is not statistically significant.

Ophthalmoscopy

Ophthalmologic examination was performed on study day 27.

No ocular anomalies were reported with Lipovenos 20 % or Intralipid 20 % infusion for 1 month.

ECG

Heart rate of the animals were recorded on study days 0 (prior to infusion), and on days 7, 14, 21, and 29 prior to the start of infusion.

The heart rate of the animals was not changed by test article or reference article infusion.

Hematology

Blood samples were obtained from animals prior to start of IV infusion on study day 0, 7, 14, 21, and 30.
Changes observed include moderate fall in red blood cell counts (RBC) in Lipovenos 20 % treated animals (from 6.27 to 5.90 X 10^{12} cells/L), and in Intralipid 20 % treated animals (from 6.52 to 6.06 X 10^{12} cells/L) when compared to control animals. The difference between the two treatment groups was not statistically significant. There were also decreases in hematocrit values from 0.49 to 0.41 ml/dl, and from 0.52 to 0.42 ml/dl in the Lipovenos 20 % and in the Intralipid 20 % animals respectively, when compared to control animals. Decreases in hemoglobin levels from 10.05 to 8.4 mM/L, and from 10.9 to 8.9 mM/L were also observed respectively, in the Lipovenos 20 % and in the Intralipid 20 % animals, when compared to control animals. However, none of the differences between the two treatment groups were statistically significant.

Other changes observed were elevation in blood sedimentation rate from 14 to 63 mm/hr and from 7 to 64 mm/hr, and elevation in reticulocyte count from 0.049 to 0.187 X 10^{12} cells/L and from 0.032 to 0.139 X 10^{12} cells/L for lipovenos 20 % and Intralipid 20 % animals respectively, when compared to control animals. However, none of the differences between the two treatment groups were statistically significant.
The platelet counts on day 21 and day 30 are identical in animals from the two lipid emulsion treatment groups, but lower than the platelet counts in the control animals.

Clinical Chemistry

For clinical chemistry, blood samples were obtained from animals prior to start of IV infusion on study day 0, 7, 14, 21, and 30.
Changes observed in clinical chemistry parameters include elevation in total cholesterol and phospholipid levels in lipovenos 20 % and Intralipid 20 % treated animals, when compared to control animals. The differences between the two treatment groups were not statistically significant.

Elevation in liver alkaline phosphatase and bilirubin levels from 1.55 to 6.53 µM/L, and 1.73 to 4.65 µM/L, respectively, in lipovenos 20 % and Intralipid 20 % treated animals were observed, in comparison to control animal values. However, differences between the two treatment groups were not statistically significant.

Urinalysis

Urine samples were collected from animals over 24 hrs, with the water supply withdrawn immediately prior to urine collection in the morning, on days 0, (prior to start of infusion), 7, 14, 21, and 30. Cytological examination of residual urine was also performed on days 0, 14, and 30.

There were no observed differences in urinalysis and urine cytology between the control and treated animal groups.

Gross Pathology

The following organs were examined at autopsy:
Liver
Kidneys
Lungs
Spleen
Stomach
Duodenum

Observed changes in the treated animals include small areas on liver lobes of 2 group A (Lipovenos 20 % treated) animals, and pale yellow coloration of the liver in group B (Intralipid 20 % treated) animal. There were areas of congestion and yellow coloration in the cortico-medullary zones of the kidneys in one animal in each of the treatment group.

There were hemorrhagic points in the lungs of 1 animal, hypertrophy of the spleen in 1 animal, and zones of inflammation in the stomach (1 animal) and duodenum (2 animals) in the lipovenos 20 % treated group. Lipid inclusions were also observed in the coronary vessel of the heart in an animal in the Intralipid 20 % treated group. However, only 2 control animals were observed with white zones and hemorrhagic points in the lungs.

Organ Weights

The following organs were weighed:

- adrenal glands
- brain
- heart
- hypophysis
- kidneys
- liver
- lungs
- pancreas
- prostate
- spleen
- testicles or ovaries
- thyroids

There were no significant differences in the organ weights between the treated groups and the control animals, with the exception of the liver weight, which was higher in the animals treated with Lipovenos 20 %, when expressed as a percentage of the body

weight. The values were within the physiological range, and the differences between the two treatment groups were not statistically significant.

Histopathology

Histopathology samples were fixed with 10 % neutralized formalin, with the exception of the liver, lungs, and the spleen, which were placed in 10 % neutralized formalin plus 1 % calcium acetate. The following organs were dissected from the animals for histopathological analysis:

Adrenal glands
Aorta (arch and abdominal section)
Bladder
Brain
Colon
Duodenum
Eye and optic nerve
Gall Bladder
Heart
Hypophysis
Ileum
Jejunum
Kidneys
Liver
Lungs
Lymph nodes (cervical and mesenteric)
Mammary gland
Esophagus
Pancreas
Prostate or uterus
Salivary gland
Sciatic nerve
Site of infusion
Skeletal muscle
Skin
Spleen
Sternum
Stomach
Testicles or ovaries
Thymus
Thyroids
Tongue
Trachea

Adequate Battery
Yes
Peer Review
Yes
Histological Findings

There were no adverse histopathological changes observed with the 30-day infusion of Lipovenos 20 % or Intralipid 20 % in Beagle dogs. The changes observed consist of hemosiderosis in Kupffer cells and splenic reticular cells and advancement of

hemocatheretic processes in the splenic red pulp. An excess of lipids in hepatocytes and in the reticular cells of the splenic red pulp was also observed. The organs of animals treated with Lipovenos 20 % or Intralipid 20 % displayed similar histological appearances, and did not show any demonstrable differences.
In conclusion, IV infusion of Lipovenos 20 % or Intralipid 20 % at a dose of 45 ml/kg or 9 g lipids/kg (3X the maximum recommended clinical volume/kg) in dogs at a rate of 150 ml/hr for 3 hrs (10X the maximum recommended clinical rate) daily for 30 days was tolerated without any mortality, adverse gross or histopathological effects. The observed decreases in RBCs, hematocrit and hemoglobin levels were within physiological range, while the increases in total cholesterol and phospholipids were due to the composition of the products infused.

The number of animals/group in the study was inadequate (2/sex/group), and so the study is inadequate.

Study title: A Tolerance Study in Dog of Fat Emulsion 73403 Administered Intravenously for One Month.

Study no.:	88-20
Study report location:	Project No. 734, Page 1107 to 1265
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 4, 1988
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat Emulsion 73403 20 %, lot # 3568-177; Intralipid 20 %, lot # 39 360-51

Key Study Findings

Daily infusion of Fat emulsion 73403 to dogs for 1 month resulted in a transient but dose-related increase in beta-hydroxybutyrate, causing metabolic acidosis in the high dose group. Transient elevations of free fatty acid and triglyceride levels in plasma were dose related in fat emulsion treated animal groups. Histopathological changes were comparable between the fat emulsion 73403 test groups and the Intralipid 20 % reference group. However, the IV infusion of Fat emulsion 73403 resulted in lower fatty acid content in the liver, when compared with the Intralipid 20 % group, but there were no differences between the two fat emulsion 73403 test groups.

Methods

Doses:	6 g TG/kg/day (Low fat emulsion 73403); 9 g TG/kg/day (High fat emulsion 73403); 9 g TG/kg/day (Intralipid 20 %)
Frequency of dosing:	6 hrs/day for 28 days
Route of administration:	Intravenous infusion
Dose volume:	45 ml/kg, 30 ml/kg; and 45 ml/kg respectively.
Formulation/Vehicle:	9 g TG/kg high fat, reference fat, or 6 g TG/kg low fat
Species/Strain:	Beagle Dogs
Number/Sex/Group:	4/sex/group
Age:	25-31 Weeks
Weight:	9.6-13.1 kg (males), 7.5-10.1 kg (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minor deviations from intended protocol doses.

Repeated IV infusion of Fat emulsion 73403 at doses of 6 g or 9 g TG/kg/day, or Intralipid 20 % at a dose of 9 g TG/kg/day resulted in lower weight gains (5-20 %) in the fat emulsion test groups than in the Intralipid 20 % reference group (10-30 %), but without noticeable difference between the two fat emulsion groups. Blood chemistry analysis showed that all dogs in all treatment groups had slight to moderately reduced blood urea throughout the treatment period, with the exception of male dogs in the high dose fat emulsion 73403 groups which showed a marked reduction in the serum urea at the end of the study.

Infusion of Fat emulsion 73403 caused significant dose-related increase in plasma beta-hydroxybutyrate and a parallel decrease in blood pH, bicarbonate and base excess, and a ketogenic response. Mild lactacidemia, and consequently metabolic acidosis developed in a few dogs in the high dose group, but only to a slight degree in the low dose fat emulsion 73403 group. These changes declined as dosing progressed, but were not observed in the Intralipid 20 % group.

The IV infusion of Fat emulsion 73403 resulted in lower fatty acid content in the liver, when compared with the Intralipid 20 % group, but there were no observed differences between the two fat emulsion 73403 test groups. There was no untreated animal control group in this study.

Study title: Omegavenous: 4 Week Intravenous Administration Sub-Chronic Toxicity Study in the Beagle Dog.

Study no.:	5820-630/5
Study report location:	Document 6, Volume 2, Page 847 - 1061
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 20, 1988
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Omegavenous, Lot #s 2-3, 10 % ; Lipovenos 10 %, Lot #s 1-4, 10 % .

Key Study Findings

The intravenous administration of Omegavenous daily at doses of 250, 1,000 and 5,000 mg/kg/day for 1 month did not result in animal deaths. There was no evidence of systemic toxicity or intra vascular irritancy in treated animals. Changes at the injection site showed that periphlebitis was more frequent in animals receiving the test article than in animals receiving the Lipovenos 20 % vehicle control. The test article was well tolerated in male and female dogs.

Methods

Doses:	0 (control) group 1, 0 (vehicle control) group 2, 250 (group 3), 1,000 (group 4) and 5,000 (group 5) mg/kg/day.
Frequency of dosing:	Daily for 34 days
Route of administration:	Intravenous injection via a cephalic or saphenous vein.
Dose volume:	5 ml/kg
Formulation/Vehicle:	Omegavenous 10 %/ Lipovenos 20 %, 0.9 % sodium chloride (saline)
Species/Strain:	Beagle Dog
Number/Sex/Group:	3/sex/group
Age:	4 to 6 months of age
Weight:	5.45 to 8.75 kg (males), 4.90 to 8.90 kg (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	In order to prevent further animal distress by repeated venipuncture, one group 2 male (days 1 and 8), one group 3 male (days 23 and 27) and one group 5 female (day 9) did not receive a full dose of drug; Group 3 and 4 animals were administered undiluted test article at dose volumes of 0.25 ml/kg and 1 ml/kg, respectively on 2 days during week 5; Group 2 animals were not dosed on 1 day during week 5; The results obtained for total lipid at week 2 were not considered reliable; Because samples were not available for a repeat analysis, samples were collected from all animals in week 3 for measurement of total lipid only. Deviations did not affect study integrity or outcome.

Observations and Results

Mortality

There were no animal mortalities in this study

Clinical Signs

Animals were observed daily in the mornings, and throughout the day, when necessary.

There were no clinical observations considered to be treatment related.

Body Weights

Individual animal body weights were recorded weekly throughout the study period.

There were no significant differences in the body weight gain of treated animals when compared to the respective control groups.

Feed Consumption

Food consumption of individual animal was estimated daily and weekly food consumption data were calculated from the estimates.

The food consumption in the treated animal groups was similar to that of the respective control groups.

Ophthalmoscopy

Ophthalmic examination of all animals were done pre-dose and in week 4.
No ocular abnormalities were observed in any of the study animals at pre-dose or during week 4 examination.

ECG

Electrocardiographic (ECG) recordings were done pre-dose and in week 4.
No abnormalities in the ECG recordings, heart rate or electrical activity of the heart were observed in any of the study animals. No changes in the systolic, diastolic or mean arterial blood pressure were seen in the animals.

Hematology

Blood samples were collected from all animals pre-dose and in weeks 2 and 5.
There were no apparent effects of treatment with the test article on the hematologic parameters in the study animals.

Clinical Chemistry

Blood samples were collected from all animals pre-dose and in weeks 2 and 5.
There were no effects of treatment with the test article on the measured clinical chemistry parameters.

Urinalysis

Urine samples were collected from all animals pre-dose and in weeks two and five by direct catheterization of the bladder.
There were no effects of treatment with the test article on the measured parameters.

Gross Pathology

Animals were killed by anesthetic (sodium thiopentone) overdose, and full necropsy of all the animals were conducted over a period of three days.
There were no treatment-related observations in the study animals at necropsy.
Injection site lesions were mostly perivenous and probably due to leakage. There were no significant reactions in the vessel lumen or wall that are suggestive of local intravascular irritation.

Organ Weights

The following organs were dissected from the animals and weighed:

adrenals	brain (including brain stem)
kidneys	heart
ovaries	liver
spleen	pituitary
thyroids (with parathyroids)	testes (with epididymides)

Paired organs were weighed separately.

Organ weights and organ/body weight ratios were unaffected by test article treatment.

Histopathology

Samples of the following tissues from each animal were fixed in neutral buffered 10 % formalin, with the exception of the eyes which were fixed in Davidson's fluid:

adrenals	brain (including brain stem)
caecum	colon
duodenum	epididymides
eyes (with optic nerves)	gall bladder
heart	ileum
injection sites	jejunum
kidneys	liver
lungs (with mainstem bronchi)	lymph nodes (mandibular and mesenteric)
oesophagus	pancreas
ovaries	prostate
pituitary	skin and mammary gland
salivary glands (submaxillary)	spleen
spinal cord (lumbar, cervical, thoracic)	sternum (with bone marrow)
stomach	testes
thymus	thyroids (with parathyroids)
trachea	uterus (corpus and cervix)
urinary bladder	
all gross lesions	

Samples of tissues were embedded in paraffin wax, and stained with hematoxylin and eosin. Frozen sections of liver, kidney and heart were stained with Oil red O.

Adequate Battery
Yes
Peer Review
Yes

Histological Findings
There were no treatment related histopathological findings observed in the study animals.
In conclusion, the daily intravenous infusion of Omegavenous at dose levels of 0 (control), 0 (vehicle control), 250, 1,000 and 5,000 mg/kg/day for one month did not show any evidence of systemic toxicity or intravascular irritancy in male and female dogs.

Study title: 4-Week Subchronic Toxicity Study of SMOF 20 % by Daily 6-Hour Intravenous Infusion to Beagle Dogs.

Study no.:	9358/1/95
Study report location:	(b) (4) Report No. 9802/1/96, Page 1 to 323
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 5, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SMOF 20 %, lot # FHFE 11; Lipovenos 20 %, lot # FG 1544

Key Study Findings

Infusion of SMOF 20 % (test article) or Lipovenos 20% (reference article) to dogs resulted in increased segmented neutrophil granulocytes, ESR, and reticulocytes, decreased lymphocytes, hemoglobin, platelet count, and hematocrit values, without noticeable differences between the two animal groups.
Similar increases were also observed in plasma alkaline phosphatase, total cholesterol, and acetoacetate in animals from both groups, when compared with predose values. However, the increased phospholipids, bile acids and direct bilirubin (but not total bilirubin) appeared more pronounced in Lipovenos 20 % treated animals than in SMOF 20 % treated animals.

Methods

Doses:	9 g TG/kg body weight/day
Frequency of dosing:	6 hrs./day X 28 days
Route of administration:	Intravenously, via leg peripheral vein
Dose volume:	45 ml/kg body weight/day
Formulation/Vehicle:	None
Species/Strain:	Beagle Dogs
Number/Sex/Group:	2/sex/group
Age:	7-10 months (males), 9-15 months (females)
Weight:	11.7-12.4 kg (males), 8.5-8.9 kg (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No significant deviation from study protocol

Intravenous infusion of 9 g TG/kg of SMOF 20 % or Lipovenos 20 % was equally well tolerated and all the animals survived till euthanasia. Soft feces were present in dogs of both treatment groups. Reduced food consumption was observed in both animal groups, but did not affect body weight gain. The increased phospholipids, bile acids and direct bilirubin (but not total bilirubin) appeared more pronounced in Lipovenos 20 % treated animals. However, only 2 animals/sex/group were used in the study which was inadequate.
In animals from both groups, there was moderate or marked presence of fat in liver acinar cells, slight to moderate foci of granulomatous pneumonia in the lungs and moderate interstitial nephritis in the kidneys, but without noticeable difference in severity.

Omegaven: 6-Week Preliminary Intravenous (16-Hour Infusion) toxicity Study in the Beagle Dog (**Study No.AA76191**)

Methods: Twenty Beagle dogs (10 males and 10 females) aged 5-6 months and weighing 6.6-8.9 kg (males) and 6.3-7.8 kg (females) were randomized into 3 groups of 2/sex/group. The animals were infused intravenously with 0.9 % NaCl (control, group 1) at a dose volume of 40ml/kg/16 hr, or Omegaven (test article) at dose levels of 2 g triglycerides/kg/16 hr (group 2) at a dose volume of 20 ml/kg, and 4 g triglycerides/kg/16hr (group 3) at a dose volume of 40 ml/kg for up to 42 days.

In a second (phase II) study, a male (No.421) and a female (No.423) dog from control group 1 in the phase 1 study were designated as phase 2 control animals and treated with 0.9 % NaCl (control, group 1) at a dose volume of 60/40 ml/kg/16 hr for up to 72 days. In the phase II study, 2 dogs/sex/group aged 6 to 7 months and weighing 7.4 to 10.2 kg (males), and 6.9-8.4 kg (females) were at first treated with Omegaven (test article) at 6 g trigycerides/kg/16 hr (group 4) at a dose volume of 60 ml/kg, or Intralipid 10 % (reference article) 6 g triglycerides/kg/16 hr (group 5) at a dose volume of 60 ml/kg for up to 42 days, but the doses were reduced to 4 g triglycerides/kg/16hr at a dose volume of 40 ml/kg on day 2 of the study for animal survival.

Morbidity/mortality checks were performed twice daily, and body weight was recorded twice weekly for each animal. Food consumption was measured daily for each animal. Blood samples for hematology and clinical chemistry was obtained once pre-test, and at various time-points after dosing on days 13, 20 and 40 for each study phase. Control animals retained for phase II study were only sampled once pre-test during phase I. Blood samples for toxicokinetic (TK) evaluation were obtained at various time-points after the start of the daily infusion on days 0, 14 and 40 for each phase. Additional blood samples were taken for TK, hematology and clinical chemistry evaluation on group 5 animals on day 3 to check for changes in clinical chemistry parameters. The animal found dead during the study was necropsied. All the surviving animals were sacrificed at the end of the treatment period of each phase (day 42). Selected organs were weighed; organ/tissue samples from all animals were fixed, and were examined histopathologically.

Results: There were no animal deaths in the Phase I study at any dose level. There were no treatment-related clinical signs observed in animals dosed at 4 g triglycerides/kg/16 hr.

In the Phase II study, a group 4 male dog (No.434) treated with Omegaven at 6 g triglycerides/kg/16 hr was found dead on treatment day 1. There were no clinical signs observed prior to its death. A group 4 female dog (No.435) had marked clinical signs such as decreased activity, slow movements, rapid breathing, hot to the touch, and no reaction to stimulation on day 1. Because of the observed changes, the doses of group 4 and 5 animals were reduced to 4 g triglycerides/kg/16 hrs. from dosing day 2. After the dose reduction, occasionally marked clinical signs noted in the animals included decreased activity, retching, labored/rapid/deep breathing and/or pale ocular and/or buccal mucous membrane, subdued behavior and intermittent tremors, observed between treatment days 4 through day 41.

In addition, group 5 (Intralipid treated) animals occasionally showed ventral decubitus, panting, noisy breathing and hyperthermia. Other clinical signs observed pre-treatment and during dosing in control and treated animals include scars, scabs,nodules, interdigit, excrescences (forelimb or hindlimb), liquid feces, vomiting food, slight foamy vomit, vaginal discharge, prolapse of the nictitating gland, red ocular mucous membrane, licking and/or induration at the implantation site.

At 4g triglycerides/kg/16 hr, 1 male (No.430) and 1 female (no.432) animal from group 3 gained more weight than controls (+1.4 kg and +1.5 kg, respectively vs. between +0.5 kg and + 1.1 kg in controls).

Dose-related reduction in mean food consumption was observed in Omegaven treated animals at a maximum of -37.2 % for males and -18.0 % for females (group 2); at a maximum of -56.5 % for males and -61.4 % for females in group 4; and at a maximum of -53.8 % for males and -41.9 % for females in group 5, when compared with controls during the treatment period.

Plasma and/or serum from group 3 to 5 animals were lipaemic at the end of the 16-hr infusion period and before the start of the next infusion (+ 24 hours), in comparison to plasma from control animals.

On dosing day 40 at the end of the infusion period, a decrease in red blood cell count, hemoglobion level and packed cell volume was noted in one group 2 male, one group 3 male and female dog each, and all group 4 and 5 animals compared with pretest and control values. The values remained lower before the start of the next infusion (+ 24 hours). The changes correlated well with an increase in reticulocyte count in these animals. A summary of the measured hematological parameters are presented in the Sponsor's table below.

	Differences in RBC, hemoglobin , PCV and reticulocyte levels on day 40 when compared with pretest							
	Group 2[a]		Group 3[a]		Group 4[c]		Group 5[b]	
	Males							
	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr
RBC (Tera/L)	-31.0 %	-38.3 %	-21.2 %	-19.7 %	-46.0 %	-43.1 %	-34.2 %	-32.1 %
Hemoglobin (Grams/L)	-33.3 %	-40 %	-24.2 %	-22.8 %	-52.0 %	-49.0 %	-35.7 %	-35.1 %
PCV (%)	-31.4 %	-39.4 %	-21.5 %	-21.9 %	-44.9 %	-43.2 %	-31.6 %	-30.1 %
Reticulocyte (%)	x5.8	x7.3	x2.2	x1.6	x13.8	x12.8	x7.5	x7.4
	Females							
	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr
RBC (Tera/L)	-	-	-25.1 %	-28.6 %	-41.2 %	-44.2 %	-34.4 %	-32.8 %
Hemoglobin (Grams/L)			-29.0 %	-31.2 %	-46.4 %	-49.2 %	-38.0 %	-38.0 %
PCV (%)	-	-	-26.1 %	-31.6 %	-39.5 %	-44.3 %	-34.8 %	-34.3 %
Reticulocyte (%)	-	-	x1.6	x2.2	x7.9	x7.1	x2.3	x2.5

a: indivual value; b: in mean terms (when applicable), c: individual values except for females (in mean terms).

-: no differences from pretest.

M: male / F: female.

Marked, treatment-related decrease in platelet count was observed on all occasions in most group 2 and group 3 animals and all the group 4 and group 5 animals, when compared to pretest and control values. In group 2 and 3 animals, the lowest values were transitory, whereas, in group 4 and 5 animals, the platelet count tended to return to pretest values on occasions corresponding to the stoppage of treatment within the sampling time.

Longer activated partial thromboplastin time (APTT) and slightly higher fibrinogen concentrations were occasionally observed in treated animals, when compared with pretest and controls. However, a group 2 female animal had shorter APTT, in comparison to pretest values. A correlation could therefore not be established.

A summary table of the decreased platelet count is shown in the Sponsor's table below.

	Differences in platelet count (Giga/L) when compared with pretest					
	Males			Females		
	Animal no.	Pretest values	Largest difference	Animal no.	Pretest values	Largest difference
Group 1	421	353	NA	423	407	NA
	422	348	NA	424	141	NA
Group 2	425	401	-84.5 %	427	285	-90.2 %
	426	322	-43.2 %	428	362	-72.1 %
Group 3	429	426	-69.0 %	431	270	-70.4 %
	430	292	-77.7 %	432	349	-10.0 %
Group 4	433	214	-88.8 %	435	299	-85.6 %
	434	NA	NA	436	262	-91.2 %
Group 5	437	277	-90.3 %	439	423	-82.3 %
	438	306	-85.3 %	440	233	-79.8 %

NA: Not applicable.

Marked, treatment-related increases in cholesterol levels (total, free and LDL) were observed in Omegaven or Intralipid treated animals at the 4g triglycerides/kg/16 hrs. dose level, when compared to pretest or control values. Although these changes were noted on day 3 in the group 5 animals, the increase was mainly noted on day 40 in group 2 animals. In all treated groups, triglyceride level was increased at the end of each 16-hour infusion period, when compared with pretest and controls. The results are summarized in the Sponsor's table below.

	Differences in cholesterol (mmol/L) and triglyceride (Grams/L) levels when compared with pretest							
	Males							
	Group 2		Group 3		Group 4		Group 5	
	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr
Total cholesterol	+35.1 %	+23.9 %	x2.5	x2.4	x2.0	x1.9	x3.1	x2.9
Free cholesterol	x2.5	x2.2	X5.9	x5.6	x4.2	x4.1	x6.3	x5.7
LDL cholesterol	x2.2	x1.7	x7.8	x5.6	x7.5	x6.4	x10.9	X7.7
Triglyceride	x2.0	-	x3.8	x1.4	x3.2	x3.2	x3.4	x1.8
	Females							
	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr	+16 hr	+24 hr
Total cholesterol	+47.3 %	+46.6 %	x2.0	x1.9	x2.0	x1.9	x2.9	x2.8
Free cholesterol	x2.9	x2.8	x4.8	x4.5	x4.4	x4.1	x5.8	x5.3
LDL cholesterol	x3.7	x3.1	x6.8	x5.0	x7.5	x5.4	x11.4	x8.0
Triglyceride	x2.0	x1.2	x2.5	x1.1	x3.6	x1.9	x3.7	x2.4

-: no differences from pretest.

On treatment day 41, urea levels were markedly higher in group 2 animals, compared to pretest levels. However, in groups' 3 to 5 animals, changes in urea levels were less pronounced or remained stable during the treatment period. A similar profile was also observed for creatinine levels.

On all occasions, decreased albumin levels associated with a higher globulin levels were generally observed at 4 g/kg/16 hr in group 3 to 5 animals, when compared with pretest and controls. As a result of this, the albumin/globulin ratio and total protein level were lower and more marked at the end of the study period. Similar finding were generally observed between days 20 and 40 in group 2 animals.

Increased alkaline phosphatase (ALP) activity was observed in group 3 and 4 animals (Omegaven treated animals), but not in group 5 (Intralipid treated) animals. A group 3 male dog (No.429) had elevated alanine aminotransferase (ALT) activity (between 1.3 X and 3.6 x) on most occasions. The differences in ALP activity in dogs is shown in the Sponsor's table below.

		Differences in alkaline phosphatase activity (International Units) when compared with pretest				
		Males		Females		
	Animal no.	Pretest values	Largest difference	Animal no.	Pretest values	Largest difference
Group 1	421	689	NA	423	478	NA
	422	316	NA	424	488	NA
Group 2	425	505	NA	427	382	NA
	426	337	NA	428	360	NA
Group 3	429	412	x6.5	431	411	x9.5
	430	260	x9.2	432	330	x4.0
Group 4	433	373	x4.7	435	328	x6.1
	434	367	NA	436	356	x4.8
Group 5	437	330	NA	439	351	NA
	438	294	NA	440	270	NA

NA: Not applicable.

At necropsy, a dead group 4 male dog (No.434) treated with Omegaven at 6 g/kg/16 hr was observed with edematous areas in the thymus, dilated right cardiac ventricle, large amount of froth in the trachea and un-collapsed lungs with dark foci in all lobes. The death was associated with changes in the respiratory tract that was histologically correlated with marked alveolar edema.

The absolute and relative liver weights were higher in all treatment groups than in control group. The higher liver weights were histologically correlated with diffuse sinusoidal inflammatory cell infiltration, and considered to be treatment related.

The absolute and relative kidney weights were higher in all treatment groups than in control group, but there were no histological correlations observed.

In the lungs, the main macroscopic finding included dark foci with variable incidences observed in control and all the treated animal groups. This finding was microscopically correlated with inflammatory changes in the treated animal groups.

In the liver, pale foci was mainly noted in group 4 (6 g/kg Omegaven) and group 5 (6 g/kg Intralipid 10 %) animals, and was correlated histologically with diffuse sinusoidal inflammation.

Histopathological changes that are treatment-related were mainly noted in the liver and lungs.

Microscopic changes observed in the liver are acute sinusoidal and hepatocytic inflammation, which is correlated with increased liver weight in treated groups versus the control group, and considered to be treatment related. Histological changes in the lungs are alveolar inflammation with vacuolated macrophages and alveolar abscess.

The changes in the liver and lungs show a variable dose-response effect in treated animals, with changes in group 4 (Omegaven) and group 5 (Intralipid 10 %) slightly more severe than in groups 2 and 3 animals. Slight to severe subendothelial necrosis were observed at the injection site in some treated animals from groups 2-5, which may have been exacerbated by the test or reference item, beyond the spontaneous background infusion effects. The findings of the test or reference article on the liver and lungs of dogs are shown in the Sponsor's table below.

Treatment-Related Microscopic findings in the Liver of Dogs

Group Treatment	Group 1		Group 2		Group3		Group 4		Group 5	
Sex	M	F	M	F	M	F	M	F	M	F
No. animals	2	2	2	2	2	2	1(*)	2	2	2
Sinusoidal inflammatory cell infiltration										
- Minimal	-	-	-	1	1	-	-	-	-	-
- Slight	-	-	2	1	1	2	-	1	1	-
- Moderate	-	-	-	-	-	-	1	1	1	2
Hepatocytic multifocal inflammation										
- Minimal	1	-	1	1	1	-	-	-	1	
- Slight	-	-	1	-	-	-	1	1	-	2

(*) excluded premature decedent.

Treatment-Related Microscopic findings in the Lungs of Dogs

Group Treatment	Group 1		Group 2		Group3		Group 4		Group 5	
Sex	M	F	M	F	M	F	M	F	M	F
No. animals	2	2	2	2	2	2	1(*)	2	2	2
Inflammation alveolar (focal/multifocal)										
- Minimal	1(f)	-	-	-	1(f)	-	-	-		
- Slight	-	1	1	1	1	1	1	2	2	2
Vacuolated macrophages										
- Minimal	-	-	-	-	1	1	-	-		
- Slight	-	-	1	-	-	-	1	2	2	2
Alveolar abscess (focal/multiple)										
- Slight	-	-	1	1(f)	-	-	1	-	-	-
- Moderate	-	-	-	-	-	-	-	1	-	-

(*) excluded premature decedent.

(f) Focal distribution.

- Incidental or non treatment-related findings

In conclusion, the intravenous infusion of Omegaven (test article) and Intralipid 10 % (reference) (60 ml/kg/16hr) at 6 g triglycerides/kg/16-hour for 6 weeks was poorly tolerated, and resulted in animal mortality during the phase II study. The infusion of the test or reference article at 2 g triglycerides/kg/16 hr was well-tolerated whereas the 4 g

triglycerides/kg/16 hr was not well tolerated during the 6 weeks administration. Based on this finding, the highest dose that will be administered to dogs in the long-term 13-week toxicity study will be 3 g triglycerides/kg/16 hr.

Omegaven: 6-Week Preliminary Intravenous (16-Hour Infusion) toxicity Study in the Beagle Dog (**Study No.AA76191, Amendment 1**)

Methods: Twenty Beagle dogs (10 males and 10 females) aged 5-6 months and weighing 6.6-8.9 kg (males), and 6.3-7.8 kg (females) were randomized into 3 groups of 2/sex/group. The animals were infused intravenously with 0.9 % NaCl (control, group 1) at a dose volume of 40ml/kg/16 hr, or Omegaven (test article) at doses of 2 g triglycerides/kg/16 hr (group 2) at a dose volume of 20 ml/kg, and 4 g triglycerides/kg/16hr (group 3) at a dose volume of 40 ml/kg for up to 42 days in a phase I study.

In a second (phase II) study, a male (No.421) and female (No.423) dog from control group 1 in the phase 1 study were designated as phase 2 control animals and treated with 0.9 % NaCl (control, group 1) at a dose volume of 60/40 ml/kg/16 hr for up to 72 days. In the phase II study, 2 dog/sex/group aged 6 to 7 months and weighing 7.4 to 10.2 kg (males),and 6.9-8.4 kg (females) were at first treated with Omegaven (test article) at 6 g trigycerides/kg/16 hr (group 4) at a dose volume of 60 ml/kg, or Intralipid 10 % (reference article) 6 g triglycerides/kg/16 hr (group 5) at a dose volume of 60 ml/kg for up to 42 days, but the doses were reduced to 4 g triglycerides/kg/16hr at a dose volume of 40 ml/kg/16 hr on day 2 of the study.

Blood samples for toxicokinetic (TK) analysis was collected from study animals on days 0 (first treatment day), 14 and 40, before the start of infusion, 2, 6 and 16 hours after the start of infusion and 0.5, 2, and 8 hours after the end of infusion. Plasma serum analysis of 10 fatty acids were performed instead of 12 because the plasma concentration of the 2 other fatty acids were too low. Plasma concentrations of fatty acids below the lower limit of quantification (LLOQ) were taken as 0.

Results: Smoflipid (test article) and Intralipid 10 % (reference article) were detected in the plasma obtained from all the study animals including the control group, but the concentrations measured in the control group were equal or lower than that of the low dose (2 g triglyceride (TG)/kg/16 hr) group.

Fatty acid C16:01 was mainly detected in the high dose and control groups while C18:03 was mainly detected in the reference article (Intralipid 10 %) dosed group. For all the other fatty acids, test/reference article was measured in all groups including control, with the exception of fatty acid C20:05 which was not detected in plasma from the control group.

The maximum plasma concentrations of fatty acids during infusion were observed at the end of the 16 hour infusion, for fatty acids 16:00, 18:01, 20:05 and 22:06. For the other fatty acids, T_{max} was variable during infusion.

For all the fatty acids, there were no sex-related differences observed in C_{max} and AUCs except on day 0 for dogs treated at 4 g TG/kg/16 h for 7 out of 10 fatty acids where male values were 1.39 to 1.93-fold higher than values in females.

For all the fatty acids, no major accumulation was observed between day 0 and 40. Between day 14 and 40 however, a slight accumulation was noted at 2 g TG/kg/16 h.

Between doses of 2 and 6/4 g TG/kg/16 h, the increase in systemic exposure for males and females was generally less than dose-proportional on day 0, dose-proportional or more than dose-proportional on day 14, and less than dose-proportional or dose-proportional on day 40.

For all the measured fatty acids at all occasions and for both sexes, the systemic exposure (C_{max} and AUCs) of the reference article (Intralipid 10 %) was higher than that of the test article (Omegaven). However, for fatty acids C16:01 and C20:05, the systemic exposure of Intralipid 10 % was lower than that of Omegaven, and for C22:05, the systemic exposures for the test and reference articles were similar.
The mean toxicokinetic parameters measured as accumulation ratios for the fatty acid eicosapentaenoic acid (C20:05) and docosapentaenoic acid (C22:05) are shown in the Sponsor's table below.

Accumulation Ratios of Eicopentaenoic Acid (C22:05) in Beagle Dogs

Dose (g triglycerides/kg/16hr)	Sex	Day 40 to day 0 C_{max} ratios	Day 40 to day 0 $AUC_{infusion}$ ratios	Day 40 to day 0 $AUC_{0\text{-}24h}$ ratios
2	Male	2.27	3.80	3.62
	Female	2.92	3.27	3.42
4	Male	1.27	1.85	1.87
	Female	2.28	3.43	3.68
6/4 (Omegaven)	Male	0.975	1.22	1.40
	Female	1.63	2.17	2.32
6/4 (Reference item)	Male	NA	NA	NA
	Female	1.02	3.09	2.57

Print date: 18 May 2011

Accumulation Ratios of Docosapentaenoic Acid (C22:05) in Beagle Dogs

Dose (g triglycerides/kg/16hr)	Sex	Day 40 to day 0 C_{max} ratios	Day 40 to day 0 $AUC_{infusion}$ ratios	Day 40 to day 0 AUC_{0-24h} ratios
2	Male	0.692	0.757	0.757
	Female	0.879	0.995	0.976
4	Male	0.719	0.742	0.758
	Female	1.12	1.24	1.30
6/4 (Omegaven)	Male	0.748	0.748	0.736
	Female	1.06	1.05	1.08
6/4 (Reference item)	Male	NA	NA	NA
	Female	NA	NA	NA

Print date: 18 May 2011

Plasma Medium Chain Dicarboxylic acids and Medium Chain 3-Hydroxy Fatty Acids in Dogs Administered Fat Emulsion 73403 Intravenously - Preliminary Study

Methods: Six Beagle dogs (3 males and 3 females) divided into 2 groups were infused over 4 hours at a dose of 4 g triglyceride (TG)/kg with Fat emulsion 73403 (2/sex, group 1) or with Intralipid 20 % (1/sex, group 2) for 13 weeks (Jönsson M. Fat emulsion 73403: 13-week intravenous study in Beagle dogs followed by a four-week recovery period. (b) (4) Document 9520999:1995). Plasma samples obtained from the 6 dogs before and at 2 and/or 4 hours after the start of infusion on day 1 was analyzed for medium chain dicarboxylic acids and medium chain 3-hydroxy fatty acids by high pressure liquid chromatography (HPLC) – mass spectroscopy

Results: The majority of the measured fatty acids have a chain length of 10 carbon atoms. Increases in the concentrations of these fatty acids were observed during the infusion of fat emulsion 73403, but there were no changes in the concentrations of the fatty acids during the infusion of Intralipid 20 %. The changes in the plasma medium chain dicarboxylic acids and medium chain 3-hydroxy fatty acids in Beagle dogs are shown in the Sponsor's table below.

Plasma Medium Chain Dicarboxylic acids and 3-hydroxy fatty acids in Beagle Dogs Infused with Fat Emulsion 73403 or Intralipid 20 %

Fat Emulsion 73403, 4 g TG/kg, 1 g/kg/h					Intralipid 20%, 4 g TG/kg, 1 g/kg/h				
Dog No.	Sex	0h	2h	4h	Dog No.	Sex	0h	2h	4h
0D104	F	1.04	10.93	29.52	0D088	F	0.56	0.29	NQ
0D107	F	0.94	6.8	11.04					
0D106	M	0.76	6.18	28.83	0D115	M	0.99	1.27	1.46
0D110	M	0.68	-	55.18					

Table 1. Plasma medium chain dicarboxylic acid concentrations (µM) in female (F) and male (M) dogs at indicated time points during infusion.
NQ = below detection level.

Plasma Medium Chain Dicarboxylic acids and 3-hydroxy fatty acids in Beagle Dogs Infused with Fat Emulsion 73403 or Intralipid 20 %

Fat Emulsion 73403, 4 g TG/kg, 1 g/kg/h					Intralipid 20%, 4 g TG/kg, 1 g/kg/h				
Dog No.	Sex	0h	2h	4h	Dog No.	Sex	0h	2h	4h
0D104	F	2.22	3.99	5.24	0D088	F	1.08	1.12	NQ
0D107	F	2.18	1.45	1.79					
0D106	M	2.14	1.82	5.75	0D115	M	1.09	1.13	1.23
0D110	M	2.14	-	8.34					

Table 2. Plasma medium chain 3-hydroxy fatty acid concentrations (µM) in female (F) and male (M) dogs at indicated time points during infusion.
NQ = below detection level.

In conclusion, the infusion of male and female Beagle dogs with Fat emulsion 73403 or Intralipid 20 % (emulsion containing medium chain fatty acids) for 13 weeks at doses of 4 g TG/kg/ for 4 hours/day produced a measurable medium chain dicarboxylic acids and medium chain 3-hydroxy fatty acids in the plasma of treated dogs.

Study title: Fat Emulsion 73403: 13-Week Intravenous Toxicity Study in the Beagle Dog Followed by a 4-Week Recovery Period.

Study no.:	90-25
Study report location:	KPO 734, Pages 1 to 235
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09-11-1990
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 73403 30 %, lot # 3560-014; Intralipid 20 %, lot # 54036-51; Ringer-acetate, lot # 90E30J63, 90E08J15

Key Study Findings

Daily IV infusion of 4 g TG/kg/day of Fat emulsion 73403 (consisting of medium chain and long chain triglycerides equimolar (b) (4) %, w/w) or Intralipid 20 % (reference article) for 13 weeks at a rate of 1 g TG/kg/hr was well tolerated in dogs. The infusion resulted in transient increases in plasma beta-hydroxybutyrate, triglycerides, free fatty acids, and a slight reduction in blood glucose. Increased serum lipids were more pronounced in the test article group than in the reference article group, whereas the hematology and clinical chemistry changes were similar in the two treatment groups. In 2 dogs from the test article group, the changes in the liver and spleen were still present at the completion of the 4 week recovery period. Pale liver was noted in the test and reference article groups as the only treatment-related macroscopic changes, which

were however absent in the recovery animals. Microscopic changes of fat pigment deposition and granulomatous reaction were observed in the liver and spleen of treated animals, and were still present in the test article treated groups after the recovery period.

Methods:

Doses:	4 g TG/kg/day Fat emulsion 73403 or Intralipid 20 %
Frequency of dosing:	4 Hours daily for 13 weeks
Route of administration:	Intravenous via catheter in leg peripheral veins.
Dose volume:	20 ml/kg/day
Formulation/Vehicle:	(b) (4) (fat emulsion 73403), (b) (4) intralipid 20 %, 1.004 g/ml ringer-acetate solution/ringer-acetate vehicle control.
Species/Strain:	Beagle Dog
Number/Sex/Group:	3 males/5 females/group (test article), 3/sex/group (Reference article and control), 2 males (untreated control)
Age:	22-27 Weeks old
Weight:	5.7-14.9 kg (males), 6.5-9.5 kg (females)
Satellite groups:	Yes, 2 female dogs from the test group were used for recovery.
Unique study design:	No
Deviation from study protocol:	Deviation reported was from technical problems with infusion pump cassette & dosing and had no effects on the study integrity.

Observations and Results

Mortality

Animals were observed daily for mortality or moribundity
There was no animal mortality during the study.

Clinical Signs

Animals were observed daily pretest, during treatment and recovery periods for clinical signs.

There were clinical signs of vomiting in 2 female dogs receiving fat emulsion 73403 (on study day 6 and 90); one male dog (on study days 17, 22, and 45), 1 female dog (on study day 28) and another female dog (on study days 35 and 64) all receiving Intralipid 20 %. Furthermore, five of the six control dogs vomited one or more times during the infusion period.
Slight swelling of infusion sites for 2 or more days were observed in dogs that were inadvertently injected subcutaneously with small amounts of emulsions or control solution.

Body Weights

Body weights were recorded three times during the pretest period, and once a week during the treatment and recovery periods. The animals were also weighed on days 1, 90, and 92 prior to infusion.

Increased body weight gain was observed in male and female animals in the fat emulsion 73403 group (mean of 38% and 40 %, respectively), and in the intralipid 20 % group (mean of 46% and 35 % respectively), when compared to male and female ringer-acetate control (mean of 29% and 23 % respectively). The weight gains for untreated control males ranged from 32 % - 41 %.

The body weight gain in the fat emulsion 73403 treatment group animals were comparable and higher than that of control animals during the study, whereas the body weights of the two recovery female animals remained constant during the 4 week recovery period.

Feed Consumption

Food consumption was recorded daily 13 days pretest and during the treatment and recovery periods.

Decreased food consumption was observed in male (with a mean of -20 to -50 %) and female (with a mean of -10 to -20 %) dogs in the fat emulsion 73403 group and in 2 male (with a mean of -15 to -20 %) and 2 female (with a mean of -10 to -20 %) dogs in the intralipid 20 % group during weeks 8-13 of the study. However, food consumption in the animals reverted back to pretest levels within the first two weeks of the recovery period.

Ophthalmoscopy

Ophthalmic examinations were performed on 5-7 days pretest, 45 and 85 days of the study period, and at the end of the recovery period.

No adverse effects linked to test or reference article infusion was observed at ophthalmoscopic examination.

ECG

ECG recordings were performed 6-9 days pretest, days 43 and 89 of the study period, and at the end of the recovery period.

There were no abnormal ECG findings on study animals, except for a male control dog with occasional ventricular premature beats in the ECG recordings on study days 42 and 88.

Hematology

Blood sample for hematology was obtained from each animal on day 14-16 pretest, on treatment days 29, 91, and on the 27th day of the recovery period. Blood sample for the determination of white blood cell counts was also obtained from animals (due to viral infection in some animals), on days 4-6 pretest and on dosing days 1, 19, 51, and 79

The observed neutrophilic leukocytosis seen pretest in some dogs from all the study groups (an indication of viral infection) was normalized with treatment by study days 29 and 51.

Slight reduction in red cell values (hemoglobin, PCV and RBC), changes in red cell morphology (anisocytosis, hypochromasia and/or polychromasia, and nucleated erythrocytes) with increased reticulocyte counts were observed in all study group animals on treatment day 29. All values reverted back to normal after the 4 week recovery period.

Moderately reduced lymphocyte counts were observed in dogs treated with fat emulsion 73403 or intralipid 20 %, when compared to pretest values, and in control animals. The reduction was similar in both treatment groups, remained unchanged through the treatment period, and tended towards normalization during the recovery period.

Clinical Chemistry

Blood samples for clinical chemistry were obtained from each animal on day 14-16 pretest, on treatment days 29, 91, and on the 27th day of the recovery period.
Blood samples for plasma triglycerides, free fatty acids (FFA), beta-hydroxybutyrate, lactate, blood gases, and glucose were collected on treatment days 1, 19, 51, and 79

Slight, but similar decrease in total protein values observed in most male and female animals from the test and reference groups on treatment days 29 and 91, rebounded back to normal range at the end of the recovery period.

Increased serum cholesterol and phospholipids observed in fat emulsion 73403 or Intralipid 20 % groups, in comparison to pretest levels and control animal values during the treatment period, were completely reversed after a 4 week recovery period. There were however, no significant changes in serum triglyceride levels in both treatment groups.
The increases seen in plasma triglycerides and free fatty acids in fat emulsion 73403 or intralipid 20 % treated animal reverted back to normal within 20 hrs after the end of infusion.

Infusion of fat emulsion 73403, but not Intralipid 20% resulted in increased beta-hydroxybutyrate level (to a mean of 1.04 mmol/l), and slightly decreased blood pH, bicarbonate, and base excess by study day 79 in male and female animals.

Urinalysis

Urine analysis was performed on each animal, from urine samples collected on day 11-15 pretest, and on treatment days 30-31, 86-87, and on recovery day 23-24.

No treatment related changes of fat emulsion 73403 or intralipid 20 % infusion were observed in urine analysis of study animals.

Gross Pathology

Following a 13-week treatment period (day 92) and a 4-week recovery period (day 120), a complete necropsy was performed on each animal.

Slightly or moderately pale livers were seen in terminal animals treated with fat emulsion 73403 or intralipid 20 %. Perivascular hemorrhages were also seen at injection sites to a similar degree in all study animals. No treatment related macroscopic observations were observed in recovery animals.

Organ Weights

The following organs were weighed:

brain	kidneys	lungs
heart	liver	spleen

There were no treatment related changes in absolute or relative organ weights observed in study animals.

Histopathology

Samples of the organs and tissues listed below were fixed in neutral buffered 10 % formalin for histopathological examinations.

adrenals	kidneys	skin
aorta (thoracic, abdominal)	liver	spinal cord (lumbar)
bone marrow from sternum	lungs	spleen
brain (cerebrum, pons, cerebellum, medulla oblongata)	lymph nodes (mesenteric, cervical, popliteal, axillary)	stomach (fundus, pylorus)
caecum	mammary gland	testes
colon	ovaries	thymus
duodenum	pancreas	thyroid
epididymides	parathyroids	tongue
esophagus	pituitary	tonsil
eye (left) with optic nerve	prostate	trachea
gall bladder	salivary gland	urinary bladder
heart*	sciatic nerve	uterus
ileum	sites of i.v. infusions	other altered tissues
jejunum	skeletal muscle (thigh)	

*Papillary muscle of left ventricle, upper and lower region of right ventricle and ventricular septum, and left and right atrium

Adequate Battery
Yes
Peer Review
Yes

Histological Findings
Histopathological findings include fine droplet fatty changes of centrilobular distribution in the liver of fat emulsion 73403 and Intralipid 20 % treated animals. Fatty change in the Kupffer cells was more evident in dogs infused with Fat emulsion 73403, while the fatty change in the hepatocytes was slightly more pronounced in the Intralipid 20 % treated animals. Vacuolated cells containing lipid or brownish, ion-negative pigment, localized periportally, or occasional necrotic cells associated with inflammatory reactions were observed in the test and reference animal dose groups.
Fatty changes in the spleen including the presence of IV fat pigments in the macrophages of the red pulp were observed in all dogs from the test and reference dose groups. In 2 dogs from the test article group, the changes in the liver and spleen were still present at the completion of the 4 week recovery period.
Fatty change of a similar degree was observed in macrophages in the lung, thymus, and mesenteric lymph nodes in dogs from all study animals. Deposition of glycogen with similar distribution was seen in all study animals.

Deposition of fat content in the liver was slightly higher in the test article group than in the control group, but lower than in the reference article group. However, the total liver fat content in the recovery dogs reverted back to normal levels after the 4 week recovery period.

In conclusion, daily IV infusion of 4 g TG/kg/day of Fat emulsion 73403 for 13 weeks at a rate of 1 g TG/kg/hr was tolerated in dogs. The infusion resulted in transient increases in plasma beta-hydroxybutyrate, triglycerides, free fatty acids, and a slight reduction in blood glucose. The observed increases in serum lipids in animals were more pronounced in the test article group than in the reference article group, but still slightly higher than that of control animals; whereas the hematology and clinical chemistry changes were similar in the two treatment groups.
In 2 dogs from the test article group, the changes in the liver and spleen were still present at the completion of the 4 week recovery period.

Study title: A Three Month Intravenous Tolerance Study in Dogs of Fat Emulsion 4501, Containing a (b) (4) Mixture of Soybean and (b) (4) Oils.

Study no.:	87-2
Study report location:	Project No. 715, Pages 1 to 190
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 2, 1987
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 4501, lot # 3576-012; Intralipid 20 %, lot # 62131; Ringer acetate, lot # LL 47177, MH 47304

Key Study Findings

IV administration of 4 g TG/kg/day of fat emulsion 4501 or Intralipid 20 % for 3 months to Beagle dogs, produced no adverse clinical signs except convulsions in a male dog from the test article group.

There were no significant differences observed in plasma or liver total fatty acids between the treatment groups and control group, except for a higher liver fat content in intralipid 20 % treated male dogs, a consequence of the differences in the lipid composition between the 2 fat emulsions.

Methods

Doses:	4 g TG/kg/day and 8 mg L-carnitine/kg/day (Fat emulsion 4501); 4 g TG/kg/day Intralipid 20 %
Frequency of dosing:	4 hrs infusion, daily for 3 months
Route of administration:	IV infusion via catheter in peripheral leg veins
Dose volume:	20 ml/kg/day
Formulation/Vehicle:	Ringer-Acetate infusion solution (vehicle/control)
Species/Strain:	Beagle Dog
Number/Sex/Group:	4/sex/group (Fat emulsion 4501, Intralipid 20 %); 2/sex/group (control)
Age:	29-32 weeks old
Weight:	7.5-10.2 kg (males), 7.1-9.7 kg (females)
Satellite groups:	Yes, 1animal/sex/group (from test and reference article dose groups) as recovery animals.
Unique study design:	None
Deviation from study protocol:	Deviation reported as a 45 min infusion interruption in a test article group animal.

Observations and Results

Mortality

The animals were observed daily for mortality or moribundity.
There were no animal deaths during the study or recovery periods.

Clinical Signs

Study animals were observed daily for clinical signs pretest, test, and recovery periods. Clinical observations consisted of isolated vomiting incidents (some animals from all dose groups), red skin eruptions on the chest/flanks (from 2 females each in the test/reference groups and 1 test group male; result of duration of animals on Pavlov sling).

A male dog in the Fat emulsion 4501 group had episodic incidents of clonic convulsions, salivation, urination, and/or defecation on treatment days 51, 67, 75, 80, 82, and 89. Incidental findings of cherry eyes were observed in 2 dogs from the test group.

Body Weights

Body weights were measured once weekly during the pretest and through the study duration.

Increased body weight gain was observed in fat emulsion 4501 animal group during treatment week 2-3, when compared to controls. All 3 study group animals did not show any significant difference in body weight gain through the rest of the study period.

Feed Consumption

Food consumption was recorded daily.

From study week 4 to the end of the study period, fat emulsion 4501 treated animals had reduced food consumption by 25 % as compared to control animals. However, after the first week of the study period, the test article treated animals had increased food consumption to the pretest levels. Control animals consumed all available food throughout the study.

Ophthalmoscopy

Ophthalmoscopy was performed on each animal once pretest, and at the end of the test period on day 87/88.
No adverse effects of ophthalmoscopic observation could be attributed to test article infusion.

ECG

ECG recordings were done on pretest day 3-4, at the end of the test period (day 84-88), and day 22/23 of the recovery period.

There were no observed effects on ECG that could be attributed to the infusion of Fat emulsion 4501 or Intralipid 20 %.

Hematology

Hematology was performed on all animals once pretest (day 7), and on dosing days 28 and 91, and on recovery day 28/29. Blood samples were obtained from the vena cephalica of the animals, in the morning after a 16 hr. fasting period.

The hemoglobin concentrations, packed cell volumes, and red blood cell counts were transiently decreased predominantly in male dogs after 4 week treatment with fat emulsion 4501, in comparison to the reference article group animals and the respective pretest values. After 13-week treatment period, the changes persisted only in one animal, but normalized after the 4 week recovery period. The animal (and 3 intralipid 20 % infused dogs) also had slight hypochromasia and nucleated erythrocytes in blood smears at 4 and 13 weeks of infusion, and also at recovery.

Decreased platelet and lymphocyte counts were observed in male dogs from the test and reference article groups after 4 weeks of infusion, but tended to normal values as dosing progressed. Changes were not different between the two treatment groups.
Increased white blood cell count (seen in a male dog in the test article group) and increased eosinophil (seen in a female dog from control group) persisted till the end of study.

Clinical Chemistry

Blood samples for clinical chemistry, fatty acids and L-carnitine determination were obtained from all animals once pretest (day 7), and on dosing days 28 and 91, and on recovery day 28/29. Blood samples were obtained from the vena cephalica of the animals, in the morning after a 16 hr. fasting period.

Observed changes in clinical chemistry parameters include increased pretest triglyceride levels in a male and female dog from the test solution group, and decreased albumin levels in a male dog from the test solution group.

Increased serum cholesterol (15-25 % at week 4, and 30-35 % at week 13) and phospholipids (15-25 % at week 4, and 40 % at week 13 in female dogs) were observed in both fat emulsion 4501 and intralipid 20 % treated animals. The serum lipid changes were similar in both treatment groups, and returned to normal after the 4 weeks recovery period. There were no changes in the serum triglyceride levels.

Plasma L carnitine levels were increased in the test animals throughout the 13 week treatment period, but remained elevated only in the male dogs at the end of the recovery period. In contrast, plasma L-carnitine levels in the reference and control group animals had decreased to 50-82 % of pretest values through 13 weeks treatment and 4 weeks of recovery periods.

Slightly elevated total plasma fatty acids at 4 and 13 weeks were observed in animals treated with fat emulsion 4501 and intralipid 20 %, when compared to pretest levels and levels found in control dogs.

Increased total liver fat content (30 % higher than in test or control animals) was observed in the male dogs from intralipid 20 % dose group.

Urinalysis

Urine analysis was performed on samples collected on pretest day 12, and also on treatment day 85/86, and on recovery days 21-23.

Slightly increased urine volume and slight to moderately decreased urinary density and osmolality were observed in animals from all the study groups. Increased urine protein was also observed in all the test group males and in 3 of the 4 male dogs from the reference article group. This change was not considered abnormal because the values did not exceed normal range, and urinary protein was not seen in female dogs.

Gross Pathology

On the last infusion day (Day 92), 3 males and 3 females from the test (Fat emulsion 4501) and reference (Intralipid 20 %) groups, and all the control animals were sacrificed for macroscopic examination. The 4 (2 males/2 females) recovery animals were also sacrificed on recovery day 28/29 for macroscopic examination.
Macroscopic observations include grayish foci and reddish discolorations of the kidneys and ileo-cecal valve in the test and reference article animals (during the study and recovery periods), and in the lungs of all study animals, including control animals. A small number of hemorrhages were observed in the mucosa of female animals from the intralipid dose group. Perivascular reddish discolorations were observed in the limbs of all study animals as a result of infusion catheter placements.

Organ Weights

The weights of the following organs were recorded after autopsy:
Liver
Spleen
Kidneys
Lungs
Brain

There were no differences in organ weights between the 3 dose groups.

Histopathology

Adequate Battery
Yes
Peer Review
Yes

Histological Findings

Histopathological findings include fine droplet fatty changes of centrilobular distribution in the liver, fine droplets fatty change mainly in the macrophages of the red pulp, and in the spleen of test and reference article dose groups. However, fine droplets fatty changes of a similar degree were observed in pulmonary macrophages and in sinusoidal macrophages in the mesenteric lymph node of all the study animals. Deposition of fat droplets observed in the kidneys of all study animals were within the normal range, and the deposition of glycogen and hemosiderin (iron containing pigment) observed in all the study animals was of a comparable degree.
Observations of hemorrhage and inflammatory changes were present at the site of IV infusion, and in the urinary bladder of all study animals due to catheterizations for injections and urine sampling.

In conclusion, the IV administration of 4 g TG/kg/day of fat emulsion 4501 or Intralipid 20 % for 3 months in Beagle dogs did not show any adverse clinical signs except for convulsions in a male dog from the test article group. All the clinical chemistry changes were slight, and similar between test and reference article animals when compared with control animals, and reversible when treatment was discontinued.

There were no significant differences observed in plasma or liver total fatty acids between the treatment and control groups, except for a higher liver fat content in intralipid 20 % treated male dogs, a consequence of the differences in the lipid composition between the 2 fat emulsions.

Study title: Omegaven – 13-Week Intravenous Infusion (16 hours per day) Toxicity Study in the Beagle Dog Followed by a 4-Week Treatment-Free Period.

Study no.:	10825/97
Study report location:	Document No. AA76192, Page 1 to 3,607
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 25, 2011
GLP compliance:	Compliance with the OECD Principles of Good Laboratory Practice.
QA statement:	Yes
Drug, lot #, and % purity:	Omegaven, Batch # 16DL0184, 10 %; Intralipid, Batch # 10DL7757, 10 %

Key Study Findings

The administration of Omegaven (1, 2 or 3 g/kg/16-h) or Intralipid (3 g/kg/16-h) by intravenous infusion to male and female dogs for 13 weeks induced acute and chronic inflammatory changes in multiple organs which were partially reversed after a 4-week recovery period. Major treatment-related changes were observed in the liver, kidneys,

heart, lungs, lymph nodes, thymus and spleen. Treatment-related changes of low severity were seen in the jugular vein, duodenum, adrenal glands, femoro-tibial joint and bone marrow. Overall, there were no clear dose-response effects in Omegaven-treated animals, because the changes observed in the animals dosed with 1 or 2 g/kg were generally of higher severity than in animals dosed with the 3 g/kg dose. The changes observed in animals dosed with 3 g/kg Omegaven were generally lower than in animals dosed with 3 g/kg Intralipid.

Methods

Doses:	0, control (0.9 % saline); Omegaven 1 g Triglyceride (TG)/kg/16 h (low dose), 2 g TG/kg/16 h Intermediate dose), 3 g TG/kg/16 h (high dose), Intralipid 10 % (reference article) 3 g TG/kg/16 h
Frequency of dosing:	16 hours daily for 13 weeks
Route of administration:	Intravenous infusion via the jugular vein or femoral vein into the anterior vena cava.
Dose volume:	0.625 to 1.875 ml/kg/h
Formulation/Vehicle:	(b) (4) Omegaven 10 %, (b) (4) Intralipid 10 %/0.9 % Saline
Species/Strain:	Beagle Dogs
Number/Sex/Group:	4/sex/group
Age:	6 – 7 Months
Weight:	8.6 to 10.4 kg (males), 7 to 9.8 kg (females)
Satellite groups:	2/sex/group was designated as recovery animals.
Unique study design:	None
Deviation from study protocol:	On several occasions, the daily infusion was less than 16 hours in some animals due to infusion incidents or for catheter re-implantation; The relative humidity and the temperature were occasionally below the indicated values (minimum of 17 % and 15.1 ^{o}C, respectively); The normal dark cycle was interrupted once or twice in a cycle for up to 7 hours 10 minutes during blood sampling for TK or to allow for the completion of scheduled procedures. These deviations were not considered to have affected the outcome of the study.

Observations and Results

Mortality

All animals were observed at least twice daily for morbidity of mortality.

Two animals (1 group 2 male No.517 treated with Omegaven at 1 g Tg/kg/16-hr; 1 group 3 male No.529 treated with Omegaven at 2 g TG/kg/16-hr) were sacrificed prematurely and necropsied due to their moribund appearance, believed to be due to technical problems with catheter re-implantation. After histological examination, the poor health status of the group 2 male animal was considered to be related to the prominent cutaneous and pulmonary inflammatory changes which were secondary to the accumulation of material consistent with the Omegaven solution. The animal has been re-implanted twice with the catether. After histological examination, the poor health status of the group 3 male animals was considered to be related to the prominent inflammatory changes in the skin (cervical and inguinal region) and the thrombotic and inflammatory changes in the vena cava.

Clinical Signs
All animals were observed daily. A physical examination was performed for the animals pretest, weekly for the first 4 weeks of treatment, then every 2 weeks for the remainder of the treatment period and the treatment-free period.

Limping (forelimb), sore, swelling and/or induration at the implantation site/ventral neck region were observed in animal groups treated with Omegaven or Intralipid. Some of these signs were also noted sporadically in the control group. The clinical signs were therefore considered to be related to the treatment procedure, but also probably exacerbated by the nature of the test and reference articles (lipids) as shown by the higher incidence/frequency in the treated groups.

Individual animals from treated groups also showed marked to severe clinical signs during the treatment period.

Male nos. 514 and 516 in the low dose group showed subdued behavior, decreased activity, lying down, slow movements, and limping, partly closed eyes, intermittent tremors from days 80and 63, respectively. Female no. 522 in the low dose Omegaven group had decreased activity, subdued behavior, slow movements and/or hyperthermia from day 35 to day 83.

In the intermediate dose group (Omegaven at 2 g TG/kg/16-hr), male nos. 525 and 526 showed decreased activity, subdued behavior, slow movements, unsteady gait, and inability to stand from day 63 to 86. In addition, the body of the male no. 525 was pale and anemic. Female no. 536 had decreased activity, subdued behavior, slow movements and/or partly closed eyes from day 65 to 91, and was sacrificed at the end of the treatment period and not allowed to be a recovery animal as previously designated.

In the high dose group (Omegaven 3 g TG/kg/16-hr), a female dog (no.547) showed decreased activity on day 72.

In the group treated with Intralipid at 3 g TG/kg/16-hr, 2 males (nos. 551, 554) and 4 females (nos. 555, 556, 557, 560) all had subdued behavior, decreased activity, slow

movement and/or rapid breathing, while one of the female also appeared thin, and another female had hyperthermia from dosing day 33 to 91.

During the treatment period, most of the treated dogs exhibited unsatisfactory clinical status and also marked reduction in food intake and/or body weight loss. These animals were therefore given hypertonic wet food in addition to their daily diet for several days.

Pale mucous membranes were observed occasionally in some animals treated with Omegaven or Intralipid, and this treatment-related observation was believed to be consistent with the hematological changes seen in these animals during the study.

The clinical signs were not observed in the animals during the recovery period, either immediately or few days after the end of the treatment period, indicating a rapid return to normal clinical condition.

Body Weights

Individual animal body weights were recorded twice weekly, starting from seven days prior to dosing initiation.

The mean body weight gain was observed to be higher in all treated male dogs than the control group during the first month of the treatment period. The increase was also observed in female dogs treated with Omegaven at 2 and 3 g/kg/day. The body weight gain was not dose-responsive.
During the second month of treatment, the mean body weight gain in the treated animal was either similar or lower than that of the control animals.
During the last month of the treatment period, the mean body weight gain was lower than the control group for all the treated groups except for the high dose Omegaven female and the Intralipid male group.

Overall, during the entire treatment duration, the reduction in body weight gain in treated dogs was considered to be toxicologically relevant in dogs treated with Omegaven at 1 or 2 g/kg/16-hr and in female dogs treated with Omegaven and Intralipid at 3 g/kg/16-h. In contrast, a higher body weight gain was observed in male animals treated with Omegaven or intralipd at 3 g/kg/16-h.
In one or two weeks after the treatment period, body weight gain in the recovery animals was comparable or higher than that of the control animals. The mean body weight gain for different groups is summarized in the Sponsor's table below.

Mean Body Weight Gain (in kg) in Dogs over selected Intervals

Group	Group 1 (0 g/kg/16 hr)		Group 2 (1 g/kg/16 hr)		Group 3 (2 g/kg/16 hr)		Group 4 (3 g/kg/16 hr)		Group 5 (3 g/kg/16 hr)	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Days 0 to 28	0.36	0.42	0.82	0.35	0.53	0.65	1.1	0.77	1.04	0.41
Days 28 to 56	0.45	0.25	0.26	0.22	0.14	0.02	0.58	-0.37	0.35	0.02
Days 56 to 91	0.2	0.43	-0.36	-0.13	-0.68	-0.44	-0.31	0.34	0.48	-0.05
Days 0 to 91	1.02	1.10	0.70	0.43	-0.04	0.23	1.37	0.73	1.87	0.38

M: male; F: female.

Feed Consumption

Individual food consumption by the animals was measured daily and reported as a weekly mean in g/animal/day.

Decreased food consumption was observed in all the animals treated with Omegaven or Intralipid during the treatment period, when compared to control animals. This decrease was noted in females from week 4, and was dose-dependent, whereas the decreased body weight noted from week 5 in males was not dose related.
In one or two weeks after the treatment period, food consumption was comparable or higher in the treated groups designated as recovery animals as compared to the control animals.

Ophthalmoscopy

All animals were examined once pretest, and during weeks 13/14 (females/males) and 17

All ophthalmological findings such as lenticular opacity, change in coloration/pigmentation and presence of spots were incidental since they were observed pretest and present in control animals.

ECG

Electrocardiographic recordings were performed on all animals once pretest (after implantation), on day 2, during week 13 and during week 17 at the end of the recovery period.

There was no evidence of any treatment related effect on blood pressure in treated dogs, when compared to control animals. The occasional differences seen in mean values between the control and treatment groups were very small in magnitude, were not dose-related and not considered to be toxicologically relevant.
There were no treatment-related effects at any dose level of Omegaven or Intralipid on heart rate, QT interval or QRS complex duration in treated animals, when compared to values in control animals.

The increase in mean QRS complex duration observed in male dogs were consistently higher in the Omegaven 2 g/kg/16-h dose group during the dosing period, when compared to the control and other treated groups. However, this difference was due to a male dog (no.528) which demonstrated high QRS complex duration values that have been observed pre-dose.

There was no treatment related observations in QTc interval duration values in any treated animals, at each scheduled time-point.

Hematology

Blood samples for hematology were collected from each animal 7 days pretest and During weeks 4 and 13, and at the end of the 16-hr infusion period. Blood sampling was also performed during week 17 (recovery period) for male and female animals, once in the morning.

Serum lipaemic appearance was observed in male and female animals treated with Omegaven or intralipid (3 g/kg/16h) during treatment week 4, and in 5 out of 6 control female and 1 control male animal in week 13.

During week 4 and 13, a significant decrease was observed in red blood cell count, hemoglobin level and packed cell volume in all treated groups versus control. The decrease was not dose related, was not accompanied by significant decrease in reticulocyte count at week 4, but was accompanied by significant decrease in reticulocyte count during week 13. However, at the end of the 4-week treatment free period, hematological parameters in the treated groups had returned to normal, when compared to historical values or the values in control animals.

During treatment week 13, reduction in platelet count, prolongation in APTT, and elevation in fibrinogen level were observed in most of the treated groups in comparison to the control group. These coagulation parameters were not dose related, and had returned to normal at the end of the treatment free period in the recovery animals, with the exception of the fibrinogen level, which was still slightly elevated in female groups. The fibrinogen level however, was comparable to the historical data, and indicative of a complete or partial recovery.

At the end of the treatment period on week 13, elevated white blood cell count related to higher relative and absolute neutrophil counts, associated with lower lymphocyte, eosinophil and basophil counts (absolute and relative values) were observed in all the treated animal groups, when compared with pretest data and/or control group. These findings are indicative of inflammatory changes partly related to technical and implantation procedures.

At the end of the treatment-free recovery period, white blood cell parameters in the treated animals groups returned to normal values and were comparable to the historical control values.

Clinical Chemistry

Blood samples for clinical chemistry were collected from each animal 7 days pretest; and once during week 2 and week 8 for male and female animals, respectively, for alkaline phosphatase (ALP) only. Blood sampling was performed 8 hours after the end of the 16-hr infusion period. During weeks 4 and 13, blood sampling was performed twice a day: at the end of the 16-hr infusion period, and a second time, 8 hours after the end of the 16-hr infusion period. Blood sampling was also performed during week 17 (recovery period) for male and female animals.

At the end of treatment week 4, the major changes observed in clinical chemistry parameters in all the treatment groups versus control group consist of:

Dose-responsive increases in total, free and LDL cholesterol levels, and also in the HDL cholesterol level for the Intralipid treated group.

Dose-responsive increase in triglyceride levels in the 2 and 3 g/kg Omegaven treated groups or the Intralipid treated group.

Decrease in lipase activity in all the treatment groups at the end of the 16 hour infusion, and before the start of the next infusion.

An elevated (non-dose-related) C-reactive protein level was observed in all the treatment groups.

At the of treatment week 13, the clinical chemistry changes were still present with a greater magnitude than was observed in week 4. However, at the end of the 4 week treatment free recovery period, the serum clinical chemistry parameters were comparable to that of the control animals and/or pretest values.

Increased Alkaline phosphatase (ALP) activity was observed in Omegaven (2 and 3 g/kg/16-h) treated groups, when compared with the Intralipid treated group, from treatment week 2 to the end of the treatment period. The increase was dose-dependent and significant, when compared to the control animal group. However, at the end of the 4-week recovery period, the ALP activity values from Omegaven treated animals were comparable to pretest values and control animal values. There were no treatment-related changes in bilirubin levels or other liver enzyme activity during the treatment period. A summary of findings for alkaline phosphatase activity in study animals is shown in the Sponsor’s table below.

Table 22 Alkaline Phosphatases

	Group 1 (0 g/kg/16 hr)	Group 2 (1 g/kg/16 hr)	Group 3 (2 g/kg/16 hr)	Group 4 (3 g/kg/16 hr)	Group 5 (3 g/kg/16 hr)
	Saline	Omegaven			Intralipid
	Males				
Pretest	333.7	360.8	294.8	296.5	318.5
D11	301.3 (-10 %)	377.3 (+5%)	373.3 (+27 %)	879.7** (x 3)	233.8 (-27 %)
D21 +16hr	331.3 (-1 %)	435 (+21%)	617.3 (x 2.1)	1204.2** (x 4)	259.5 (-19 %)
D21 +24hr	330.7 (-1 %)	445.3 (+23%)	615 (x 2.1)	1180.2** (x 4)	267.3 (-16 %)
D53	332.8	378.8 (+5%)	498* (+69 %)	1838.5** (x 6.2)	333.5 (+5 %)
D85 +16hr	302.8 (- 9%)	339.4 (-6%)	463 (+57 %)	1543.3** (x 5.2)	257 (-19 %)
D85 +24hr	292 (-13 %)	334.6 (-7%)	458.8 (+56 %)	1469** (x 5)	261.3 (-18 %)
D113	266.5	261.5	245.5	366.5	201.5
	Females				
Pretest	307.2	378.2	393	355.8	372.2
D12	280 (-9 %)	349.8 (-8 %)	642.7** (+64 %)	834.7** (+135 %)	288.5 (-23 %)
D21 +16hr	300.7 (-2 %)	438.8 (+16 %)	725.7** (+85 %)	1120.3** (x 3.1)	303.2 (-19 %)
D21 +24hr	303 (-1 %)	448.3* (+19 %)	694.2** (+77 %)	1044.7** (+194 %)	296.8 (-20 %)
D54	291.7 (-5 %)	387.7 (+3 %)	560.8* (+43 %)	867.8** (+144 %)	320 (-14 %)
D85 +16hr	291.8 (-5 %)	387.7 (+3 %)	638.7* (+63 %)	1791** (x 5)	332.7 (-11 %)
D85 +24hr	298.7 (-3 %)	387.7 (+3 %)	627.5* (+60 %)	1729.8** (x 4.9)	326.8 (-12 %)
D112	269.5	256.5	334.5	358.5	229

* or **: $p \leq 0.05$ or 0.01 versus group 1.
hr: hours.

Urinalysis

Urine samples were collected from animals once 7 days pretest, during weeks 13 and 14 and during week 17 from recovery animals.

Decreased urinary pH (below 7) was observed in animal groups treated with Omegaven (2 and 3 g/kg/16-h) and in the low dose Omegaven, and Intralipid-treated female groups, when compared to pretest values or with control group values ($p \leq 0.01$), at the end of the 13-week treatment period. However, at the end of the 4-week recovery period the acid urinary pH had normalized to pretest and control values.

Gross Pathology

At the end of treatment week 13 and after the week 17 recovery period, all surviving animals were randomly necropsied after fasting. All the animals sacrificed for ethical reasons were also necropsied. The following tissues were examined:
External surface
All orifices
Cranial cavity
External surface of the brain and spinal cord
Thoracic and abdominal cavities and organs
Cervical tissues and organs
The carcass
The injection site(s)

The two moribund male dogs dosed with Omegaven (1 and 2 g/kg/16-h) that were sacrificed on day 29 and day 56 due to poor health from repeated cannula re-implantation showed swelling and edematous appearance of skin in the ventral neck and inguinal region, with cutaneous/subcutaneous and pulmonary inflammatory changes.

Liver
At terminal sacrifice, the liver showed mottled appearance or multiple pale foci correlated histopathologically with congestion and/or sinusoidal leukocytosis in 3 female dogs (555, 556, and 557) treated with 3 g/kg Intralipid. The left median lobe and the right lobes of the liver appeared thickened in a male dog (525) given 2 g/kg Omegaven and correlated with moderate leukocytosis. The right medium liver lobe had pale foci in a male dog dosed with Intralipid, and had no histopathological correlate. After the recovery period, none of the treated animals had any macroscopic liver changes.

Lungs
Macroscopic changes of dark foci was observed in lungs of all the treated animals and also seen at a lower incidence in the control animals. The dark foci correlated histopathologically with hemorrhages, perivascular inflammation and alveolar accumulation of eosinophilic material with mixed cell infiltration.

Lymph nodes
Dark appearance which is generally associated with the enlargement of lymph nodes was seen in control and Omegaven or Intralipd treated animals, with treated animals affected more than controls. The dark foci are correlated histopathologically with congestion and/or hemorrhages and/or erythrophagocytosis in the mesenteric and mandibular and other lymph nodes. After the recovery period, dark appearance of the lymph nodes occurred in a few of the Omegaven treated females (but not in males) and one Intralipid treated female.

Implantation sites (injection site, catheterized and non-catheterized vein)

After the treatment free recovery period, firm appearance of the catheterized vein was seen in the treated animals and a few control animals. There were no necropsy changes seen at the injection site or in the non-catheterized vein. However, histopathological examination showed exacerbation of inflammatory changes in the implanted vein in treated animals in comparison to control animals.

The prostate gland was small in a male (no.513) dosed with Omegaven at 1 g/kg and 2 males (nos. 525 and 526) given Omegaven at 2 g/kg. The small prostate size correlated histopathologically with immature appearance.

Organ Weights

Paired organs were weighed together and organ weights were expressed as absolute or relative values in g per 100 g body weight.

Liver
Weight differences were observed in the liver, spleen, kidneys and thymus between Omegaven/Intralipid treated and control animals that were considered to be treatment related. Male animals given Omegaven at 2 g/kg had mean absolute and relative liver weights 50 % and 80 % higher than control, respectively, in males and approximately 60 % and 70 % in females. In Intralipid 3 g/kg dosed animals, the mean absolute and relative liver weights were higher than in controls and the differences were greater than in animals dosed with 3 g/kg Omegaven. The higher liver weights were considered related to sinusoidal congestion from sinusoidal leukocytosis. After the treatment free period, the absolute and relative liver weights in all the treatment groups were still higher than the concurrent controls, without any dose relationship and no histopathological correlates.

Spleen
The mean absolute and relative spleen weights in Omegaven (1 or 2 g/kg) treated males and Omegaven (2 or 3 g/kg) treated females were higher than that of control animals. The differences were dose-related but not significant in male animals whereas, the differences were not dose-related but significant ($p \leq 0.05$ or $p \leq 0.01$) in female animals. In Intralipid treated animals, the spleen weights were lower than in control animals. The higher spleen weights were considered to be secondary to the treatment-related increased cellularity of the red pulp and increased development of the white pulp. After the treatment free recovery period, the mean absolute and relative spleen weights in the treated animals were lower than in control animals, and there was no histopathological correlate for the differences.

Kidneys
The mean absolute and relative kidney weights in Omegaven or Intralipid treated male and female animals were higher than in control animals, with the highest weights seen in animals treated with 2 g/kg Omegaven. The weight differences were considered secondary to treatment-related inflammatory renal changes. After the treatment free recovery period, the absolute and relative kidney weights were still higher than controls in all the treated females, whereas, there were no relevant differences between treated males and controls. The kidney weight differences seen in the female animals were considered to correlate with inflammatory changes seen histopathologically.

Thymus
The mean absolute and relative thymus weights were slightly lower than controls in all Omegaven treated males, females treated with 1 or 2 g/kg Omegaven, and all Intralipid treated animals. After the 4-week recovery period, the mean absolute and relative thymus weights were higher than controls in all Omegaven treated groups, and slightly higher than controls in the Intralipid treated group. There was no histopathological correlate seen for the higher thymus weights.

Adrenal glands
The mean absolute and relative adrenal gland weights in Omegaven or Intralipid treated male and female animals were higher than in control animals. The differences from

controls were significantly greater in males given Omegaven at 2 or 3 g/kg ($p \leq 0.01$). A relationship between the increased weight and increased vacuolation seen histopathologically in the zona fasciculate and glomerulosa in treated animals was considered unlikely because of its low severity and sporadic occurrence. After the 4-week recovery period, the absolute and relative weight of adrenal glands in Omegaven or Intralipid treated animals was still higher than in controls. Although these differences were not dose-related, increased vacuolation was seen histopathologically in the zona fasciculate and glomerulosa in a few of the Omegaven treated animals.

Histopathology

Samples of the organs or tissues listed below were fixed and preserved in 10 % neutral formalin with the exception of the testes, epididymis, eyes and optic nerve which were fixed in modified Davidson's fluid:

Organs	Organ weight	Preservation	Tissue preparation	Microscopic examination
Animal identification		X		
Macroscopic lesions		X	X	X
Adrenal glands	X	X	X	X
Aorta		X	X	X
Bone (femur) and articulation		X	X	X
Bone (sternum) with bone marrow		X	X	X
Bone marrow smears		X		
Brain	X	X	X	X
Bronchus		X		
Caecum		X	X	X
Colon		X	X	X
Duodenum		X	X	X
Epididymides	X	X	X	X
Eyes		X	X	X
Gall bladder		X	X	X
Heart	X	X	X	X
Ileum		X	X	X
Injection site[1]		X	X	X
Jejunum		X	X	X
Kidneys	X	X	X	X
Liver	X	X	X	X
Lungs		X	X	X
Lymph node (mandibular)		X	X (left only)	X (left only)
Lymph node (mesenteric)		X	X	X
Mammary gland		X	X (females)	X (females)
Oesophagus		X	X	X
Optic nerves		X	X	X
Ovaries	X	X	X	X
Oviducts		X		
Pancreas		X	X	X
Parathyroid glands		X	X	X
Pituitary gland	X	X	X	X
Prostate		X	X	X
Rectum		X		
Salivary glands (mandibular, parotid, sublingual)		X	X (left only)	X (left only)
Sciatic nerve (left)		X	X	X
Skeletal muscle		X	X	X
Skin		X	X	X
Spinal cord (cervical, thoracic, lumbar)		X	X	X
Spleen	X	X	X	X

[1]. A sample was taken from the catheterised vein (corresponding to the end of the catheter) and another sample was taken 1 cm beyond the catheter tip. One section was cut and examined from each block.

Organs	Organ weight	Preservation	Tissue preparation	Microscopic examination
Stomach		X	X	X
Testes	X	X	X	X
Thymus	X	X	X	X
Thyroid glands	X	X	X	X
Tongue		X		
Trachea		X	X	X
Urinary bladder		X	X	X
Uterus (horns + cervix)	X	X	X	X
Vagina		X	X	X

Histopathological examinations were performed for all the tissues/organs listed after sections were stained with hematoxylin and eosin (except bone marrow smears, which were stained using the May Grünwald Giemsa method). Additional staining such as Perl's stain for hemosiderin, Schmorl's stain for lipofuscin, Fouchet's stain for bile pigment, Oil red O's stain for lipids were performed for selected organs (liver, kidney, lung, spleen, mandibular lymph node and subcutis).
Adequate Battery
Yes
Peer Review
Yes

Histological Findings

Treatment related histopathological changes were seen in the liver, kidneys, heart, lungs, lymph nodes, thymus, duodenum, spleen, femoro-tibial joint, bone-marrow, injection site and non-catheterized vein. There was no relationship between the treatment-related changes and the dose level in Omegaven-treated animals.

Liver
Treatment-related changes observed consist of diffuse sinusoidal accumulation of neutrophils, sinusoidal congestion, perivascular accumulation of lymphocytes, plasma cells and hematopoietic precursors and formation of macrophage aggregates. The changes were severe in Intralipid treated animals than in Omegaven treated animals. The histopathological changes observed in the liver of animals are shown in the Sponsor's table below.

Microscopic Changes in Liver of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	4	4	3	4	3	4	4	4	4	4
Inflammatory cells (mainly neutrophils) in sinusoids										
- Minimal	-	-	-	2	-	-	1	4	2	-
- Slight	-	-	2	1	-	1	3	-	1	1
- Moderate	-	-	1	1	3	2	-	-	1	2
- Marked	-	-	-	-	-	1	-	-	-	1
Lympho-plasma cells and/or hematopoiesis (perivascular)										
- Minimal	-	-	-	2	1	2	4	4	4	3
- Slight	-	-	3	1	2	2	-	-	-	1
Diffuse sinusoidal congestion										
- Minimal	-	1	1	1	-	2	1	3	-	1
- Slight	1	-	1	1	1	1	-	1	3	2
- Moderate	-	-	-	1	2	1	-	-	1	1
Macrophage aggregates										
- Minimal	-	-	1	2	1	3	1	-	1	2
- Slight	-	-	-	1	-	-	-	-	2	1
Brown pigments, Perl's positive in macrophages (isolated or aggregates)										
- Minimal	-	-	3	3	3	4	4	1	1	-
- Slight	-	-	-	-	-	-	-	-	-	2
Brown pigments, Perl's negative and anisotropic, hepatocytes										
- Minimal	2	1	2	-	3	1	3	2	-	1
- Slight	-	-	-	-	-	-	-	-	3	3
- Moderate	-	-	-	-	-	-	-	-	1	-

Microscopic Changes in Liver of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	4	4	3	4	3	4	4	4	4	4
Brown pigments, Perl's negative and anisotropic, Kupffer cells										
- Minimal	2	1	2	3	-	3	3	1	1	3
- Slight	-	-	-	-	1	-	-	-	3	1
Brown pigments, Perl's negative and anisotropic, macrophage aggregates										
- Minimal	-	-	-	3	-	1	-	-	1	3
- Slight	-	-	-	-	-	2	-	-	3	-

M: male/F: female.

After the treatment free recovery period, none of the treated animals showed any evidence of sinusoidal accumulation of neutrophils. However, other changes such as perivascular infiltration by lymphocytes, plasma cells and hematopoietic precursor cells, macrophage aggregates were observed at a greater severity than in terminally killed animals.

Minimal bile duct hyperplasia and intra-canalicular accumulation of bile occurred in a male and a female Omegaven treated dog and in a female dog given Intralipid.

Microscopic Changes in Liver of Dogs at Recovery Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	2	2	2	2	2	2	2	2	2	2
Lympho-plasma cells and/or hematopoiesis (perivascular)										
- Minimal	-	-	-	-	2	1	-	2	-	-
Macrophage aggregates										
- Minimal	-	-	2	2	1	-	2	-	-	-
- Slight	-	-	-	-	1	2	-	-	2	-
- Moderate	-	-	-	-	-	-	-	2	-	2
Brown pigments, Perl's positive in macrophages (isolated or aggregates)										
- Minimal	-	-	1	2	-	2	2	-	-	1
- Slight	-	-	1	-	2	-	-	-	-	-
- Moderate	-	-	-	-	-	-	-	2	-	-
Brown pigments, Perl's negative and anisotropic, hepatocytes										
- Minimal	1	1	2	1	2	1	1	2	1	1
- Slight	-	-	-	-	-	-	-	-	1	1
Brown pigments, Perl's negative and anisotropic, Kupffer cells										
- Minimal	1	-	2	-	2	1	1	-	2	1
Brown pigments, Perl's negative and anisotropic, macrophage aggregates										
- Minimal	-	-	2	2	2	-	1	1	-	1
- Slight	-	-	-	-	-	1	-	-	2	1

M: male/F: female.

Kidneys

Treatment-related changes observed in the kidneys of all the treated animals consist of interstitial inflammation and glomerular degenerative changes. The treatment-related changes in animals dosed with 3 g/kg Omegaven was not dose responsive and was generally lower than in animals dosed with 3 g/kg Intralipid. Other treatment related changes observed were of lesser severity.

Multifocal lymphoid cell infiltration from inflammatory changes was observed in the cortical region of animals dosed with 3 g/kg Omegaven. In contrast, in animals dosed with 1 or 2 g /kg Omegaven and 3 g/kg Intralipid, there was a multifocal subacute or chronic interstitial inflammation characterized by multifocal accumulation of lymphocyte and plasma cells, but sometimes associated with interstitial fibrosis and surrounding

dilated or basophilic tubules with luminal proteinaceous material consistent with hyaline casts.
In the papilla and/or medullary region of the kidneys, the inflammation-associated hypercellular foci that were seen consist of interstitial and/or intratubular aggregates of macrophage- and/or fibrocyte-like cells associated with deposits of eosinophilic or slightly granular material. Although the inflammation-associated renal changes may be observed spontaneously in Beagle dogs, the higher incidence and severity in treated animals compared to controls was considered to be treatment (Omegaven or Intralipid) related.

In the cortico-medullary junction of animals treated with either of the 2 lipids, dark brown pigments were occasionally observed, and were considered to represent interstitial and/or intra-macrophagic hemosiderin deposits observed in a male dog (no.518) given 1 g/kg Omegaven.

In the glomerulus, the degenerative changes seen in several Omegaven and Intralipid-treated animals, characterized by a diffuse, homogenous thickening of the mesangium and the basement membrane of the glomerular capillaries, were considered to be consistent with a membranous or membrano-proliferative glomerulonephritis.

In the proximal convoluted tubules in several animals treated with the two test lipids, a cytoplasmic granular appearance which may have been related to the endocytotic accumulation of excreted material could be related to the glomerular changes seen in the animals.

At the junction between the cortical and medullary renal regions in treated animals, foci of thickening and/or vacuolation and/or inflammation of the vascular wall and/or thrombus formation in small/medium sized arteries were observed. These changes may be related to the treatment and part of the vascular changes observed in other organs (i.e. heart), although acute arteritis/periarteritis may be observed in untreated Beagle dogs.

At the end of the treatment period, subacute interstitial inflammation was still present in all renal compartments. Hypercellular foci of the papilla and/or medulla were seen in the males given 2 g/kg Omegaven. A few animals dosed with 1 or 2 g/kg Omegaven or 3 g/kg Intralipid had foci of minimal or slight dilatation of the Bowman’s capsule with glomerular atrophy and luminal accumulation of eosinophilic material. This suggests that the glomerulopathy seen in several treated animals at the end of the treatment period may have induced an atrophic glomerular change after the 4-week recovery period only in a few animals and at a low severity.

At the end of the treatment-free recovery period, the changes observed did not completely resolve, as shown in the Sponsor’s table below.

Treatment-Related Microscopic Findings in the Kidneys of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	4	4	3	4	3	4	4	4	4	4
Cortical region: lymphoid cell infiltration										
- Minimal	-	-	-	-	-	-	4	3	-	2
- Slight	-	-	-	-	-	-	-	1	-	-
Cortical region: interstitial inflammation, subacute										
- Minimal	-	-	2	1	2	4	-	-	3	-
- Slight	-	-	-	1	1	-	-	-	-	1
- Moderate	-	-	-	-	-	-	-	-	-	1
Medullary region and papilla: interstitial inflammation, acute or subacute										
- Minimal	-	-	-	1	2	1	-	-	1	-
- Slight	-	-	1	1	-	2	1	-	-	3
Calyx: mixed cell infiltration										
- Minimal	-	-	-	2	3	1	-	-	1	1
- Slight	-	-	-	1	-	1	-	-	-	1
Papilla and/or medullary region: hypercellular focus/i										
- Minimal	-	-	-	1	2	2	-	-	-	2
- Slight	-	-	1	-	-	-	-	-	-	-
Pigment deposits in cortico-medullary junction										
- Minimal	-	-	-	1	-	1	-	-	1	1
Glomeruli: thickening of mesangium and basement membrane (with or without glomerular enlargement)										
- Minimal	-	-	2	3	2	1	3	3	4	2
- Slight	-	-	1	-	-	3	-	-	-	1
- Moderate	-	-	-	1	1	-	-	-	-	-
Proximal tubules: granular appearance										
- Minimal	-	-	1	2	1	2	-	1	-	2
- Slight	-	-	-	1	1	2	-	-	-	-

M: male/F: female.

Heart

The observed changes in the heart of animals treated with Omegaven or Intralipid were associated with inflammation, and consist of acute inflammation of the atrial and ventricular wall and vascular changes in small or medium sized vessels such as intimal thickening and/or arteritis/periarteritis.

The inflammatory changes were not dose related, and the severity was higher in animals dosed with 1 or 2 g/kg Omegaven than in animals dosed with 3 g/kg Omegaven, which was in turn less severe than was seen in animals dosed with 3 g/kg Intralipid.

The atrial and ventricular inflammation was acute, generally observed in the right atrium (except in 3 animals where it was seen in one or both ventricles), and was characterized by endocardial and myocardial mixed cell infiltration, edema, congestion and fibrin deposits.

An acute left atrio-ventricular valvulitis was observed in a female dog (no.555) dosed with 3 g/kg Intralipid, and also showing prominent inflammatory changes such as atrial and ventricular myocardial inflammation and arteritis/periarteritis in the other cardiac compartments. Foci of arteritis/periarteritis were also seen in 2 females (nos. 556, 557) in the Intralipid group. The arterial inimal thickening was generally observed in the wall of the right atrium and in the ventricular wall in only a few of the animals.

Overall, the inflammatory changes observed in the heart wall and in the vessels were concentrated in the atria, suggesting a secondary effect of the inflammatory changes observed in the implanted jugular vein, or to a direct effect of the test items on the endocardial lining cells.

At the end of the treatment-free recovery period, the inflammation in the atrio-ventricular wall or in the coronary vessels (arteritis/periarteritis) or in the atrio-ventricular valves was seen in a few animals previously dosed with Omegaven at 2 or 3 g/kg or Intralipid at 3 g/kg.
The findings in the heart of treated and control animals at the end of the treatment-free recovery period are summarized in the Sponsor’s table below.

Treatment-Related Microscopic Findings in the Heart of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	4	4	3	4	3	4	4	4	4	4
Inflammation (atrial and/or ventricular wall)										
- Minimal	-	-	1	-	-	1	-	-	-	-
- Slight	-	-	-	-	-	1*	-	-	-	2
- Moderate	-	-	-	2	2	1*	-	-	-	1
- Marked	-	-	-	-	-	-	-	-	-	1
Arterial intimal thickening (atrium)										
- Minimal	-	-	-	-	2	2	-	1	-	-
- Slight	-	-	-	-	-	-	-	-	-	1
- Moderate	-	-	-	-	1	-	2	-	1	-

M: male/F: female; * = female no. 536, severity slight in the ventricle and moderate in the atrium.

Lungs

Histopathological findings in the lungs of animals consists of inflammatory changes such as the intra-alveolar accumulation of eosinophilic material (consistent with fibrin), mixed cell infiltration (neutrophils), hemorrhages and accumulation of vacuolated/pigmented macrophages, and were observed in all Omegaven and Intralipid treated groups. There was no evidence of dose-response in Omegaven dosed animals, but the severity of changes in animals dosed with 3 g/kg Omegaven or Intralipid were similar. Control animals showed similar inflammatory changes, but these were generally at lower incidence and severity than in the test article treated groups.

The intra-alveolar eosinophilic stained fibrin was assessed in 2 male dogs (nos. 527 and 539) dosed with 2 or 3 g/kg Omegaven, respectively. The vacuolated/pigmented macrophages had dark brown cytoplasmic hemosiderin deposits shown to be positive perl's staining in a female dog (no. 556) dosed with 3 g/kg Intralipid.

There was an increased cytoplasmic staining in macrophages (perivascular and/or septal and/or intralveolar) and in epithelial cells lining the terminal bronchioles in some animals treated with Omegaven at 2 or 3 g/kg and to a lesser extent in Intralipid-treated animals, when compared to controls. The cytoplasm in both cell types contained several small globules or vacuoles. A few stained macrophages which were minimal in severity were seen in some control animals.

In a control male dog (no. 502), it was observed that the prominent vascular changes associated with intra-alveolar fibrin deposits and large number of macrophages with

vacuolated cytoplasm, did not show remarkable ORO-stained lung section. On the other hand, there was a slightly increased ORO staining in Omegaven and Intralipid treated animals in comparison to controls, which suggests a moderate lung accumulation of fatty material following treatment.

At the end of the recovery period, the inflammatory and other pulmonary changes had only been partially resolved, with the differences in terms of incidence and severity between the two test items being minor. The two recovery control males (nos. 505 and 506) both had inflammatory lung changes. A summary of the findings in the lungs of the study animals is shown in the Sponsor's table below.

Microscopic Findings in the Lungs of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	**0**		**1**		**2**		**3**		**3**	
	Saline		**Omegaven**						**Intralipid**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals	**4**	**4**	**3**	**4**	**3**	**4**	**4**	**4**	**4**	**4**
Intimal/media thickening/inflammation										
- Minimal	2	-	3	-	-	2	3	2	-	1
- Slight	1	1	-	-	2	1	-	-	3	-
- Moderate	1	1	-	-	-	1	-	-	-	-
Thrombus (recent or partly organized or chronic)										
- Minimal	-	1	-	-	-	1	1	-	1	-
- Slight	-	-	-	-	-	1	-	-	-	1
- Moderate	-	1	1	-	-	-	-	2	-	-
- Marked	1	1	-	-	-	-	-	-	-	-

M: male/F: female.

Microscopic Findings in the Lungs of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	4	4	3	4	3	4	4	4	4	4
Intra-alveolar eosinophilic material/mixed cell infiltration										
- Minimal	-	1	2	1	-	2	2	3	3	3
- Slight	-	-	-	1	2	2	2	1	-	1
- Moderate	1	-	1	2	-	-	-	-	-	-
- Marked	-	-	-	-	1	-	-	-	-	-
Hemorrhage										
- Minimal	-	-	1	3	1	3	3	3	-	1
- Slight	-	-	-	-	-	-	-	-	-	1
- Moderate	1	-	1	1	-	-	1	-	-	-
- Marked	-	-	-	-	1	-	-	-	-	-
Vacuolated/pigmented macrophages										
- Minimal	-	1	-	1	1	-	-	2	2	3
- Slight	-	-	-	-	-	-	-	1	2	-
- Moderate	-	-	-	-	-	-	-	-	-	1
ORO-positive macrophages (perivascular/septal/intralveolar)										
- Minimal	1	2	2	1	1	2	3	3	3	1
- Slight	-	-	-	-	1	1	-	1	-	2
Increased ORO-positive staining in terminal bronchioles										
- Minimal	-	-	-	-	-	2	3	4	-	1
Perivascular inflammation (acute or subacute or chronic)										
- Minimal	2	1	2	-	2	1	2	3	4	1
- Slight	1	1	-	1	-	2	1	-	-	3
- Moderate	1	-	1	1	1	-	-	1	-	-
- Marked	-	1	-	1	-	-	-	-	-	-

Microscopic Findings in the Lungs of Dogs at the Recovery Period

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	2	2	2	2	2	2	2	2	2	2
Intra-alveolar eosinophilic material/mixed cell infiltration										
- Minimal	-	-	-	1	-	-	-	-	-	-
- Slight	-	-	1	-	1	-	-	1	-	-
- Moderate	1a	-	1	1	-	-	1	1	-	-
Hemorrhage										
- Slight	-	-	-	-	-	-	-	1	-	-
- Moderate	1a	-	1	-	-	-	1	-	-	-
- Marked	-	-	-	1	-	-	-	-	-	-
Vacuolated/pigmented macrophages										
- Minimal	1b	-	-	1	1	1	1	2	1	1
- Moderate	-	-	-	-	-	-	1	-	-	-
ORO-positive macrophages (perivascular/septal/intralveolar)										
- Minimal	-	1	1	-	-	-	1	1	1	-
- Slight	-	-	-	-	-	-	-	-	-	1
Increased ORO-positive staining in terminal bronchioles										
- Minimal				2			1	1	1	

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	2	2	2	2	2	2	2	2	2	2
Perivascular inflammation (acute or subacute or chronic)										
- Minimal	1b	1	2	1	1	1	-	2	-	1
- Slight	-	-	-	-	1	-	1	-	-	-
- Moderate	1a	-	-	-	-	1	-	-	-	-
- Marked	-	-	-	1	-	-	-	-	-	-
Intimal/media thickening/inflammation										
- Minimal	-	-	-	-	-	-	-	1	-	-
- Slight	-	-	-	-	-	-	1	-	1	-
- Marked	1b	-	-	-	-	-	-	-	-	-
Thrombus (recent or partly organized or chronic)										
- Moderate	-	-	-	-	1	-	-	1	2	-
- Marked	1a	-	1	-	-	-	-	-	-	-

M: male/F: female; a = male no. 506; b = male no. 505.

Lymph nodes

In the mesenteric and mandibular lymph nodes, at the end of treatment, atrophic changes of the lymphoid parenchyma was observed, including the presence of necropsy changes in other lymph nodes (thymic, trachea-bronchial, ilio-lumbar and renal lymph nodes).There was no dose-response relationship in Omegaven-treated animals, and only minor differences were noted in the comparison of the animal groups receiving either of the test articles. The atrophic changes were more severe in the mesenteric lymph node than on the mandibular node. These changes were accompanied by granulocyte infiltration, congestion and/or hemorrhage and/or erythrophagocytosis in the lymph nodes. At the end of the treatment-free recovery period, the decreased lymphoid size of lymphoid follicles was still present in the mesenteric lymph node in Omegaven-treated males and in Omegaven or Intralipid treated females. In the mandibular lymph node, the decreased size of lymphoid follicles was was seen in all the treated groups, and further accompanied by granulocyte infiltration (Omegaven-treated animals) and accumulation of vacuolated histiocytes in a male dog given Intralipid. A summary of the findings in the lymph nodes of the study animals is shown in the Sponsor's table below.

Microscopic Findings in the Mandibular Lymph Node of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	4	4	3	4	3	4	4	4	4	4
Lymphoid follicle development, decreased										
- Minimal	-	-	-	-	1	-	1	1	-	1
- Slight	-	-	-	-	-	1	1	-	1	1
- Moderate	-	-	1	-	1	-	-	-	-	-
Paracortical region, decreased size										
- Slight	-	-	-	-	1	2	1	1	-	-
Plasmacytosis										
- Minimal	-	-	3	1	1	2	2	-	1	2
- Slight	-	-	-	1	1	-	1	-	-	
- Moderate	-	-	-	-	-	-	-	-	-	2
- Marked	-	-	-	-	-	1	-	-	-	-
Histiocytosis										
- Minimal	1	1	2	2	1	1	2	3	1	2
- Slight	-	-	-	1	-	1	1	1	-	2
Histiocytes, cytoplasmic vacuolation										
- Minimal	-	-	2	1	2	2	1	1	3	1
- Slight	-	-	-	-	1	2	1	3	1	3
- Moderate	-	-	-	-	-	-	2	-	-	-
Granulocyte infiltration (medullary/cortical/sinusoidal)										
- Minimal	1	2	3	3	2	2	3	3	3	2
- Slight	-	1	-	-	1	1	1	1	1	1
- Moderate	-	-	-	1	-	-	-	-	-	1
Congestion and/or hemorrhage and/or erythrophagocytosis										
- Minimal	-	1	1	1	-	2	1	1	-	1
- Moderate	-	-	-	-	1	-	-	-	-	-

M: male/F: female.

Microscopic Findings in the Mesenteric Lymph Node of Dogs at Terminal Sacrifice

Group Treatment (g/kg/16 hrs)	0		1		2		3		3	
	Saline		Omegaven						Intralipid	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals	4	4	3	4	3	4	4	4	4	4
Lymphoid follicle development, decreased										
- Minimal	-	-	1	1	-	1	-	1	2	1
- Slight	-	-	-	-	2	1	1	-	-	-
- Moderate	-	-	1	3	1	2	-	1	1	2
- Marked	-	-	-	-	-	-	-	-	-	1
Paracortical region, decreased size										
- Minimal	-	-	1	-	-	1	1	1	3	-
- Slight	-	-	-	2	-	-	-	2	1	-
- Moderate	-	-	-	1	3	2	-	-	-	3
- Marked	-	-	-	-	-	1	-	-	-	1
Granulocyte infiltration (medullary/cortical/sinusoidal)										
- Minimal	-	-	1	1	1	-	2	1	-	1
- Slight	-	-	-	-	-	-	-	-	-	2
Congestion and/or hemorrhage and/or erythrophagocytosis										
- Minimal	3	4	-	2	-	2	1	-	-	1
- Slight	-	-	2	1	-	1	3	1	2	2
- Moderate	-	-	1	1	3	-	-	3	2	1

M: male/F: female.

Thymus

Minimal to marked thymus atrophy was seen in Omegaven and Intralipid treated animals at the end of the dosing period. The severity of the atrophy was not dose-related in Omegaven dosed animals, and the differences in changes observed between the Omegaven and Intralipid dosed animals were minor. Thymus atrophy of minimal severity was also seen in control animals. At the end of the recovery period, there was evidence of complete recovery of the thymus atrophy in treated animals.

Spleen

Treatment-related changes were seen in the white and red pulp of the spleen in Omegaven or Intralipid treated animals at the end of the dosing period, with a lower severity in the Omegaven high dose group than in the Intralipid group. The increased cellularity of the red pulp was due to an accumulation of plasma cells and bone marrow precursor cells (extramedullary hematopoiesis), whereas the white pulp was characterized by an increased size of periarteriolar lymphoid sheaths with an increased size/number of secondary lymphoid follicles. Minimal or slight Perl's positive iron-containing deposits were seen in the red pulp in control and treated animals without treatment-related differences. The severity was minimal in control and Omegaven-treated animals, and was slight or moderate in Intralipid treated animals. At the end of the recovery period, occasional animals treated with either of the test article had a minimal increase of development of the white pulp and increased cellularity of the red pulp. Perl's negative pigment deposits were still observed in control and treated animals, with greater severity in Intralipid treated animals, whereas Omegaven treated animals showed minimal differences from the control animals. The Perl's positive

pigment deposits were observed at similar severity in control and treated animals at terminal sacrifice.

Duodenum

Histopathological changes observed in the duodenum at the end of dosing consist of foci of mixed cell infiltration in the superficial mucosa of the proximal duodenum near the gastro-duodenal junction. There were evidence of an acute inflammation and the mixed cell infiltration was associated with a dystrophic appearance of the duodenal glands and/or villous atrophy in some animals. These findings were minimal in severity and infrequent in Omegaven or Intralipid treated animals. At the end of the recovery period, only a female dog (no.535) given Omegaven at 2 g/kg had minimal focal mixed cell infiltration in the duodenal mucosa.

In conclusion, Omegaven (1, 2 or 3 g/kg/16-h) and Intralipid (3 g/kg/16-h) infusion administration to male and female dogs for 13 weeks induced acute and chronic inflammatory changes in multiple organs which were partially reversed after a 4-week recovery period. Major treatment-related changes were observed in the liver, kidneys, heart, lungs, lymph nodes, thymus and spleen. Treatment-related changes generally at a low severity were seen in the jugular vein, duodenum, adrenal glands, femoro-tibial joint and bone marrow. Overall, there were no clear dose-response effects in Omegaven-treated animals, because the changes observed in the animals dosed with 1 or 2 g/kg were generally of higher severity than in animals dosed with 3 g/kg. However, the changes observed in animals dosed with 3 g/kg Omegaven were generally lower than in animals dosed with 3 g/kg Intralipid.

Toxicokinetics

Blood samples for toxicokinetic (TK) analysis were collected on the first day of dosing, during week 4, and during week 13. Blood samples were also collected on week 17 for the recovery animals. Two ml blood samples were taken just before the start of the daily infusion, 8 hours after the end of the 16 hour infusion (on day 0), 4, 8, 16 and 20 hours after the start of infusion, and 4 hours after the end of the 16 hour infusion. A blood sample was also taken from recovery animals during week 17.

Individual fatty acid plasma concentration profiles from male and female dogs were analyzed by gas chromatography. The lower limit of quantification (LOQ) for each fatty acid was 0.08 mmol/L. Plasma concentrations of 8:0, 10:0, 12:0, 14:0 and 20:1 were below LOQ at all sampling times and were not included in the toxicokinetic analysis. The TK evaluation was performed on 10 fatty acids: 16:0, 16:1, 18:0, 18:1, 18:2, 18:3c, 20:4, 20:5, 22:5 and 22:6.

Following single day (day 0) and repeated (weeks 4 and 13) daily doses of Omegaven at 3 g TG/kg/16-h, systemic exposure to fatty acids 16:1, 20:5, 22:5 (weeks 4 and 13 only) and 22:6 in male and female dogs tended to be greater than that after the administration of Intralipid at 3 g TG/kg/16-h. In contrast, systemic exposure to 16:0,

18:0, 18:1, 18:2, 18:3 and 20:4 (weeks 4 and 13 only) in male and female dogs after the administration of Intralipid at 3 g TG/kg/16-h tended to be greater than that after the administration of Omegaven at 3 g TG/kg/16-h. Following single and repeated administration of Omegaven or Intralipid during weeks 4 and 13, the systemic exposure to all the fatty acids was greater than after administration of control formulation.

After repeated administration of Omegaven at 1, 2, or 3 g TG/kg, systemic exposure to 16:0, 16:1, 18:1 (weeks 3 and 13 only), 20:5 and 22:6 tended to increase with increasing dose. However, the increase was less than dose-proportional. In contrast, there was no appreciable increase in systemic exposure to 18:0, 18:1 (day 0 only), 18:2, 20:4 and 22:5 after the administration of Omegaven.

Following the single and repeated doses of control, Omegaven or Intralipid, systemic exposure to each fatty acid in male dogs was not appreciably different from to that in female dogs.

Following repeated daily dosing of 1-3 g/kg Omegaven and 3 g/kg Intralipid, systemic exposure (C_{max}, AUC_{0-16h} and AUC_{0-24h}) to 16:0, 16:1, 18:0, 18:1 and 20:4 (Intralipid at 3 g/kg only) during weeks 4 and 13 was not appreciably different to that on day 0. However, systemic exposure to 20:5 and 22:6 during weeks 4 and 13 appeared to be greater than that on day 0, suggesting that these fatty acids accumulated in plasma after repeated dosing. In contrast, following repeated daily dosing with Omegaven or Intralipid, the concentrations of 18:2, 18:3 20:4 and 22:5 fatty acids during weeks 4 and 13 decreased in plasma.

Following repeated daily intravenous infusions of Omegaven at 3 g TG/kg, plasma concentrations of 20:5 and 22:6 during the recovery week 17 were 5-fold and 2-fold greater, respectively than plasma concentrations of the corresponding fatty acid during week 17 in control and Intralipid-treated animals. There were no appreciable differences in the plasma concentration of all other fatty acids during the recovery period, for control, Omegaven or Intralipid-treated animals.

In summary, following the single or repeated doses of control, Omegaven or Intralipid, there is no gender difference in the systemic exposure to the corresponding fatty acids, and the systemic exposure appeared to be less than dose proportional in the animals. A summary of the measured TK parameters is shown in the Sponsor's table below.

TK Parameters in Dogs following IV Infusion of 3 g TG/kg Omegaven (Week 4)

Males

Animal Number	C_{max} (mmol/L)	t_{max} (h)	t_{last} (h)	AUC_{0-t} (mmol.h/L)	AUC_{0-16h} (mmol.h/L)	AUC_{0-24h} (mmol.h/L)
537	3.06	16.0	24.0	64.3	42.1	64.3
538	2.56	16.0	24.0	56.8	38.0	56.8
539	2.93	16.0	24.0	59.6	38.5	59.6
540	2.54	16.0	24.0	52.6	34.6	52.6
541	2.18	0	24.0	47.3	32.6	47.3
542	3.23	16.0	24.0	71.8	48.5	71.8
Geometric Mean	2.73	NC	NC	58.2	38.7	58.2
Geometric Mean CV (%)	14.7	NC	NC	14.8	14.2	14.8
Arithmetic Mean	2.75	13.3	24.0	58.7	39.1	58.7
Arithmetic Mean SD	0.391	6.53	0	8.65	5.67	8.65
Arithmetic Mean CV (%)	14.2	49.0	0	14.7	14.5	14.7
Median	2.75	16.0	24.0	58.2	38.3	58.2
Minimum	2.18	0	24.0	47.3	32.6	47.3
Maximum	3.23	16.0	24.0	71.8	48.5	71.8

Females

Animal Number	C_{max} (mmol/L)	t_{max} (h)	t_{last} (h)	AUC_{0-t} (mmol.h/L)	AUC_{0-16h} (mmol.h/L)	AUC_{0-24h} (mmol.h/L)
543	3.17	16.0	24.0	69.5	46.6	69.5
544	2.34	16.0	24.0	54.5	37.0	54.5
545	2.83	16.0	24.0	56.4	37.6	56.4
546	2.18	16.0	24.0	48.1	32.0	48.1
547	2.36	4.00	24.0	54.1	35.5	54.1
548	2.30	16.0	24.0	53.4	35.5	53.4
Geometric Mean	2.51	NC	NC	55.6	37.1	55.6
Geometric Mean CV (%)	14.6	NC	NC	12.2	12.5	12.2
Arithmetic Mean	2.53	14.0	24.0	56.0	37.4	56.0
Arithmetic Mean SD	0.385	4.90	0	7.17	4.92	7.17
Arithmetic Mean CV (%)	15.2	35.0	0	12.8	13.2	12.8
Median	2.35	16.0	24.0	54.3	36.3	54.3
Minimum	2.18	4.00	24.0	48.1	32.0	48.1
Maximum	3.17	16.0	24.0	69.5	46.6	69.5

SD Standard deviation
CV Coefficient of variation
NC Not calculated

TK Parameters in Dogs following IV Infusion of 3 g TG/kg Intralipid (Week 4)

Males

Animal Number	C_{max} (mmol/L)	t_{max} (h)	t_{last} (h)	AUC_{0-t} (mmol.h/L)	AUC_{0-16h} (mmol.h/L)	AUC_{0-24h} (mmol.h/L)
549	4.94	16.0	24.0	110	73.7	110
550	7.23	16.0	24.0	161	108	161
551	5.66	20.0	24.0	126	82.2	126
552	6.15	16.0	24.0	134	89.9	134
553	6.91	16.0	24.0	157	105	157
554	6.74	16.0	24.0	133	83.5	133
Geometric Mean	6.22	NC	NC	136	89.6	136
Geometric Mean CV (%)	14.4	NC	NC	14.2	14.9	14.2
Arithmetic Mean	6.27	16.7	24.0	137	90.4	137
Arithmetic Mean SD	0.861	1.63	0	19.2	13.5	19.2
Arithmetic Mean CV (%)	13.7	9.80	0	14.0	15.0	14.0
Median	6.45	16.0	24.0	134	86.7	134
Minimum	4.94	16.0	24.0	110	73.7	110
Maximum	7.23	20.0	24.0	161	108	161

Females

Animal Number	C_{max} (mmol/L)	t_{max} (h)	t_{last} (h)	AUC_{0-t} (mmol.h/L)	AUC_{0-16h} (mmol.h/L)	AUC_{0-24h} (mmol.h/L)
555	6.04	20.0	24.0	127	82.5	127
556	7.62	16.0	24.0	166	108	166
557	4.89	4.00	24.0	112	75.3	112
558	6.28	16.0	24.0	134	85.9	134
559	6.39	16.0	24.0	137	90.9	137
560	6.07	16.0	24.0	134	88.9	134
Geometric Mean	6.16	NC	NC	134	88.1	134
Geometric Mean CV (%)	14.3	NC	NC	12.9	12.1	12.9
Arithmetic Mean	6.22	14.7	24.0	135	88.6	135
Arithmetic Mean SD	0.874	5.47	0	17.8	11.0	17.8
Arithmetic Mean CV (%)	14.1	37.3	0	13.2	12.4	13.2
Median	6.18	16.0	24.0	134	87.4	134
Minimum	4.89	4.00	24.0	112	75.3	112
Maximum	7.62	20.0	24.0	166	108	166

SD Standard deviation
CV Coefficient of variation
NC Not calculated

Dosing Solution Analysis

All the measured dosing values delivered by the pumps were within ± 10 % of the theoretical values.

Study title: 13-Week Subchronic Toxicity Study of SMOF 20 % by Daily 6-Hour Intravenous Infusion to Beagle Dogs.

Study no.:	10825/97
Study report location:	FE-SM-PT-01, Page 1-589
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 25, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SMOF 20 %, batch #s HMFE 13, 14 and 15, 100.7 % – 101.0 %.

Key Study Findings

The intravenous infusion of 3 and 6 g/kg of SMOF 20 % in Beagle dogs at 6 hours/day for 13 weeks was well tolerated and did not result in animal mortality. The slight increase in the number of leucocytes, PTT and the decrease in MCV, MCH, hematocrit and the number of platelets observed in the high dose animals were due to the hypervolemic effect of the large volume and high doses of the lipid emulsion. Similarly, the increase in α_2-globulin, cholesterol, phospholipids, acetoacetate, and decrease in urea, lipase activity and some liver enzymes were also related to the hypervolemic lipid emulsion effect. Histopathological changes observed in the liver parenchyma of the high dose animals were considered to be associated with the administration of large amounts of lipid. Changes of slight to moderate indurations of similar severity were observed at the infusion site of control and treated animals. All the observed changes were completely reversible after the 4-week recovery period, with the exception of the fatty change which was still present in the liver parenchyma of all the animals.

Methods

Doses:	0 (Saline Control), 3 g TG/kg/day, 6 g TG/kg/day
Frequency of dosing:	6 Hours daily for 13 weeks
Route of administration:	Intravenous infusion via the left or right cephalic vein of the forelimbs, or the left or right saphenous vein of the hindlimbs.
Dose volume:	15 ml to 30 ml/kg/day
Formulation/Vehicle:	(b) (4) (Smoflipid)
Species/Strain:	Beagle Dog
Number/Sex/Group:	3/sex/group
Age:	8-9 months
Weight:	7.4 – 8.3 kg (males), 7.0 – 7.8 kg (females)
Satellite groups:	Yes, 2/sex/group from control and high dose group designated as recovery animals.
Unique study design:	No
Deviation from study protocol:	No deviations considered to affect the intergrity of the study.

Observations and Results

Mortality

Animals were checked twice a day for premortal symptoms or deaths

There were no animal mortalities in this study.

Clinical Signs

Animals were examined individually for clinical signs daily, prior to dosing, and at suitable intervals after dosing for any treatment signs.

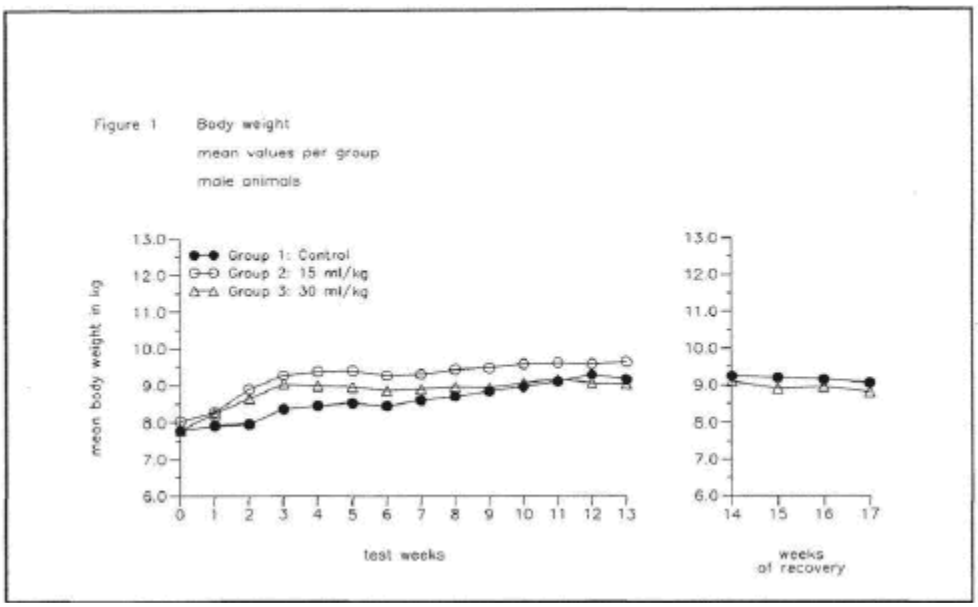
There were no treatment related clinical signs of toxicity in treated animals.

Body Weights

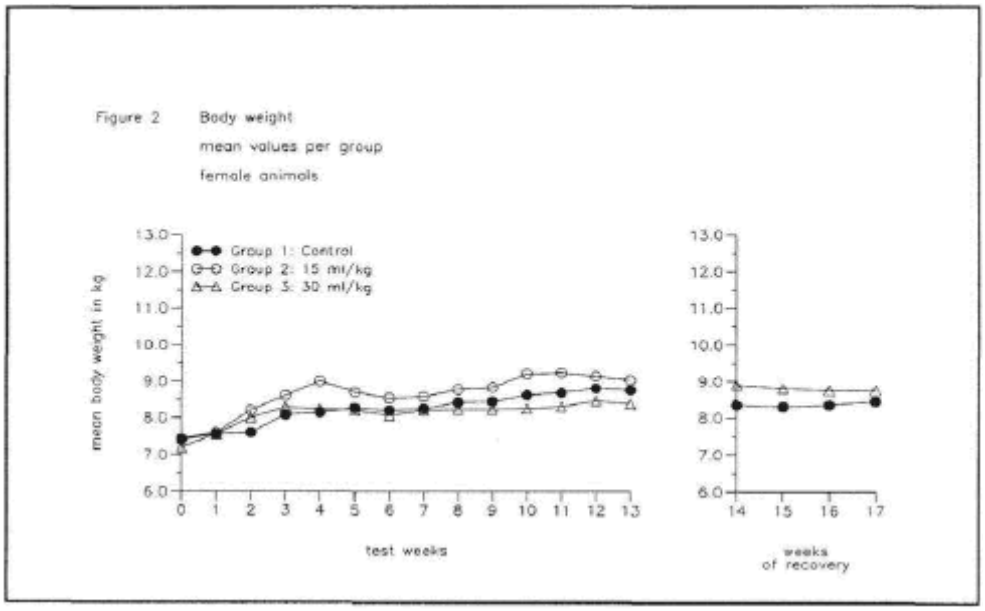
Body weight of the animals was recorded at study initiation and at weekly intervals during the dosing period.

All the treated animals showed increased body weight gain during the first 3 to 4 weeks of dosing. The body weight of the 3 g/kg Smoflipid dose group was consistently increased (0.5 to 5 %) above that of the control animals for the entire study period, whereas, the body weight of the 6 g/kg Smoflipid dose group was higher (3.9 to 4.7 %) than that of the control group during the first half of the study. The increased body weight was considered to be due to the dietary effect of the test article. Figures showing the body weights of study animals are presented below from the Sponsor's submission.

Body Weights of Male Dogs treated with SMOF 20 % for 13 Weeks



Body Weights of Female Dogs treated with SMOF 20 % for 13 Weeks



Feed Consumption

Food consumption in animals was recorded daily.

Decreased food consumption was observed from the first test week or the second test week in 3 g/kg SMOF 20 % treated animals. The decreased food consumption was

considered treatment-related, due to the high caloric value of the administered test article. A summary table of the differences in food intake between the treated and control animals is presented below from the Sponsor's submission.
During the recovery period, the observed decrease in food intake in treated animals appeared to normalize, although the food intake was still lower than in the control animals at the end of the 4 week recovery period.

Ophthalmoscopy

Ophthalmoscopic examinations were performed on the animals prior to dosing initiation, at the end of dosing period (day 92), and at the end of the recovery period (day 120).

There were no test article related lesions of the eyes or the optic region in animals treated for 13 weeks or in the recovery animals 4 weeks post dose on ophthalmic examinations, when compared to control animals.

ECG

Electrocardiographic recordings were performed on all animals on test day 1 and in the 6^{th} (day 41) and 13^{th} test week (day 91) and at the end of the recovery period (day 118).

There was no test article related changes observed in ECG recordings of dogs treated for 13 weeks with 3 and 6 g/kg SMOF 20 %.
During the 4 week recovery period, there were no differences observed from the ECG recordings in the high dosed animals when compared to that of the control animals.

Hematology

Blood samples were collected from the cephalic veins of animals after an overnight fast, prior to dosing, at the end of the 6^{th} (day 42) and 13^{th} (day 85) test week, and 17^{th} (day 118) dose-free, recovery period.

Several hematological parameters measured were affected in a dose-response manner with 3 or 6 g/kg SMOF 20 % treatment.

While the levels of leucocytes, PTT, hematocrit, MCV and MCH were increased in treated animals in comparison to control values, the platelet count was decreased in treated animals, when compared to values in control animals.

The changes in hematological parameters are considered due to the hypervolemic effect of the large volume infusion of the test article (150 to 300 ml/animal/day).

The erythrocyte sedimentation rate (ESR) was slightly increased in individual animals during weeks 6 and 13, but without a dose-response effect.

There was no treatment related effect observed for hemoglobin, erythrocyte or differential blood count, the bleeding time, PTT and MCHC.

After the 4 week recovery period, there were no treatment-related changes observed between the hematological parameters of previously treated SMOF 20 % (6 g/kg) and control animals.

Clinical Chemistry

Blood samples were collected from the cephalic veins of animals after an overnight fast, prior to dosing, at the end of the 6th (day 42) and 13th (day 85) test week, and 17th (day 118) dose-free, recovery period.

Significantly decreased α1-globulin values were observed in treated male animals during treatment week 0 or 13 and in 3 female dogs during treatment week 0 and 13. The globulin concentration was also increased after dosing weeks 6 and 13, consequently, the A/G ratio was slightly decreased. This was mainly the result of an increase in the α2-globulin fraction.

At the end of the 4 week recovery period, all the clinical chemistry parameters measured tended to return to normal values, or are within the normal range in the recovery animals.

Urinalysis

Urine sample was collected from animals fasted overnight prior to dosing, and at the end of the 6th (day 42) and 13th (day 85) test week, and 17th (day 118) dose-free, recovery period.
There were no changes in urinary composition or urinary specific gravity with 13 week treatment of animals with 3 or 6 g/kg SMOF 20 %. However, urinary pH value was slightly increased in the treated animals in test week 6 and 13. Urinalysis in the recovery animals at the end of the recovery period (week 17) did not show any substance-related changes in the previously high-dosed dogs designated as recovery animals.

Gross Pathology

After the 13 week treatment period (day 92) and at the end of the recovery period, (day 120), animals were sacrificed and a complete necropsy performed.

The macroscopic changes observed in animals treated with 3 g/kg SMOF 20 % are considered to be spontaneous and within the range of normal limits.
The macroscopic findings in the liver of animals treated with 6 g/kg SMOF 20 % are treatment related and considered due to the large volume of the infused lipid emulsion.
A summary of the macroscopic findings during the treatment and recovery periods in animals treated with 3 and 6 g/kg SMOF 20 % are summarized in the Sponsor's table below.

Summary of Macroscopic Findings in Dogs treated with SMOF 20 %

Group/ Dose	Animal No./Sex	Affected Organ/Tissue / Findings	Related Histopathology
1 Control	6 ♀	**urinary bladder:** slightly reddened	no corollary change detected
		liver: slightly yellowish discoloured	no corollary change detected
	7 ♀	**urinary bladder:** slightly reddened	no corollary change detected
		small intestine: slightly reddened	no corollary change detected
	8 ♀	**intestinal tract:** slightly reddened	no corollary change detected
2 15 ml SMOF 20%/kg	13 ♂	**small intestine:** slightly reddened	no corollary change detected
3 30 ml SMOF 20%/kg	19 ♂	**liver:** slightly yellowish discoloured	lipid droplets, hepatocellular, microvesicular, GRADE 1
	21 ♂ (R)	**liver:** slightly yellowish discoloured; bile gritty	lipid droplets, hepatocellular, microvesicular, GRADE 3
	22 ♀	**liver:** slightly yellowish discoloured	lipid droplets, hepatocellular, centrilobular, GRADE 3
	23 ♀	**liver:** slightly yellowish discoloured	lipid droplets, hepatocellular, microvesicular, GRADE 2
	24 ♀	**liver:** slightly yellowish discoloured	lipid droplets, hepatocellular, centrilobular, GRADE 1
	26 ♀ (R)	**liver:** slightly yellowish discoloured **uterus (incl. horns):** thickened walls, congestion	lipid droplets, hepatocellular, microvesicular, GRADE 3 dilation, GRADE 3

(R) = recovery animal

Organ Weights

The weights of the following organs of all the study animals were determined before fixation, with the exception of the pituitary, which was fixed before weighing:

adrenal (2)	liver	prostate	thymus
brain	lungs	salivary gland	thyroid (2)
heart	ovary (2)	spleen	uterus
kidney (2)	pituitary	testicle (2)	

The adrenals, gonads, kidneys and thyroids were weighed individually and identified as left or right.

Increased relative and absolute organ weights were observed in animals treated with 3 and 6 g/kg SMOF 20 % for 13 weeks. However, at the end of the 4-week recovery period, all organ weights were within the normal limits in high dose treated animals, when compared to control, as shown in the Sponsor's table below.

Comparison of the Relative Organ Weights in Dogs Treated with SMOF 20 %

Organ/ Dose/ Sex		TW 13 M ± SD	TW 13 %	TW 13 Range	TW 17 M#	TW 17 %
Kidney (left)						
Control	♂	2.7 ± 0.0		2.7	2.9	
	♀	2.9 ± 0.2		2.7-3.0	2.7	
15 ml/kg	♂	3.4 ± 0.3	+26	3.2-3.7		-
	♀	2.8 ± 0.3	+3	2.6-3.1		-
30 ml/kg	♂	3.4 ± 0.5	+26	2.8-3.7	3.0	+3
	♀	3.5 ± 0.2	+21	3.3-3.6	2.7	none
Kidney (right)		TW 13 M ± SD	TW 13 %	TW 13 Range	TW 17 M#	TW 17 %
Control	♂	2.6 ± 0.1		2.6-2.8	3.1	
	♀	2.6 ± 0.2		2.5-2.8	2.7	
15 ml/kg	♂	3.3 ± 0.3	+27	3.1-3.7		-
	♀	2.8 ± 0.2	+8	2.6-2.9		-
30 ml/kg	♂	3.5 ± 0.5	+35	2.9-3.9	3.1	none
	♀	3.4 ± 0.2	+31*	3.1-3.6	2.7	none

Comparison of the Relative Organ Weights in Dogs Treated with SMOF 20 %

Organ/ Dose/ Sex		TW 13 M ± SD		Range	TW 17 M#	%
Lungs						
Control	♂	9.4±0.8		8.5-9.9	12.9	
	♀	8.8±0.6		8.2-9.3	9.3	
15 ml/kg	♂	11.1±1.3	+18	9.9-12.4		-
	♀	8.9±0.5	+1	8.4-9.3		-
30 ml/kg	♂	11.9±3.8	+27	8.8-16.2	14.9	+16
	♀	11.4±2.1	+30	9.3-13.6	9.5	+2

Histopathology

Samples (or the whole) of all the organs or tissues listed below were preserved in neutral buffered 7 % formalin. All the tissues were examined histopathologically after hematoxylin-eosin staining of thin paraffin sections, with the exception of those marked with an asterisk (*).

adrenal (2)
*aorta abdominalis
blood smears (*in case of anaemia, enlarged thymus, lymphadenopathy*)
bone (*os femoris*)
bone marrow (*os femoris*)
brain (*coronal sections at 3 levels*)
epididymis (2)
eye with
optic nerve (2)
gall bladder
gross lesions
heart
infusion site (*earlier / last*)
intestine, small (*duodenum, jejunum, ileum*)
intestine, large (* *caecum, colon, rectum*)
kidney (2)
lacrimal gland (2)
liver
lungs (*with mainstem bronchi*)
lymph node (*cervical, mesenteric*)
mammary gland
muscle (*skeletal, leg*)
nerve (*sciatic*)
oesophagus
ovary (2)
pancreas
pharynx
pituitary
prostate
salivary gland
*seminal duct
skin (*left flank*)
spinal cord
spleen
stomach
*teeth (*two incisors, two molars*)
testicle (2)
thymus
thyroid (2) (*incl. parathyroid*)
tissue masses or tumours (*incl. regional lymph nodes*)
tongue (*incl. base*)
trachea
urinary bladder
uterus (*incl. cervix*)
vagina

In addition, frozen sections of the heart, liver and kidneys (2) were made and stained with scarlet R.

Adequate Battery
Yes
Peer Review
Yes
Histological Findings
Histopathological findings in dogs treated with 6 g/kg SMOF 20 % showed a fatty change in the liver parenchyma of all animals treated with 6 g/kg of SMOF 20 %. The fatty change observed was as a result of lipid accumulation, which was of a minimal to moderate degree, and considered to be related to the treatment with SMOF 20 %. The fatty change was observed in treated and recovery animals.

Other findings observed at the infusion site in the animals include; focal thrombosis, intima proliferation and fibrosis, perivascular hemorrhage and pigment accumulation. These findings were noted to be of similar severity in treated and control animals.

In conclusion, the intravenous infusion of 3 and 6 g/kg of SMOF 20 % in Beagle dogs at 6 hours/day for 13 weeks with a 4 week recovery period was well tolerated and did not result in animal mortality. The hematological and biochemical parameter changes observed were due to the hypervolemic effect of the large volume and high doses of the lipid emulsion. Histopathological changes observed in the liver parenchyma of the high dose animals were considered to be associated with the administration of large amounts of lipid from SMOF 20 %. The changes observed at the infusion site were caused by the mechanical irritation of the vascular wall and were observed in treated and control animals. All the observed changes were completely reversible after the 4-week recovery period, with the exception of the fatty change which was still present in the liver parenchyma of all the animals.

7 Genetic Toxicology

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

Study title: Mutagenicity Study of SMOF 20 % in the *Salmonella typhimurium* reverse mutation assay *in vitro*.

Study no.:	9357/95
Study report location:	Pages 000173 to 000206
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 1, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SMOF 20 %, lot # FHFE 11; Water for Injection, lot # 5293A41A

Key Study Findings

SMOF 20 % at a maximum concentration of 200 µl/plate did not produce any mutagenic effect in any of the 5 bacterial strains tested, with and without metabolic activation.

Methods

Strains:	TA 1537, TA 102, TA 98, TA 1535, TA 100
Concentrations in definitive study:	2.0 to 200 µl
Basis of concentration selection:	Cytotoxicity
Negative control:	Water for Injection
Positive control:	Sodium azide, 2-nitro-9H-fluorene, 9-amino-acridine, methyl methane sulfonate, 2-aminoanthracene. (with metabolic activation)
Formulation/Vehicle:	Water for Injection
Incubation & sampling time:	48 hours

Study Validity

The following criteria were used for determining the study validity:
None of the bacterial strains, when streaked, should grow on media plates containing biotin only.
All the bacterial strains, when streaked, should show excessive growth on media plates with L-Histidine and biotin.
Application of 0.1 % crystal paper disc on media plates seeded with bacteria should give clear zones of inhibition in all tester strains.
A 10 µg ampicillin paper disc placed on plates seeded with bacteria should not show zones of inhibition with strains TA98 or TA 100 (indicating ampicillin resistance), but should show zones of inhibition with strains TA 1535, TA 1537, and TA 102.
The solvent control revertants/plate should be within the historical control range.

Results

None of the tested concentrations (ranging from 2.0 to 200 µl) of SMOF 20 % from the two independent experiments (with and without metabolic activation) possessed cytotoxic effects.

There was no mutagenic effect observed for SMOF 20 % in any of the 5 tester strains in the two independent experiments with and without metabolic activation.
The findings of the study are summarized in the Sponsor's Tables below.

Mutagenicity Study of SMOF 20 % in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (First experiment without metabolic activation).

Substance (µl/plate)		Summarized data without metabolic activation revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
			1st experiment			
SMOF 20%						
200.0	M	20.3	103.0	324.7	10.7	6.7
	±SD	0.58	7.00	5.85	3.06	3.06
63.2	M	19.0	109.0	327.0	7.3	5.7
	±SD	1.00	18.03	14.11	2.08	2.52
20.0	M	20.7	117.7	330.7	6.3	5.7
	±SD	2.89	11.02	23.12	4.16	0.58
6.32	M	23.7	102.3	309.3	9.0	5.7
	±SD	3.51	6.51	24.03	4.58	2.52
2.0	M	22.3	105.7	312.3	7.3	5.3
	±SD	0.58	2.08	11.24	2.08	1.15
Solvent control: 200 µl/plate	M	20.7	108.0	343.0	11.3	5.3
	±SD	1.15	9.17	7.00	4.73	0.58
Positive control: substance		2-Nitro-fluorene	Sodium-azide	Methyl methane sulfonate	Sodium-azide	9-Amino-acridine
Concentration: (µg/plate)		10	10	1300	10	100
	M	599.7	1425.7	957.7	2035.7	3305.3
	±SD	49.07	59.18	95.50	106.64	111.55

M = mean number of revertants
SD = standard deviation

Mutagenicity Study of SMOF 20 % in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (Second experiment without metabolic activation).

Substance (µl/plate)		Summarized data without metabolic activation revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
2nd experiment						
SMOF 20%						
200.0	M	20.7	101.7	319.3	6.0	4.7
	±SD	2.08	1.53	15.04	1.73	0.58
63.2	M	21.7	101.0	318.3	5.3	5.0
	±SD	0.58	1.73	29.19	1.15	1.73
20.0	M	21.0	101.3	315.3	6.0	5.0
	±SD	1.00	6.81	4.51	2.00	1.00
6.32	M	20.7	101.0	322.3	4.0	4.7
	±SD	2.08	1.00	16.56	0.00	3.79
2.0	M	21.0	102.0	328.7	6.7	6.0
	±SD	1.00	1.00	22.03	2.08	1.73
Solvent control: 200 µl/plate	M	20.7	103.7	332.0	6.3	5.7
	±SD	0.58	3.79	11.27	1.15	0.58
Positive control: substance		2-Nitro-fluorene	Sodium-azide	Methyl methane sulfonate	Sodium-azide	9-Amino-acridine
Concentration: (µg/plate)		10	10	1300	10	100
	M	847.7	1819.3	858.3	1862.3	4195.7
	±SD	112.04	101.16	78.44	216.18	64.3

M = mean number of revertants
SD = standard deviation

Mutagenicity Study of SMOF 20 % in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (First experiment with metabolic activation).

Substance (µl/plate)		Summarized data with metabolic activation revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
		1st experiment				
SMOF 20%						
200.0	M	23.7	113.3	232.7	9.7	5.3
	±SD	3.79	13.50	19.22	1.15	1.15
63.2	M	24.7	111.7	308.0	10.0	4.7
	±SD	5.69	3.06	9.85	3.61	2.08
20.0	M	23.0	103.7	307.7	12.0	6.3
	±SD	3.46	4.04	10.12	6.00	2.52
6.32	M	30.0	118.3	319.7	11.0	7.0
	±SD	1.73	17.39	5.69	1.00	1.00
2.0	M	29.3	104.0	312.3	10.0	6.0
	±SD	2.89	6.93	15.37	1.00	3.46
Solvent control: 200 µl/plate	M	25.7	108.3	327.0	10.3	6.7
	±SD	3.21	6.43	13.0	4.04	1.53
Positive control: substance			2-Aminoanthracene			
Concentration: (µg/plate)		2	2	2	2	2
	M	993.0	968.3	709.7	154.0	144.7
	±SD	112.05	70.07	122.40	7.55	5.03

M = mean number of revertants
SD = standard deviation

Mutagenicity Study of SMOF 20 % in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (Second experiment with metabolic activation).

Substance (µl/plate)		Summarized data with metabolic activation revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
		2nd experiment				
SMOF 20%						
200.0	M	24.7	105.3	309.3	7.7	4.7
	±SD	4.04	3.51	12.50	0.58	0.58
63.2	M	25.0	103.7	335.0	6.7	4.0
	±SD	3.46	5.69	35.0	1.53	1.00
20.0	M	24.0	105.7	321.7	7.7	5.3
	±SD	4.36	9.29	24.58	1.15	2.52
6.32	M	26.0	107.0	335.0	5.3	5.0
	±SD	6.93	11.79	30.27	2.31	4.36
2.0	M	22.0	106.7	348.3	4.7	4.0
	±SD	1.73	6.65	21.55	0.58	0.00
Solvent control: 200 µl/plate	M	25.3	107.0	343.0	8.7	6.0
	±SD	3.21	6.00	15.10	0.58	1.00
Positive control: substance		2-Aminoanthracene				
Concentration: (µg/plate)		2	2	2	2	2
	M	1326.3	1365.3	858.0	154.7	133.3
	±SD	246.27	116.62	42.93	11.37	10.69

M = mean number of revertants
SD = standard deviation

Study title: Mutagenicity Study of Omegavenos in the *Salmonella Typhimurium* reverse Mutation Assay *In Vitro*.

Study no.:	8197/93
Study report location:	Electronic submission; Pages 1136 - 1167
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 11, 1993.
GLP compliance:	Yes, OECD guidelines
QA statement:	Yes
Drug, lot #, and % purity:	Omegavenos, batch # DBFE 02

Key Study Findings

Omegavenos at a maximum concentration of 100 µl/plate did not produce any mutagenic effect in any of the five bacterial strains tested with and without metabolic activation.

Methods

Strains:	TA98, TA100, TA102, TA1535, TA1537
Concentrations in definitive study:	1 µl to 100 µl
Basis of concentration selection:	Cytotoxicity
Negative control:	DMSO
Positive control:	Sodium azide, 2-nitro-9H-fluorene, 9-amino-acridine, methyl methane sulfonate, 2-aminoanthracene
Formulation/Vehicle:	Water, DMSO, Ethanol.
Incubation & sampling time:	48 Hours

Study Validity

The following criteria were used for determining the study validity:
None of the bacterial strains, when streaked, should grow on media plates containing biotin only.
All the bacterial strains, when streaked, should show excessive growth on media plates with L-Histidine and biotin.
Application of 0.1 % crystal paper disc on media plates seeded with bacteria should give clear zones of inhibition in all tester strains.
A 10 µg ampicillin paper disc placed on plates seeded with bacteria should not show zones of inhibition with strains TA98 or TA 100 (indicating ampicillin resistance), but should show zones of inhibition with strains TA 1535, TA 1537, and TA 102.
The solvent control revertants/plate should be within the historical control range.

Results

None of the five tested concentrations (ranging from 1 µl to 100 µl) of Omegavenos from the two independent experiments (with and without metabolic activation) showed any cytotoxic effects.

There was no mutagenic effect observed for Omegavenos tested up to 100 µl/plate in any of the five tester strains in two independent experiments with and without metabolic activation. The study findings are summarized in the Sponsor's table below.

Mutagenicity Study of Omegavenos in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (First experiment without metabolic activation).

Summarized data
without metabolic activation

Substance (µl/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
1st experiment						
Omegavenös:						
100	M	25.7	108.3	248.3	12.7	3.0
	± SD	1.5	3.1	6.5	3.1	1.0
31.6	M	25.3	120.0	240.7	10.3	1.3
	± SD	4.7	6.6	9.1	0.6	0.6
10	M	23.0	110.7	240.3	11.7	3.0
	± SD	2.6	3.1	12.1	1.5	1.0
3.16	M	22.7	102.7	237.0	12.3	4.7
	± SD	2.1	5.5	6.2	2.5	3.8
1.0	M	23.3	111.0	244.0	14.3	4.3
	± SD	4.2	1.7	11.4	4.0	1.5
Solvent control: 100 µl/plate	M	24.3	113.0	277.3	10.7	2.3
	± SD	2.5	4.6	11.6	2.5	0.6
Positive control: substance		2-Nitro-fluorene	Sodium-azide	Methyl methane sulfonate	Sodium-azide	9-Amino-acridine
Concentration: (µg/plate)		10	10	1300	10	100
	M	842.0	1607.3	1273.7	1225.7	1306.7
	± SD	90.4	412.0	120.6	117.4	34.3

M = mean number of revertants
SD = standard deviation

Mutagenicity Study of Omegavenos in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (Second experiment without metabolic activation).

Summarized data
without metabolic activation

Substance (µl/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
2nd experiment						
Omegavenös:						
100	M	22.3	105.0	263.3	22.7	8.7
	± SD	2.1	3.0	6.1	4.0	2.1
31.6	M	26.7	104.3	257.0	18.7	11.7
	± SD	4.0	1.5	18.2	2.3	2.1
10	M	21.7	107.0	253.0	25.0	10.7
	± SD	3.8	3.0	16.8	3.0	1.5
3.16	M	21.3	110.7	243.7	21.0	11.7
	± SD	3.8	5.7	14.2	6.2	1.2
1.0	M	24.3	100.7	247.0	23.3	6.7
	± SD	2.3	0.6	12.1	0.6	2.9
Solvent control: 100 µl/plate	M	21.7	102.0	258.7	22.7	9.0
	± SD	1.5	1.0	15.4	3.8	1.0
Positive control: substance		2-Nitro-fluorene	Sodium-azide	Methyl methane sulfonate	Sodium-azide	9-Amino-acridine
Concentration: (µg/plate)		10	10	1300	10	100
	M	1164.3	1667.3	1957.0	1421.3	1038.7
	± SD	59.2	68.1	24.9	108.8	82.6

M = mean number of revertants
SD = standard deviation

Mutagenicity Study of Omegavenos in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (First experiment with metabolic activation).

Summarized data
with metabolic activation

Substance (µl/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
1st experiment						
Omegavenös:						
100	M	22.0	102.7	286.3	13.7	4.0
	± SD	3.5	11.2	14.3	2.5	2.0
31.6	M	22.3	117.7	283.3	17.7	2.3
	± SD	2.3	2.9	17.1	7.6	1.5
10	M	21.7	105.0	292.3	10.7	4.3
	± SD	2.5	8.7	6.8	2.3	0.6
3.16	M	26.3	105.7	301.0	15.7	5.0
	± SD	0.6	5.1	3.0	1.2	1.0
1.0	M	26.0	101.3	293.7	10.0	3.7
	± SD	3.0	8.5	7.6	2.0	1.5
Solvent control: 100 µl/plate:	M	21.7	110.3	313.3	22.3	6.3
	± SD	0.6	10.1	3.5	7.5	1.5
Positive control: substance		2-Aminoanthracene				
Concentration: (µg/plate)		2	2	2	2	2
	M	1290.0	2071.7	1071.3	95.0	123.0
	± SD	176.7	351.7	80.6	4.0	17.6

M = mean number of revertants
SD = standard deviation

Mutagenicity Study of Omegavenos in the *Salmonella typhimurium* Reverse Mutation Assay *In vitro* (Second experiment with metabolic activation).

Summarized data
with metabolic activation

Substance (µl/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
2nd experiment						
Omegavenös:						
100	M	23.7	107.7	327.3	13.7	9.0
	± SD	2.1	6.7	24.2	1.2	3.5
31.6	M	21.7	104.3	320.7	22.7	11.0
	± SD	1.2	1.2	20.2	3.2	2.6
10	M	21.0	114.7	330.0	17.0	12.0
	± SD	1.0	4.9	19.7	2.0	1.7
3.16	M	25.7	105.0	324.7	22.0	10.3
	± SD	2.3	1.7	6.0	1.0	4.9
1.0	M	21.7	109.0	322.0	16.0	10.0
	± SD	2.1	5.2	7.0	2.0	1.7
Solvent control: 100 µl/plate:	M	24.3	106.0	333.7	16.3	10.7
	± SD	2.3	6.1	2.5	6.1	2.9
Positive control: substance		2-Aminoanthracene				
Concentration: (µg/plate)		2	2	2	2	2
	M	1175.3	1722.0	1323.0	145.0	171.0
	± SD	61.7	71.5	93.4	7.5	20.4

M = mean number of revertants
SD = standard deviation

Study title: Study to Determine the Ability of Fat Emulsion 73403 to Induce Mutation in Four Histidine-Requiring Strains of *Salmonella Typhimurium* and Two Tryptophan-Requiring Strains of *Eschericia Coli*.

Study no.:	982/3
Study report location:	9296848, Pages 1 to 59
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 12, 1992.
GLP compliance:	Yes, OECD guidelines.
QA statement:	Yes
Drug, lot #, and % purity:	Fat Emulsion 73403, batch # 3560-025.

Key Study Findings

Fat emulsion 73403 induced mutation in *Eschericia coli* strain WP2 uvrA pKM101 when tested up to 5,000 µg/plate (a precipitating dose), both in the absence and presence of a rat liver metabolic activating system (S-9). Subsequent experiments using this *E.coli* strain failed to detect similar increases in revertant numbers. No mutagenic activity was demonstrated in the 4 Salmonella strains or the *E.coli* strain WP2 pKM101. Precipitation was observed on all plates treated with the maximum (fat emulsion 73403) test dose of 5,000 µg/plate, and also on treatments of 2,500 µg/plate in the absence of S-9 only.

Methods

Strains:	TA98, TA100, TA1535, TA1537, WP2 pKM101, WP2 uvra pKM101.
Concentrations in definitive study:	312.5 to 5,000 µg
Basis of concentration selection:	Toxicity
Negative control:	Acetone, Butylated hydroxyl-anisole (BHA)
Positive control:	2-Nitrofluorene (2NF), Sodium azide (NaN_3), 9-Aminoacridine (AAC), 4-Nitroquinoline-1-oxide (NQO), 2-Aminoanthracene (AAN).
Formulation/Vehicle:	Acetone
Incubation & sampling time:	72 hours

Study Validity

The following criteria were used for determining the study validity
The assay was considered valid if:

(1) The mean negative control counts fell within the normal range of the historical negative (solvent) control values for *Salmonella Typhimurium* and *E.coli* test strains.

(2) The positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S-9 preparation.

(3) No more than 5 % of the plates were lost through contamination or some other unforeseen event.

Results

None of the 5 concentrations of Fat emulsion 73403 up to 5,000 µg/plate from two independent experiments (with and without metabolic activation), showed mutagenic effects, (which would be indicated by thinning of the background bacterial lawn or marked reductions in revertant numbers) in the 4 *Salmonella typhimurium* and 1 *Eschericia.coli* tester strains.

However, precipitation was observed on all plates treated with the maximum (fat emulsion 73403) test dose of 5,000 µg/plate, and also on treatments of 2,500 µg/plate in the absence of S-9 only.

Fat emulsion 73403 was able to induce mutation in *Eschericia coli* strain WP2 uvrA pKM101 when tested up to 5,000 µg/plate (a precipitating dose), both in the absence and presence of a rat liver metabolic activating system (S-9). However, subsequent experiments using this *E.coli* strain failed to detect similar increases in revertant numbers. No mutagenic activity was demonstrated in the 4 Salmonella strains or the *E.coli* strain WP2 pKM101. The study findings are summarized in the Sponsor's table below.

Mutagenicity Study of Fat Emulsion 73403 in the *E.coli* Reverse Mutation Assay *in vitro* (First Experiment without metabolic activation)

Study Number	:KVS 11/S			Experiment Number	:1		
Positive Control	:NQO			S-9 Present	:NO		
Compound Name	:Fat Emulsion 73403			Strain Number	:WP2 uvrA pKM101		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	8	40	200	1000	5000	2
Revertants	129	104	110	107	141	279P	989
	109	112	100	108	126	318P	946
	127	90	100	100	127	295P	923
	115						
	133						
Mean	122.6	102.0	103.3	105.0	131.3	297.3	952.7
Increase over Control		0.8	0.8	0.9	1.1	2.4^	7.8
Standard Deviation	10.1	11.1	5.8	4.4	8.4	19.6	33.5
R-Coefficient			0.52	0.33	0.53	0.98	
Gradient			-0.40	-0.05	0.02	0.04	
Degrees of freedom			9	12	15	18	
Significance			NS	NS	*	***	
Dunnett's t value			-3.08	-2.85	-2.59	1.24	19.51
Significance			NS	NS	NS	NS	***
M Statistic	0.75						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

Mutagenicity Study of Fat Emulsion 73403 in the *E.coli* Reverse Mutation Assay *in vitro* (First Experiment with metabolic activation)

Study Number	:KVS 11/S			Experiment Number	:1		
Positive Control	:N/A			S-9 Present	:YES		
Compound Name	:Fat Emulsion 73403			Strain Number	:WP2 uvrA pKM101		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	0
Dose ug/plate	0	8	40	200	1000	5000	0
Revertants	137	124	112	133	99	284P	
	141	129	131	118	128	291P	
	154	144	143	140	156	289P	
	144						
	142						
Mean	143.6	132.3	128.7	130.3	127.7	288.0	
Increase over Control		0.9	0.9	0.9	0.9	2.0^	
Standard Deviation	6.3	10.4	15.6	11.2	28.5	3.6	
R-Coefficient			0.50	0.31	0.24	0.94	
Gradient			-0.33	-0.04	-0.01	0.03	
Degrees of freedom			9	12	15	18	
Significance			NS	NS	NS	***	
Dunnett's t value		-1.07	-1.44	-1.26	-1.61	11.01	
Significance		NS	NS	NS	NS	***	
M Statistic	1.52						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

Mutagenicity Study of Fat Emulsion 73403 in the *E.coli* Reverse Mutation Assay *in vitro* (Second) Experiment without metabolic activation)

Study Number	:KVS 11/S			Experiment Number	:2		
Positive Control	:NQO			S-9 Present	:NO		
Compound Name	:Fat Emulsion 73403			Strain Number	:WP2 uvrA pKM101		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	312.5	625	1250	2500	5000	2
Revertants	109	137	162	157	205P	271P	785
	122	130	128	165	208P	274P	692
	116	127	135	168	212P	335P	773
	124						
	108						
Mean	115.8	131.3	141.7	163.3	208.3	293.3	750.0
Increase over Control		1.1	1.2	1.4	1.8	2.5^	6.5
Standard Deviation	7.3	5.1	18.0	5.7	3.5	36.1	50.6
R-Coefficient			0.77	0.90	0.97	0.98	
Gradient			0.04	0.04	0.04	0.04	
Degrees of freedom			9	12	15	18	
Significance			**	***	***	***	
Dunnett's t value		1.81	2.91	5.21	9.48	16.37	
Significance		NS	*	***	***	***	
M Statistic	1.16						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

Mutagenicity Study of Fat Emulsion 73403 in the *E.coli* Reverse Mutation Assay *in vitro* (Second) Experiment with metabolic activation)

Study Number	:KVS 11/S			Experiment Number	:2		
Positive Control	:N/A			S-9 Present	:YES		
Compound Name	:Fat Emulsion 73403			Strain Number	:WP2 uvrA pKM101		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	0
Dose ug/plate	0	312.5	625	1250	2500	5000	0
Revertants	159	166	195	165	169	294P	
	175	144	156	173	208	279P	
	147	158	178	191	221	255P	
	135						
	153						
Mean	153.8	156.0	176.3	176.3	199.3	276.0	
Increase over Control		1.0	1.1	1.1	1.3	1.8^	
Standard Deviation	14.8	11.1	19.6	13.3	27.1	19.7	
R-Coefficient			0.54	0.57	0.72	0.93	
Gradient			0.03	0.02	0.02	0.02	
Degrees of freedom			9	12	15	18	
Significance			NS	*	**	***	
Dunnett's t value		0.20	1.83	1.84	3.56	8.80	
Significance		NS	NS	NS	**	***	
M Statistic	1.70						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

Mutagenicity Study of Fat Emulsion 73403 in the *Salmonella Typhimurium* Reverse Mutation Assay *in vitro* (First Experiment without Metabolic Activation)

Study Number	:KVS 11/S			Experiment Number	:1		
Positive Control	:2NF			S-9 Present	:NO		
Compound Name	:Fat Emulsion 73403			Strain Number	:TA98		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	8	40	200	1000	5000	50
Revertants	25	23	35	19	21	11P	1681
	23	14	22	19	18	21P	1749
	22	20	20	31	17	20P	2181
	17						
	21						
Mean	21.6	19.0	25.7	23.0	18.7	17.3	1870.3
Increase over Control		0.9	1.2^	1.1	0.9	0.8	86.6
Standard Deviation	3.0	4.6	8.1	6.9	2.1	5.5	271.2
R-Coefficient			0.40	0.14	0.24	0.33	
Gradient			0.12	0.01	-0.00	-0.00	
Degrees of freedom			9	12	15	18	
Significance			NS	NS	NS	NS	
Dunnett's t value		-0.75	0.96	0.31	-0.80	-1.27	
Significance		NS	NS	NS	NS	NS	
M Statistic	1.22						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

Mutagenicity Study of Fat Emulsion 73403 in the *Salmonella Typhimurium* Reverse Mutation Assay *in vitro* (First Experiment with Metabolic Activation)

Study Number	:KVS 11/S			Experiment Number	:1		
Positive Control	:AAN			S-9 Present	:YES		
Compound Name	:Fat Emulsion 73403			Strain Number	:TA98		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	8	40	200	1000	5000	5
Revertants	15	16	9	12	20	19P	831
	13	20	15	14	17	7P	L
	14	20	9	17	9	12P	950
	19						
	22						
Mean	16.6	18.7	11.0	14.3	15.3	12.7	890.5
Increase over Control		1.1^	0.7	0.9	0.9	0.8	53.6
Standard Deviation	3.8	2.3	3.5	2.5	5.7	6.0	84.1
R-Coefficient			0.63	0.25	0.04	0.23	
Gradient			-0.16	-0.01	-0.00	-0.00	
Degrees of freedom			9	12	15	18	
Significance			NS	NS	NS	NS	
Dunnett's t value		0.64	-1.87	-0.68	-0.46	-1.39	
Significance		NS	NS	NS	NS	NS	
M Statistic	1.22						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

Mutagenicity Study of Fat Emulsion 73403 in the *Salmonella Typhimurium* Reverse Mutation Assay *in vitro* (Second Experiment without Metabolic Activation)

Study Number	:KVS 11/S			Experiment Number	:2		
Positive Control	:2NF			S-9 Present	:NO		
Compound Name	:Fat Emulsion 73403			Strain Number	:TA98		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	312.5	625	1250	2500	5000	50
Revertants	38	L	29	26	25P	21P	1796
	C	33	29	38	13P	31P	1624
	17	23	32	27	24P	32P	1658
	20						
	L						
Mean	25.0	28.0	30.0	30.3	20.7	24.7	1692.7
Increase over Control		1.1	1.2	1.2^	0.8	1.0	67.7
Standard Deviation	11.4	7.1	1.7	6.7	6.7	5.5	91.1
R-Coefficient			0.33	0.30	0.28	0.23	
Gradient			0.01	0.00	-0.00	-0.00	
Degrees of freedom			6	9	12	15	
Significance			NS	NS	NS	NS	
Dunnett's t value		0.55	0.97	0.99	-0.73	0.05	
Significance		NS	NS	NS	NS	NS	
M Statistic	2.00						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

Mutagenicity Study of Fat Emulsion 73403 in the *Salmonella Typhimurium* Reverse Mutation Assay *in vitro* (Second Experiment with Metabolic Activation)

Study Number	:KVS 11/S			Experiment Number	:2		
Positive Control	:AAN			S-9 Present	:YES		
Compound Name	:Fat Emulsion 73403			Strain Number	:TA98		
Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	312.5	625	1250	2500	5000	5
Revertants	25	19	15	25	19	22P	1047
	15	16	14	15	18	15P	803
	23	13	17	15	18	22P	713
	17						
	13						
Mean	18.6	16.0	15.3	18.3	18.3	19.7	854.3
Increase over Control		0.9	0.8	1.0	1.0	1.1^	45.9
Standard Deviation	5.2	3.0	1.5	5.8	0.6	4.0	172.8
R-Coefficient			0.38	0.04	0.08	0.22	
Gradient			-0.01	-0.00	0.00	0.00	
Degrees of freedom			9	12	15	18	
Significance			NS	NS	NS	NS	
Dunnett's t value		-0.85	-1.07	-0.09	0.01	0.40	
Significance		NS	NS	NS	NS	NS	
H Statistic	0.89						

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Mutagenicity Study of SMOF 20 % in mammalian cells (V79) in the in vitro gene mutation assay (HPRT Test).

Study no.:	10554/97
Study report location:	EDR; Pages 000245 to 000272
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 12, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SMOF 20 %, lot # EFC 385; Aqua ad iniectabilia, lot # 6291A41A

Key Study Findings

SMOF 20 % at a concentration of 5.0% (in the presence and absence of metabolic activation in two independent experiments) did not induce any mutation in the HPRT-V79 mammalian cells in conditions where positive controls exerted potent mutagenic effects.

Methods

Cell line:	HPRT-V79 mammalian cells
Concentrations in definitive study:	0.313 % to 5.0 % (v/v) SMOF 20 %
Basis of concentration selection:	Highest concentration of test compound was tolerated by the cells.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	Ethyl methanesulfonate (EMS) (absence of metabolic activation); 9,10-dimethyl-1,2-benzanthracene (DMBA) (presence of metabolic activation)
Formulation/Vehicle:	Water for Injection
Incubation & sampling time:	24 hrs, and 4 hrs

Study Validity

If the negative control (DMSO) and positive controls (EMS, DMBA) show normal results, and if the test compound does not increase the mutation frequency 2-fold above the mean of the negative control or if the mutation frequency is always lower than 20 x 10^{-6} cells in both independent experiments, the compound is considered as negative in the test.
If there is a dose dependent increase of the mutation frequency in both independent experiments (at similar concentrations) to at least 2-fold the solvent control, or if the mutation frequency is always lower than 20 x 10^{-6} cells in the presence and/or absence of S9 mix, the compound is considered as positive in the test.

Results

The mutation frequency for SMOF 20 % at concentrations of 0.313, 0.625, 1.25, 2.5, and 5.0 % (without metabolic activation) ranged from 4.2 to 15.8 x 10^{-6} clonable cells. Since these results are within the normal range of the negative controls (1.1 and 4.3 x 10^{-6}), no significant difference was observed according to the criteria for the assay evaluation.

The mutation frequency of SMOF 20 % at concentrations of 0.313, 0.625, 1.25, 2.5, and 5.0 % (with metabolic activation) ranged from 1.5 to 14.0 x 10^{-6} clonable cells. Since these results are within the normal range of the negative controls (2.3 and 10.9 x 10^{-6}), no significant difference was observed according to the criteria for assay evaluation.

According to the criteria for this assay evaluation, SMOF 20 % did not induce any mutations in the HPRT-V79 mammalian cells.

Results are shown in the Sponsor's tables below.

Mutagenicity Study of SMOF 20 % in the *In Vitro* HPRT Point Mutation Assay (First experiment without S9 mix)

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	Plating Efficiencies PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells x 10^6 exposed to thioguanine (d)	Mutation frequency x 10^{-6} (e)
Control: Aqua ad iniectabilia											
0	5.0	1 : 20	0.91	0.92	3	1	0	1	0	4.60	1.1
SMOF 20%											
0.313	5.0	1 : 20	1.35	1.16	7	11	5	4	4	5.80	5.3
0.625	5.0	1 : 20	0.82	0.94	6	17	17	11	9	4.70	12.8
1.25	5.0	1 : 20	0.53	0.96	14	15	22	12	12	4.80	15.8
2.5	5.0	1 : 20	0.35	0.88	3	14	9	6	1	4.40	7.5
5.0	5.0	1 : 20	0.23	1.00	11	4	14	10	9	5.00	9.6
EMS (µg/ml)											
600	1.0	1 : 20	0.19	0.41	488	463	422	399	455	2.05	1086.3
700	1.0	1 : 20	0.04	0.30	499	511	520	530	491	1.50	1700.7

for a, b, c, d, and e please see page 18

Mutagenicity Study of SMOF 20 % in the *In Vitro* HPRT Point Mutation Assay (Second experiment without S9 mix)

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	Plating Efficiencies PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells x 10^6 exposed to thioguanine (d)	Mutation frequency x 10^{-6} (e)
Control: Aqua ad iniectabilia											
0	5.0	1 : 20	1.11	0.87	3	2	4	5	5	4.40	4.3
SMOF 20%											
0.313	5.0	1 : 20	0.96	0.98	6	3	3	4	9	4.90	4.5
0.625	5.0	1 : 20	0.50	0.95	2	2	7	4	5	4.75	4.2
1.25	5.0	1 : 20	0.87	0.98	15	8	16	13	3	4.90	11.2
2.5	5.0	1 : 20	0.77	1.07	16	9	12	10	3	5.35	9.3
5.0	5.0	1 : 20	0.25	1.00	14	12	9	14	11	5.00	12.0
EMS (µg/ml)											
600	1.0	1 : 20	0.19	0.36	549	534	491	560	499	1.80	1462.8
700	1.0	1 : 20	0.06	0.25	491	464	500	495	539	1.25	1991.2

for a, b, c, d, and e please see page 18

Mutagenicity Study of SMOF 20 % in the *In Vitro* HPRT Point Mutation Assay (First experiment with S9 mix)

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells x 10^8 exposed to thioguanine (d)	Mutation frequency x 10^{-6} (e)
Control: Aqua ad iniectabilia											
0	5.0	1 : 20	0.88	1.21	2	4	4	1	3	6.05	2.3
SMOF 20%											
0.313	5.0	1 : 20	0.62	1.07	0	2	1	1	4	5.35	1.5
0.625	5.0	1 : 20	0.70	0.89	0	4	1	2	2	4.45	2.0
1.25	5.0	1 : 20	0.75	1.05	3	0	5	4	3	5.25	2.9
2.5	5.0	1 : 20	0.78	0.78	3	1	1	1	6	3.90	3.1
5.0	5.0	1 : 20	0.93	0.95	0	2	2	3	0	4.75	1.5
DMBA (µg/ml)											
20	1.0	1 : 20	0.10	0.78	339	337	335	360	333	3.90	437.7
30	1.0	1 : 20	0.68	0.75	299	258	274	273	291	3.75	372.0

for a, b, c, d, and e please see page 18

Mutagenicity Study of SMOF 20 % in the *In Vitro* HPRT Point Mutation Assay (Second experiment with S9 mix)

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells x 10^8 exposed to thioguanine (d)	Mutation frequency x 10^{-6} (e)
Control: Aqua ad iniectabilia											
0	5.0	1 : 20	0.95	1.01	13	5	10	18	9	5.05	10.9
SMOF 20%											
0.313	5.0	1 : 20	0.74	0.99	8	3	7	5	1	4.95	4.8
0.625	5.0	1 : 20	0.76	1.04	1	6	10	7	10	5.20	6.5
1.25	5.0	1 : 20	0.84	1.03	16	23	23	7	3	5.15	14.0
2.5	5.0	1 : 20	1.03	0.97	2	2	2	10	10	4.85	5.4
5.0	5.0	1 : 20	0.71	1.01	8	2	3	3	3	5.15	3.7
DMBA (µg/ml)											
20	1.0	1 : 20	0.81	1.01	524	511	533	539	516	5.05	519.4
30	1.0	1 : 20	0.83	1.06	421	401	410	421	405	5.30	388.3

for a, b, c, d, and e please see page 18

Study title: *In Vitro* assessment of the Clastogenic Activity of SMOF 20 % in Cultured Human Peripheral Lymphocytes.

Study no.:	10555/97
Study report location:	EDR; Pages 000273 to 000309
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 6, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SMOF 20 %, lot # EFC 385; Aqua ad Iniectabilia, lot # 6291A41A

Key Study Findings

SMOF 20 % at a concentration of 2.5 % in the presence and absence of metabolic activation, with two exposure times without S9, and one exposure time with S9, did not induce chromosomal or chromatic damage in human lymphocytes.

Methods

Cell line:	Human lymphocyte cells
Concentrations in definitive study:	0.313 - 2.5 % SMOF 20 % or 0.625 - 5 mg/ml of exposure medium
Basis of concentration selection:	Cytotoxicity
Negative control:	Water for Injection
Positive control:	Mitomycin C, Cyclophosphamide
Formulation/Vehicle:	125 µl/5 ml or 2.5 %/ Water for Injection
Incubation & sampling time:	24 hrs, and 4 hrs

Study Validity

The criteria for positive results and method of analysis are adequate.
The highest concentration employed in the main study (2.5 % SMOF 20 %) should suppress the mitotic index by 50 – 80 %, while the lowest dose (0.313 % SMOF 20 %) should be in the region of the solvent (negative) control.

Results

In the 4-hr exposure of 0.313, 0.625, and 1.25 % (at non-cytotoxic) concentrations of SMOF 20 % to lymphocyte cells in the absence of S9 metabolic activation, the mean incidence of chromosomal aberrations (excluding gaps) in dividing cells ranged from 1.0 % to 2.5 %. These results are within normal range of the negative control where an incidence of chromosomal aberrations (excluding gaps) of 1.5 % was observed after 4-hr exposure.

At a cytotoxic SMOF 20 % concentration of 2.5 % (mitotic index 0.68), chromosomal aberrations increased to 6.0 % (not significant at $p \leq 0.05$).
In the 4-hr exposure of 0.313, 0.625, and 1.25 % (at non-cytotoxic) concentrations of SMOF 20 % to lymphocyte cells in the presence of S9 metabolic activation, the mean incidence of chromosomal aberrations (excluding gaps) in dividing cells ranged from 1.5 % to 2.0 %. These results are within normal range of the negative control where an incidence of chromosomal aberrations (excluding gaps) of 2.0 % was observed after 4-hr exposure, as shown in the Sponsor's table below.

SMOF 20 % Chromosome Aberrations in Human Peripheral Lymphocytes
In Vitro

Treatment (%)	4-h exposure		
	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps
with metabolic activation			
Aqua ad iniectabilia			
2.5	1.00	200	2.0
SMOF 20%			
0.313	1.08	200	1.5
0.625	1.07	200	1.5
1.25	0.80	200	2.0
2.5	0.78	200	4.0
Cyclophosphamide (µg/ml)			
10.0	0.99	200	12.0 *

\# = mitotic index: number of metaphases/1000 cells; negative control = 1.00
* = significantly different from negative control ($p \leq 0.05$)

SMOF 20 % at a concentration of 2.5 % (mitotic index 0.78), caused a small increase of 4.0 % (not significant at $p \leq 0.05$) of chromosomal aberrations to lymphocyte cells.
In the 24-hr exposure of 0.313 % SMOF 20 % (at a non-cytotoxic concentration) caused a small increase in chromosomal aberration to lymphocyte cells in the absence of S9 metabolic activation. The mean incidence of chromosomal aberrations (excluding gaps) in dividing cells was 3.5 %. This result is within normal range of the negative control where an incidence of chromosomal aberrations (excluding gaps) of 1.5 % was observed after 24-hr exposure.

SMOF 20 % Chromosome Analysis in Human Peripheral Lymphocytes
In Vitro

Treatment (%)	4-h exposure			24-h exposure		
	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps
without metabolic activation						
Aqua ad iniectabilia						
2.5	1.00	200	1.5	1.00	200	1.5
SMOF 20%						
0.313	0.93	200	1.0	1.02	200	3.5
0.625	0.91	200	1.5	0.48	200	6.0
1.25	0.93	200	2.5	0.23	200	5.5
2.5	0.68	200	6.0	0.16	144	5.5
Mitomycin C (µg/ml)						
0.1	0.95	200	12.0 *	0.59	200	16.0 *

= mitotic index: number of metaphases/1000 cells; negative control = 1.00
* = significantly different from negative control ($p = 0.05$)

At cytotoxic concentrations (0.625, 1.25, and 2.5 %) of SMOF 20 % (mitotic indices 0.48 to 0.16), an increase in chromosomal aberrations to 5.5 % and 6.0 % (not significant at $p \leq 0.05$) was observed.

The incidence of chromosomal aberrations (excluding gaps) of the negative controls was 0.0 % to 5.0 % for the last 30 experiments.
Thus, SMOF was not considered genotoxic under the conditions of the assay.

Study title: In Vitro Assessment of the Clastogenic Activity of Omegavenos in Cultured Human Peripheral Lymphocytes.

Study no.:	8199/93
Study report location:	Document 8, Volume 3, Page 1168-1210
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 12, 1993
GLP compliance:	Yes, OECD Guidelines.
QA statement:	Yes
Drug, lot #, and % purity:	Omegavenos, batch # DBFE 02.

Key Study Findings

Omegavenos tested in two independent tests at cytotoxic concentrations of up to 5.0 % in the presence and absence of metabolic activation did not show any indication of mutagenesis with respect to chromosomal or chromatic damage in human lymphocytes. In the same test, mitomycin C and cyclophosphamide induced significant damage.

Methods

Cell line:	Human lymphocyte cells
Concentrations in definitive study:	0.078 % - 5.0 % Omegavenos
Basis of concentration selection:	Cytotoxicity
Negative control:	0.9 % NaCl (Saline)
Positive control:	Mitomycin C, Cyclophosphamide
Formulation/Vehicle:	0.9 % NaCl (Saline)
Incubation & sampling time:	24 hours, and 2 hours.

Study Validity

The criteria for positive results and method of analysis are adequate.
The highest concentration of employed in this study (5.0 % Omegavenos) should suppress the mitotic index, while the lowest dose (0.078 % Omegavenos) should be in the region of the solvent (negative) control.

Results

In the 24 hours exposure of Omegavenos (without metabolic activation, absence of S9) at non-cytotoxic concentrations of 0.078 %, 0.156 %, 0.3131 %, the mean incidence of chromosomal aberrations (excluding gaps) ranged from 1.0 % to 4.0 % in the two independent experiments. These results are within the normal range of the negative controls where a mean incidence of chromosomal aberrations (excluding gaps) of 0 % to 1.0 % was observed.

At cytotoxic Omegavenos concentrations of 0.625 % and 1.25 % in the medium without metabolic activation, a slight but non-significant increase in the mean incidence of chromosomal aberrations (excluding gaps) to 6.5 % and 9.1 % was observed in the second experiment, but not in the first experiment. Hence, the increase can be considered as non-specific cytotoxic artefact at distinctly cytotoxic concentrations.

In the 2 hours exposure of Omegavenos (with metabolic activation, presence of S9) at non-cytotoxic concentrations of 0.078 %, 0.156 %, 0.3131 %, the mean incidence of chromosomal aberrations (excluding gaps) ranged from 0 % to 2.0 % in the two independent experiments. These results are within the normal range of the negative controls (0 % to 1.0 %), and hence no significance was observed.

At cytotoxic Omegavenos concentration of 5.0 % in the medium with metabolic activation, a marginal increase in the mean incidence of chromosomal aberrations (excluding gaps) to 4.0 % and 5.0 % was observed in both experiments, respectively.

This increase can be considered as non-specific cytotoxic artefact at cytotoxic concentrations and/or a non-specific effect resulting from a test substance concentration of 5.0 % (v/v) in the medium. A summary of the results is presented from the Sponsor's table below.

Omegavenos Chromosome Analysis in Human Peripheral Lymphocytes *In Vitro*

Treatment (% in the medium; v/v)	1st experiment			2nd experiment		
	mitotic index*	number of metaphases scored	% of cells with aberrations excluding gaps	mitotic index*	number of metaphases scored	% of cells with aberrations excluding gaps
without metabolic activation						
0.9% NaCl-solution						
5.0	1.00	100	1.0	1.00	100	0.0
Omegavenös						
0.078	0.67	100	2.0	0.91	100	1.0
0.156	0.46	100	2.0	0.89	100	1.0
0.313	0.37	100	4.0	0.68	100	3.0
0.625	0.30	17#	0.0	0.23	31#	6.5
1.25	0.20	7#	0.0	0.20	11#	9.1
Mitomycin C (µg/ml)						
0.1	0.41	100	11.0##	0.73	100	12.0##

\# = no more metaphases of sufficient quality for evaluation due to cytotoxicity of Omegavenös
= significantly different from negative control ($p \leq 0.05$)
* = negative control = 1.00

Omegavenos Chromosome Analysis in Human Peripheral Lymphocytes *In Vitro*

Treatment (% in the medium; v/v)	1st experiment			2nd experiment		
	mitotic index*	number of metaphases scored	% of cells with aberrations excluding gaps	mitotic index*	number of metaphases scored	% of cells with aberrations excluding gaps
with metabolic activation						
0.9% NaCl-solution						
5.0	1.00	100	1.0	1.00	100	0.0
Omegavenös						
0.313	0.65	100	0.0	1.02	100	0.0
0.625	0.68	100	0.0	0.95	100	0.0
1.25	0.60	100	1.0	0.84	100	2.0
2.5	0.45	100	3.0	0.57	100	2.0
5.0	0.47	100	5.0	0.50	100	4.0
Cyclophosphamide (µg/ml)						
10	0.95	100	11.0#	0.89	100	12.0#

\# = significantly different from negative control ($p \leq 0.05$)
* = negative control = 1.00

Study title: Study to Evaluate the Chromosome Damaging Potential of Fat Emulsion 73403 by its Effects on Cultured Human Lymphocytes using an In Vitro Cytogenetics Assay.

Study no.:	KVS11/HLC
Study report location:	Document No. 9296849, Pages 1 to 44
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 28, 1991.
GLP compliance:	Yes, compliance with OECD guidelines.
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 73403, batch # 3560-025.

Key Study Findings

Fat emulsion 73403 at a maximum test concentration of up to 1250 µg/ml (a dose above the solubility limit) in the absence and presence of metabolic activation with S-9 did not induce chromosome aberration in cultured human lymphocyte cells.

Methods

Cell line:	Human lymphocyte cells
Concentrations in definitive study:	703.1, 937.5, 1250 µg/ml
Basis of concentration selection:	Cytotoxicity
Negative control:	Acetone
Positive control:	4-Nitroquinoline 1-oxide (NQO), Cyclophosphamide (CPA).
Formulation/Vehicle:	Fat emulsion 73403 diluted with Acetone
Incubation & sampling time:	20 hours or 44 hours, and 3 hours.

Study Validity

The human lymphocyte assay was to be considered valid if the following criteria were met:
The top dose (1250 µg fat emulsion/ml) should be able to reduce the mitotic index (MI) by 50 – 80 %, or a concentration close to the solubility limit in the treated medium, while the lowest dose (703.1 µg fat emulsion/ml) should be in the region of the solvent control. The proportion of cells with structural aberrations (without gaps) in negative control cultures should fall within the normal range.

Results

Cultures receiving fat emulsion 73403 in the absence and presence of S-9 had a frequency of aberrant cells which was not significantly different from those in the concurrent solvent controls under all the sampling/treatment conditions.

Although the frequencies of cells with aberration exceeding the normal range were seen after 44 hours of treatment in the absence of S-9, this was not considered to be biologically significant because the increase was not statistically significant.

The frequencies of cells with numerical chromosome aberrations which fell within historical negative control ranges were observed following all the treatment conditions.

Fat emulsion 73403 was unable to induce chromosomal aberration in cultured human lymphocytes, when tested at a high concentration of up to 1250 µg/ml, a dose which was above its solubility limit, in both the absence and presence of S-9 activation. A summary of the results is shown below.

Mutagenicity Study of Fat Emulsion 73403 in Human Lymphocyte Cells in the Absence of S-9

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic Index (Mean)
Solvent	A	100	4	1		1.8
	B	100	5	2		2.2
	Totals	200	9	3		(2.0)
Untreated	A	100	6	3		4.4
	B	100	1	1		4.4
	Totals	200	7	4	NS	(4.4)
703.1	A	100	6	1		1.3
	B	100	4	1		2.1
	Totals	200	10	2	NS	(1.7)
937.5	A	100	6	3		1.0
	B	100	4	0		1.7
	Totals	200	10	3	NS	(1.4)
1250	A	100	5	2		1.6
	B	99	2	2		1.3
	Totals	199	7	4	NS	(1.5)
NQO, 2.5	A	25	**10**	8		
	B	25	**12**	**11**		
	Totals	50	22	19	p ≤0.001	

§ Statistical significance (Appendix 5a)
NS = not significant
Numbers in bold typeface exceed historical negative control ranges (Appendix 6)

Mutagenicity Study of Fat Emulsion 73403 in Human Lymphocyte Cells in the Presence of S-9

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic Index (Mean)
Solvent	A	100	4	2		3.6
	B	100	3	2		3.6
	Totals	200	7	4		(3.6)
Untreated	A	100	3	3		3.9
	B	100	2	1		3.3
	Totals	200	5	4	NS	(3.6)
703.1	A	100	7	1		4.0
	B	100	5	3		3.1
	Totals	200	12	4	NS	(3.6)
937.5	A	100	2	2		3.7
	B	100	0	0		3.3
	Totals	200	2	2	NS	(3.5)
1250	A	100	5	0		3.8
	B	100	0	0		3.7
	Totals	200	5	0	NS	(3.8)
CPA, 25	A	25	**18**	**17**		
	B	25	**9**	**9**		
	Totals	50	27	26	p ≤0.001	

§ Statistical significance (Appendix 5a)
NS = not significant
Numbers in bold typeface exceed historical negative control ranges (Appendix 6)

Mutagenicity Study of Fat Emulsion 73403 in Human Lymphocyte Cells in the Absence of S-9

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic Index (Mean)
Solvent	A	100	8	3		1.8
	B	100	11	3		1.1
	Totals	200	19	6		(1.5)
Untreated	A	100	6	1		1.7
	B	100	5	1		1.6
	Totals	200	11	2	NS	(1.8)
1250	A	100	9	5		0.7
	B	100	8	5		1.4
	Totals	200	17	10	NS	(1.1)

§ Statistical significance (Appendix 5b)

Numbers in bold typeface exceed historical negative control ranges (Appendix 6)

NS = not significant

Mutagenicity Study of Fat Emulsion 73403 in Human Lymphocyte Cells in the Presence of S-9

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic Index (Mean)
Solvent	A	100	2	0		2.8
	B	100	3	1		2.5
	Totals	200	5	1		(2.7)
Untreated	A	100	3	0		3.3
	B	100	5	0		2.6
	Totals	200	8	0	NS	(3.0)
1250	A	100	3	3		1.5
	B	100	2	1		2.5
	Totals	200	5	4	NS	(2.0)

§ Statistical significance (Appendix 5b)

NS = not significant

Study title: Study to Determine the Ability of Fat Emulsion 73403 to Induce Mutations at the Thymidine Kinase (*tk*) Locus in Mouse Lymphoma L5178Y Cells using a Fluctuation Assay.

Study no.:	KVS11/TK
Study report location:	9296850 Pages 1 - 43
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 20, 1992
GLP compliance:	Yes, Compliance with OECD guidelines.
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 73403, batch # 3560-025.

Key Study Findings

Fat emulsion 73403 at concentrations of 62.5 - 1,000 µg/ml (beyond its limit of solubility) was unable to induce mutation at the *tk* locus of mouse lymphoma L5178Y cells in the presence and absence of S-9 metabolic activation.

Methods

Cell line:	L5178Y
Concentrations in definitive study:	62.5 - 1,000 µg/ml
Basis of concentration selection:	Cytotoxicity
Negative control:	Acetone
Positive control:	4-nitroquinoline 1-oxide (NQO), Benzo (a) pyrene (BP).
Formulation/Vehicle:	Fat emulsion 73403 diluted with Acetone
Incubation & sampling time:	48 hours, and 3 hours

Study Validity

The following criteria were used to determine study validity:
The mutant frequencies in the negative (solvent) control cultures were within the normal range (not more than 3 times the historical mean value).
At least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies is greater than half the historical mean value).

Results

Following 3 and 48 hours of treatment with Fat emulsion 73403 at doses from 31.25 to 1,000 µg/ml, with and without metabolic activation, there was no relevant toxic effect as indicated by a relative total growth of 32 % in the parallel cultures.

In the absence of S-9, a statistically significant increase in mutant frequency was observed following treatment with 500 µg/ml of Fat emulsion 73403 in experiment 1, but at no other doses. Therefore, the increase in mutant frequency seen at 500 µg/ml in experiment 1 was not reproducible, and was considered a chance effect.

Precipitation of the test emulsion was observed at 500 and 1,000 µg/ml in the presence and absence of S-9.

In the first experiment after 2 days of treatment, the high dose (1,000 µg/ml) of Fat emulsion 73403 yielded 119.4 % survival in the absence of S-9 and 86. 6 % in the presence of S-9. Similarly, in the second experiment after 2 days of treatment, the high dose (1,000 µg/ml) of Fat emulsion 73403 yielded relative survival values of 105.2 % in the absence of S-9, and 96% % in the presence of S-9.

When tested at concentrations up to its limit of solubility, Fat emulsion 73403 failed to induce mutation at the *tk* locus of mouse lymphoma L5178Y cells. A summary of the study results is shown in the Sponsor's tables below.

% of relative survival in the Cytotoxicity Range-finder experiment

Treatment µg/ml	Survival (1) at day 0*	
	In the absence of S-9	In the presence of S-9
0	32	32
31.25	32	32
62.5	32	32
125	32	32
250	32	32
500	32	32
1000	32	32

(1) 160 cells/well plated (see Appendix 9)

* 32 wells scored

Mutagenicity study of Fat emulsion 73403 in Mouse Lymphoma L5178Y Cells

Experiment 1

Treatment (μg/ml)	-S-9 %RS	-S-9 Mutant frequency		Treatment (μg/ml)	+S-9 %RS	+S-9 Mutant frequency	
0	100.0	185.72		0	100.0	318.30	
62.5	87.8	202.33	NS	62.5	93.8	197.75	NS
125	100.0	183.81	NS	125.5	102.9	143.63	NS
250	105.1	197.82	NS	250	91.9	226.73	NS
500	108.1	328.57	*	500	92.5	194.57	NS
1000	119.4	204.07	NS	1000	86.8	226.72	NS
Linear trend		NS		Linear trend		NS	
NQO				BP			
0.05	104.4	513.64		2	66.7	1004.49	
0.1	95.8	491.68		3	41.9	1136.72	

Experiment 2

Treatment (μg/ml)	-S-9 %RS	-S-9 Mutant frequency		Treatment (μg/ml)	+S-9 %RS	+S-9 Mutant frequency	
0	100.0	160.01		0	100.0	161.90	
125	124.0	180.45	NS	125	97.9	207.02	NS
250	103.0	146.11	NS	250	109.7	224.96	NS
500	100.0	186.34	NS	500	101.4	168.02	NS
1000	105.2	166.51	NS	1000	96.5	162.46	NS
Linear trend		NS		Linear trend		NS	
NQO				BP			
0.05	99.3	450.40		2	68.5	957.31	
0.1	61.6	504.69		3	26.9	915.78	

NS Not significant
*,**,*** Significant at 5%, 1% and 0.1% level respectively

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

Study title: Study to evaluate the Potential of Fat Emulsion 73403 to Induce Micronuclei in the Polychromatic Erythrocytes of CD-1 Mice.

Study no:	KVS11/NMT
Study report location:	9296851, Pages 1 - 34
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 4, 1991
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 73403, batch # 3560-025

Key Study Findings

Fat emulsion 73403 at a maximum dose of 25 ml/kg did not induce micronuclei in the polychromatic erythrocytes (PCEs) of the bone marrow of CD-1 male and female mice.

Methods

Doses in definitive study:	6.25, 12.5 and 25 ml/kg
Frequency of dosing:	Daily
Route of administration:	Intravenous
Dose volume:	25 ml/kg
Formulation/Vehicle:	0.9 % NaCl
Species/Strain:	CD-1 Mice
Number/Sex/Group:	6/sex/group
Satellite groups:	None
Basis of dose selection:	Maximum tolerated dose (MTD)
Negative control:	0.9 % NaCl
Positive control:	Cyclophosphamide (CPA)

Study Validity

The following criteria were used for determining the study validity:
Limited heterogeneity with acceptable variability between animals receiving the low and intermediate doses sampled at 24 hours.
The incidence of micronucleated polychromatic erythrocytes (PCEs) in negative control groups fell within or close to the historical vehicle control range.

Results

The mean frequency of micronucleated PCEs (per 1,000 cells) of the cells treated with Fat emulsion 73403 (6.25 – 25 ml/kg) ranged from 0.25 to 1.05 % at 24 and 48 after administration, and are within the historical negative control range (0 – 1.80 %).
When comparisons were made between test emulsion 6.25 or 12.5 ml/kg treated mice and saline treated mice, small but statistically significant increases were apparent for

animals receiving the low and intermediate doses sampled at 24 hours. This was because of a very low frequency of micronuclei seen in the concurrent saline control. However, the absence of a dose-response relationship in the test emulsion treated mice and the absence of any bone marrow toxicity may show that the differences do not convey the induction of micronuclei.
A summary of the study results from the Sponsor's submission is presented in the tables below.

Micronuclei Assay in Bone Marrow Cells of CD-1 Mice with Fat Emulsion 73404

24 hour sampling:

Treatment (ml/kg x 4)	PCE scored	MN observed	MN/ 1000 cells	Hetero-geneity χ^2	Significance	2 x 2 Contingency χ^2	Significance
Untreated	20064	14	0.70	7.42	NS		
6.25	20055	21	1.05	17.59	p ≤0.05	1.03	NS
12.5	20055	20	1.00	20.06	p ≤0.05	0.74	NS
25	20075	5	0.25	4.99	NS	3.37	NS
CPA,40 (mg/kg x 1)	20051	280	13.97			240.8	p ≤0.001

Linear trend: z = -2.180, p ≤ 0.05 (significant negative trend)

48 hour sampling:

Treatment (ml/kg x 4)	PCE scored	MN observed	MN/ 1000 cells	Hetero-geneity χ^2	Significance	2 x 2 Contingency χ^2	Significance
Untreated	20059	14	0.70	5.99	NS		
25	20054	13	0.65	4.70	NS	0.00	NS

NS = not significant

MN = micronuclei

Note: No statistically significant differences were observed between untreated and treated groups when males and females were analysed separately so only analyses on combined data are presented

Micronuclei Assay in Bone Marrow Cells of CD-1 Mice with Fat Emulsion 73404

24 hour sampling:

Treatment (ml/kg x 4)	PCE scored	MN observed	MN/ 1000 cells	Hetero-geneity χ^2	Significance	2 x 2 Contingency χ^2	Significance
Negative (saline)	20050	8	0.40	7.00			
6.25	20055	21	1.05	17.59	$p \leq 0.05$	4.97	$p \leq 0.05$
12.5	20055	20	1.00	20.06	$p \leq 0.05$	4.32	$p \leq 0.05$
25	20075	5	0.25	4.99	NS	0.31	NS
CPA,40 (mg/kg x 1)	20051	280	13.97			256.8	$p \leq 0.001$

Linear trend: z = -1.246, not significant

48 hour sampling:

Treatment (ml/kg x 4)	PCE scored	MN observed	MN/ 1000 cells	Hetero-geneity χ^2	Significance	2 x 2 Contingency χ^2	Significance
Negative (saline)	20055	6	0.30	4.01	NS		
25	20054	13	0.65	4.70	NS	1.90	NS

NS = not significant

MN = micronuclei

Note: A statistically significant difference was only observed between control and treated groups at a single data point (24 hour females receiving 6.25 ml/kg x4) when males and females were analysed separately. Only analyses on combined data are presented

Intravenous CPA administration (positive control) in CD-1 mice at 40 mg/kg showed a statistically significant increase in induced micronucleus frequency.

Study title: In Vivo Bone Marrow Cytogenetics Test of SMOF 20 % by Intravenous Administration to Sprague-Dawley Rats (Chromosomal Analysis).

Study no:	10556/97
Study report location:	EDR, Pages 1 to 38
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 30, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SMOF 20 % lot # EFC 385; 0.9 % NaCl, Lot # 7091P14F; CPA, lot # 73H 0846

Key Study Findings

SMOF 20 % at 10 g TG/kg (50 ml/kg) was not mutagenic and did not induce any chromosomal abberations in a rat bone marrow cytogenetic study.

Methods

Doses in definitive study:	10 g TG/kg
Frequency of dosing:	Single IV dose
Route of administration:	IV
Dose volume:	50 ml/kg
Formulation/Vehicle:	NaCl vehicle solution
Species/Strain:	Crl: CD Sprague-Dawley Rat
Number/Sex/Group:	5/sex/group
Satellite groups:	No
Basis of dose selection:	Dose selected (50 ml/kg) was based on the maximum reasonable application volume for an IV bolus injection.
Negative control:	0.9 % NaCl
Positive control:	Cyclophosphamide (CPA), 27 mg/kg, IP

Study Validity

The analysis for structural aberrations (chromosome and chromatid type) was recorded in 50 cells (50 metaphases) per animal, and cells with an incomplete number of centromeres or insufficient spreading were not used for analysis. Metaphases which differed from the normal diploid complement were excluded from evaluation.

Results

The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with SMOF 20 % ranged from 0.6 % to 0.8 % at 6, 24, and 48 hrs after administration, and are within the normal range of the negative control and not significant.

The number of cells with gaps was also within the range of the negative controls (treated groups: 7.4 % to 9.0 %; controls: 9.6 %)

In Vivo Rat Bone Marrow Cytogenetic Test of Intravenous SMOF 20 %

Compound ml/kg b.w. i.v.	Sampling time (h)	Number of metaphases analysed	Mitotic index*	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi²-test (aberrations excluding gaps)
0.9% NaCl solution 50 ml/kg b.w. i.v.							
	24	500	1.00	9.6	10.2	0.6	-
SMOF 20%							
50	6	500	0.85	9.0	9.6	0.8	n.s.
50	24	500	0.78	7.4	8.0	0.8	n.s.
50	48	500	0.76	7.8	8.0	0.6	n.s.
Cyclophosphamide							
27 mg/kg b.w. i.p.	24	500	0.30	28.6	53.8	45.6	s.

s. significant at $p \leq 0.01$
n.s. not significant at $p \leq 0.01$
* negative control = 1.00

In conclusion, SMOF 20 % did not reveal any mutagenic properties with respect to structural chromosomal aberrations in the rat bone marrow cytogenetic study tested at a dose level of 50 ml/kg IV (10 g/TG/kg).

Study title: In Vivo Bone Marrow Cytogenetic Test of Omegavenos by Intravenous Administration in Sprague-Dawley Rats (Chromosomal Analysis).

Study no:	8200/93
Study report location:	Document 10, Vol. 3, Page 1242 - 1280
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 12, 1993
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Omegavenos, batch # DBFE 02

Key Study Findings

Omegavenos at 40 ml/kg body weight I.V. was not mutagenic in a rat bone marrow cytogenetic study.

Methods

Doses in definitive study:	40 ml/kg
Frequency of dosing:	Single intravenous dose
Route of administration:	Intravenous
Dose volume:	40 ml/kg
Formulation/Vehicle:	0.9 % Nacl (saline)
Species/Strain:	Crl: CD Sprague-Dawley Rat
Number/Sex/Group:	5/sex/group
Satellite groups:	No
Basis of dose selection:	Dose selection was based on the maximum reasonable dosage.
Negative control:	0.9 % NaCl (Saline)
Positive control:	Cyclophosphamide (CPA), 27 mg/kg, IP

Study Validity

The analysis for structural aberrations (chromosome and chromatic type) was recorded in 50 cells (50 metaphases) per animal, and cells with an incomplete number of centromeres or insufficient spreading were not used for analysis. Metaphases which differed from the normal diploid complement were excluded from evaluation.

Results

The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with Omegavenos ranged from 0.2 % to 0.6 % at 6, 24 and 48 hours after administration, and are within the normal range of the negative control and not significant.

The number of cells with gaps was also within the range of the negative controls (treated groups: 3.2 % to 3.8 %; controls: 4.2 %). Omegavenos did not depress the mitotic index. A summary of the findings is shown in the Sponsor's table below.

In Vivo Rat Bone Marrow Cytogenetic Test of Intravenous Omegavenos

Compound ml/kg b.w. i.v.	Sampling time (h)	Number of metaphases analysed	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi²-test (aberrations excluding gaps)
0.9% NaCl-solution						
40	24	500	4.0	4.2	0.2	-
Omegavenös						
40	6	500	3.0	3.6	0.6	n.s.
40	24	500	3.6	3.8	0.2	n.s.
40	48	500	2.8	3.2	0.4	n.s.
Cyclophosphamide						
27 mg/kg b.w. i.p.	24	500	17.2	41.2	31.0	s.

n.s. not significant
s. significant at $p \leq 0.05$

In conclusion, Omegavenos did not reveal any mutagenic properties with respect to structural chromosomal aberrations in the rat bone marrow cytogenetic study.

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Study title: Fat Emulsion 73403: Teratology Study in the Rabbit

Study no.:	94/KAB021/0212
Study report location:	EDR; Document 2153OF, Pages 832 to 975.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 30, 1992
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 73403, lot # 3561-001; Intralipid 20 %, lot # 64615-51; Ringer acetate, lot # 91J30J59

Key Study Findings

The IV infusion of Fat emulsion 73403 to pregnant rabbits during the period of organogenesis, at doses of 3, and 4.5 g TG/kg/day and Intralipid 20 % at a dose of 4.5

g TG/kg/day had no adverse effect on fetal survival, and fetal growth, and development *in utero* were unaffected by the fat emulsions in all treatment groups.

Fat emulsion 73403 administration was associated with reduced food and water intake, and fecal output. There was increased plasma levels of triglycerides and free fatty acids on study days 6 and 18, with both lipid emulsion treatment, but was more pronounced in the Intralipid treatment group. Increased embryo-fetal loss was seen in Fat emulsion 73403 (4.5 g TG/kg/day dose group) treated animals, as indicated by abortion and increased post-implantation loss observed in one female rabbit. However, this incidence was not dose responsive and does not appear to be significant.

Methods

Doses:	0 (control), 3, 4.5 g TG/kg/day (Fat emulsion 73403); 4.5 g TG/kg/day Intralipid 20 %.
Frequency of dosing:	4 hr/day for 13 days (days 6 to 19 of gestation)
Dose volume:	22.5 ml/kg/day Control, (Intralipid 20 %) 15, and 22.5 ml/kg/day (Fat emulsion 73403).
Route of administration:	Intravenous (IV) via ear vein
Formulation/Vehicle:	500 ml stock/Ringer acetate vehicle control
Species/Strain:	New Zealand White Rabbits
Number/Sex/Group:	18 pregnant females/group (Control, group 1); 16 and 17 pregnant females/ group (3, 4.5 g Fat emulsion 73403, dose groups 2 and 3); 13 pregnant females/group (Intralipid 20 %, group 4).
Satellite groups:	4 pregnant females from groups 2 and 4 for TK
Study design:	4 groups of pregnant female rabbits, with 3, and 4.5 mg/kg Fat emulsion 73403 (dose groups 2 and 3); and control and Intralipid 20 % (reference article) (dose groups 1 and 4).
Deviation from study protocol:	A TK rabbit from the Intralipid dose group 4 died on study day 12 from accidental overdose of the reference lipid emulsion at an unintended dose of 7.9 mg/kg/h (31.6 ml/kg/day).

Observations and Results

Mortality

All the study animals were examined twice daily at the beginning and end of the infusion for mortality and morbidity.

One female rabbit in group 3 (4.5 g TG/kg/day) was found dead on day 28 post-coitus, without any untoward clinical signs, while 3 females were euthanized; one group 2 female (3 g TG/kg/day) with strangulated abdominal hernia was sacrificed on day 29 post coitus; one group 3 female sacrificed on day 28 was presented with weight loss, reduced food intake, fecal output, bradypnea; one group 3 female was sacrificed on day 10 post-coitus due to the poor ear vein condition, since dosing has to be discontinued.

Necropsy of all the unscheduled dead animals revealed only non-specific findings, and was therefore not related to fat emulsion 73403 dosing.
A satellite group female rabbit from the intralipid dose group 4 died on day 12 from accidental overdose of the reference article at an unintended dose of 7.9 ml/kg/hr (31.6 ml/kg/day).

Clinical Signs

All animals were examined at least once daily for clinical signs of ill health or toxicity.
The clinical conditions of the Fat emulsion 73403 treated animals and the volume control females were similar, although increased incidence of loose or reduced fecal output was recorded in females dosed with Fat emulsion 73403 or Intralipid 20 %.

Body Weight

All animals were weighed and examined daily throughout the study duration.

Body weights of treated and control animals were similar in spite of a slight loss in body weight by all animals at the start of infusion.

Feed Consumption

Food consumption was recorded daily for each of the study animal.

Significant reduction in food intake was observed during the treatment period for the treatment group animals versus the control animals. The decreased food consumption was dose related in the test article dose groups. The food consumption in all the animals was essentially similar during the post-treatment phase.

Toxicokinetics

Blood samples were obtained from the central auricular artery of each animal prior to, and 2 to 4 hrs after the start of dosing on gestational day 6 and on gestational day 18. Blood was analyzed for β-Hydroxybutyrate (β-HBD), Triglycerides, and lactate levels.

The plasma triglyceride levels increased during the infusion of both the test and reference articles on both gestational day 6 and 18, with a mean triglyceride level of 32 and 26 mmol/L, following infusion of Fat emulsion 73403 for 4 hrs on the respective days. Similarly, the mean triglyceride levels for 4 hrs of Intralipid 20 % infusion on gestational day 6 and day 18 were 22 and 16 mmol/L, respectively.

Infusion of fat emulsion 73403 resulted in increased plasma β-HBD, which was more pronounced on day 18 (mean of 1.28 mmol/l at 4 hrs) than on day 6 (mean of 0.51 mmol/l at 4 hrs). There were no significant changes in β-HBD with the infusion of Intralipid 20 % on day 6, whereas increased values were observed on day 18 (mean of 1.07 mmol/l at 4 hrs).

The measured plasma free fatty acids (FFA) in rabbits increased during day 6 and day 18 of the test and reference fat emulsions treatment, with higher FFA levels (range 5.3-9.3 mmol/L at 4 hrs) for the test article animals than in the reference group animals (range 1.9-3.8 mmol/L at 4 hrs). The increase was generally less obvious with repeated lipid infusions.
The infusion of Fat emulsion 73403 resulted in the elevation of medium-chain free fatty acids (MCFFA), both octanoic acid (C8:0) and decanoic acid (C10:0), with values ranging from 3.3 to 5.8 mmol/L at 4 hrs after the beginning of infusion.

There were no consistent changes in plasma lactate levels observed with any of the fat emulsion dose groups.

Necropsy

At the end of dosing on day 19 of gestation, or day 29 for satellite animals, rabbits were euthanized prior to macroscopic examination.
All animals found dead, moribund, or animals which aborted were also sacrificed and subjected to detailed examination.

Necropsy of the female rabbits on day 29 of gestation did not show any macroscopic changes considered related to fat emulsion 73403 treatment

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The ovaries and uteri of all the animals were dissected out and examined for:
1. Number of corpora lutea in each ovary
2. Number of implantation sites
3. Number of early or late resorption sites
4. Number and distribution of live and dead fetuses in each uterine horn.

A slight increase in post-implantation loss was observed in group 3 Fat emulsion 73403 (4.5 g TG/kg/day) females, in comparison to the volume control and historical control values, with the volume controls at the upper limit of the historical control range. The increase was not dose responsive and does not appear to be significant.

The number of corpora lutea, implantations, viable fetuses, pre- and post-implantation loss in group 2 (3.0 g TG/kg/day) females were unaffected by dosing. A summary of the findings is shown in the Sponsor’s table below.

Group Mean Litter Data in Rabbits

Group mean litter data - females killed on Day 29 of gestation

Group	1	2	3	4
Compound	Ringer acetate	Fat emulsion 73403		Intralipid® 20%
Dosage (ml/kg/day)	22.5	15	22.5	22.5

Group	Number of pregnant animals		% Abortion and total litter loss[σ]	Corpora lutea count	Implant-ations	Viable young M	Viable young F	Viable young Total	Resorptions Early	Resorptions Late	Resorptions Total	Implantation loss (%) Pre-	Implantation loss (%) Post-
1	13	Mean	0.0	10.7	8.7	3.2	4.0	7.2	1.1	0.5	1.5	18.7	17.7
		SD		2.3	3.0	2.3	2.2	2.7	1.0	0.7	1.2		
2	15	Mean	6.7	9.9	7.9	3.4	3.7	7.1	0.4	0.3	0.7	21.4	9.1
		SD		1.7	3.2	2.0	2.1	3.0	0.7	0.5	0.8		
3	15	Mean	13.3	10.0	8.5	2.9	3.4	6.3	2.1	0.1	2.2	15.4	25.5
		SD		1.6	2.3	1.9	1.9	2.9	1.4	0.3	1.5		
4	18	Mean	0.0	9.8	7.9	4.1	2.9	7.1	0.8	0.1	0.9	18.8	11.2
		SD		2.2	3.2	2.2	2.2	2.6	0.9	0.2	0.9		
Background control data (69 studies)													
Mean			0.2	10.7	8.8	4.1	4.0	8.0	0.4	0.5	0.9	17.3	8.9
Low			0.0	9.1	6.7	2.9	2.7	5.9	0.1	0.1	0.2	2.2	2.8
High			20.0	12.8	11.1	5.4	6.0	10.3	0.9	1.3	1.9	29.2	19.7

Means are derived from females which survived to term and bore viable young.

σ See Appendix 2.
SD Standard deviation.

Offspring (Malformations, Variations, etc.)

Fetuses were euthanized by subcutaneous injection of Pentobarbitone sodium, and for each live fetus, and dead fetus, the following data were recorded:
1. Individual fetal weight.
2. Individual placental weight
3. Fetal sex
4. Skeletal or visceral abnormalities (fixed in 95 % alcohol, and examined using Alizarin standing technique).

Reduced fetal weights observed in group 3 and 4 animal dose groups were considered due to isolated litters with exceptionally small fetuses, and not related to treatment.
The fetal anomalies observed after skeletal examinations in all groups showed incidences that have been previously seen in historical controls, and bear no consistent relationship to the Fat emulsion treatment. Summary of findings are presented in the Sponsor's tables below.

Mean Fetal and Placental Weights (g) in Rabbits

Group	: 1	2	3	4
Compound	: Ringer acetate	Fat emulsion	73403	Intralipid® 20%
Dosage (ml/kg/day)	: 22.5	15	22.5	22.5

Group		Foetal weight (g)			Placental weight (g)
		Male	Female	Overall	Overall
1	Mean	42.0	41.9	42.5	5.7
	SD	3.0	2.9	2.0	0.3
	n	11	13	13	13
2	Mean	43.3	40.0	42.3	5.9
	SD	1.9	2.3	1.9	0.3
	n	14	13	14	14
3	Mean	39.0	38.6	38.8	5.9
	SD	1.7	2.4	2.0	0.3
	n	12	13	13	13
4	Mean	36.2*	35.5*	36.9*	6.1
	SD	3.3	2.8	1.9	0.3
	n	17	17	18	18
Background control (69 studies)					
Mean				40.4	5.5
Low				36.1	4.9
High				45.3	6.5

SD Standard deviation.
n Number of litters.
* Significantly different from Controls; P<0.05 (t-test).

Summary of foetal observations at skeletal examination

Group	: 1	2	3	4
Compound	: Ringer acetate	Fat emulsion	73403	Intralipid® 20%
Dosage (ml/kg/day)	: 22.5	15	22.5	22.5

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	93(13)	100(14)	82(13)	127(18)	7608 foetuses	69 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Vertebrae						
Incomplete ossification of one thoracic vertebral centrum	0.0(0)	0.0(0)	2.4(2)	0.0(0)	0.18	0.0- 2.4
Thoracic vertebra with bipartite centra or agenesis of a hemicentrum	0.0(0)	2.0(1)	1.2(1)	0.0(0)	#	#
Incompletely ossified or asymmetric costal elements of sacral vertebrae	1.1(1)	6.0(6)	9.8(6)	4.7(5)	5.31	0.0-11.8
One caudal vertebra offset, tail tip kinked	0.0(0)	1.0(1)	0.0(0)	1.6(2)	0.53	0.0- 3.1
Caudal vertebrae fused, short and kinky tail	0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.04	0.0- 1.1
26 pre-sacral vertebrae	77.4(13)	84.0(13)	62.2(13)	69.3(18)	80.13	61.4-93.5
27 pre-sacral vertebrae	22.6(8)	16.0(8)	36.6(10)	30.7(13)	16.98	5.2-38.6
Anomalous lumbar vertebrae$^{\sigma}$	0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.03	0.0- 1.2
Anomalous thoracic vertebrae resulting in scoliosis$^{\sigma}$	0.0(0)	0.0(0)	0.0(0)	0.8(1)	0.03	0.0- 1.4

ϕ One foetus may have more than one observation.
No previous record in background control data.
σ See key to Appendix 8.

Summary of foetal observations at skeletal examination

Group	: 1	2	3	4
Compound	: Ringer acetate	Fat emulsion 73403		Intralipid^{R} 20%
Dosage (ml/kg/day)	: 22.5	15	22.5	22.5

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	93(13)	100(14)	82(13)	127(18)	7608 foetuses	69 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Limbs and girdles						
Incomplete ossification of heads of limb long-bones	33.3(12)	33.0(10)	48.8(11)	56.7(15)	56.65	0.0-78.0
Additional ossification of one or both olecranon processes	3.2(2)	12.0(5)	2.4(2)	2.4(3)	1.63	0.0-10.3
One or both centrales incompletely ossified or unossified	0.0(0)	3.0(2)	8.5(2)	4.7(3)	1.10	0.0- 7.3
Metacarpals and/or phalanges incompletely ossified or unossified	8.6(5)	18.0(5)	20.7(6)	21.3(10)	14.33	2.1-32.7
Acromion processes elongated	0.0(0)	2.0(2)	1.2(1)	0.0(0)	0.26	0.0- 4.0
Pubic bones incompletely ossified or unossified	0.0(0)	3.0(2)	6.1(1)	3.9(2)	1.46	0.0- 7.1
Asymmetric pelvis, ilial bones associated with different sacral vertebrae	1.1(1)	5.0(5)	8.5(6)	3.9(4)	4.38	0.0-11.2
Double association pelvis, ilial bones associated with both sacral vertebrae	2.2(2)	0.0(0)	1.2(1)	1.6(2)	2.10	0.0-15.5

ϕ One foetus may have more than one observation.

In conclusion, IV infusion of Fat emulsion 73403 to pregnant rabbits during the period of organogenesis, at doses of 3, and 4.5 g TG/kg/day and Intralipid 20 % at a dose of 4.5 g TG/kg/day had no adverse effect on fetal survival, and fetal growth, and development *in utero* were unaffected by the fat emulsion at the administered doses in all treatment groups.

Developmental Toxicity Study in Rats and Rabbits Administered an Emulsion Containing Medium Chain Triglycerides as an Alternative Caloric Source. S. Henwood, D. Wilson, R. White, and S. Trimbo. Fundamental and Applied Toxicology 40, p185-190 (1997)

Groups of pregnant Crl:CD BR rats (25 or 29/group) and Hra:(NZW)SPF rabbits were randomly assigned to 1 of 3 groups. Animals in group 1 (control) received 0.9 % NaCl at a dose volume of 21.4 ml/kg. Animals in groups 2 and 3 received a 20 % lipid emulsion (containing medium chain triglyceride (MCT) and long chain triglyceride (LCT) at a 3:1 ratio, respectively) at a lipid dose of 1 or 4.28 g/kg and dose volumes of 5 or 21.4 ml/kg, respectively. The dose was administered daily to the rats by intravenous (IV) infusion for 4 hours/day from gestational day (GD) 6 through GD 15 through a caudal vein. The dose was administered daily to the rabbits through a marginal ear vein for 5 hours/day from GD 7 through GD 19. Animals were observed twice daily (AM and PM) for mortality and/or moribundity, and once daily, predose and postdose for clinical signs of toxicity. Body weights were recorded daily from GD 5 through GD 20 for rats and from GD 7 through GD 29 for rabbits. Feed consumption data were collected daily beginning on the day of receipt for rats and on GD 6 for rabbits.

On GD 20 (for rats) and GD 29 (for rabbits), the animals were euthanized and complete necropsy performed. Lesions were preserved in 10 % phosphate buffered formalin. The ovaries were removed and examined, and the number of corpora lutea recorded. The uterus was excised, weighed, and the number and placement of implantation sites (live and dead fetuses and early and late resorptions) were recorded. Animals that died or had unscheduled euthanasia were also examined. Conceptuses were removed, and live fetuses were weighed, examined externally, sexed (rats only), and then euthanized. The internal organs of the rabbit fetuses were examined for variations and malformations. Fetuses were examined for visceral, skeletal and soft tissue variations or malformations.

There were no mortalities in the rat; the maternal clinical signs observed are tail lesions, red-tinged urine and vaginal bleeding. The incidences of tail discoloration and ulceration in the rat dams are 1 of 25, 14 of 25, and 23 of 29 in the control, low and high dose groups, respectively, and ranged from mild to severe with some necrosis and partial tail loss. This finding is related to the extravasation of the MCT:LCT lipid test article into perivascular areas. The occurrence of red tinged urine (8 of 29) and vaginal bleeding (1 of 29) was occasional in the high dose (4.28 g/kg lipid) animals. Lower body weight and decreased food consumption were consistently observed in the high dose group during the dosing period. The decreased feeding in the high dose group is due to the high-caloric nature of the test article. Apart from the tail effects, necropsy findings in the high dose group include enlarged lymph nodes, enlarged lymph, hydronephrosis/enlarged renal pelvis, small thymus, and small red lung foci.

There was a slight dose-response trend of decreasing mean gravid uterine weights with increasing test article dose; however, because of large group variability, the group mean uterine weights appeared similar. All females were pregnant (with the exception of one control dam) and had at least one viable fetus/litter (i.e. no dams had total resorption).

There were no significant group differences in preimplantation or postimplantation loss or in the mean percentage of live or resorbed fetuses; no dead fetuses were present. Mean fetal sex ratios of the test article-treated groups were comparable to that of controls, and there were no effects of the test article on mean fetal body weight. There were no test article-related fetal external, soft tissue, or skeletal changes observed in the rats. Omphalocele and cleft palate were observed in one control and in one high dose fetus. Malformed/misshapen skull bone skeletal malformation was observed in one fetus each from two control litters.

In the rabbit study, there were no deaths in the 1 g/kg lipid dose group. 1 rabbit in the control group died on gestational day (GD) 11, and one rabbit given 4.28 g/kg lipid was sacrificed after abortion on GD 20. The euthanized rabbit had the lowest food consumption in the group. The single rabbit abortion was within the range of historical control incidence.

Clinical observations in the surviving rabbit dams was limited to decreased or no fecal output intermittently in the high dose group, during the dosing period. Mean body weight

changes and feed consumption were also found to be decreased in the high dose animals, and were attributable to the high caloric nature of the test-article. There were no test-article-related findings at necropsy, and all the pregnant rabbits had at least one viable fetus at scheduled cesarean section on GD 29 (i.e. no dams had total resorption).

The NOEL for developmental toxicity of a 20 % lipid emulsion administration in pregnant rats was ≥ 4.28 g lipid/kg.

In the rabbits, the mean percentage of total resorptions/litter (postimplantation loss) and the proportion of fetuses and litters with external morphological abnormalities were significantly higher in the high dose group than in the control group; whereas, the mean percentage of live fetuses/litter and mean adjusted fetal body weights were significantly lower in the high dose groups, when compared to control animals. Notable morphological abnormalities observed include rachischisis (3 high dose litters) and short tail (2 high dose litters) and single incidences of other abnormalities all in the high dose group.

The total incidence of litters in the 1 g/kg lipid group with fetuses having soft tissue abnormalities was higher than in the control group, but not statistically significant. The incidences of visceral abnormalities were higher in the 4.28 g/kg lipid group than in the control group, but not statistically significant. In general soft tissue abnormalities were present as single fetal or litter incidences in 3 or 4 litters/group in treated rabbits. The proportions of fetuses and litters in the high dose group with skeletal abnormalities (skull bones unossified, presacral vertebrae, misaligned sternebrae) were significantly higher in the high dose group than in the control animal group. The incidence of any of these findings was not markedly higher than that of controls because, usually, one litter was affected with a given abnormality.
The NOEL for developmental toxicity of a 20 % lipid emulsion administration in pregnant rabbits was ≥ 1 g lipid/kg.

In conclusion, the oral administration the IV administration of a 20 % lipid emulsion (containing medium chain triglyceride (MCT) and long chain triglyceride (LCT) at a 3:1 ratio, respectively) resulted in expected lower food consumption in rats and rabbits at the high dose level, but also in lower maternal mean body weights in the high dose rats. Cesarean section examinations in rabbits showed higher postimplantation loss and, correspondingly, fewer live fetuses in the high dose group. Similar observations were not seen in treated rats. The NOEL dose for developmental toxicity of the 20 % lipid emulsion infusion in rats is 4.28 g/kg, and 1 g/kg in rabbits.

9.3 Prenatal and postnatal development

Study on the Perinatal and Postnatal Toxicity in the Rat of AG 103, a Mixture Based on Eicosapentaenoic Acid and Docosahexaenoic Acid at 85 %. M. De Bernardi., S. Contos., and F. Feletti. University of Pavia, Institute of Pharmacology II, Department of Toxicology II

Groups of 80 sexually matured female Wistar rats weighing 280 – 300 g were mated successfully to adult male Wistar rats, and placed in individual cages. The animals were dosed from gestational day (GD) 16 up to 3 weeks of lactation. The dams were randomly divided into 4 groups of 20 animals each and treated with AGP 103, the test article at doses of 0 (control), 100, 350 and 600 mg/kg/day. Treated females had AGP-103 added to their daily diet from the GD 16 up to 3 weeks of lactation. The body weight of the female rats was recorded from GD 14 to GD 21 and postnatal day (PD) 14 and 21. The food consumption was measured daily. After birth, the newborn animals were weighed and examined macroscopically for any malformations in the live or dead fetuses. If birth did not take place on the expected day, the females were sacrificed at GD 24 and the fetuses were evaluated for vitality, malformations, and the number of implantations and resorptions. The newborn pups were also weighed on GD 14 and 21. The maternal mortality rates were recorded throughout the study period, while the weight of the offspring was recorded during suckling.

There was no animal mortality in this study. The control and treated rat dams all gained weight during GD 14 to GD 21, and there was no difference in the food consumption between control rats and rats treated with AGP 103 at the respective doses.
There were no statistically significant differences observed in the percentage of pregnancies between the control and treatment groups. The impregnation rate was between 88 and 100%, with a mean gestational duration of 22 days.

The mean number of live fetuses per female and the number of dead fetuses recorded at birth were similar for the control and treatment groups. No abnormalities or macroscopically perceptible malformations were found at the time of birth or while the offspring were suckling. The mean weight of the live fetuses at birth and the survival rate of the offspring during lactation were similar in the control and treated animal groups. The weight gain in the surviving newborn animals was similar in control and treated animals. Treatment of the rat dams with AGP 103 did not produce any toxic effects in the fetuses either directly or through suckling. A summary of findings is shown in the Sponsor's table below.

Group Mean Data in the Treatment of Pregnant rats with AGP 103

TREATMENT	CONTROLS	100 mg/kg/day	t	p	350 mg/kg/day	t	p	600 mg/kg/day	t	p
pregnancy (%)	97.75 ± 4.06	96.50 ± 4.81	0.89	≥ 0.05	97.40 ± 3.84	0.28	≥ 0.05	95.95 ± 4.91	1.26	≥ 0.05
weight gain wk 3	59.33 ± 1.26	59.65 ± 1.18	0.83	≥ 0.05	58.19 ± 1.83	2.29	≥ 0.05	46.74 ± 1.34	30.61	≤ 0.001
food consumed 16-22 days (g)	200.60 ± 4.75	198.55 ± 5.37	1.28	≥ 0.05	194.80 ± 5.43	3.59	≤ 0.001	146.95 ± 5.61	33.19	≤ 0.001
food consumed 3rd wk lact.	1059.60 ± 4.32	1060.40 ± 3.83	0.62	≥ 0.05	1057.25 ± 5.51	1.5	≥ 0.05	912.55 ± 2.89	126.5	≤ 0.001
gestation (days)	22 ± 0	22 ± 0	-	-	22 ± 0	-	-	22 ± 0	-	-
mortality (%)	0 ± 0	0 ± 0	-	-	0 ± 0	-	-	0 ± 0	-	-
survival (%)	100 ± 0	100 ± 0	-	-	100 ± 0	-	-	100 ± 0	-	-
live foetuses (No.)	0.85 ± 1.23	9.90 ± 1.25	0.13	≥ 0.05	9.70 ± 1.22	0.39	≥ 0.05	9.80 ± 1.11	0.13	≥ 0.05
dead foetuses (No.)	0.35 ± 0.49	0.40 ± 0.50	0.32	≥ 0.05	0.40 ± 0.50	0.32	≥ 0.05	0.45 ± 0.51	0.63	≥ 0.05
mean weight live foetuses (g)	5.52 ± 0.25	5.47 ± 0.24	0.64	≥ 0.05	5.55 ± 0.24	0.39	≥ 0.05	5.50 ± 0.25	0.25	≥ 0.05
mean weight males at 14 days (g)	24.07 ± 0.49	24.09 ± 0.59	0.12	≥ 0.05	24.09 ± 0.39	0.14	≥ 0.05	24.12 ± 0.52	0.31	≥ 0.05
mean weight females at 14 days (g)	24.05 ± 0.55	24.12 ± 0.55	0.4	≥ 0.05	24.16 ± 0.47	0.68	≥ 0.05	24.10 ± 0.45	0.31	≥ 0.05
mean weight males 3rd wk of lactation	42.94 ± 0.57	42.89 ± 0.61	0.27	≥ 0.05	42.96 ± 0.58	0.11	≥ 0.05	42.90 ± 0.57	0.22	≥ 0.05
mean weight females 3rd wk of lactation	43.38 ± 0.33	43.57 ± 0.37	1.71	≥ 0.05	43.49 ± 0.35	1.02	≥ 0.05	43.13 ± 0.47	1.95	≥ 0.05
survival at 21 days(%)	93.80 ± 4.30	94.05 ± 3.97	0.19	≥ 0.05	94.75 ± 3.86	0.73	≥ 0.05	92.20 ± 3.81	1.24	≥ 0.05
major malformations	-	-	-	-	-	-	-	-	-	-

In conclusion, in perinatal and postnatal toxicity study of AGP (a mixture of eicosapentaenoic and docosahexaenoic acid at 85 %) in pregnant rats at doses of 0, (control), 100, 350 and 600 mg/kg/day, there were no evidences of test article-related toxicity in dams or offspring from any treatmrnt group.. However, a reduction in the amount of feed consumed by the treated animals was proportional to the administered dose, which also resulted in a parallel reduction in the weight gain of the dams during the last week of gestation.

10 Special Toxicology Studies

Local Tolerance Studies

Local Tolerance Test of Lipovenos 20 % in Rabbits after a Single Intravenous, Intra-arterial, Paravenous, Intramuscular and Subcutaneous Administration
(**RPT # 9803/1/96**

The objective of this study was to determine the local tolerance of rabbits to Lipovenos 20 % after a single intravenous, intra-arterial (IA), paravenous (PV), Intramuscular (IM), and subcutaneous (SC) injection.

Methods:

Doses: Lipovenos 20 % (lot # FG 1544) was administered by single dose infusion at 18.8 ml/hr for 4 hrs (75.2 ml/animal) IV, and IA; at 0.5 ml/animal for the PV and SC injection; and 1 ml/animal for the IM injections. 0.9 % NaCl solution was used as the negative vehicle control.

Study design: 12 Male and female (6/sex) Himalayan rabbits, 14-16 weeks old, and weighing 2.7-3.2 kg were infused with lipovenos 20 %, 75.2 ml IV (via marginal ear vein); 75.2 ml IA (via central ear artery); 0.5 ml PV (beside the *vena saphena parva*); 0.5 ml SC (under the dorsal skin); and 1 ml IM (into the gastrocnemius muscle).

The test solution was administered once on the left side of each animal, and for the IV and IA infusion, two separate animals were used.
As a control, each animal's right side was treated with 0.9 % NaCl solution.
The IV infusion was performed first, followed by the IM, PV, and SC injections at the end of the 4 hr infusion, one after the other, but the IA infusion was done in separate rabbits.

For the IV and IA infusion of 18.8 ml Lipovenos 20 %, a corresponding 18.8 ml of 0.9 % NaCl was also infused. Dosing for the IM, PV, and SC were similarly administered.
In all cases, the dosing started with the test solution on the animal's left side, followed by the 0.9 % NaCl solution on the rabbit's right side.

Clinical signs and mortality were checked and recorded daily, and local reactions were inspected macroscopically 2, 24, 48, 96 hrs, and 14 days after administration in the appropriate rabbit.

Two rabbits/sex/group were sacrificed 48 hrs, 96 hrs, and 14 days after dosing. The injection sites were dissected out along with samples of affected tissues and control tissues (approx., 5 mm thickness) fixed in 10 % buffered formalin, stained with hematoxylin-eosin after paraffin sectioning, and examined histologically.

Results

The IV, and IA infusion sites and the PV, IM, and SC injection sites did not show any macroscopically visible changes in both groups of study animals.

Histopathological examinations of the tissues did not show any local changes at the test article-treated and control IV infusion sites in both groups.

Microscopic examination of the IA infusion sites showed a slight thrombosis in the two animals dissected after 48 hrs in the test group. A slight thrombosis was also observed in one of the IA control sites at the 48 hr time point.

Histopathological examination of the PV injection sites revealed minimal or moderate inflammation, mild/moderate mesenchymal activation, mild edema and minimal or mild hemorrhage in both test group and control animals.

Microscopic examination of the IM injection showed mild, moderate or marked necrosis and mild mesenchymal activation in 3 animals from the test group and 2 animals from the control group.

Histopathology of the SC group showed mild or moderate edema in 3 animals, a mild mesenchymal activation in one animal from the test group, and only a mild edema in a 48 hr exposed animal from the control group.

None of the tested dose levels caused any systemic intolerance reactions or body weight changes, and none of the animals died prematurely.

In conclusion, there appeared to be no local intolerance reactions following the IV administration of 75.2 ml (18.8 ml/animal/hr for 4 hrs) of lipovenos 20 % in rabbits. Moderate but up to 14 days subsiding intolerance reactions were observed following IM injections (administrations made in error) in rabbits.

Local Tolerance Test of Omegavenos (10 % fat emulsion) in Rabbits after a Single Intravenous, Intra-arterial, Paravenous, Intramuscular and Subcutaneous Administration (**RPT No. 8876/94)**

The objective of this study was to determine the local tolerance of rabbits to Lipovenos 20 % after a single intravenous (IV), intra-arterial (IA), paravenous (PV), Intramuscular (IM), and subcutaneous (SC) injection.

Methods:

Doses: Omegavenos 10 % (batch # DH 1030) was administered by single dose infusion at 30 ml/animal/hr intravenous (IV) and IA; at 0.5 ml/animal/hr paravenous (PV) and subcutaneous (SC), and 1 ml/animal/hr for 4 hours; 0.9% NaCl solution was used as the negative vehicle control.
Study design: 6 male and female (3/sex) Himalayan rabbits, 5.5 to 6.5 months old and weighing 3.0 kg each were infused with Omegavenos 10 %, 30 ml IV (via marginal ear vein); 30 ml IA (via central ear artery); 0.5 ml PV (beside the vena saphena parva); 0.5 ml SC (under the dorsal skin); and 1 ml IM (into the gastrocnemius muscle).

The test emulsion was administered once on the left side of each animal. The application areas were sheared and disinfected with 70 % ethanol before administration. As a control, each animal's right side was treated with the same volumes of 0.9 % NaCl solution accordingly. The IM, PV and SC injection sites were marked with Indian ink. The duration of infusion was 4 hours; speed of injection for IM, PV and SC injection was dose/30 sec.

Clinical signs and mortality were checked and recorded daily, and local reactions were inspected macroscopically 2, 24, 48, 96 hrs, and 14 days after administration in the appropriate rabbit.

One rabbit/sex/group was sacrificed 48 hrs, 96 hrs, and 14 days after dosing. The injection sites were dissected out, along with samples of affected tissues and control tissues (approx., 5 mm thickness), fixed in 10 % buffered formalin, stained with

hematoxylin-eosin after paraffin sectioning of 3-5 µm tissue, and examined histologically.

Results

The rabbits did not show any test article-related clinical signs, and their body weight was within the normal range.

The IV and IA infusion sites did not show any macroscopically visible changes in both groups of study animals.

A single PV injection of 0.5 ml Omegavenos caused macroscopically, a slight edema at the test article injection site of male rabbit no.1 assigned to dissection after 48 hours. Fatty deposits were found in the musculature and fasciae of the test article treatment site of female rabbit no.4 (96 hour dissection). The PV control site of female rabbit no.2 after 48 hour dissection showed a slight hematoma.

A single IM injection of 1 ml Omegavenos/animal caused fatty deposits in female rabbit no. 4 assigned to 96 hour dissection. There were no changes observed at the IM control sites in the animals.

After a single SC injection of 0.5 ml Omegavenos/animal, fatty deposits were detected in the musculature and fasciae of male rabbit no.3 and female rabbit no.4, both assigned to the 96 hour dissection. The SC control site of male rabbit no.1 assigned to 48 hour dissection showed a very small hematoma.

There were no test article-related histopathological findings observed at the infusion sites after a single IV and IA infusion of 30 ml Omegavenos /hr/animal for 4 hours, or the same volume of the control solution. A slight thrombosis was found at the IV infusion site of a female rabbit no.4 assigned to 96 hour dissection. This effect was however related to the 4 hour infusion.

After PV injection of 0.5 ml/animal, a moderate edema was seen in one of the two test article-treated sites after 48 hours; a moderate inflammation in the 4 rabbits assigned to dissection after 48 and 96 hours, and a mild or very mild inflammation in the 2 rabbits with 14 day dissection. One of the test article sites showed a slight necrosis after 96 hour treatment, whereas, two test article-treated sites show a very mild mesenchymal activation. In the PV control injection sites, a small hematoma was detected in one animal after 48 hour dissection, a mild inflammation after a 48 and 96 hr dissection, and a slight necrosis and moderate edema in one animal after a 96 hour dissection.

Administration of 1 ml Omegavenos/animal IM caused a slight to marked necrosis in 3 rabbits after 48 and 96 hours. A minimal, slight or moderate mesenchymal activation was observed after 96 hours and 14 days in two of four animals; a minimal to slight inflammation was seen after 48 hours in both animals, and 96 hours in one animal. In addition, a test article-treated site in a male rabbit no.1 showed a small edema after 48

hours. In the control site after IM injection, a female rabbit showed a slight necrosis and a very slight inflammation after 48 hours, and the control sites of a male (no.3) and a female (no.4) showed mini mesenchymal activation after 96 hours.

The SC injection of 0.5 ml Omegavenos/animal showed a mild to moderate inflammation after 48 and 96 hours at the treated sites. One of the test article-treated sites also showed a mild mesenchymal activation after 14 days of treatment.
In the injection control site of a male rabbit (no.1), a small hematoma was observed after 48 hours, and a mild necrosis and inflammation was seen at the injection site of a female rabbit (no.4) 96 hours after treatment.

In conclusion, IV and IA infusion with 30 ml of Omegavenos/animal for 4 hours did not lead to any test article-related local intolerance reactions, when compared to the 0.9 % NaCl solution. The PV, IM and SC administration resulted in mild test article-related local intolerance reactions, when compared to the 0.9 % NaCl control solution.

Local Tolerance Test of SMOF 20 % in Rabbits after a Single Intravenous, Intra-arterial, Paravenous, Intramuscular and Subcutaneous Administration (**RPT # 9802/1/96)**

The objective of this study was to determine the local tolerance of rabbits to SMOF 20 % after a single intravenous (IV), intra-arterial (IA), paravenous (PV), Intramuscular (IM), and subcutaneous (SC) injection.

Methods:

Doses: SMOF 20 % (batch # FHFE 11) was administered by single dose infusion at 18.8 ml/hr for 4 hours (75 ml/animal) IV and IA; at 0.5 ml/animal for the PV and SC injection; and at 1 ml/animal for the IM injections. 0.9 % NaCl was used as the negative vehicle control.
Study design: 12 male and female (6/sex) Himalayan rabbits, 14-16 weeks old, and weighing 2.7 to 3.2 kg were infused with SMOF 20 %, 75 ml IV (via marginal ear vein); 75 ml IA (via central ear artery); 0.5 ml PV (beside the *vena saphena parva*); 0.5 ml SC (under the dorsal skin); and 1 ml IM (into the gastrocnemius muscle).

The test solution was administered once on the left side of each animal, and for the IV and IA infusion, two separate animals were used.
As a control, each animal's right side was treated with 0.9 % NaCl solution.
The IV infusion was performed first, followed by the IM, PV, and SC injections at the end of the 4 hr infusion, one after the other, but the IA infusion was done in separate rabbits.

For the IV and IA infusion of 18.8 ml Lipovenos 20 %, a corresponding 18.8 ml of 0.9 % NaCl was infused. Dosing for the IM, PV, and SC were similarly administered.
In all cases, the dosing started with the test solution on the animal's left side, followed by the 0.9 % NaCl solution on the rabbit's right side.

Clinical signs and mortality were checked and recorded daily, and local reactions were inspected macroscopically 2, 24, 48, 96 hrs, and 14 days after administration in the appropriate rabbit.

Two rabbits/sex/group were sacrificed 48 hrs, 96 hrs, and 14 days after dosing. The injection sites were dissected out, and along with samples of affected tissues and control tissues (approx., 5 mm thickness) fixed in 10 % buffered formalin, stained with hematoxylin-eosin after paraffin sectioning, and examined histologically.

Treatment and time of sacrifice

Animal No. and Sex	Left Side	Right Side	Time of sacrifice
1 m, 7 m 2 f, 8 f	SMOF 20%	0.9% NaCl solution	after 48 h
3 m, 9 m 4 f, 10 f			after 96 h
5 m, 11 m 6 f, 12 f			after 14 days

m = male
f = female

Results

The rabbits did not show any test article-related clinical signs, and their body weight was within the normal range. None of the rabbits died prematurely.

The IV, and IA infusion sites and the PV, IM, and SC injection sites did not show any macroscopically visible changes in both groups of study animals.

Histopathological examinations of the tissues showed a slight thrombosis, mild edema and moderate inflammation in a male rabbit (no.9) 96 hours after treatment in the test group. The IA control sites did not reveal any changes.

After the PV injection, histopathology of the injection sites showed a minimal mesenchymal activation in 2 rabbits, 96 hours and 14 days after treatment and a minimal edema in another control site 96 hours after treatment in the test group.

Following IM injection, microscopic examination of the injection site of 3 animals revealed a mild or marked necrosis (I rabbit at 48 h, and 2 rabbits at 96 h); a mild

mesenchymal activation was seen in 2 animals (at 96 h and 14 d); a minimal or mild inflammation in 4 animals (at 48 h and 96 h) in the test group.
It was noted that one of the IM control sites showed a mild necrosis (at 96 h), and another site showed a mild mesenchymal activation and calcification at 14 days after injection.

Examination of the SC injection sites revealed a minimal or mild edema (2 animals, 48 h and 96 h), a mild mesenchymal activation (1 animal, 96 h) and a minimal inflammation (2 animals, 48 h and 96 h) in the test group. It was noted that the control sites showed a mild edema (1 animal, 96 h), a mild hemorrhage (1 animal, 48 h), and a mild mesenchymal activation (1 animal, 14 days).

In conclusion, following IV infusion with 18.8 ml SMOF 20 %/animal for 4 hours, there were no test article-related local changes, except for a spontaneous thrombosis in one of 6 rabbits. Following application made in error (IA, PV, and SC administration), SMOF 20 % injection sites in the treated animals showed changes comparable to the injection sites of the control article (0.9% NaCl solution). All the changes observed were similar in frequency and intensity. More distinct local changes in form of inflammation and necrosis were observed after IM injection (application made in error). The effect disappeared in 14 day, and the changes can be graded as moderate.

Functional Activity of the Reticuloendothelial System of the Rat after Total Parenteral Nutrition with Intralipid or Fat Emulsion 73403 (**Document No. 92 96 104**)

The objective of this study was to determine the effects of total parenteral nutrition (TPN) containing Intralipid (a long chain triglyceride emulsion) or Fat emulsion 73403 (a mixture of medium and long chain triglyceride on a common glycerol backbone) on the activity of the reticuloendothelial system (RES) in the healthy rat.

Methods:

Rats were divided into 3 dose groups, with group 1 receiving total parenteral nutrition (TPN) consisting of amino acids (vamin N) at 1 g nitrogen/kg/day, as well as vitamins, minerals, trace elements, including 25 % glucose and 75 % Intralipid 20 % (20 g fat/kg/day) with a total calorie intake of 313 kcal/kg/day. The group 2 animals received amino acids (vamin N) at 1 g nitrogen/kg/day, as well as vitamins, minerals, trace elements, including 25 % glucose and 75 % Fat emulsion 73403 (20 g fat/kg/day) with a total calorie intake of 305 kcal/kg/day. The group 3 (control) animals received isovolemic infusion of 0.9 % NaCl, including standard diet ad libitum. Infusions were given for 20 hrs each day consecutively for 7 days.

Overnight culture of bacterial *E. coli* (ATCC 8739) was inoculated into a ^{59}Fe-citrate (2 µCi/ml) radiolabeled broth. Animals were anesthetized on the morning of the eighth day, and the catheter flushed with saline prior to the infusion of 1 ml of ^{59}Fe-labeled E. coli into each animal, during a 30 sec period. Timing was begun when 50 % of the E. coli

had been infused, and 25 min later, the rats were sacrificed. Blood samples were obtained from each animal via the tail vein after 2, 5, 8, 12 and 25 min, and total blood volume (estimated at 6.4 % of the body weight) was used to calculate ^{59}Fe remaining in the blood.

Liver lung and spleen of each animal was harvested, dissected out into 200 mg pieces, weighed and processed, and solubilized, the radioactivity counted in a scintillation counter to determine the amount of radioactivity uptake by each organ. The results are expressed as % of infused ^{59}Fe taken up by each tissue.

Results

There were no significant differences in weight gain between the three dose groups over the last 4 days of the study, and there were no observed adverse effects of the IV TPN (Intralipid 20 % or Fat emulsion 73403) nutrition during the study duration.

There was no difference in liver weight (g/kg body weight) between the control group and the TPN groups, but the Fat emulsion 73403 group had significantly elevated liver weight, in comparison to the Intralipid 20 % group.

The lung weight of the TPN groups was significantly increased, when compared with control group lung weight; although the Fat emulsion 73403 animals had significantly lower lung weight than the Intralipid 20 % infused animals.
There were no significant changes in relative spleen weights between the study animals.

The clearance of an IV dose of *E. coli* from the blood was almost identical in all dose groups, with approximately 0.001 % of the infused live *E. coli* remaining in the blood. A stable E. coli count of 1 x 10^3 cfu/ml remained in the blood at the end of the 25 min study period.
The uptake of ^{59}Fe-labeled E. coli by the liver of control rats (62.4 ± 5.4 %) was higher than uptake in Intralipid 20 % rats (54.8 ± 3.9 %) and in Fat emulsion 73403 rats (55.7 ± 5.6 %), although not significant. The uptake of bacteria from the bloodstream by the liver appears to be uniform throughout the liver, from the count of radioactivity obtained from 5 liver samples.

The uptake of E. coli from the spleen of the Intralipid 20 % dosed rats (2.3 ± 0.9 %) and the Fat emulsion 73403 dosed rats (2.3 ± 1.1 %) was not significantly different from that of the control rats (3.1 ± 1.0 %).

A considerably significant increase in the E. coli uptake by the lungs of the Intralipid 20 % dosed rats (15.3 ± 3.7 %) and the Fat emulsion 73403 dosed rats (16.5 ± 5.2 %) was observed, in comparison to uptake by the control animals (3.0 ± 0.9 %).

The combined capacity of the liver, lungs and spleen to clear bacteria from the blood of rats from the control group (at 68.5 ± 5.6 %), Intralipid 20 % dose group (at 72.4 ± 5.2

%), and the Fat emulsion 73403 dose group (at 74.5 ± 7.5 %) of the infused dose, still leaves about 25 % of the infused dose of bacterial E. coli to be accounted for. Uptake by other organs may account for the residue.

In conclusion, TPN infusion with a high dose (20 g fat/kg/day) of Fat emulsion 73403 or Intralipid 20 % concurrently for 7 days in healthy rats sustained normal growth without observable side effects, and did not affect the ability of the animal to clear bacteria from the blood. Lipid emulsion based TPN however, significantly increased bacterial uptake by the lung in comparison to controls.

The Effect of Infusions of Intralipid 20 % and Fat Emulsion 73403 on Reticuloendothelial Function in the Rat (Document No. **92 96 106**)

Methods:

Intralipid 20 % at doses of 8 g and 20 g fat/kg body weight (b.w.), and 0.9 % NaCl were infused into rats for 20 hrs each day for 7 days.
In the first of two experiments, two groups of 6 male Sprague-Dawley rats weighing 166-202 g were first anesthetized and a catheter placed into the *vena cava* via the *vena jugularis,* prior to the infusion of each group of animals with 8 g or 20 g of Intralipid 20 %

In the second experiment 3 groups of 8 animals/group were dosed either with 20 g Intralipid 20 %, Fat emulsion 73403, or 0.9 % NaCl (control group).
Overnight culture of bacterial *E. coli* (ATCC 8739) was inoculated into a ^{59}Fe-citrate (2.3 µCi/ml) radiolabeled broth.
The animals were anesthetized on the morning of the eighth day, and the catheter flushed with saline prior to the infusion of 1 ml of ^{59}Fe-labeled E. coli into each animal, during a 30 sec period. Timing was begun when 50 % of the E. coli had been infused, and 25 min later, the rats were sacrificed. Blood samples were obtained from each animal, and the total blood volume (estimated at 6.4 % of the body weight) was used to calculate ^{59}Fe remaining in the blood.

Liver lung and spleen of each animal was harvested, dissected out into 200 mg pieces, weighed, processed, and the radioactivity counted in a scintillation counter to determine the amount of radioactivity uptake by each organ. The results are expressed as % of infused ^{59}Fe taken up by each tissue.

Results

Three rats in the second experiment were lost from the study, 2 as a result of catheter occlusion, and one rat was septic.
Growth rates of all emulsion treated animals were comparable to the saline-infused rats, but the treated rats had hypophagia as a result of calories infused from the emulsions, and maintained normal body weight during the infusion period.

There were no differences in the relative liver, lung and spleen weights of rats infused with 8 g or 20 g fat/kg/day intralipid. However, high fat doses (20 g fat/kg/day) of Intralipid 20 % and Fat emulsion 73403 resulted in significant elevations in relative liver weights compared to saline control animals. Rats infused with Fat emulsion 73403 also showed significantly higher relative spleen weights compared to control animals. Lung weights were unaffected by either 20 g fat/kg/day Intralipid 20 % or Fat emulsion 73403.

There were no significant differences in bacterial uptake by the liver, lung, or spleen of rats infused with 8 or 20 g fat/kg/day Intralipid 20 %, and the amount of bacteria remaining in the blood was also similar, in spite of the change in Intralipid dose.

There were no differences in bacterial uptake by the liver, lung, and spleen of animals infused with 20 g fat/kg/day Intralipid or Fat emulsion 73403, when compared to saline infused control animals. However, the bacterial uptake by the spleen was higher in the Fat emulsion 73403 group compared to the Intralipid 20 % group, and the blood bacteria was significantly lower in the fat-infused animals compared to the saline-infused control animals.

The combined total bacterial uptake by the liver, lungs and spleen were significantly higher in the high dose Intralipid 20 % and Fat emulsion 73403 groups, in comparison to both the control group and the low dose Intralipid 20 % group.

In conclusion, the infusion of Intralipid 20 % or Fat emulsion 73403 at high doses for 7 days does not affect reticular activating system (RES) function in the liver, spleen and lungs of the rat.

Systemic Antigenicity test of Omegavenos 10 % (**Study No. 90C-05014-00**)

The objective of this study was to determine the anaphylactogenic potential of Omegaven 10% following multiple injections in healthy guinea pigs.

Methods:

Ten female (Hartley strain) albino guinea pigs weighing 300 – 500 grams were randomized into 2 groups of six treated female (group 1), and four control females (group 2). The animals were injected intraperitoneally (IP) with Omegavenos 10 % (group 1), or 0.9 % NaCl (group 2) at a dose of 10 ml/kg body weight, three times a week (Monday, Wednesday, and Friday) for two consecutive weeks. Fresh test article and 0.9 % NaCl (saline) controls were prepared for each of the six injections, and body weights of the animals were recorded prior to the first and fourth injections. Eleven days after the last induction injection, 2 ml of fresh Omegaven 10 % was injected into the ear vein of each test and control animal. The animals were then observed for 30 min for signs of anaphylaxis following the challenge injection.

Results:

There were no significant reactions observed in the test or control animals following the challenge injection. All the guinea pigs appeared normal throughout the test period. There was no significant evidence that the test article produced systemic anaphylaxis in the guinea pig.
In conclusion, the induction injection of Omegaven 10 % at 10 mL/kg into the peritoneal cavity of female guinea pigs three times a week for two weeks, with a subsequent challenge injection of the test article 11 days later, did not induce systemic anaphylactic reaction in any of the guinea pigs.

Examination of SMOFLIPID for Compatibility and Hemolytic Properties in EDTA-Anticoagulated Human Blood *In Vitro* **(Report No. 29103)**

The study objective was to determine the biocompatibility and hemolytic properties of SMOFlipid in human red blood cells *in vitro*.

Method:

Fresh $EDTA^2$-anticoagulated human blood was obtained from three healthy volunteers and each volunteer's blood was mixed with SMOFlipid at concentrations of 100 % (native), 50 %, 25 %, 10 % and 5%, or 0.9 % NaCl (negative control) prior to the incubation of the mixture (in triplicate) at 37 ^{o}C for 30 min. After incubation, duplicate microhematocrit (capillary) tubes were filled with each preparation, and following centrifugation, the hematocrits were determined and the supernatant was inspected visually for color and the presence of precipitate. Additionally, erythrocyte morphology and the presence of precipitate were evaluated microscopically using blood smears. Due to the chemical and physical properties of SMOFlipid and its opacity, a blank control of the test item without mixing with the blood was also measured. The difference between both samples (test article mixed with blood and the negative control) represents the degree of hemolysis by SMOFlipid.

For hemolysis determination, blood was diluted with five volumes of 0.9 % NaCl solution, centrifuged, and the erythrocytes separated as pellets. The pelleted erythrocytes were diluted 1:9 with sterile 0.9 % NaCl solution and 2 ml of the erythrocyte solution were mixed with 2 ml of the test article or the control solution (0.9 % NaCl) and the samples were incubated at 37 ^{o}C for 30 min.

SMOFlipid compatibility with human plasma was determined by mixing equal volumes of plasma with the 0.9 5 NaCl (vehicle) and the undiluted (native) test article formulation. The mixtures were observed both macroscopically and microscopically for any formation of precipitate or coagulum.

The osmolality of the samples of the test and and control solutions before and after the addition of blood was measured with a semi-micro osmometer.

Results:

There were no precipitates observed from the incubation of different concentrations of SMOFlipid with human blood. The morphology of the erythrocytes examined microscopically using blood smears was not influenced by SMOFlipid at any of the concentrations tested. There was no effect of SMOFlipid (5 – 50 % and undiluted: 100%) on the hematocrit of the erythrocyte suspensions.
There were no signs of hemolysis from any of the concentrations of SMOFlipid tested. There was also no incompatibility noted for SMOFlipid with human plasma. The osmolality of the erythrocyte suspensions increased with increasing SMOFlipid concentrations in a dose-responsive manner.

In conclusion, the incubation of human blood, with SMOFlipid at concentrations of 5 % to 50 %, or with the undiluted 100 % test article did not result in any hematocrit precipitation, or any blood hemolysis, or change in blood morphology or plasma incompatibility. The osmolality of the erythrocyte suspensions increased with increasing SMOFliopid concentrations in a dose-responsive manner.

Safety Evaluation of the Leachable and Extractable Migrants from the (b) (4) Container 1 Chamber bag (1-CB) System

Smoflipid is packed in a flexible plastic packaging system called the (b) (4) one-chamber bag (1-CB). The submission include the qualitative composition of the 1-CB packaging system, extraction and migration studies, and the toxicological qualification of each extractable and migrant based on the anticipated maximum daily exposure to the drug product. The (b) (4) 1-CB consists of a printed primary bag, ports, a secondary (b) (4) bag, an oxygen absorber and an integrity indicator. (b) (4) For protection during storage, the primary container is placed in a secondary (b) (4) bag/overpouch along with an oxygen absorber and integrity indicator (b) (4) In the event that the integrity of the secondary barrier bag is compromised unintentionally, the package is also fitted with an integrity indicator (b) (4) The components of the (b) (4) bag and those in direct contact with the drug product are listed in the Applicant's table below.

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INTEGRATED SUMMARY AND SAFETY EVALUATION

Smoflipid 20 % is a fat emulsion supplement for parenteral nutrition as a source of energy and essential/non-essential fatty acids in patients unable to obtain nutrients orally. The constituents of Smoflipid 20 % are long chain triglycerides (soybean oil), medium chain triglycerides (MCT), olive oil and fish oil mixture, supplied in a (b) (4) single chamber plastic container of 100 ml, 200 ml or 500 ml.

The recommended maximum dose for Smoflipid 20 % in adults is (b) (4) g lipids/kg/day, (b) (4) Smoflipid is to be administered by intravenous infusion into a central or peripheral vein.

The Sponsor has submitted nonclinical studies with Smoflipid and its different lipid emulsion constituents present in some of their other products such as lipovenos 20 %, Omegaven 10 %. Nonclinical studies with Intralipid 20 %, another soybean-derived long chain triglyceride (LCT) lipid emulsion is also presented in comparison to Smoflipid 20 %. The concept used in this submission is that, the pharmacological and toxicological effects of the individual lipid constituents of Smoflipid will not change in the drug product since their quality in the Smoflipid 20 % mixture remain unchanged.

In the nonclinical safety assessment of Smoflipid 20 %, the pharmacology and toxicology of the constituents of the lipid emulsion mixture was reviewed along with the Smoflipid drug product.

In a primary pharmacodynamics study in dogs, where LCT and MCT parenteral administration in dogs were supplemented with omega 3 fatty acids from fish oil, there was incorporation of omega 3 fatty acids into tissue phospholipids, especially in the liver of the study animals within 2 weeks of administration.

Safety pharmacology studies were conducted with lipovenos 20 % (which contains soybean oil (a LCT) in a similar concentration as Smoflipid 20 %) in cats and dogs. The injection of Lipovenos 20 % at 5 ml/kg for over 30 seconds did not induce a drop in blood pressure in anesthetized male cats. Similarly, the infusion of lipovenos 20 % of Intralipid 20 % at 4 g/kg (a dose higher than the maximum clinical dose of 3 g/kg) did not induce any undesirable cardiovascular effects in healthy female dogs after 30 minutes of treatment.

Pharmacokinetic (PK) studies on the elimination of different lipid emulsions in rabbits and dogs were conducted, along with the determination of the elimination half lifes of Intralipid emulsion in different animal species and man. No PK studies were conducted with Smoflipid 20 %.
In PK studies in dogs, the plasma elimination of 3 g/kg Intralipid 20 % and lipovenos 20 % infusion in dogs are biphasic, following a zero order elimination rate at low exogenous lipid concentration, and a first order elimination rate at the higher (3 g/kg) lipid concentration. In studies to determine the elimination rate of 100 mg and 200 mg lipids (Intralipid 20 %, fat emulsion (b) (4), 4501) in rabbits, the half-lives and fractional clearance rates were not significantly different for the lipids, with a $t_{1/2}$ of 7.66 min and a K_2 of 9.05 for a 200 mg dose of Intralipid 20 %. However, lipid emulsion 73403 was found to be eliminated significantly faster than Intralipid 20 %, at a similar concentration of 100 mg/kg in male rabbits. There were no differences in the half-lives of Intralipid 10 % in adult dog, cat, rabbit, rat, mouse and human. A mean half-life of 10 min was the mean elimination rate of Intralipid 10 % in all species.

Single dose intravenous (IV) toxicity study was conducted with Smoflipid 20 % in rats, along with single dose acute IV toxicity studies with other lipid emulsions (lipovenos 20 %, Intralipid 20 % and Omegaven) in mice and rats.
The single dose IV toxicity of Smoflipid in rats was very low, and well tolerated at the 45 ml/kg (9 g/kg triglycerides) and 90 mg/kg (18 g/kg triglycerides) dose level. However, at the 180 ml/kg (36 g/kg triglyceride) dose, the rats all died within 18 hours of dosing from volume overload. Necropsy findings of pale liver and spleen were observed in the dead animals. In rats, the maximum single dose of 5,000 mg/kg of Omegaven 10 % was well tolerated and did not result in any animal mortality. The LD_{50} values of IV lipovenos 20 % and Intralipid 20 % administration in mice were determined to be 125 and 112 mg/kg, respectively. When fat emulsion 73403 was administered IV in single doses of 45ml/kg (0.5 ml/min) to mice and 60 ml/kg (2 ml/min) in rats via the tail vein, macroscopic observations of pale kidneys and histopathological lesions in the kidney cortex were observed.

Repeated dose subchronic and chronic IV toxicity studies of up to 13 weeks were conducted with Smoflipid 20 % and with the individual constituents soybean oil (lipovenos 20 %, Intralipid 20 %) and triglycerides in dogs, and for up to 4 weeks in rats.

The 24 hour continuous IV infusion of Smoflipid 20 % was tolerated only up to 4 weeks at a high dose of 18 g/kg/day (3.8 ml/kg/hr) in rats with mortalities and a wide range of severe toxicities and histopathological findings of vessel wall necrosis, pulmonary inflammation, lung abcessess and infusion infections were observed. The toxicity seen was as a result of the 24 hour/day continuous exposure, in combination with the flow rate of administration and the physical nature of the test material, and not due to the physical nature of Smoflipid. It was concluded that the rat continuous infusion model is not suitable for the chronic administration of parenteral lipid emulsion. In toxicokinetic analysis of the 4-week dosing of Smoflipid in rats, there was no strong correlation seen between the administered dose of Smoflipid 20 % and plasma concentration of measured fatty acids in the animals.

Repeat dose IV toxicity studies with Smoflipid 20 % and other lipid emulsions were studied for up 13 weeks in dogs.

The administration of Smoflipid 20 % by IV infusion for 6 hours per day at a dose of 45 ml/kg (at 9 g triglycerides/kg body weight/day) in dogs for 4 weeks was generally well tolerated. The observation of reduced food intake in the animals did not lead to body weight loss, suggesting adequate calorie uptake from Smoflipid administration. The increased phospholipids, bile acids and direct bilirubin observed were less pronounced in the dogs. Histopathological changes observed in liver lungs and kidneys were due to the administration of large amounts of lipids.

When high dose Omegaven and Intralipid 10 % (at 60 ml/kg/16 hr) corresponding to 6 g triglycerides/kg/16 hr was administered by continuous 16 hour intravenous infusion for 6 weeks, the lipids were not tolerated by the dogs, and it resulted in acute mortality in treated dogs. There were no clinical signs observed prior to death in the animals, but at necropsy, treatment-related findings were observed in the thymus, heart, trachea and lungs. Death in the animals was histopathologically correlated with alveolar edema of the respiratory tract. However, the lipid emulsions at dose level of 4 g triglycerides were borderline tolerated, and were well tolerated at 2 g triglycerides for the prolonged 16 hour continuous daily administration for 6 weeks. Therefore, it was determined from the study that 3 g triglyceride continuous infusion for 16 hours was a safe dose in dogs.

In the 13-week administration of Omegaven, Intralipid 20 % or fat emulsion 73403 by intravenous infusion with a 4-week recovery period in dogs, most of the acute and chronic inflammatory and severe changes observed in target organs such as the liver, spleen, lungs and thymus were associated with the increased lipid, free fatty acids and triglyceride load, and were partially or completely reversed after the 4-week recovery period in dogs.

Similarly, the 13-week administration of Smoflipid 20 % for 6 hours/day by IV infusion at 3 and 6 g/kg (6 hours/day) with a 4-week recovery period was also well tolerated in dogs with no mortality in the study animals. There were no hemodynamic or ECG changes observed in treated animals. The clinical chemistry changes, increased kidney and lung weights, and liver discoloration noticed in treated animals had subsided after the 4-week recovery period. However, at the end of the 4-week recovery period, 2 dogs still show slight discoloration of the liver that is associated histopathologically with fatty change in the liver parenchyma. Changes of slight to moderate indurations observed at the infusion site for control and treated dogs were due to the catheter placement site, and are of no consequence in the clinical administration of Smoflipid 20 % at a central or peripheral vein.

In general, toxicity studies with Smoflipid 20 % and its individual lipid constituents were well tolerated in mice and/or rats after single intravenous administration at the highest meaningful doses with regard to lipid load and/or fluid volume.
In subchronic repeat dose intravenous toxicity studies with Smoflipid and with individual lipid constituents for up to 13 weeks duration in dogs, there were no adverse clinical signs or evidence of organ toxicity seen, beyond the lipid and free fatty acid overload effects.

In a battery of genotoxicity tests (bacterial reverse mutation assays, *in vitro* clastogenicity tests in mammalian cells or human peripheral lymphocytes, and in rat *in vivo* bone marrow cytogenetic tests), Smoflipid 20 % was non mutagenic.

There were no reproductive or developmental toxicology studies performed with Smoflipid 20 % in animals. The IV infusion fat emulsion 73403 at doses of 15 and 22.5 ml/kg/day and Intralipid 20 % at 22.5 ml/kg/day to pregnant rabbits during the period of organogenesis did not result in any meaningful embryo-fetal or post-implantation loss in the rabbit dams. Fetal growth and development were not affected by the lipid emulsion infusion in all the treated rabbit groups.
From a published study submitted by the Sponsor, the oral administration of medium and long chain triglycerides MCT and LCT) at a 3:1 ratio, respectively, in rats and rabbits resulted in lower food consumption in the high dose (4.28 g/kg) animal groups. Higher post implantation loss and fewer live fetuses were observed in the rabbits treated with the high dose emulsion, but not observed in the high dose rat group. The NOEL dose for developmental toxicity of the administration of MCT/LCT containing lipid emulsion in rats is 4.28 g/kg, and 1 g/kg in rabbits. In a second published study submitted, the perinatal and postnatal toxicity study on the administration of AG 103 (a mixture of eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) at doses of up to 600 mg/kg/day in pregnant rats did not show any evidence of test article-related toxicity in dams or offsprings of the study animals.

In local tolerance studies, the single dose administration of Smoflipid 20 % at 18.8 ml/hr for 4 hours (75 ml/animal) IV and dosing by alternative paravenous (PV), intra-arterial (IA), intramuscular (IM), and subcutaneous routes were well tolerated in rabbits. More distinct local inflammatory and necrotic changes observed after the IM injection

disappeared in 14 days after the injection. There was no systemic intolerance observed post administration of the test article in rabbits. Similar results were obtained in rabbits after the administration of lipovenos 20 % and Omegaven 10 % in rabbits.

When Smoflipid 20 % was examined for compatibility and hemolytic properties in human blood (erythrocyte suspension, hematocrit or in plasma), the test article did not result in hematocrit precipitation, blood hemolysis, or in any changes in blood morphology or plasma incompatibility in the study.

In functional activity studies for the rat endothelial system following TPN administration of Fat emulsion 73403 or Intralipid 20 % (20 g fat/kg/day for 7 days at 20 hours per day), the rats sustained normal normal growth without any observable side effects. The lipid emulsions did not affect the ability of the healthy rats in clearing bacteria from the blood. However, the high lipid emulsion doses resulted in significant elevation in liver and spleen weight, and in higher combined total bacterial uptake by the liver, lungs and spleen. The reticular activating system function in the liver, lungs and spleen were unaffected by the lipid emulsion administration.
The systemic antigenicity test in guinea pigs by the intraperitoneal injection of Omegaven 10 % at a dose of 10 ml/kg/body weight three times a week for 2 consecutive weeks did not result in systemic anaphylactic reactions when a challenge dose (2 ml) of the test article was injected into the ear vein of the animals 11 days after the initial treatment.

The safety assessments of the potential migration of component materials of the (b) (4) packaging system into the Smoflipid 20 % emulsion parenteral infusion product and the associated risk for the recipient patients were evaluated. In the safety assessment of the potential migrants (leachables and extractables), there was a separation or clear separation between calculated patient exposures and the permitted daily exposure (PDE) as calculated according to animal or human toxicity data from toxicological information or as calculated according to the ICH Q3C(R5) guidance.

This non-clinical safety assessment show that there are no safety concern with potential and established migrants from the 1-CB (b) (4) container system into the Smoflipid parenteral nutrition product indicated for use in adult and children.

In addition, there are no safety concerns with potential and established migrants from (b) (4) the storage of the raw material oils used in the synthesis of Smoflipid.

In conclusion, based on nonclinical safety information provided for the Smoflipid drug product and the 1-CB packaging system, the TPN infusion of Smoflipid 20 % in adults and children via a central or peripheral vein at the proposed doses appear to be safe, and there are no significant safety concerns.

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/s/

BABATUNDE E AKINSHOLA
06/30/2015

SUSHANTA K CHAKDER
06/30/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 207648 **Applicant: Fresenius Kabi** **Stamp Date: 09/26/2014**

Drug Name: Smoflipid 20 % **NDA/BLA Type: 505(b)(1)**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The proposed labeling sections relevant to nonclinical studies may need to be revised during the labeling review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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

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

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter. **None**

Babatunde Emmanuel Akinshola, Ph.D. November 19, 2014
Reviewing Pharmacologist Date

Sushanta K. Chakder, Ph.D. November 19, 2014
Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

BABATUNDE E AKINSHOLA
11/19/2014

SUSHANTA K CHAKDER
11/19/2014