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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 204-508
Supporting document/s: 0001
Applicant's letter date: January 03, 2013
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Product: Clinolipid 20%
Indication: Parenteral nutrition when oral or enteral nutrition
is not possible, insufficient, or contraindicated.
Applicant: Baxter Healthcare Corporation, IL
Review Division: Division of Gastroenterology product (HFD-180)
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Executive Summary

1.1 Recommendations

1.1.1 Approvability

From a nonclinical standpoint, approval of the NDA application is recommended.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

The draft labeling of Clinolipid 20% generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR (Physician's Labeling Rule) Prescription Drug Labeling. However, the following changes in the proposed label are recommended.

8.1 Pregnancy

Sponsor's version:

Teratogenic Effects

Pregnancy Category C

Animal reproduction studies have not been conducted with ClinOleic 20%. It is also not known whether ClinOleic 20% can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. ClinOleic 20% should be given to a pregnant woman only if clearly needed.

Evaluation: No changes are recommended in this section; however, the trade name should be changed from ClinOleic to Clinolipid.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's version:

[REDACTED] (b) (4)

Evaluation: The following changes are recommended in this section.

Proposed version:

Evaluation: The following changes in Section 13.2 is recommended to reflect the adverse findings in animals (rats and dogs) and doses at which these effects were observed.

Proposed version:

Clinolipid 20% was well tolerated in toxicity studies conducted in rats and dogs for up to 3 months. The principle signs of toxicity noted in these studies were:

- Slight hemolytic anemia at 12 g/kg/day in rats and at 6 g/kg/day in dogs. These doses in rats and dogs are 4.8 and 2.4 times higher, respectively, than the recommended adult dose (2.5 g/kg/day) of Clinolipid.
- Dose-dependent decrease in urea levels in rats at 6 and 12 g/kg/day dose levels and in dogs at 3, 4.5 and 6 g/kg/day dose levels.
- Hypercholesterolemia in dogs at 3, 4.5 and 6 g/kg/day dose levels.
- Hepatic pathology of lipid and pigmentary overload in male and female rats at 3, 6 and 12 g/kg/day dose levels and in male dogs at 6 g/kg/day and female dogs at 3, 4.5 and 6 g/kg/day dose levels.
- Splenic hemosideration and vacuolization in rats at 3, 6 and 12 g/kg/day dose levels, and dogs in 4.5 and 6 g/kg/day dose levels.

At a dose of 3 g/kg/day, slight lipid and pigmentary overload of the liver and vacuolization of Kupffer cells were observed in rats and dogs. At a dose of 12 g/kg/day in rats, hepatocellular vacuolation, granulomatous inflammation of the liver, hepatocellular necrosis and hemosideration of the liver and splenic hemosiderosis,

associated with a strong lipid load hemosiderin cells were observed. In dogs, at a dose of 6 g/kg/day, brownish-yellow pigmentation in the Kupffer cells of liver and spleen, hyperplasia, vacuolization, and a slight increase in the number of lipid storage cells in the liver and macrophage vacuolization of the spleen were observed.

1.2 Brief Discussion of Nonclinical Findings

This NDA was submitted under section 505(b)(2) of the Federal Food, Drug and Cosmetic Act. The applicant provided pharmacology, pharmacokinetics and toxicology studies of Clinolipid in rodents and/or nonrodents. Compared to Intralipid®, Clinolipid contains lower unsaturated fatty acid content, higher oleic acid content, and higher Vitamin E (α -tocopherol, active form of Vitamin E) level. The levels of essential fatty acids (EFAs) derivatives in the blood were similar in rats after intravenous administration of Clinolipid or Intralipid®. The *in vitro* hemolytic potential of Clinolipid was significantly lower than Intralipid® in rabbit red blood cells, indicating lower peroxidation for Clinolipid. Clinolipid was not hypotensive in anesthetized cats following IV administration. In mice, following intravenous administration of Clinolipid and Intralipid®, the distribution of triglyceride in the liver, spleen and lungs were low and were identical for both lipids. The plasma clearance kinetics of Clinolipid was found to be comparable with Intralipid® in rats and dogs.

The toxicity profile of Clinolipid was assessed in single-dose toxicity studies in mice and rats, and in repeat-dose toxicity studies for up to 3-months in rats and dogs. The toxicity profile of Clinolipid was compared with several soybean based lipid emulsions (Intralipid®, Ivelip® and Endolipide®). Clinolipid was well-tolerated and the toxicity profiles were comparable to other lipid emulsions (Intralipid®, Ivelip® and Endolipide®) when administered intravenously to dogs and rats. Common pathological observations related to parenteral nutrition were noticed in the liver, spleen, kidneys and adrenal glands, and included pale discoloration of the livers, increased liver weight, kidney weight and adrenal glands weight, and sometimes decreases in the weight of the thyroid glands. Lipid treated animals also showed yellow-brown lipid pigment in the sinusoidal macrophages (Kupffer cells) in the livers and in splenic macrophages, and hepatocellular vacuolation (lipid) along with vacuolation of splenic macrophages. In addition, a slight hemolytic anemia and decreased urea levels were observed in rats and dogs receiving intravenous infusions of lipid emulsions, including Clinolipid. Hypercholesterolemia was noted in dogs at 3, 4.5 and 6 g/kg/day dose levels of Clinolipid.

A local tolerance study showed that extravascular administration of Clinolipid has no potential to cause tissue necrosis if administered subcutaneously or intradermally due to procedural mistake. An *in vitro* immune assay in peripheral blood mononuclear cells (PBMCs) of humans or rats, suggests that Clinolipid could modulate immune response, and reduce inflammatory response.

Safety assessment of the extractable and leachables showed that the potential extractables and leachables from the Clinolipid 20% container closure system have no significant safety concerns, and are within the recommended safety limit.

2 Drug Information

2.1 Drug: Clinolipid 20% intravenous lipid emulsion

2.1.1 CAS Registry Number (Optional)

None

2.1.2 Generic Name

None

2.1.3 Code Name

CSW 6.3

2.1.4 Chemical Name

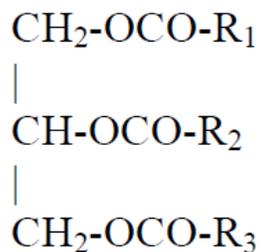
None

2.1.5 Molecular Formula/Molecular Weight

Clinolipid 20% is a lipid emulsion of refined olive oil and refined soybean oil in an approximate ratio of 4:1 (olive:soy). Soybean Oil USP and Olive oil NF are vegetable fatty oils. The main components of fatty oils are 1, 2, 3-triacylglycerols (triglycerides). In soybean oil, the predominant fatty acid components are linoleic acid, linolenic acid, oleic acid and palmitic acid. In olive oil, the predominant fatty acid components are oleic acid, linoleic acid and palmitic acid. The molecular mass depends on the fatty acid composition.

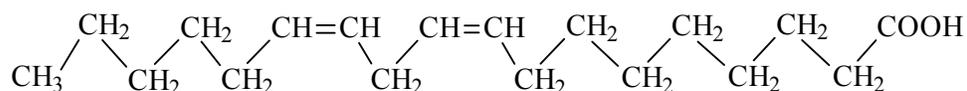
2.1.6 Structure

Both soybean and olive oil consists of glycerol molecules substituted with a heterogeneous but characteristic mixture of saturated and unsaturated fatty acid of carbon chain length 14 to 24. Fatty acids of different chain lengths and degrees of saturation are esterified to the three positions of the glycerol backbone. R_1 , R_2 and R_3 represent the fatty acids linked to the glycerol moiety of the triglyceride. In the example below, R_1 is palmitic acid, R_2 is linoleic acid and R_3 is oleic acid.

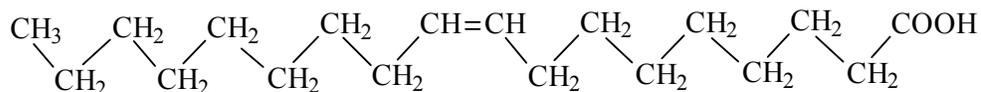


Structures of major fatty acids are given below.

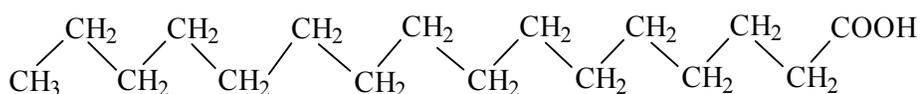
Linoleic acid
 $\text{C}_{18}\text{H}_{32}\text{O}_2$



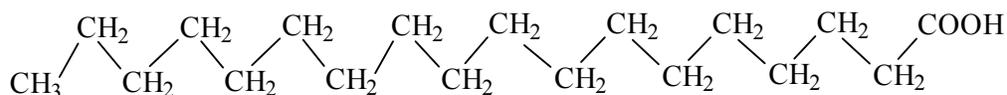
Oleic acid
 $\text{C}_{18}\text{H}_{34}\text{O}_2$



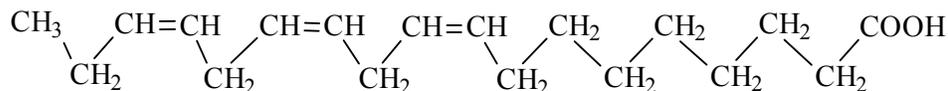
Palmitic acid
 $\text{C}_{16}\text{H}_{32}\text{O}_2$



Stearic acid
 $\text{C}_{18}\text{H}_{36}\text{O}_2$



Linolenic acid
 $\text{C}_{18}\text{H}_{30}\text{O}_2$



2.1.7 Pharmacologic class

Intravenous lipid emulsion

2.2 Relevant IND/s, NDA/s, and DMF/s

None

2.3 Clinical Formulation

2.3.1 Drug Formulation

Clinolipid 20% is a lipid emulsion comprising a mixture of refined olive oil and refined soybean oil in a ratio of 4:1. The content of essential fatty acids (linoleic plus α -linolenic acid) in the finished product is 20% of the total fatty acids. The lipid content (olive and soybean oil) is 0.20 g/mL. Each 100 mL of Clinolipid 20% contains approximately 16g of olive oil NF and 4g of soybean oil USP, 1.2g egg phospholipids NF, 2.25g glycerin USP, 0.03g sodium oleate, and Water for Injection USP, and sodium hydroxide NF for pH adjustment to 6.0-9.0. The major components of fatty acids are linoleic (13.8-22.0%), oleic (44.3-79.5%), palmitic (7.6-19.3%), linolenic (0.5-4.2%) and stearic (0.7-5.0%) acids. The Table below lists the components of Clinolipid 20%.

Table 2.
Composition of the Dosage Form

Component	Quality Standard	Function	Component Quantity per 1000 mL ¹
Soybean Oil	USP	Active Pharmaceutical Ingredient	40 g ²
Olive Oil	NF	Active Pharmaceutical Ingredient	160 g ²
Glycerin	USP	(b) (4)	22.5 g
Sodium Oleate	In-house	(b) (4)	0.30 g
Egg Phospholipids	NF	(b) (4)	12 g
Sodium Hydroxide ³	NF	(b) (4)	As needed
(b) (4)			
Water for Injection	USP	(b) (4)	(b) (4)

USP: United States Pharmacopeia; NF: National Formulary

¹ Labeled volume 1000 mL, fill volume (b) (4)

² The lipid emulsion contains Olive oil, NF and Soybean oil, USP in a ratio corresponding respectively to approximately 4:1. The respective proportions of Olive oil and Soybean oil are adjusted as a function of the content of essential fatty acids in the raw materials.

³ Sodium Hydroxide, NF is used (b) (4) for use as a pH adjuster.

2.3.2 Comments on Novel Excipients

Sodium Oleate is a novel excipient in Clinolipid 20%. Sodium Oleate is the sodium salt of oleic acid from vegetable origin. It is also called as Sodium 9-octadecenoate with the CAS Registry Number: 143-19-1, molecular formula C₁₈H₃₃O₂.Na, molecular mass 304.4. According to 21CFR186.1770, Sodium Oleate is specified as generally recognized as safe (GRAS). Sodium Oleate is not mutagenic, genotoxic or carcinogenic, and is not a reproductive or developmental toxicant. In a 24-week oral toxicity study in rats, no adverse effects were observed at doses up to 7,500 mg/kg/day. Thus, there are no safety concerns for the amount of sodium oleate present in Clinolipid.

2.3.3 Comments on Impurities/Degradants of Concern

The impurities in drug substances are the residual solvents which are listed in the Table below.

Table 1: Composition of ClinOleic 20% Lipid Injectable Emulsion, USP

Product Components/Raw Materials	Amount of Component (g/L)	Material Specification	Residual Solvents
Soybean oil, USP	40		(b) (4)
Olive oil, NF	160		
Glycerin, USP	22.5		
Sodium Oleate	0.30		
Egg Phospholipids, NF	12		
Sodium Hydroxide, NF	pH adjuster		
Water for Injection, USP	(b) (4)		

^aNMT=not more than

According to the ICH Q3C (R5) guidance, (b) (4) belong to (b) (4) solvent and (b) (4) is a (b) (4) solvent. (b) (4) solvents have permitted daily exposures (PDEs) of 50 mg (5000 ppm) or more per day. The PDE of (b) (4) is (b) (4) mg/day or (b) (4) ppm limit. Thus, based on ICH Q3C(R5) guidance, these impurities are within acceptable limits.

The Agency requested the Applicant to determine the levels of elemental impurities (b) (4) and any other (b) (4) in the finished drug product. The Applicant's Table below (column 4) shows the amount of different elemental impurities (ng/mL) detected in ClinOleic emulsion. The anticipated human daily exposure (HDE, ug/day) to each of the elemental impurities were calculated (column 5) based on a maximum daily dose of 625 mL for a 50 kg body weight. Permissible daily exposures (PDEs) proposed in the ICH Q3D draft is shown in column 6 of the Table.

Summary of Elemental Impurities Risk Assessment

Code Number	Elemental Impurity	Experimental Value (ng/mL) ¹	Quantitation Limit (QL, ng/mL) ¹	HDE (µg/day) ²	PDE (µg/day)	Is the HDE < PDE?
(b) (4)	(b) (4)					

QL = Quantitation limit for the standards prepared on the day of analysis.

HDE = Human Daily Exposure

PDE = Permissible Daily Exposure. Reference 2.

¹ Testing was performed on three units from each of the 3 respective primary stability batches manufactured in support of NDA 204508. Reference 8. No values were observed above the QL.

² Calculated as [(QL (ng/mL) x 625 mL/day) / (1000 ng/µg)]

The permissible daily exposures (PDEs) of the (b) (4) impurities proposed in the ICH Q3D draft show that these impurities in ClinOleic are several fold lower than their PDEs. Since (b) (4) are not listed in the ICH Q3D draft guidance, the current EMEA guidance was consulted for their PDEs. According to EMEA guidance, (b) (4) are also several folds lower than the PDE values. Thus, the levels of these (b) (4) impurities are within acceptable limits and there are no safety concerns.

2.4 Proposed Clinical Population and Dosing Regimen

For adult (b) (4), when oral or enteral nutrition is not possible, insufficient, or contraindicated. The maximum daily dose of ClinOleic 20% should be based on individual total nutritional requirements and patient tolerance. The usual dosage is 1 to 1.5 g/kg/day (equivalent to 5 to (b) (4) mL/kg/day). The daily dose should not exceed 2.5 g/kg/day. The initial infusion rate should not exceed 0.1 g (equal to 0.5 mL) per minute for the first 15 to 30 minutes.

(b) (4)

2.5 Regulatory Background

A Type B pre-IND meeting was held on July 13, 2011 regarding possible submission of the NDA and to obtain an agreement with the Agency on the requirements to support registration of ClinOleic 20% in the US.

3 Studies Submitted

1. PHARMACOLOGY TABULATED SUMMARY

1.1 Pharmacology: Overview

Test Article: ClinOleic 20% (Code: CSW 6/3)

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Primary and Secondary Pharmacodynamics				
Modification of fatty acids in the rat on parenteral nutrition as a function of the type of lipid intake	Rat, Sprague-Dawley (male)	Continuous intravenous infusion	(b) (4)	B 92 04
Study of hemolysis induced in vitro by the emulsions ClinOleic and CSW 6/2 in comparison with Intralipid	Rabbit red blood cells <i>in vitro</i>	Incubation with the emulsion		B 03 87 02
Influence of intravenous lipid intake on peroxidation induced by pro-oxidation shock in rats	Rat, Sprague-Dawley (male)	Intravenous infusion		B 92 06
Study of lipid peroxidation during the administration of lipid emulsions in beagle dogs: Intralipid, CSW 6/2, ClinOleic	Dog, Beagle (male & female)	Intravenous infusion		B 88 01
Vitamin E: development of an assay method and application to Vitamin E status in rats	Rat CD (male)	Intravenous infusion		B 92 03
Study of hepatic Vitamin E status in rats undergoing different oral and intravenous diets	Rat	Intravenous infusion		B 92 05
Effects of parenteral nutrition on biliary secretion in rats	Rat, Sprague Dawley OFA	Intravenous infusion		B 89 07
Effect of lipid composition on hepatobiliary complications under total parenteral nutrition	Rat, Sprague Dawley	Intravenous infusion		A 91 23

1. PHARMACOKINETICS TABULATED SUMMARY**1.1 Pharmacokinetics: Overview**

Test Article: ClinOleic 20% (Code: CSW 6/3)

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption – Not applicable.				
Distribution				
Study of tissue distribution of CSW 6/2 and ClinOleic lipid emulsions versus Intralipid	3 groups of 10 male mice	Single IV injection of 16 g lipid/kg	(b) (4)	B 03 87 04
Metabolism				
Biotransformation – lipolysis <i>in vitro</i> of CSW 6/1, CSW 6/2, ClinOleic, Ivelip versus Intralipid	Rat plasma	Incubation of emulsion and rat plasma		B 88 02
Biotransformation – lipolysis <i>in vitro</i> of different injectable lipid emulsions using an automated method	Rat plasma	Incubation of emulsion and rat plasma		A 91 03
Biotransformation – apolipoprotein binding capacity and lipoprotein lipase substrate function	Artificial triglyceride-rich particles incubated with lipoprotein lipase of cow milk	Incubation with cow milk lipoprotein lipase		B 92 02
Excretion				
Plasma elimination kinetics of ClinOleic versus Intralipid	Rat	IV bolus		B 89 04
Plasma elimination kinetics of CSW 6/2 and ClinOleic versus Intralipid	Dog, Beagle	IV infusion		A 89 07

1. TOXICOLOGY TABULATED SUMMARY

1.1 Toxicology: Overview

Test Article: ClinOleic 20% (Code: CSW 6/3)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mL/kg/day)	GLP	Testing Facility	Study Number
Single-Dose Toxicity							
LD ₅₀ in the Mouse	Mouse CD1 (b) (4)	Intravenous	Single dose	100, 112.5, 125, 137.5 at 1 mL/min	Yes	(b) (4)	A 87 15
LD ₅₀ in the Rat	Rat Sprague Dawley CD (b) (4)	Intravenous	Single dose	100, 110, 120, 125, 130, 135 at 1 mL/min	Yes		A 88 14
Repeat-Dose Toxicity							
30 day toxicity study in the rat	Rat Sprague Dawley CD (b) (4)	Intravenous infusion	30 days	90 2 mL/kg/min	Yes	(b) (4)	A 90 11
25 day toxicity study in the rat	Rat Sprague Dawley CD (b) (4)	Central Intravenous infusion	24 or 25 days	90 2 mL/kg/min	Yes		A 90 14
30 day toxicity study in the rat	Rat Sprague Dawley CD (b) (4)	Intravenous infusion	30 days	90 1.2 mL/kg/min	Yes		A 91 04
30 day toxicity study in the rat	Rat Sprague Dawley CD (b) (4)	Intravenous infusion	30 days	75 1.5 mL/kg/min	Yes		A 92 03
90 day toxicity study in the rat	Rat Sprague Dawley CD (b) (4)	Intravenous infusion	90 days	15, 30 and 60 2 mL/kg/min	Yes		A90 09
14 day toxicity study in the rabbit	Rabbit Hy/cr	Intravenous infusion	14 days	45 0.5 mL/kg/min	No		A 89 11

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mL/kg/day)	GLP	Testing Facility	Study Number
30 day toxicity study in the dog	Dog Beagle	Intravenous infusion	30 days	45 0.2 mL/kg/min	Yes	(b) (4)	A 01 87 18
30 day toxicity study in the dog	Dog Beagle	Intravenous infusion	30 days	60 0.2 mL/kg/min	Yes		A 91 15
30 day toxicity study in the dog	Dog Beagle	Intravenous infusion	30 days	60 0.2 mL/kg/min	Yes		A 92 02 A 92 04
90 day toxicity study in the dog	Dog Beagle	Intravenous infusion	90 days	45 0.2 mL/kg/min	No		A 88 06
91 day toxicity study in the dog	Dog Beagle	Intravenous infusion	91 days	15, 22.5, 30 0.2 mL/kg/min	Yes		1495 TTC/096.90 A 90 01
Local Tolerance							
Local tolerance in rats	Rat Sprague Dawley CD (b) (4)	Subcutaneous and Intradermal	Single dose	sc 2.5 mL id 0.1 mL	No	A 88 13	
Other Toxicity Studies							
Study to determine <i>in vitro</i> the effect of lipid emulsion on human peripheral white blood cells	Human peripheral white blood cells	<i>In vitro</i>	48 hr incubation period	0.00188 – 0.3% (v/v) concentration	Not required	P 99 01E	
<i>In vivo</i> study to determine the effect of lipid emulsion on rat spleen lymphocyte cells	Rat, Wistar	Intravenous infusion	6 days	18 mL/kg/day (12% energy supplied as lipid)	Yes	P 99 02E	

3.1 Studies Reviewed

All studies listed in Section 3.0 were reviewed except Study # A 92 04 (Thirty-Day Repeat-Dose Toxicity Study at a Dose of 60 mL/kg (0.2 mL/kg/min Infusion Rate)).

3.2 Studies Not Reviewed

Study # A 92 04 (Thirty-Day Repeat-Dose Toxicity Study of ClinOleic at a Dose of 60 mL/kg (0.2 mL/kg/min Infusion Rate) is not reviewed because this is combination of two studies (study # A 91 15 and Study # A 92 02). These two 30-day repeated-dose toxicity studies in dogs (Study # A 91 15 and Study # A 92 02) have been reviewed separately.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

1. Modification of fatty acids in rats in parenteral nutrition as a function of the type of lipid intake: Intralipid®- CSW 6.3 comparative study (Study # B 92 04).

This study was conducted to determine the effects of CSW 6.3 (ClinOleic) on essential fatty acid (EFA) status in rats relative to the EFA profile associated with the administration of Intralipid®, a soybean oil-based lipid emulsion. Male Sprague-Dawley rats were infused intravenously either with ClinOleic (6 animals) or Intralipid® (5 animals) for a period of seven days. The dose was 48 kcal per day (50% as lipids) with infusion rate of 2 mL/h. Control animals (n = 4) were catheterized but were fed enterally. At the end of the study, blood samples were collected and the liver and perineal adipose tissues were isolated. After extraction of lipids from red blood cells and liver samples, phospholipids were separated by thin-layer chromatography, and analyzed by gas chromatography, whereas adipose tissue was analyzed by gas chromatography without prior separation of lipid fractions.

The data in the Table below shows that palmitic acid remained unchanged in the plasma and erythrocytes, whereas it decreased significantly in the hepatic and adipose tissues for both lipid treated groups in comparison with the control group. Stearic acid was significantly decreased in the RBC of both lipid treated groups but remained unchanged in the plasma, liver and adipose tissues. Palmitoleic acid was lower in the plasma, RBC, liver and adipose tissues in both lipid treated groups. Hepatic and adipose phospholipids showed significant decreases of vaccenic acid in the Intralipid® group. Oleic acid was increased significantly in the plasma, RBC, and hepatic and adipose tissues of animals treated with ClinOleic. It was also significantly increased in the plasma and RBC but not in the liver and adipose tissues of the Intralipid® group. Linoleic acid was significantly lower in the plasma and RBC of both lipid treated groups and was also lower in the liver of ClinOleic group only. However, it was significantly higher in the adipose tissue of Intralipid® group. Arachidonic acid was significantly higher in the plasma and hepatic phospholipids of the ClinOleic and Intralipid® groups, but there was no variation in the RBC and adipose tissues. Docosahexaenoic acid was significantly increased in the plasma and hepatic phospholipids of both lipid treated groups.

In conclusion, ClinOleic was able to maintain similar levels of EFA compared to Intralipid® when infused to rats, while ClinOleic contributed three times less saturated fat compared to Intralipid®.

Table: Essential fatty acid after ClinOleic or Intralipid® administration to rats

Lipid name	Common name	Fatty acid composition of phospholipids in---											
		Plasma			Red blood cell			Liver			Adipose tissue		
		Control	ClinOleic	Intralipid	Control	ClinOleic	Intralipid	Control	ClinOleic	Intralipid	Control	ClinOleic	Intralipid
C 16: 0	Palmitic acid	25.7±0.9	27.1±0.6	25.5±1.2	39±1.3	36.7±1.6	38.6±0.3	22.0±0.5	19.8±0.5**	18.5±0.4**	25.2±1.0	21.9±0.5**	20.9±0.7**
C 16:1 (n-7)	Palmitoleic acid	0.5±0.2	tr	tr	0.4±0.0	0.2±0.0**	0.2±0.0**	1.1±0.3	0.3±0.0	0.2±0.0	5.9±0.4	4.8±0.3	4.3±0.4**
C 18: 0	Stearic acid	19.2±0.8	20.2±0.8	22.0±0.9	21±0.3	13±0.3*	13.7±0.0**	20.8±0.7	21.2±0.6	22.6±1.1	2.9±0.1	2.9±0.1	3.0±0.4
C 18: 1 (n-9)	Oleic acid	4.8±0.3	12.1±0.5*	7.2±1.4*	6.5±0.0	11.6±0.0**	8.9±0.3**	4.5±0.4	7.5±0.2**	3.7±0.5	24±0.3	29.1±0.1**	24±1.0
C 18: 1 (n-7)	Vaccenic acid	2.2±0.1	2.5±0.1	1.8±0.1	3.5±0.1	3.2±0.1	2.9±0.3	2.3±0.2	2.3±0.0	1.6±0.0**	3.7±0.1	3.5±0.1	3.0±0.2*
C 18: 2 (n-6)	Linoleic acid	27.3±0.7	12.3±0.8**	19.1±0.9**	13.8±0.3	8.6±0.2*	11.9±0.3*	19.8±0.8	11.7±0.8**	19.2±1.6	35.2±1.2	35.2±0.7	41.2±1.6**
C 18: 3 (n-6)	γ-linolenic acid	ND	ND	ND	nm	nm	Nm	0.2±0.1	tr	tr	nm	nm	Nm
C 18: 3 (n-3)	α-Linolenic acid	tr	tr	tr	nm	nm	Nm	nm	nm	nm	2.0±0.1	1.7±0.12	2.6±0.3**
C 20: 3 (n-9)	Mead acid	0.6±0.2	0.7±0.14	0.9±0.11	0.7±0.0	0.7±0.1	0.6±0.0	0.5±0.0	0.6±0.0	0.6±0.0	nm	nm	nm
C 20: 3 (n-6)	Dihomogammalinolenic acid	1.6±0.7	0.7±0.14	0.9±0.11	1.0±0.0	0.7±0.1*	0.7±0.0*	2.4±0.1	1.2±0.1**	0.8±0.1**	nm	nm	nm
C 20: 4 (n-6)	Arachidonic acid	13.8±1.0	20.4±1.4**	18.1±1.4*	19.7±1.4	21.7±1.2	18.6±0.6	19.0±1.1	28.5±1.2**	26.4±1.4**	0.6±0.1	0.5±0.1	0.5±0.1
C 20: 5 (n-3)	Eicosapentaenoic acid	0.6±0.32	tr	tr	0.7±0.0	0.5±0.1	0.5±0.1	nm	nm	nm	nm	nm	nm
C 22: 6 (n-3)	Docosahexaenoic acid	2.7±0.2	4.1±0.2*	4.7±0.6**	2.8±0.2	3.1±0.2	3.2±0.2	5.3±0.3	6.7±0.4*	6.8±0.4*	0.4±0.1	0.3±0.0	0.3±0.0

* = p < 0.5; ** = p < 0.1; ND = not detected; tr = traces; nm = not measured

2. *In vitro* test of hemolysis of rabbit red blood cells by lipid emulsions (CSW6-3 [ClinOleic], CSW6-2) and 20% Intralipid (Study # B 03 87 02).

The purpose of this study was to determine the *in vitro* hemolytic potential of lipid emulsions by measuring the hemoglobin released by the rabbit RBC. Rabbit RBCs (25 mL) were incubated with 100 ul of lipid emulsions (ClinOleic or Intralipid®) at 37°C. The samples were collected into Eppendorf tubes at every 30 min from 0 to 2:30 h, and then every 15 min from 2:30 h to 8 h. A spectrophotometer was used to determine the time to hemolyze 50% of RBCs (H₅₀).

The results showed that the mean H₅₀ of rabbit RBCs for ClinOleic was significantly longer (5.22 h) than that of Intralipid® (3.45 h).

3. Influence of intravenous lipid intake on peroxidation induced by pro-oxidant shock in rats (Study # B 92 06).

The purpose of this study was to investigate the effect of polyunsaturated fatty acids (PUFAs) and Vitamin E on the response to a pro-oxidant shock in Sprague-Dawley rats. Pro-oxidant shock was induced by intraperitoneal injection of carbon tetrachloride. Expired pentane (product of oxidative degradation of linoleic acid) was measured as the peroxidation marker. In this study, the diets provided to mice were deficient of Vitamin E to ensure that the lipid emulsions were the only source of Vitamin E.

In the first lipid emulsion study, groups of rats (n=12) were intravenously infused with ClinOleic or Ivelip® (soy oil). In the second study, rats (n=12) received intravenous infusions of Intralipid® (soy oil) or ClinOleic. The lipids were infused for four days along with the food without Vitamin E. The doses of intravenous lipids were 6 g/kg, corresponding to 30 mL/kg. On the 12th day of both studies, the animals were dosed intraperitoneally with carbon tetrachloride (500 µL/kg) and expired pentane was measured for 90 minutes.

Both studies did not demonstrate a relationship between pentane expiration and Vitamin E content of the lipid emulsions. Thus, the findings of the studies did not support the hypothesis that higher levels of Vitamin E (in the form of α-tocopherol) in ClinOleic afforded any additional protection against pro-oxidant shock.

4. Study of lipid peroxidation during administration of lipid emulsions to beagle dogs: Intralipid®, CSW 6-2, CSW 6-3 (Study # B 88 01).

After intravenous injection, the saturated fatty acids oxidize to various compounds including conjugated dienes. The purpose of this study was to evaluate accumulation of plasma conjugated dienes in Beagle dogs after intravenous administration of 3 different lipids (Intralipid®, CSW6-2 [11.5% soybean oil and 8.5% olive oil] and CSW6-3 [ClinOleic, 15% soybean oil and 85% olive oil]).

Male and female Beagle dogs (2/sex/dose) were intravenously infused with 9 g/kg/day (45 mL/kg/day) of lipid emulsions (Intralipid®, CSW6-2 or CSW6-3) for 30 days. A control group received 45 mL/kg/day of saline. Blood samples were collected on Days 0, 14 and 28, and conjugated dienes were measured. After extraction of the lipids from the serum, the extract was evaporated and redissolved with a known volume of heptane. The quantities of conjugated dienes were assessed by measurement of absorbance (OD [optical density]) at 232 nm. The results are expressed in OD/mL.

After 14 and 28 days of infusion, conjugated dienes significantly increased in all 3 groups that received lipid emulsion compared to control. However, animals treated with ClinOleic produced the lowest levels of conjugated dienes. Results are presented in the Table below.

CONJUGATED DIENES (OD/mL)

	Day 00		Day +14		Day +28	
	Before infusion	After infusion	Before infusion	After infusion	Before infusion	After infusion
Control						
TM01	0.71	-	0.99	-	1.00	-
TF02	1.04	-	1.06	-	1.04	-
TM03	0.96	-	0.74	-	1.23	-
TF04	1.10	-	0.95	-	0.89	-
Intralipid						
AM05	0.97	6.46	1.44	5.35	1.61	5.96
AF06	1.16	7.12	1.80	5.44	1.93	7.53
AM07	0.81	6.32	1.84	5.15	1.88	5.47
AF08	0.72	7.54	1.44	4.33	1.95	5.31
CSW6						
BM09	1.20	4.72	1.72	4.04	1.53	4.71
BF10	1.18	5.62	1.61	4.37	1.51	5.15
BM11	0.75	5.62	1.45	4.01	1.67	4.37
BF12	1.13	6.04	1.56	4.80	1.66	5.59
ClinOleic						
CM13	0.78	4.84	1.48	3.44	1.61	4.62
CF14	1.21	5.76	1.47	3.63	1.65	4.22
CM15	0.99	5.27	1.62	3.43	1.68	3.67
CF16	0.89	4.50	1.17	3.66	1.40	4.03

Intralipid 20%

CSW6-2 = 11.5% soybean oil and 8.5% olive oil

CSW6-3 = ClinOleic (soybean oil 15% and olive oil 85%)

5. Evaluation of the vitamin E status in animals (rats) as a function of diet (Study # B 92 03).

Intravenous administration of lipid emulsion could modify the isomers of Vitamin E. The purpose of this study was to evaluate different isomers of Vitamin E after IV administration of lipid emulsions in rats.

Male rats (10/treatment group) were intravenously infused with either CSW6-3 (ClinOleic, rich in α -tocopherol) or Ivelip® (rich in β - and γ -tocopherols) for 4 days at

dose of 6 g/kg/day (30 mL/kg/day). During the experimental period, the diet provided to rats was deficient of Vitamin E. At the end of the experiment, blood samples were collected from the abdominal aorta, and analyzed for plasma tocopherol levels. The four isomers of tocopherol (α , γ , β and δ) were extracted from the plasma using high performance liquid chromatography, and detected and quantified by fluorescence.

The plasma tocopherol content correlated with the content of the infused lipid; α -tocopherol level increased in the ClinOleic group and γ -tocopherol level increased in the Ivelip® group. Results are presented in the Table below.

Treatment	Tocopherols (ng/mL)			
	α	β	γ	δ
Diet without Vitamin E	3.02 ± 0.15	0.09 ± 0.02	0.07 ± 0.00	-
Ivelip® + Diet without Vitamin E	3.16 ± 0.27	0.13 ± 0.02	0.51 ± 0.13	-
ClinOleic + Diet without Vitamin E	5.48 ± 0.49	0.09 ± 0.01	0.15 ± 0.09	(0.06)

The tocopherol levels were also measured in liver samples (Study #B 92 05; continuation of study B 92 03).

High levels of α -tocopherol and γ -tocopherol were determined in the liver of ClinOleic and Ivelip® treated mice, respectively. The data are presented in the Table below.

Treatment	Tocopherols (μ g/g tissue)			
	α	β	γ	δ
Diet without Vitamin E	10.55 ± 0.77	0.0178 ± 0.034	0.035 ± 0.015	-
Ivelip® + Diet without Vitamin E	25.34 ± 3.87	0.885 ± 0.188	2.326 ± 0.513	0.107 ± 0.037
ClinOleic + Diet without Vitamin E	68.76 ± 10.72	1.237 ± 0.145	0.974 ± 0.370	0.049 ± 0.034

4.2 Secondary Pharmacology

1. Effects of parenteral nutrition on biliary secretion in the rat (Study # B 89 07):

The purpose of this study was to evaluate the biliary secretion, bile composition and hepatic cholesterol in rats after IV administration of lipids (Intralipid® and ClinOleic [CSW 6/3]).

Male Sprague-Dawley rats (n=10-14) were intravenously infused with ClinOleic (CSW 6/3) or Intralipid® for 6 days to provide 25-50% of non-protein calories. A control group was infused with 0.9% saline solution. The bile was collected at every 30 min for 2 h and the levels of cholesterol, phospholipids, total bile acids and bilirubin were determined in the total amount collected. The total serum and biliary bile acids were determined by an enzymatic method using α -hydroxysteroid dehydrogenase. The bile cholesterol was determined by an enzymatic method in kinetics with cholesterol oxidase. Bile phospholipids were determined by a colorimetric method for the presence of phosphates. The livers were lyophilized, grinded and total cholesterol and free cholesterol were determined by an enzymatic method (Biomerieux®).

The results showed that animals administered Intralipid® at either 25% or 50% of caloric intake had decreased biliary flow and increased bilirubin and phospholipids. Similarly, rats administered 25% caloric intake as Intralipid® also showed increased

biliary calcium, cholesterol and bile acid levels. The bile flow in the ClinOleic groups (25% or 50% caloric intake) was decreased compared to control group but it was higher than animals in the Intralipid® groups. Bilirubin levels were increased in the ClinOleic at either 25 or 50% of the caloric intake groups. Rats that received 25% caloric intake as ClinOleic also exhibited increased biliary calcium and bile acids levels compared to control. However, there was no change in biliary phospholipids and cholesterol levels in the rats receiving ClinOleic. The results are presented in the Table below.

Parameters	50% of Calories			25% of Calories		
	Control	Intralipid®	ClinOleic (CSW 6/3)	Control	Intralipid®	ClinOleic (CSW 6/3)
Bile flow (mL/h)	0.906±0.042	0.624±0.048**	0.732±0.048**	1.01±0.08	0.666±0.048**	0.726±0.042**
Bilirubin (µmol/L)	109±6	375±80**	390±60**	61±15	145±88*	142±56*
Calcium (mmol/L)	Not measured			2.24±0.12	2.39±0.10*	2.43±0.06*
Phospholipids (mmol/L)	3.77±0.44	5.07±0.81*	3.66±0.95	3.88±0.65	4.83±0.55*	4.09±0.97
Cholesterol (mmol/L)	0.348±0.099	0.426±0.237	0.330±0.109	0.355±0.048	0.448±0.100*	0.314±0.049
Bile acids (mmol/L)	14.57±1.53	16.73±2.05	16.73±2.73	9.44±2.15	14.05±2.13*	15.90±3.79**

Hepatic lipid analysis showed significantly higher levels of lipid accumulation at 50% of caloric intake for both Intralipid® and ClinOleic groups. Hepatic cholesterol was higher for ClinOleic than Intralipid® at either 25% or 50% of caloric intake groups compared to the control, as shown in the Table below.

Parameters	50% of Calories			25% of Calories		
	Control	Intralipid®	ClinOleic (CSW 6/3)	Control	Intralipid®	ClinOleic (CSW 6/3)
Total lipid (mg/gm liver)	116±6	114±24*	167±18**	105±9	126±18	132±18
Total cholesterol (mg/gm liver)	5.89±0.37	9.27±1.13*	11.54±1.85**	5.82±1.51	7.91±1.05*	9.00±1.80**

In conclusion, in rats, ClinOleic modulate biliary secretion with reduced biliary phospholipids and cholesterol more than that with Intralipid®.

2. Hepatobiliary complications of total parenteral nutrition - effect of the components of lipid emulsion (Study # A 91 23):

The purpose of this study was to evaluate the hepatobiliary effects of lipids and emulsifiers used in lipid emulsions for total parenteral nutrition in rats.

Male Sprague-Dawley rats were maintained on total parenteral nutrition (2.5mL/h) for 7 days as 30% non-protein calories in the form of lipids, either ClinOleic (CSW 6/3), Ivelip®, Ivelip® Z40 or Intralipid®. A control group was infused with 0.9% saline solution. The lipid emulsions also differed in the ratio of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the purified egg phospholipid used as an emulsifier. Intralipid® and Ivelip® Z40 emulsion contained egg phospholipid with a low PC/PE ratio (5.3), while ClinOleic (CSW 6/3) and Ivelip® contained egg phospholipid with a high PC/PE ratio (9.4), as shown in the Table below.

Composition of the Lipid Emulsions Tested

	Group A= CSW 6.3 Batch 92122	Group B † IVELIP Batch 94958	Group C= IVELIP Z40 Batch 92188	Group D Intralipid Batch 51663.51 KABI VITRUM
Soybean oil	30 g/L	200 g/L	200 g/L	200 g/L
Olive oil	170 g/L	-	-	-
Egg lecithin	12 g/L PC/PE = 9.4	12 g/L PC/PE = 9.4	12 g/L PC/PE = 5.3	12 g/L PC/PE = 5.3
Na Oleate	0.3 g/L	0.3 g/L	0.3 g/L	-
Glycerol	22.5 g/L	25 g/L	25 g/L	22.5 g/L

Collection of bile and other analytical methods were same as mentioned above (Study # B 89 07).

The animals that received a total parenteral nutrition as ClinOleic (CSW 6/3) had higher bile output than the Intralipid®, Ivelip® Z40 and Ivelip® groups. Animals that received lipid emulsions containing egg phospholipid with a low PC/PE ratio showed significantly reduced biliary flow relative to lipid emulsions containing phospholipid with a high PC/PE ratio (CSW 6/3 and Ivelip®). Results are presented in the Sponsor's table below.

	µl/min
Reference (n = 11)	18.0 ± 0.41
CSW 6.3 lot 92122 † (A) (n=11)	16.4 ± 0.37
IVELIP lot 94958 † (B) (n=13)	15.5 ± 0.27
IVELIP Z40 lot 92118 • (C) (n=13)	14.6 ± 0.36
INTRALIPID lot 51663.51 • (D) (n=13)	14.2 ± 0.36

KEY: .
 † : PC/PE 9.4 lot = batch
 • : PC/PE 5.3
 * p < 0.05 ** p < 0.01

In conclusion, this study showed that the composition of the emulsifying agent used in the lipid emulsions can modulate hepatobiliary secretion.

4.3 Safety Pharmacology

Study: CSW 6.3, batch 72002-Search for hypotensive substances (Study # A 01 87 14):

Methods: The aim of this study was to determine the potential hypotensive effect of CSW 6.3 (ClinOleic). Two male anesthetized cats were catheterized in the carotid artery to monitor arterial blood pressure and heart rate. Histamine (positive control) and test article (CSW 6.3) were administered via a jugular vein catheter. Sensitivity of the animals to histamine injection was determined and the animals were only used if the intensity of the hypotensive response was proportional to the dose administered. The hypotensive response to 0.1 µg/kg injections of histamine was reproducible and was at least 20 mmHg. The hypotensive response of CSW 6.3 was determined after intravenous administration of 5 mL CSW 6.3 over 30 sec.

Results: The administration of 5 mL of CSW 6.3 over 30 sec had no hypotensive effect in cats.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

ABSORPTION:

No absorption studies with ClinOleic were conducted.

DISTRIBUTION:

Study of the tissue distribution of lipid emulsions, CSW6-2 and CSW6-3, compared to 20% Intralipid (Study # B 03 87 04).

Methods: Groups of 10 male mice received 16 g/kg (80 mL/kg) of ClinOleic (CSW 6/3; 3% soybean oil: 17% olive oil), Intralipid®, or CSW 6/2 (11.5% soybean oil: 8.5% olive oil) as intravenous injections at a rate of 1 mL/min. After injection, the animals were fed with their normal diet, and were sacrificed 24 h after administration of the lipids. The liver, spleen, and lungs were isolated for measurement of triglycerides.

Results: The triglyceride concentrations in the liver, spleen, and lung were identical for all three groups of rats treated with lipid emulsions. The results are presented in the Sponsor's Table below.

TISSUE TRIGLYCERIDES
(in mg/g of wet tissue)

	LIVER	SPLEEN	LUNG
CSW6-2 emulsion	10.5 ± 2.2	2.6 ± 0.9	4.1 ± 0.5
CSW6-3 emulsion	11.5 ± 4.0	2.1 ± 0.6	3.9 ± 0.3
20% INTRALIPID®	10.3 ± 2.5	2.2 ± 0.8	3.9 ± 0.6

METABOLISM:

1. Determination of the post-heparin lipolytic activity (PHLA) of the emulsions, Ivelip®, CSW6-3 (ClinOleic), CSW6-2, and CSW 6-1 compared to Intralipid® (Study # B 88 02).

Methods: Ivelip® (soybean oil 20%, olive oil 0%), CSW6-3 (soybean oil 3%, olive oil 17%), CSW6-2 (soybean oil 8.5%, olive oil 11.5%), CSW 6-1 (soybean oil 0%, olive oil 20%) and Intralipid® (soybean oil 20%, olive oil 0%) were incubated with rat plasma at 27-28°C, and free fatty acid content was measured following 0, 5, 10, 15, and 20 minutes of incubation. Plasma lipolysis was evaluated in post-heparin plasma with respect to various emulsions.

Results: Lipolysis of CSW 6-1 was slightly lower (24% less) than the reference emulsion Intralipid®. Lipolysis of Ivelip® under the same conditions was similar to CSW 6-1. The result showed that all lipid emulsions were slightly less hydrolyzed than Intralipid®. Results are presented in the Sponsor's table below. The Sponsor stated that this could be due to the presence of inhibitors of lipolytic activity in post-heparin plasma such as oxidation derivatives and of sterols. To verify this unpredicted result, another lipolytic experiment was conducted (study # A 91 03) to standardize the lipolytic protocol. The study showed that ClinOleic (CSW 6/3) can be lipolyzed faster than Intralipid®.

	20% INTRALIPID (82444)	20% CSW 6/3 (72002)	20% CSW 6/2 (72001)	20% IVELIP (7404601)	CSW 6/1 (CER 86010)
Composition %:					
olive	0	17	11.5	0	20
soybean	20	3	8.5	20	0
sodium oleate	-	0.03	0.03	0.03	0.03
glycerol	2.25	2.25	2.25	2.5	2.25
PHLA activity μ M free fatty acids/min/mL of plasma \pm IC	0.322 \pm 0.018	0.278 \pm 0.029	0.278 \pm 0.020	0.271 \pm 0.024	0.246 \pm 0.018
% of error	5.624	10.347	7.292	8.915	7.461
Significance	<p>Diagram illustrating statistical significance between groups:</p> <ul style="list-style-type: none"> Intralipid vs CSW 6/3: ** Intralipid vs CSW 6/2: NS Intralipid vs Ivelip: NS Intralipid vs CSW 6/1: * CSW 6/3 vs CSW 6/2: ** CSW 6/3 vs Ivelip: ** CSW 6/3 vs CSW 6/1: NS CSW 6/2 vs Ivelip: ** CSW 6/2 vs CSW 6/1: ** Ivelip vs CSW 6/1: ** 				

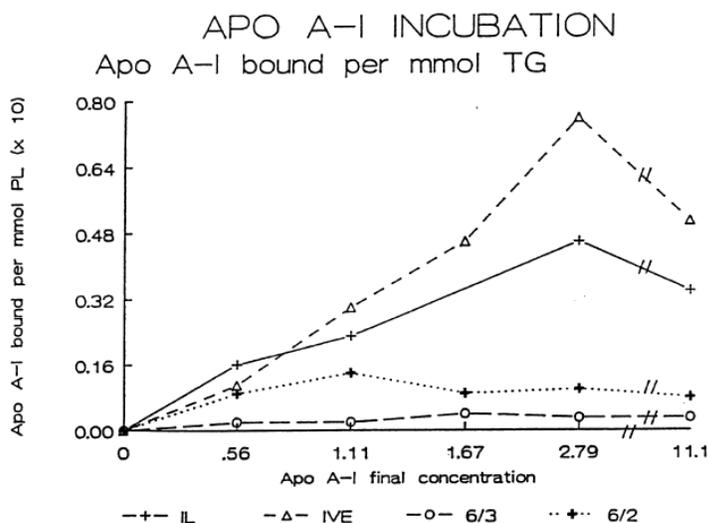
NS = Not significant - * = P < 0.05 - ** = P < 0.01 - (Duncan test)

Correction coefficient for Ivelip and CSW 6/1 = 1.028

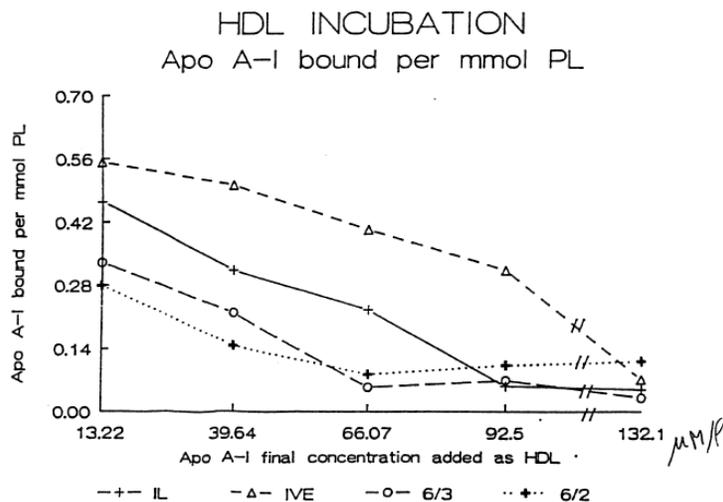
2. Study: Comparing soybean oil and olive oil emulsions for their apolipoprotein binding capacity and lipoprotein lipase substrate function (Study # B 92 02).

Methods: Artificial triglyceride-rich particles (ATRP) were prepared from Intralipid® (20 % soybean oil), Ivelip® (20 % soybean oil), CSW 6/2 (11.5 % olive oil - 8.5 % soybean oil) and CSW 6/3 (17 % olive oil - 3 % soybean oil) by ultracentrifugation. These ATRPs were incubated for 2 hours at 37°C in the presence of apolipoprotein (Apo) A-I or high density lipoproteins (HDLs). Lipolysis was activated by the addition of purified milk lipoprotein lipase and incubated for another 30 min at 37°C. Triglycerides were measured with an enzymatic colorimetric assay. Phospholipids were measured by the Fiske Subbarow phosphate analysis method. Apolipoprotein A-1 in HDL and purified Apo A-1 in solution were measured by the immunoturbidimetry method. Apolipoprotein A-1 in the incubation medium was measured by a Sandwich ELISA technique.

Results: The results show that Apo A-I binding was greater for the lipid particles from soybean oil (Intralipid®, Ivelip®) than the emulsions containing soybean and olive oil (CSW 6/3, CSW 6/2). The findings are presented in the Figure below.



The incubation of ATRP with various lipid emulsions (Intralipid®, Ivelip® and CSW 6/2 and CSW 6/3) with different amounts of High Density Lipoprotein (HDL) as a source of Apo A-1 was sufficient to obtain maximum lipolysis. The findings are presented in the Figure below.



In conclusion, the substrate function of the ATRPs from CSW 6/2, CSW 6/3 and the reference emulsions (Intralipid®, Ivelip®) for lipoprotein lipase were similar following preincubation with HDLs.

ELIMINATION:

1. Study: Kinetics of plasma clearance of exogenous lipids in the rat: comparison of two 20% lipid emulsions Intralipid® and CSW 6/3 (Study # B 89 04).

Methods: Plasma elimination kinetics was evaluated in 33 male Sprague-Dawley rats following intravenous administration of 0.2 g/kg (1 mL/kg) of either CSW 6/3 (ClinOleic) or Intralipid®. Blood samples were collected prior to injection and at 3, 5, 10, 15, 20, and 30 minutes after injection to determine triglyceride levels by turbidimetry methods. The elimination kinetics was determined as the fractional clearance (K_2) as a percentage per min, as well as the initial concentration extrapolated C_0 to T_0 .

Results: The fractional clearance (K_2) was $11.1 \pm 1.36\%$ per min for CSW 6/3 (ClinOleic) compared to $12.4 \pm 0.76\%$ per min for Intralipid®. The initial concentrations (C_0 extrapolated to T_0) were 6.38 ± 0.45 g/L and 6.60 ± 0.56 g/L for CSW 6/3 (ClinOleic) and Intralipid®, respectively. The findings are shown in the Table below.

**Elimination Kinetics of Lipid Emulsions
Following Intravenous Administration to Rats**

Lipid Emulsion	K_2 (%/min)	Initial concentration (C_0) (g/L)
ClinOleic	11.1 ± 1.36	6.38 ± 0.45
Intralipid	12.4 ± 0.76	6.60 ± 0.56

K_2 = fractional clearance; C_0 = initial concentration extrapolated to t_0

In conclusion, both fat emulsions, Intralipid® and CSW 6/3 (ClinOleic), were cleared in a comparable fashion.

2. Study: Plasma clearance kinetics of two 20% lipid emulsions: CSW 6.2 and CSW 6.3 in the dog; comparison with 20% Intralipid® (Study # A 89 07).

Methods: Plasma elimination kinetics were evaluated in groups of 3 male and 3 female Beagle dogs following one hour intravenous infusions of 3 g/kg (15 mL/kg) of CSW 6.3 (ClinOleic), CSW 6/2, or Intralipid®. Blood samples were collected from the cephalic vein or a saphenous vein to determine the triglyceride levels prior to infusion, and at every 10-minute intervals for 3 hours, then at every 15-minute intervals for 4 hours following infusion. The maximum clearance (K_1), fractional clearance (K_2) and critical clearance were determined.

Results: The maximum clearances (K_1) were 0.255 ± 0.023 g/L/min for CSW 6/3 (ClinOleic) and 0.249 ± 0.034 for CSW 6/2 compared to 0.308 ± 0.033 g/L/min for Intralipid®. The fractional clearance (K_2) were 1.128 ± 0.116 % per min for CSW 6/3 (ClinOleic) and 1.096 ± 0.066 for CSW 6/2 compared to $2.038 \pm 0.560\%$ per min for Intralipid®. The critical concentrations were 23.04 ± 1.75 g/L, 22.72 ± 2.79 g/L and 18.67 ± 3.67 g/L for CSW 6/3 (ClinOleic), CSW 6/2 and Intralipid®, respectively. The results are presented in the Table below.

**Elimination Kinetics of Lipid Emulsions
Following Intravenous Administration to Dogs**

Lipid Emulsion	K ₁ (g/L/min)	K ₂ (%/min)	Critical concentration (g/L)
CSW 6/2	0.249 ± 0.034	1.096 ± 0.066	22.72 ± 2.79
ClinOleic	0.255 ± 0.023	1.128 ± 0.116	23.04 ± 1.75
Intralipid	0.308 ± 0.033	2.038 ± 0.560	18.67 ± 3.67

K₁ = maximum clearance capacity, K₂ = fractional clearance

In conclusion, all three lipid emulsions, Intralipid®, CSW 6/2 and CSW 6/3 (ClinOleic), were cleared in a comparable fashion in dogs. However, the fractional clearance (K₂) of Intralipid® was higher than ClinOleic and CSW 6/2.

5.2 Toxicokinetics

N/A

6 General Toxicology

6.1 Single-Dose Toxicity

1. Study title: Acute toxicity by intravenous route in male and female mice - determination of LD₅₀ CSW 6.3 (ClinOleic) batch 72002 (study # A 87 15).

Key study findings: Groups of CD1 mice (10 per group) received intravenous infusions of lipid emulsion at doses of 100, 112.5, 125, and 137.5 mL/kg body weight, at an infusion rate of 1 mL/min. Animals were observed for 14 days after lipid administration. Mortality occurred for up to three days after administration. Highest mortality was observed in the high and mid-high dose groups. The causes of death were not determined. The symptoms observed were non-specific neuromuscular signs, and most of the dead animals were not necropsied due to tissue autolysis. Gross observations in mice that were necropsied involved lesions in the liver and spleen. Mice euthanized after 14 days of observation showed lesions in the liver, spleen, and kidneys. The LD₅₀ was between 100 mL/kg and 112.5 mL/kg in male mice, corresponding to 20 - 22.5 g/kg. In female mice, the LD₅₀ was 111.4 mL/kg (104.8 -118.4 mL/kg), corresponding to 22.3 g/kg.

Study No.: A 87 15

Volume and pages: Electronic submission

Conducting laboratory and location: [REDACTED]

(b) (4)

Date of study initiation: April 10, 1987

GLP compliance: Yes

QA report: Yes

Drug lot and purity: Drug lot 72002, purity not provided.

Methods:

Doses: 100, 112.5, 125, and 137.5 mL/kg
 Species/strain: CD1 mice
 Number/sex/group or time point (main study): 10/sex/group
 Route, formulation, volume and infusion rate:
 Route: Intravenous
 Formulation: 20% lipid emulsion
 Infusion rate: 1 mL/min
 Satellite groups: No
 Age: Not specified
 Weight: Male 26g and female 23g
 Unique study design or methodology (if any): none

Observations and Results:**Mortality:**

Animals were observed daily for mortality. Mortalities occurred up to three days after intravenous injection. Table below shows the mortality pattern of animals. All survived animals were euthanized on Day 15.

Dose (mL/kg)	Male mortality 2 days after injection (n=10)	LD ₅₀ in males	Female mortality 3 days after injection (n=10)	LD ₅₀ in females
100	0	100 mL/kg and 112.5 mL/kg (20 - 22.5 g/kg)	3	111.4 mL/kg (104.8 - 118.4 mL/kg; 22.3 g/kg)
112.5	6		3	
125	9		9	
137.5	10		10	

Clinical signs:

Clinical signs were observed daily for 14 days. Both male and female mice exhibited diminished activities, ataxia, tonic and clonic convulsions, loss of reflex and increased respiration.

Body weight:

Body weights were measured before injection and on Day 14. Both male and female mice showed gains in body weight from 3 to 7g. The body weights for different groups are shown in the Table below.

Dose (mL/kg)	MALE				FEMALE			
	No of animal on Day 14	Weight on Day 1 (g)	Weight on Day 14 (g)	Weight gain (g)	No of animal on Day 14	Weight on Day 1 (g)	Weight on Day 14 (g)	Weight gain (g)
100	10	26.6±0.5	34.4±0.8	7.8	7	22.3±0.3	25.4±0.5	3.1
112.5	4	24.6±0.6	31.2±0.5	6.6	7	21.3±0.5	24.0±0.7	2.7
125	1	27.4	31.1	3.7	1	21.6	24.3	2.7

Gross Pathology:

Mice that died on day 2 and 3 showed pale lesions in the kidney and white spots and marbled liver. All surviving animals were euthanized on Day 15. The euthanized mice showed pale, swollen and dilated kidneys, marbled liver and splenomegaly.

Summary of individual findings: Single IV dose of CSW 6.3 in mice showed 100% mortality at the high dose, followed by 90% mortality at the medium high dose within 2 to 3 days in both males and females. Main organs of toxicity were the kidney, spleen and liver. The LD₅₀ was between 100 mL/kg and 112.5 mL/kg in male mice, corresponding to 20 - 22.5 g/kg. In female mice, the LD₅₀ was 111.4 mL/kg (104.8 - 118.4 mL/kg), corresponding to 22.3 g/kg.

2. Study title: Acute toxicity by intravenous route in male and female rats - determination of LD₅₀ CSW 6.3 (ClinOleic) batch 72068 (Study # A 88 14).

Key study findings: Mortalities occurred for up to 7 days after lipids administration. The cause or causes of death was not determined. The symptoms observed were non-specific neuromuscular signs, and most of the dead animals were not necropsied because of tissue autolysis. Gross observations in rats that were necropsied at the end of the study period included lesions in the liver, kidney, lungs and spleen. The LD₅₀ was estimated to be around 100 mL/kg in male and female rats, corresponding to 20 g/kg. No control group was included in this study.

Study No.: A 88 14

Volume and pages: Electronic submission

Conducting laboratory and location: (b) (4)

Date of study initiation: April 6, 1988

GLP compliance: Yes

QA report: Yes

Drug lot and purity: Drug lot 72068, purity not provided.

Methods:

Doses: Male: 100, 110, 120 and 130 mL/kg, and Female: 100, 125, 130, and 135 mL/kg

Species/strain: Sprague Dawley Rats

Number/sex/group or time point (main study): 6/sex/group

Route, formulation, volume and infusion rate:

Route: Intravenous

Formulation: 20% lipid emulsion

Infusion rate: 1mL/min

Satellite groups: None

Age: Not specified

Weight: Male 266±18g and female 202±13g

Unique study design or methodology (if any): none

Observations and Results:**Mortality:**

Animals were observed daily for mortality. Mortalities occurred up to 7 days after intravenous injection. The mortality pattern for different doses is shown in the Table below. All survived animals were euthanized on Day 15.

Dose (mL/kg) Male	Male mortality 4 days after injection (n=6)	LD ₅₀ in males	Dose (mL/kg) Female	Female mortality 7 days after injection (n=10)	LD ₅₀ in females
100	3	About 100 mL/kg (20g/kg)	100	4	About 100 mL/kg (20g/kg)
110	5		125	6	
120	6		130	6	
130	6		135	6	

Clinical signs:

Clinical signs were observed daily for 14 days. Both male and female rats exhibited diminished activities, ataxia, loss of reflex and increased respiration.

Body weight:

Body weights were measured before injection and on Day 14. Both male and female surviving rats showed a gain in body weight from 18 to 41g. However, the numbers of animals are very small due to early mortality of animals. Results are presented in the Table below.

Dose (mL/kg)	MALE				FEMALE			
	No of animal on Day 14	Weight on Day 1 (g)	Weight on Day 14 (g)	Weight gain (g)	No of animal on Day 14	Weight on Day 1 (g)	Weight on Day 14 (g)	Weight gain (g)
100	3	288±10	309±7	21	2	195±11	213±24	18
110	1	260	309	49				

Gross Pathology:

The rats that died on day 4 and 7 showed pale lesions in the kidney and marbled liver with white spots on the liver. All surviving animals were euthanized on Day 15. Euthanized rats showed pale, swollen, dilated kidneys, marbled liver, splenomegaly and hemorrhagic lungs.

Summary of individual findings: In summary, single IV dose of CSW 6.3 in rats showed high mortality at all doses. Only 2 females and 3 males survived at the low dose and 1 male on 110 mg/kg. All remaining male and female rats died within 4 to 7 days after injection. Main organs of toxicity were the kidney, spleen, liver and lungs. The LD₅₀ was estimated to be around 100 mL/kg in male and female rats, corresponding to 20 g/kg.

6.2 Repeat-Dose Toxicity

Study title: Toxicity at repeated doses by intravenous administration to rats of 20% Endolipide, 20% Ivelip batch 92050.01 and CSW 6.3 batch 92001.01 (30

Days of administration).

Study no.:	A 90 11
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 16, 1990
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ivelip® batch 92050.01 and CSW 6.3 batch 92001.01; purity not provided

Key Study Findings

Eight rats died and 3 were terminated before the completion of the study (3, 4 and 4 rats from Endolipide®, Ivelip® and ClinOleic groups, respectively). The mortalities occurred during the first 10 days of the study. At necropsy, these rats showed white organ discoloration and yellow deposits in the renal pelvis. Morbidity was attributed to renal failure secondary to renal papillary necrosis. Rats in all lipid emulsion groups had anemia with reticulocytosis and thrombocytopenia. Serum chemistry included decreased urea concentrations and increased alanine aminotransferase and aspartate aminotransferase activities in all lipid emulsion groups. Creatinine concentrations increased in rats in the Ivelip® and ClinOleic groups. Cholesterol, triglyceride, phospholipids, and bilirubin concentrations increased in rats which received Endolipide®. Treatment-related histopathologic changes in livers, adrenal, kidney and spleen were noticed. Due to mortalities and organ toxicities, the dose of lipid emulsions in this study, 90 mL/kg/day at a rate of 2 mL/kg/day, was considered excessive and unsuitable for the comparison to different lipid emulsions.

Methods

Doses:	18 g/kg/day (90 mL/kg/day)
Frequency of dosing:	Once daily for 45 min
Route of administration:	Intravenous
Dose volume:	2 mL/kg/min
Formulation/Vehicle:	Ivelip® and Endolipide® (20% soybean oil based emulsion) and CSW 6/3 (Olive oil and soybean oil)
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	Group A (4 males): 0.9% NaCl Group B (6 males- 6 females): 20% Endolipide® Group C (7 males- 7 females): 20% Ivelip® Group D (6 males- 7 females): CSW 6/3 (ClinOleic) Group T (3 males- 1 female): No treatment
Age:	Not specified
Weight:	Males – approximately 270g and females –

approximately 200g.
Satellite groups: None
Unique study design: None
Deviation from study protocol: None

Observations and Results

Mortality

Eight animals died during the course of the study, and 3 animals were terminated before the completion of the study. They are 3 rats from Endolipide® (group B), 4 rats from Ivelip® (group C), and 4 rats from ClinOleic (group D). The mortality occurred during the first 10 days of the study. Female rats died in at higher numbers (8 females vs. 2 males).

Clinical Signs

Hematuria was noticed in groups B, C, and D. The frequency of hematuria was higher in male animals of the Endolipide® group and in a female animal of the Ivelip® group. Sporadic diarrhea was also noted in some animals of all groups.

Body Weights

The variation of body weight was similar in the 3 groups of animals treated with lipids (Endolipide®, Ivelip® and CSW 6/3).

Feed Consumption

Food consumption (g/day) was reduced for all animals treated with lipids. The reduction of food consumption was approximately 60% starting the first week of treatment, and it was similar in the 3 lipid treated groups (B, C, and D).

Hematology

Blood samples were collected by abdominal aorta under halothane anesthesia. Food and water were removed overnight prior to blood collection.

Decreased hemoglobin and thrombopenia (decreased platelets) were observed in all lipid treated groups. Several animals in the Endolipide® group showed neutrophilia (increased neutrophils). Results are presented in the Sponsor's Table below.

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PARAMETERS	UNITS	Endolipide®		Ivelip®		CSW 6/3 (ClinOleic)							
		Male	Female	Male	Female	Male	Female						
Red blood cells	$10^6/mm^3$	6.47	0.38	6.18	0.31	7.01	0.16	7.03	0.30	6.98	0.17	6.51	0.10
Hematocrit	%	37.6	1.6	34.0	1.0	39.9	1.2	40.4	1.5	39.1	1.2	37.3	0.8
Hemoglobin	g/dl	13.8	1.4	11.8	0.5	14.1	0.5	14.4	0.5	13.8	0.4	13.1	0.3
M.C.V.	f.l	58	1.5	55	1.2	57	1.5	58	1.4	56	1.3	57	1.2
M.C.H.	pg	20.5	0.3	18.8	1.0	20.0	0.5	20.5	0.5	19.8	0.4	20.2	0.3
M.C.H.C.	g/100 ml	35.3	2.15	34.0	0.35	35.3	0.16	35.6	0.27	35.3	0.47	35.2	0.25
Reticulocytes	%	10.4	1.5	7.2	1.1	6.6	0.7	5.0	0.3	7.5	1.2	5.4	0.7
Reticulocytes	$10^6/mm^3$	0.656	0.091	0.441	0.054	0.459	0.061	0.349	0.017	0.515	0.076	0.348	0.051
White blood cells	$10^3/mm^3$	6.7	1.0	6.5	1.6	6.2	1.1	4.9	1.0	6.0	0.5	2.9	0.3
Neutrophils	%	31	2.6	44	8.6	26	2.0	25	3.9	32	4.2	34	4.1
Neutrophils	$10^3/mm^3$	2.70	0.38	2.98	0.97	1.56	0.23	1.20	0.27	1.90	0.26	0.98	0.14
Eosinophils	%	0	0.4	0	0.0	0	0.2	0	0.3	0	0.2	0	0.3
Basophils	%	0	0.0	0	0.0	0	0.2	0	0.0	0	0.0	0	0.0
Lymphocytes	%	67	2.7	54	8.0	73	1.9	73	4.4	67	4.3	52	17.0
Lymphocytes	$10^3/mm^3$	5.82	0.66	3.45	0.98	4.54	0.64	3.95	0.78	4.04	0.45	13.83	13.73
Monocytes	%	1	0.4	2	1.0	1	0.4	2	0.5	2	0.5	1	0.5
Platelets	$10^3/mm^3$	786	68	623	103	825	44	597	95	735	79	631	59
P. time	sec.	12.8	0.7	12.0	0.6	11.5	0.3	10.8	0.1	11.4	0.2	10.9	0.1

Clinical Chemistry

Following clinical chemistry parameters were determined.

PARAMETRES PARAMETERS	UNITES UNITS
Glucose	mmol/l
Uree/B.U.N.	mmol/l
Bilirubine totale	umol/l
Acides bilaires	ummol/l
Cholesterol total	mmol/l
Cholesterol HDL	mmol/l
Triglycerides	mmol/l
Phospholipides	mmol/l
S.G.O.T./A.S.A.T.	UI/l
S.G.P.T./A.L.A.T.	UI/l
Phosphatases alcalines	UI/l
Creatinine	umol/l
Proteines totales	g/l
Albumine	g/l
Globulines	g/l
Rapport A/G. A/G Ratio	
Sodium	mmol/l
Potassium	mmol/l

Decreased urea levels and increased creatinine levels were noticed in the Ivelip® and CSW 6-3 treated groups. The levels of transaminase SGPT were elevated in all the lipid treated groups. The levels of cholesterol, phospholipids, triglycerides and bilirubin were increased only in the Endolipide® group. The sodium levels were lower in Endolipide® group than other 2 lipid treated groups. Glucose level was higher in the CSW 6-3 group than the Ivelip® and Endolipide® groups. Results are presented in the Sponsor's Table below.

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PARAMETRES PARAMETERS	UNITS	Endolipide®		Ivelip®		CSW 6/3 (ClinOleic)							
		Male	Female	Male	Female	Male	Female						
		MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.
Glucose	mmol/l	6.03	0.45	4.68	0.80	6.95	0.53	6.37	0.40	6.32	0.67	7.74	0.70
Uree/B. U. N.	mmol/l	3.7	0.45	3.0	0.47	2.9	0.35	4.4	0.33	3.2	2.15	4.5	0.08
Bilirubine totale	umol/l	4.6	0.83	4.7	1.24	2.4	0.57	1.6	0.65	2.3	0.32	1.1	0.45
Acides biliaires	umol/l	13.28	7.44	21.01	4.63	8.06	4.60	4.45	2.40	20.39	6.24	16.82	5.49
Cholesterol total	mmol/l	10.94	5.19	7.67	4.15	1.57	0.19	1.10	0.13	1.11	0.20	0.54	0.06
Cholesterol HDL	mmol/l	0.54	0.05	0.85	0.17	0.53	0.05	0.56	0.13	0.44	0.05	0.34	0.09
Triolycerides	mmol/l	10.26	4.86	6.36	5.13	0.64	0.09	0.54	0.05	0.80	0.26	0.53	0.04
Phoronolipides	mmol/l	3.29	0.27	3.66	0.68	2.21	0.17	1.80	0.14	1.60	0.14	1.51	0.10
S. B. D. T. /A. S. A. T.	UI/l	305	75	218	27	151	8	120	11	579	265	268	84
S. B. P. T. /A. L. A. T.	UI/l	228	115	103	39	82	21	33	6	755	442	155	56
Phosphatases alcalines	UI/l	170	31	123	30	126	7	54	6	201	37	130	19
Creatinine	umol/l	48	8.1	61	8.3	91	9.1	67	4.3	96	22.9	69	8.6
Proteines totales	g/l	59	1.2	59	2.1	58	0.9	63	0.3	59	1.5	61	1.9
Albumine	g/l	37	1.0	36	1.6	36	0.7	37	1.3	37	1.5	34	1.5
Globulines	g/l	21	0.5	23	1.3	22	1.1	25	1.2	22	2.1	26	1.3
Rapport A/G. A/G Ratio		1.76	0.06	1.56	0.10	1.66	0.10	1.49	0.13	1.75	0.20	1.31	0.09
Sodium	mmol/l	141	1.7	144	3.5	149	1.9	148	1.3	143	1.3	147	1.4
Potassium	mmol/l	5.4	0.1	4.8	0.1	5.2	0.1	4.7	0.3	5.5	0.2	5.4	0.1

Urinalysis

Urine analysis was conducted once at the end of the experiment. Urine volume, density and pH were measured.

Diuresis was noticed in the Ivelip® group compared to the 2 other lipid treated groups. In addition, ketone bodies and traces of hemoglobin were noticed in the urine of most of the animals.

Gross Pathology

Animals were euthanized at the end of the experiment following an overnight period without food and/or water. For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

The liver of all animals of the Endolipide® group and few animals of the two other lipid treated groups showed yellow or white spots. The kidneys of most of the animals had congested or swollen appearance with a nonvisible corticomedullary hypertrophy. Yellow spots were noted on the adrenal glands of all female animals of the Endolipide® group and a few from other groups.

Organ Weights

For all animals, liver, heart, kidney, lungs and adrenal glands were dissected, trimmed free of fat and weighed:

The weight of the liver was higher than the normal range in the lipid treated groups but the hypertrophy was more extensive in the Endolipide® and CSW 6-3 treated groups. The weight of the spleen was increased in the Endolipide® group. The findings are presented in the Sponsor's Table below.

PARAMETRES PARAMETERS	Endolipide®				Ivelip®				CSW 6/3 (ClinOleic)			
	Male		Female		Male		Female		Male		Female	
	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.						
Poids / Weight (g)	302.26	17.62	243.15	5.82	343.80	12.26	232.58	17.41	322.44	17.93	225.43	4.27
Foie / Liver	14.233	0.820	13.236	1.398	12.681	0.548	9.585	1.284	16.010	1.286	11.017	0.731
Rate / Spleen	1.311	0.143	0.942	0.066	0.848	0.056	0.714	0.094	0.737	0.041	0.713	0.036
Rein d. / R. kidney	1.472	0.101	1.140	0.096	1.741	0.040	0.967	0.067	1.304	0.077	0.888	0.106
Rein g. / L. kidney	1.514	0.108	1.165	0.122	1.713	0.029	0.977	0.064	1.303	0.068	0.935	0.077
Poumons / Lung	1.309	0.051	1.276	0.097	1.375	0.063	1.130	0.127	1.248	0.052	1.122	0.073
Coeur / Heart	1.168	0.055	1.071	0.079	1.256	0.054	0.989	0.104	1.173	0.076	0.891	0.032
Surrenal e. / R. adrenal	0.0339	0.0012	0.0511	0.0242	0.0344	0.0042	0.0485	0.004	0.0333	0.0038	0.0430	0.0049
Surrenal e. / L. adrenal	0.0384	0.0064	0.0375	0.0266	0.0435	0.0036	0.0469	0.004	0.0397	0.0034	0.0630	0.0365

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Histopathology

Adequate Battery: No

Peer Review: No

Histological Findings

On completion of the gross pathology examination, the liver, spleen, lungs, heart, kidneys and adrenal glands were fixed in 10% neutral buffered formalin for the histological studies.

Histopathological findings are mainly associated with the liver, kidneys and spleen. Intralobular inflammatory granulomas were present in most of the animals. Foci of hepatocytic fatty degeneration were observed in all the lipid treated groups. Foci of hemorrhagic necrosis were noted in 2 animals of the CSW 6-3 group. Lipid deposits in the hepatocytes and in the Kupffer cells were noted in all lipid treated animals. In the spleen, high myelopoietic activities of similar pattern were observed in all the lipid treated groups. In the kidneys, various stages in the development of epithelial, interstitial or chronic sclerocystic nephritis are observed in all 3 lipids treated groups. They are more extensive in the Ivelip® and CSW 6.3 groups than in the Endolipide® group. In the adrenal glands, a high lipid overload was noted in most of the animals. In the lungs, densifications of the alveolar septa associated with emphysema were observed in all the treated animals.

Study title: Toxicity due to repeated intravenous doses of CSW 6/3 (ClinOleic) emulsion lot 1120170 in the rat - comparison with Intralipid 20% lot 62121-51 (30 days of administration).

Study no.:	A 92 03
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 24, 1991
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Intralipid 20% lot 62121-51 and CSW 6/3 batch 1120170; purity not provided

Key Study Findings

A group of Sprague Dawley rats (5 rats/sex) were administered either CSW 6/3 (ClinOleic) or Intralipid® by intravenous infusion at a rate of 1.5 mL/kg/min and at a dose of 15 g/kg/day (75 mL/kg/day) for 30 days. A control group (5 rats/sex) received 75 mL/kg/day saline for 30 days. One male from ClinOleic group died on day 4. Three females from Intralipid® group and 1 male from ClinOleic group were sacrificed before day 8 due to technical difficulties, and were replaced with new animals. Two more females from the ClinOleic group were terminated on day 18 and 22, respectively due to technical reasons that made continued dosing impossible. Rats in both treatment groups showed reduced food intake without an adverse effect on body weight gains. Rats in both lipid emulsion groups showed hematuria and anemia with reticulocytosis, accompanied by mild thrombocytopenia and increased fibrinogen concentrations. Serum albumin and total protein concentrations decreased in rats that received ClinOleic, and bilirubin concentrations increased in rats that received Intralipid. Aspartate aminotransferase activity increased in both lipid emulsion groups. Treatment-related histopathologic changes were observed in liver (hemosiderosis of the Kupffer cells), adrenal (necrotic coagulation foci), kidney (hemosiderosis of the nephrocytes) and spleen (hemosiderosis with excess lipid). The systemic effects of ClinOleic were comparable to Intralipid®.

Methods

Doses:	15 g/kg/day (75 mL/kg/day)
Frequency of dosing:	Once daily for 45 min
Route of administration:	Intravenous infusion
Dose volume:	2 mL/kg/min
Formulation/Vehicle:	Intralipid® 20% (soybean oil) and CSW 6/3 (ClinOleic; Olive oil and soybean oil)
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	Group T (5 rats/sex): 0.9% NaCl Group A (5 rats/sex): CSW 6/3 Group C (5 rats/sex): Intralipid® 20%

Age: Not specified
Weight: Males: 140-150 g, females: 160-170 g
Satellite groups: None
Unique study design: None
Deviation from study protocol: None

Observations and Results

Mortality

The animals were observed every day at regular intervals of about 1 hour; the last observation of the day was conducted at 4:00PM. The animals that died or were sacrificed before day 8 were replaced. The animals sacrificed after day 8 were not replaced.

One male died on Day 4 and another male was eliminated (difficulty in infusion) on Day 8 from the CSW 6/3 group. Three females from the Intralipid® group were sacrificed on Days 3, 6 and 7, respectively due to difficulties in intravenous infusion. Two females were sacrificed from the CSW 6/3 group on Day 18 and 22, respectively.

Clinical Signs

Hematuria was noticed in animals from both Intralipid® and CSW 6/3 treated groups. Sporadic diarrhea was also noted in some of the animals in all groups.

Body Weights

The animals were weighed twice a week.

The variation of body weight gain was similar in all 3 groups of animals. However, female animals in the Intralipid® group showed slightly higher body weight. It could be due to the replacement of the sacrificed animals.

Feed Consumption

Food intake was measured twice a week.

Food consumption (g/day) was reduced by 40% in the beginning in the CSW 6/3 and Intralipid® treated groups compared to control. However, the variations were comparable between the two lipid treated groups.

Hematology

Blood samples were collected via abdominal aorta after being anaesthetized with halothane. Following hematological parameters were determined.

PARAMETRES PARAMETERS	UNITES UNITS
Hematies/Red blood cells	10 ⁶ /mm ³
Hematocrit	%
Hemoglobin	g/dl
Vol.glob.moy./M.C.V.	fl
Hem.glob.moy./M.C.H.	pg
C.G.M.H. /M.C.H.C.	g/100ml
Reticulocytes	%
Reticulocytes	10 ⁶ /mm ³

The number of red blood cells, and hemoglobin and hematocrit levels, and the mean corpuscular hemoglobin concentration decreased and the reticulocyte levels increased significantly in the two lipid treated groups. The increase was more significant in CSW 6/3 group. The findings are presented in the Sponsor's Table below.

PARAMETRES PARAMETERS	UNITES UNITS	NaCl 0.9%				CSW 6/3 (ClinOleic)				Intralipid			
		Male		Female		Male		Female		Male		Female	
		MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.
Hematies/Red blood cells	10 ⁶ /mm ³	0.39	0.17	7.69	0.15	7.12	0.09	6.76	0.28	7.18	0.16	6.83	0.19
Hematocrit	%	44.0	0.8	40.9	0.7	38.2	0.8	35.2	1.8	39.6	0.5	36.8	0.8
Hemoglobin	g/dl	15.4	0.3	14.6	0.1	12.9	0.2	11.7	0.5	13.2	0.1	12.1	0.4
Vol.glob.moy./M.C.V.	fl	52	0.5	54	0.3	54	0.6	52	1.2	55	0.7	54	0.9
Hem.glob.moy./M.C.H.	pg	18.3	0.2	18.9	0.2	18.1	0.2	17.2	0.3	18.3	0.3	17.7	0.3
C.G.M.H. /M.C.H.C.	g/100ml	34.9	0.32	35.6	0.38	33.7	0.28	33.1	0.15	33.3	0.49	32.8	0.44
Reticulocytes	%	0.5	0.2	0.1	0.1	2.0	0.4	1.8	0.5	3.1	0.7	1.7	0.6
Reticulocytes	10 ⁶ /mm ³	0.046	0.016	0.006	0.006	0.141	0.027	0.122	0.031	0.255	0.052	0.111	0.034

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Clinical Chemistry

Following clinical chemistry parameters were determined.

Parameters	Units
Glucose	mmol/L
Urea	mmol/L
Creatinine	µmol/L
Proteins	g/L
A bumin	g/L
Globulin	g/L
A bumin-Globulin ratio	
Total bilirubin	µmol/L
A kaline phosphatase	U/L
SGOT	U/L
SGPT	U/L
Cholesterol	mmol/L
Phospholipids	mmol/L
Triglycerides	mmol/L
Calcium	mmol/L
Sodium	mmol/L
Potassium	mmol/L
Chlorides	mmol/L

There was a decrease in serum albumin and total protein levels by approximately 10% in CSW 6/3 group compared to the control group. The total bilirubin level increased in both the Intralipid® and CSW 6/3 treated groups, and both lipid treated groups showed an increase in SGOT levels. The phospholipid was higher in the males of CSW 6/3 group compared to control group. The findings are summarized in the Sponsor's Table below.

PARAMETRES PARAMETERS	UNITES UNITS	NaCl 0.9%				CSW 6/3 (ClinOleic)				Intralipid			
		Male		Female		Male		Female		Male		Female	
		MOYENNES MEANS	ECARTS N. S.E.M.	MOYENNES MEANS	ECARTS N. S.E.M.	MOYENNES MEANS	ECARTS N. S.E.M.	MOYENNES MEANS	ECARTS N. S.E.M.	MOYENNES MEANS	ECARTS N. S.E.M.	MOYENNES MEANS	ECARTS N. S.E.M.
Glucose	mmol/l	10.73	1.18	9.02	1.07	10.46	0.61	9.77	0.36	9.07	0.41	8.94	0.41
Uree/B.U.N.	mmol/l	5.5	0.26	5.6	0.57	4.2	0.49	4.8	0.45	9.7	5.13	4.8	0.49
Total bilirubin	umol/l	2.1	0.10	1.5	0.48	3.2	0.50	2.0	0.10	4.3	0.98	2.8	0.61
Total cholesterol	mmol/l	1.06	0.16	1.17	0.14	2.08	0.41	1.34	0.16	1.91	0.40	1.16	0.16
Triglycerides	mmol/l	0.61	0.07	0.44	0.04	0.68	0.09	0.45	0.06	0.89	0.12	0.46	0.03
Phospholipids	mmol/l	1.52	0.151	1.65	0.197	2.76	0.502	1.96	0.194	2.49	0.376	1.62	0.176
S.G.O.T./A.S.A.T.	UI/l	79	7	87	9	103	8	111	7	257	157	104	17
S.G.P.T./A.L.A.T.	UI/l	25	2	20	2	18	1	20	4	193	173	16	2
Alkaline phosphatase	UI/l	140	10	85	5	125	10	101	17	185	57	77	5
Creatinine	umol/l	50	2.5	47	3.0	45	5.0	52	3.5	77	38.2	44	2.2
Total proteins	g/l	65	0.9	67	2.0	61	1.5	60	2.9	63	1.1	66	1.1
Albumin	g/l	36	1.0	38	0.8	36	1.1	30	1.9	37	0.8	37	0.9
Globulins	g/l	28	1.0	29	1.3	26	1.0	30	4.4	26	0.5	30	1.7
Rapport A/G-A/G Ratio		1.28	0.07	1.29	0.04	1.41	0.06	1.05	0.17	1.42	0.04	1.25	0.10
Sodium	mmol/l	142	1.7	140	1.2	143	1.7	142	1.5	143	1.2	142	0.5
Potassium	mmol/l	5.5	0.2	4.9	0.2	5.0	0.2	4.7	0.2	5.4	0.2	4.7	0.0
Calcium	mmol/l	2.60	0.087	2.45	0.141	2.57	0.071	2.59	0.059	2.64	0.070	2.34	0.163
Chlorides	mmol/l	98	1.4	99	1.7	100	0.5	101	0.8	99	0.9	100	0.9

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Urinalysis

Urine analysis was conducted on Day -6 and Day +28. Urine volume, density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin, blood and leukocytes were determined.

Urine density decreased and urine pH increased significantly between day -06 and day +28 in the control group. In lipid treated groups, urine density increased and the pH decreased. The increase in density and the decrease in pH were not significant. Traces of blood or hemoglobin were detected in the urine of all male animals in the lipid treated groups.

Gross Pathology

Animals were euthanized at the end of the study following an overnight period without food and/or water. For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

Both lipid-treated groups showed hypertrophy (3 animals from each group) and a mottled appearance (5 animals from each group) in the liver. The liver of 2 male animals

from Intralipid® group showed lipid inclusions with a massive lipid hepatitis. The kidneys of 6 animals from the CSW 6/3 treated group (3 males and 3 females) and of 4 animals from the Intralipid® treated group (2 males and 2 females) had a discolored, yellow or mottled area. The adrenal glands hypertrophy was noticed in 2 females from the CSW 6/3 and one male from the Intralipid® group.

Organ Weights

For all animals, brain, heart, liver, adrenal glands, lungs, spleen and kidneys were dissected, trimmed free of fat and weighed.

The absolute and relative weights of the liver and of the spleen were increased in the lipid treated groups when compared to the control group. The relative weight of the kidneys and adrenal glands increased in both lipid treated groups compared to control group. The Sponsor's Table below shows the relative weight of different organs.

PARAMETRES PARAMETERS	NaCl 0.9%				CSW 6/3 (ClinOleic)				Intralipid			
	Male		Female		Male		Female		Male		Female	
	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.
Poids / weight (g)	367.0	13.50	227.8	7.50	356.2	9.47	234.0	7.67	370.8	8.35	254.2	6.38
Cerveau / Brain	2.161	0.025	2.029	0.036	2.206	0.013	2.029	0.035	2.136	0.030	2.058	0.056
Coeur / Heart	1.318	0.032	0.776	0.037	1.314	0.046	0.946	0.025	1.328	0.025	1.018	0.024
Foie / Liver	10.638	0.566	6.386	0.374	13.650	0.525	10.161	0.587	15.512	1.580	10.067	0.438
Poumons / Lung	1.342	0.050	1.063	0.033	1.297	0.045	1.182	0.026	1.312	0.101	1.190	0.051
Rate / Spleen	0.670	0.030	0.520	0.033	0.854	0.068	0.769	0.019	0.991	0.063	0.800	0.051
Rein d. / R. kidney	1.414	0.039	0.887	0.029	1.789	0.148	1.205	0.091	1.769	0.248	1.193	0.073
Rein g. / L. kidney	1.383	0.058	0.853	0.039	1.800	0.126	1.226	0.109	1.819	0.257	1.199	0.092
Surrenale d./r. adrenal	0.0357	0.0031	0.0367	0.0034	0.0429	0.0026	0.0449	0.0060	0.0408	0.0035	0.0497	0.0038
Surrenale g./l. adrenal	0.0356	0.0037	0.0375	0.0032	0.0436	0.0035	0.0510	0.0089	0.0470	0.0051	0.0486	0.0035

Histopathology

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Adequate Battery: Yes

Peer Review: No

Histological Findings:

On completion of the gross pathology examination, the following organs (listed below) were fixed in 10% neutral buffered formalin for histological examination.

- Aorta (arch and abdominal part)
- Heart
- Brain
- Colon
- Duodenum
- Stomach
- Liver
- Mesenteric ganglion
- Adrenal glands
- Hypophysis
- Ileum
- Jejunum
- Tongue
- Skeletal muscle
- Sciatic nerve
- Eyes and optical nerves
- Esophagus
- Bone (femur)
- Pancreas
- Skin
- Lungs
- Prostate or uterus
- Spleen
- Kidneys
- Infusion site
- Sternum
- Testicles or ovaries
- Thyroid
- Trachea
- Bladder

Histopathological findings were mainly observed in the liver, spleen and kidney. In the liver, a hemosiderosis of the Kupffer cells with excess lipids were observed in all animals of the lipid treated groups. Both lipid treated groups showed heavy red pulp reticular cell hemosiderosis with excess lipid in the spleen and hemosiderosis of the nephrocytes in the kidneys. These observations were more pronounced in the Intralipid® treated animals. Animals also showed extended cystic nephritis lesions (up to 80% of the renal parenchyma), possibly associated with a renal medullary fibrosis. Animals treated with Intralipid® showed necrotic foci in the liver (2/10 animals) and on the adrenal glands (5/10 animals), and the presence of free lipid droplets in the bone marrow (9/10 animals).

Study title: Toxicity, with repeated doses by central intravenous route in rats, of CSW 6.3 (ClinOleic) batch 92122 and 20% Intralipid® batch 51663.51 (25 days of administration).

Study no.:	A 90 14
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 31, 1991
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Intralipid 20% batches 51663-51 and CSW 6.3 batch 92122; purity not provided.

Key Study Findings

Male Sprague Dawley rats (n=6) were administered either CSW 6.3 (ClinOleic) or Intralipid® by jugular vein at a rate of 2 mL/kg/min at 18 g/kg/day (90 mL/kg/day) for 24-25 days. There were no deaths and treatment related clinical observations. However, 3

animals were sacrificed due to catheter-related issues. Decreased food consumption and body weight were noticed in both lipid treated groups. Rats treated with CSW 6.3 had slight anemia with reticulocytosis. Serum chemistry showed a decrease in urea concentrations in both groups, and an increase in alanine aminotransferase and aspartate aminotransferase activities in some rats in the CSW 6.3 group. Urinalysis showed decreased pH, ketonuria, and hematuria in most rats in the both lipid treated groups. Macroscopic examination showed discolored areas on the livers and a lack of corticomedullary demarcation in the kidneys in both lipid treated groups. Liver, spleen, kidney, lung, and adrenal gland weights were higher in the CSW 6.3 group than in the Intralipid® group. Treatment-related histopathologic changes were lipid overload in the liver and spleen, and interstitial or embolic nephritis in the corticomedullary plexus in both the groups. Only male animals were used in the study, and no control group was included.

Methods

Doses:	18 g/kg/day (90 mL/kg/day)
Frequency of dosing:	Once daily for 45 min
Route of administration:	Intravenous (Jugular vein)
Dose volume:	2 mL/kg/min
Formulation/Vehicle:	Intralipid® 20% (soybean oil) and CSW 6/3 (Olive oil and soybean oil)
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	Group A (6 males): 90 mL/kg/day of CSW 6.3 Group B (6 males): 90 mL/kg/day of Intralipid® 20%
Age:	Not specified
Weight:	Not provided
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

No mortality related to the treatment with lipid emulsions was observed. However, 3 animals (1 from Intralipid® and 2 from CSW 6.3) were sacrificed due to technical errors related to the catheter and one animal from the Intralipid® group died before initiation of the experiment.

Clinical Signs

One animal from CSW 6.3 group showed hematuria on Day 1. No other clinical signs were noticed.

Body Weights

Body weight was measured twice a week.

The body weight of the animals was reduced during the first week of treatment and then resumed a normal growth. Body weight gain of the animals of the CSW 6.3 group remained less than the animals of the Intralipid® group. The body weights at different time points are presented in the Sponsor's Table below.

PARAMETRES PARAMETERS	Body weight (g)			
	CSW 6.3 (90 mL/kg)		Intralipid (90 mL/kg)	
	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.
Jour / Day -04	217.4	1.54	217.8	2.01
Jour / Day -02	202.3	5.56	210.0	9.72
Jour / Day +01	218.6	13.26	223.3	11.79
Jour / Day +04	207.5	20.34	219.4	12.39
Jour / Day +08	213.0	29.70	247.0	17.79
Jour / Day +11	218.4	35.32	261.3	25.50
Jour / Day +15	258.8	32.67	295.1	20.10
Jour / Day +19	276.6	35.10	315.8	19.12
Jour / Day +22	277.9	36.13	317.7	19.94
Jour / Day +25	306.9	36.50	336.6	20.76

Feed Consumption

Food intake was measured once a week.

Food consumption was reduced for most of the animals starting with the first week of treatment as shown in the Sponsor's Table below.

PARAMETRES PARAMETERS	Food Consumption (g/day/rat)			
	CSW 6.3 (90 mL/kg)		Intralipid (90 mL/kg)	
	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.
Semaine / Week -01	15	2.0	18	3.2
Semaine / Week +01	9	3.8	12	4.1
Semaine / Week +02	12	4.4	16	2.8
Semaine / Week +03	14	1.9	15	0.8
Semaine / Week +04	16	2.1	14	0.8

Hematology

Blood samples were collected from the abdominal aorta following anesthesia with halothane on Day 26. The animals from the CSW 6.3 group sacrificed at the end of the study showed a slight anemia.

Clinical Chemistry

Serum chemistry analysis showed decreased urea level (2.2 ± 0.58 nmol/L in CSW 6.3 group and 2.4 ± 0.74 nmol/L in Intralipid® group) in all animals in both lipid treated groups. The other parameters were normal in the 2 groups of animals.

Urinalysis

Urine density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood were determined at the end of the study.

Animals in both lipid treated groups showed decreased pH, ketonuria and presence of nitrites or traces of proteins.

Gross Pathology

For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

The liver of the animals in CSW 6.3 treated group was large, pale, and/or marbled (3/4). Yellow or white spots in the liver were noticed in 3 animals of the Intralipid® group. There was a lack of corticomedullary demarcation in the kidneys of most rats in both groups.

Organ Weights

The weights of the following organs were recorded- liver, spleen, kidneys, lungs, heart and adrenal glands.

The absolute and relative weight of the liver, spleen, kidneys lungs, and adrenal glands were higher in the CSW 6.3 group than Intralipid® group. The findings are shown in the Sponsor's Table below.

PARAMETRES PARAMETERS	Organ weight (g)			
	CSW 6.3 (90 mL/kg)		Intralipid (90 mL/kg)	
	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.
Poids / Weight (g)	290.20	33.51	323.63	19.61
Foie / Liver	13.121	0.991	11.751	1.674
Rate / Spleen	1.285	0.324	0.909	0.098
Rein d. / R.kidney	1.919	0.271	1.577	0.164
Rein g. / L.kidney	1.871	0.227	1.399	0.127
Poumons / Lung	1.615	0.189	1.530	0.078
Coeur / Heart	1.119	0.098	1.154	0.074
Surrenale d. /R.adrenal	0.0416	0.0041	0.0298	0.0013
Surrenale g. /L.adrenal	0.0502	0.0058	0.0313	0.0013

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

Histopathologic changes in livers consisted of hepatocellular vacuolation (lipid) and pigmentation (hemosiderin), in sinusoidal macrophages (Kupffer cells) in both lipid emulsion treated groups. Pigment (hemosiderin) and lipid vacuolation were observed in macrophages in the spleen, and the kidneys showed chronic polycystic interstitial nephritis in both lipid treated groups.

Study title: Emulsion CSW 6/3 toxicity due to repeated intravenous doses in the rat - comparison with 20% Intralipid® (30 day administration).

A 91 04

Study no.:
 Study report location: Electronic submission (EDR)
 Conducting laboratory and location: (b) (4)
 Date of study initiation: June 27, 1990
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NaCl 0.9% batch 2377, CSW 6/3 batch 92122 and Intralipid® batch 52327.51; purity not provided

Key Study Findings

Sprague Dawley rats (5 rats/sex) were intravenously infused with either CSW 6/3 (ClinOleic) or Intralipid® at a rate of 1.2 mL/kg/min at a dose of 18 g/kg/day (90 mL/kg/day), for 30 days. A control group (5 rats/sex) received 90 mL/kg/day saline for 30 days. Six females and 1 male from the CSW 6/3 group died between day 01 and day 10. One female from the same group was sacrificed on day 24 due to technical difficulties. Hematuria was observed in both lipid treated groups. Animals of group A (CSW 6/3), that survived up to the end of the study had a weight gain comparable to that of the control group. Anemia and thrombopenia was observed in both lipid treated groups. Blood chemistry showed decreased urea level in both lipid treated groups, increased ALT and AST, especially in CSW 6.3 group and increased cholesterol and phospholipids in males of Intralipid® group were observed. Urinalyses showed decreased pH and ketonuria in lipid treated rats with slight hematuria in rats in the Intralipid® group. Gross pathology observations included discolored livers in all lipid treated rats, a variety of renal lesions and gastric ulcers in some rats from both groups. Some rats in the Intralipid® group showed discolored spots on the adrenal glands and lungs. Liver weights were increased in rats receiving lipid emulsions. Treatment-related histopathologic changes in livers consisted of hepatocellular vacuolation (lipid), foci of necrosis, and pigment, interpreted as hemosiderin, in sinusoidal macrophages (Kupffer cells) in both lipid treated groups. Pigment (hemosiderin) and lipid vacuolation were also present in macrophages in the spleens of lipid treated animals. The adrenal cortex of male rats treated with CSW 6/3 had excess lipid and some rats in the Intralipid® group had focal necrosis in the adrenal gland.

The infusion rate used in this study, 1.2 mL/kg/min, appeared to limit the renal changes seen at higher infusion rates (2 mL/kg/min) in previous studies. However, in this study, CSW 6/3 was less tolerated than Intralipid® and was associated with higher mortality in rats.

Methods

Doses:	18 g/kg/day (90 mL/kg/day)
Frequency of dosing:	Once daily for 1h 15min
Route of administration:	Intravenous
Dose volume:	1.2 mL/kg/min
Formulation/Vehicle:	Intralipid® 20% (soybean oil) and CSW 6/3 (Olive oil and soybean oil)
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	Group T: (5 rats/sex) 0.9% NaCl Group A: (5 rats/sex) CSW 6/3 Group B: (5 rats/sex) Intralipid
Age:	Not specified
Weight:	Males: 270 g, Females: 220 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

The animals were observed every day at regular intervals of about 1 hour. The animals that died during the study were necropsied.

Six female animals and 1 male animal treated with CSW 6/3 died after 1 to 10 days of infusion. In addition, a female animal from the same group was sacrificed on Day 24 due to technical difficulties to administer the lipid emulsion.

Clinical Signs

The animals were observed every day at a regular interval of about 1 hour; the last observation of the day was at 4:00PM.

Hematuria was noticed in most of the animals treated with lipids.

Body Weights

The animals were weighed twice a week.

The male and female animals from Intralipid® group showed significantly higher body weight than animals in the control group. The male and female animals which survived in the CSW 6/3 group gained comparable body weight to that of the control animals. The body weight data are shown in the Sponsor's Table below.

Body weight Male Rats (g)							
		Control (0.9% NaCl)		CSW 6.3 (90 mL/kg)		Intralipid (90mL/kg)	
PARAMETRES PARAMETERS	MOYENNES MEANS	ECARTS P. S. E. M.	MOYENNES MEANS	ECARTS P. S. E. M.	MOYENNES MEANS	ECARTS P. S. E. M.	
Jour / Day -7	202.3	1.43	203.6	1.96	201.8	1.39	
Jour / Day -5	225.0	5.84	219.3	2.88	222.2	2.44	
Jour / Day +1	267.6	3.00	269.6	3.57	271.8	5.02	
Jour / Day +3	275.1	4.55	271.5	4.14	273.4	7.58	
Jour / Day +7	289.7	6.28	291.0	5.61	293.4	8.61	
Jour / Day +10	302.6	9.58	301.9	4.19	309.0	10.23	
Jour / Day +14	316.5	11.58	311.0	7.49	326.9	12.42	
Jour / Day +17	329.1	12.67	331.0	5.83	342.3	13.27	
Jour / Day +21	345.5	15.05	349.7	6.89	362.0	14.81	
Jour / Day +24	356.6	15.39	366.8	8.23	381.0	16.41	
Jour / Day +28	369.6	16.19	376.4	8.60	397.3	18.18	
Jour / Day +30	376.5	16.72	383.5	9.46	404.6	19.11	

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Body Weight Female rats (g)					
		Control (NaCl 0.9%)		Intralipid (90mL/kg)	
PARAMETRES PARAMETERS	MOYENNES MEANS	ECARTS P. S. E. M.	MOYENNES MEANS	ECARTS P. S. E. M.	
Jour / Day -7	195.0	1.89	197.4	2.30	
Jour / Day -5	201.3	2.30	200.8	3.15	
Jour / Day +1	217.5	6.66	220.3	3.63	
Jour / Day +3	225.4	5.08	219.6	5.14	
Jour / Day +7	226.6	4.81	231.5	5.17	
Jour / Day +10	231.7	3.73	243.2	6.67	
Jour / Day +14	236.1	2.99	256.0	6.74	
Jour / Day +17	238.8	5.00	259.7	7.35	
Jour / Day +21	244.1	5.11	269.5	7.96	
Jour / Day +24	251.5	4.01	275.0	8.75	
Jour / Day +28	252.2	3.97	286.9	10.93	
Jour / Day +30	255.4	3.62	292.2	9.40	

Feed Consumption

Food intake was measured once a week.

Food consumption (g/day) was reduced in both sexes and both lipid treated groups from the first week of the study. By the end of the study, the food intake was reduced by half in the CSW 6/3 and Intralipid® groups compared to control (0.9% NaCl group). The food intake data for different groups are shown in the Sponsor's Table below.

Food intake Male Rats (g/day/rat)							
		Control (NaCl 0.9%)		CSW 6.3 (90 mL/kg)		Intralipid (90 mL/kg)	
PARAMETRES PARAMETERS	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	
Seaine / Week -01	28	1.0	29	0.2	30	0.3	
Seaine / Week 01	30	3.7	18	2.8	18	2.1	
Seaine / Week 02	23	3.1	16	2.6	16	0.9	
Seaine / Week 03	27	0.3	15	2.2	15	0.6	
Seaine / Week 04	28	0.1	13	1.5	14	1.4	

Food intake Female rats (g/day/rat)					
		Control (NaCl 0.9%)		Intralipid (90 mL/kg)	
PARAMETRES PARAMETERS	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	
Seaine / Week -01	21	0.5	20	1.3	
Seaine / Week 01	21	0.9	12	1.3	
Seaine / Week 02	20	0.4	11	1.3	
Seaine / Week 03	26	5.1	10	0.5	
Seaine / Week 04	20	0.1	8	1.0	

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Hematology

Blood samples for hematology were collected via the abdominal aorta after being anaesthetized with halothane.

Both lipids treated groups had comparable anemia (a hematocrit, hemoglobin and red blood cell decrease of around 20%) associated with reticulocytosis, as well as a slight leukocytosis. In addition, male animals of both lipids treated groups, as well as the female animals from Intralipid® group, showed slight thrombopenia,

Clinical Chemistry

Following clinical chemistry parameters were determined on Day 31.

Parameters	Units
Glucose	mmol/L
Urea	mmol/L
Creatinine	µmol/L
Proteins	g/L
A bumin	g/L
Globulin	g/L
A bumin-Globulin ratio	
Total bilirubin	µmol/L
A kaline phosphatase	U/L
SGOT	U/L

SGPT	U/L
Cholesterol	mmol/L
Phospholipids	mmol/L
Triglycerides	mmol/L
Sodium	mmol/L
Potassium	mmol/L

Serum chemistry analysis showed a slight elevation in glycemia, cholesterol, phospholipid and ALAT in male animals of Intralipid® group. Both male and female rats in the CSW 6/3 treated group showed significantly higher levels of SGPT transaminase associated with an increase in the ASAT. Clinical chemistry changes are shown in the Sponsor's Table below.

PARAMETRES PARAMETERS	UNITES UNITS	Control (NaCl 0.9%)		CSW 6.3		Intralipid®					
		MOYENNES MEANS	ECARTS % S.E.M.	MOYENNES MEANS	ECARTS % S.E.M.	MOYENNES MEANS	ECARTS % S.E.M.	MOYENNES MEANS	ECARTS % S.E.M.		
Glucose	mmol/l	6.46	0.24	5.76	0.38	7.02	0.49	8.22	0.40	6.32	0.61
Uree/B.U.R.	mmol/l	5.7	0.25	7.4	0.87	4.1	0.24	3.5	0.31	3.4	0.82
Bilirubine totale	mmol/l	2.0	0.35	1.2	0.33	5.3	2.85	2.7	0.27	0.9	0.12
Acides biliaires	mmol/l	14.29	8.97	4.92	1.65	12.12	3.78	16.42	8.79		
Cholesterol total	mmol/l	1.21	0.05	1.08	0.08	1.45	0.29	2.32	0.39	1.28	0.15
Triglycerides	mmol/l	0.48	0.04	0.40	0.03	1.31	0.69	0.61	0.04	0.70	0.15
Phospholipides	mmol/l	1.65	0.01	1.57	0.05	1.79	0.18	2.44	0.13	1.68	0.16
S.G.U.T./P.S.A.T.	U/l	157	12	113	15	199	12	165	30	124	13
S.S.P.T./P.L.A.T.	U/l	34	4	24	1	131	31	79	27	26	3
Phosphatases alcalines	U/l	155	11	102	7	157	12	168	28	110	12
Creatinine	mmol/l	46	2.1	58	5.0	44	2.4	36	1.4	47	2.4
Proteines totales	g/l	58	1.3	48	0.9	58	0.4	59	1.8	47	2.0
Albumine	g/l	39	0.7	32	0.5	38	1.1	39	1.4	32	1.3
Globulines	g/l	19	0.5	16	0.6	19	0.9	19	0.8	15	1.0
Rapport A/G .A/G Ratio		2.02	0.09	2.03	0.08	2.08	0.16	2.15	0.07	2.09	0.11
Sodium	mmol/l	145	0.7	143	0.9	145	0.5	146	0.9	147	2.0
Potassium	mmol/l	5	0.2	5	0.1	5	0.1	5	0.3	5	0.2

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Urinalysis

Urine analysis was conducted at the end of the study. Urine density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood were determined.

Male and female rats from lipid treated groups showed a slight decrease in pH and ketonuria. Hematuria (7 /10 animals) and the presence of nitrites (3 /5 males) were observed in animals from the Intralipid® treated group. Nitrites were also detected in the male control animals (4/5).

Gross Pathology

Animals were euthanized at the end of the experiment. For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

Gross pathology observations included discolored livers in all lipid treated rats, gastric ulcers in some rats from both lipid treated groups, and discolored spots on the adrenal glands and lungs of some rats treated with Intralipid®. Renal lesions include bloated

kidneys (1 male from CSW 6.3 group, 1 male from Intralipid® group), mottled appearance (1 male from CSW 6.3 group, 1 female from Intralipid® group), pale or discolored cortical area or at the corticomedullary junction (1 male from CSW 6.3 group, 2 males and 1 female from Intralipid® group), and hemorrhagic spot on the papilla (1 female from CSW 6.3 group). The adrenal glands of most of the animals from lipid treated group had brown or white spots. The lungs of 3 males and 2 females from the Intralipid® treated group had a heterogeneous (white, brown or red) coloration or spots.

Organ Weights

The weights of the following organs were recorded: brain, heart, liver, adrenal glands, lungs, spleen and kidneys.

Liver weights increased in rats infused with lipid emulsions (Male: Saline, 9.45 ± 0.36 g; Clinolipid, 17.28 ± 0.55 g and Intralipid, 16.12 ± 1.14 g; Female: Saline 6.3 ± 0.16 g and Intralipid 12.53 ± 0.77 g). Adrenal gland weights (Saline 0.031 ± 0.001 g, Clinolipid 0.050 ± 0.006 g and Intralipid 0.037 ± 0.002 g) increased in males in the Clinolipid group and heart weights (Saline 0.82 ± 0.03 g and Intralipid® 1.33 ± 0.05 g) increased in females in the Intralipid® group. The findings are shown in the Sponsor's Table below.

Organ Weight (g) Male Rats							
		Control (0.9% NaCl)		CSW 6.3 (90mL/kg)		Intralipid (90mL/kg)	
PARAMETRES PARAMETERS	MOYENNES MEANS	ECARTS % S. E. %	MOYENNES MEANS	ECARTS % S. E. %	MOYENNES MEANS	ECARTS % S. E. %	
Poids / Weight (g)	343.70	15.21	365.40	8.11	389.84	18.58	
Foie / Liver	9.453	0.363	17.281	0.552	16.124	1.145	
Rate / Spleen	0.736	0.029	0.947	0.054	1.089	0.071	
Rein d. / R. kidney	1.441	0.043	1.672	0.154	1.733	0.076	
Rein g. / L. kidney	1.431	0.061	1.662	0.152	1.732	0.102	
Poumons / Lung	1.310	0.045	1.403	0.059	1.502	0.051	
Coeur / Heart	1.188	0.039	1.479	0.085	1.470	0.051	
Surrenaie d. / R. adrena	0.0319	0.0019	0.0500	0.0065	0.0372	0.0025	
Surrenaie g. / L. adrena	0.0349	0.0035	0.0489	0.0023	0.0429	0.0024	
Cerveau / Brain	2.056	0.042	2.153	0.034	2.220	0.067	

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Organ Weight (g) Female Rats					
		Control (0.9%)		Intralipid	
PARAMETRES PARAMETERS	MOYENNES MEANS	ECARTS % S. E. %	MOYENNES MEANS	ECARTS % S. E. %	
Poids / Weight (g)	229.24	3.38	275.05	6.09	
Foie / Liver	6.329	0.166	12.532	0.771	
Rate / Spleen	0.540	0.050	0.791	0.051	
Rein d. / R. kidney	0.914	0.030	1.145	0.048	
Rein g. / L. kidney	0.878	0.027	1.145	0.078	
Poumons / Lung	1.119	0.036	1.253	0.031	
Coeur / Heart	0.820	0.037	1.338	0.057	
Surrenaie d. / R. adrena	0.0393	0.0006	0.0546	0.0054	
Surrenaie g. / L. adrena	0.0424	0.0013	0.0640	0.0066	
Cerveau / Brain	2.089	0.064	2.062	0.015	

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

Following organs were examined for potential histopathological changes: liver, spleen, lung, trachea, bladder, skin, bone marrow (femur, sternum), digestive tract (tongue, esophagus, stomach, duodenum, jejunum, ileum, colon), pancreas, lymphatic ganglion (mesenteric), adrenal glands, thyroid, aorta (abdominal arch), heart, skeletal muscle,

sciatic nerve, encephalon (brain, cerebellum, bulb), eyes (R and L), infusion site, testicles or ovaries, prostate or uterus.

Histopathological findings were mainly associated with the liver, spleen, kidney, adrenals and the heart. In the liver, lipid deposits in hepatocytes were observed in case of both lipid treated groups, which was associated with necrosis, especially in the male animals. The necrotic lesions were less significant in female animals of Intralipid® group. In addition, hemosiderin in sinusoidal macrophages (Kupffer cells) was observed in both lipid emulsion groups. Pigment (hemosiderin) and lipid vacuolation were also present in macrophages in the spleens of lipid treated animals. In the kidney, chronic interstitial and epithelial nephritic lesions, polycystic sclerosis and inflammatory obliteration of the capillary plexus of the corticomedullary area were observed in the lipid treated groups. The adrenal cortex of male rats treated with CSW 6.3 had excess lipid and some rats in the Intralipid® group had focal necrosis in the adrenal glands.

Study title: Repeated-dose toxicity by intravenous administration of CSW 6.3 in the rat (90 days of administration) - comparison with 20% Intralipid®

Study no.:	A 90 09
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 14, 1988
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Intralipid® 20% batches 35052-51, 69809-51 and 83081-51 and CSW 6.3 (ClinOleic) batch 82044; purity not provided

Key Study Findings

Eight rats died or were eliminated from experiment; 2 from Intralipid® group, 1 from CSW 6.3 group (6 g/kg/day) and 5 from CSW 6.3 group (12 g/kg/day). Most of the deaths were accidental, related to technical difficulties during dosing. Hematuria was more frequent in animals treated with Intralipid® at 60 mL/kg/day dose. A slight reduction of the erythrocyte parameter was noticed in the high dose lipid treated groups. Blood chemistry showed a reduction in urea levels, increased cholesterol and phospholipid concentrations in males treated with Intralipid®; and increased serum alkaline phosphatase in the high dose (12 g/kg/day) lipid treated groups. Urinalysis revealed presence of blood, ketone bodies and protein in both lipid treated groups at all doses. Macroscopic examination showed marbled appearance and white spots on the livers of high dose of lipids (12 g/kg/day) treated rats; liver and spleen weights were increased. Treatment-related histopathologic changes in livers consisted of hepatocellular vacuolation (lipid), foci of granulomatous inflammation, focal hepatocellular necrosis, and pigment (hemosiderin) in sinusoidal macrophages (Kupffer cells). The severity of the hepatic changes were similar in rats at the high dose (12 g/kg/day) of lipid emulsions and decreased in rats given lower doses of CSW 6.3. At the

end of the 1-month recovery period, the inflammatory granulomas and pigment persisted in the livers, and splenic vacuolations were still present in rats at the high dose (12 g/kg/day) lipid emulsions. The effects of CSW 6.3 were comparable to Intralipid® at equivalent doses.

Methods

Doses: 3, 6 and 12 g/kg/day (15, 30, or 60 mL/kg/day)
 Frequency of dosing: Once daily for 50 min
 Route of administration: Intravenous
 Dose volume: 2 mL/kg/min
 Formulation/Vehicle: Intralipid® 20% (soybean oil) and CSW 6.3 (Olive oil and soybean oil)
 Species/Strain: Sprague Dawley rats
 Number/Sex/Group: Group T (6/sex): 60 mL/kg/day of 0.9% NaCl
 Group A (9/sex): 60 mL/kg/day of 20% Intralipid®
 Group B (2 males and 4 females): 60 mL/kg/day of 20% Intralipid®
 Group C (3/sex): 15 mL/kg/day of CSW 6.3
 Group D (9/sex): 30 mL/kg/day of CSW 6.3
 Group E (9/sex): 60 mL/kg/day of CSW 6.3
 Group F (3/sex): 60 mL/kg/day of CSW 6.3
 The animals of groups B and F were sacrificed after a period of reversibility of 1 month.
 Age: Not specified
 Weight: Males: 140 - 150 g, Females: 160 - 170 g
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: None

Observations and Results

Mortality

The animals were observed every day at regular intervals of about 1 hour. The animals that died during the study were necropsied.

Three male animals from the CSW 6.3 study group died. One male from CSW 6.3 (30 mL/kg) group died on Day 81 and two males from 60 mL/kg/day CSW 6.3 dose group died on Days 81 and 88, respectively. Two males and one female from 60 mL/kg/day CSW 6.3 dose group and 2 females from Intralipid® group (60 mL/kg/day) were eliminated or sacrificed due to technical difficulties in infusion.

Clinical Signs

The animals were observed every day at regular intervals of about 1 hour.

Hematuria was noticed in all animals at the high dose (60 mL/kg) of Intralipid® or CSW 6.3. Hematuria was more frequent in animals infused with Intralipid®.

Body Weights

The animals were weighed twice a week.

There were no effects on body weight at any dose levels after 90 days of lipid infusion.

Feed Consumption

Food intake was measured once a week.

The food consumption (g/day) was lower in both sexes in all dose groups of Intralipid® or CSW 6.3 compared to control (0.9% NaCl). The reduction of food consumption was proportional to the doses of lipids infused. The food consumption was comparable to the control group after 3 to 4 weeks of recovery period.

Ophthalmoscopy

Ophthalmoscopy examination was conducted with indirect-light ophthalmoscope (Luneau type).

There were no abnormal ophthalmologic effects.

Hematology

Blood samples for hematology were collected from the abdominal aorta after being anaesthetized with halothane on Day 91 and Day 120 (recovery groups).

Erythrocyte counts were reduced in rats in 12 g/kg/day lipid emulsion dose group. A significant reduction of the hematocrit and hemoglobin levels in male and female animals were noticed in both lipid emulsions treated high dose groups. An increase in reticulocytes and leukocytes were observed in males that were proportional to the doses.

Clinical Chemistry

Following clinical chemistry parameters were determined on Day 91 and Day 120.

Parameters	Units
Glucose	mmol/L
Urea	mmol/L
Creatinine	µmol/L
Proteins	g/L
A bumin	g/L
Globulin	g/L
A bumin-Globulin ratio	
Total bilirubin	µmol/L
A kaline phosphatase	U/L

SGOT	U/L
SGPT	U/L
Cholesterol	mmol/L
Phospholipids	mmol/L
Triglycerides	mmol/L
Sodium	mmol/L
Potassium	mmol/L

Serum chemistry analysis showed decreased urea concentrations (dose dependent in rats in the CSW 6.3 group), increased cholesterol and phospholipid concentrations in males treated with Intralipid®; and increased serum alkaline phosphatase activity in rats treated with 12 g/kg/day (60 mL/kg) Intralipid and CSW 6/3. Male rats at the high dose of lipid showed an increase in SGOT levels. Changes in clinical chemistry parameters are shown in the Sponsor's Table below.

Table: Serum Chemistry of male rats.

PARAMETRES PARAMETERS	UNITES UNITS	Control (0.9% NaCl)		Intralipid (60 ML/kg)		CSW 6/3 (15 mL/kg)		CSW 6/3 (30 mL/kg)		CSW 6/3 (60 mL/kg)	
		MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.	MOYENNES MEANS	ECARTS M. S.E.M.
Glucose	mmol/l	8.06	0.42	6.90	0.29	8.21	1.33	7.67	0.28	7.14	0.39
Uree/B.U.N.	mmol/l	5.5	0.42	3.9	0.64	5.7	0.25	4.2	0.37	3.1	0.29
Creatinine	umol/l	43	2.5	46	6.3	52	0.5	44	3.3	36	3.0
Total Bilirubin	umol/l	2.3	0.29	3.1	0.25	1.6	0.55	2.0	0.15	3.1	0.59
Total cholesterol	mmol/l	1.30	0.07	2.61	0.33	1.01	0.27	0.86	0.05	1.27	0.40
Cholesterol n.D.L.	mmol/l	0.78	0.04	0.38	0.05	0.60	0.04	0.57	0.04	0.60	0.06
Phospholipids	mmol/l	1.51	0.03	2.79	0.40	1.34	0.12	1.20	0.05	1.34	0.12
Triglycerides	mmol/l	0.62	0.07	0.82	0.20	0.50	0.18	0.53	0.12	0.99	0.55
Alkaline phosphatases	U/l	73	4	114	20	57	7	55	3	100	9
S. G. O. T. /A. S. A. T.	U/i	98	9	142	31	94	24	100	11	142	20
S. G. P. T. /A. L. A. T.	U/i	25	3	49	24	26	1	20	3	35	8
Total proteins	g/l	58	1.2	64	1.7	60	1.5	60	1.6	64	3.1
Albumin	g/l	38	1.0	40	1.3	42	0.5	39	1.7	41	1.8
Globulins	g/l	20	1.0	24	1.7	18	1.0	21	1.4	23	2.6
Ratio: A/G Ratio		1.89	0.12	1.79	0.14	2.31	0.10	2.01	0.19	1.93	0.23
Sodium	mmol/l	144	1.9	147	0.6	143	1.5	146	0.7	147	0.6
Potassium	mmol/l	5.1	0.06	5.3	0.15	5.1	0.05	5.1	0.09	5.3	0.14

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Table: Serum Chemistry of female rats.

PARAMETRES PARAMETERS	UNITES UNITS	Control (0.9% NaCl)		Intralipid (60 ML/kg)		CSW 6/3 (15 mL/kg)		CSW 6/3 (30 mL/kg)		CSW 6/3 (60 mL/kg)	
		MOYENNES MEANS	ECARTS M S. E. M.	MOYENNES MEANS	ECARTS M S. E. M.	MOYENNES MEANS	ECARTS M S. E. M.	MOYENNES MEANS	ECARTS M S. E. M.	MOYENNES MEANS	ECARTS M S. E. M.
Glucose	mg/dl	7.16	0.38	7.47	0.42	7.96	0.72	7.17	0.45	7.38	0.51
Uree/B.U.N.	mg/dl	6.2	0.38	4.3	0.37	6.3	0.40	5.7	0.38	3.9	0.10
Creatinine	mg/dl	50	3.4	49	2.4	49	3.2	48	2.5	45	2.7
Total Bilirubin	mg/dl	2.7	0.11	2.4	0.17	2.1	0.03	2.3	0.27	1.8	0.20
Total cholesterol	mg/dl	1.79	0.12	1.07	0.15	1.43	0.16	1.12	0.07	1.03	0.11
Cholesterol H.D.L.	mg/dl	1.12	0.07	0.65	0.07	1.08	0.20	0.75	0.09	0.73	0.12
Phospholipids	mg/dl	2.52	0.14	1.61	0.19	1.91	0.37	2.03	0.10	1.65	0.13
Triglycerides	mg/dl	0.54	0.06	0.39	0.06	0.54	0.09	0.39	0.05	0.37	0.05
Alkaline phosphatases	U/l	36	5	49	6	34	11	35	4	40	7
S.G.P.T./A.S.A.T.	U/l	128	14	115	13	82	3	119	14	98	9
S.G.P.T./A.L.A.T.	U/l	43	9	40	12	31	2	34	6	21	1
Total proteins	g/l	68	0.7	65	1.6	66	3.1	71	1.4	69	1.1
Albumin	g/l	47	1.1	44	1.2	48	2.3	47	1.3	45	1.0
Globulins	g/l	22	1.0	21	0.7	18	0.9	24	1.2	24	0.7
Ratio A/G Ratio		2.20	0.14	2.15	0.08	2.60	0.09	2.05	0.13	1.86	0.08
Sodium	mg/dl	144	2.3	145	1.2	147	0.9	146	1.1	144	2.1
Potassium	mg/dl	4.8	0.12	5.2	0.18	4.7	0.18	4.7	0.18	4.6	0.16

Urinalysis

Urine analysis was conducted on Day 89. Urine density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood were determined.

In all the groups, the presence of blood, proteins and ketone bodies were detected. The presence of blood was less frequent in animals in the control groups and low dose CSW 6.3 (15 mL/kg) group.

Gross Pathology

Animals were euthanized at the end of the dosing period with halothane following an overnight period without food and/or water. For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

Treatment-related gross pathology observations consisted of marbled appearance and the presence of white spots in the livers of rats that received 12 g/kg/day lipid emulsion.

Organ Weights

The weights of the following organs were recorded: brain, heart, liver, adrenal glands, lungs, spleen and kidneys.

There was a dose-dependent increase of liver and spleen weights in rats that received CSW 6.3. Liver and spleen weights also increased in rats that received Intralipid®. Rats

infused with CSW 6.3 or Intralipid® also showed increased adrenal gland weights. The Sponsor's Table below shows the relative weight of different organs.

Table: Organ weights of male rats.

PARAMETRES PARAMETERS	Control (0.9% NaCl)		Intralipid (60 ML/kg)		CSW 6/3 (15 mL/kg)		CSW 6/3 (30 mL/kg)		CSW 6/3 (60 mL/kg)	
	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.
Poids / Weight (g)	454.0	10.64	405.0	18.49	404.1	39.21	431.5	14.55	452.0	27.90
Cerveau / Brain	2.245	0.090	2.146	0.025	2.149	0.062	2.124	0.065	2.165	0.022
Coeur / Heart	1.278	0.027	1.274	0.026	1.171	0.077	1.285	0.032	1.444	0.084
Foie / Liver	10.818	0.545	12.642	0.614	10.058	2.048	11.947	0.561	16.500	1.246
Rate / Spleen	0.657	0.036	1.129	0.067	0.589	0.007	0.972	0.059	1.352	0.121
Rein d. / R. kidney	1.535	0.073	1.530	0.123	1.259	0.144	1.547	0.068	1.616	0.119
Rein g. / L. kidney	1.355	0.217	1.503	0.096	1.271	0.148	1.517	0.052	1.606	0.127
Surrenale d./R. adrenal	0.0263	0.0028	0.0327	0.0024	0.0267	0.0033	0.0334	0.0025	0.0397	0.0026
Surrenale g./L. adrenal	0.0266	0.0024	0.0375	0.0047	0.0292	0.0025	0.0335	0.0028	0.0386	0.0026
Poumon / Lung	1.394	0.015	1.437	0.054	1.157	0.052	1.324	0.046	1.528	0.142

Table: Organ weights of female rats.

PARAMETRES PARAMETERS	Control (0.9% NaCl)		Intralipid (60 ML/kg)		CSW 6/3 (15 mL/kg)		CSW 6/3 (30 mL/kg)		CSW 6/3 (60 mL/kg)	
	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.	MOYENNES MEANS	ECARTS M. S. E. M.
Poids / Weight (g)	244.2	16.65	250.4	10.51	223.6	6.74	247.9	7.65	268.4	11.57
Cerveau / Brain	1.967	0.066	1.993	0.043	1.946	0.039	1.944	0.042	1.982	0.074
Coeur / Heart	0.853	0.051	0.955	0.024	0.802	0.008	0.865	0.045	1.013	0.045
Foie / Liver	6.809	0.416	10.012	0.850	5.967	0.081	8.432	0.542	11.233	0.676
Rate / Spleen	0.424	0.032	0.689	0.055	0.365	0.013	0.614	0.060	0.807	0.052
Rein d. / R. kidney	1.019	0.068	1.031	0.057	0.831	0.047	0.993	0.058	1.115	0.066
Rein g. / L. kidney	0.991	0.061	1.074	0.062	0.843	0.046	0.996	0.046	1.127	0.074
Surrenale d./R. adrenal	0.0326	0.0044	0.0418	0.0031	0.0313	0.0004	0.0444	0.0026	0.0509	0.0027
Surrenale g./L. adrenal	0.0343	0.0036	0.0454	0.0037	0.0344	0.0012	0.0461	0.0025	0.0526	0.0022
Poumon / Lung	1.051	0.064	1.163	0.064	0.952	0.043	1.069	0.041	1.172	0.059

Histopathology

Adequate Battery: Yes

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Peer Review: No

Histological Findings

On completion of the gross pathology examination, the following organs (listed below) were fixed in 10% neutral buffered formalin for histological examination.

- Aorta (transverse and abdominal part)
- Brain
- Heart
- Colon
- Duodenum
- Stomach
- Liver
- Mesenteric ganglion
- Adrenal glands
- Pituitary glands
- Ileum
- Jejunum
- Tongue
- Skeletal muscle
- Sciatic nerve
- Eye and optical nerve
- Oesophagus
- Bone (femur)
- Pancreas
- Skin
- Lungs
- Prostate or uterus
- Spleen
- Kidneys
- Sternum
- Testicles or ovaries
- Thyroid glands
- Trachea
- Bladder

Histopathological findings were mainly associated with the liver and the spleen. In the liver, hepatocellular vacuolation (lipid), foci of granulomatous inflammation, focal hepatocellular necrosis, and pigment, interpreted as hemosiderin in Kupffer cells, and pronounced splenic hemosiderosis were observed in both lipid treated groups. The severity of the hepatic changes was similar in rats infused with 12 g/kg/day of either lipid emulsions. Rats with 6 g/kg/day CSW 6.3, the changes in the liver described above were very mild and at 3 g/kg/day CSW 6.3, only a moderate overload in small lipid deposits in Kupffer cells was observed. At the end of the 1-month recovery period, the inflammatory granulomas and pigment persisted in the livers and splenic vacuolation was still present in rats at 12 g/kg/day lipid emulsion dose groups.

Study title: Toxicity from repeated intravenous doses in rabbits of two 20% lipid emulsions: Intralipid and CSW 6.3 (14 days of administration).

Study no.:	A 89 11
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February, 1989
GLP compliance:	No
QA statement:	Not available
Drug, lot #, and % purity:	CSW 6.3 lot 82044 and Intralipid lot 82758, 35275 and 69089; purity not provided

Key Study Findings

CSW 6.3 (ClinOleic) or Intralipid® was administered intravenously to male rabbits (3/dose group) at a dose of 45 mL/kg (0.5 mL/kg/min infusion rate) once daily for 14 days. There were no deaths or abnormal clinical signs, and the weight gain was normal, but food consumption was reduced in both groups. A decrease in red blood cell (about 10%) and hematocrit in the CSW 6.3 group were noticed. Levels of alkaline phosphatase and SGPT were increased in both lipid treated groups. Postmortem examination revealed increased liver weights (4.9% and 5.7% of body weight in CSW 6.3 and Intralipid® groups, respectively). Histopathological changes, such as lipid overload of splenic reticular cells and hepatocytes were observed in both groups. Macrophages with lipid deposits in the pulmonary artery (sub-endothelial stratum) were more pronounced in CSW 6.3 treated animals.

Methods

Doses:	45 mL/kg/day
Frequency of dosing:	Once daily for 1h 30min
Route of administration:	Intravenous
Dose volume:	0.5 mL/kg/min
Formulation/Vehicle:	Intralipid® 20% (soybean oil) and CSW 6.3 (Olive oil and soybean oil)
Species/Strain:	Hy/cr Rabbit
Number/Sex/Group:	Group A: (3 males) Intralipid® Group B: (3 males) CSW 6.3
Age:	Not specified
Weight:	Not provided
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

No mortalities were observed in any group.

Clinical Signs

No treatment-related clinical signs were observed.

Body Weights

The animals were weighed twice a week.

Body weight gain of the animals in group A (Intralipid®) was higher (+ 0.5 kg) than that of the animals in the CSW 6.3 treated group (+ 0.39 kg).

Feed Consumption

Food intake was measured once a week.

The food consumption (g/day) was reduced in both groups and was comparable.

Hematology

Following hematological parameters were determined on Day1 and Day 15.

PARAMETRES PARAMETERS	UNITES UNITS
Heaaties / R. B. C.	$10^6/mm^3$
Hematocrite	%
Hemoglobine	g/dl
Vol. glob. moy. / M. C. V.	f1
Hen. glob. moy. / M. C. H.	pg
C. B. M. H. / M. C. H. C.	g/100ml
Reticulocytes	%
Reticulocytes	$10^6/mm^3$
Leucocytes / W. B. C.	$10^3/mm^3$
Neutrophiles	%
Neutrophiles	$10^3/mm^3$
Eosinophiles	%
Basophiles	%
Lymphocytes	%
Lymphocytes	$10^3/mm^3$
Monocytes	%
Plaquettes / Platelets	$10^3/mm^3$
Temps de Quick/P. Time	sec.

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Hematology showed a reduction in the number of red blood cells (-10%) and hematocrit percentage (-15%) in animals infused with CSW 6.3. The number of platelets was slightly reduced in both groups (Intralipid® -19% and CSW 6.3 -15%).

Clinical Chemistry

Following clinical chemistry parameters were determined.

PARAMETRES PARAMETERS	UNITES UNITS
Glucose	mmol/l
Uree/B. U. N.	mmol/l
Creatinine	umol/l
Proteines totales	g/l
Albumine	g/l
Globuline	g/l
Rapport A/G .A/G Ratio	
Bilirubine totale	umol/l
Phosphatases alcalines	U/l
S. G. O. T. /A.S. A. T.	U/l
S. G. P. T. /A.L. A. T.	U/l
Cholesterol libre	mmol/l
Cholesterol total	mmol/l
Phospholipides	mmol/l
Triglycerides	mmol/l
Sodium	mmol/l
Potassium	mmol/l
Fer	umol/l
Haptoglobine	mg/dl

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The levels of alkaline phosphatase (Intralipid® -28%, CSW 6.3 -33%) and SGPT decreased and total protein levels increased in both lipid treated groups. Lipid parameters increased in both groups, which are summarized in the Sponsor's Table below.

Increases in Lipid Parameters Following 14 Days of Treatment in Rabbits

Lipid parameter	ClinOleic	Intralipid
Total cholesterol (%)	+ 705	+ 475
Free cholesterol (%)	+ 1050	+870
Phospholipids (%)	+ 345	+ 325
Triglycerides (%)	+120	+184

Urinalysis

Not conducted.

Gross Pathology

Gross pathology observations showed that the organs of all animals appeared normal. One rabbit in the Intralipid® group showed viscous bile and one rabbit in the CSW 6.3 group showed liquid bile.

Organ Weights

The weight of the liver, expressed as percentage of body weight, was 5.7% higher in the Intralipid® group and 4.9% higher in the CSW 6.3.

Histopathology

Adequate Battery: No

Peer Review: No

Histopathological findings showed lipid overload of splenic reticular cells and hepatocytes in both lipid treated groups. Macrophages with lipid deposits in the branches of the pulmonary artery (sub-endothelial stratum) were common in animals that received CSW 6.3.

Study title: Toxicity with repeated doses in beagle dogs of two lipid emulsions (CSW 6.2 and CSW 6.3) administered by intravenous route (30 days) - comparison with 20% Intralipid®.

Study no.:	A 01 87 18
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 5, 1987
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NaCl batch 2043; Intralipid® 20% batch not provided; CSW 6.2, batch 72001 and CSW 6.3 (ClinOleic), batch 72002; purity not provided

Key Study Findings

There were no mortalities and treatment-related clinical signs in dogs that received any of the lipid emulsions. Serum chemistry changes included decreased urea concentrations, increased cholesterol and phospholipid concentrations, increased serum alkaline phosphatase activity and bilirubin concentration in all lipid treated groups. Gross pathology observations showed pale discoloration of the livers with increased hepatic weights in all lipid treated animals. Histopathologic changes consisted of hepatocellular vacuolation (lipid) and pigment (hemosiderin), and sinusoidal macrophages (Kupffer cells) in the livers. Vacuolation and pigmentation were also present in splenic macrophages of treated animals. These changes were comparable in all groups receiving lipid emulsions. CSW 6.3 and CSW 6.2 were well tolerated. The effects of CSW 6.3 were comparable to Intralipid®.

Methods

Doses:	45 mL/kg/day (9 g/kg/day)
Frequency of dosing:	Once daily
Route of administration:	Intravenous (saphenous or cephalic vein)
Dose volume:	0.2 mL/kg/min
Formulation/Vehicle:	NaCl, CSW 6.2 and CSW 6.3 (Olive oil and soybean oil), and Intralipid® 20% (soybean oil)
Species/Strain:	Beagle dogs
Number/Sex/Group:	Group T (2 /sex): 45 mL/kg/day NaCl Group A (2/sex): 45 mL/kg/day Intralipid® 20% Group B (2/sex) 45 mL/kg/day CSW 6.2 Group C (2/sex) 45 mL/kg/day CSW 6.3
Age:	9 months
Weight:	8 - 10 kg for both males and females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results**Mortality**

The animals were observed several times a day.

There was no mortality in any group.

Clinical Signs

The animals were observed several times a day for clinical signs.

No abnormal clinical signs were observed in any group.

Body Weights

Body weight was measured twice a week starting on Day -7.

Body weights were comparable in each group including the control (NaCl) group. However, due to the small number of animals (2 males and 2 females/group) the result was inconclusive.

Feed Consumption

Food intake was measured every day.

Food consumption was reduced in animals infused with the lipid emulsions. Food consumption was comparable in the 3 lipid infused groups.

Ophthalmoscopy

Ophthalmoscopy examination was conducted before and at the end of the dosing period.

There were no abnormal ophthalmologic effects.

Hematology

Blood samples for hematology were collected before treatment and on Day 15 and Day 29 of the treatment either from saphenous or cephalic vein.

There were no changes in the hematological parameters.

Clinical Chemistry

Following clinical chemistry parameters were determined before treatment and on Day 15 and Day 29 of the treatment.

Parameters	Units
Glucose	mmol/L
Urea	mmol/L
Creatinine	μmol/L
Proteins	g/L
A bumin	g/L
Globulin	g/L
A bumin-Globulin ratio	
Total bilirubin	μmol/L
A kaline phosphatase	U/L
SGOT	U/L
SGPT	U/L
Total cholesterol	mmol/L
Phospholipids	mmol/L
Triglycerides	mmol/L
Inorganic phosphorus	mmol/L
Chloride	mmol/L
Sodium	mmol/L
Potassium	mmol/L
Calcium	mmol/L

Serum chemistry analysis showed decreased urea concentrations, increased cholesterol and phospholipid concentrations, increased serum alkaline phosphatase activity and bilirubin concentration when compared Day 0 levels with that of Day 30 in all lipid treated groups. Results are presented in the Table below.

Parameters	Saline		Intralipid 20%		CSW 6.2		CSW 6.3	
	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
Urea (mmol/L)	4.12±0.19	4.60±0.59	5.72±0.22	1.65±0.27	4.31±0.11	1.49±0.09	3.67±0.28	1.79±0.43
Cholesterol (mmol/L)	4.47±0.49	4.56±0.41	3.54±0.39	11.36±0.96	4.12±0.71	12.54±1.15	3.68±0.34	12.88±0.56
Phospholipids (mmol/L)	4.60±0.26	4.22±0.05	3.90±0.32	7.26±0.40	4.34±0.60	7.50±0.28	3.90±0.26	7.50±0.13
Alkaline phosphatase (U/L)	82±19	74±13	72±7	131±9	69±6	126±21	69±10	122±10
Total bilirubin (µmol/L)	1.2±0.14	1.5±0.15	1.2±0.17	2.3±0.26	1.2±0.11	2.7±0.26	1.2±0.05	2.4±0.05

Urinalysis

Urine collection was conducted over 18h after removing water from the cage. Urine density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood were determined before treatment and on Day 15 and Day 30.

Urinalysis showed no effects on the urine parameters in all lipid treated groups.

Gross Pathology

For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

Gross pathology observation revealed yellowish brown liver in all the animals that received lipid emulsions.

Organ Weights

At necropsy, the weights of the following organs were recorded.

- Brain
- Heart
- Liver
- Adrenal glands
- Pituitary
- Pancreas
- Prostate
- Lungs
- Spleen
- Kidneys
- Testicles or ovaries
- Thyroids

The absolute and relative weights of the liver were higher in the lipid treated groups as compared to the control (NaCl) group. The changes in the liver weights are shown in the Table below.

Organ	Saline		Intralipid 20%		CSW 6.2		CSW 6.3	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver (g) Absolute	307±54.6	264±63	361±5.0	361±26.7	338±27.8	381±2.3	314±2.9	382±51.8
Liver (%) Relative	2.55±0.34	2.50±0.22	3.15±0.27	3.22±0.05	2.90±0.20	3.84±0.02	2.64±0.01	3.43±0.28

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

On completion of the gross pathology examination, organs were fixed in 10% neutral buffered formalin for histological examination. The following organs were examined:

- Aorta (transverse and abdominal part)
- Brain
- Heart
- Colon
- Duodenum
- Stomach
- Liver
- Lymphatic ganglia (cervical and mesenteric)
- Mammary gland
- Salivary gland
- Adrenal glands
- Pituitary
- Ileum

- Jejunum
- Tongue
- Skeletal muscle
- Sciatic nerve
- Eye and optical nerve
- Esophagus
- Pancreas
- Skin
- Lung
- Prostate or uterus
- Spleen
- Kidneys
- Infusion site
- Sternum
- Testicles or ovaries
- Thymus
- Thyroids
- Trachea
- Gall bladder
- Bladder

Histopathologic changes in the liver consisted of hepatocellular vacuolation (lipid) and pigment (hemosiderin) in sinusoidal macrophages (Kupffer cells) in the livers. Vacuolation and pigmentation were also present in splenic macrophages of lipid treated animals. These changes were comparable in all groups receiving lipid emulsions.

Study title: Toxicity of the lipid emulsion 20% CSW 6.3 batch 92122 administrated intravenously in dogs in repeated doses; comparison with 20% Intralipid® batch 55472.51 (administered for 30 days).

Study no.:	A 91 15
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 20, 1990
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NaCl batch 2444; CSW 6.3, batch 92122 and Intralipid® 20% batch 55472.51; purity not provided

Key Study Findings

CSW 6.3 (ClinOleic) or Intralipid® were administered to Beagle dogs (2/sex/group) by intravenous infusion at a rate of 0.2 mL/kg/min at a dose of 12 g/kg/day, corresponding to 60 mL/kg/day, for 30 days. A control group (2/sex) received 60 mL/kg/day saline. Clinical observation revealed sporadic episodes of vomiting or diarrhea. Both male dogs infused with Intralipid® were euthanized on Days 23 and 29, respectively due to anorexia and deteriorating general condition accompanied by icterus,

hypertriglyceridemia, hyperbilirubinemia, and increased bile acid concentrations. Dogs in both treatment groups had mild anemia, thrombocytopenia with increased sedimentation rate and increased fibrinogen concentrations. Serum chemistry showed decreased urea concentrations, increased cholesterol and phospholipid concentrations and increased serum alkaline phosphatase and alanine aminotransferase activities in dogs that received lipid emulsions. Gross pathology observation revealed pale discoloration of the livers with increased liver weights and gastric mucosal lesions (ulcers, erosions, black spots) in most lipid treated dogs. Histopathological finding included hepatocellular vacuolation (steatosis) in animals in all lipid treated dogs and was more pronounced in dogs in the Intralipid® group. Sinusoidal macrophages (Kupffer cells) in the livers and in splenic macrophages of treated animals were also vacuolated and pigmented (hemosiderin). Additional treatment-related changes in the Intralipid group included steatosis of renal cortical tubules and thymic involution. The findings suggest that CSW 6.3 emulsion is better tolerated in dogs than 20% Intralipid® emulsion.

Methods

Doses:	60 mL/kg/day
Frequency of dosing:	Once daily
Route of administration:	Intravenous (saphenous or cephalic vein)
Dose volume:	0.2 mL/kg/min
Formulation/Vehicle:	NaCl, CSW 6.3 (Olive oil and soybean oil) and Intralipid 20% (soybean oil)
Species/Strain:	Beagle dogs
Number/Sex/Group:	Group T (2 /sex): 60 mL/kg/day NaCl Group A (2/sex): 60 mL/kg/day CSW 6.3 Group B (2/sex) 60 mL/kg/day 20% Intralipid®
Age:	8-9 months
Weight:	Approximately 11 kg for both males and females
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

The animals were observed for clinical signs and mortality several times a day. During weekend, animals were observed only in the morning.

Both males from the Intralipid® group were sacrificed on day 23 and day 29, respectively, due to their deteriorated health conditions. The cause of death was assessed to be a massive hepatic disorder for both animals.

Clinical Signs

Vomiting and sporadic diarrhea was common in both lipid treated groups.

Body Weights

Body weight was measured twice a week starting with Day -7.

Body weight gain was comparable in females between Intralipid® and CSW 6.3 treated groups. However, for males, the body weight gain was higher in the CSW 6.3 treated group compared to Intralipid® group.

Feed Consumption

Food intake was measured every day and an average food intake was calculated per week.

Food consumption was reduced significantly in Intralipid® group compared to CSW 6.3 group.

Hematology

Blood samples for hematology were collected before treatment (Day -11) and on days 8, 15, 22 and 30 from the saphenous or cephalic vein.

Hematological findings included mild anemia at the end of the second week of treatment which tended to regress thereafter in both lipid treated groups. The anemic condition was slightly higher in group B (20% Intralipid) than group A (CSW 6.3). A slight reduction of the number of platelets and increased sedimentation rate and fibrinogen levels were noted in both lipid treated groups on the first week, and then remained stable thereafter.

Clinical Chemistry

Following clinical chemistry parameters were determined before treatment (Day -11) and on days 8, 15, 22 and 30 of treatment.

Parameters	Units
Bile Acid	mmol/L
Glucose	mmol/L
Urea	mmol/L
Creatinine	μmol/L
Proteins	g/L
A bumin	g/L
Globulin	g/L
A bumin-Globulin ratio	
Total bilirubin	μmol/L
A kaline phosphatase	U/L
SGOT	U/L
SGPT	U/L
Total cholesterol	mmol/L
Phospholipids	mmol/L
Triglycerides	mmol/L
Chloride	mmol/L
Sodium	mmol/L
Potassium	mmol/L
Calcium	mmol/L

Serum chemistry analysis showed decreased urea, albumin, and total protein levels in both lipid treated groups. Alkaline phosphatase level increased sharply on day 2 and day 3 in both lipid treated groups. The increase was higher in the CSW 6.3 group. The values then decreased in both groups up to day 15 (but still remained higher than the control group) and then increased again up to day 30 in both lipid treated groups. The transaminase levels increased up to day 8 but were comparable to those of the Intralipid® group on day 30. An increase in the phospholipids and cholesterol levels were noticed in the Intralipid® group. Bile analysis revealed an increase in the biliary cholesterol levels in the Intralipid® group compared to the CSW 6.3 group.

Urinalysis

Urine was collected over 18h in the metabolism cage after removing water from the cage. Urine density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood were determined before treatment and on Days 15 and 30 of treatment.

Urinalysis showed decreases in the urinary volume and pH in both lipid treated groups.

Gross Pathology

For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

Gross pathology observation revealed pale discoloration of the livers in all treated animals and gastric mucosal lesions (ulcers, erosions, black spots) in most treated dogs.

Organ Weights

At necropsy, the weights of the following organs were recorded.

- Brain
- Heart
- Liver
- Adrenal glands
- Hypophysis
- Prostate
- Lungs
- Spleen
- Kidneys
- Testicles or ovaries
- Thyroids and parathyroids.

The weight of the liver was higher in the lipid treated animals. The hypertrophy was more pronounced in the Intralipid® group than the CSW 6.3 group. The weight of the prostate was reduced in the lipid treated animals, particularly in the Intralipid® group.

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

Histopathological examinations of the following organs were conducted:

- Aorta (transverse and abdominal part)
- Brain
- Heart
- Colon
- Duodenum
- Stomach
- Liver
- Lymphatic ganglia (cervical and mesenteric)
- Mammary gland
- Salivary gland
- Adrenal glands
- Pituitary
- Ileum
- Jejunum
- Tongue
- Skeletal muscle
- Sciatic nerve
- Eye and optical nerve
- Esophagus
- Pancreas
- Skin
- Lung
- Prostate or uterus
- Spleen
- Kidneys
- Infusion site
- Sternum
- Testicles or ovaries
- Thymus
- Thyroids
- Trachea
- Gall bladder
- Bladder

Histopathologic changes in the liver consisted of hepatocellular vacuolation (steatosis) in all lipid treated dogs. However, it was more pronounced in dogs in the Intralipid® group as compared to the CSW 6.3 group. Sinusoidal macrophages (Kupffer cells) in the livers and splenic macrophages with vacuolation and pigmentation (hemosiderin)

were noticed in both lipid treated groups. Additional treatment-related changes included steatosis of renal cortical tubules and thymic involution.

Study title: CSW 6/3 (ClinOleic) emulsion batch 1120170 toxicity due to repeated intravenous doses in the dog - comparison with Intralipid 20% batch 62121-51 (30 days of administration).

Study no.:	A 92 02
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August, 1991
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NaCl batch 2510; CSW 6.3, batch 1120170 and Intralipid® 20% batch 62121-51; purity not provided

Key Study Findings

The purpose of the study was to evaluate the tolerance of CSW 6.3 in dogs, compared with Intralipid® 20%. This study was conducted in order to bring the total number of animals studied to 4 males and 4 females per group by combining the animals with those from study # A 91 15. The results of these two studies (# A 91 15 and # 92 02) were later combined into a single study (study # 92 04).

There were no mortalities in this study. However, one female and one male from Intralipid® group were sacrificed on Days 28 and 29, respectively due to advanced jaundice. Dogs in both lipid treatment groups had mild thrombocytopenia with increased sedimentation rate and increased fibrinogen concentrations. Serum chemistry showed decreased urea, creatinine, proteins, potassium and calcium, increased cholesterol and phospholipid concentrations, and increased serum alkaline phosphatase in both lipid treated groups. Treatment-related gross pathology changes consisted of pale discoloration of the livers in all treated animals and gastric mucosal lesions (ulcers, erosions, black spots) in most treated dogs. Liver weights increased in dogs receiving lipids. In addition, dogs in the Intralipid® group had higher renal weights and lower prostate weights compared to controls. Hepatocellular vacuolation (steatosis) was present in the livers of all treated dogs and was more pronounced in the Intralipid® group. Sinusoidal macrophages (Kupffer cells) in the livers and splenic macrophages with vacuolation and pigmentation (hemosiderin) were noticed in the lipid treated animals. Additional treatment-related changes included steatosis of renal cortical tubules and thymic involution in Intralipid® group.

Methods

Doses:	60 mL/kg/day
Frequency of dosing:	Once daily
Route of administration:	Intravenous (saphenous or cephalic vein)
Dose volume:	0.2 mL/kg/min

Formulation/Vehicle: NaCl, CSW 6.3 (Olive oil and soybean oil) and Intralipid® 20% (soybean oil)
Species/Strain: Beagle dogs
Number/Sex/Group: Group T (2 /sex): 60 mL/kg/day NaCl
Group A (2/sex): 60 mL/kg/day CSW 6.3
Group B (2/sex) 60 mL/kg/day Intralipid® 20%
Age: 8-9 months
Weight: 11 kg for both males and females
Satellite groups: None
Unique study design: None
Deviation from study protocol: None

Observations and Results

Mortality

The animals were observed several times a day; the last observation of the day was at 4:00 pm. During weekend, animals were observed only in the morning.

One female and one male animal from the Intralipid® group were sacrificed on Day 28 and Day 23, respectively due to deteriorated general condition, vomiting, dehydration and jaundice (yellow mucosa, dark urine).

Clinical Signs

Occasional vomiting was common in all 3 groups including the control.

Body Weights

Body weight was measured twice a week starting on Day -7.

Body weight gain was higher in the males of both lipid treated groups compared with the saline treated group. However, it was similar among the 3 groups for the female dogs.

Feed Consumption

Food intake was measured every day and an average food intake was calculated per week.

Food consumption was reduced in both lipid treated groups.

Hematology

Blood samples for hematology were collected before treatment (Day -11) and on days 8, 15, 22 and 30 of treatment from the saphenous or cephalic vein.

The results showed slight anemia, thrombocytopenia, lymphopenia, and an increase in sedimentation rate and fibrinogen levels in the lipid treated groups.

Clinical Chemistry

Following clinical chemistry parameters were determined before treatment (Day -11) and on days 8, 15, 22 and 30 of treatment.

Parameters	Units
Bile Acid	mmol/L
Glucose	mmol/L
Urea	mmol/L
Creatinine	μmol/L
Proteins	g/L
A bumin	g/L
Globulin	g/L
A bumin-Globulin ratio	
Total bilirubin	μmol/L
A kaline phosphatase	U/L
SGOT	U/L
SGPT	U/L
Total cholesterol	mmol/L
Phospholipids	mmol/L
Triglycerides	mmol/L
Chloride	mmol/L
Sodium	mmol/L
Potassium	mmol/L
Calcium	mmol/L

Serum chemistry analysis showed decreased urea, creatinine, protein, potassium, calcium, and increased cholesterol and phospholipid levels in both the lipid treated groups. Alkaline phosphatase levels significantly increased in the Intralipid® group at the beginning of the study, and in the CSW 6.3 group at the end of the study.

Urinalysis

Urine was collected over 18h, after removing water from the cage. Urine density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood were determined before treatment and on Days 15 and 30 of treatment.

No changes in the urinalysis parameters were observed.

Gross Pathology

For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

Gross pathology observation revealed pale discoloration of the livers in all treated animals and gastric mucosal lesions (ulcers, erosions, black spots) in most treated dogs. The cortical area of the kidney was striated in two animals in the Intralipid® group and one animal in the CSW 6.3 group.

Organ Weights

At necropsy, the weights of the following organs were recorded.

- Brain
- Heart
- Liver
- Adrenal glands
- Hypophysis
- Prostate
- Lungs
- Spleen
- Kidneys
- Testicles or ovaries
- Thyroids and parathyroids.

The weight of the liver was higher in the lipid treated animals, and the weight of the prostate was lower, particularly in the Intralipid® group. The weights of the liver and prostate are shown in the Table below.

Organ	Saline		Intralipid 20%		CSW 6.3	
	Male	Female	Male	Female	Male	Female
Liver (g) Absolute	351±61.7	225±0.4	685±30.3	522±55.5	587±21.8	505±35.1
Liver (%) Relative	2.83±0.18	2.12±0.12	4.67±0.409	5.10±1.11	3.92±0.39	4.54±0.07
Prostate (g) Absolute	4.85±0.05		2.35±0.05		3.5±0.90	
Prostate (%) Relative	0.040±0.0041		0.016±0.0025		0.023±0.0045	

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings:

On completion of the gross pathology examination, organs were fixed in 10% neutral buffered formalin for histological examinations. Following organs were examined.

- Aorta (transverse and abdominal part)
- Brain
- Heart
- Colon
- Duodenum
- Stomach
- Liver
- Lymphatic ganglia (cervical and mesenteric)
- Mammary gland
- Salivary gland
- Adrenal glands
- Pituitary
- Ileum

- Jejunum
- Tongue
- Skeletal muscle
- Sciatic nerve
- Eye and optical nerve
- Esophagus
- Pancreas
- Skin
- Lung
- Prostate or uterus
- Spleen
- Kidneys
- Infusion site
- Sternum
- Testicles or ovaries
- Thymus
- Thyroids
- Trachea
- Gall bladder
- Bladder

Histopathological changes in the livers consisted of hepatocellular vacuolation (steatosis) in all lipid treated dogs and were more pronounced in dogs in the Intralipid® group. Sinusoidal macrophages (Kupffer cells) in the liver and splenic macrophages with vacuolation and, pigmentation (hemosiderin) were noticed in all lipid treated animals. Additional treatment-related changes included steatosis of renal cortical tubules and thymic involution in the Intralipid® group.

Study title: Toxicity from repeated doses of CSW 6.3 in beagle dogs (90 days) comparison to Ivelip® 20%

Study no.:	A 88 06
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 1987
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CSW 6.3 and Ivelip® 20%; batch and purity information were not provided

Key Study Findings

CSW 6.3 (ClinOleic) or Ivelip® 20% were administered to Beagle dogs (1/sex/group) by intravenous infusion at a rate of 0.2 mL/kg/min, at a dose of 9 g/kg/day, corresponding to 45 mL/kg/day, for 90 days. Three animals died during the study (2 animals from the Ivelip® group and 1 animal from the CSW 6-3 group). Clinical observation revealed vomiting, and diarrhea with blood in the stool for both groups. After 30 days of infusions, blood chemistry revealed a decrease in the level of urea, total protein and albumin, a significant increase in the level of total cholesterol and phospholipids, and a

significant increase in the activity of alkaline phosphatase. The presence of blood and bilirubin was noted in the urine of all animals. Histopathological examination of the organs showed very high hepatic lipid in animals treated with Ivelip®. However, since this study was conducted on a small number of animals, the above findings are inconclusive.

Methods

Doses:	45 mL/kg/day (9 g/kg/day)
Frequency of dosing:	Once daily
Route of administration:	Intravenous
Dose volume:	0.2 mL/kg/min
Formulation/Vehicle:	CSW 6.3 (Olive oil and soybean oil) and Ivelip® 20% (soybean oil)
Species/Strain:	Beagle dogs
Number/Sex/Group:	Group A (1/sex): 45 mL/kg/day Ivelip® 20% Group B (1/sex): 45 mL/kg/day CSW 6.3
Age:	Not known
Weight:	Not known
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

Three animals died during the study. Two animals from Ivelip® group died after 48 and 61 infusions, respectively. One male from CSW 6.3 group died after the 70th infusion.

Clinical Signs

Clinical signs revealed occasional vomiting, diarrhea with blood in the stool. Two animals that received Ivelip® showed an icteric phase (yellow coloration of the mucous) prior to death.

Body Weights

No changes in the body weight were observed. However, a female animal that died on Day 48, showed a decrease in body weight.

Feed Consumption

Food consumption was significantly reduced in both lipid treated groups.

Ophthalmoscopy:

Ophthalmology examinations were conducted on 3 animals after 51 days of treatment. No ocular anomalies were observed.

Hematology

On day 30, animals that received Ivelip® showed a 58% reduction in the level of platelets, and the level of fibrinogen increased from 1.55 to 3.40 g/L.

Clinical Chemistry

After 30 infusions, both groups of animals showed a decrease in the levels of urea, total proteins and albumin, a significant increase in the level of total cholesterol and phospholipids, and a significant increase in the level of alkaline phosphatase.

Urinalysis

The presence of blood and bilirubin was noted in the urine of all animals.

Gross Pathology

Gross pathology observation revealed pale discoloration of the livers in all treated animals. Ivelip® treated dogs showed hemorrhagic ulceration in the stomach and colon, and a yellow pancreas. CSW 6.3 treated dogs showed splenomegaly and yellow medullary cortex of the kidney.

Organ Weights

The weights of the liver and spleen of the animals treated with Ivelip® were higher than animals treated with CSW 6.3.

Histopathology

Adequate Battery: No. Liver, lungs, heart, pancreas, spleen, kidneys, stomach, duodenum, jejunum, ileum, mesenteric lymph nodes and thyroid glands were histologically examined.

Peer Review: No

Histological Findings

Histopathological findings revealed higher levels of lipid in the livers of the animals that received Ivelip® than in animals that received CSW 6.3. Two animals infused with

Ivelip® (that died after 48 and 61 infusions, respectively) showed acute interstitial pancreatitis.

Study title: Evaluation of the toxicity of the CSW 6/3 product administered intravenously to dogs over 3 months.

Study no.:	A 1495 TTC/096.90
Study report location:	Electronic submission (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 21, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NaCl batch 225; CSW 6.3, batches 82055 and 92001.01, and Intralipid® 20% batch 39900.51; purity not provided

Key Study Findings

CSW 6.3 (ClinOleic) or Intralipid was administered to Beagle dogs (3/sex/group) by intravenous infusion at a rate of 0.2 mL/kg/min, at doses of 3, 4.5 and 6 g/kg/day, corresponding to 15, 22.5 and 30 mL/kg/day, for 3 months. A reference group received 6 g/kg/day of Intralipid® and a control group received 30 mL/kg/day of saline. Additional recovery animals (2/sex) were included in the high-dose CSW 6.3 and Intralipid® groups to evaluate the reversibility of any treatment-related effects.

One female in the Intralipid® group was terminated on day 63 due to excessive weight loss and deteriorating general health condition. Mild anemia was observed in some dogs that received 6 g/kg/day of CSW 6.3 or Intralipid®. Serum chemistry changes included decreased urea and triglyceride concentrations, and increased cholesterol and phospholipid concentrations in all groups receiving the lipid emulsions. Biliary cholesterol levels were higher in dogs at 6 g/kg/day Intralipid® compared to dogs that received 6 g/kg/day CSW 6.3. In addition, serum alanine aminotransferase activities were increased (2-3 fold) in dogs that received 4.5 or 6 g/kg/day of CSW 6.3. Gross pathological observations consisted of pale discoloration of the livers in all lipid treated animals. Liver weights (absolute) increased in males and female dogs that received Intralipid®. Histopathological changes included yellow-brown lipid pigment in the sinusoidal macrophages (Kupffer cells) in the livers and in splenic macrophages of most treated animals. Hepatocellular and splenic vacuolation were noticed in 6 g/kg/day CSW 6.3 and Intralipid® groups. Reversibility study showed that the pigmentation and vacuolation in the liver persisted at the end of the 1-month recovery period. The effects of CSW 6.3 were comparable to Intralipid® at equivalent doses (6 g/kg/day).

Methods

Doses:	15, 22.2 and 30 mL/kg/day (3, 4.5 or 6 g/kg/day)
Frequency of dosing:	Once daily
Route of administration:	Intravenous
Dose volume:	0.2 mL/kg/min

Formulation/Vehicle: NaCl, CSW 6.3 (Olive oil and soybean oil) and Intralipid® 20% (soybean oil)
Species/Strain: Beagle dogs
Number/Sex/Group: Group 1 (3/sex): 30 mL/kg/day NaCl
Group 2 (5/sex): 30 mL/kg/day Intralipid® 20%
Group 3 (3/sex): 15 mL/kg/day CSW 6.3
Group 4 (3/sex): 22.5 mL/kg/day CSW 6.3
Group 5 (5/sex): 30 mL/kg/day CSW 6.3
Age: 8 to 8.5 months
Weight: Male: 7.7 to 10 kg and Female: 7 to 9 kg
Satellite groups: None
Unique study design: None
Deviation from study protocol: None

Observations and Results

Mortality

No mortality was observed in any group. However, a female animal treated with Intralipid® was sacrificed on Day 63 due to excessive weight loss and poor clinical condition.

Clinical Signs

No adverse clinical observations were noticed.

Body Weights

Body weight was measured every week starting on Day -7.

Body weight gain was not affected. However, one control male and one female in the Intralipid® group (sacrificed early) showed reductions in body weight gain.

Feed Consumption

Food intake was measured every day and average food intake was calculated per week.

A dose-dependent reduction in food consumption was noticed in the lipid treated groups. Specifically, the food consumption was decreased in all male animals at the high dose CSW 6.3. Females in the Intralipid® group showed a significant decrease in food intake compared to the CSW 6.3 group.

Ophthalmoscopy

Ophthalmoscopy examinations were conducted before treatment and at the end of the treatment period. No ocular abnormalities were observed.

ECG

ECG was conducted prior to treatment (Day -5), and during treatment (Days 50 and 90). The electrocardiographic examinations did not reveal any changes associated to the administration of the lipid products.

Hematology

Blood samples were collected before treatment (Day -7) and on days 50 and 87 of treatment. On Day 50, no noticeable changes were observed in the males. However, a decrease in the erythrocyte parameters (RBC, HGC, HCT) were observed in 3/5 females treated with Intralipid® (12 to 18%, 15 to 22%, 10 to 15%, respectively), and 3/3 females treated with CSW 6.3 at the 15 mL/kg/day dose (9 to 13%, 10 to 16%, 5 to 9%, respectively), and 1/3 females treated with CSW 6.3 at the 22.5 mL/kg/day dose showed 22%, 25%, 17% decreases, respectively. All lipid treated groups showed slight neutrophilia, and an increased sedimentation rate was observed in all groups, including control. Anemia, associated with a reactive thrombocytopenia was observed in CSW 6/3 at 30 mL/kg/day group. The changes are shown in the Sponsor's Table below.

(1) Day 50

Males	No biologically significant anomaly			
	Intralipid® (mL/kg/day)		CSW 6/3 (mL/kg/day)	
Females	30	15	22.5	30
Number of animals affected	3/5	3/3	1/3	-
Number of red blood corpuscles (%)	-12 to -18	-9 to -13	-22	-
Hemoglobin level (%)	-15 to -22	-10 to -16	-25	-
Hematocrit (%)	-10 to -15	-5 to -9	-17	-

(2) Day 87

	Males		Females		
	Saline Control	CSW 6/3 (30mL/Kg /day)	Saline Control	Intralipid® (30 mL/kg/day)	CSW 6/3 (30mL/kg /day)
Number of animals affected	1/3	1/5	2/3	3/4	2/5
Number of red blood corpuscles (%)	-45	-19	-10	-10 to -17	-13 and -17
Hemoglobin level (%)	-45	-29	-10	-16 to -21	-15 and -17
Hematocrit (%)	-44	-26	-10	-11 to -19	-11 and -15

Clinical Chemistry

Following clinical chemistry parameters were determined before treatment (Day -7) and on days 50 and 87 of the treatment.

			<u>Unités/Units</u>
Glucose	-	GLU	g/l
Urée	Urea	UREA	g/l
Créatinine	-	CREA	mg/l
Cholestérol	-	CHOL	g/l
Cholestérol libre	Free cholesterol	FCHO	g/l
Triglycérides	-	TRIG	g/l
Triglycérides vrais	True triglycerides	T_TG	g/l
Phospholipides	Phospholipids	PHOS	g/l
Protéines	Proteins	PT_S	g/l
Albumine	Albumin	ALB	g/l
Globulines	Globulins	GLOB	g/l
Rapport albumine/globuline	Albumin/globulin ratio	A_G	
Phosphatase alcaline	Alkaline phosphatase	ALP	UI/l
Alanine aminotransférase	-	ALAT	UI/l
Aspartate aminotransférase	-	ASAT	UI/l
Bilirubine totale	Total bilirubin	BILI	mg/l
Fer sérique	Iron	IRON	ug/dl
Capacité de fixation du fer	Total iron binding capacity	TIBC	ug/dl
Pourcentage de saturation	Saturation percentage	%SAT	%
Sodium	-	NA	mEq/l
Potassium	-	K	mEq/l
Chlore	-	Cl	mEq/l
Calcium	-	CA	mg/l
Phosphore	-	P	mg/l

Serum chemistry analysis showed a decrease in uric acid and increased free cholesterol level in all animals treated with Intralipid® and CSW 6.3. The increase was dose dependent in the CSW 6.3 group. An increase in the phospholipid level and a decrease in triglycerides level were noted in all lipid treated groups. An increase in ALAT activity was observed in animals treated with CSW 6.3 at the medium and high doses, and a decrease in serum iron was observed in animals treated with CSW 6.3, regardless of the doses. These changes were not observed 4 weeks after the recovery period. Results are presented in the Sponsor's Table below.

	Intralipid®				CSW 6/3			
	D50		D87		D50		D87	
	M	F	M	F	M	F	M	F
Decrease (%) in uremia (as a function of the dose administered)	47	66	50	57	18-32	24-50	29-43	34-59
Increase (%) in cholesterolemia (as a function of the dose administered)	125	140	128	123	16-71	33-73	15-61	7-114
Increase (%) in free cholesterol level (as a function of the dose administered)	284	252	320	288	22-98	16-57	24-100	7-114
Increase (%) in phospholipid level (only at the 30 mL/kg/day dose)	56	60	59	58	39	-	17	41
Decrease (%) in the triglyceride level (only at the 2 lower doses)	-	41	-	46	28&30	33&31	15&39	24&27
Decrease (%) in the true triglyceride level (with no relation to the doses administered)	16	49	38	57	40-65	38-57	51-68	19-60
ALAT transaminase (increase factor) (only at 2 higher doses)	-	-	-	-	x 1.5 and 3.5	x 1.5 and 1.5	x 1.9 and 3.2	x 2.0 and 2.2
Decrease (%) of the serum iron level (on the average without relation to the doses administered)	-	-	-	-	20	30	40	30

Urinalysis

Urine was collected over 18h in the metabolism cage after removing water from the cage. Urine density, pH, nitrites, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood were determined on Days 43 and 90 of treatment.

Urinalysis on day 92, revealed the presence of blood pigments and proteins in a male treated with Intralipid®. On day 43, presence of magnesium ammonium phosphate crystals was noted in animals treated with CSW 6.3 at the medium dose (22.5 mL/kg/day). After the period of reversibility, the blood pigments were observed in two males of the Intralipid® group.

Gross Pathology

For all animals, gross pathology consisted of an external examination, identification of all clinically-recorded lesions, as well as a detailed internal examination.

Gross pathology observation revealed pale discoloration of the livers in all lipid treated animals which was more pronounced in the 30 ml/kg/day Intralipid® and CSW 6.3 groups. At the end of the reversibility period, the paleness of the liver was observed in the CSW 6.3 group only (1 male and 2 females). One male in the Intralipid® group showed greenish liver.

Organ Weights

At necropsy, the weights of the following organs were recorded: thyroids, heart, lungs, liver, spleen, pancreas, kidneys, adrenal glands, brain and pituitary gland.

Males treated with the lipids (Intralipid® at 30 mL/kg; CSW 6.3 at 15, 22.5 and 30 mL/kg) showed a decrease in relative thyroid weight (50, 40, 30 and 40%, respectively). A decrease in absolute thyroid weight was noticed in the 3 lipid treated groups (Intralipid® at 30 mL/kg, 38%; CSW 6/3 at 15 mL/kg, 13% and CSW 6/3 at 30 mL/kg, 16%). The absolute weight of the liver increased in all 4 lipid treated male groups (15, 18, 24 and 46%, respectively) and in relative weight in the high dose group of CSW 6/3 was also higher (14%). Females in the Intralipid® group showed increase in absolute and relative liver weights (20 and 24%, respectively). The relative weight (22%, 22%, 21% and 29%, respectively) and absolute weights (25%, 50%, 50% and 50%, respectively) of the spleen were lower in the all 4 lipid treated male groups. The absolute weight of the adrenal gland of the male animals was increased in the Intralipid® group and at the three doses of CSW 6/3 (35, 32, 14 and 10%, respectively), but the relative weight increased with Intralipid® (20%) and only in the low-dose CSW 6/3 male animals (10%). The organ weights are shown in the Sponsor's Table below.

(1) in relationship with the controls

Organs and sex	Intralipid® (mL/kg/day)		CSW 6/3 (mL/kg/day)		Intralipid® (mL/kg/day)		CSW 6/3 (mL/kg/day)	
	30	15	22.5	30	30	15	22.5	30
	Absolute value				Value related to body weight			
Thyroid (%)								
Males	-38	-13	-	-16	-50	-40	-30	-40
Liver (%)								
Males	+15	+18	+24	+46	-	-	-	+14
Females	+20	-	-	-	+24	-	-	-
Adrenal glands (%)								
Males	+35	+32	+14	+10	+20	+10	-	-

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

On completion of the gross pathology examination, organs were fixed in 10% neutral buffered formalin for histological examination. Following organs were examined histologically:

- Aorta (transverse and abdominal part)
- Brain
- Heart
- Colon
- Duodenum
- Stomach
- Liver
- Lymphatic ganglia (cervical and mesenteric)
- Mammary gland
- Salivary gland
- Adrenal glands
- Pituitary
- Ileum
- Jejunum
- Tongue
- Skeletal muscle
- Sciatic nerve
- Eye and optical nerve
- Esophagus
- Pancreas
- Skin
- Lung
- Prostate or uterus
- Spleen
- Kidneys
- Infusion site
- Sternum
- Testicles or ovaries
- Thymus
- Thyroids
- Trachea
- Gall bladder
- Bladder

Histopathological changes in the livers included brownish-yellow pigmentation in the Kupffer cells and vacuolation in the liver in most of the lipid treated animals. It was more pronounced in the Intralipid® group and the CSW 6/3 group females at all doses. The

spleen showed brownish-yellow pigmentation in both sexes in the Intralipid® and high-dose CSW 6.3 groups. The thymus showed lipid involution in all treated females, and in the mid and high dose CSW 6.3, and Intralipid® groups males. All these effects were reversed at the end of the 1-month recovery period except pigments and lipids in hepatic and splenic macrophages in the animals treated with high dose of Intralipid® and CSW 6.3.

7 Genetic Toxicology

No genetic toxicology studies were submitted.

8 Carcinogenicity

No carcinogenicity studies were submitted.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicology studies were submitted.

10 Special Toxicology Studies

Local Tolerance:

Study: Evaluation of the local tolerance of CSW 6.3, lot 72002 in rats (Study # A 88 13).

The purpose of this study was to evaluate the effects of CSW 6.3 when administered extravascularly (SC or intradermal) by an accident.

Methods: Male Sprague Dawley rats (n=18) received injections of CSW 6.3 according to the following schedule:

- 2.5 mL CSW 6.3 subcutaneously into the crural space (inner thigh);
- 0.1 mL CSW 6.3 intradermally into the dorsal median area;
- 0.1 mL 0.9% saline solution intradermally into the contra-lateral areas.

Groups of 6 rats were then examined (2 from each group) after 48 hours, and 7 or 14 days. Skin was excised from the site of subcutaneous administration and examined macroscopically and the degree of absorption was evaluated. Skin at the intradermal site was excised and placed into formalin for histological examination.

Results: Forty-eight hours after subcutaneous administration, saline administered sites appeared normal. Partial absorption of CSW 6.3 was noted at the SC and intradermal injection sites after 48 hours. After 7 days of injection, CSW 6.3 was absorbed partially

in 2/6 animals and completely in 4/6 animals. After 14 days of injection (SC or intradermal), CSW 6.3 sites appeared normal. Histopathologically saline administered sites were normal after 48h of injection. The CSW 6.3 injected sites showed congestion, edema with fibrin, and a macrophage infiltrate after 48h of injection. These inflammatory reactions were slightly reduced by day 7, and disappeared by day 14.

Tissue necrosis did not occur following subcutaneous or intradermal administration of CSW 6.3, and absorption from the injection sites was complete by 14 days after administration. Thus, an accidental extravascular administration of CSW 6.3 may not have any serious side effects.

Immune Function Test:

Study: Effects of Parenteral Lipid Emulsions with Different Fatty Acid Composition on Immune Cell Function *in vitro* (study # P 99 01E).

The objective of the study was to verify the *in vitro* stimulatory or inhibitory effects of ClinOleic on different immune functions (lymphocyte proliferation, T-cell activation markers expression, and cytokine release). The effects of ClinOleic on different immune functions were compared with soybean oil-based emulsions (Intralipid® and/or Ivelip®).

Peripheral white blood cells were collected from healthy volunteers. Three different lipid emulsions (Intralipid® 20%, Ivelip® 20% and ClinOleic 20%) were used for *in vitro* human immune cell function tests.

Lymphocyte proliferation assay: Peripheral blood mononuclear cells (PBMCs) were separated from red blood cells, washed, and re-suspended in RPMI medium. PBMCs were then stimulated by 10 µg/mL phytohemagglutinin (PHA) in the presence of freshly prepared lipid emulsions (ClinOleic, Ivelip® or Intralipid®). After 48h in culture, cell proliferation was estimated by (³H)- thymidine (1 µCi/well, 2 Ci/mmol) incorporation. The radioactivity incorporated was measured by liquid scintillation counting. An antibody-based T-cell stimulation was also evaluated in the presence of 5% autologous human plasma. Combinations of anti-CD3 and anti-CD28 monoclonal antibodies were used to trigger the proliferative response.

Lymphocyte subsets and T-cell activation markers expression: The cultured PMBCs were stimulated with PHA alone or PHA with 0.03% (v/v) of either lipid emulsions. After three days of culture, lymphocytes were harvested and the expressions of activation markers on different lymphocyte subpopulations were measured by flow cytometry using conjugated monoclonal antibodies. Cells were stained with a combination of the following conjugated monoclonal antibodies: CD45/CD3 (T-cells); CD4/CD3 (T-helper); CD8/CD3 (cytotoxic T-cells); CD16/CD3 (NK-cells/T-cells); CD19/CD3 (B-cells/T-cells); CD25/CD4 (IL-2 receptor α-chain on T-helper cells); HLA-DR/CD4 (MHC class II antigen, up-regulated on activated T-cells); CD25/CD8 (IL-2 receptor α-chain on cytotoxic T-cells); HLA-DR/CD8 (activation of cytotoxic T-cells). The percentages of positive cells were calculated using the Lysis II software which compared the fluorescence of stimulated versus unstimulated lymphocytes.

IL-2 release: The cultured PMBCs were stimulated with PHA in the presence or absence of the lipid emulsions as described above. After 48 hours of culture, supernatants were harvested and assayed for IL-2 content, determined by enzyme-linked immunosorbent assay (ELISA).

Assays on monocytes/macrophages: Monocytes/macrophages were collected by density centrifugation and fibronectin-adherence in gelatin coated flasks. The purified monocytes/macrophages were stimulated using 1 µg/mL LPS from *E. coli* in the absence or presence of lipid emulsions (ClinOleic, Ivelip® or Intralipid®). After 24 hours, the cell culture supernatant was analyzed for secreted TNFα and IL-1β using ELISA.

Results:

Lymphocyte proliferation assay: A dose-dependent inhibition of (³H)- thymidine incorporation was observed with the soybean oil based emulsions (incorporation reduced to 39.9%, p<0.001) whereas ClinOleic showed no inhibitory effect.

Lymphocyte subsets and T-cell activation markers expression: Antigen activation expression on both CD4⁺ and CD8⁺ T-cells were decreased with Intralipid® (CD25: -53.4% on CD4⁺ and -57.4% on CD8⁺; HLA-DR: -61.5% on CD4⁺ and -58.5% on CD8⁺) but not with ClinOleic (from -2.9% for CD25 on CD4⁺ to +16.7% for HLA-DR on CD4⁺).

IL-2 release: Intralipid® decreased IL-2 production (-39.0%, p<0.05) whereas ClinOleic had little effect (-13.0%, NS).

Assays on monocytes/macrophages: Intralipid® and ClinOleic inhibited the release of pro-inflammatory cytokines to a similar extent (TNFα: -21.5% and -34.8%, IL-1β: -45.1% and -40.3%; respectively).

These findings suggest that an olive oil-based lipid emulsion could modulate immune response and thus reducing the inflammatory response. Olive oil may offer an immunologically neutral alternative to soybean oil for use in parenteral lipid emulsions.

Study: In vivo effects of Olive oil based lipid emulsion on lymphocyte activation in rats (study # P 99 02E).

Weaning Wister rat pups (n=24) were fed for 4 weeks with a diet that contained 12% of total energy intake as lipids from soybean oil. Animals then received total parenteral nutrition for a period of 6 days (260 kcal/kg/day; 228 mL/kg/day) in which 12% of total energy was supplied by ClinOleic (n=12) or Ivelip® (n=12). The rats were sacrificed after 6 days of treatment, and the spleens were removed.

Lymphocyte proliferation assay: Spleen was homogenized and mononuclear cells were isolated by centrifugation. The cells were washed and re-suspended in RPMI medium. Mononuclear cells were stimulated by addition of concanavalin A. After 48

hours of incubation, lymphocyte proliferation was measured by flow cytometry, and by a colorimetric Mossman's technique with tetrazolium salts (MTT).

Phospholipid analysis: Lymphocytes were prepared as described above then extracted with chloroform/methanol technique. Total phospholipids were separated by thin layer chromatography and gas chromatography was used to analyze total phospholipids fatty acids.

Lymphocyte populations: Cells were incubated for 30 minutes at 4⁰C with monoclonal antibodies: anti CD4, anti CD8, MHC class II as β lymphocyte marker (phycoerythrin). Fluorescence activated cell sorter analysis was used to measure markers on lymphocyte surface.

IL-2 receptor α -chain expression: Lymphocytes were cultured as mentioned above, and incubated for 30 minutes at 4⁰C with monoclonal antibody against the α -chain of IL-2 receptor (CD25). Flow cytometry was used to measure the expression of IL-2 receptor α -chain (CD25).

Results:

Lymphocyte proliferation assay: The lymphocyte proliferation index tended to be lower in the Ivelip® group (0.445 ± 0.115 and 0.869 ± 0.108 respectively) compared to the group that received ClinOleic, although the differences were not statistically significant.

Phospholipid analysis: Phospholipid analysis showed higher content of oleic acid and lower linoleic acid in the ClinOleic treated group than Ivelip® treated group.

Lymphocyte populations: Lymphocyte populations from spleen consisted of approximately 60% T-cells (35% of cells were CD4⁺ and 28% were CD8⁺) and 30% B-cells. There was no significant effect of diet on lymphocyte populations in the spleen.

IL-2 receptor α -chain expression: IL-2 receptor α -chain (CD25) measured by the percentage of CD25 positive cells were expressed significantly higher ($p < 0.05$) in ClinOleic group than the Ivelip® group.

The findings suggested that ClinOleic could offer an immunologically neutral alternative to soybean oil-based lipid emulsions for use in total parenteral nutrition.

Safety Assessment of the Container-Closure system:

Description of container-closure system:

The container closure (packaging) system for ClinOleic 20% is a multi-layer, lipid compatible, (b) (4) (PL2401) plastic bag. The plastic bag consists of PL2401-1 (b) (4) for the container body, (b) (4) port tubes (b) (4) an

administration closure port, called "Twist-Off Protector", made from (b) (4) material, and an injection site (IS) closure made from (b) (4) material (b) (4) (b) (4) (b) (4). The film that makes up the container body is (b) (4) polyolefin, Baxter PL2401-1. The composition of the film is provided below:



The closure system consists of the following (b) (4) components:

- (b) (4) port tubes (b) (4)
- Administration closure port called "Twist-Off Protector" made from (b) (4) material.
- Injection site closure made from (b) (4) (b) (4) (b) (4).

Extractables Profile:

The packaging system used for Pediatric 3CB (study 33270) is compositionally equivalent to the packaging used for ClinOleic 20%, except the bar code printing, which is unique to ClinOleic 20%, and a secondary port tube, which is unique to Pediatric 3CB. Thus, the target leachables chosen for Pediatric 3CB are also appropriate for ClinOleic 20%. These target leachables associated to ClinOleic 20% are listed in the Table below. (b) (4) is a leachable (b) (4) from ClinOleic 20%.

Table IV. Targeted Leachables, ClinOleic 20% in Clarity Packaging. Also see Figure 1.		
Target Leachable	Source	Compound Class
(b) (4)		

Note: ¹Substitution of this target for (b) (4) is discussed in reference 10.

With reference to study 33270 (extractables and leachables in the Pediatric 3CB product), the table below summarizes the list of potential extractable substances associated with the multi-chambered packaging system.

Plastic Materials Summary Sheet



(b) (4)

Plastic Materials Summary Sheet



(b) (4)

(B). (b) (4) (Port tubes):

The (b) (4) port tube of the container closure system is made of (b) (4). Following standard tests were performed to evaluate any potential toxicity of port tubes of the container closure system (b) (4). (b) (4) passed the *in vitro* and *in vivo* biological reactivity tests (USP <87> and <88>), *in vitro* hemolysis test (ASTMF756), sensitization tests in guinea pigs and physicochemical tests (USP<661>). The results are presented in the Sponsor's Table below.

Plastic Materials Summary Sheet

(b) (4)

(C). (b) (4) (Administration and injection site closures):

Administration closure port and injection site (IS) closure system are made from (b) (4) material. Following standard tests were performed to evaluate any potential toxicity of (b) (4). The results showed that (b) (4) of the container closure system passed *in vitro* and *in vivo* biological reactivity tests (USP <87> and <88>), *in vitro* hemolysis test (ASTMF756), sensitization tests in guinea pigs and physicochemical tests (USP<661>). The results are presented in the Sponsor's Table below.

Plastic Materials Summary Sheet

(b) (4)

(D). (b) (4) (b) (4) of injection site):

The (b) (4) of injection site is made of (b) (4). Following standard tests were performed to evaluate any potential toxicities of (b) (4). The results showed that (b) (4) passed the *in vitro* and *in vivo* biological reactivity tests (USP <87> and <88>), *in vitro* hemolysis test (ASTMF756), sensitization tests in guinea pigs, Ames test and physicochemical tests (USP<661>). The results are presented in the Sponsor's Table below.

Rubber Materials Summary Sheet



Accumulation of leachables in ClinOleic:

For the target leachables in ClinOleic, the Applicant referred study report 33270 for multi-chambered pediatric 3CB packaging product. According to report 33270, an accumulation level of leachables in study 7 is appropriate for ClinOleic 20%. In this study, 100 mL lipid was filled in the container, autoclaved and stored for 24 months, and the targeted leachables were determined. The Sponsor's Table for the leachables under simulated condition is shown below.

Table 3. Measured Accumulation Levels of Leachables Under Simulated Use Conditions.

(b) (4)

Safety evaluation of the leachables:

Safety evaluations of individual leachable were performed based on the recommended daily dose of ClinOleic 20%. The recommended maximum daily dose of ClinOleic 20% is 2.5 g/kg/day for adults, (b) (4)

. Therefore, in order to evaluate the safety profile of individual leachable, the estimation of maximum daily exposure for each leachable was calculated based on a 50 kg body weight with the daily dose of 3 g/kg/day of ClinOleic 20% (i.e. 150 g/day). ClinOleic 20% contains 20 g lipids per 100 ml. Thus, the total volume required for a 50 kg individual is 750 ml.

In this review, the PDE (Permitted Daily Exposure) is calculated based on the following formula, which is similar to the formula used in the ICH guidance Q3C(R4) (Impurities: Guideline for Residual Solvents):

$$\text{PDE} = \text{NOEL}^* \text{ or } \text{NOAEL}^{**} \div (\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5} \times 10)$$

F1: A factor to account for extrapolation between species

F2: A factor of 10 to account for variability between individuals

F3: A variable factor to account for toxicity studies of short-term exposure

F4: A factor that may be applied in cases of severe toxicity (e.g. nongenotoxic carcinogenicity, neurotoxicity or teratogenicity)

F5: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

* NOEL: No observed effect level

** NOAEL: No observed adverse effect level

(b) (4)

In the leaching study, the level of (b) (4) in ClinOleic 20% was below the limit of detection (b) (4). Thus, the maximum exposure to (b) (4) would be less than (b) (4) µg/day based on the maximum daily administration volume of (b) (4) ml ((b) (4) µg/day).

Toxicology information for (b) (4) is summarized below. (b) (4) was evaluated to be overall negative for mutagenicity in *Salmonella* strains TA100, TA1535, TA97 and TA98 with and without metabolic activation. No *in vivo* genotoxicity studies were conducted with (b) (4).

In a 13-week toxicity study followed by a 4-week recovery period, Fischer 344 rats were given (b) (4) through dietary administration at doses of 0, 61, 303 and 917 mg/kg/day for males, and 0, 71, 360 and 1068 mg/kg/day for females. The NOEL was (b) (4) mg/kg/day for males and 71 mg/kg/day for females (b) (4).

In a 13-week toxicity study followed by 4-week recovery period, B6C3F₁ mice were given (b) (4) through dietary administration at doses of 0, 180, 885 and 2728 mg/kg/day for males, and 0, 205, 1038 and 3139 mg/kg/day for females. The NOEL was (b) (4) mg/kg/day for males and (b) (4) mg/kg/day for females (b) (4).

Thus, the calculated PDE for (b) (4) was (b) (4) µg/day (details of calculation are shown below). The maximum potential exposure to (b) (4) (b) (4) µg/day) from ClinOleic 20% is 1.6-fold lower than the calculated PDE. Therefore, there is a reasonable assurance of safety for the potential leaching of (b) (4) in ClinOleic 20%.

$$\text{PDE for (b) (4)} = \frac{(b) (4) \text{ mg/kg/day}}{(b) (4) \text{ µg/kg/day}} = \frac{(b) (4) \text{ mg/kg/day}}{(b) (4) \text{ µg/kg/day} \times (b) (4) \text{ kg (body weight)}} = (b) (4) \text{ µg/day}$$

(b) (4)

(b) (4)

(b) (4)

In the leaching study, the level of (b) (4) in ClinOleic 20% was below the limit of detection ((b) (4) µg/ml). Thus, the maximum exposure to (b) (4) would be less than (b) (4) µg/day based on the maximum daily administration volume of (b) (4) ml ((b) (4) x (b) (4) µg/day).

Toxicology information from the available literature for (b) (4) is discussed below.

(b) (4) was negative in the Ames test, the *in vivo* Chinese Hamster micronucleus test, and the dominant lethal assay in mice. (b) (4)

In a two-generation dietary reproductive toxicity study in rats, F1 and F2 offspring NOAELs were determined to be (b) (4) ppm ((b) (4) mg/kg/day), and the NOAEL for parental toxicity was (b) (4) ppm ((b) (4) mg/kg/day). In an embryo-fetal development study in rats, the NOAELs for maternal toxicity and teratogenicity were reported to be (b) (4) mg/kg/day and greater than (b) (4) mg/kg/day, respectively. (b) (4) was not considered to be teratogenic in rats. In mice, the NOAELs for maternal toxicity and teratogenicity were reported to be (b) (4) mg/kg/day (b) (4)

(b) (4) Based on the NOAEL ((b) (4) mg/kg/day) for parental toxicity in the two-generation rat reproductive toxicity study, the PDE for (b) (4) was determined to be (b) (4) mg/kg/day ((b) (4) µg/day) (details of calculation are shown below). The maximum potential exposure to (b) (4) ((b) (4) µg/day) from ClinOleic 20% is 4.5-fold lower than the calculated PDE value of (b) (4) µg/day. Therefore, there is a reasonable assurance of safety for the potential leaching of (b) (4) in ClinOleic 20%.

$$\text{PDE for (b) (4)} = \frac{(b) (4) \text{ mg/kg/day}}{(b) (4) \text{ µg/kg/day}} = \frac{(b) (4) \text{ mg/kg/day}}{(b) (4) \text{ µg/kg/day} \times (b) (4) \text{ kg (assumed body weight)}} = (b) (4) \text{ µg/day}$$

(b) (4)

F1 = 5: for extrapolation from rats to human

F2 = 10: for variability between individuals

F3 = 1: for reproductive studies in which the whole period of organogenesis is covered

F4 = Omitted due to lack of severe toxicity (e.g. nongenotoxic carcinogenicity, neurotoxicity or teratogenicity)

F5 = 1: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

(b) (4)

In the leaching study, the level of (b) (4) in ClinOleic 20% was below the limit of detection (b) (4) µg/ml). Thus, the maximum exposure to (b) (4) would be less than (b) (4) µg/day based on the maximum daily volume of (b) (4) ml ClinOleic 20% ((b) (4) µg/day).

(b) (4) did not induce a genotoxic response in *S. typhimurium* strains TA100, TA1530, TA98 and TA1537 either with or without rat liver S9 mixture. When sister-chromatid exchanges (SCEs) were quantitated after exposure to (b) (4), the results reflected no significant genotoxic effects on Chinese hamster V79 cells (b) (4).

In 2-week toxicity studies in rats and mice, (b) (4) was administered intravenously at doses of (b) (4) to (b) (4) mg/kg/day. The NOAEL was (b) (4) mg/kg/day for both rats and mice (b) (4). The maximum potential exposure to (b) (4) ((b) (4) µg/day) from ClinOleic 20% is (b) (4)-fold lower than the calculated PDE value ((b) (4) µg/day). Therefore, there is no safety concern for the potential leaching of (b) (4) in ClinOleic 20%.

PDE for (b) (4) = (b) (4) mg/kg/day ÷ ((b) (4)) = (b) (4) mg/kg/day = (b) (4) µg/kg/day; (b) (4) µg/kg/day × (b) (4) kg (assumed body weight) = (b) (4) µg/day

(b) (4)

- F1 = 5: for extrapolation from rats to human
- F2 = 10: for variability between individuals
- F3 = 10: for shorter study duration
- F4 = Omitted due to lack of severe toxicity (e.g. nongenotoxic)
- F5 = 1: A variable factor that may be applied if the NOEL was not established
- 1: safety factor for oral to intravenous conversion

(b) (4)

In the leaching study, the level of (b) (4) was (b) (4) µg/ml. Thus, the maximum exposure to (b) (4) would be (b) (4) µg/day based on the maximum daily administration volume of (b) (4) ml ClinOleic 20% ((b) (4) µg/day).

(b) (4) was negative in the Ames test, chromosome aberration assay with CHO cells, and sister chromatid exchange (SCE) assay. The *in vivo* micronucleus assays with mice, cytogenetic assays with rats and mice, and dominant lethal assays with rats and mice were also negative. Thus, (b) (4) is not mutagenic. (b) (4) was not carcinogenic in 2-year bioassays in rats and mice (GDCh) (b) (4).

In a two-generation 'carcinogenicity study' ((b) (4)), (b) (4) was administered in the diet to male and female Wister rats at doses of 0, 25, 100 or 500 mg/kg/day (F0 generation) until mating (week 13) and, in the case of female rats, until the end of the lactation period. The F1 generation groups received treatment with 25, 100, or 250 mg/kg/day until the age of 141 - 144 weeks (500 mg/kg/day was not used due to nephrotoxicity in the F0 females). The NOAEL was determined to be 25 mg/kg/day. Based on the NOAEL in the two-generation carcinogenicity study in rats, the PDE was calculated to be (b) (4) µg/day (details of calculation are shown below). Thus, the estimated maximum human exposure to (b) (4) from ClinOleic 20% ((b) (4) µg/day) is 3.8-times lower than the calculated PDE. Therefore, there is a reasonable assurance of safety for the potential leaching of (b) (4) in ClinOleic 20%.

$$\text{PDE for } (b) (4) = \frac{(b) (4) \text{ mg/kg/day}}{(b) (4) \text{ µg/kg/day} \times (b) (4) \text{ kg (assumed body weight)}} = \frac{(b) (4)}{(b) (4)} \text{ mg/kg/day} = \frac{(b) (4)}{(b) (4)} \text{ µg/kg/day}$$

(b) (4)

F1 = 5: for extrapolation from rats to human

F2 = 10: for variability between individuals

F3 = 1: for studies that last at least one half-lifetime (1 year for rodents)

F4 = Omitted due to lack of severe toxicity observed (e.g. nongenotoxic carcinogenicity, neurotoxicity or teratogenicity)

F5 = 1: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

(b) (4)

In the leaching study, the level of (b) (4) was below the limit of detection ((b) (4) /ml). Thus, the maximum exposure to (b) (4) would be < (b) (4) µg/day based on the maximum daily administration volume of (b) (4) ml ClinOleic 20% ((b) (4) µg/day).

(b) (4) was negative with and without activation in an Ames mutagenicity assay using *Salmonella typhimurium* (TA98, TA 100, TA1535, TA1537, TA1538) at concentrations of 4, 20, 100, 500, 2500 ug/plate (b) (4)

In an oral toxicity study, Wister rats were administered (b) (4) by gavage once a day, 5 days a week at doses of 0, 100, 500, 1000 mg/kg/day for 28 days. No mortality, symptoms of intoxication, clinical effects or substance related injury of organs was recorded. The NOEL was (b) (4) mg/kg/day (b) (4)

(b) (4). Based on the NOAEL, the PDE was calculated to be (b) (4) µg/day (details of calculation are shown below). Thus, the estimated maximum human exposure to (b) (4) from ClinOleic 20% ((b) (4) µg/day) is 133.3-times lower than the calculated PDE. Therefore, there is a reasonable assurance of safety for the potential leaching of (b) (4) in ClinOleic 20%.

$$\text{PDE} = \frac{(b) (4) \text{ mg/kg/day}}{(b) (4) \text{ mg/kg/day}} = \frac{(b) (4)}{(b) (4)} \text{ µg/kg/day}$$

$$\text{(b) (4)} \mu\text{g/kg/day} \times \text{(b) (4)} \text{ kg (assumed body weight)} = \text{(b) (4)} \mu\text{g/day}$$

(b) (4)

F1 = 5: for extrapolation from rats to human

F2 = 10: for variability between individuals

F3 = 10: for shorter study duration

F4 = Omitted due to lack of severe toxicity observed (e.g. nongenotoxic carcinogenicity)

F5 = 1: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

(b) (4)

In the leaching study, the level of (b) (4) in ClinOleic 20% was below the limit of detection ((b) (4) $\mu\text{g/ml}$). Thus, the maximum exposure to (b) (4) would be less than (b) (4) $\mu\text{g/day}$ based on the maximum daily administration volume of (b) (4) ml ClinOleic 20% (b) (4) $\mu\text{g/day}$.

(b) (4) was negative in gene mutation tests. Studies in rat lymphocytes ((b) (4)) and in rat liver epithelial cells were negative. (b) (4)

In a 46-day toxicity study in rats, (b) (4) was administered orally at doses of 20, 60 and 200 mg/kg/day. The NOAEL was 200 mg/kg/day. Based on the NOAEL, the PDE was calculated to be (b) (4) $\mu\text{g/day}$ (details of calculation are shown below). The maximum potential exposure to (b) (4) from ClinOleic 20% is 53.3-fold lower than the calculated PDE value (b) (4) $\mu\text{g/day}$). Therefore, there is a reasonable assurance of safety for the potential leaching of (b) (4) in ClinOleic 20%.

$$\text{PDE for (b) (4)} = \text{(b) (4)} \text{ mg/kg/day} \div \text{(b) (4)}$$

$$\text{mg/kg/day} = \text{(b) (4)} \mu\text{g/kg/day}; \text{(b) (4)} \mu\text{g/kg/day} \times \text{(b) (4)} \text{ kg (assumed body weight)} = \text{(b) (4)} \mu\text{g/day}$$

(b) (4)

F1 = 5: for extrapolation from rats to human

F2 = 10: for variability between individuals

F3 = 10: for shorter study duration

F4 = Omitted due to lack of severe toxicity (e.g. nongenotoxic)

F5 = 1: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

(b) (4)

In the leaching study, the level of (b) (4) in ClinOleic 20% was below the limit of detection ((b) (4) /ml). Thus, the maximum exposure to (b) (4) would be less than (b) (4) $\mu\text{g/day}$ based on the maximum daily administration volume of (b) (4) ml ((b) (4) $\mu\text{g/day}$).

(b) (4) was negative in the Ames test, the *in vivo* chromosomal aberration test in CHO cells and was also negative in the *in vivo* micronucleus assay in the mouse ((b) (4))

In a 90-day toxicity study, Beagle dogs were given (b) (4) through dietary administration at doses up to (b) (4) mg/kg/day. The NOEL was (b) (4) mg/kg/day in dogs. Thus, the calculated PDE for (b) (4) was (b) (4) mg/kg/day (details of calculation are shown below). The maximum potential exposure to (b) (4) ((b) (4) µg/day) from ClinOleic 20% is (b) (4)-fold lower than the PDE value ((b) (4) µg/day). Therefore, there is a reasonable assurance of safety for the potential leaching of (b) (4) in ClinOleic 20%.

$$\text{PDE for (b) (4) mg/kg/day} \div \text{(b) (4) mg/kg/day} = \text{(b) (4) µg/kg/day}; \text{(b) (4) µg/kg/day} \times \text{(b) (4) kg} = \text{(b) (4) µg/day}$$

F1 = 2: for extrapolation from dogs to human

F2 = 10: for variability between individuals

F3 = 10: for shorter study duration

F4 = Omitted due to lack of severe toxicity (e.g. nongenotoxic)

F5 = 1: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

In the leaching study, (b) (4) level in ClinOleic 20% was (b) (4) µg/ml. Thus, the maximum exposure to (b) (4) would be (b) (4) µg/day ((b) (4) mg/day) based on the maximum daily administration volume of (b) (4) ml ClinOleic 20% ((b) (4) µg/day).

The Ames assay ((b) (4)), the CHO/HGPRT-forward mutation assay and the mouse micronucleus assay ((b) (4)) demonstrated that (b) (4) is unable to induce gene mutations in bacterial or mammalian cells *in vitro*, or micronuclei *in vivo*. It is also negative in the Sister Chromatid Exchange (SCE) test in Chinese hamster lung fibroblasts ((b) (4)).

According to ICH Q3C guidance, (b) (4) belongs to the class 3 solvent. Solvents in this class are regarded as less toxic and lower risk to human health with PDE up to (b) (4) mg/day. Thus, the potential leachable amount of (b) (4) in ClinOleic 20% appears to be safe.

In the leaching study, (b) (4) level in ClinOleic 20% was (b) (4) µg/ml. Thus, the maximum exposure to (b) (4) would be (b) (4) µg/day based on the maximum daily administration volume of (b) (4) ml ClinOleic 20% ((b) (4) µg/day).

According to CFR 201.32325, a level of (b) (4) ug/L (b) (4) is a valid level for LPV (large volume parenterals). Therefore, there is no safety concern for the potential leaching of (b) (4) in ClinOleic 20%.

(b) (4)

In the leaching study, the level of (b) (4) in ClinOleic 20% was (b) (4) µg/ml (Ref. letter 003 received from the Sponsor on Feb 22, 2013). Thus, the maximum exposure to (b) (4) would be (b) (4) µg/day based on the maximum daily administration volume of (b) (4) ml of ClinOleic 20% ((b) (4) = (b) (4) µg/day).

Aqueous extracts of steam sterilized (b) (4) film were evaluated in the *Salmonella*/mammalian-microsome reverse mutation assay (Ames test). Extracts of the film were prepared by autoclaving according to the USP guideline. Under the conditions of this study, extracts of (b) (4) did not increase the number of histidine revertants for any of the tester strains in the presence or absence of microsomal enzymes. In addition, Toxtree version 2.5.0 was utilized using the Benigni/Bossa rule base for the assessment of mutagenicity and carcinogenicity of (b) (4). The Toxtree study showed that (b) (4) was negative for genotoxic and nongenotoxic carcinogenicity.

(b) (4) was also evaluated (CDER Computational Toxicology consult) for potential genetic toxicity using (quantitative) structure-activity relationship [(Q)SAR] models. (b) (4) was predicted to be negative for genetic toxicity tests recommended in the ICH S2 battery and it was also predicted to be negative for genetic toxicity that correlate with rodent carcinogenicity results.

As per the ICH Q3A guidance (impurities in new drug substances), the PDE of (b) (4) is calculated to be (b) (4) mg/day, which is more than 100-fold higher than the maximum exposure from 20% ClinOleic emulsion ((b) (4) µg/day). Thus, the level of (b) (4) as a leachable in ClinOleic 20% appears to be safe.

(b) (4)

The (b) (4) are the (b) (4) of the ClinOleic bag. The (b) (4) include (b) (4). In order for the (b) (4) to accumulate in lipid-containing solutions in the packaging system, they would have to migrate through all (b) (4) of the bag. The migration of (b) (4) were determined using exaggerated contact conditions including high heat (b) (4) conditions). Diffusion of the (b) (4), (b) (4) in lipid-containing solutions was also assessed by octanol-water partition coefficient (log P). The larger the log P value, the lower the propensity of the compound to migrate from the container and accumulate in the lipid product. This is an indirect approach to speculate the diffusion of leachable compounds. The octanol/water partition coefficient (log P) for (b) (4) was higher ((b) (4)) than (b) (4) but lower than (b) (4). All these compounds are from the (b) (4) of the packaging system. The

Applicant measured the accumulation of (b) (4) and (b) (4) in the lipid solution. They are migrated into the lipid from the (b) (4) of the bag. However, (b) (4) were only detected (b) (4) $\mu\text{g/mL}$ in container filled with (b) (4) mL water (study # CHR-90-0468). Both (b) (4) migration into the lipid reflect the (b) (4) behavior in terms of migration and accumulation because the (b) (4) share a common migration pattern and have a similar chemical nature. Considering migration patterns, the Applicant stated that the (b) (4) do accumulate in lipid products stored in the packaging system, and the accumulation levels would be less $<0.6 \mu\text{g/mL}$.

The Applicant assessed the safety of (b) (4) from estimated tolerable intakes (TI) and highest tolerable concentrations. The estimated level of (b) (4) from the container film, (b) (4) $\mu\text{g/mL}$, falls below the highest tolerable concentrations of (b) (4) $\mu\text{g/mL}$ and (b) (4) $\mu\text{g/mL}$ (for (b) (4), respectively) for the maximum clinical dose of ClinOleic.

(b) (4)

The migration pattern of (b) (4) in the lipid emulsion may be similar to (b) (4) as both of them are derived from the (b) (4) of the bag. No direct information on the levels of (b) (4) in the lipid emulsion is available. Its log P value ((b) (4)) suggests that it is water soluble and thus it could be accumulated in lipid solutions similar to water solutions. In the leaching study, the level of (b) (4) in the aqueous solution following (b) (4) and storage was (b) (4) $\mu\text{g/mL}$. Based on this, the maximum exposure to (b) (4) would be (b) (4) $\mu\text{g/day}$ considering the maximum daily administration volume of (b) (4) mL ClinOleic 20% ((b) (4) $\mu\text{g/day}$).

(b) (4) was negative in the Ames test and the *in vivo* chromosomal aberration test (b) (4)

In a 13-week toxicity study in rats, (b) (4) was administered orally through drinking water at doses of 0, 256, 479 or 861 mg/kg/day in males and 0, 440, 754 or 1754 mg/kg/day in females, respectively. The NOAEL was (b) (4) mg/kg/day for males and (b) (4) mg/kg/day for females (b) (4). Based on the NOAEL of male rats, the PDE was calculated to be (b) (4) $\mu\text{g/day}$ (details of calculation are shown below). The maximum potential exposure to (b) (4) from Clinolipid 20% is (b) (4) than the calculated PDE value (b) (4) $\mu\text{g/day}$). Therefore, there is no safety concern for the potential leaching of (b) (4) in Clinolipid 20%.

PDE for (b) (4) = (b) (4) mg/kg/day = (b) (4) $\mu\text{g/kg/day}$; (b) (4) $\mu\text{g/kg/day} \times$ (b) (4) kg (assumed body weight) = (b) (4) $\mu\text{g/day}$

(b) (4)

F1 = 5: for extrapolation from rats to human

F2 = 10: for variability between individuals

F3 = 10: for shorter study duration

F4 = Omitted due to lack of severe toxicity (e.g. nongenotoxic)

F5 = 1: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

(b) (4)

The (b) (4) is associated with the (b) (4) layer of the ClinOleic bag. Thus it could leach into the solution due to its direct contact with lipid. In the leaching study, the maximum level of (b) (4) in aqueous solution was (b) (4)/ml (data provided by the Applicant on June 11, 2013). Thus, the maximum exposure to (b) (4) would be (b) (4) µg/day based on the maximum daily administration volume of (b) (4) ml Clinolipid 20% ((b) (4) µg/day).

(b) (4) was negative in the Ames test, mouse micronucleus test, mouse lymphoma assay using L5178Y TK^{+/−}, *in vivo* dominant lethal assay in male and female mice and an *in vivo* Chromosomal aberration assay (EPA, 40 CFR Part 180).

In a 3-month toxicity study in rats, (b) (4) was administered orally to male and female rats at doses of 0, 25, 125, 250 and 500 mg/kg 5 days/week. The NOAEL was (b) (4) mg/kg, and from the NOAEL, the PDE was calculated to be (b) (4) µg/day (details of calculation are shown below). The maximum potential exposure to (b) (4) from Clinolipid 20% is (b) (4)-fold lower than the calculated PDE value ((b) (4) µg/day). Therefore, there is a reasonable assurance of safety for the potential leaching of (b) (4) in Clinolipid 20%.

PDE for (b) (4) = (b) (4) mg/kg/day ÷ (b) (4) mg/kg/day = (b) (4) µg/kg/day; (b) (4) µg/kg/day × (b) (4) kg (assumed body weight) = (b) (4) µg/day

(b) (4)

F1 = 5: for extrapolation from rats to human

F2 = 10: for variability between individuals

F3 = 10: for shorter study duration

F4 = Omitted due to lack of severe toxicity (e.g. nongenotoxic)

F5 = 1: A variable factor that may be applied if the NOEL was not established

10: safety factor for oral to intravenous conversion

Conclusion: All potential extractables and leachables from the ClinOleic 20% container closure system are within the recommended safety limit and appear to be acceptable.

11 Integrated Summary and Safety Evaluation

In the current submission, the applicant is seeking approval of ClinOleic 20% (the nomenclature of ClinOleic has been changed to Clinolipid; emulsion of refined olive oil and refined soybean oil) for parenteral nutrition, when oral or enteral nutrition is not

possible, insufficient, or contraindicated. ClinOleic is an intravenous lipid emulsion formulation containing refined olive oil and refined soybean oil in an approximate ratio of 4:1 (olive : soy). The recommended dose of ClinOleic for adult patients is 1 to 1.5 g/kg/day (equivalent to 5 to ^(b)₍₄₎ mL/kg/day). The daily dose should not exceed 2.5 g/kg/day. (b) (4)



ClinOleic is a combination of olive oil and soybean oil which has a capacity to preserve the antioxidant status by contributing fewer polyunsaturated fatty acids. *In vitro* studies indicated that the hemolytic potential of ClinOleic was significantly lower than that of the soybean oil based comparator product, Intralipid®. The distribution, metabolism and elimination of ClinOleic in animals (rats and dogs) are very similar to that of soybean oil-based emulsions such as Intralipid® and Ivelip®. ClinOleic is currently approved as a parenteral nutrition in European countries and in Canada.

This NDA is submitted under section 505(b)(2) of the Federal Food, Drug and Cosmetic Act. The applicant provided pharmacology, pharmacokinetics, single-dose IV toxicity studies in mice and rats, and repeated-dose IV toxicity studies of up to 90 days duration in rats and dogs. The applicant also provided an assessment of the safety profiles of the container closure system for the product.

ClinOleic was tolerated in toxicity studies conducted in rats and dogs for up to three months, and was generally comparable to that of the reference emulsions containing soybean oil alone. The major signs of toxicity were: slight hemolytic anemia, transitory thrombocytopenia, hypercholesterolemia and hepatic pathology of lipid and pigmentary overload. At a dose of 15 mL/kg/day in dogs, only very slight lipid and pigmentary overload of the liver were noticed. In rats, the dose of 30 mL/kg/day was well tolerated but in the dogs, the same dose was associated with adverse effects (pigmentation and vacuolation in the liver). The dose of 15 mL/kg/day may be considered the highest dose without significant adverse events in dogs. The hepatic adverse effects observed in dogs with ClinOleic were comparable with other reference emulsions like Intralipid®, Ivelip® and Endolipide®. However, blood biochemistry parameters (especially transaminases and alkaline phosphatases) tended to increase more in the ClinOleic group than in the comparator group (Intralipid®) in rats. In a 30-day repeated dose toxicity study in dogs, 4 dogs receiving Intralipid® were terminated before the end of the study (days 23-29) due to anorexia and deteriorating general condition accompanied by icterus, hypertriglyceridemia, hyperbilirubinemia, and increased bile acid concentrations. Food consumption decreased in Intralipid® treated dogs beginning week 2 and remained low at the end of the study. However, these effects were not observed in the ClinOleic treated dogs. In the 90-day repeated dose toxicity study in dogs, one female in the Intralipid® group was terminated on day 63 due to excessive weight loss and deteriorating general condition. These effects were not observed in the dogs in the ClinOleic treated group. Pharmacological studies showed that the hemolytic potential of

ClinOleic was significantly lower than Intralipid®. ClinOleic was also shown to modulate biliary secretion with reduced biliary phospholipids and cholesterol compared to Intralipid®.

Studies were conducted to determine if there is a potential safety concern for the extractables and leachables released in the product from the container closure system. Target extractables from the (b) (4) components of the container closure system were assessed in the lipid extracts. These studies indicated that all potential extractables and leachables from the ClinOleic 20% container closure system are within the recommended safety limit and appear to be acceptable.

- In conclusion, the findings of the submitted nonclinical studies indicated that overall, the toxicity profile of ClinOleic was better or comparable to that of its comparators containing soybean oil only (Intralipid®, Ivelip® or Endolipide®). In repeated dose toxicity studies in rats and dogs of up to 90 days, ClinOleic showed no overt clinical findings and serious toxicity and was comparable to or better than other soybean-based lipid emulsions.

Thus, there are no significant safety concerns for the proposed doses of ClinOleic in the proposed patient population.

12 Appendix/Attachments: None

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/s/

DINESH C GAUTAM
08/08/2013

SUSHANTA K CHAKDER
08/08/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204-508

Applicant: Baxter Healthcare Corp.

Stamp Date: January 13, 2013

Drug Name: ClinOlic 20%

NDA/BLA Type: 505(b)2

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?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
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)?	Yes		
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 <u>submitted</u> a rationale to justify the alternative route?	Yes		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	Yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	Yes		

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?	Yes		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	Yes		
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.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Dinesh Gautam, Ph.D.

Reviewing Pharmacologist

Date

Sushanta Chakder, Ph.D.

Team Leader/Supervisor

Date

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

/s/

DINESH C GAUTAM
01/30/2013

SUSHANTA K CHAKDER
01/30/2013