

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

200656Orig1s000

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/BLA REVIEW AND EVALUATION

Application number: NDA 200656
Supporting document/s: 030
Applicant's letter date: January 31, 2014
CDER stamp date: January 31, 2014
Product: Kabiven and Perikabiven (b) (4)
Indication: Kabiven is a source of calories, protein and essential fatty acids for patients requiring parenteral nutrition, (b) (4) prevention of essential fatty acid deficiency (b) (4) or treatment of negative nitrogen balance.
Applicant: Fresenius Kabi USA, LLC
Review Division: Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewer: B. Emmanuel Akinshola, Ph.D.
Supervisor/Team Leader: Sushanta K. Chakder, Ph.D.
Division Director: Donna Griebel, M.D.
Project Manager: Matthew Brancazio, Pharm.D.

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1 Executive Summary

1.1 Introduction

Kabiven and (b) (4) are proposed to be used as sources of calories, protein and essential fatty acids for patients requiring parenteral nutrition (b) (4) prevention of essential fatty acid deficiency, (b) (4) or treatment of negative nitrogen balance. The Applicant submitted the NDA 200656 for Kabiven and Perikabiven on January 28, 2011. A Complete Response (CR) letter was issued due to Clinical, Product Quality, Device Performance, Usability and other safety issues. In subsequent communications, the Applicant was told that in addition to the deficiencies listed in the CR letter, the Applicant needs to submit a safety assessment of the extractables and leachables from the 3-CB (b) (4) packaging system in the resubmission. On January 31, 2014, the Applicant submitted (SEQ # 0024) information on the safety assessment of leachables and extractables for the 3-CB (b) (4) packaging system. Additionally, on February 20, 2014, the Applicant submitted (SEQ-0029) a safety assessment of two migrants, (b) (4) and (b) (4) from the (b) (4) 3-CB packaging system for Kabiven/Perikabiven.

1.2 Brief Discussion of Nonclinical Findings

In the current submission, the Applicant has submitted a complete safety assessment of leachables/extractables from the 3-CB (b) (4) packaging system. Safety assessment of the potential leachables from the 3-CB packaging system was performed following (b) (4) and storage of the 3-CB (b) (4) bags with components of the drug product (lipid, glucose and amino acid), or treatments with comparable solvents, such as saline, water or 13 % ethanol stored in the (b) (4) container for extended period at different temperatures after (b) (4) to allow leaching of the migrants from the container into the liquids. The levels of leachables and extractables and their degradation products were measured, analyzed, and an assessment of the safety was performed. For some components, for which the levels were not detectable because of insolubility or for some other reason, the safety assessment of that component was performed based on the assumption that 100% of it will be released into the IV fluid.

The calculated maximum potential exposure to the leachables/migrants under the worst case scenario was lower than or at least, equal to the PDE (permitted daily exposure) calculated from the available nonclinical data for each component of the (b) (4) 3-CB packaging system. There is a clear separation between the estimated maximum patient exposure to the migrants (or their degradation products) and the exposures considered to be safe based on animal data. Thus, there are no safety concerns for the potential leachables/extractables from the (b) (4) 3-CB packaging system into Kabiven/Perikabiven IV fluids.

1.3 Recommendations

1.3.1 Approvability

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

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

8.1 Pregnancy

Proposed Version:

Pregnancy Category C. Animal reproduction studies have not been conducted with (b) (4). It is not known whether Kabiven (b) (4) can cause fetal harm when administered to a pregnant woman (b) (4). Kabiven (b) (4) should be given to a pregnant woman only if clearly needed.

Evaluation: The text should be modified as proposed below.

Animal reproduction studies have not been conducted with Kabiven (b) (4). It is not known whether Kabiven (b) (4) can cause fetal harm when administered to pregnant women. Kabiven (b) (4) should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers

Proposed Version

Caution should be exercised when Kabiven (b) (4) is administered to a nursing woman.

Evaluation: This section should be modified as recommended below.

Recommended Version

It is not known whether any component of Kabiven (b) (4) is excreted in human milk. Caution should be exercised when Kabiven (b) (4) is administered to a nursing woman.

13 NONCLINICAL TOXICOLOGY

Proposed Version

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Evaluation: The text is not in accordance with 21CFR 201.57(f)(6)(i)(c), and should be modified as proposed below.

Recommended Version

Long-term animal studies have not been conducted to evaluate the carcinogenic potential of Kabiven (b) (4), or its effects on fertility. Genotoxicity studies have not been conducted with Kabiven (b) (4) to assess its mutagenic potential.

2 Drug Information

2.1 Drug

Kabiven and (b) (4).

2.1.1 CAS Registry Number

N/A

2.1.2 Generic Name

N/A

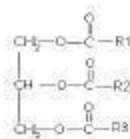
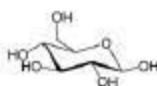
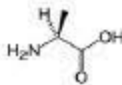
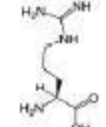
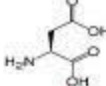
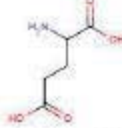
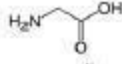
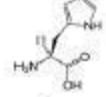
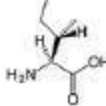
2.1.3 Code Name

N/A

2.1.4 Chemical Name

N/A

2.1.5 Molecular Formula/Structure

Name	Molecular Formula	Structure
<i>Lipids</i>		
Soybean oil	Triacylglycerol with fatty acid chains mainly C16:0, C18:1, C18:2	 R=Fatty Acid Chains
<i>Carbohydrate</i>		
Glucose monohydrate (Dextrose)	$C_6H_{12}O_2 \cdot H_2O$	
<i>Amino Acids</i>		
L-Alanine	$C_3H_7NO_2$	
L-Arginine	$C_6H_{14}N_4O_2$	
L-Aspartic acid	$C_4H_6NO_4$	
L-Glutamic acid	$C_5H_8NO_4$	
Glycine	$C_2H_5NO_2$	
L-Histidine	$C_6H_9N_3O_2$	
L-Isoleucine	$C_6H_{13}NO_2$	

Name	Molecular Formula	Structure
L-Leucine	$C_6H_{13}NO_2$	
L-Lysine hydrochloride	$C_6H_{14}N_2O_2 \cdot HCl$	
L-Methionine	$C_5H_{11}NO_2S$	
L-Phenylalanine	$C_9H_9NO_2$	
L-Proline	$C_5H_9NO_2$	
L-Serine	$C_3H_7NO_3$	
L-Threonine	$C_4H_9NO_3$	
L-Tryptophan	$C_{11}H_{12}N_2O_2$	
L-Tyrosine	$C_9H_9NO_3$	
L-Valine	$C_5H_{11}NO_2$	
<i>Electrolytes</i>		
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	-
Sodium glycerophosphate	$C_3H_5(OH)_2PO_4Na_2 \cdot xH_2O$	-
Magnesium (b) (4) heptahydrate	$MgSO_4 \cdot 7H_2O$	-
Potassium chloride	KCl	-
Sodium acetate trihydrate	$CH_3COONa \cdot 3H_2O$	-

2.1.7 Pharmacologic class

Total Parenteral Nutrition (TPN)

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

NDA 018449, NDA 020248 (Intralipid 20 %; Fresenius)

NDA 020678 (Clinimix E Sulfite free with electrolytes in Dextrose with calcium; Baxter Healthcare),

NDA 019683 (Aminosyn II with electrolytes in dextrose injection with calcium; Hospira),

NDA 017957 (Novamine 11.4 % injection; Hospira)

2.2 Clinical Formulation

Kabiven and PeriKabiven TPN formulations are packaged in a 3 chamber bag in which the lipid, amino acids and dextrose components are separated from one another. Prior to administration, the three components are to be mixed by rupturing the separating seals. The composition of each component is shown in the Applicant's Table below.

(b) (4)

(b) (4)

(b) (4)

2.3.1 Drug formulation.

Kabiven is presented as four packaging sizes, 1026 ml, 1540 ml, 2053 ml, and 2556 ml in a 3 chamber bag consisting of lipid (b) (4) %, amino acids (b) (4) %, and glucose (dextrose) (b) (4) %. (b) (4) for administration via peripheral veins is available in 3 packaging sizes, 1440 ml, 1920 ml, and 2400 ml in a 3 chamber bag consisting of lipid (b) (4) %, amino acids (b) (4) %, and glucose (dextrose) (b) (4) %.

2.3.2 Comments on Novel Excipients

There are no novel excipients in the Kabiven drug products

Safety Evaluation of the Leachable and Extractable Migrants from the (b) (4) Container 3-CB System.

The submissions include the qualitative composition of the (b) (4) 3-CB packaging system, extraction and migration studies, and the toxicological qualification of each extractable and migrant based on the anticipated maximum daily exposure to the drug product. The (b) (4) 3-(b) (4) container closure system includes a plastic bag consisting of (b) (4), three fill-ports, and pre-assembled caps and stoppers designed to close the filling ports. These three components ((b) (4), the fill-port and the stopper or septum) of the container closure system are in direct contact with the drug product. All components of the (b) (4) 3-CB packaging system are listed in the Applicant's table below.

Components of the (b) (4) Packaging System

Component	Packaging material in direct contact with product (Yes/No)
(b) (4)	Yes
Fill-port	Yes
Cap	No
Stopper (also called septum)	Yes
(b) (4)	No
(b) (4)	No
(b) (4)	No
Oxygen absorber	No

The (b) (4) (b) (4) The Applicant stated that all the additives and (b) (4) included in the (b) (4) are acceptable according to the European Pharmacopeia monograph 3.1.6. The estimated maximum daily exposure and the permitted daily exposure (PDE) values for the potential migrants of the (b) (4), the fill-port, stopper,

printing ink ((b) (4)), (b) (4) are shown in the
Applicant's table below.

Migrant	Estim. Exp. (µg/kg/d)	PDE (µg/kg/d)	TDI /ADI*** (µg/kg/d)	Comment
Components of the	(b) (4) fill-ports and caps			

Patient exposure to migrants is influenced by the extent of exposure of the container material with the administered volume. Since the largest amount of container material in contact with 1 ml of infusion solution comes from the container with the smallest volume, the 1026 ml container for Kabiven was chosen as the model product to represent the worst-case exposure. The weight of the (b) (4) g, and the weights of the two different sizes of stoppers used are (b) (4) g and (b) (4) g, respectively. The weight of the (b) (4) placed on the surface of the primary 3-CB container is approximately (b) (4) g. The maximum recommended dose used in estimating patient exposure to migrants is 40 ml/kg/day. This value has been used in estimating the exposures/worst case exposures to migrants not analyzed, migrants analyzed but not detected, and migrants analyzed and detected.

Migrants analyzed, detected or not detected:

Worst case exposure = dose (40 ml/kg) x concentration in infusion solution or limit of detection (LOD) in mg/ml

For the target leachables, the migration and hence patient exposure of several substances/degradation products/impurities of the 3-CB (b) (4) packaging system has been estimated from the migration into solutions (b) (4) and stored for 24 months prior to their determination. Wherever possible, the safety of patient exposures to migrants were assessed based on human experience, or animal experimentation using the no observed effect level (NOEL) or the no observed adverse effect level (NOAEL) to calculate a safe permitted daily exposure (PDE) level. The PDE is calculated according to a generally used procedure in the ICH guidance Q3C(R4):

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

F1: A factor to account for extrapolation between species

F2: A factor 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. genotoxicity, 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

Safety evaluation of components of the (b) (4), fill-ports and caps**Summary of Extraction and Migration Studies ((b) (4))****3.2.P.7.5- 1. Migrants from (b) (4) / fill ports /caps**

Migrant	Source of migrant	Extraction/ migration performed	Analytical results	Analytical method	Analytical method validation	Comment and/or theoretical evaluation
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(b) (4)

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/s/

BABATUNDE E AKINSHOLA
05/13/2014

SUSHANTA K CHAKDER
05/13/2014

**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: NDA 200-656

Supporting document/s: 01

Applicant's letter date: January 28, 2011

CDER stamp date: January 28, 2011

Product: Kabiven/ (b) (4)
(b) (4)

Indication: Kabiven is a source of calories, protein and essential fatty acids for patients requiring parenteral nutrition, (b) (4) prevention of essential fatty acid deficiency (b) (4)
(b) (4) or treatment of negative nitrogen balance.

Applicant: APP Pharmaceuticals, LLC

Review Division: Division of Gastroenterology and Inborn Errors Products (DGIEP)

Reviewer: B. Emmanuel Akinshola, Ph.D.

Supervisor/Team Leader: Sushanta K. Chakder, Ph.D.

Division Director: Donna Griebel, M.D.

Project Manager: Frances G. Fahnbulleh, Pharm. D.

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as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that APP Pharmaceuticals does not own or from FDA reviews or summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 200,656

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1 Executive Summary

1.1 Recommendations

The applicant has provided adequate nonclinical information in support of the safety of sodium glycerophosphate, an NME used in Kabiven products. Other ingredients are present in currently approved TPN products.

1.1.1 Approvability

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

1.1.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended.

1.1.3 Labeling

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

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Proposed Version:

Pregnancy Category C. Animal reproduction studies have not been conducted with (b) (4)

It is not known whether Kabiven (b) (4) can cause fetal harm when administered to a pregnant woman (b) (4) Kabiven (b) (4) should be given to a pregnant woman only if clearly needed.

Evaluation: The text is not in accordance with 21CFR 201.57(f)(6)(i)(c), and should be modified as proposed below.

8.1 Pregnancy

Recommended Version:

Animal reproduction studies have not been conducted with Kabiven (b) (4). It is not known whether Kabiven (b) (4) can cause fetal harm when administered to pregnant women or can affect reproduction capacity in women. Kabiven (b) (4) should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers

Proposed Version

Caution should be exercised when Kabiven (b) (4) is administered to a nursing woman.

Evaluation: This section should be modified as recommended below.

Recommended Version

It is not known whether any component of Kabiven (b) (4) is excreted in human milk. Caution should be exercised when Kabiven (b) (4) is administered to a nursing woman.

13 NONCLINICAL TOXICOLOGY

Proposed Version

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Evaluation: The text is not in accordance with 21CFR 201.57(f)(6)(i)(c), and should be modified as proposed below.

Recommended Version

Long-term animal studies have not been conducted to evaluate the carcinogenic potential of Kabiven (b) (4) or its effects on fertility. Genotoxicity studies have not been conducted with Kabiven (b) (4) to assess its genotoxic potential.

1.2 Brief Discussion of Nonclinical Findings

The applicant has provided adequate nonclinical pharmacology and toxicology information in support of the safety of Kabiven and (b) (4).

The nonclinical studies submitted are for individual lipid, amino acid, and sodium glycerophosphate constituents of the macronutrient product. In the nonclinical studies, with the lipid and amino acid components, they were well-tolerated at the maximum doses administered to the study animals, both rodents and non-rodents, with slight and reversible adverse effects. The safety of sodium glycerophosphate, a new molecular entity, has been established in several repeat dose toxicity studies (IV and oral) and genotoxicity studies, and there are no safety concerns for this compound.

Repeat-dose IV toxicity studies have been conducted with the lipid and amino acid constituents of Kabiven (Intralipid 20 %, (b) (4)) for up to 3 months. Administration of the lipid and amino acid components at doses up to the maximum tolerated dose was tolerated well in these studies. Following administration of the lipid component, changes in lipid load in some organs, such as fine lipid droplets in the liver, kidneys and spleen, with acidosis and inflammation were transient and reversible. Following repeated administration of the amino acid component to dogs at the maximum tolerated dose, changes in blood cell parameters were observed, which were reversible during the recovery period. Other changes observed at the hyperosmolar concentration were hyperemia in the liver, kidney and lung of male rats, and the finding might be related to the osmolarity of the solution.

Several single and repeat dose toxicity studies in rodents and non-rodents were submitted in support of the safety of sodium glycerophosphate. In single dose IV toxicity studies in rats, the LD₅₀s for α - and β -glycerophosphates were very high (3800 and 3400 mg/kg, respectively), suggesting very low toxicological profiles. In intravenous toxicity studies with sodium glycerophosphate in rats and dogs for up to 4 weeks at doses up to 13 times the proposed clinical dose, it was tolerated well without any target organ toxicity. In addition, dietary administration of sodium glycerophosphate at very high doses for 33 months in rats, and drinking water administration for 15 months in primates (squirrel monkeys) was not associated with any remarkable toxicity. Sodium glycerophosphate was not genotoxic in a standard battery of genotoxicity assays. Thus, from the nonclinical toxicity studies, there are no significant safety concerns for sodium glycerophosphate at the proposed dose.

The safety assessment of the three chamber bag packaging system appears to be adequate and acceptable, and does not raise any safety concerns.

2 Drug Information

2.1 Drug

Kabiven and (b) (4)

2.1.1 CAS Registry Number

N/A

2.1.2 Generic Name

N/A

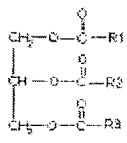
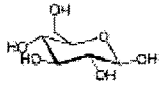
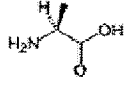
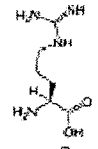
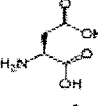
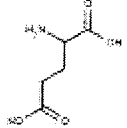
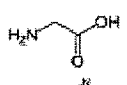
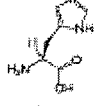
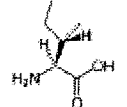
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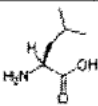
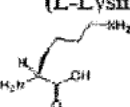
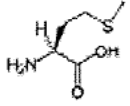
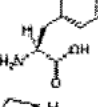
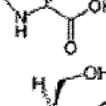
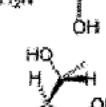
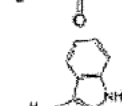
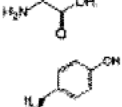
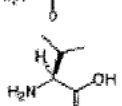
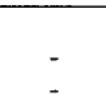
N/A

2.1.4 Chemical Name

Amino Acids	(b) (4) 2-Aminopropanoic acid 2-amino-5-guanidinopentanoic acid 2-aminobutanedioic acid 2-aminopentanedioic acid 2-Aminoacetic acid (b) (4) (b) (4) 2-amino-3-phenylpropanoic acid pyrrolidine-2-carboxylic acid
	(b) (4) 2-amino-3-hydroxypropanoic acid (b) (4) 2-amino-3-hydroxybutanoic acid (b) (4) (b) (4) 2-amino-3-(4-hydroxyphenyl)propanoic acid 2-amino-3-methylbutanoic acid
Electrolytes	Calcium chloride dihydrate Magnesium (b) (4) heptahydrate Potassium chloride (b) (4)
Soybean Oil	Soybean oil
Glucose Monohydrate	(b) (4)

2.1.5 Molecular Formula/Structure

Name	Molecular Formula	Structure
<i>Lipids</i>		
Soybean oil	Triacylglycerol with fatty acid chains mainly C16:0, C18:1, C18:2	 R=Fatty Acid Chains
<i>Carbohydrate</i>		
Glucose monohydrate (Dextrose)	$C_6H_{12}O_2 \cdot H_2O$	
<i>Amino Acids</i>		
L-Alanine	$C_3H_7NO_2$	
L-Arginine	$C_6H_{14}N_4O_2$	
L-Aspartic acid	$C_4H_7NO_4$	
L-Glutamic acid	$C_5H_9NO_4$	
Glycine	$C_2H_5NO_2$	
L-Histidine	$C_6H_9N_3O_2$	
L-Isoleucine	$C_6H_{13}NO_2$	

Name	Molecular Formula	Structure
L-Leucine	$C_6H_{13}NO_2$	
L-Lysine hydrochloride	$C_6H_{14}N_2O_2 \cdot HCl$	
L-Methionine	$C_5H_{11}NO_2S$	
L-Phenylalanine	$C_9H_9NO_2$	
L-Proline	$C_5H_9NO_2$	
L-Serine	$C_3H_7NO_3$	
L-Threonine	$C_4H_9NO_3$	
L-Tryptophan	$C_{11}H_{12}N_2O_2$	
L-Tyrosine	$C_9H_9NO_3$	
L-Valine	$C_5H_{11}NO_2$	
<i>Electrolytes</i>		
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	-
Sodium glycerophosphate	$C_3H_5(OH)_2PO_4Na \cdot xH_2O$	-
Magnesium ^{(b) (4)} heptahydrate	$MgSO_4 \cdot 7H_2O$	-
Potassium chloride	KCl	-
Sodium acetate trihydrate	$CH_3COONa \cdot 3H_2O$	-

2.1.7 Pharmacologic class

Total Parenteral Nutrition (TPN)

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

NDA 018449, NDA 020248 (Intralipid 20 %; Fresenius), NDA 020678 (Clinimix E Sulfite free with electrolytes in Dextrose with calcium; Baxter Healthcare), NDA 019683 (Aminosyn II with electrolytes in dextrose injection with calcium; Hospira), NDA 017957 (Novamine 11.4 % injection; Hospira)

2.2 Clinical Formulation

	Kabiven	(b) (4)
Pack sizes (ml)	2566 2053 1540 1026	2400 1920 1440
Composition		
Individual Chamber (per 1000 ml)		
(b) (4)		
Combined mixture (mg/ml)		
Purified Soybean Oil	39	35
Glucose	98	68
Amino acids		
Alanine	4.67	3.33
Arginine	3.30	2.35
Aspartic Acid	0.99	0.71
Glutamic Acid	1.64	1.16
Glycine	2.31	1.64
Histidine	1.99	1.41
Isoleucine	1.64	1.16
Leucine	2.31	1.64
Lysine	2.63	1.87
Methionine	1.64	1.16
Phenylalanine	2.31	1.64
Proline	1.99	1.41
Serine	1.31	0.94
Threonine	1.64	1.16
Tryptophan	0.55	0.40
Tyrosine	0.067	0.048
Valine	2.13	1.52
Electrolytes		(b) (4)
(b) (4)		

N.A. Not applicable

2.3.1 Drug formulation.

The applicant is seeking marketing approval under the current NDA for Kabiven and (b) (4). Kabiven for administration via central veins has 4 packaging sizes, 1026 ml, 1540 ml, 2053 ml, and 2556 ml in a 3 chamber bag consisting of lipid 20 %, amino acids (b) (4) %, and glucose (dextrose) (b) (4) %. (b) (4) for administration via peripheral veins is available in 3 packaging sizes, 1440 ml, 1920 ml, and 2400 ml in a 3 chamber bag consisting of lipid 20 %, amino acids (b) (4) %, and glucose (dextrose) (b) (4) %.

2.3.2 Comments on Novel Excipients

There are no novel excipients in the Kabiven drug products

2.3.3 Comments on Impurities/Degradants of Concern

Impurities and degradants from glucose (b) (4), amino acids (b) (4), and soybean oil lipids (b) (4) that may be formed during (b) (4) and storage were tested during stability studies, and meet USP standards. Other impurities such as (b) (4) phytosterols present in the soybean oil lipid emulsion were also within the USP standard concentrations, and are qualified.

2.4 Proposed Clinical Population and Dosing Regimen

Kabiven and (b) (4) are proposed to be used as sources of calories, protein and essential fatty acids for patients requiring parenteral nutrition; and indicated for prevention of essential fatty acid deficiency, (b) (4) or treatment of negative nitrogen balance (b) (4).

Dosing is in the form of (b) (4) after mixing the contents of a 3 chamber bag. Proposed dosing regimen for Kabiven and (b) (4) is shown in the Sponsor's Table below.

	Kabiven	(b) (4)
Mode of Administration	Central Vein	Peripheral Vein
Adult Energy Requirement	Depends on clinical condition; most often (b) (4) kcal/kg body weight/day	Depends on clinical condition; most often 20 to 30kcal/kg body weight/day
Dosing	(b) (4)	
	(b) (4)	
	(b) (4)	
Infusion rate	Not more than 2.6 ml/kg body weight/hr (corresponding to 0.25 g glucose, 0.09 g amino acid and 0.1 g (b) (4) kg body weight) Recommended infusion period: 12 to 24 hours	Not more than 3.7 ml/kg body weight/hr (corresponding to 0.25 g glucose, 0.09 g amino acids, 0.13 g (b) (4) kg body weight) Recommended infusion period: 12 to 24 hours

2.5 Regulatory Background

At a pre-NDA type B meeting (July 20, 2009) between FDA and sponsor (APP pharmaceuticals), there was an agreement that Kabiven and (b) (4) products may be filed as a 505(b)(2) application, and that the nonclinical safety studies submitted for sodium glycerophosphate, the electrolyte component of kabiven, different from the reference listed drug (Intralipid) product are adequate.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology Studies

Primary pharmacodynamics:

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

Safety pharmacology:

1. (b) (4) Comparison with Intralipid 20 % (batch (b) (4)), (Experiment code: (b) (4)).
2. Investigation for (b) (4) (European pharmacopoeia): Study on (b) (4) % (Experiment code: (b) (4)).
3. Cardiovascular investigation of (b) (4) % in the anesthetized cat (**Study No.** (b) (4)).
4. Cardiovascular investigation of fat emulsion 73403 (a mixture of long and medium chain fatty acid) in anesthetized cat (**Study No. 89-9**)

Pharmacokinetic Studies (ADME)

1. The suitability of DL-glycerol-3-phosphate for parenteral substitution of inorganic phosphate (Bässler and Hassinger, Infusion therapy 3: 138-142 (1976). Published study
2. Plasma elimination kinetics of (b) (4) comparison with Intralipid 20 % (fat emulsion) (Experiment code (b) (4)).
3. Elimination test from blood serum of intravenous infusion of 30 % fat emulsion 4315 (**Study No. 84-7**)
4. Elimination test from blood serum of intravenous infusion of fat emulsion 4501 20 % (a mixture of long and medium chain fatty acids) (**Study No. 86-6**)
- 5 Elimination test from blood serum of intravenous infusion of intralipid 10 % (SSPC) (**Study No. 88-9**)
6. Elimination from blood after intravenous infusion of fat emulsion 73403, 20 % (**Study No. 89-5**)
7. Half-lives of intralipid particles in mammals (Document No. **82 99 026**)
8. Estimation of the elimination rate of fat particles from the blood stream in rabbits after injection of intralipid 20 %

ToxicologySingle dose:

1. Acute toxicity via the intravenous route in the male and female mouse: Determination of the LD₅₀; comparison between (b) (4) % - (b) (4) (Experiment codes: (b) (4))
2. Acute toxicity in mice after single intravenous administration of sodium glycerophosphate solution (**Study No. 90-2**)
3. Acute toxicity in mice and rats after single intravenous administration of (b) (4) % (**Study** (b) (4))
4. Estimation of acute toxicity in mice of amino acid solution AA 4964
5. Estimation of acute toxicity (LD₅₀) of intralipid 20 %
6. Acute toxicity via the intravenous route in the male and female rat: Determination of the LD₅₀; study on (b) (4) % (Experiment codes: (b) (4))

7. Acute and subacute toxicity of intravenously administered α - and β -sodium glycerophosphate in rats and dogs.

Repeat Dose Toxicology Studies

1. Repeated dose toxicity of (b) (4) % via the intravenous route in the Beagle dog (30 days) (Experiment code: (b) (4))
2. 28-Day toxicity of (b) (4) infusion solution ((b) (4) %), Lot No. KI 145 and Intralipid 20 % vitrium (preparation for comparison), Lot No. (b) (4) – called for short (b) (4) % and Intralipid 20 % - by intravenous infusion in Beagle dogs.
3. A tolerance study in dogs of the amino acid solution 4203, containing 13.5 G nitrogen (N)/L and electrolytes, given intravenously for 28 days (**Study No. 82-5**)
4. A tolerance study in dogs of L-Carnitine solution (200 mg/ml), added to Intralipid 20 %, with intravenous administration for 28 days (**Study No. 84-10**)
5. A tolerance study in dogs of fat emulsion 4501, containing a purified mixture of soybean and borage oils, with intravenous administration for 28 days (**Study No. 86-2**)
6. A tolerance study in dogs of fat emulsion 73403 administered intravenously for one month (**Study No. 88-20**)
7. A tolerance study in dogs of (b) (4) % administered intravenously for one month (**Study** (b) (4))
8. 4-Week subchronic toxicity study of SMOF 20 % (a mixture of long and medium chain triglycerides and soybean oil) by daily 6-hr intravenous infusion in beagle dogs (Report No. **9358 1 95**)
9. Repeated dose toxicity of (b) (4) % via the intravenous route in the rat (30 days): Comparison with (b) (4) % (Experiment code: (b) (4))
10. Utilization and tolerance of new amino acid solutions with high nitrogen content (4959 and 4964) compared to the commercial high nitrogen amino acid solution Azonutril: TPN studies in the rat (Document No. **8398016**)
11. Tolerance and nutritional studies of AA 4959 (STARK) solution (Document No. **8399005**)
12. A tolerance study in rats of a new fat emulsion 4501 infused intravenously during 28 days (**Study No. 86-1**)
13. Safety and long-term tolerance of (b) (4) % (b) (4) in the rat after intravenous administration for 28 days (**Study** (b) (4))
14. An intravenous tolerance study in rats of fat emulsion 73403 administered at a high dose during 27 days (**Study No. 90-12**)
15. Effects of chronic β -glycerophosphate administration on growth rate and on serum, liver and bile lipid composition in the squirrel monkey- A toxicity study (Raheja et al., **Toxicology, 11:1-4, 1978**)
16. (b) (4): 13-Week intravenous (12-hr infusion) toxicity study in the beagle dog followed by a 4 week treatment-free period (**Study No.** (b) (4))
17. Estimation of long term toxicity in dogs of two amino acid solutions with high contents of nitrogen (Document No. **80 99 115**)
18. A three months intravenous tolerance study in dogs of fat emulsion 4501 containing a purified mixture of soybean and borage oils. (**Study No. 87-2**)

19. Fat emulsion 73403: 13-Week intravenous toxicity study in the beagle dog followed by a four-week recovery period (**Study No. 90-25**)
20. Effects of chronic β -glycerophosphate administration on growth rate and on serum, liver and bile lipid composition in the rat- A toxicity study (**Toxicology, 8:115-119, 1977**)
21. A two weeks intravenous tolerance study in the rat of fat emulsion 73403 (**Study No. 89-11**)
22. A tolerance study in dogs of fat emulsion 73403 administered intravenously for eight days (**Study No. 90-6**)

Genetic Toxicology Studies

1. Mutagenicity study of SMOF 20 % in the Salmonella Typhimurium reverse mutation assay *in vitro* (Report No. **935/95**)
2. Mutagenicity study of SMOF 20 % in mammalian cells (V79) in the *in vitro* gene mutation assay (HPRT Test) (Report No. **10554/97**)
3. *In Vitro* assessment of the clastogenic activity of SMOF 20 % in cultured human peripheral lymphocytes (Report No. **10555/97**)
4. Salmonella Typhimurium and Escherichia Coli reverse mutation assay according to the treat and plate method (**Study No. 1128801**)
5. Salmonella Typhimurium and Escherichia Coli reverse mutation assay with Glycophos (Study No. **1225401**)
6. Cell mutation assay at the Thymidine Kinase locus (TK +/-) in mouse lymphoma L5178Y cells with Neoven (**Study No. 1128802**)
7. Cell mutation assay at the Thymidine Kinase locus (TK +/-) in mouse lymphoma L5178Y cells with Glycophos (sodium glycerophosphate) (**Study No. 1225402**)
8. Micronucleus assay in bone marrow cells of the mouse with Glycophos (**Study No. 1225403**)
9. *In Vivo* Bone marrow cytogenetic test of SMOF 20 % by intravenous administration to Sprague-Dawley rats (chromosomal analysis) (**Report No. 10556/97**)

Reproductive and Developmental Toxicology Studies

1. DP-Gln (dipeptide, glycyl- glutamine) intravenous teratogenicity study in the rabbit (Report No. **1051-1021-002**)
2. Fat emulsion 73403: Teratology study in the rabbit (Report No. **94/0212**)

Local Tolerance Studies

1. Local tolerance test of (b) (4) in rabbits after a single intravenous, intra-arterial, Para venous, intramuscular and subcutaneous administration (Report No. (b) (4))
2. Local tolerance test of (b) (4) in rabbits after a single intravenous, intra-arterial, Para venous, intramuscular and subcutaneous administration (Report No. **23837**)

Special Toxicology Studies

1. Functional activity of the reticuloendothelial system of the rat after total parenteral nutrition with intralipid or fat emulsion 73403 (Document No. **92 96 104**)
2. The effect of infusions of intralipid 20 % and fat emulsion 73403 on reticuloendothelial function in the rat (Document No. **92 96 106**)
3. Toxicological Evaluation of the 3-CB (Chamber Bag) (b) (4) Packaging System

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology**4.1 Primary Pharmacology****Fifteen-day infusion of fish oil supplemented emulsions in dogs**

The study objective was to determine the effects of 4 different lipid emulsions (a long chain triglyceride (LCT) soy emulsion, a mixed 50 % medium chain triglyceride (MCT)/50 % LCT emulsion, an LCT soy emulsion supplemented with 10 % fish oil triglycerides, and a mixed 50 % MCT/50 % LCT supplemented with 10 % fish oil triglycerides), on the fatty acid (FA) composition of dog tissues, when infused daily over 15 days as substantial components of total parenteral nutrition.

Sixteen healthy beagle dogs (mean body weight of 14.7 ± 1.1 kg) randomly divided into 4 groups, with each group assigned to one of 4 different lipid emulsion (TPN transfusion groups). Two weeks before the start of TPN, the dogs were surgically implanted with a jugular vein catheter under anesthesia.

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

Admixture 1: 900 ml phospholipid-reduced 10% soy derived LCT emulsion
 + 100 ml 10% LCT emulsion ((b) (4))

Admixture 2: 900 ml phospholipid-reduced 10% MCT/LCT emulsion
 +100 ml 10% MCT/LCT emulsion (Fresenius AG).

Admixture 3: 900 ml phospholipid-reduced 10% LCT emulsion
 +100 ml 10% fish oil emulsion (Omegavenoes, Fresenius AG).

Admixture 4: 900 ml phospholipid-reduced 10% MCT/LCT emulsion
 +100 ml 10% fish oil emulsion (Omegavenoes, Fresenius AG).

All the lipid preparations contained 100 g triglycerides and 6.6 g egg yolk phospholipids per liter of emulsion. The preparations were mixed in all-in-one 3 liter bags that were infused via implanted central catheter, with infusion rates controlled by volumetric pumps.

Fatty Acid Pattern of Emulsion Components

	TG (100g/l)				PL (6.6g/l)
	LCT	LCT + FO	MCT/LCT	MCT/LCT + FO	
C8:0			32.3	29.4	
C10:0			20.3	18.6	
Saturated	16.6	17.2	7.6	8.3	47.7
Monounsatur.	20.9	20.7	9.9	10.6	27.1
C18:2n-6	54	49.8	25.3	23.7	16.6
C20:4n-6		0.2		0.1	4.7
C18:3n-3	7	6.6	3.2	3.1	
C20:5n-3	0.1	1.8		2.1	
C22:6n-3		1.7		2.0	3.9

The results are expressed in weight %

Serum samples were obtained on days 1, 8, and 15, from which hepatic and renal function parameters were measured, and hematological status determined. Surgical biopsies from the liver, muscle (rectus abdominis), and adipose tissue (greater momentum) were obtained 2 weeks before the start of TPN infusion. In the morning following the last infusion, the second biopsy was performed, with the removal of the spleen.

Tissue biopsies were immediately frozen in liquid nitrogen, subsequently lyophilized and extracted in chloroform/methanol, prior to the determination of triglycerides (TG), phospholipids (PL) total free cholesterol in aliquot extract of tissues.

There were no mortalities and no clinical or laboratory test abnormalities observed in any of the 16 study animals.

A marked decrease in arachidonic acid level (from 23.5 ± 0.2 % to 16.6 ± 4.1 %) was observed after 2 weeks infusion with LCT emulsion, in contrast to the other 3 groups where this effect was less pronounced. A significant rise of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in erythrocyte phospholipids (PL) was observed in the 2 groups supplemented with fish oil.

Fatty Acid pattern of Erythrocyte Phospholipids in Dogs following TPN for 15 Days

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

Although liver triglycerides were low, initial individual variability (differences) were quite high in the initial samples, prior to TPN initiation, and became more homogenous in biopsies obtained from all animal groups after 2 weeks of TPN. The free cholesterol measured in initial samples became esterified by 25-40 % in all groups on day 16. Changes in liver triglyceride fatty acids (TGFA) pattern consists of increased linoleic in both LCT treated groups (1 and 3), unchanged linoleate in both MCT/LCT groups, and marked reduction in the MCT/LCT groups (2 and 4). EPA (C20:5n-3), and to a lesser extent DHA (C20:6n-3) were slightly incorporated into liver triglycerides fatty acids of group 3 and 4 animals at 3 % and 1.2 % respectively. The increase in n-3FA in liver phospholipid was associated with a significant decrease in 20:4n-6 (arachidonic acid), which was not observed in the non-supplemented groups. Medium chain fatty acids (MCFA) were also incorporated into liver triglycerides of both MCT/LCT groups (2 and 4) where they accounted for 10 % of TGFA.

There was no significant change in muscle TG, PL, TC, and FC in any group after 15 days of TPN administration. LCT treatment induced changes in TGFA pattern in muscle, consisting of increase in 18:2n-6 (linoleic), which was however suppressed in the LCT/MCT group. Similar to liver TG findings, MCFA were also found in muscle TGFA of dogs infused with LCT/MCT, but in contrast, EPA and DHA were not detected in muscle TG, even in fish-oil supplemented groups.

After 15 days of TPN, 18:2n-6 was unchanged in muscle PL in all groups, whereas 20:4n-6 decreased, particularly in the LCT group. EPA and DHA content however, increased significantly in muscle PL only in the fish oil treatment groups.

There were no differences in TG, PL, TC, and FC contents in the spleen of all the study animals. Fatty acid (FA) pattern in spleen TG was influenced by the composition of the infused emulsions, in that, dogs infused with LCT showed higher concentration of 18:2n-6 than the corresponding LCT/MCT groups. Dogs receiving MCT showed substantial amounts of MCFA (5-13 %), while n-3LCPUFA was present in animals receiving fish-oil supplementation. The only difference in FA pattern in spleen PL between the groups consisted of marked rise in EPA and DHA content in the fish-oil supplemented animals.

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

In summary, following a two week (15 day) total parenteral nutrition (TPN) in 4 groups of 4 dogs each infused with LCT, or LCT and 10 % fish oil, or LCT/MCT, or LCT/MCT and 10 % fish oil, there were no differences in safety or tolerance to the different TPN preparations. However, linoleate content of tissue triglycerides (TG) was markedly increased with the LCT emulsions, but reduced with LCT/MCT preparations.

Omega 3 fatty acids (n-3FA) were incorporated (slightly) into liver TG, but remained undetected in extra hepatic tissue TG, in contrast to medium chain fatty acid (FA), which was found in tissue TG after infusion of the mixed MCT/LCT emulsions.

Changes in tissue phospholipids (PL) involved increased linoleate in liver PL, but not in other tissues with infusion of long chain triglyceride (LCT) soy emulsion. MCT/LCT infusion did not markedly affect phospholipids fatty acid (PLFA) pattern in any tissue, but supplementation with fish-oil was associated with the incorporation of n-3FA into tissue PL, especially in the liver.

4.3 Safety Pharmacology

Action of (b) (4) % (Batch (b) (4)) on the Hemodynamics, Respiration, and Acid-Base Equilibrium of the Anesthetized Dog: Comparison with Intralipid 20 % Batch 71135-51 (Experiment code: (b) (4))

Six healthy female beagle dogs (mean weight of 11 kg) assigned (3 animals/group) to 1 of 2 groups (b) (4) % or Intralipid 20 %) were fasted for 16 hours and anesthetized IV with 30 mg/kg sodium pentobarbital, prior to dosing with 20 ml/kg (4 g (b) (4)/intralipid/kg) for 30 minutes. The animals were implanted with catheters for the recording of cardiovascular parameters of arterial blood pressure (systolic and diastolic), heart rate (HR), left intraventricular pressure (LIVP), isometric myocardial contractility (dp/dt), electrocardiogram, respiration tidal volume rate (TV), pH of arterial blood, PO₂-PCO₂ of arterial blood. Other vascular parameters calculated from the study

are, differential arterial pressure (DAP), mean arterial pressure (MAP), Ejection time (ET), tension-time index (ET•MAP•HR), total CO₂ (tCO₂), base excess (BE), oxygen saturation (O₂ sat), standard bicarbonate (HCO₃).

At the end of the 30 min infusion of (b) (4) % or intralipid 20 %, cardiovascular parameters such as the HR, ET, and the electrocardiogram, and the acid-base equilibrium all remained unchanged. The moderate MAP elevation seen in dogs was ascribed to the rapid infusion of the emulsions and was not considered biologically significant.

In two dogs infused with (b) (4) %, there was elevation of the LVP by 20 % and 24 % during the infusion, with a subsequent decrease by 9 % and 5 %, respectively after the 30 mins infusion period. Similarly, elevation of dp/dt occurred in 3 animals receiving (b) (4) % by 40 %, 12 %, and 25 % during infusion, but decreased to values identical to the 2 groups of animals at the end of the 30 mins infusion period.

The measured tension-time index showed an increase of 13 %, 22 %, and 10 % in the animals receiving (b) (4) % during the infusion period, but the changes were low and not considered pharmacologically significant.

A single animal developed irregular respiration during the study period, and was administered artificial respiration. Other animals with observed changes in respiration were considered affected by the degree of anesthesia, and not by the TPN infusion.

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

Investigation for (b) (4) (European pharmacopoeia): Study on (b) (4) % in the Anesthetized Cat (Experiment code: (b) (4))

Two healthy male cats were anesthetized, and fitted with a common carotid cannula through the trachea, to allow for continuous blood pressure monitoring. A second cannula was introduced into the jugular or femoral vein to allow the injection of histamine or (b) (4) %.

Histamine at 0.1 and 0.15 µg (dissolved in 0.9 % saline to a volume of 1 ml or 1.5 ml/kg body weight) was injected IV at regular intervals four times, prior to the injection of 5 ml/kg body weight of (b) (4) %. The histamine test injection was repeated 2 times in each study animal, to elicit a drop in blood pressure with each injection, prior to the test substance injection.

The injection of 5 ml/kg body weight of (b) (4) % failed to induce a drop in arterial blood pressure in any of the study animals. In contrast, histamine injection elicited a drop in arterial blood pressure of – 50, and – 47 mm/Hg in test animal # 1, and – 53, and – 47 mm/Hg in test animal #2.

Cardiovascular Investigation of (b) (4) % in the Anesthetized Cat (Study No. (b) (4))

Six healthy male cats, 7-8 month old, and weighing 3.0 – 3.7 kg, were randomly assigned (at 3 animals/group) to 1 of 2 groups ((b) (4) % or Vamin 18 EF), and anesthetized with Saffan (intramuscularly). Cannulas were inserted into the cephalic vein for infusion; into the trachea for respiration monitoring; and into the right femoral artery for monitoring of blood pressure. Blood flow in the central mesenteric artery was measured electromagnetically through a flow meter probe, and ECG was registered through electrodes attached to the animal extremities.

Each animal was either infused IV with 216 mg/kg (b) (4) % (2102 mosm/kg) at a dose volume of 27.24 ml/kg (infusion rate of 6.81 ml/kg) or 216 mg/kg Vamin 18 EF (1130 mosm/kg) at a dose volume of 48 ml/kg (infusion rate of 12 ml/kg) with an infusion pump for 4 hours, and a follow-up period of 1 hr. The infusion dose rate of amino acids (measured as 31.7 g/l nitrogen (N) in (b) (4) % and 18.0 g/l in vamin 18 EF) is about seven fold the human maximal dose rate of 18 g/l N administration to a 70 kg person (257 mg N/kg) for 8 hrs.

Blood samples were taken at intervals of 30 mins for measurement of blood gases and hematocrit.

The observed cardiovascular effect in the test animals was a 30 % decrease in blood pressure, which was stabilized during the post-infusion period. There were compensatory (20 - 30 %, and 10 - 15 %) increases in heart rate for the test article ((b) (4) %) and the reference article (Vamin 18 % EF) groups, respectively. The stable blood pressure appeared to be maintained by increased intravascular volume and high osmolality of the infused articles. There was also increased blood flow, which was maintained for the duration of the infusion period.

The electrocardiographic (ECG) changes observed include increases in R- and T-wave amplitudes, which were attributable to the increased blood volume, heart rate and/or prolonged anesthesia. An additional increase in T-wave amplitude seen at 180 min in ECG recordings from an animal in the (b) (4) % group might suggest a slight elevation in the serum K⁺ level. Since the ECG changes did not indicate a major abnormality, and because there were no differences noted between the test and reference article, the changes were considered clinically insignificant.

Hematocrit values initially decreased one hour into the infusion period as a result of hemodilution, but returned to pre-infusion values after the infusion, in both animal groups.

The respiratory frequency was twice increased from the pre-infusion values in test article animals, and more than twice increased in animals infused with the reference article, with a concomitant 70 % and 35 % increase in tidal volume for the test and

reference articles, respectively. Breathing remained augmented, but declined at 60 min post-infusion. Increased respiration is frequently observed during IV infusion of large volumes in animals under anesthesia.

Changes in blood gases were qualitatively similar, but quantitatively different, with a less pronounced decrease in PCO₂ after the infusion of the test article compared to the after infusion of the reference article. The infusion of the reference article resulted in pH changes towards basic values, in contrast to a pH of 7.1 and absolute base excess (ABE) of -14 to -15 mmol/l and slight metabolic acidosis with test article infusion. Acidosis might be induced by chloride and potassium ions present in (b) (4) %, but not present in the reference article (Vamin 18 EF) or DP-Gln 20. The slight acidosis observed in the animals during the last 2 hrs of (b) (4) % infusion, started to return to normal values after the end of infusion, with blood flow, respiration and blood gases normalization, showing that the acidosis is reversible.

In conclusion, the infusion of 216 mg/kg (b) (4) % (at a rate of 6.81 ml/kg) or Vamin EF 20 % (at a rate of 12 ml/kg) during the first 2 hours of a 4 hr infusion in male cats resulted in similar (b) (4) but non adverse cardiovascular changes. During the last two hours of (b) (4) % infusion, a slight acidosis was observed in treated animals. The acidosis was reversed on completion of the infusion in the animals.

Cardiovascular Investigation of Fat Emulsion 73403 in the Anesthetized Cat (Study No. 89-9)

Eight healthy 6-10 months old male cats weighing 2.6 to 4 kg were anesthetized with 1.3 ml/kg Saffan, Glaxovet intramuscularly (IM). Cannulas were inserted into the trachea to monitor respiration; into the right femoral artery to monitor blood pressure. Heart rate was monitored through a tachygraph connected to an amplifier, and blood flow was measured electromagnetically through a flow probe inserted around the central mesenteric artery. ECG was recorded from electrodes attached to the extremities of the animals. Blood gas measurements were obtained through a pH/blood gas analyzer, and hemoglobin levels were determined with a spectrophotometer.

Blood samples were obtained before the start of infusion, 90 mins after the start of infusion, immediately after the end of infusion (180 mins), and 60 mins after the end of infusion (240 mins). Serum lipids were determined by enzymatic analysis. Prior to the start of infusion, all the basic cardiovascular parameter measurements were obtained. The cats were randomly divided into 2 groups of 4 animals/group, prior to fat emulsion 73403 or Intralipid 20 % administration by intravenous infusion (at a rate of 0.15 ml/kg/min for 180 mins or 27 ml/kg body weight) through the cephalic vein at 1.8 g TG/kg/hour or 5.4 g TG/kg/day to group 1 or 2.

The increases in blood pressure, heart rate, blood flow, respiratory frequency and tidal volume were similar in the two groups of animals during the infusion of similar doses of

fat emulsion 73403 or Intralipid 20 %. There was a slight increase in the S-T segment duration of ECG after the infusion of fat emulsion 73403 or Intralipid 20 %.

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

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

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

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

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

In conclusion, following 3 hrs of infusion of 1.8 g TG/kg of fat emulsion 73403 and Intralipid 20 % in groups of male cats, the cardiovascular events observed were similar in the two groups. However, the pH and ABE values were slightly lower, while the lactate and β -hydroxybutyrate levels were higher after Fat emulsion 73403 infusion than after Intralipid 20 % infusion.

The pH, ABE and β -hydroxybutyrate value differences were only observed in a single cat (#1), whereas, the difference in lactate levels were observed in 3 of 4 group 1 cat. However, since the observed differences did not affect respiration or other cardiovascular parameters, the slight metabolic acidosis induced may not be significant. With a 4.8X higher infusion dose than the maximum clinical dose of 3 g TG/kg body weight in an 8 hr period, the fat emulsion 73403 may be considered safe for clinical use.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Distribution

The Suitability of DL-Glycerol-3-Phosphate for Parenteral Substitution of Inorganic Phosphate (Bässler and Hassinger, Infusion Therapy, 3:138-142, 1976)

Methods: The study objective was to determine the utilization of sodium glycerophosphate as a substitution for inorganic phosphate during parenteral administration.

Twenty-one Male SIV rats weighing 220 to 280 g were catheterized under anesthesia through the inferior vena cava for the infusion study, and for large blood volume collection for incubation studies. Urine was collected from a catheter placed in the genitalia. Seven rats were used as control animals and 14 rats were infused with glycerol. The mixture for the measurement of the hydrolysis of DL-glycerol-3-phosphate from purified phosphatases consists of 0.1 mmol/l tromethamine, 24 mM alkaline phosphatase (from calf intestine), 70 mU acid phosphatase (from potatoes), and 1 μ mol DL-glycerol-3-phosphate. The mixture for measurement of hydrolysis of DL-glycerol-3-phosphate from plasma contained 250 μ l of plasma from heparinized rat blood instead of tromethamine. After the incubation period, the reactions were stopped by perchloric acid, and the formation of inorganic phosphate measured from the supernatant. The amount of glycerol phosphate, glycerol, and phosphate fractions were also measured from the supernatant.

Results: Glycerol-3-phosphate (L-form) was rapidly eliminated from the blood following intravenous injection of 0.5 mmol DL-glycerol-3-phosphate into the vena cava of the rat. The elimination half-life ($t_{1/2}$) was 11 mins, with an elimination constant (c) of 0.063 min^{-1} . The amount of L-form of glycerol-3-phosphate eliminated in the urine was 40 %, considerably larger than the amount attributed to metabolic elimination. However, equal metabolic hydrolysis of the D and L forms of glycerol-3-phosphate was attributed to non-specific alkaline phosphatase in the plasma of blood vessels, as opposed to acid phosphatase due to the pH conditions in the blood plasma.

There was a stoichiometric 1:1 increase in the value of glycerol (0.412 μ mol, and 0.741 μ mol) versus increase in value of inorganic phosphate (0.450 μ mol, and 0.721 μ mol), respectively with glycerol-3-phosphate preparations from plasma. Plasma serum activities of alkaline phosphatase from rat are between 45 and 100 mU/ml, and comparable to normal values of alkaline phosphatase in humans, at 30 to 35 mU/ml. It appears that the introduction of phosphate via sodium glycerophosphate in infusion solutions offers an advantage of not leading to phosphate precipitation.

Elimination

Plasma Elimination Kinetics of (b) (4) % in the Dog: Comparison with Intralipid 20 % (Experiment code: (b) (4))

Methods: Six one year old male Beagle dogs (with an average weight of 12 kg) were intravenously infused with 15 ml/kg (3 g lipid/kg) of (b) (4) % and Intralipid 20 % for 60 minutes, with one week interval between the administrations of each lipid to each dog. Blood samples were taken prior to infusion, at the end of infusion, and every 15 mins for 7 hours after the infusion. Samples are diluted in saline (0.9 % NaCl), erythrocytes separated by centrifugation, and triglyceride levels in the supernatant determined by nephelometry. The method allowed the calculation of maximum elimination capacity (K1), the fractional clearance rate (K2), and the critical concentration (C.C) of each lipid from dog plasma.

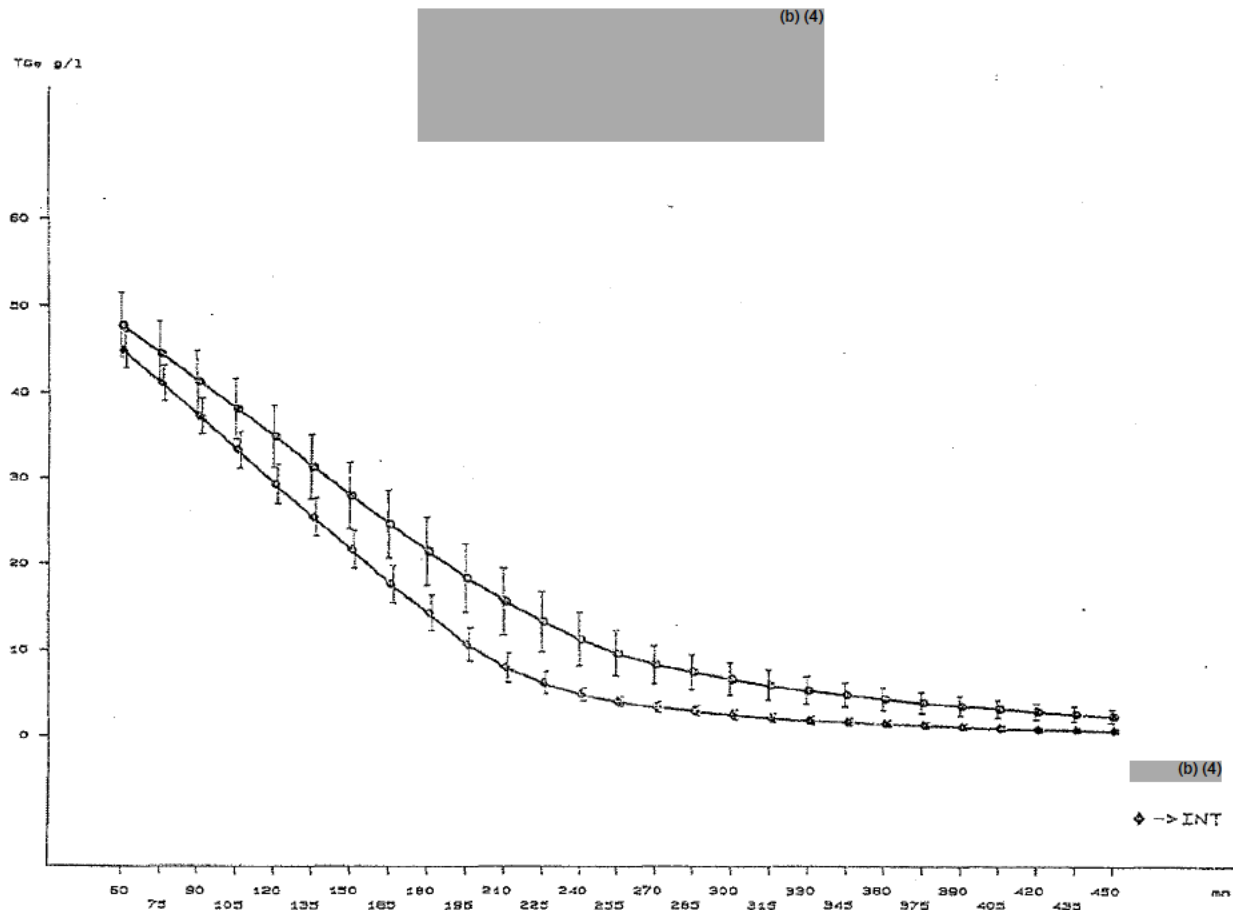
Results: The plasma elimination curve of the lipids obtained was (a mixed linear-exponential model) biphasic and allowed the calculations of K1, K2, and C.C.

The low plasma exogenous lipid concentration obtained at an infusion rate of 15 ml/kg in 1 hr (is 10X the rate used clinically) followed the zero order elimination kinetics; where the rate is constant, and independent of the lipid concentration.

At lower lipid concentration, the elimination was a first order (exponential elimination) kinetics, where the elimination rate is proportional to the plasma lipid concentration.

The concentration of exogenous triglycerides at which the kinetics changed from zero order kinetics to first order kinetics, is termed the critical concentration. This value is expressed in g/l, and is obtained using the formula $(K1/K2) \times 100$.

Average Elimination of (b) (4) % and Intralipid 20 % from the Plasma of Beagle Dogs



It can be concluded from the study that the plasma elimination kinetics of 3 g/kg (b) (4) % and Intralipid 20 % administered in one hour infusion are biphasic and identical.

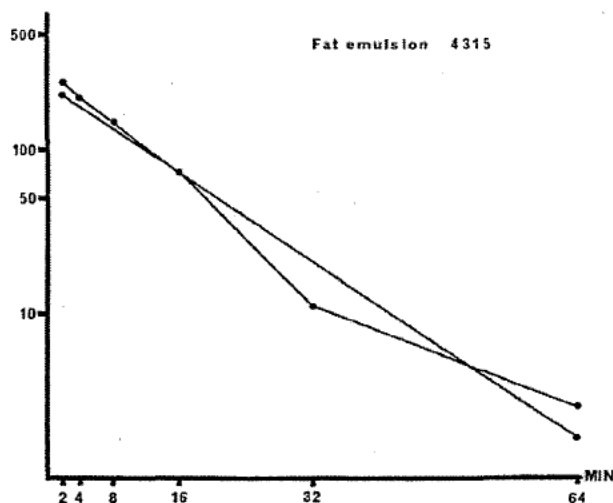
Elimination from Blood Serum of Intravenous Infusion of 30 % Fat Emulsion 4315 (Study No. 84-7)

Methodology: Four adult female rabbits weighing 2.45 kg to 3 kg were administered IV infusion of a 30 % fat emulsion 4315, at a dose of 100 mg fat/kg (0.33 ml/kg) via the marginal ear vein at a rate of 1 ml/min.

Blood samples of 0.1 ml were taken from a central ear artery before dosing (at time 0), and then at 2, 4, 8, 16, 32, and 64 mins after the end of infusion. Each blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and triglyceride levels in the supernatant determined by nephelometry.

Results: The plasma lipid clearance rate was expressed in light scattering index (LSI) in the table below. The elimination curve of the lipids was estimated as a regression line in the graph below, with a half-life ($t_{1/2}$) of 9.00 ± 0.64 min and a fractional clearance rate (K_2) of 5.51 ± 0.39 %/min. For comparison, the $t_{1/2}$ of Intralipid 20 % in an earlier study was measured as 12.58 ± 0.88 , and the K_2 measured as 5.51 ± 0.39 %/min.

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



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

Min. after end of inf.	Rabbit No 1	Rabbit No 2	Rabbit No 3	Rabbit No 4
2	(b) (4)			
4				
8				
16				
32				
64				

Elimination Rate from Blood Serum of Intravenous Infusion of 20 % Fat Emulsion 4501 (Study No. 86-6)

Methods: Four adult male rabbits weighing 2.1 kg to 2.9 kg were administered IV infusion of a 20 % fat emulsion 4501, at a dose of 100 mg fat/kg (0.50 ml/kg), with a total fat concentration of 241 ± 17 mg or Intralipid 20 % at a dose of 200 mg fat/ml, with a total fat concentration of 238 ± 16 mg via the marginal ear vein at a rate of 1 ml/min. The interval time between the administrations of the lipids to the animals was 2 days.

Blood samples of 0.1 ml were taken from a central ear artery before dosing (at time 0), and then at 2, 4, 8, 16, 32, and 64 mins after the end of infusion. Each blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and triglyceride levels in the supernatant determined by nephelometry.

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

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

Min. after end of inf.	Rabbit No 1	Rabbit No 2	Rabbit No 3	Rabbit No 4
2	(b) (4)			
4				
8				
16				
32				
64				

Nephelometric Values of Intralipid 20 % Elimination from Plasma of Rabbits

Min. after end of inf.	Rabbit No 1	Rabbit No 2	Rabbit No 3	Rabbit No 4
2	(b) (4)			
4				
8				
16				
32				
64				

Elimination Rate from Blood Serum of Intravenous Infusion of Intralipid 10 % (SSPC) (Study No. 88-9)

Methods: Four (6-8 month old) New Zealand white male Rabbits weighing 1.9 - 2.3 kg were randomly injected each with 3 batches (# 70043 (or A), # 70045 (or B), and # 68241-71 (or C)) of Intralipid 10 % containing 100 mg fat/ml, as single IV infusions in the marginal ear veins at a rate of 1 ml/min.

Blood samples of 0.1 ml were taken from a central ear artery before dosing (at time 0), and then at 2, 4, 8, 16, 32, and 64 mins after the end of infusion. Each blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and lipid levels in the supernatant determined by nephelometry. The interval time between the administrations of each lipid to the animals was a 24 hr fasting period for each animal.

Results: The plasma lipid clearance rates for the batches of Intralipid 10 % are expressed in light scattering index (LSI) in tables below. The elimination curves of the lipids are also estimated as regression lines, and the $t_{1/2}$ and K_2 values expressed in tables below. There were no differences (at $p > 0.05$) between the results of the administration of three batches of Intralipid 10 % to rabbits.

LSI Values of Intralipid 10 %, Batch 70043 in Male Rabbits

Min after end of infusion	Rabbit no 1	Rabbit no 2	Rabbit no 3	Rabbit no 4
2	(b) (4)			
4				
8				
16				
32				
64				

* No value, because of difficulties with the blood sampling.

LSI Values of Intralipid 10 %, Batch 70045 in Male Rabbits

Min after end of infusion	Rabbit no 1	Rabbit no 2	Rabbit no 3	Rabbit no 4
2	(b) (4)			
4				
8				
16				
32				
64				

LSI Values of Intralipid 10 %, Batch 68241-71 in Male Rabbits

Min after end of infusion	Rabbit no 1	Rabbit no 2	Rabbit no 3	Rabbit no 4
2				
4				
8				
16				
32				
64				

(b) (4)

Total Fat Administered and the Half Life and K₂ of Intralipid 10 % Batches

	Total fat mg	T _{1/2} min	K ₂ %/min	n
Intralipid® 10% batch 70043 (China)	208 ±8	11.83 ±1.68	5.86 ±0.83	4
Intralipid® 10% batch 70045 (China)	210 ±9	10.21 ±0.44	6.79 ±0.29	4
Intralipid® 10% batch 68241-71	213 ±9	11.26 ±0.75	6.16 ±0.41	4

In conclusion, there were no differences (at $p > 0.05$) between the results of the administration of three batches of Intralipid 10 % to rabbits.

Elimination from Blood after Intravenous Infusion of Fat Emulsion 73403, 20 % (Study No. 89-5)

Methods: Four (6-8 month old) New Zealand white male Rabbits weighing 2.4 - 2.7 kg were randomly infused each with a 20 % fat emulsion 73403 and Intralipid 20 %, at a dose of 100 mg fat/kg body weight (0.5 ml/kg) in a marginal ear vein at a rate of 1 ml/min. The lipids were each administered following a 24 hr fasting period.

Blood samples of 0.1 ml were taken from a central ear artery before dosing (at time 0), and then at 2, 4, 8, 16, 32, and 64 mins after the end of infusion. Each 0.1 ml blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and lipid levels in the supernatant measured by light scattering index (LSI) nephelometry.

Results: The results show that the 20 % fat emulsion 73403, as measured with LSI, is eliminated faster than Intralipid 20 % with the given dose.

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

Min after end of inj.	Rabbit no 1	Rabbit no 2	Rabbit no 3	Rabbit no 4	Mean value (n=4)
2	(b) (4)				103
4					66
8					38
16					17
32					3
64					-

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

Min after end of inj.	Rabbit no 1	Rabbit no 2	Rabbit no 3	Rabbit no 4	Mean value (n=4)
2	(b) (4)				119
4					96
8					62
16					34
32					13
64					3

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

	Fat mg	T _{1/2} min	K ₂ %/min
Fat emulsion 73403	263±8	5.7±0.4 *	12.2±0.9 *
Intralipid 20 %	260±7	9.2±0.6	7.6±0.5

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

In conclusion, the results show that the 20 % fat emulsion 73403, as measured with LSI, is eliminated faster than Intralipid 20 % at the given dose of 100 mg fat/kg body weight (0.5 ml/kg).

Half-Lives of Intralipid Particles in Mammals (Document No. 82 99 026)

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

Venous blood samples were drawn before dosing at time 0, and at 2, 4, 8, 16, 32, and 64 min after injections. In the mouse, retroocular blood vessels were used for blood sampling, which limited the number of samples from each animal to 2. Each 0.1 ml blood sample was diluted with 5 ml of saline (0.9 % NaCl), erythrocytes separated by centrifugation, and lipid levels in the supernatant measured by light scattering unit (LSU) nephelometry. Values obtained were used in calculating slopes of regression lines for the determination of Intralipid half-life in each animal.

Results: The combined results of the calculated half-lives of Intralipid 10 % for all the animals, except the cat are shown in the table below. A comparison of all the animal results was made to human half-life values for Intralipid 10 %.

Half-Lives for Intralipid 10 % Fat Particles in Different Species

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

[#]Calculated from (b) (4) (5)

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

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

Methods: Nine white New Zealand rabbits weighing between 2.2 kg and 3.4 kg were deprived of food, but not water for 18 hrs prior to lipid infusion. 100 mg fat/kg body weight of Intralipid 20 % was infused via the marginal ear vein at a rate of 1.0 ml/min. Venous blood samples from the central ear vein of the opposite ear were drawn at 2, 4, 8, 16, and 64 min after injections, and the light scattering indexes (LSI) analyzed by nephelometry. The elimination rate K_2 and the $t_{1/2}$ were calculated from the slope of the estimated regression line.

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

6 General Toxicology

6.1 Single-Dose Toxicity

Acute Toxicity of (b) (4) % Via the IV route in the Male and Female Rat:
Determination of the LD₅₀ (Experiment codes: (b) (4))

Methods: (b) (4) % at doses of 87.50, 100, 112.50 and 137.50 ml/kg were randomly administered IV to CD rats (in groups of 10/sex/dose) at 1ml/min, to determine the toxicity of the product. The animals were observed for signs of acute toxicity for 14 days prior to autopsy at the end of the study period. Mortality was also recorded, and a linear dose-response regression curve was used to calculate the LD₅₀ dose of the lipid in the animals.

Results: Total mortality in male rats dosed with (b) (4) % were, 1, 7, and 9 animals in the 87.50, 100, and 112.50, ml/kg dose groups, respectively. The animals in the 100 and 112.50 ml/kg dose groups showed clinical signs of moribundity, whereas the 100 ml/kg dose groups showed paralysis, irregular breathing, tonic spasm, loss of righting reflex, and muscular tonus. Macroscopic observations seen in all the dose groups in the animals include mottled spleen, white, greenish and swollen kidneys, and white/yellowish liver spots. The LD₅₀ of (b) (4) % infused IV in male rat was 98 ml/kg, and ranges between 93-103 ml/kg.

Mortality in the female rats dosed with (b) (4) % was observed to occur within a variable period following IV dosing, with total mortality of 2, 6, and 7 rats occurring at 100, 112.50, and 137.50 ml/kg dose groups, respectively. Only female animals in the 137.50 ml/kg dose groups showed clinical signs of moribundity, loss of righting reflex, muscular tone, irregular breathing and catatonia. Macroscopic observations seen in all the dose groups include mottled spleen, white, greenish and swollen kidneys, and white/yellowish liver spots. The LD₅₀ of (b) (4) % infused IV in female rats is 116 ml/kg, and ranges between 103-130 ml/kg.

In conclusion, the acute toxicities of 87.50, 100, 112.50, and 137.50 ml/kg (b) (4) % infused IV at 1 ml/min in male and female rats provided LD₅₀ values of 98 ml/kg (with a range of 93-103 ml/kg) in male rats, and an LD₅₀ value of 116 ml/kg (with a range of 103-130 ml/kg) in female rats.

Acute and Subacute Toxicity of IV Administered α - and β - Sodium Glycerophosphate in Rats and Dogs

α - and β - sodium glycerophosphate were injected IV at a range between 10 and 40 ml/kg and at the highest dose of 6 g/kg into the caudal vein of male Sprague-Dawley rats weighing 180-200 g over a 2 min period. Control animals were also injected IV with 50 ml/kg of sodium chloride. The animals were observed for 5 days for signs of acute toxicity.

The control animals behaved normally and survived the study. The calculated LD₅₀ for the treated rats, presented in the table below, showed that the toxicity of the two test articles is similar.

0.5 g/kg of β - sodium glycerophosphate was injected IV into 2 dogs weighing 18 and 20 kg for 5 min each morning for 14 days. The first dog received an injection volume of 60 ml while the second dog got an injection volume of 66.6 ml. The animals' behaviors were completely normal throughout the study period.

2 g/kg of β - sodium glycerophosphate (20 % solution) was injected IV in divided doses in the morning (1 g/kg) and in the afternoon (1 g/kg) for 14 days to a female dog weighing 19 kg. The animal tolerated the injection, without showing any signs of toxicity during the observation period.

In a dog weighing 17 kg, 4g/kg of α - sodium glycerophosphate was injected in divided doses of 2 g/kg in the morning and afternoon with an injection volume of 170 ml of a 20 % solution. Clinical observations in this dog include vomiting in the morning after the first injection on day 1. The first injection on day 2 resulted in extensive spasms in the animal, and after the second injection, the animal became severely spastic, and lapsed into a temporary stupor. Injection of the test compound on the third day resulted in vomiting, extension spasm, and loss of consciousness. The study was terminated on the fourth day because it was believed that the dog would have died.

The IV LD₅₀ for Sodium Glycerophosphate in Male Sprague Dawley Rats

α -Sodium glycerophosphate: 3.8 g/kg (3.39 - 4.26)

β -Sodium glycerophosphate: 3.4 g/kg (2.66 - 4.34)

At the highest dose of sodium glycerophosphate at 6 g/kg, all the rats died with tonic-clonic convulsion shortly after the injection. At the lower dose, when death occurred, it happened within 3 hrs with attendant convulsions. All surviving animals were in good condition, even on day 5 of the observation period.

In conclusion, in the acute IV toxicity of sodium glycerophosphate in rats, the LD₅₀ was identified as 3.4 to 3.8 g/kg. Similarly, in the dogs, the LD₅₀ was 2 g/kg in a 14-day study.

Acute Toxicity via the IV Route in the Male and Female Mouse: Determination of the LD₅₀; Comparison between (b) (4) % and Intralipid 20 % (Experiment codes: (b) (4))

Methods: (b) (4) % and Intralipid 20 % at doses of 112.5, 125, and 137.5 ml/kg were administered IV at 1 ml/min to male and female CD-1 mice weighing 22-23 g (10/sex/group), randomly assigned to the 3 (b) (4) % dose groups or the 3 intralipid 20 % dose groups, to determine the toxicity in the animals. The animals were

observed for signs of acute toxicity for 14 days. Mortality was also recorded, and a linear dose-response regression curve was used to calculate the LD₅₀ dose of the lipid formulations.

Results: Mortality in male mice dosed with (b) (4) % was observed within a maximum period of 2 days following IV dosing, with the exception of one animal receiving 125 ml/kg of (b) (4) %, which died on day 6. Total mortality in male mice dosed with (b) (4) % were, 1 mouse (112.5 ml/kg); 4 mice (125 ml/kg); and 9 mice (137.5 ml/kg). The dosed animals were observed to be moribund, developed clonic convulsions, loss of righting reflex and irregular breathing at the 125 and 137.5 ml/kg dose. Macroscopic observations included mottled spleen, pale, swollen kidneys, with white and yellow liver spots. The LD₅₀ for (b) (4) % in male mice was determined to be 129 ml/kg, with a range between 122 – 137 ml/kg.

Mortality in male mice dosed with 137.5 and 112.5 ml/kg intralipid 20 % was observed to occur within a maximum period of 3 days following IV dosing, with total mortality at 3 mice and 7 mice in the 112.5 ml/kg and the 137 ml/kg dose groups, respectively. The dosed animals were observed to be moribund, and with diminished respiratory activity in all the dose groups. At the 137.5 mL/kg (high) dose group, the male mice were present with catatonia, loss of righting reflex, opisthotonos and tonic convulsion. Macroscopic observations include mottled spleen, clear swollen kidneys, and mottled and yellow spotted liver. The LD₅₀ for intralipid 20 % in male mice could not be determined because the percentage mortality was not proportional to the administered dose. The LD₅₀ however, could be postulated at the range of the administered dose of 112 – 137 ml/kg. Mortality in the female mice dosed with (b) (4) % was observed to occur within a maximum period of 6 days following IV dosing, with total mortality at 4, 1, and 9 mice in the 100, 112.5, and 125 ml/kg dose groups, respectively. The only clinical observation of diminished activity was seen in the 125 ml/kg dose group; however, macroscopic observations of mottled spleen, swollen kidneys, and white/yellow liver spots were present in the animals. The LD₅₀ for (b) (4) % in female mice could not be determined because the percentage mortality was not proportional to the administered dose. The LD₅₀ however, could be postulated at the range of the administered dose of 100 – 125 ml/kg.

Mortality in the female mice dosed with intralipid 20 % was observed to occur within a period of five days following IV dosing, with the exception of one animal dosed at 100 ml/kg which died on day 9. Total mortality in female mice dosed with intralipid 20 % were 1, 8, 8 mice in the 100, 112.5, and 125 ml/kg dose groups respectively. The animals in the 112.5 and 125 ml/kg dose groups were observed to be moribund, with irregular breathing, with loss of righting reflex and tonic convulsions. Macroscopic observations include mottled spleen, swollen kidneys, and yellow mottled liver. The LD₅₀ for intralipid 20 % in female mice could not be determined because the percentage mortality was not proportional to the administered dose. The LD₅₀ however, could be postulated at the range of the administered dose of 100 – 125 ml/kg.

LD₅₀ Values in Mice Administered (b) (4) % and Intralipid 20 %

	(b) (4) %	INTRALIPID® 20 %
male mice	129	112 < LD ₅₀ < 137
female mice	100 < LD ₅₀ < 125	100 < LD ₅₀ < 112

In conclusion, the acute toxicities of (b) (4) % and Intralipid 20 % administered IV at doses of 100, 112.5, 125, and 137.5 ml/kg to male and female CD-1 mice are comparable, although mortalities observed are not always proportional to the administered dose.

Acute Toxicity in Mice after Single IV Administration of Sodium Glycerophosphate Solution (Study No. 90-2)

Methods: Sodium glycerophosphate (1 mmol/ml) was administered IV at 60 ml/kg to 40 day-old 5 male and 5 female NMRI mice, weighing 24-34 g through the tail vein at 0.5 ml/min. The animals were weighed at dosing days 0, 2, 7 14 and observed for signs of acute toxicity for 14 days. Autopsy was performed on the animals under pentobarbital anesthesia at the end of the 14 day observation period.

Results: No mortality or signs of toxicity were observed in any of the animals, and no significant changes in body weight gain or macroscopic changes were observed. In conclusion, no toxic effects were observed in NMRI mice following single IV administration of 60 ml/kg of 1 mmol/ml sodium glycerophosphate solution.

Acute Toxicity in Mice and Rats after Single IV Administration of (b) (4) % (Study No. (b) (4))

Methods: (b) (4) % was administered IV at 17 ml/kg (0.54 g N/kg) to 10 (40-day old) NMRI mice (5/sex) weighing 24-34 g and 10 (45-day old) Sprague Dawley rats (5/sex) weighing 152-217 g, via a tail vein. 10 mice (5/sex) and 10 rats (5/sex) served as control animals, receiving saline (9 mg/ml) at a dose level of 24 ml/kg body weight. Both control and study animals were dosed through the tail vein at a constant infusion rate of 0.14 ml/min. The animals were weighed at dosing days 0, 2, 7 and 14, and observed for signs of toxicity for 14 days. Autopsy was performed on the animals at the end of the 14 day observation period.

Results: No mortality occurred in the animals and no drug-related clinical signs were observed. No changes in body weight were observed in the mice or rats, and no macroscopic changes were seen at autopsy. In conclusion, no toxicity was observed in mice and rats following single IV administration of 17 ml/kg of (b) (4) %.

Estimation of Acute Toxicity in Mice of Amino Acid Solution AA 4964

Methods: Amino acid solution (AA 4964) at a dose of 50 ml/kg was administered IV through the dorsal tail vein to 30 NMRI male mice weighing 16-18 g at an infusion rate of 0.11 ml/min. The mice were fasted for 16 hrs, but allowed access to water prior to the start of the study.

Results: All the animals tolerated the amino acid solution AA 4964 well, without any observed toxicity for the entire 24 hr study duration.

In conclusion, no acute toxicity was observed in mice following IV administration of 50 mL/kg of the amino acid solution.

Estimation of Acute Toxicity (LD₅₀) of Intralipid 20 %

Methods: Intralipid 20 % at doses of 72.9, 91.6, and 115.2 ml/kg IV at 0.25 ml/min or at doses of 78.5, 98, or 122.2 ml/kg IV at 0.50 ml/min were administered through the dorsal tail vein to male NMRI mice (20 animals/dose). The mice were fasted for 16 hrs, but allowed access to water prior to the start of the study, and were observed for 72 hrs for signs of toxicity.

Results: The LD₅, LD₅₀ and LD₉₅ values for the two groups are shown in the Table below.

LD₅, LD₅₀ and LD₉₅ Values in Mice Administered Intralipid 20 %

Injection rate	LD50	LD5	LD95
0.25 ml/min. Batch No. 290712	91.6 ml/kg : (86.1 - 96.7)	72.9 ml/kg (68.5 - 76.9)	115.2 ml/kg (108.2 - 121.5)
0.5 ml/min. Batch No. 291042	98.0 ml/kg (92.1 - 104.3)	78.5 ml/kg (73.8 - 83.6)	122.4 ml/kg (115.0 - 130.2)

Figures within brackets are the confidence limits, given at a probability level of 0.05

In conclusion, with the IV infusion of intralipid 20 % at doses 72.9, 91.6, and 115.2 ml/kg (at 0.25 ml/min), or 78.5, 98, or 122.2 ml/kg (at 0.50 ml/min), it was possible to predict the LD₅₀ in male NMRI mice at a probability level of 0.05.

6.2 Repeat-Dose Toxicity

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

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

Key Study Findings

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

Methods

Doses:	9 g TG/kg/day
Frequency of dosing:	20 Hrs infusion daily for 2 weeks
Route of administration:	IV via a central venous Jugular catheter
Dose volume:	45 ml/kg/day
Formulation/Vehicle:	200 g Triglyceride (fat emulsion 73403), 200 g soybean oil (intralipid 20 %)
Species/Strain:	CD: Sprague-Dawley Rats
Number/Sex/Group:	9 males/group
Age:	37 days old
Weight:	142.8-162.4 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minor protocol deviation reported as technical problems with catheter.

Intravenous administration of Fat emulsion 73403 at 9 g TG/kg for 20hrs/day or Intralipid 20 % to male rats for 2 weeks was well tolerated.

The platelet counts were similarly reduced in test and reference group animals, and individual blood parameter values for some rats in both groups differed slightly, but were not of toxicological significance.

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

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

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

Study title: Repeated dose toxicity of (b) (4) % via the Intravenous route in the Rat (30 days): Comparison with Intralipid 20 %

Study no.: (b) (4)

Study report location: Project (b) (4), Page 1 to 188

Conducting laboratory and location: (b) (4)

Date of study initiation: August 6, 1985

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) %, lot # (b) (4); Intralipid 20 %, lot # (b) (4); Isotonic Saline, lot # (b) (4)

Key Study Findings

There was decreased food intake in the two lipid emulsion treated groups, compared to control, without changes in animal weight gain. Mottled liver was observed in 5 (b) (4) and 6 Intralipid treated animals, with slight increases in liver and spleen weight of treated animals, when measured as a percentage of body weight.

Excessive lipid and pigment hemosiderosis, and hypertrophy of the reticular cells of the liver and spleen were observed in treated animals in comparison to control animals.

Methods

Doses: 9 g TG/kg/day of (b) (4) % or Intralipid 20 %

Frequency of dosing: Daily for 30 days

Route of administration: Intravenous

Dose volume: 45 ml/kg/day

Formulation/Vehicle: 9 g TG/kg /Isotonic NaCl

Species/Strain: CD: Sprague-Dawley Rats
Number/Sex/Group: 4/sex/group
Age: 6 weeks old
Weight: 200-250 g
Satellite groups: None
Unique study design: None
Deviation from study protocol: No major deviations

Observations and Results

Mortality

There was no animal mortality in this study.

Clinical Signs

Animals were observed daily from the start of infusion to the end at regular intervals. Clinical observation in the animals included pink urine coloration in (b) (4) % and Intralipid 20 % treated animals, and on a single occasion in 3 control animals, at the end of the infusion period. The pink coloration, which is indicative of hemoglobinuria, disappeared after the end of the infusion.

Body Weights

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

Feed Consumption

Food consumption was recorded twice weekly for each animal. The food intake in (b) (4) % and Intralipid 20 % treated animals was reduced, when compared with food intake in control animals. The decreased food intake was attributable to calories from the lipid emulsion. However, the changes were not statistically significant.

Ophthalmoscopy

No ophthalmological changes were observed.

ECG

No ECG recordings were done.

Hematology

Blood samples were taken 18 hrs after the last infusion.

There were no observed differences in hematological parameters between the control and treated animals, with the exception of diminished hematocrit values in one animal in each of the 3 groups.

Clinical Chemistry

Blood samples were taken 18 hrs after the last infusion.

There were no changes attributable to the treatment with (b) (4) % or Intralipid 20 %. All clinical chemistry results from all the study animals were comparable.

Urinalysis

Urine samples were taken on day 30, following diuresis on day 29 for each animal.

There were no observed differences in urine analysis between the control and treated animals.

Gross Pathology

(b) (4) % treated animals had mottled liver (5 rats), and 1 animal each with spleen showing clear zone and heart with a zone of infarct. Intralipid treatment group also showed 6 animals with mottled liver and 1 animal each with white inclusions in the spleen, clear zone in the spleen, and congested zone in the kidney. All other organs appeared normal.

Organ Weights

The weights of the following organs were recorded:

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

In comparison to control animals, the hepatic weight expressed as a percentage of body weight was slightly higher in animals treated with (b) (4) % (by 1 %), and higher in animals treated with Intralipid 20 % (by 1-5 %).

Similarly, in comparison to control animals, the splenic weight expressed as a percentage of body weight is slightly higher in animals treated with (b) (4) % or Intralipid 20 % (by 1 %).

Histopathology

The following organs were examined histopathologically:

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

Adequate Battery: Yes

Peer Review No

Histological Findings

Histopathological observations in both Intralipid 20 % and (b) (4) % treated animals include an excess of lipids and pigment hemosiderosis, and hypertrophy of the reticular cells of the liver and spleen. There were no significant differences between the appearances of the organs in the two treatment groups.

The number of rats/sex/group was inadequate at 4/sex/group, instead of 10/sex/group

Study title: Utilization and Tolerance of New Amino Acid Solutions with a High Nitrogen Content (4959 and 4964) Compared to the Commercial High Nitrogen Amino Acid Solution Azonutril: TPN Studies in the Rat

Study no.: 8398016

Study report location: Pages 1352 to 1374

Conducting laboratory and location:

(b) (4)

Date of study initiation: January 23, 1983

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: Azonutril 25; Amino acid solution 4959;
Amino acid solution 4964

Key Study Findings

In a comparison of Azonutril 25 infusion at doses of 1 and 3 g N/kg/day for 10 and 14 days respectively to Amino acid 4959 at 1 g N/kg/10 days and amino acid 4964 at 3 g N/kg/14 days in male rats, the quality of the 4959 amino acid solution appeared to be higher than that of Azonutril 25 (at the 1.0 g N/kg/10 days). The growth rate of the male rats in the 1.0 g solution 4959 group at 3.9 g/day, more than doubled the growth rate of the 1.0 g Azonutril 25 group at 1.7 g/day, with a higher degree of nitrogen utilization at 99 % versus 79 % respectively. There were no differences observed in the growth rate of both Azonutril 25 and solution 4964 high dose (3.0 g N/kg/14 days) groups at 6.1 g/day during the last seven days of the study.

There were no changes in clinical signs, hematology, clinical chemistry, organ weights or histopathological parameters.

Methods

Doses:	1.0 g N/kg; 3.0 g N/kg (Azonutril 25); 1.0 g N/kg solution 4959); 3.0 g N/kg (solution 4964)
Frequency of dosing:	Daily for 10 days (1.0 g N/kg); Daily for 14 days (3.0 g N/kg)
Route of administration:	Intravenous
Dose volume:	25 g N/l (Azonutril 25); 13.5 g N/l (solution 4959); 18 g N/l (solution 4964).
Formulation/Vehicle:	1.0 g or 3.0 g N/kg / vehicle was not given
Species/Strain:	Sprague-Dawley Rats
Number/Sex/Group:	7 or 8 males/group
Age:	Not provided
Weight:	155-165 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No major deviation from study protocol reported.

The infusion of the amino acid solutions was well tolerated by all the study animals, with slight differences between the dose levels in hemoglobin, serum chloride, ALP and A/G-ratio, which were lower on the high dose level for both solutions, whereas packed cell volume and serum phosphates were higher.

The increased level of gamma-globulin at 70 % between the high/low dose Azonutril 25 groups is an exception to the minor changes seen in the blood parameters between the dose groups, which were still not significant at an increased level of 20 % between the high dose solution 4964 and the low dose solution 4959.

The increased amino acid load also produced slightly elevated thymus, spleen and liver weights, but reduced relative heart weight at the higher dose levels (ascribed to a much faster growth rate).

Rats infused with high dose Azonutril and high dose solution 4964 had higher centrolobular fat deposition in the livers, but the increase in the solution 4964 group was much smaller than in the Azonutril group.

In conclusion, the quality of the amino acid solution 4959 and 4964 was found to be higher than that of Azonutril 25, which only had a slight amino acid imbalance, since no changes were noted at the high dose level for most parameters studied.

Both solutions were well tolerated by study animals, with the exception of a slight hypoxia during the infusion of the high dose Azonutril 25.

There was no control animal group in this study.

Study title: Tolerance and Nutritional Studies of AA 4959 (STARK) Solution in Rats

Study no.:	703
Study report location:	Doc. No. 8399005, Pages 1234-1260
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 10, 1983
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	AA 4959, lot # lot #1979; 285001

Key Study Findings

In the evaluation of tolerance to amino acid solution AA 4959 at 3.6 or 1.4 g N/kg for 28 days in male rats, two animals in the 3.6 g N/kg (high dose group) died on study days 3 and 28. Although no clinical abnormalities were recorded, the animals had histopathological changes of hyperemia of the liver, lungs, and kidneys, suggesting circulatory disturbances, possibly from the hyperosmolality of AA 4959 solution. Other findings include significantly lower alkaline phosphatase (ALP) and lactate dehydrogenase (LD) in the high dose test group, elevated (+ 65 %) AST in the low dose group, and decreased total protein (- 14 %) and alpha-globulin (-38 %) at the high dose AA 4959 group, when compared to control group.

Methods

Doses:	1.4 g or 3.6 g N/kg AA 4959 (high or low dose); 30 % protein or 10 % protein as control groups
Frequency of dosing:	Daily for 28 days
Route of administration:	Intravenous via a catheter, or oral (Control animals)
Dose volume:	78 ml/kg (1.4 g N/kg), or 200 ml/kg (3.6 g N/kg)
Formulation/Vehicle:	Electrolytes- Na acetate
Species/Strain:	Sprague-Dawley Rats
Number/Sex/Group:	7 or 8 male rats/group
Age:	37-38 days old

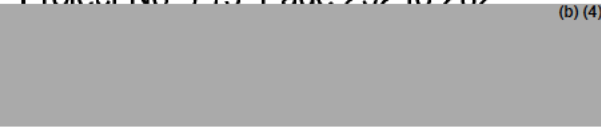
Weight: 155-175 g
Satellite groups: None
Unique study design: None
Deviation from study protocol: No major deviations.

AA 4959 solution at doses of 3.6 and 1.4 g N/kg/day for 28 days were tolerated by male rats without any severe side effects. The two animal deaths from the high dose groups (the only significant change noted between the two test groups) were present with histopathological changes of hyperemia of the liver, lungs, and kidneys, suggesting circulatory disturbances, possibly from the hyperosmolality of AA 4959 solution.

Observed differences in weight gain (growth) between the two test groups in comparison to the two reference (control diet of 30 and 10 % protein) groups were only observed during the first two weeks of the study period, but were not present in the last two weeks of the study period.

Slightly decreased hemoglobin levels observed in the low dose AA 4959 rats, coupled with the differences in total protein, alpha-globulin and liver enzymes between the treatment groups and the reference groups were within normal range.
There was no (untreated) control animal group in this study.

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

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

Key Study Findings

In the evaluation of tolerance to 18 g TG/kg of fat emulsion 4501 20 % (consisting of 85 % soybean oil and 15 % borage oil), or Intralipid 20 % in male rats, all study animals were in good clinical conditions without any significant differences in food consumption or weight gain between the test and reference groups.

There were no changes in organ weights, clinical chemistry, and total fatty acid contents in the liver, macro or microscopic pathology between the test and reference animals.

There were similarities in weight gain, food consumption, organ weights, clinical chemistry and histopathology between the animals infused with fat emulsion 4501 20 %

or Intralipid 20 %. Thus, Lipid emulsion 4501 20 % was well tolerated for 28 days by male rats.

Methods

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

The twenty-hr/day infusion for 28 days of 18 g TG/kg/day fat emulsion 4501 20 % or Intralipid 20 % was well tolerated by male Sprague-Dawley rats. There were macroscopic observations of patechial bleeding in the thymus, pulmonary, kidney, and one cranial lobe in 4 test animals respectively, whereas, there were bilateral dilatations of the renal pelvis, and bleeding of a lobe of the lung observation in the reference dose animals. The macroscopic observations did not show significant differences between the two groups.

Observed microscopic changes include generally slight focal inflammation of the lungs, spleen vacuolation, and yellowish pigment deposition in the spleen of most rats, focal hemorrhage and/or venous congestion in the lungs of some animals from both group, vacuolated cells with mononuclear infiltration, and glycogen deposition in the liver of all rats; fat deposition in liver hepatocytes and kupffer cells in all rats. The observed changes following the infusion of Fat emulsion 4501 20 % in male rats was no more severe than for Intralipid 20 % in the 28 day-treatment. There was no control group in this study.

Study title: Safety and Long-Term Tolerance of (b) (4) % in the Rat After Intravenous Administration for 28 Days

Study no.: (b) (4)

Study report location: Project No. 738, Page 1-68; 13 page appendix

Conducting laboratory and location: (b) (4)

Date of study initiation: November 7, 1989

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) %, lot # (b) (4) A; Vamin 18EF, lot # (b) (4); NaCl, lot # PC

(b) (4)

Key Study Findings

Intravenous infusion of 3 g N/kg/day of (b) (4) % for 28 days in rats did not result in adverse effects on weight gain, food intake, clinical chemistry, or organ/tissue morphology when compared to Vamin 18EF (a high quality commercial amino acid solution), or a physiological saline control infusion.

There were significant differences between the two formulations for all amino acids evaluated in plasma and urine of treated animals. The plasma and urine amino acid variations between the two treatment groups were attributed to the different amino acid compositions of the solutions and the diet.

Methods

Doses: 3 g N/kg/day (b) (4) %, or Vamin 18EF)
 Frequency of dosing: 20 hrs/day
 Route of administration: Intravenous via central venous catheter
 Dose volume: 94.6 ml/kg/day (b) (4), 166.7 ml/kg/day (Vamin EF, Saline Control).
 Formulation/Vehicle: 3 g N/kg/Saline
 Species/Strain: Sprague-Dawley Rats
 Number/Sex/Group: 10 male rats (b) (4) test group), 8 male rats (Vamin 18EF, Saline Control)
 Age: 39 days old
 Weight: 140.1-160.5 g
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: Deviations from the intended daily infusion doses were recorded.

Observations and Results

Mortality

Animals were checked daily for mortalities.
There were no animal deaths in this study.

Clinical Signs

Clinical observations were performed daily.
There were no adverse clinical signs observed in any group.
Four animals were excluded from the study for phlebitis in the catheter vessel, catheter obstruction, (2 animals), and technical problems respectively.

Body Weights

Body weight changes were recorded daily.
There were no significant differences in initial and final body weights between the dose groups. Similarity in weight gain was observed between the (b) (4) % and Vamin 18EF dose groups. Both treatment groups had slightly reduced, but insignificant final body weights than the saline control group.

Feed Consumption

Food consumption was recorded daily.
There were no differences in food consumption between the (b) (4) % and Vamin 18EF dose groups. However, both the test and reference product dose groups had reduced food intake compared to the control animals.

Ophthalmoscopy

Ophthalmoscopic examination was not performed in this study

ECG

ECG recordings were not conducted in this study.

Hematology

Blood samples for hematology were collected from the orbital plexus of the animals under light ether anesthesia prior to autopsy at the conclusion of infusion.
No statistically significant differences were observed in hematological parameters between the test and reference product groups. The normotest (NT) values were slightly higher in the test and reference animal groups than in the control animal group.

Clinical Chemistry

Blood samples for clinical chemistry were collected prior to autopsy.

The mean bilirubin concentration was decreased in animals dosed with (b) (4) % when compared to Vamin 18EF dosed animals.

Slightly decreased total serum proteins and decreased urea, as well as increased cholesterol levels were observed in both treatment groups, in contrast to the control group. In addition, reduced liver ALP levels was observed in the (b) (4) treated animals, while slightly elevated AST and bilirubin values were observed in the Vamin 18EF dosed animals. These changes in the liver enzymes and bilirubin between the treated groups were still within normal range, and not considered to be of toxicological significance.

Urinalysis

Urine samples were collected from animals during the fasting period on day 28 and day 29. Urine samples were also collected for dipeptide and amino acid analysis 24 hrs before autopsy.

Positive occult blood reactions observed on urine samples from all animals were likely due to blood contamination.

There were significant differences noted between the test group and the reference group for all the plasma amino acid evaluated, because they occurred as a result of the different amino acid compositions of the solutions and the diet. Glutamine, tyrosine and glycine levels were higher and branched-chain amino acids and phenylalanine (PE) were lower following the infusion of (b) (4) % compared to Vamin 18EF, whereas, in the control group, branched-chain amino acids, glycine and PE levels were lower compared to both the test and reference groups.

Urine concentrations of glutamine and glycine were higher, and isoleucine and leucine were lower in the (b) (4)-treated group compared to the Vamin 18EF group.

Gross Pathology

Besides the 3 animals sacrificed prior to end of study, macroscopic changes observed at autopsy include slight hydronephrosis in one of the kidneys of one animal each in the two treatment groups; hyperemic lymph nodes adjacent to the thymus in 2 animals from the Vamin 18EF dose group; hemorrhage in one testicle of a control animal. These changes were considered incidental or spontaneous in the study animals.

Organ Weights

The weight of kidneys, lungs, liver, myocardium, spleen, and thymus were recorded.

There were no significant differences observed in organ weights between the (b) (4) test group and the Vamin 18EF group. The relative liver weight was significantly

lower in the (b) (4) test group and the Vamin 18EF group, when compared to the control group.

Histopathology

For histopathology, the following organs and tissues were fixed in neutral buffered 10 % formalin solution, prior to paraffin embedding and sectioning and staining with hematoxylin and eosin.

tongue	salivary gland
oesophagus	stomach*
duodenum	loop of small intestine*
caecum*	colon*
thyroid* and parathyroid*	pituitary gland*
adrenal*	liver*
pancreas*	myocardium*
aorta*	trachea
lung*	skin* (flank)
skeletal muscle* (thigh)	femur (including bone marrow)
kidney*	urinary bladder
testis*	epididymis*
seminal vesicle	prostate*
sciatic nerve	thymus*
spleen*	lymph node* (mesenteric)
brain* (cerebrum*, pons, cerebellum* and medulla oblongata)	
organs and tissues with macroscopically visible changes*	
spinal cord (if neurological signs, e.g. ataxia, were observed clinically)	

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

There were no observed histopathological changes believed to be related to the treatment with the test or reference product. The periportal fine droplets of fatty acid changes observed in the liver were comparable in all three study groups.

In conclusion, the IV infusion of 3 g N/kg/day of (b) (4) % for 28 days in rats did not result in adverse effects on weight gain, food intake, clinical chemistry, or organ/tissue morphology when compared to Vamin 18EF (a high quality commercial amino acid solution), or a physiological saline control infusion.

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

Study no.: 90-12
Study report location: Project No. 1992-06-04. Page 387 to 467
Conducting laboratory and location: (b) (4)

Date of study initiation: April 19, 1990
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Fat Emulsion 73403 20 %, lot #356900-1;
Intralipid 20 %, lot #50782-91; Sodium
chloride, lot #84056-51

Key Study Findings

Intravenous infusion of 18 g TG/kg/day for 27 days in rats resulted mainly in a decrease in platelet count in treated rats when compared to animals infused with 18 g TG/kg/day Intralipid 20 % or saline infused control animals for 27 days. Eleven of the 12 study animals (1 from the test group, 4 from the reference group, and 7 from the control group) were excluded from the study due to microbial infection. The histopathological findings and fatty changes in the liver and spleen were comparable in the lipid emulsion treated groups. The study demonstrated a good tolerance for the high dose Fat emulsion 73403 20 % for 27 days in the study animals.

Methods

Doses: 18 g TG/kg
Frequency of dosing: 20 hrs daily for 27 days
Route of administration: Intravenous via a central venous catheter
Dose volume: 90 ml/kg
Formulation/Vehicle: 200 mg/ml (Fat emulsion 73403 20 % or
Intralipid 20 %)/0.9 % NaCl (Saline)
Species/Strain: Sprague-Dawley Rats (Male)
Number/Sex/Group: 10 males/group
Age: 40 days of age.
Weight: 158.1-182.6 g
Satellite groups: None
Unique study design: None
Deviation from study protocol: Deviation from study protocol was due to
microbial infection of some animals and
technical problems with infusion catheter

In summary, intravenous infusion of a high dose (18 g TG/kg) fat emulsion (test article) (Fat emulsion 73403 20 %) or a similarly high dose (18 g TG/kg) (reference article) (Intralipid 20 %) were similarly tolerated by Sprague-Dawley rats for 27 days. There were no significant differences observed between the test and reference animal groups in body weight gain and organ weights, clinical chemical and morphological findings.

Eleven of the 12 study animals (1 from the test group, 4 from the reference group, and 7 from the control group) were excluded from the study due to microbial infection. Most of the clinical, hematology, and blood chemistry changes were slight, similar in the two treatment groups, not considered to be of toxicological importance, and were found mainly in the animals excluded from the study.

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

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

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

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

Study title: Repeated Dose Toxicity of (b) (4) % via the Intravenous route in the Beagle Dog: 30 Days Comparison with Intralipid 20 %

Study no.: (b) (4)

Study report location: Pages 2 to 299 of the electronic submission

Conducting laboratory and location: (b) (4)

Date of study initiation: August 1985

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) %, lot # (b) (4); Intralipid 20 %, lot # (b) (4)

Key Study Findings

(b) (4) % and Intralipid 20 % IV infusion in Beagle dogs, at a daily dose of 45 ml/kg (9 g/kg) for 30 days were well tolerated, without adverse changes. Mild, but similar changes observed for both (b) (4) % and Intralipid 20 % include decreased levels of RBCs, hemoglobin, hematocrit, increase in reticulocytes, alkaline phosphatase, bilirubin, cholesterol and phospholipids.

Methods

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

Observations and Results

Mortality

No mortality was recorded.

Clinical Signs

Animals were observed daily from the start of infusion to its completion.
Sporadic vomiting in 2 female dogs dosed (b) (4) % on days 24, 26, and 28

Body Weights

The animals were weighed twice weekly at regular intervals from study day 7.
Increased mean weight gain (+ 1.20 kg) in animals dosed with (b) (4) % (between day 1 and day 29) was higher than control animals (+ 0.18 kg), or animals dosed with Intralipid 20 % (+ 0.15 kg). The differences are not statistically significant.

Feed Consumption

Food consumption of the animals was recorded daily.

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

Ophthalmoscopy

Ophthalmologic examination was performed on study day 27.

No ocular anomalies were reported with (b) (4) % or Intralipid 20 % infusion for 1 month.

ECG

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

The heart rate of the animals was not changed by test article or reference article infusion, when compared to that of control animals.

Hematology

Blood samples were obtained from animals prior to start of IV infusion on study day 0, and on days 7, 14, 21, and 30.

Changes observed include moderate fall in red blood cell counts (RBC) in (b) (4) % treated animals (from 6.27 to 5.90×10^{12} cells/L), and in Intralipid 20 % treated animals (from 6.52 to 6.06×10^{12} cells/L) when compared to control animals. The difference between the two treatment groups was not statistically significant. There were also decreases in hematocrit values from 0.49 to 0.41 ml/dl, and from 0.52 to 0.42 ml/dl in the (b) (4) % and in the Intralipid 20 % animals respectively, when compared to control animals. Decreases in hemoglobin levels from 10.05 to 8.4 mM/L, and from 10.9 to 8.9 mM/L were also observed respectively in the (b) (4) % and in the Intralipid 20 % animals, when compared to control animals. However, none of the differences between the two treatment groups were statistically significant.

Other changes observed were elevation in blood sedimentation rate from 14 to 63 mm/hr and from 7 to 64 mm/hr, and elevation in reticulocyte count from 0.049 to 0.187×10^{12} cells/L and from 0.032 to 0.139×10^{12} cells/L for (b) (4) % and Intralipid 20 % animals respectively, when compared to control animals. However, none of the differences between the two treatment groups were statistically significant.

The platelet counts on day 21 and day 30 are identical in animals from the two lipid emulsion treatment groups, but lower than the platelet counts in the control animals.

Clinical Chemistry

For clinical chemistry, blood samples were obtained from animals prior to start of IV infusion on study day 0, and on days 7, 14, 21, and 30.

Changes observed in clinical chemistry parameters include elevation in total cholesterol and phospholipid levels in (b) (4) % and Intralipid 20 % treated animals, when

compared to control animals. The differences between the two treatment groups were not statistically significant.

Elevation in liver alkaline phosphatase and bilirubin levels from 1.55 to 6.53 $\mu\text{M/L}$, and 1.73 to 4.65 $\mu\text{M/L}$, respectively in (b) (4) % and Intralipid 20 % treated animals were observed, in comparison to control animal values. However, differences between the two treatment groups were not statistically significant.

Urinalysis

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

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

Gross Pathology

Each animal was sacrificed by injection of sodium pentobarbital and bleeding by catheterization of the carotid artery.

The following organs were examined at autopsy:

Liver, Kidneys, Lungs, Spleen, Stomach, and Duodenum

Observed changes in the treated animals include small areas on liver lobes of 2 group A ((b) (4) % treated) animals, and pale yellow coloration of the liver in group B (Intralipid 20 % treated) animal. There were areas of congestion and yellow coloration in the cortico-medullary zones of the kidneys in one animal in each of the treatment group.

There were hemorrhagic points in the lungs of 1 animal, hypertrophy of the spleen in 1 animal, and zones of inflammation in the stomach (1 animal) and duodenum (2 animals) in the (b) (4) % treated group. Lipid inclusions were also observed in the coronary vessel of the heart in an animal in the Intralipid 20 % treated group. However, only 2 control animals were observed with white zones and hemorrhagic points in the lungs.

Organ Weights

The following organs were weighed:

- adrenal glands
- brain
- heart
- hypophysis

- kidneys
- liver
- lungs
- pancreas
- prostate
- spleen
- testicles or ovaries
- thyroids

There were no significant differences in the organ weights between the treated groups and the control animals, with the exception of the liver weight, which was higher in the animals treated with (b) (4) %, when expressed as a percentage of the body weight. The recorded values were within the physiological range, and the differences between the two treatment groups were not statistically significant.

Histopathology

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

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

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

There were no adverse effects observed with the 30-day infusion of (b) (4) % or Intralipid 20 % in Beagle dogs. The treatment related changes observed consist of hemosiderosis in Kupffer cells and splenic reticular cells and advancement of hemocatheretic processes in the splenic red pulp. An excess of lipids in hepatocytes and in the reticular cells of the splenic red pulp was also observed. The organs of animals treated with (b) (4) % or Intralipid 20 % displayed similar histological appearances, and did not show any demonstrable differences dependent on the product administered.

In conclusion, IV infusion of (b) (4) % or Intralipid 20 % at a dose of 45 ml/kg or 9 g lipids/kg (3X the maximum recommended clinical volume) in dogs at a rate of 150 ml/hr for 3 hrs (10X the maximum recommended clinical rate) daily for 30 days was tolerated without any mortality, adverse gross or histopathological effects. The observed decreases in RBCs, hematocrit and hemoglobin levels were within physiological range, while the increases in total cholesterol and phospholipids were due to the composition of the products infused.

This is not a valid toxicology study because the number of animals/group was inadequate at 2/sex/group.

Study title: 28-Day Toxicity of (b) (4) % and Intralipid 20 % Intravenous infusion to Beagle Dogs.

Study no.:	Not provided
Study report location:	Pages 1 to 121
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 5, 1985
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) %, lot # (b) (4); Intralipid 20 %, lot # (b) (4)

Key Study Findings

In (b) (4) %-treated animals, increased serum alkaline phosphatase, total lipids, phospholipids, free cholesterol and esterified fatty acids were observed, with fatty deposits in the liver, lungs and spleen. Minor, but similar findings were observed with (b) (4) % and Intralipid 20 % administration in Beagle dogs.

Methods

Doses: 45 ml/kg or 9 g/kg body weight, 4 hr/day
Frequency of dosing: Daily for 28 days
Route of administration: Intravenous
Dose volume: 45 ml/kg
Formulation/Vehicle: 9 g lipid/kg/ Ringer's solution
Species/Strain: Beagle Dog
Number/Sex/Group: 3/sex/group
Age: 12 to 16 months
Weight: 8.0 to 8.7 kg (male); 7.0 to 9.2 kg (female)
Satellite groups: None
Unique study design: None
Deviation from study protocol: N/A

Observations and Results

Mortality

No animal mortality was recorded in this study

Clinical Signs

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

Body Weights

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

Food Consumption

Food consumption was estimated daily.
Food consumption by the animals in the treatment groups was lower in comparison to the control animals for the entire test period. At the end of the infusion period, the food intake was reduced by 44 % in the male dogs, and by 50 % in the female dogs treated with (b) (4) %. The food intakes were reduced by 53 % in the male dogs and by 37 % in the female dogs treated with Intralipid 20 %.

Ophthalmoscopy

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

ECG

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

No effects of (b) (4) % or Intralipid 20 % were observed on the electrocardiogram in any of the treatment animals. Minimal changes in the heart rate of control and treated animals are attributed to the lack of relaxation during the recording period.

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

Hematology

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

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

Clinical Chemistry

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

In both (b) (4) % and Intralipid 20 % treatment groups, the activity of liver alkaline phosphatase was significantly increased, and moderate to markedly significant increases were also observed for total lipids, phospholipids, total and free cholesterol, and esterified fatty acids in the treated animals in contrast to control animals. There were also increases in bile acids in Intralipid 20 % treated animals versus control animals.

However, the values of glucose, blood urea, total protein, bilirubin, ALT and AST activities, and plasma LDH activity remain unchanged in all study animals.

Urinalysis

Urine was collected from all animals before the first infusion and in test week 3 over 5 hrs.

There were no observed treatment related changes in urinalysis in any treatment group.

Gross Pathology

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

Organ Weights

After macroscopic inspection, the weights of the following organs were determined: Heart, Liver, Brain, Lungs, Kidneys, Adrenals, Gonads, Thyroids, Thymus, Spleen and Pituitary

There were no differences in organ weights between control and treated animals.

Histopathology

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

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

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

In both (b) (4) % and Intralipid 20 % treated animals, there were accumulation of fat in the liver and mononuclear cellular proliferation (partly with siderosis, as well as infiltration of mononuclear cells), including lesions of the spleen, (sinus histiocytosis), and lungs (slight to moderate bronchopneumonia). Other changes occurring in single animal organs are regarded as spontaneous occurrences.

Histopathological Findings in the Liver of (Group 2) Male and Female Dogs Treated with (b) (4) %

- 1 m diffuse fine- to coarse-droplet fatty deposits in hepatocytes+, diffuse mononuclear cellular proliferation+, mainly periportal mononuclear cell infiltration+, congestion+
- 2 m fine-droplet fatty deposits in hepatocytes++, focal to diffuse mononuclear cellular proliferation+, focal infiltration of mononuclear cells+, siderosis of mononuclear cells(+)
- 3 m diffuse and focal proliferation of mononuclear cells+, focal infiltration of mononuclear cells+, siderosis of mononuclear cells(+), peripheral fine-droplet fatty deposits in hepatocytes++
- 4 f focal fine-droplet fatty deposits in hepatocytes++, diffuse and focal proliferation of mononuclear cells(+), individual mononuclear cellular infiltrates(+)
- 5 f moderate- to fine-droplet peripheral fatty deposits in hepatocytes+, diffuse mononuclear cellular proliferation+ with siderosis+, periportal mononuclear cell infiltration+
- 6 f fine- to moderate-droplet fatty deposits+, congestion+, diffuse mononuclear cellular proliferation+

Histopathological Findings in the Liver of (Group 3) Male and Female Dogs Treated with Intralipid 20%

- 1 m diffuse fine- to coarse-droplet fatty deposits in hepatocytes+, nodular mononuclear cellular proliferation and histiocytes with single cell necrosis+, diffuse mononuclear cellular proliferation with siderosis+
- 2 m congestion+, diffuse mononuclear cellular proliferation++, periportal infiltrates of lympho- and mononuclear cells++, diffuse fine- to coarse-droplet fatty deposits in hepatocytes+++
- 3 m diffuse fine- to coarse-droplet fatty deposits in hepatocytes+++, congestion, mononuclear cellular proliferation with siderosis+

Histopathological Findings in the Liver of (Group 3) Male and Female Dogs Treated with Intralipid 20%

- 4 f diffuse mononuclear cellular proliferation with siderosis++, periportal mononuclear cellular infiltration+, diffuse fine-droplet fatty deposits in hepatocytes++
- 5 f diffuse fine-droplet fatty deposits in hepatocytes++, diffuse and focal mononuclear cellular proliferation++ with siderosis+, periportal mononuclear cellular infiltration+
- 6 f diffuse fine-droplet fatty deposits in hepatocytes+++, diffuse and focal mononuclear cellular proliferation++ with siderosis+, mononuclear periportal cell infiltration+

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

Study title: A Tolerance Study in Dogs of the Amino Acid Solution 4203, Containing 13.5 G N/L and Electrolytes, given IV for 28 Days

Study no.: 82-5

Study report location: Page 1 to 42

Conducting laboratory and location:

(b) (4)

Date of study initiation: May 26, 1982

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Amino Acid Solution 4203, lot # 2502-001; Vamin N, lot # 181014

Key Study Findings

There were no drug-related organ toxicity in dogs treated IV for 28 days with 0.4 g N/kg/day of amino acid solution 4203 or Vamin N.

The only clinical chemistry changes observed in dogs infused with amino acid solution 4203 or Vamin N was increased bilirubin levels. There were no histopathological changes observed in the liver of the treated dogs related to the increased bilirubin levels. A slight centrilobular reticulated appearance of the hepatocytes in treated animals was observed, with a reversible liver cytoplasmic accumulation of glycogen.

Methods

Doses: 31.3 ml/kg (0.4 g N/kg/day) Amino Acid Solution 4203; 45 ml/kg (0.4 g N/kg/day Vamin N
Frequency of dosing: Daily for 6 hrs/day for 28 consecutive days
Route of administration: IV infusion via a peripheral vein in the leg
Dose volume: 31.3 ml/kg (Amino Acid Solution); 45 ml/kg (Vamin N)
Formulation/Vehicle: 0.4 g Nitrogen/kg/Ringer-Acetate Solution
Species/Strain: Beagle Dogs
Number/Sex/Group: 2/sex/group
Age: 5.5 to 8 months old.
Weight: 7.4 to 11 kg (males), 8.4 to 12.1 kg (females)
Satellite groups: None
Unique study design: None

Observations and Results

Clinical Signs

Animals were observed daily for clinical signs and symptoms of test or reference article infusion.

There was no animal mortality in this study. The observed clinical signs were vomiting in 2 male dogs (1 from the amino acid solution 4203 group, and 1 from the Vamin N group) on treatment day 22, and diarrhea on day 4 and day 22 in 2 dogs from the Vamin N group.

Body Weights

Body weight was recorded three times a week during pretest period of 14 days and throughout the study period.

There were no adverse effects on body weight gain in any treatment groups.

Feed Consumption

Food consumption was recorded daily during pretest and throughout the study period.

Food consumption was decreased to a similar extent in females from both treatment groups, and also slightly decreased in a male dog infused with Amino Acid solution 4203. Food consumption in other treated male dogs was unaffected.

Ophthalmoscopy

Ophthalmology was not performed.

ECG

ECG was not performed on the animals.

Hematology

Blood samples were collected pretest, and after 28 days of dosing, prior to animal euthanasia. Blood samples were collected 16 hours after the last IV infusion.

Hematological parameter values were within normal range, with the exception of a male and a female dog in the Vamin N group where the predose lymphocyte and eosinophil counts were at the upper normal limits, and were still increased at the end of the test period.

Clinical Chemistry

Blood samples for clinical chemistry were collected pretest, and after 28 days of dosing, prior to animal euthanasia. Blood samples were collected 16 hours after the last IV infusion.

Blood samples were also collected on day 28, just before the start of infusion, and 0.5, 1.0, and 2 hrs after completion of infusion for analysis of plasma amino acids.

The bilirubin levels were increased to a similar extent in all dogs treated with Amino Acid solution 4203 or Vamin N. Alkaline phosphatase values were also increased predose in 2 male dogs from the two treatment groups. A slight increase in urea values were also observed in all treated dogs.

Urinalysis

Urinalysis was performed on urine samples collected 1-2 days before dosing initiation, and 21-22 days after dosing. Drinking water was withdrawn from the animals prior to sampling.

There was no treatment effects observed in any of the study animal urine analysis.

Gross Pathology

Focal pneumonia was observed in 4 dogs, and believed to be related to parasitic migration. In two of the dogs, focal dark red enlargements of the spleen were seen. A congenital intestinal anomaly (Meckel's diverticulum) was seen in one dog, while a renal infarction was seen in the other dog. None of these findings were treatment-related.

Organ Weights

After macroscopic analysis, the weights of the following organs were recorded: Liver, Lungs, Kidneys, Spleen, Heart, and Brain.

All the organ weights measured were within normal limits.

Histopathology

Tissue samples were taken from tongue, salivary gland, esophagus, stomach (fundic and pyloric regions), duodenum, jejunum, ileum, caecum, colon, mesenteric lymph node, thyroid, parathyroid, pituitary gland, adrenal, liver, pancreas, myocardium, aorta, trachea, lung, skin, skeletal muscle, bone marrow, kidney, urinary bladder, testicle, epididymis, prostate (ovary, uterus, mammary gland), brain (cerebrum, pons, cerebellum and medulla oblongata), N. ischiadicus, spinal cord, thymus, spleen, cervical lymph node, tonsil and eye. Additional samples were taken from organs and tissues with pathological changes.

The tissue samples were fixed in 10 % formalin. Liver, Kidney and myocardium were also fixed in Histofix, in addition to a small sample of liver fixation in Carnoy's fluid. The right eye with the optical nerve was fixed in Zenker's fluid. Tissue samples were then dehydrated in alcohol, and embedded in paraffin, sectioned, and stained with hematoxylin-eosin and periodic acid for histopathology.

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Slight centrilobular reticulated appearance of the hepatocytes observed in all dogs was indicative of glycogen accumulation in the liver. Slight lipid deposits were also observed in perilobular hepatic cells of a male dog in the Amino Acid solution 4203 group, and in a female dog in the Vamin N treated group.

Slight lipid deposits were observed in the intermediary tubular epithelium of 3 dogs in the test article group, and 2 dogs in the reference article group. A solitary renal infarction was seen in the amino acid solution 4203 group.

Parasitic granuloma of the lungs was recorded in dogs from the test article and the reference article groups.

Slight lipid phagocytosis in the reticuloendothelial cells of the mesenteric lymph nodes was present in three dogs from the test article group and in 2 dogs from the reference group. Other findings include splenic hemorrhage in one dog from each treatment group, and a focal inflammation of the tongue in one dog from the amino acid solution 4203 group.

In conclusion, there were no treatment-related organ toxicity observed in dogs treated IV 6 hrs daily for 28 days at a dose equivalent to 0.4 g N/L of either amino acid solution 4203 (31.3 ml/kg) or Vamin N (45 ml/kg).

The number of animals used in this study was inadequate (2/sex/group), and thus, this is not a valid toxicology study.

Study title: A Tolerance Study in Dogs of L-Carnitine Solution (200 mg/ml),
Added to Intralipid 20 %, with IV Administration for 28 Days.

Study no.: 84-10
Study report location: Project No. 732, Pages 1 to 110 (b) (4)
Conducting laboratory and location: [REDACTED]
Date of study initiation: March 19, 1984
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Intralipid 20 %, lot # 293478; L-carnitine, lot # 2022, lot # 224, 104 %

Key Study Findings

Intravenous infusion of 9 g fat/kg/day Intralipid 20% only or in admixture with L-carnitine to dogs at doses of 90 mg/kg/day or 270 mg/kg/day for 28 days resulted in lower lipid content in the liver than after the administration of intralipid 20 % only.

No treatment related clinical signs were observed in dogs as a result of repeated infusion for 28 days of intralipid 20 % with or without L-carnitine. The clinical chemistry and histopathological findings were comparable between the two test groups.

Methods

Doses: Intralipid 20 % 9 g/kg; L-carnitine 90 mg/kg +
Intralipid 20 % 9 g/kg or L-carnitine 270 mg/kg +
Intralipid 20 % 9 g/kg; Control Ringer acetate 45 ml/kg
Frequency of dosing: Daily for 28 days
Route of administration: Intravenous (IV)
Dose volume: Intralipid 20 % 500 ml; L-carnitine 46.6 ml/kg, or
L-carnitine 45.5 ml/kg
Formulation/Vehicle: 1 g or 3 (1 g) ampoule of L-carnitine added to
500 ml of Intralipid 20 %/Ringer-acetate infusion
solution, 1000 ml.
Species/Strain: Beagle Dog
Number/Sex/Group: 2/sex/group
Age: 8-10 months
Weight: 7.2 to 10 kg (males), 6.9 to 8 kg (females)
Satellite groups: None
Unique study design: None
Deviation from study protocol: N/A

Observations and Results

Mortality

No animal mortality was reported in this study.

Clinical Signs

The dogs were observed daily for clinical signs.

There were no treatment-related clinical signs observed in the test animals. In the control group, a male dog vomited on treatment day 6, and a female dog had diarrhea on treatment day 24.

Body Weights

Body weight was measured twice a week during the pretest period and for the study duration. During the first two weeks of the treatment period, all treatment group male dogs had increased mean weight of 20 % (15–24 %), while the treatment group female dogs had increased mean weight of 17 % (10–21 %). The body weight remained constant during the last two weeks of the treatment period, without any weight difference between the treated groups. There were no changes in the body weight of control animals.

Feed Consumption

Food consumption was recorded daily during a 14 day pretest period, and prior to daily infusion during the treatment period.

A gradual decreased in food consumption was observed in all the 3 treatment groups during the last three weeks of treatment. However, two male dogs in the High dose L-carnitine group and a male dog in the low dose L-carnitine group ate all of their food. There were no observed differences in food consumption between the Intralipid 20 %, Intralipid 20 % + 90 mg/kg L-carnitine, and the Intralipid 20 % + 270 mg/kg L-carnitine treatment groups.

Ophthalmoscopy

Ophthalmologic examination was performed on each dog during a 5–6 days pretest period, and once at the end of the test period, prior to that day's infusion.

No treatment-related abnormality of the eyes was detected in any of the study animals.

ECG

No ECG recordings were conducted in this study

Hematology

Hematological analysis was performed on all animals once during a 14-15 day pre-treatment period, and after the 28 day dosing period, on the day of euthanasia. Blood samples were obtained from the vena cephalica of the animals in the morning, after a 16 hour fasting period.

Decreased serum iron concentrations were observed in female dogs from the L-carnitine treated groups, and in one control female dog, but the values were within normal range. Decreased total iron binding capacity (TIBC), but still within normal range was observed in most of the study animals, but more predominantly in the 3 treatment groups.

Nucleated erythrocytes were observed in blood smears from single dogs in both treated and control groups, but appear pronounced after administration of test and reference emulsion solutions. Polychromasia was observed in a male dog, and a high non-segmented neutrophil count was recorded in another male dog infused with 90 mg/kg L-carnitine.

Decreased lymphocyte counts were observed in all the treated animals, with all but two values at or below the normal limits. Increased neutrophil counts were recorded in all treated male and female animals and in one male and a female control animal. The changes in the white blood cell (WBC) count was believed to have resulted in a slight increase in leukocyte counts in most female dogs, with no changes in the WBC counts of male dogs.

Clinical Chemistry

Clinical chemistry analysis was performed on all animals once during a 14-15 day pre-treatment period, and after the 28 day dosing period, on the day of euthanasia.

Plasma L-carnitine determination was also made from blood samples collected from animals 6-7 days pretest, and 18 hrs before the last IV infusion on the 28th day. Esterified P-L-carnitine was calculated as the difference between the values of total and free P-L-carnitine.

Decreased but comparable urea values were seen in treated animals, with one animal from each of the treatment groups showing values below the normal limit. Slightly decreased urea values were also observed in three dogs from the control group.

Decreased phosphate values were observed in most of the fat emulsion treated male dogs and in 2 female dogs (one from the intralipid 20 % group and one from the L-carnitine high dose group), when compared to pretest values and control values. Increased total serum protein values was measured in a female dog from the intralipid

20 % group and in a male dog from the L-carnitine (low dose) group due to the elevation of albumin values.

Comparable (2-4 folds) increases in serum cholesterol and phospholipids of both low density lipoprotein (LDL) and high density lipoprotein (HDL) were observed in all lipid emulsion treated animals, but no significant changes in serum triglycerides were seen. Traces of lipoprotein X (LpX) were seen in serum samples from 5 dogs (2 from the high dose L-carnitine group, and 3 from the intralipid 20 % group).

At the end of the study period, elevated fasting plasma levels of free and esterified L-carnitine as compared to pre-test values were observed in all dogs receiving intralipid 20 % supplemented with 90 or 270 mg/kg L-carnitine. The increase in free L-carnitine was 10- and 6- fold respectively in the high and low dose group, while the increase in esterified L-carnitine was 6-fold in the high dose group and 5-fold in the low dose group. The infusion of Intralipid 20 % only and of Ringer-acetate did not result in any changes in plasma L-carnitine values.

Urinalysis

Urine samples for urinalysis were collected from animals once during a 13 day pretest period, and again after 22-23 days of infusion. Urine was collected in the morning after a 4 hr water withdrawal period (pretest) or withdrawal from the start of the infusion preceding the urine collection.

At the end of the study period, a slight increase in urinary volume and moderate to markedly decreased urinary density and osmolality were observed in most of the treated and control dogs. Increased number of red blood cells was seen in the urine of two female dogs, one in the L-carnitine (90 mg/kg) dose group and in one in the intralipid 20 % dose group. The red blood cells were not seen again after study day 6.

Gross Pathology

Inflammatory changes were observed in the lungs of 3 control animals, and 2 animals in each of the treated groups. Venous congestion was seen in the lymph nodes of a comparable number of control and treated animals.

Venous congestion and thrombosis was seen in the spleen of 2 control animals.

Duodenal ulceration was seen in a dog in the L-carnitine low dose group. Hemorrhage and inflammation were observed in the bladder of two 90 mg/kg L-carnitine dose group, two intralipid 20 % dose group, and one 270 mg/kg L-carnitine dose group.

Organ Weights

After macroscopic examination, the relative weights of the Liver, Spleen, Kidneys, Heart, Lungs and Brain were calculated for each animal.

There were no observed changes in organ weights.

Histopathology

Samples of the following organs and tissues were fixed in 10% neutral buffered formalin: liver (4)*, gall bladder*, pancreas*, spleen*, kidneys (2)*, adrenals*, ventricle* - fundic and pyloric regions, duodenum*, jejunum*, ileum*, caecum*, colon*, mesenteric lymph node*, skin*, mammary gland*, uterus*, ovaries*, urinary bladder*, prostate*, testes*, epididymes*, proximal femur with bone marrow*, skeletal muscle* - thigh, sciatic nerve*, tongue*, cervical lymph node*, oesophagus*, thyroid* with parathyroids, salivary gland*, trachea*, thymus*, pituitary gland*, myocardium (3)*, aorta (2)*, lungs (2)*, (tonsilla palat.), brain* - cerebrum, pons, cerebellum and medulla oblongata, spinal cord* (left eye) and i.v. injection sites*.

The right eye with the optic nerve from each dog was fixed in Davidson's fluid. Paraffin embedded sections from the tissue samples were stained in Hematoxylin-eosin and examined histopathologically. In addition, frozen sections of the liver, myocardium, kidney, lung, mesenteric lymph node and spleen were stained with oil Red O stain for fat and examined by light microscopy.

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Deposition of fine fat droplets were noticed in interstitial macrophages of the myocardium in all treated dogs (except a dog in the high dose L-carnitine group), and in macrophage of lung and mesenteric lymph nodes of all control and treated animals.

Deposition of a yellowish pigment (positive for iron in control and treated dogs, but positive for iron and fat in treated dogs) were observed in the red pulp of the spleen

Other observed changes include focal necrosis (of parasitic origin) in the liver of a dog in the intralipid 20 % dose group; foci of mononuclear cells of the liver in all treated dogs; deposition of fat containing yellow pigments in hepatocytes and kupffer cells of the liver in all treated dogs, and iron containing yellow pigments in control animals. There were no differences between the high and low L-carnitine treated groups and the reference Intralipid 20 % dose groups for the observed yellow pigment deposition. All control and treated dogs were also observed to have glycogen deposition in centrilobular regions of the liver.

Granulomatous inflammatory changes (related to the macroscopic urinary bladder lesions) were observed in 2 dogs treated with intralipid 20 % and one dog treated with low dose L-carnitine. Cysts of the pituitary (one animal each from all the dose groups), thyroid (intralipid 20 % dose group), and parathyroid (control animal) were also recorded. Partial involution of the thymus was presented in all the study animals.

In conclusion, daily infusion of Intralipid 20 % (at 9 g TG/kg/day) with or without L-carnitine at doses of 90 or 270 mg/kg/day for 28 days in dogs resulted in comparable clinical chemical and histopathological changes between the two test groups and the reference (Intralipid 20 %) group. However, the addition of L-carnitine to intralipid 20 % resulted in a lower lipid content of the liver than what was obtained with infusion of Intralipid 20 % only.

Study title: A Tolerance study in Dogs of Fat Emulsion 4501, Containing a Purified Mixture of Soybean and Borage Oils, with Intravenous Administration for 28 Days.

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

Key Study Findings

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

In the fat emulsion 4501 treated dogs, the plasma levels of gamma-linolenic acid and its chain elongation product homo-gamma-linolenic acid were significantly increased, as compared to the Intralipid 20 % treated dogs, but there was no difference in the arachidonic acid content.

Methods

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

In summary, IV infusion of 9 g TG/kg/day of fat emulsion 4501 (containing a purified mixture of 85 % soybean oil and 15 % borage oil) or 9 g TG/kg/day Intralipid 20 % for 28 days in dogs were well tolerated and did not result in any deaths or treatment related clinical signs. All the fat emulsion treated dogs showed initial increased body weight during the first two weeks of the test period, but later showed body weight gain similar to that of the pre-test period, in spite of reduced food intake. Slight anulocytosis and/or anisocytosis of no biological significance were present in blood smears from some female dogs treated with fat emulsion.

However, the clinical chemical and histopathological findings from the two treatment groups were similar to that of control group, with the exception of significantly lower lipid contents in the liver of fat emulsion 4501 treated dogs in comparison to Intralipid 20 % treated dogs.

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

Study no.: 88-20
 Study report location: Project No. 734. Page 1107 to 1265
 Conducting laboratory and location: (b) (4)
 S-112 87 Stockholm, Sweden
 Date of study initiation: November 4, 1988
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Fat Emulsion 73403 20 %, lot # 3568-177; Intralipid 20 %, lot # 39 360-51

Key Study Findings

Daily infusion of Fat emulsion 73403 to dogs for 28 days resulted in a transient but dose-related increase in beta-hydroxybutyrate, causing metabolic acidosis in the high dose group. Transient elevations of free fatty acid and triglyceride levels in plasma were dose related in fat emulsion treated animal groups. Histopathological changes were comparable between the fat emulsion test groups and the Intralipid 20 % reference group. However, the IV infusion of Fat emulsion 73403 resulted in lower fatty acid content in the liver, when compared with the Intralipid 20 % reference group, but there were no differences between the two fat emulsion 73403 test groups.

Methods

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

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

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

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

There was no untreated animal control group in this study.

Study title: A Tolerance Study in Dogs of (b) (4) % Administered Intravenously for One Month.

Study no.: (b) (4)

Study report location: Project No. 738, Page 1106 to 1233

Conducting laboratory and location: (b) (4)

Date of study initiation: October 31, 1989

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) %, lot # (b) (4)

Ringer Acetate, lot # (b) (4)

Key Study Findings

Administration of (b) (4) % (0.48 g N/kg/day) IV for 28 days in dogs resulted in moderately reduced food intake (especially in male dogs) without adverse effects on body weight. Transiently observed elevation of plasma dipeptides and branched chain amino acids became stable after one hour of (b) (4) % infusion and all administered glycyl peptides, dipeptides and corresponding free amino acids were rapidly cleared from the plasma without accumulation.

Methods

Doses: 0.48 g N/kg/day (b) (4)
 Frequency of dosing: 6 hrs daily for 28 days.
 Route of administration: Intravenous via a Peripheral leg vein
 Dose volume: 15 ml/kg/day (b) (4) %; 21 ml/kg/day (Ringer Acetate)
 Formulation/Vehicle: 0.48 g dipeptide N/kg /Ringer Acetate
 Species/Strain: Beagle Dogs
 Number/Sex/Group: 3/sex/group
 Age: 29-39 weeks old.
 Weight: 11.5-16.7 kg (males); 8.2-15.0 kg (females)
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: Minor deviation from protocol by inadvertent injection of test article subcutaneously.

Observations and Results

Mortality

Animals were checked daily for mortality. There were no mortalities during the treatment period.

Clinical Signs

Animals were observed for clinical signs prior to, during, and after daily infusion. Occasional vomiting during or shortly after the end of infusion were observed in both treatment and control animals, but most prevalent in control animals.

Body Weights

Body weight was recorded once a week during the pre-test and test periods. There were no differences observed in body weight gain between animals from the test group and the control group.

Feed Consumption

Food consumption was recorded once a week during the pre-test and test periods, and on the first and last day of infusion and on the day of autopsy. All male dogs treated with (b) (4) had reduced food intake (during the last 3 weeks of treatment) by a mean of 20-40 % in comparison to pre-test food intake, whereas two of the treated female dogs reduced their food intake by 15-20 % during the same time period. A slight to moderate reduction in food consumption was observed in one male and one female dog receiving Ringer acetate.

Ophthalmoscopy

Ophthalmoscopic examinations were performed on each animal once before the treatment start day, and again during the last week of treatment (day 25), prior to the infusion of that day.

No abnormalities or changes seen in ophthalmoscopic examination could be attributed to treatment with (b) (4).

ECG

ECGs were recorded on each animal, once before the treatment start day, and during the last week of treatment (day 25), prior to the infusion of that day.

There were no abnormalities of ECG recordings before or after the dosing period, with the exception of tall P-waves seen in on day 25 for dog 9 of the control group.

Hematology

Hematological analysis was performed on blood samples taken from prior to the start of the dosing period (14-15 days) and on the day after the last dosage (day 29).

At the end of the treatment period, lymphocyte counts were low to moderately decreased (20 and 40 %, respectively) in 2 male dogs from the (b) (4) % treated group, and comparable reductions (10-35 %) in lymphocyte count were also observed in all males and one female dog from the control group, when compared to their pre-test values.

Clinical Chemistry

Clinical chemistry analysis was performed on blood samples taken prior to the start of the dosing period (14-15 days) and on the day after the last dosage (day 29).

Blood samples were obtained to determine ammonia levels from study animals on days 1, 10, and 20 and blood samples from dogs in the treatment (test) groups for the determination of Plasma concentrations of amino acids, dipeptides and glycyl-glutamic were also obtained.

Slight to moderately increased urea values were observed in the test group (47-142 %, mean 89 %), which were more pronounced than the increase in the control group (15-66 %, mean 36 %). The urea levels in two females treated with (b) (4) % were at the upper normal limit.

Slightly increased alanine aminotransferase (ALT) activities were observed in (b) (4) treated female dogs and in male control animals. A slight increase in the alkaline phosphatase (ALP) activities was also seen in most of the treated male dogs. Slightly elevated calcium and reduced potassium values, with changes in albumin (increase) and globulin (decrease) were observed in test and control animals, but all the values were within normal limits.

There were no consistent changes observed in blood ammonia levels after (b) (4) % administration

Transient elevation of branched chain amino acids, with steady state values obtained 1 hr after the start of infusion were observed for glycyl-glutamine (140-170 $\mu\text{mol/l}$), glycyl-tyrosine (30-40 $\mu\text{mol/l}$), glycyl-valine (40-60 $\mu\text{mol/l}$), glycyl-leucine, and glycyl-isoleucine.

Urinalysis

Urine sample for urinalysis was taken from each animal (10-14 days) before the start of treatment, and after 22-23 days of dosing.

Increased urinary density and osmolality were seen in dogs from the test group when compared to the respective pretest values, towards the end of the treatment period, but

values were within normal range. However, this was accompanied by increased urine volume in the female dogs.

Other observed changes include increased red and white blood cell counts in the urine of 2 test group dogs and 3 control group dogs; proteinuria in 2 dogs receiving (b) (4) % . These changes were attributed to the catheterization procedure for urine collection.

Gross Pathology

Ascariasis seen in all but three dogs was considered mild and unrelated to the administration of test article. Vascular/perivascular reactions seen at injection sites did not show any differences between test and control animal groups. All other changes observed were considered to be incidental or spontaneous and not related to test article treatment.

Organ Weights

The weights of the liver, kidneys, spleen, lungs, heart, and brain were recorded. There were no treatment related changes observed in the absolute and relative organ weights.

Histopathology

For histopathology, samples of the following tissues from each dog were fixed in 4 % neutral buffered formaldehyde:

liver	skin	pituitary gland
gall bladder	mammary tissue	heart - papillary muscle
pancreas	ovaries	of left ventricle, septum
spleen	uterus	and right ventricle
kidneys	urinary bladder	aorta - thoracic and (abdominal)
adrenals	prostate	lungs
stomach - fundic and	testes	(tonsilla palatina)
pyloric regions	epididymides	(left eye with optic nerve)
duodenum	bone marrow from sternum	brain - cerebrum, pons,
jejunum	skeletal muscle - thigh	cerebellum and medulla
ileum	sciatic nerve	oblongata
caecum	tongue	spinal cord at lumbar region
colon	oesophagus	injection sites
mesenteric lymph node	thyroid with parathyroids	tissues with
cervical lymph node	salivary gland - mandibular	macroscopically visible
(popliteal lymph node)	trachea	pathological lesions
(axillary lymph node)	thymus	

A small piece of liver was preserved in Carnoy's fixative while the right eye with optic nerve from each dog was preserved in Davidson's fixative. Tissues were preserved in formaldehyde, embedded in paraffin wax, and thin (4-5 µm) sections were cut and stained with hematoxylin-eosin (HE) for analysis or oil red O for fat analysis.

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Intravenous injection sites had slight to moderate perivascular hemorrhage and inflammatory changes with thrombotic masses or granulation tissue on the vessel wall, but without noticeable differences between test and control animals. Liver glycogen deposition (staining negative with diastase-PAS) was observed in all dogs from both groups.

In conclusion, IV (b) (4) % infusion at 0.48 g N/kg/day for 28 days was well tolerated in dogs with no observed treatment related clinical signs, clinical chemical or morphological evidence of organ toxicity. IV injection sites had slight perivascular hemorrhage, and inflammatory changes.

All administered glycy peptides, dipeptides and corresponding free amino acids were rapidly cleared from the plasma without accumulation.

Study title: 4-Week Subchronic Toxicity Study of SMOF 20 % by Daily 6-Hr Intravenous Infusion in Beagle Dogs

Study no.: (b) (4)

Study report location: Pages 000106 to 000145

Conducting laboratory and location: (b) (4)

Date of study initiation: October 5, 1995

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SMOF 20 %, lot # FHFE 11; (b) (4) %
(b) (4) %, lot # FG 1544

Key Study Findings

Infusion of SMOF 20 % (test article) or (b) (4) % (reference article) to dogs resulted in increased segmented neutrophil granulocytes, ESR, and reticulocytes, decreased lymphocytes, hemoglobin, platelet count, and hematocrit values, without noticeable differences between the two animal groups.

Similar increases were also observed in plasma alkaline phosphatase, total cholesterol, and acetoacetate in animals from both groups, when compared with predose values. However, the increased phospholipids, bile acids and direct bilirubin (but not total bilirubin) appeared more pronounced in (b) (4) % treated animals than in SMOF 20 % treated animals.

Methods

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

Intravenous infusion of 9 g TG/kg of SMOF 20 % or (b) (4) % was equally well tolerated, and all the animals survived till euthanasia. Clinical observation of soft feces was present in dogs of both treatment groups. Observation of reduced food consumption was observed in both animal groups, but did not affect body weight gain. The increased phospholipids, bile acids and direct bilirubin (but not total bilirubin) appeared more pronounced in (b) (4) % treated animals. However, only 2 animals/sex/group reduced the importance of this difference, since experimental animal number was inadequate.

In animals from both groups, there was moderate or marked presence of fat in liver acinar cells, slight to moderate foci of granulomatous pneumonia in the lungs and moderate interstitial nephritis in the kidneys, but without noticeable difference in severity.

Study title: (b) (4) A 13 Week Intravenous (12 hr infusion) toxicity in the Beagle Dog Followed by a 4 Week treatment-free period.

Study no.: (b) (4)

Study report location: Page 1 to 765

Conducting laboratory and location: (b) (4)

Date of study initiation: February 3, 1995

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4), lot #s (b) (4)
 Novamine
 (b) (4)

Key Study Findings

Treatment of beagle dogs with 0.94 g N/kg/day (b) (4) daily for 13 weeks resulted in slight decreases in red blood cell and platelet count that is frequently observed during long term IV infusions. Similar but transient effects were induced in the animals with Novamine 11.4 % reference article. The observed changes were reversible.

Methods

Doses:	0.94 g N/kg/day (b) (4), Novamine 11.4 %; 0 g N/kg/day (control)
Frequency of dosing:	12 hrs/day/13 weeks
Route of administration:	Intravenous infusion
Dose volume:	24 ml/kg/day (control), 42 ml/kg/day (b) (4), 52 ml/kg/day (novamine 11.4 %)
Formulation/Vehicle:	gln-gln 1.26 g, gly-tyr 0.14 g/kg/day (dipeptides) (b) (4) / 0.9 % NaCl
Species/Strain:	Beagle Dogs
Number/Sex/Group:	4/sex/group
Age:	5 months old
Weight:	5 to 9 kg (males); 6-8 kg (females)
Satellite groups:	Yes, 2/sex from the test, and control groups.
Unique study design:	No
Deviation from study protocol:	Documented technical problem with the infusion delivery system.

Observations and Results

Mortality

Mortality or morbidity checks were performed daily.
There was no mortality in any of the animal study groups.

Clinical Signs

Clinical examination was performed daily.
There were no treatment related changes in clinical observations in the test or reference groups.

Body Weights

Individual animal weights were measured once weekly.
All the animals showed normal weight increase during the study.

Feed Consumption

Food consumption by individual animal was measured daily.

All animals infused with the test article and the reference article consumed less food than the control animals, with the (b) (4) treated animals eating less food than the Novamine 11.4 % treated animals.

Ophthalmoscopy

Ophthalmological examinations was performed once pretest and during weeks 13 and 17. There were no treatment-related ophthalmological changes observed in the animals.

ECG

ECG recordings were performed pretest and at weeks 12 and 17 or 18. There were no treatment related effects on the ECG.

Hematology

Blood samples for amino acids and dipeptide plasma concentrations was collected on day 1, and at weeks 4 and 12

A slight decrease in hemoglobin concentration and packed cell volume was observed in the (b) (4) and Novamine 11.4 % treated female animals at week 4 of the treatment period. At treatment week 13, the decrease in hemoglobin and packed cell volume was observed in (b) (4) treated animals only, whereas the levels had normalized in Novamine 11.4 % treated animals. There was less than total recovery of the blood parameters in the (b) (4) treated animals at the end of the 4 week recovery period.

Slight reduction in platelet counts was observed in the (b) (4) group at week 4 and 13, but was normal at the end of the recovery period. Increased erythrocyte sedimentation rate (measured at week 13) was seen in the (b) (4) group when compared to the reference and control groups, with the difference detectable at the end of the treatment free period in male dogs only.

Clinical Chemistry

Similarly increased blood urea concentrations were observed during the first nine hours of daily infusion with the test and reference articles in comparison to control animals, but blood ammonia concentration was stable during infusion in all groups.

Slightly decreased cholesterol and phospholipids levels were observed in male dogs receiving the test and reference articles, with marked reduction in the Novamine 11.4 % group. The slight reduction in plasma albumin (relative to β and γ globulin) observed in dogs treated with (b) (4), was not present at the end of the recovery period.

Increased plasma glycyl-L-glutamine (gly-gln), but not glycyl-L-tyrosine (gly-tyr) was recorded after infusion of (b) (4), but constituent amino acids glycine, glutamine and tyrosine levels were increased in the plasma within 6 hrs of infusion.

Urinalysis

Urine collection for amino acid and dipeptide concentrations was performed at weeks 1, 5, and 11 or 12.

Urinalysis did not show any treatment related effects in the study animals.

The urinary excretion of gly-gln was less than 0.1 % of the dose infused in animals.

Gross Pathology

Clinical pathology examinations were performed pretest, during weeks 4 or 5 at the end of the treatment period, and at the end of the recovery period.

Treatment of animals with (b) (4) or Novamine 11.4 % did not induce any macroscopic changes in any group. Majority of dogs from all groups had dark areas of the lungs, which were linked to lung lobe adhesions, and enlarged thymic lymph nodes.

Organ Weights

The following organs were weighed at scheduled necropsy for all animals:

- adrenals
- brain
- heart
- kidneys
- liver
- ovaries
- pituitary
- prostate
- spleen
- testes
- thymus
- thyroids
- uterus.

The observed differences in organ weights were considered incidental.

Histopathology

Histopathological evaluation was performed at the end of the treatment period, and at the end of the recovery period.

Samples from the following organs/tissues were collected from all animals:

- adrenals
- aorta
- bone (sternum) with bone marrow
- bone marrow smears (1)
- bronchi (mainstem)
- trachea
- urinary bladder
- uterus (horns and cervix)
- all gross lesions.
- brain
- caecum
- colon
- duodenum
- epididymides
- eyes
- gall bladder
- heart
- ileum
- injection site (area surrounding the catheter tip)
- jejunum
- kidneys
- liver
- lungs
- lymph node (cervical)
- lymph node (submaxillary)
- lymph node (mesenteric)
- mammary gland
- oesophagus
- optic nerves
- ovaries
- pancreas
- parathyroids
- pituitary
- prostate
- salivary gland (submaxillary)
- sciatic nerve
- skeletal muscle
- skin
- spinal cord (cervical, thoracic, lumbar)
- spleen
- stomach
- testes
- thymus (where identified)
- thyroids

All organs/tissues were fixed and preserved in 10 % neutral formalin, with the exception of bone marrow smears (fixed in methanol), eyes (fixed in Davidson's fluid), and the lungs which were infused via the airways with 10 % neutral formalin.

Thin sections of tissues embedded in paraffin were stained with hematoxylin and eosin for microscopy.

Adequate Battery: Yes

Peer Review: No

Histological Findings

There were no histopathological changes considered related to the test or reference article.

Observed pulmonary infarcts, alveolitis, pulmonary artery thrombi and interstitial pneumonia were found in animals from all study groups.

In conclusion, treatment of beagle dogs with 0.94 g N/kg/day (b) (4) daily for 13 weeks resulted in slight decreases in red blood cell and platelet count that is frequently observed during long term IV infusions. Similar but transient effects were induced in the animals with Novamine 11.4 % (reference article). The observed changes were reversible and not sufficiently adverse to be physiologically relevant in this study.

Study title: Estimation of Long Term Toxicity in Dogs of Two Amino Acid Solutions with High Contents of Nitrogen.

Study no.:	002
Study report location:	Project Nos AA 4959, 4964, Pages 1375 to 1420
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 26, 1979
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	AA 4959, lot #s 285901, 285902 (with electrolytes; AA 4964, lot #s 288001, 288002, 288003 (without electrolytes); Vamin N, lot #s 188017, 188013, 189100

Key Study Findings

In the 56 days IV infusion of Beagle dogs with 0.4 g N/kg/day of either AA 4959 11.4 %, AA 4964 11.4 % (test articles), or Vamin N 7 % (reference article), the dogs in all three dose groups tolerated the infusion solutions well without differences. Due to high osmolality of the test solutions, differences were noted between the test and reference groups. A key finding is that amino acid solutions with high nitrogen content should be administered via a central vein, instead of a peripheral vein.

Methods

Doses:	0.4 g N/kg/day AA 4959 11.4 %, AA 4964 11.4 %, Vamin N 7 %
Frequency of dosing:	6 hrs/day/56 days
Route of administration:	Intravenous infusion via a leg peripheral vein

Dose volume: 18 g/l AA 4959 and AA 4964
Formulation/Vehicle: AA 4959 (with electrolytes) 25 ml/kg, AA 4964, Vamin N (electrolytes free) 25 ml/kg, Vamin N 45 ml/kg / Na acetate
Species/Strain: Beagle Dogs
Number/Sex/Group: 4/sex/group (AA 4959); 2/sex/group (AA 4964, Vamin N).
Age: 6.5-13 months (Male); 6.5-29 months (Female)
Weight: 9.7-13.1 kg (Males); 7.3-15.3 kg (Females)
Satellite groups: None
Unique study design: None
Deviation from study protocol: Deviation from study protocol reported as technical difficulty with animal infusion.

Observations and Results

Mortality

Mortality or morbidity checks were performed daily.

There was no mortality in any study groups.

Two female dogs in the AA 4959 group were excluded from the study and replaced because, one had problems with venipuncture, and the other dog was found to be pregnant.

Clinical Signs

Clinical examination was performed daily.

There were problems in the last half (28 days) of the test period with performing the daily leg venipunctures in the dogs given AA 4959 due to extravasations in the leg vein to be infused. To a lesser extent, the dogs receiving AA 4964 (electrolyte free) also had venipuncture problems. However, the dogs given Vamin N had no problems with venipuncture.

Body Weights

Body weight was recorded daily prior to the daily infusion.

Feed Consumption

Food intake was recorded daily.

Ophthalmoscopy

Not done

ECG

Not done

Hematology

Blood samples were taken twice before the start of the test period, and once every second week during the test.

Most blood parameters measured in the dogs were within normal limits in comparison to normal values. However, the mean corpuscular hemoglobin concentration (MCHC) show values outside of the normal range both before and during the test period in the two test groups and reference group. No changes were attributed to the test solution infusion.

Clinical Chemistry

Blood samples were taken twice before the start of the test period, and once every second week during the test.

Most clinical chemistry parameters measured in the dogs were within normal limits in comparison to normal values. However, a few parameters such as the phosphate and gamma globulin show values outside of the normal range both before and during the test period in the two test groups and reference group. No changes were attributed to the test solution infusion.

Urinalysis

Urine analysis was performed once a week after the day's infusion.

There were no presence of glucose, protein, ketone bodies and blood in the urine. The urine pH was 6-7.

Gross Pathology

The dogs were sacrificed on the day after the last infusion, and autopsy were performed on all the animals.

Macroscopic findings include well demarcated dark-red pneumonic foci in nine dogs from all the 3 groups, intestinal parasites in a few dogs, and parasitic granulomas in the kidney of one dog in the AA 4964 group and in the kidney of another dog in the Vamin N group.

Organ Weights

Ratios of Organ weight in grams/body weight in kilograms (g/kg) were recorded for the Liver, Spleen, Kidneys, Lungs, Heart, and Thymus.

There were no significant differences noted between the AA 4959 and AA 4964 groups and the group given the vamin N reference solution.

Histopathology

Histopathological examinations of Liver, Spleen, Kidneys, Lungs, Heart, Thymus, lymph nodes, pancreas, stomach, intestines, skeletal muscle, thyroid, parathyroid, adrenal gland, brain, hypophysis, salivary gland, testicle, prostate and bone marrow were performed.

Tissue samples were fixed in 10 percent neutral formalin, and liver samples were fixed in Carnoy's fluid. After fixation, tissue blocks were sectioned, alcohol dehydrated, and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin, iron stain, periodic acid Schiff (PAS), Kumick's methyl green and Ziehl-Neelsen.

Adequate Battery: Yes

Peer Review: No

Histological Findings

Histopathological changes observed, include very slight centrilobular cellular edema of the hepatocytes in all dogs, slight to moderate fattening of the epithelium of the bile ducts in two dogs in the AA 4959 group, and in three dogs in the AA 4964 group, and in one dog in the vamin N group. Pneumonic foci of parasitic origin in dogs from all groups, parasitic granulomas were observed in other organs and in the mesenteric lymph nodes (one AA 4959 dog) and in the liver (of two AA 4959 treated dogs), and one vamin N treated dog)

In conclusion, the dogs tolerated the amino acid solution AA 4959 and AA 4964 well without any observed differences in body weight, food consumption, hematology, blood chemistry and urine analysis.

The histopathological findings of slight edema of the hepatocytes were the similar and found in all dogs.

The noticeable difference between the test groups and the reference group is that the high osmolality of the Amino acid test solutions were causing extravasations of the leg veins and difficulties in the venipuncture of the leg veins to allow solution infusion.

The osmolality of AA 4959 11.4 % (test solution with electrolytes) was higher than AA 4964 11.4 % (test solution without electrolytes). The osmolality of the 2 test solutions was also higher than that of the Vamin N 7 % (the reference solution).

There was no control animal group in this study.

Study title: A Three Month Intravenous Tolerance Study in Dogs of Fat Emulsion 4501, Containing A Purified Mixture of Soybean and Borage Oils.

Study no.: 87-2
Study report location: Project No. 715. Pages 917 to 1106
Conducting laboratory and location: (b) (4)
Date of study initiation: February 2, 1987
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Fat emulsion 4501, lot # 3576-012;
Intralipid 20 %, lot # 62131; Ringer
acetate, lot # LL 47177, MH 47304

Key Study Findings

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

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

Methods

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

Observations and Results

Mortality

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

Clinical Signs

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

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

Body Weights

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

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

Feed Consumption

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

Ophthalmoscopy

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

ECG

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

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

Hematology

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

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

Decreased platelet and lymphocyte counts were observed in male dogs from the test and reference groups after 4 weeks of infusion, but tended to normal values as dosing progressed. Changes were not different between the two treatment groups.

Increased white blood cell count (seen in a male dog in the test group) and increased eosinophil (seen in a female dog from control group) persisted till the end of study.

Pretest observation of blood samples show that Normotest (NT) values of one female control dog and a male and female reference group animal were abnormal.

Clinical Chemistry

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

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

Slightly decreased urea values, but within normal limits (10-30 %) were observed in both fat emulsion 4501 and intralipid 20 % groups after the first month of the study. The urea values of the recovery dogs were however comparable to pretest values.

Increased serum cholesterol (15-25 % at week 4, and 30-35 % at week 13) and phospholipids (15-25 % at week 4, and 40 % at week 13 in female dogs) were observed

in both fat emulsion 4501 and intralipid 20 % treated animals. The serum lipid changes were similar in both treatment groups, and returned to normal after the 4 weeks recovery period. There were no changes in the serum triglyceride levels.

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

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

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

Urinalysis

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

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

Gross Pathology

On the last infusion day (Day 92), 3 males and 3 females from the test (Fat emulsion 4501) and reference (Intralipid 20 %) groups, and all the control animals were sacrificed for macroscopic analysis. The 4 (2 males/2 females) recovery animals were also sacrificed on recovery day 28/29 for macroscopic examination.

Macroscopic observations include grayish foci and reddish discolorations of the kidneys and ileo-cecal valve in the test and reference animals (during the study and recovery period), and in the lungs of all study animals, including control animals. A small number of hemorrhages were observed in the mucosa of female animals from the intralipid dose group. Perivascular reddish discolorations were observed in the limbs of all the study animals as a result of infusion catheter placements.

Organ Weights

The weights of the following organs were recorded after autopsy: Liver, Spleen, Kidneys, Heart, Lungs and Brain. Relative organ weights were calculated as percentage of body and brain weight.

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

Histopathology

Histopathological analysis of adequate battery of organs and tissues were conducted.

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Histopathological findings include fine droplet fatty changes of centrilobular distribution in the liver, fine droplets fatty change mainly in the macrophages of the red pulp in the spleen of test and reference article dose groups. However, fine droplets fatty changes of a similar degree were observed in pulmonary macrophages and in sinusoidal macrophages in the mesenteric lymph node of all the study animals. Deposition of fat droplets observed in the kidneys of all study animals were within the normal range, and the deposition of glycogen and hemosiderin (iron containing pigment) observed in all the study animals was of a comparable degree.

Observations of hemorrhage and inflammatory changes were present at the site of IV infusion, and in the urinary bladder of all study animals due to catheterizations for injections and urine sampling.

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

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

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

Study no.: 90-25

Study report location: KPO 734. Pages 871 to 1105

Conducting laboratory and location:

(b) (4)

Date of study initiation: 09-11-1990

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Fat emulsion 73403 20 %, lot # 3560-014; Intralipid 20 %, lot # 54036-51; Ringer-acetate, lot # 90E30J63, 90E08J15

Key Study Findings

Daily IV infusion of 4 g TG/kg/day of Fat emulsion 73403, consisting of medium chain and long chain triglycerides (equimolar 36:64 %, w/w) for 13 weeks at a rate of 1 g TG/kg/hr was tolerated in dogs. The infusion resulted in transient increases in plasma beta-hydroxybutyrate, triglycerides, free fatty acids, and a slight reduction in blood glucose. Increased serum lipids in animals were more pronounced in the test group than in the reference group, whereas the hematology and clinical chemistry changes were similar in the two treatment groups.

In 2 dogs from the test group, the changes in the liver and spleen were still present at the completion of the 4 week recovery period. The differences in the liver, spleen, and serum lipid levels between test and reference group animals are related to the higher molar dose of triglycerides (30 %) in fat emulsion 73403 and Intralipid 20 %, when compared with control animals.

Methods

Doses: 4 g TG/kg/day Fat emulsion 73403 20 % or Intralipid 20 %

Frequency of dosing: 4 Hrs daily for 13 weeks

Route of administration: Intravenous via catheter in leg peripheral veins.

Dose volume: 20 ml/kg/day

Formulation/Vehicle: 0.998 g/ml (fat emulsion 73403), 0.983 g/ml intralipid 20 %, 1.004 g/ml ringer-acetate solution/ringer-acetate vehicle control.

Species/Strain: Beagle Dog

Number/Sex/Group: 3 males/5 females/group (test article), 3/sex/group (Reference article and control), 2 males (untreated control)

Age: 22-27 Weeks old

Weight: 5.7-14.9 kg (males), 6.5-9.5 kg (females)
Satellite groups: Yes, 2 female dogs from the test group used for recovery.
Unique study design: No
Deviation from study protocol: Deviation was reported due to technical problems with infusion pump cassette & dosing.

Observations and Results

Mortality

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

Clinical Signs

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

There were clinical signs of vomiting in 2 female dogs receiving fat emulsion 73403 (on study day 6 and 90); one male dog (on study days 17, 22, and 45), 1 female dog (on study day 28) and another female dog (on study days 35 and 64) all receiving Intralipid 20 %. Furthermore, five of the six control dogs vomited one or more times during the infusion period.

Slight swelling of infusion sites for 2 or more days were observed in dogs that were inadvertently injected subcutaneously with small amounts of emulsions or control solution.

Body Weights

Animal body weights were recorded three times during the pretest period, and once a week during the treatment and recovery period.

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

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

Feed Consumption

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

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

Ophthalmoscopy

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

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

ECG

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

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

Hematology

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

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

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

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

Clinical Chemistry

Blood samples for clinical chemistry were obtained from each animal on days 14-16 pretest, on treatment days 29, 91, and on the 27th day of the recovery period.

Blood samples for plasma triglycerides, free fatty acids (FFA), beta-hydroxybutyrate, lactate, blood gases, and glucose were collected on treatment day 1, 19, 51, and 79

The decreased urea concentrations observed in males from the test and reference groups on study day 29 and in males and females on study day 91 were insignificant due to its similarities with pretest values.

The slight decrease in phosphate concentrations observed in females in both treatment groups on study day 29, and in both male and female animals on study day 91 were within normal range and comparable between the two treatment groups.

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

Increased serum cholesterol and phospholipids observed in fat emulsion 73403 or Intralipid 20 % groups, in comparison to pretest levels and control animal values during the treatment period, were completely reversed after a 4 week recovery period. There were however, no significant changes in serum triglyceride levels in both treatment groups.

The increases seen in plasma triglycerides and free fatty acids in fat emulsion 73403 or intralipid 20 % treated animal reverted back to normal within 20 hrs after the end of infusion.

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

Urinalysis

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

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

Gross Pathology

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

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

Organ Weights

The following organs were weighed:

brain	kidneys	lungs
heart	liver	spleen

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

Histopathology

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

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

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

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Histopathological findings include fine droplet fatty changes of centrilobular distribution in the liver of fat emulsion 73403 and Intralipid 20 % treated animals. Fatty change in

the Kupffer cells was more evident in dogs infused with Fat emulsion 73403, while the fatty change in the hepatocytes was slightly more pronounced in the Intralipid 20 % treated animals. Vacuolated cells containing lipid or brownish, iron-negative pigment, localized periportally, or occasional necrotic cells associated with inflammatory reactions were observed in the test and reference animal dose groups.

Fatty changes in the spleen including the presence of IV fat pigments in the macrophages of the red pulp were observed in all dogs from the test and reference dose groups. In 2 dogs from the test group, the changes in the liver and spleen were still present at the completion of the 4 week recovery period.

Fatty change of a similar degree was observed in macrophages in the lung, thymus, and mesenteric lymph nodes in dogs from all study animals. Deposition of glycogen with similar distribution was seen in all study animals.

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

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

In 2 dogs from the test group, the changes in the liver and spleen were still present at the completion of the 4 week recovery period when compared to control animals. The differences in the liver, spleen, and serum lipid levels between test and reference group animals, may be related to the higher molar dose of triglycerides (30 %) in fat emulsion 73403 versus Intralipid 20 %.

Study title: A Tolerance Study in Dogs of Fat Emulsion 73403 Administered IV for Eight Days.

Study no.:	90-6
Study report location:	Project No. 734. Pages 165 to 299
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 23, 1990
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat Emulsion 73403, lot # 356900-1; Intralipid 20 %, lot # 49499-51; Vasolipid 20 %, lot # 913282

Summary:

Intravenous administration of Fat emulsion 73403 (consisting of long and medium chain fatty acids, as well as medium and long chain triglycerides) at concentrations of 6, 9, or 12 g TG/kg/day, or Vasolipid 20 % at 9 g TG/kg/day, or Intralipid 20 % at 12 g TG/kg/day in male Beagle dogs resulted in vomiting in one dog from the test article high dose (12 g TG) group, and clinical signs of frequent drowsiness and vomiting were seen in dogs from the vasolipid 20 % dose group at the end of infusion day 1. There were no clinical signs seen in dogs dosed with Intralipid 20%.

Food consumption decreased in a dose responsive manner in all treatment groups, with a 50 % reduction in the high dose (12 g TG) fat emulsion 73403 and vasolipid 20 % dose groups, a 25 to 35 % reduction in the low and intermediate (6, 9 g TG) fat emulsion 73403 dose groups and the intralipid 20% group.

The observed increase in serum cholesterol and phospholipid levels were also dose related, with the test article intermediate dose group values comparable to values from the reference groups.

Infusion of the test article caused a transient increase in plasma beta-hydroxybutyrate, which was comparable at the two lower dose levels. Infusion of vasolipid 20 % resulted in metabolic effects comparable to the high dose fat emulsion 73403 effect.

6.2 Repeat-Dose Toxicity of Sodium Glycerophosphate

Effects of Chronic β -Glycerophosphate (β -GP) Administration on Growth Rate and Serum, Liver and Bile Lipid Composition in the Rat – A Toxicity Study. K.L. Raheja, W.G. Linscheer and C. Cornwall. Toxicology, 8; 115-119, 1977

In this study, male rats were administered β -Glycerophosphate (β -GP) as a dietary mixture at 190 mg/100 g body weight (1900 mg/kg) for 33 weeks. Oral administration of

190 mg/100 g body weight β -Glycerophosphate to male rats daily for 33 weeks had no effect on survival rate, body weight, hematology and liver function tests, or on serum and liver lipid concentrations. All measured organ weights were similar in control and treated animals, except for the kidneys which were heavier in β -GP fed rats. Although biliary cholesterol and bile acid concentrations were not changed, biliary phospholipids and liver phospholipids concentration significantly increased in β -GP fed rats.

Seven out of nine control rats and 8 out of 9 study group rats survived the 33 week study duration, with enlargements of the kidneys observed in the treated rats, but without any histopathological changes. The hyperplasia of the kidneys in the treated rats was attributed to the high (1.27 g/day) sodium intake

β -GP significantly increased biliary phospholipid concentrations (4.79 ± 0.20 vs. 3.55 ± 0.20 $\mu\text{mol/ml}$), and liver phospholipids concentrations (5117.0 ± 169.6 vs. 4180.8 ± 132.5 mg/100 g) in treated versus control rats, respectively.

In conclusion, β -GP given orally at a dose of 1900 mg/kg body wt. for 33 weeks had no detrimental effects in rats; growth rate, liver enzymes, hematology and blood chemistry parameters were all normal in study animals.

Effects of Chronic β -Glycerophosphate (β -GP) Administration on Growth Rate and on Serum, Liver and Bile Lipid Composition in the Squirrel Monkey – A Toxicity Study. K.L. Raheja, W.G. Linscheer and C. Cho. Toxicology, 11; 1-4, 1978

Oral administration of 20 mg/100 g β -Glycerophosphate (β -GP) in the drinking water of squirrel monkeys daily for 15 months did not have any adverse effects on body weight, hematological or liver function tests. Although serum and hepatic bile phospholipids were increased, their concentrations were not significantly changed. Except for a slight increase in kidney weight, organ weights expressed as percent of body weight were not changed. There were no observed histopathological changes in liver or kidneys, except for a slight renal hypertrophy attributed to the sodium content of β -Glycerophosphate.

Thus, squirrel monkeys receiving β -GP at a dose of 20 mg/100 g (200 mg/kg) body weight had no adverse effects on animal growth rate, histopathological, hematological and clinical chemistry determinants. The only organ changes observed was a significant increase in kidney weight of treated animals, compared to control animals, which was attributed to the sodium salt intake from β -GP (administered orally as the disodium salt).

There was tendency for increased serum phospholipids, hepatic bile phospholipids (a 20 % increase), and bile acids, when compared to control animal values. There was a tendency also for decreased liver triglycerides and increased liver cholesterol in treated animals. Sections of livers from the two animal groups showed golden brown pigment deposition confined to the Kupffer cells, and identified as hemosiderin pigments due to Prussian blue stains.

The study concluded that the addition of 200 mg/kg of β -GP orally to the drinking water of squirrel monkeys daily for 15 months did not show any significant adverse effects in the study animals.

7 Genetic Toxicology

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

Study title: Mutagenicity Study of SMOF 20 % in the Salmonella Typhimurium Reverse Mutation Assay

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

Key Study Findings

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

Methods

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

Study Validity

The following criteria were used for determining the study validity:

None of the bacterial strains, when streaked, should grow on media plates containing biotin only.

All the bacterial strains, when streaked, should show excessive growth on media plates with L-Histidine and biotin.

Application of 0.1 % crystal paper disc on media plates seeded with bacteria should give clear zones of inhibition in all tester strains.

A 10 μ g ampicillin paper disc placed on plates seeded with bacteria should not show zones of inhibition with strains TA98 or TA 100 (indicating ampicillin resistance), but should show zones of inhibition with strains TA 1535, TA 1537, and TA 102.

Results

None of the tested 5 concentrations (ranging from 2.0 to 200 μ l) of SMOF 20 %/plate from the two independent experiments (with and without metabolic activation) possessed cytotoxic effects.

There was no mutagenic effect observed for SMOF 20 % in any of the 5 tester strains in the two independent experiments with and without metabolic activation.

The findings of the study are summarized in the sponsor's Tables below.

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

Substance (μ l/plate)	Summarized data without metabolic activation				
	revertants per plate				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
1st experiment					
SMOF 20%					
200.0	N $\pm$ SD	20.3 0.58	103.0 7.60	324.7 5.85	10.7 3.66
63.2	N $\pm$ SD	19.0 1.00	109.0 18.03	327.0 14.11	7.3 2.08
20.0	N $\pm$ SD	20.7 2.89	117.7 11.02	330.7 21.12	6.3 4.16
6.32	N $\pm$ SD	23.7 3.51	102.3 6.51	309.3 24.03	9.0 4.58
2.0	N $\pm$ SD	22.3 0.58	105.7 2.08	312.3 11.24	7.3 2.08
Solvent control: 200 μ l/plate					
	N $\pm$ SD	20.7 1.15	108.0 9.17	343.0 7.09	11.3 4.73
Positive control:					
substance	2-Nitro- fluorene	Sodium- azide	Methyl methane sulfonate	Sodium- azide	9-Amino- acridine
Concentration: (μ g/plate)	10	10	1300	10	100
	N $\pm$ SD	599.7 49.07	1425.7 59.18	557.7 95.50	2035.7 106.64
					3305.3 211.55

N = mean number of revertants
SD = standard deviation

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

Summarized data without metabolic activation						
Substance (μ l/plate)	revertants per plate					
	TA 98	TA 100	TA 102	TA 1535	TA 1537	
2nd experiment						
SMOF 20%						
200.0	H $\pm$ SD	20.7 2.08	101.7 1.53	319.3 15.04	6.0 1.73	4.7 0.58
63.2	H $\pm$ SD	21.7 0.58	101.0 1.73	318.3 29.19	5.3 1.15	5.0 1.73
20.0	H $\pm$ SD	21.0 1.00	101.3 6.81	315.3 4.51	6.0 2.00	5.0 1.00
6.32	H $\pm$ SD	20.7 2.08	101.0 1.00	322.3 16.56	4.0 0.00	4.7 3.79
2.0	H $\pm$ SD	21.0 1.00	102.0 1.00	328.7 22.03	6.7 2.08	6.0 1.73
Solvent control: 200 μ l/plate						
	H $\pm$ SD	20.7 0.58	102.7 3.79	332.0 11.27	6.3 1.15	5.7 0.58
Positive control:						
substance	2-Nitro- fluorene	Sodium- azide	Methyl methane sulfonate	Sodium- azide	9-Amino- acridine	
Concentration: (μ g/plate)	10	10	1300	10	100	
	H $\pm$ SD	847.7 112.04	1819.3 101.16	858.3 78.44	1862.3 216.18	4195.7 64.3

M = mean number of revertants
SD = standard deviation

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

Summarized data with metabolic activation						
Substance (μ l/plate)	revertants per plate					
	TA 98	TA 100	TA 102	TA 1535	TA 1537	
1st experiment						
SMOF 20%						
200.0	H	23.7	113.3	232.7	9.7	5.3
	$\pm$ SD	3.79	13.50	19.22	1.15	1.15
63.2	H	24.7	111.7	308.0	10.0	4.7
	$\pm$ SD	5.69	3.05	9.85	3.61	2.03
20.0	H	23.0	103.7	307.7	12.0	6.3
	$\pm$ SD	3.46	4.64	10.12	6.00	2.52
6.32	H	30.0	118.3	319.7	11.0	7.0
	$\pm$ SD	1.73	17.39	5.69	1.00	1.00
2.0	H	29.3	104.0	312.3	10.0	6.0
	$\pm$ SD	2.69	6.93	15.37	1.00	3.46
Solvent control: 200 μ l/plate						
	H	25.7	100.3	327.0	10.3	6.7
	$\pm$ SD	3.21	6.43	13.0	4.04	1.53
Positive control: substance						
2-Aminanthracene						
Concentration: (μ g/plate)						
	2	2	2	2	2	
	H	993.0	968.3	709.7	154.0	144.7
	$\pm$ SD	112.05	70.07	122.40	7.55	5.03

N = mean number of revertants
SD = standard deviation

H = mean number of revertants
SD = standard deviation

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

Substance (μ l/plate)	Summarized data with metabolic activation				
	revertants per plate				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
2nd experiment					
SMOF 20%					
200.0	H $\pm$ SD	24.7 4.04	105.3 3.51	309.3 12.50	7.7 0.58
63.2	H $\pm$ SD	25.0 3.46	103.7 5.69	335.0 35.0	6.7 1.53
20.0	H $\pm$ SD	24.0 4.36	105.7 9.29	321.7 24.58	7.7 1.15
6.32	H $\pm$ SD	26.0 6.93	107.0 11.79	335.0 30.27	5.3 2.31
2.0	H $\pm$ SD	22.0 1.73	106.7 5.65	348.3 21.05	4.7 0.58
Solvent control: 200 μ l/plate					
	H $\pm$ SD	25.3 3.21	107.0 6.00	343.0 15.10	8.7 0.58
Positive control: substance					
2-Aminanthracene					
Concentration: (μ g/plate)					
	2	2	2	2	2
	H $\pm$ SD	1328.3 246.27	1365.3 116.62	858.0 42.93	154.7 11.37
					133.3 10.69

H = mean number of revertants
SD = standard deviation

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Mutagenicity Study of SMOF 20 % in Mammalian Cells (V79) in the *in vitro* Gene Mutation Assay (HPRT Test)

Study no.: 10554/97

Study report location: Pages 000245 to 000272

Conducting laboratory and location:

(b) (4)

Date of study initiation: September 12, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SMOF 20 %, lot # EFC 385; Aqua ad
iniectabilia, lot # 6291A41A

Key Study Findings

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

Methods

Cell line: HPRT-V79 mammalian cells

Concentrations in definitive study: 0.313 % to 5.0 % (v/v) SMOF 20 %

Basis of concentration selection: Highest concentration of test compound was tolerated by the cells.

Negative control: Dimethylsulfoxide (DMSO)

Positive control: Ethyl methanesulfonate (EMS) (absence of metabolic activation); 9,10-dimethyl-1,2-benzanthracene (DMBA) (presence of metabolic activation)

Formulation/Vehicle: Water for Injection

Incubation & sampling time: 24 hrs, and 4 hrs

Study Validity

If the negative control (DMSO) and positive controls (EMS, DMBA) show normal results, and if the test compound does not increase the mutation frequency 2-fold above the mean of the negative control or if the mutation frequency is always lower than 20×10^{-6} cells in both independent experiments, the compound is considered as negative in the test.

If there is a dose dependent increase of the mutation frequency in both independent experiments (at similar concentrations) to at least 2-fold the solvent control, or if the mutation frequency is always lower than 20×10^{-6} cells in the presence and/or absence of S9 mix, the compound is considered as positive in the test.

Results

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

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

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

Results are shown in the sponsor's tables below.

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

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies		Thioguanine- resistant colonies					Total number of viable cells x 10 ⁶ exposed to thioguanine (d)	Mutation frequency x 10 ⁻⁶ (e)
			PE ₁ (a)	PE ₂ (b)							
Control: Aqua ad Infectabilia											
0	5.0	1 : 20	0.91	0.92	3	1	0	1	0	4.60	1.1
SMOF 20%											
0.313	5.0	1 : 20	1.35	1.18	7	11	5	4	4	5.80	5.3
0.625	5.0	1 : 20	0.82	0.84	6	17	17	11	9	4.70	12.8
1.25	5.0	1 : 20	0.53	0.96	14	15	22	12	12	4.90	15.8
2.5	5.0	1 : 20	0.35	0.88	3	14	9	6	1	4.40	7.5
5.0	5.0	1 : 20	0.23	1.00	11	4	14	10	9	5.00	9.8
EMS (µg/ml)											
500	1.0	1 : 20	0.19	0.41	498	483	422	399	455	2.05	1086.3
700	1.0	1 : 20	0.04	0.30	499	511	520	530	491	1.50	1700.7

for a, b, c, d, and e please see page 18

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

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE ₁ PE ₂ (a) (b)		Thioguanine- resistant colonies (c)					Total number of viable cells x 10 ⁶ exposed to thioguanine (d)	Mutation frequency x 10 ⁻⁶ (e)
Control: Aqua ad infectabilia											
0	5.0	1 : 20	1.11	0.87	3	2	4	5	5	4.40	4.3
SMOF 20%											
0.313	5.0	1 : 20	0.96	0.98	6	3	3	4	9	4.90	4.5
0.625	5.0	1 : 20	0.50	0.95	2	2	7	4	5	4.75	4.2
1.25	5.0	1 : 20	0.87	0.98	15	8	15	13	3	4.90	11.2
2.5	5.0	1 : 20	0.77	1.07	16	9	12	10	3	5.35	9.3
5.0	5.0	1 : 20	0.25	1.00	14	12	9	14	11	5.00	12.0
EMS (µg/ml)											
800	1.0	1 : 20	0.19	0.36	549	534	491	380	499	1.80	1482.8
700	1.0	1 : 20	0.06	0.25	491	464	500	495	539	1.25	1991.2

for a, b, c, d, and e please see page 18

Mutagenicity Study of SMOF 20 % in the *In Vitro* HPRT Point Mutation Assay (First experiment with S9 mix)

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE ₁ PE ₂ (a) (b)		Thioguanine- resistant colonies (c)					Total number of viable cells x 10 ⁶ exposed to thioguanine (d)	Mutation frequency x 10 ⁻⁶ (e)
Control: Aqua ad infectabilia											
0	5.0	1 : 20	0.98	1.21	2	4	4	1	3	6.05	2.3
SMOF 20%											
0.313	5.0	1 : 20	0.62	1.07	0	2	1	1	4	5.35	1.5
0.625	5.0	1 : 20	0.70	0.99	0	4	1	2	2	4.45	2.0
1.25	5.0	1 : 20	0.75	1.05	3	0	5	4	3	5.25	2.9
2.5	5.0	1 : 20	0.78	0.78	3	1	1	1	6	5.80	3.1
5.0	5.0	1 : 20	0.93	0.85	0	2	2	3	0	4.75	1.5
DMBA (µg/ml)											
20	1.0	1 : 20	0.10	0.78	338	337	335	360	333	3.90	437.7
30	1.0	1 : 20	0.68	0.75	298	258	274	273	291	3.75	372.0

for a, b, c, d, and e please see page 18

Mutagenicity Study of SMOF 20 % in the *In Vitro* HPRT Point Mutation Assay (Second experiment with S9 mix)

Compound (% concentration in the medium, v/v)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE ₁ PE ₂ (a) (b)		Thioguanine- resistant colonies (c)					Total number of viable cells x 10 ⁶ exposed to thioguanine (d)	Mutation frequency x 10 ⁻⁶ (e)
Controls: Aqua ad iniectionis											
0	5.0	1 : 20	0.95	1.01	13	5	10	18	9	5.05	10.9
SMOF 20%											
0.313	5.0	1 : 20	0.74	0.99	8	3	7	5	1	4.95	4.8
0.625	5.0	1 : 20	0.76	1.04	1	6	10	7	10	5.20	6.5
1.25	5.0	1 : 20	0.84	1.08	16	23	23	7	3	5.15	14.0
2.5	5.0	1 : 20	1.03	0.97	2	2	2	10	10	4.95	5.4
5.0	5.0	1 : 20	0.71	1.01	8	2	3	3	3	5.15	3.7
DMBA (µg/ml)											
20	1.0	1 : 20	0.81	1.01	524	511	533	539	516	5.05	512.4
30	1.0	1 : 20	0.83	1.06	421	401	410	421	405	5.30	388.3

for a, b, c, d, and e please see page 18

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: *In Vitro* assessment of the Clastogenic Activity of SMOF 20 % in Cultured Human Peripheral Lymphocytes

Study no.: 10555/97

Study report location: Pages 000273 to 000309

Conducting laboratory and location:

(b) (4)

Date of study initiation: October 6, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SMOF 20 %, lot # EFC 385; Aqua ad Iniectionis, lot # 6291A41A

Key Study Findings

SMOF 20 % at a concentration of 2.5 % in the presence and absence of metabolic activation, with two exposure times without S9, and one exposure time with S9, did not induce chromosomal or chromatic damage in human lymphocytes.

Methods

Cell line: Human lymphocyte cells
Concentrations in definitive study: 0.313 - 2.5 % SMOF 20 % or 0.625 - 5 mg/ml of exposure medium
Basis of concentration selection: Cytotoxicity
Negative control: Water for Injection
Positive control: Mitomycin C, Cyclophosphamide
Formulation/Vehicle: 125 µl/5 ml or 2.5 %/ Water for Injection
Incubation & sampling time: 24 hrs, and 4 hrs

Study Validity

The criteria for positive results and method of analysis are adequate.

The highest concentration employed in the main study (2.5 % SMOF 20 %) should suppress the mitotic index by 50 – 80 %, while the lowest dose (0.313 % SMOF 20 %) should be in the region of the solvent (negative) control.

Results

In the 4-hr exposure of 0.313, 0.625, and 1.25 % (at non-cytotoxic) concentrations of SMOF 20 % to lymphocyte cells in the absence of S9 metabolic activation, the mean incidence of chromosomal aberrations (excluding gaps) in dividing cells ranged from 1.0 % to 2.5 %. These results are within normal range of the negative control where an incidence of chromosomal aberrations (excluding gaps) of 1.5 % was observed after 4-hr exposure.

At a cytotoxic SMOF 20 % concentration of 2.5 % (mitotic index 0.68), chromosomal aberrations increased to 6.0 % (not significant at $p \leq 0.05$).

In the 4-hr exposure of 0.313, 0.625, and 1.25 % (at non-cytotoxic) concentrations of SMOF 20 % to lymphocyte cells in the presence of S9 metabolic activation, the mean incidence of chromosomal aberrations (excluding gaps) in dividing cells ranged from 1.5 % to 2.0 %. These results are within normal range of the negative control where an incidence of chromosomal aberrations (excluding gaps) of 2.0 % was observed after 4-hr exposure, as shown in the sponsor's table below.

SMOF 20 % Chromosome Aberrations in Human Peripheral Lymphocytes *In Vitro*

Treatment (%)	4-h exposure		
	Mitotic index [#]	Number of metaphases scored	% of cells with aberrations excluding gaps
<u>with metabolic activation</u>			
<u>Aqua ad infectabilia</u>			
2.5	1.00	200	2.0
<u>SMOF 20%</u>			
0.313	1.08	200	1.5
0.625	1.07	200	1.5
1.25	0.90	200	2.0
2.5	0.78	200	4.0
<u>Cyclophosphamide (µg/ml)</u>			
10.0	0.99	200	12.0 *

= mitotic index: number of metaphases/1000 cells; negative control = 1.00
 * = significantly different from negative control (p = 0.05)

SMOF 20 % at a concentration of 2.5 % (mitotic index 0.78), caused a small increase in chromosomal aberrations to 4.0 % (not significant at $p \leq 0.05$) was observed. In the 24-hr exposure of 0.313 % SMOF 20 % (at a non-cytotoxic concentration) to lymphocyte cells in the absence of S9 metabolic activation, the mean incidence of chromosomal aberrations (excluding gaps) in dividing cells was 3.5 %. This result is within normal range of the negative control where an incidence of chromosomal aberrations (excluding gaps) of 1.5 % was observed after 24-hr exposure.

SMOF 20 % Chromosome Analysis in Human Peripheral Lymphocytes *In Vitro*

Treatment (X)	4-h exposure			24-h exposure		
	Mitotic index [†]	Number of metaphases scored	% of cells with aberrations excluding gaps	Mitotic index [†]	Number of metaphases scored	% of cells with aberrations excluding gaps
<u>without metabolic activation</u>						
Aqueous of infectabilite						
2.5	1.00	200	3.5	1.00	200	1.5
SMOF 20%						
0.313	0.92	200	1.0	1.02	200	3.5
0.625	0.91	200	1.5	0.48	200	6.0
1.25	0.93	200	2.5	0.23	200	5.5
2.5	0.68	200	6.0	0.16	144	5.5
Karyomycin C (µg/ml)						
0.1	0.95	200	12.0 *	0.59	200	16.0 *

[†] = mitotic index: number of metaphases/1000 cells; negative control = 1.00
 * = significantly different from negative control (p = 0.05)

At cytotoxic concentrations (0.625, 1.25, and 2.5 %) of SMOF 20 % (mitotic indices 0.48 to 0.16), an increase in chromosomal aberrations to 5.5 % and 6.0 % (not significant at $p \leq 0.05$) was observed.

The incidence of chromosomal aberrations (excluding gaps) of the negative controls was 0.0 % to 5.0 % for the last 30 experiments.

Thus, SMOF was not considered genotoxic under the conditions of the assay.

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

Study title: Salmonella typhimurium and Eschericia coli Reverse Mutation Assay according to the treat and Plate Method with Neoven

Study no.: 1128801

Study report location: Pages 1 to 31

Conducting laboratory and location:

(b) (4)

Date of study initiation: October 18, 2007

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Neoven, lot # G090042, 10 %; De-ionized water, lot # 260907, 241007.

Key Study Findings

Neoven is considered to be non-mutagenic in the Salmonella typhimurium and Eschericia coli reverse mutation assay.

Methods

Strains:	Salmonella typhimurium strains, TA 1535, TA 1537, TA 98, TA 100 ; E. coli strain WP2 uvrA
Concentrations in definitive study:	20; 30; 40; 60; 80; and 100 %
Basis of concentration selection:	Highest concentration of test compound was tolerated by the cells.
Negative control:	Solvent control, and concurrent untreated cells
Positive control:	Sodium azide (NaN ₃), 4-nitro-o-phenylene-diamine (4-NOPD), methyl methane sulfonate (MMS), 2-aminoanthracene (2-AA)
Formulation/Vehicle:	Dissolved in de-ionized water or DMSO
Incubation & sampling time:	3 Hr

Study Validity

The Bacterial Reverse Mutation Assay showed regular background growth in the negative and solvent control groups with spontaneous reversion rates of the negative and solvent control in the historical data range. The positive control substances produced a significant increase in mutant colony frequencies.

Results

There was no substantial increase in the revertant colony numbers of any of the five tester strains following treatment with Neoven at any concentration level, either in the presence or absence of metabolic activation with S9 mix. There was no tendency of higher mutation rates with increasing concentrations.

Positive controls used as positive controls showed a distinct increase in induced revertant colonies

Although the number of colonies did not reach the lower limit of historical control data for strain TA 98 (in the solvent control with S9 mix) and strain TA 100 (in the untreated control without S9 mix), the deviation is rather small (based on statistical control) and has no detrimental impact on the study outcome.

Mutagenicity Study of Neoven in Bacterial Strains of Salmonella Typhimurium and Eschericia coli (Study 1)

Metabolic Activation	Test Group	Dose Level (%/plate)	Revertant Colony Counts (Mean $\pm$ SD)				
			TA 1535*	TA 1537*	TA 98	TA 100	WP2 uvrA
Without Activation	Deionised water		10 $\pm$ 3	7 $\pm$ 1	51 $\pm$ 6	107 $\pm$ 7	42 $\pm$ 6
	Untreated Neoven		11 $\pm$ 4	11 $\pm$ 3	48 $\pm$ 6	94 $\pm$ 9	41 $\pm$ 8
		10 %	11 $\pm$ 3	7 $\pm$ 1	50 $\pm$ 5	115 $\pm$ 19	34 $\pm$ 3
		20 %	12 $\pm$ 4	7 $\pm$ 1	54 $\pm$ 3	109 $\pm$ 15	41 $\pm$ 3
		40 %	11 $\pm$ 4	8 $\pm$ 1	43 $\pm$ 11	113 $\pm$ 7	42 $\pm$ 6
		60 %	12 $\pm$ 3	8 $\pm$ 2	45 $\pm$ 2	112 $\pm$ 6	41 $\pm$ 6
		80 %	10 $\pm$ 2	8 $\pm$ 1	46 $\pm$ 3	112 $\pm$ 6	36 $\pm$ 9
		100 %	11 $\pm$ 2	7 $\pm$ 1	31 $\pm$ 3	108 $\pm$ 3	34 $\pm$ 7
	NaN3	10 μ g	201 $\pm$ 20			1650 $\pm$ 132	
	4-NOPD	10 μ g			476 $\pm$ 17		
	4-NOPD	50 μ g		99 $\pm$ 5			
	MMS	3.0 μ L					437 $\pm$ 14
With Activation	Deionised water		11 $\pm$ 4	8 $\pm$ 1	29 $\pm$ 6	124 $\pm$ 8	43 $\pm$ 3
	Untreated Neoven		13 $\pm$ 5	9 $\pm$ 2	29 $\pm$ 6	107 $\pm$ 8	48 $\pm$ 4
		10 %	11 $\pm$ 4	9 $\pm$ 2	28 $\pm$ 6	113 $\pm$ 12	40 $\pm$ 4
		20 %	13 $\pm$ 3	7 $\pm$ 1	37 $\pm$ 4	110 $\pm$ 7	42 $\pm$ 9
		40 %	12 $\pm$ 4	7 $\pm$ 1	31 $\pm$ 3	117 $\pm$ 19	44 $\pm$ 4
		60 %	10 $\pm$ 3	8 $\pm$ 2	33 $\pm$ 4	119 $\pm$ 13	50 $\pm$ 9
		80 %	11 $\pm$ 3	9 $\pm$ 3	35 $\pm$ 7	112 $\pm$ 12	50 $\pm$ 9
		100 %	12 $\pm$ 3	7 $\pm$ 1	33 $\pm$ 4	97 $\pm$ 2	35 $\pm$ 3
	2-AA	2.5 μ g	132 $\pm$ 5	132 $\pm$ 18	1008 $\pm$ 24	1543 $\pm$ 53	
	2-AA	10.0 μ g					305 $\pm$ 10

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Mutagenicity Study of Neoven in Bacterial Strains of Salmonella Typhimurium and Eschericia coli (Study 2)

Metabolic Activation	Test Group	Dose Level (%plate)	Revertant Colony Counts (Mean $\pm$ SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Deionised water		12 $\pm$ 3	10 $\pm$ 2	19 $\pm$ 2	94 $\pm$ 3	39 $\pm$ 7
	Untreated Neoven		12 $\pm$ 4	9 $\pm$ 3	19 $\pm$ 5	96 $\pm$ 1	49 $\pm$ 4
		20%	10 $\pm$ 2	11 $\pm$ 2	26 $\pm$ 9	104 $\pm$ 18	42 $\pm$ 7
		30%	13 $\pm$ 2	12 $\pm$ 3	20 $\pm$ 5	92 $\pm$ 4	42 $\pm$ 5
		40%	11 $\pm$ 2	12 $\pm$ 3	23 $\pm$ 6	105 $\pm$ 9	38 $\pm$ 8
		60%	14 $\pm$ 4	8 $\pm$ 2	23 $\pm$ 3	100 $\pm$ 6	47 $\pm$ 6
		80%	9 $\pm$ 3	11 $\pm$ 5	24 $\pm$ 3	112 $\pm$ 4	40 $\pm$ 3
		100%	13 $\pm$ 2	12 $\pm$ 4	24 $\pm$ 3	121 $\pm$ 9	29 $\pm$ 2
	NaN3	10 μ g	182 $\pm$ 15		479 $\pm$ 15	2158 $\pm$ 116	
	4-NOPD	10 μ g					
	4-NOPD	50 μ g		146 $\pm$ 7			
	MMS	3.0 μ L					329 $\pm$ 20
With Activation	Deionised water		16 $\pm$ 6	6 $\pm$ 2	23 $\pm$ 3	120 $\pm$ 12	49 $\pm$ 7
	Untreated Neoven		14 $\pm$ 2	10 $\pm$ 4	23 $\pm$ 6	102 $\pm$ 6	60 $\pm$ 11
		20%	15 $\pm$ 3	9 $\pm$ 4	21 $\pm$ 2	117 $\pm$ 17	52 $\pm$ 8
		30%	16 $\pm$ 5	10 $\pm$ 4	25 $\pm$ 3	131 $\pm$ 9	53 $\pm$ 7
		40%	19 $\pm$ 3	9 $\pm$ 2	23 $\pm$ 7	117 $\pm$ 11	51 $\pm$ 1
		60%	16 $\pm$ 3	12 $\pm$ 2	24 $\pm$ 2	126 $\pm$ 9	45 $\pm$ 5
		80%	10 $\pm$ 3	12 $\pm$ 3	26 $\pm$ 8	126 $\pm$ 9	41 $\pm$ 5
		100%	17 $\pm$ 3	14 $\pm$ 1	33 $\pm$ 2	120 $\pm$ 11	51 $\pm$ 11
	2-AA	2.5 μ g	134 $\pm$ 4	169 $\pm$ 6	567 $\pm$ 18	1074 $\pm$ 76	
	2-AA	10.0 μ g					292 $\pm$ 13

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

It can be concluded that the test item (Neoven) did not induce gene mutations by base pair changes or frame shifts in the genome of the strains used in this study.

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

Study title: Salmonella Typhimurium and Eschericia coli Reverse Mutation Assay with Glycophos

Study no.:	1225401
Study report location:	Pages 1 to 33
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 9, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Glycophos, lot # 12BIL92

Key Study Findings

Glycophos is considered to be non-mutagenic in the Salmonella typhimurium and Eschericia coli reverse mutation assay

Methods

Strains: Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100; Escherichia coli strain WP2 uvrA

Concentrations in definitive study: 33; 100; 333; 1000; 2500; and 5000 µg/plate

Basis of concentration selection: Cytotoxicity

Negative control: DMSO, De-ionized water, and concurrent untreated cells

Positive control: Sodium azide (NaN₃), 4-nitro-o-phenylene-diamine (4-NOPD), methyl methane sulfonate (MMS), 2-aminoanthracene (2-AA)

Formulation/Vehicle: 1 ml Glycophos (216 mg Na Glycerophosphate)/Dissolved in de-ionized water; or DMSO (positive controls)

Incubation & sampling time: 1 Hr.

Study Validity

The Study was considered valid, as the Reverse Mutation Assay showed regular background growth in the negative and solvent control; with spontaneous reversion rates of the negative and solvent control in the historical data range. In addition, the positive control substances produced a significant increase in mutant colony frequencies.

Results

There was no substantial increase in the revertant colony numbers for any of the five tester strains following treatment with Glycophos at any concentration level, either in the presence or absence of metabolic activation with S9 mix. There was no tendency of higher mutation rates with increasing concentrations.

Reference mutagens used as positive controls showed a distinct increase in revertant colonies

Mutagenicity Study of Na Glycerophosphate in Bacterial Strains of Salmonella Typhimurium and Eschericia coli

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 <i>uvrA</i>
Without Activation	Deionised water		16 ± 5	7 ± 0	34 ± 6	178 ± 11	48 ± 5
	Untreated		18 ± 3	9 ± 3	25 ± 3	168 ± 22	53 ± 9
	Glycophos	3 µg	15 ± 1	7 ± 2	31 ± 6	176 ± 15	49 ± 6
		10 µg	14 ± 5	8 ± 2	35 ± 8	175 ± 6	55 ± 6
		33 µg	15 ± 1	7 ± 2	35 ± 7	163 ± 7	52 ± 9
		100 µg	18 ± 2	7 ± 1	58 ± 5	164 ± 3	53 ± 8
		333 µg	15 ± 1	6 ± 1	35 ± 8	171 ± 9	50 ± 11
		1000 µg	17 ± 2	9 ± 2	35 ± 3	181 ± 16	60 ± 8
		2500 µg	17 ± 1	7 ± 2	28 ± 8	159 ± 5	55 ± 8
		5000 µg	16 ± 3	7 ± 2	35 ± 7	165 ± 17	55 ± 10
	NaN3	10 µg	2227 ± 88		497 ± 33	2628 ± 33	
	4-NOPD	10 µg					
	4-NOPD	50 µg		125 ± 11			
	MMS	3.0 µL					1081 ± 22
With Activation	Deionised water		18 ± 5	12 ± 3	37 ± 4	187 ± 13	55 ± 12
	Untreated		15 ± 1	10 ± 6	39 ± 5	182 ± 5	60 ± 16
	Glycophos	3 µg	17 ± 3	8 ± 2	37 ± 6	179 ± 12	46 ± 7
		10 µg	15 ± 0	11 ± 5	31 ± 5	171 ± 22	54 ± 1
		33 µg	16 ± 6	6 ± 1	29 ± 1	169 ± 22	62 ± 13
		100 µg	19 ± 2	14 ± 1	40 ± 11	179 ± 7	62 ± 7
		333 µg	15 ± 3	6 ± 1	36 ± 6	190 ± 4	54 ± 9
		1000 µg	18 ± 5	9 ± 2	42 ± 6	182 ± 14	57 ± 11
		2500 µg	16 ± 4	11 ± 1	39 ± 3	180 ± 21	54 ± 7
		5000 µg	17 ± 2	15 ± 2	36 ± 4	188 ± 21	53 ± 10
	2-AA	2.5 µg	375 ± 25	194 ± 13	1438 ± 68	2078 ± 280	
	2-AA	10.0 µg					519 ± 0

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Mutagenicity Study of Na Glycerophosphate in Bacterial Strains of Salmonella Typhimurium and Eschericia coli

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 <i>uvrA</i>
Without Activation	Deionised water		14 ± 3	10 ± 3	27 ± 7	146 ± 15	49 ± 2
	Untreated		15 ± 3	12 ± 3	23 ± 3	158 ± 6	48 ± 6
	Glycophos	33 µg	18 ± 3	9 ± 3	25 ± 11	151 ± 11	57 ± 8
		100 µg	14 ± 4	12 ± 4	22 ± 4	146 ± 10	47 ± 6
		333 µg	14 ± 4	12 ± 3	25 ± 2	160 ± 25	57 ± 8
		1000 µg	18 ± 2	11 ± 2	29 ± 6	149 ± 10	49 ± 8
		2500 µg	12 ± 7	7 ± 2	28 ± 7	140 ± 14	52 ± 6
		5000 µg	13 ± 6	13 ± 4	19 ± 3	149 ± 11	55 ± 7
	NaN3	10 µg	2804 ± 62		542 ± 26	2179 ± 32	
	4-NOPD	10 µg					
	4-NOPD	50 µg		104 ± 4			
	MMS	3.0 µL					554 ± 9
With Activation	Deionised water		21 ± 2	14 ± 5	26 ± 6	167 ± 11	61 ± 11
	Untreated		18 ± 5	16 ± 5	27 ± 10	177 ± 12	63 ± 10
	Glycophos	33 µg	16 ± 5	13 ± 3	26 ± 4	171 ± 10	63 ± 4
		100 µg	15 ± 5	11 ± 1	26 ± 6	164 ± 6	54 ± 3
		333 µg	16 ± 6	13 ± 3	31 ± 4	170 ± 24	60 ± 15
		1000 µg	20 ± 4	11 ± 1	26 ± 5	161 ± 15	60 ± 11
		2500 µg	13 ± 2	13 ± 1	31 ± 8	168 ± 8	59 ± 4
		5000 µg	18 ± 1	10 ± 2	29 ± 2	179 ± 12	63 ± 11
	2-AA	2.5 µg	327 ± 12	219 ± 23	1856 ± 55	2308 ± 172	
	2-AA	10.0 µg					218 ± 21

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

It can be concluded that the test item (Glycophos) did not induce gene mutations by base pair changes or frame shifts in the genome of the strains used in this study.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Cell Mutation Assay at the Thymidine Kinase Locus (TK[±]) in Mouse Lymphoma L5178Y Cells with Neoven

Study no.:	1128802
Study report location:	Pages 1 to 43
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 24, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Neoven, lot # G090042; De-ionized water, lot #s 280807, 120907, 121007

Key Study Findings

Neoven, a (10 %) dipeptide amino acid solution at a maximum concentration of 10 mg/ml (10 % v/v) is non-mutagenic in the mouse lymphoma thymidine kinase locus assay.

Methods

Cell line:	L5178Y
Concentrations in definitive study:	312.5 – 10,000 µg/ml
Basis of concentration selection:	Dose related toxic effect manifestation, with the highest concentration producing low level (10-20 %) cell culture growth
Negative control:	Solvent (de-ionized water) controls, concurrent untreated controls
Positive control:	methyl methane sulfonate (MMS), Cyclophosphamide (CPA) in the presence and absence of S9 mix.
Formulation/Vehicle:	Test article diluted with de-ionized water
Incubation & sampling time:	24-hrs, and 4-hrs

Study Validity

The reference mutagens used were appropriate, and showed distinct increase in induced mutant colonies, indicating assay sensitivity and validity.

Results

There was no reproducible dose-dependent increase of the mutation frequency in both main experiments up to the maximum concentration with and without metabolic activation.

In this study the range of the untreated and solvent controls was from 81 up to 167 mutant colonies per 10^6 cells; the range of the groups treated with the test item were from 71 up to 202 mutant colonies per 10^6 cells.

MMS (at 19.5 $\mu\text{g/ml}$ and 13.0 $\mu\text{g/ml}$ in experiments 1 and 2, respectively), and CPA (at 3.0 and 4.5 $\mu\text{g/ml}$) were used as positive controls, and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies.

Mutagenicity Study of Neoven (10 % dipeptide amino acid solution) in Mouse Lymphoma L5178Y Cells.

			relative	relative	mutant		relative	relative	mutant	
	conc. μg	99	cloning	total	colonies/		cloning	total	colonies/	
	per mL	mix	efficiency 1	growth	10^6 cells	threshold	efficiency 1	growth	10^6 cells	threshold
Column	1	2	3	4	5	6	7	8	9	10
Experiment I / 4 h treatment			culture I				culture II			
Untreated control with medium	-	-	100.0	100.0	133		100.0	100.0	136	
Solvent control with water	-	-	100.0	100.0	114	240	100.0	100.0	99	225
Positive control with MMS	19.5	-	49.8	33.3	333	240	52.1	18.5	321	225
Neoven	312.5	-	109.5	culture was not continued*			121.6	culture was not continued*		
Neoven	625.0	-	111.8	179.7	71	240	121.6	85.9	117	225
Neoven	1250.0	-	105.5	124.8	82	240	98.5	81.4	108	225
Neoven	2500.0	-	126.2	95.0	149	240	115.5	90.7	122	225
Neoven	5000.0	-	123.5	54.5	182	240	94.1	69.6	150	225
Neoven	10000.0	-	111.6	138.4	87	240	123.8	99.1	114	225
Untreated control with medium	+	+	100.0	100.0	112		100.0	100.0	155	
Solvent control with water	+	+	100.0	100.0	167	293	100.0	100.0	126	252
Positive control with CPA	3.0	+	9.6	10.7	554	293	10.3	10.0	326	252
Positive control with CPA	4.5	+	1.0	1.4	575	293	1.0	1.4	347	252
Neoven	312.5	+	58.5	culture was not continued*			108.5	culture was not continued*		
Neoven	625.0	+	81.0	88.0	162	293	125.1	100.5	140	252
Neoven	1250.0	+	96.3	95.8	143	293	93.9	73.5	148	252
Neoven	2500.0	+	98.3	87.9	172	293	147.5	98.5	126	252
Neoven	5000.0	+	102.0	119.1	138	293	112.3	77.3	120	252
Neoven	10000.0	+	102.0	75.0	202	293	95.4	92.8	102	252
Experiment II / 24 h treatment			culture I				culture II			
Untreated control with medium	-	-	100.0	100.0	112		100.0	100.0	133	
Solvent control with water	-	-	100.0	100.0	81	207	100.0	100.0	151	277
Positive control with MMS	13.0	-	8.8	8.3	711	207	21.5	12.0	597	277
Neoven	312.5	-	122.0	culture was not continued*			100.0	culture was not continued*		
Neoven	625.0	-	105.0	74.3	112	207	98.2	68.2	150	277
Neoven	1250.0	-	90.5	71.0	101	207	89.9	96.4	113	277
Neoven	2500.0	-	110.7	54.8	122	207	89.9	84.1	150	277
Neoven	5000.0	-	95.8	62.6	107	207	84.9	71.5	136	277
Neoven	10000.0	-	75.3	32.2	135	207	41.7	43.4	135	277

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126

culture was not continued since a minimum of four concentrations is required by the guidelines

The values printed in bold are judged as invalid, since the acceptance criteria (page 21) are not met.

In conclusion, Neoven, dipeptide amino acid (10 %) solution did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence or presence of metabolic activation.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Cell Mutation Assay at the Thymidine Kinase Locus (TK $\pm$) in Mouse Lymphoma L5178Y Cells with Glycophos

Study no.: 1225402

Study report location: Pages 1 to 43

Conducting laboratory and location:

(b) (4)

Date of study initiation: January 14, 2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Glycophos, lot # 12BIL92

Key Study Findings

Glycophos at a maximum concentration of 2160 $\mu\text{g/ml}$ (10 % v/v) was non-mutagenic in the mouse lymphoma thymidine kinase locus assay.

Methods

Cell line: L5178Y

Concentrations in definitive study: 135 – 2160 $\mu\text{g/ml}$

Basis of concentration selection: Dose related toxic effect manifestation, with the highest concentration producing low level (10-20 %) cell culture growth

Negative control: Solvent (de-ionized water) controls, concurrent untreated controls

Positive control: Methyl Methane Sulfonate (MMS) 19.5, 10.5 $\mu\text{g/ml}$ Cyclophosphamide (CPA) 3.0, 4.5 $\mu\text{g/ml}$ in the presence and absence of S9 mix.

Formulation/Vehicle: 2160 $\mu\text{g/ml}$ (glycophos) /de-ionized water

Incubation & sampling time: 24-hrs, and 4 -hrs

Study Validity

The reference mutagens used were appropriate, and showed distinct increases in induced mutant colonies, indicating assay sensitivity and validity.

Results

Following 4 and 24 hrs of treatment, and up to the maximum concentration with and without metabolic activation, there was no relevant toxic effect, as indicated by a relative total growth of less than 50 % of survival in both parallel cultures.

There was no substantial or reproducible dose dependent increase of the mutation frequency observed in both main experiments up to the maximum concentration of 2160 µg/ml, with and without metabolic activation.

In this study, the range of the mutant colonies was from 97 to 213/10⁶ cells for the solvent control, and from 61 up to 371/10⁶ cells for glycyphos treatment.

MMS (at 19.5 and 10.5 µg/ml) and CPA (at 3.0 and 4.5 µg/ml) used as positive controls, showed a distinct increase at 455 to 509/10⁶ cells and 406 to 445/10⁶ cells in induced total mutant colonies, respectively, and an increase of the relative quantity of small versus large induced colonies.

Mutagenicity Study of Na Glycerophosphate in Mouse Lymphoma L5178Y Cells.

			relative	mutant		relative	mutant	
	conc. µg	S9	total	colonies/		total	colonies/	
	per mL	mix	growth	10 ⁶ cells	threshold	growth	10 ⁶ cells	threshold
Column	1	2	3	4	5	6	7	8
Experiment I / 4 h treatment			culture I			culture II		
Solv. control with water		-	100.0	165	291	100.0	141	287
Pos. control with MMS	19.5	-	10.8	455	291	9.3	377	287
Test item	67.5	-	culture was not continued [#]			culture was not continued [#]		
Test item	135.0	-	109.6	202	291	79.1	168	287
Test item	270.0	-	103.3	243	291	70.4	166	287
Test item	540.0	-	83.3	255	291	60.3	161	287
Test item	1080.0	-	97.5	221	291	109.9	121	287
Test item	2160.0	-	98.6	239	291	111.5	136	287
Solv. control with water		+	100.0	195	321	100.0	117	243
Pos. control with CPA	3.0	+	34.2	445	321	38.9	302	243
Pos. control with CPA	4.5	+	21.8	408	321	23.9	346	243
Test item	67.5	+	culture was not continued [#]			culture was not continued [#]		
Test item	135.0	+	93.6	178	321	93.8	117	243
Test item	270.0	+	90.6	185	321	101.1	99	243
Test item	540.0	+	119.2	128	321	78.8	114	243
Test item	1080.0	+	82.5	199	321	108.4	96	243
Test item	2160.0	+	84.0	195	321	113.6	124	243
Experiment II / 24 h treatment			culture I			culture II		
Solv. control with water		-	100.0	213	339	100.0	97	223
Pos. control with MMS	10.5	-	61.8	509	339	43.1	267	223
Test item	67.5	-	culture was not continued [#]			culture was not continued [#]		
Test item	135.0	-	109.5	239	339	90.5	61	223
Test item	270.0	-	92.9	371	339	81.8	85	223
Test item	540.0	-	88.7	205	339	93.5	74	223
Test item	1080.0	-	114.4	235	339	84.4	84	223
Test item	2160.0	-	129.4	253	339	77.6	102	223

threshold = number of mutant colonies per 10⁵ cells of each solvent control plus 126

culture was not continued since a minimum of four concentrations is required by the guidelines

In conclusion, Glycophos (the test item) did not induce mutations in the mouse lymphoma thymidine kinase locus assay using L5178Y cell line, in the absence or presence of metabolic activation.

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

Study title: Micronucleus Assay in Bone Marrow Cells of the Mouse with Glycophos

Study no.:	1225403
Study report location:	Pages 1 to 32
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 17, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Glycophos, lot # 12BIL92

Key Study Findings

Glycophos at a maximum dose of 2160 mg/kg did not induce micronuclei in polychromatic erythrocytes in mouse bone marrow cells.

Methods

Doses in definitive study:	540, 1080, 2160 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Intravenous
Dose volume:	20 ml/kg
Formulation/Vehicle:	0.9 % NaCl vehicle
Species/Strain:	NMRI mouse
Number/Sex/Group:	6/sex/group
Satellite groups:	No
Basis of dose selection:	Maximum tolerated dose (MTD)
Negative control:	0.9 % NaCl, 20 ml/kg
Positive control:	Cyclophosphamide (CPA)

Study Validity

The negative and positive controls were in the range of historical control data, and the ratio of polychromatic erythrocyte to erythrocyte ratio was not less than 20 % of the negative control.

Results

The mean number of polychromatic erythrocytes was not decreased after treatment with Glycophos at a maximum concentration of 2160 mg/kg for 48 hrs in mice.

In comparison to the vehicle controls, there was no enhancement in the frequency of the detected micronuclei at the maximum 48 hr time interval and the maximum dose level of (2160 mg/kg) glycophos treatment.

Micronucleus Assay in Bone Marrow Cells of the Mouse with Na Glycerophosphate

test group	dose mg/kg b.w.	sampling time (h)	PCEs with micronuclei (%)	range	PCE per 2000 erythrocytes
vehicle	0	24	0.104	0 - 6	1175
test item	540	24	0.129	0 - 8	1102
test item	1080	24	0.083	0 - 6	1172
test item	2160	24	0.042	0 - 3	1111
positive control	40	24	2.050	16 - 95	1130
test item	2160	48	0.067	0 - 3	1206

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

Vehicle control versus test group	Significance	p
540 mg Glycophos/kg b.w.; 24 h	-	0.3481
1080 mg Glycophos/kg b.w.; 24 h	n.t.	-
2160 mg Glycophos/kg b.w.; 24 h	n.t.	-
40 mg CPA/kg b.w.; 24 h	+	< 0.0001
2160 mg Glycophos/kg b.w.; 48 h	n.t.	-

- = not significant

+ = significant;

n.t. = not tested, as the mean micronucleus frequency was not above the vehicle control value

Intravenous CPA (positive control) at 40 mg/kg showed a statistically significant increase in induced micronucleus frequency.

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

Study title: In Vivo Bone Marrow Cytogenetic Test of SMOF 20 % by IV
Administration to Sprague-Dawley Rats (Chromosomal Analysis)

Study no.: 10556/97

Study report location: Pages 1 to 38

Conducting laboratory and location:

(b) (4)

Date of study initiation: June 30, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SMOF 20 % lot # EFC 385; 0.9 % NaCl,
Lot # 7091P14F; CPA, lot # 73H 0846

Key Study Findings

SMOF 20 % at 10 g TG/kg (50 ml/kg) did not induce micronuclei in polychromatic erythrocytes in the rat bone marrow cytogenetic study.

Methods

Doses in definitive study: 10 g TG/kg

Frequency of dosing: Single IV dose

Route of administration: IV

Dose volume: 50 ml/kg

Formulation/Vehicle: NaCl vehicle solution

Species/Strain: Crl: CD Sprague-Dawley Rat

Number/Sex/Group: 5/sex/group

Satellite groups: No

Basis of dose selection: Dose selected (50 ml/kg) was based on the maximum reasonable application volume for an IV bolus injection.

Negative control: 0.9 % NaCl

Positive control: Cyclophosphamide (CPA), 27 mg/kg, IP

Study Validity

The analysis for structural aberrations of chromosome was recorded in 50 cells (50 metaphases) per animal, and cells with an incomplete number of centromeres or insufficient spreading were not used for analysis. Metaphases which differed from the normal diploid complement were excluded from evaluation.

Results

The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with SMOF 20 % ranged from 0.6 % to 0.8 % at 6, 24, and 48 hrs after administration, and are within the normal range of the negative control and not significant.

The number of cells with gaps was also within the range of the negative controls (treated groups: 7.4 % to 9.0 %; controls: 9.6 %)

In Vivo Rat Bone Marrow Cytogenetic Test of Intravenous SMOF 20 %

Compound ml/kg b.w. i.v.	Sampling time (h)	Number of metaphases analysed	Hitotic index*	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi ² -test (aberrations excluding gaps)
0.9% NaCl solution							
50 ml/kg b.w. i.v.	24	500	1.00	9.6	10.2	0.6	-
SMOF 20%							
50	6	500	0.85	9.0	9.6	0.8	n.s.
50	24	500	0.78	7.4	8.0	0.8	n.s.
50	48	500	0.76	7.8	8.0	0.6	n.s.
Cyclophosphamide							
27 mg/kg b.w. i.p.	24	500	0.30	28.6	53.8	45.6	s.
s. significant at $p \leq 0.01$ n.s. not significant at $p \leq 0.01$ * negative control = 1.00							

In conclusion, SMOF 20 % did not reveal any mutagenic properties with respect to structural chromosomal aberrations in the rat bone marrow cytogenetic study.

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Study title: Intravenous Teratogenicity Study of dipeptide, glycyl-glutamine (DP-Gln) in the Rabbit

Study no:	1021-002
Study report location:	Pages 1463 to 1788
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 14, 1992
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DP-Gln 20 lot # 3311322; 0.9 % Saline, lot #s 1228222, 1211522; Vamin 18 EF (isonitrogenous reference amino acid), lot #s 91085/91 and 91126/51

Key Study Findings

Intravenous infusion of DP-Gln 20 at 135, 270, and 540 mg N/kg or Vamin 18 EF at 540 mg N/kg for 4 hrs daily, from day 6 to day 18 (13 days) post-coitus did not induce embryotoxicity or teratogenicity in the study animals.

Methods

Doses: 0 (control); 135, 270, 540 mg N/kg/day DP-Gln 20 or Vamin 18 EF 540 mg N/kg

Frequency of dosing: 4-hrs/day/13 days

Dose volume: 6, 12, and 24 ml/kg/day (DP-Gln 20); 30 ml/kg/day Vamin 18 EF, and saline control.

Route of administration: Intravenous infusion via ear vein with infusion pumps.

Formulation/Vehicle: 13.6 % DP-Gln 20/0.9 % saline control vehicle

Species/Strain: New Zealand White Rabbit

Number/Sex/Group: 12 female rabbits/group

Satellite groups: Yes; 3 female rabbits/group for pharmacokinetics

Study design: 12 female rabbits were randomized into 5 dose groups with group 1 as control, group 2 dosed with Vamin 18 EF (reference article), and groups 3, 4, and 5 dosed with low, medium, and high concentrations of DP-Gln 20 (test article).

Deviation from study protocol: Protocol deviations were reported as inadvertent reduction in dose time and increment in dose volume in 2 treatment animals.

Study Design

Group number	Group designation	Colour code	Number of inseminated females*	Dose level ml/kg/hour	Dose level ml/kg/day	Nitrogen mg/kg/day
1	normal saline	white	18**	7.5	30	0
2	Vamin 18 EF	yellow	18**	7.5	30	540
3	DP-Gln 20-Low	blue	18**	1.5	6	135
4	DP-Gln 20-Intermediate	green	18**	3.0	12	270
5	DP-Gln 20-High	red	18**	6.0	24	540

infusion period: 4 hours/day

Observations and Results**Mortality**

All the study animals were examined twice daily at the beginning and end of the infusion daily for mortality and morbidity.

Two control animals were found dead on day 18 post-coitus (with respiratory tract signs of pneumonia), and one animal in group 3 (dosed with 135 mg/kg/day DP-Gln 20) had

subcutaneous edema on the neck, enlarged liver, dark red edema of the trachea, and heart (epicardium) ecchymosis at necropsy.

These mortalities were considered incidental.

Two moribund animals from group 3 (dosed with 135 mg/kg/day DP-Gln 20) were euthanized on days 6 and 7 post-coitus, respectively, due to vertebral column and pelvic injury.

Two control animals aborted on day 22 and day 26 post-coitus, and one animal in the Vamin 18 EF dosed group aborted on day 28 post-coitus. All abortions were post infusion and considered to be incidental.

Clinical Signs

All animals were examined at least once daily for clinical signs of ill health or toxicity.

There were no treatment related clinical signs observed in any of the study groups. Hematoma was observed at the injection site in all study animal groups, but higher incidences were noted in the control group and in the Vamin 18 EF dose group.

Body Weight

Body weight of each animal was recorded daily on day 0, day 6 to day 19, and on day 24 and day 28 post-coitus.

Mean body weight decreased in all study groups from day 6 to 7 post-coitus, especially in the Vamin 18 EF dose group; and similarly, from day 6 to 7 post-coitus, the difference in mean body weight gain from the control group was statistically significant for the DP-Gln 20 dose group 5.

Inter group differences were observed for all the study groups from days 7 to 19 post-coitus, but not dose responsive.

Statistically significant differences in mean body weight gain was observed between the DP-Gln 20 treated groups 4 and 5 when compared to the control group from day 11 to 12 post-coitus.

Feed Consumption

Food consumption of each inseminated female were recorded on days 0 to 6, day 6 to day 19, and on days 19 to 24 and 24 to 28 post-coitus.

The mean food consumption in the 540 mg/kg/day Vamin 18 EF and the DP-Gln 20 dose groups were slightly lower than in the concurrent control group during the dosing phase. Group mean food consumption was generally similar in all the other groups.

Toxicokinetics

Blood samples for toxicokinetics were collected from three animals/dose group (satellite animals) on days 6 and 18 post-coitus, prior to infusion at 2, 4, and 4.5 hrs after the start of infusion

Infusion of DP-Gln 20 at doses of 135, 270, and 540 mg N/kg/day for 13 days or Vamin 18 EF at a single dose of 540 mg N/kg/day for 13 days in treated animals resulted in increased plasma amino acids, with profiles reflecting the infused solutions, and no observed change in animals receiving the physiological saline solution.

A dose-dependent increase in amino acids and the dipeptide glycyl-glycine (gly-gln) was observed in groups receiving DP-Gln 20, with mean steady state plasma levels of 215 $\mu\text{mol/l}$ (dose level 135 ml/kg/day), 450 $\mu\text{mol/l}$ (dose level 270 ml/kg/day), and 975 $\mu\text{mol/l}$ (dose level 540 ml/kg/day) on day 6 post-coitus. For both amino acids and the dipeptides, steady state levels were reached within 2 hrs of infusion.

The corresponding plasma levels of gly-gln for the DP-Gln 20 treated animals, were approximately, 245 $\mu\text{mol/l}$, 600-750 $\mu\text{mol/l}$ and 1100 $\mu\text{mol/l}$ respectively on day 18 post-coitus.

In all the study animals, the gly-gln levels decreased when the infusion was stopped, but were still measurable at 30 min post-infusion, and low levels of gly-gln (21-60 $\mu\text{mol/l}$) were detected pre-infusion on day 18 in 4 rabbits from the two highest dose groups.

In conclusion, there were no differences in dipeptide or free amino acid plasma profiles with repeated infusions of the test or reference solutions.

There was no reported accumulation of the infused amino acids or dipeptides in pregnant rabbits receiving DP-Gln 20 or Vamin 18 EF at daily dosage up to 540 mg N/kg/day.

Necropsy

All animals were sacrificed on day 28 post-coitus, and were dissected and examined macroscopically for any pathological changes.

Animals found dead or killed after showing signs of abortion were also necropsied and examined.

The liver and kidneys were removed, weighed, and preserved in 10 % formalin.

Group mean weight of the kidneys and liver were comparable in all the study groups.

There were no treatment related changes observed in the animals during necropsy.

100 % intra-uterine deaths were observed in 3 animals from the Vamin 18 EF dose group, and one animal from the 270 mg/kg/day DP-Gln 20 dose group.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The ovaries and uteri of all the animals were removed and examined for:

Pregnancy status, number of corpora lutea in each ovary, number and position of implantations, subdivided into: Live fetuses, early resorptions, late resorptions, dead fetuses.

There was no effect of treatment on the implantation in the rabbits.

Post-implantation losses in treated animal groups 2-5 were higher than in control animals, but the values were still within the normal range.

The mean number of fetuses per doe was comparable in all groups.

The sex distribution of fetuses was similar in all groups.

Mean fetal weights for treated animal groups 2-5 were slightly lower than in control animals, although values were still within normal range, and not dose-responsive. The mean placental weight was comparable in all groups.

Offspring (Malformations, Variations, etc.)

Fetuses were euthanized, and for each live fetus, and dead fetus, the following data were recorded: external fetal abnormalities, individual fetal weight, fetal sex, and skeletal or visceral abnormalities (fixed in 95 % alcohol, and examined using Alizarin standing technique). Malformations are classified as rare and/or probably lethal. e.g. hydrocephaly.

Malformed fetuses were observed in all the study groups, including control animals.

In the control group, 5 fetuses from 5 litters showed malformations, with 4 fetuses showing skeletal malformations and 1 fetus with fused caudal vertebrae.

In the Vamin 18 EF dose group 2, two fetuses from 2 litters had malformations, with 1 fetus showing malformations such as displaced ear, malformed nose rudimentary tail, hydrocephaly, and the other fetus showing malformed skeletal vertebrae.

In the 135 mg/kg DP-Gln 20 dose group 3, three fetuses from three litters had malformations, with one fetus showing malformed head and extremities, second fetus showing rudimentary kinked tail, with skeletal abnormalities of the skull bones and vertebrae, and the 3rd fetus showing retinal dysplasia on visceral examination.

In the 270 mg/kg DP-Gln 20 dose group 4, seven fetuses from seven litters had malformations. Two fetuses showed externally kinked tail, three fetuses were present with skeletal malformations of the ribs and/or vertebrae, one fetus showed cleft palate and fused skeletal sternbrae, and the 7th fetus showing internal hydrocephaly.

In the 540 mg/kg DP-Gln 20 dose group 5, eight fetuses from five litters were observed with malformations. One fetus had acaudia, including fused skeletal ribs; one fetus showed internal hydrocephaly of the brain, and the other 6 fetuses were present with skeletal malformations of sternbrae, and/or ribs, and/or vertebrae.

The total incidences of affected fetuses and litters were slightly increased in group 4 (270 mg/kg DP-Gln 20), and in group 5 (540 mg/kg DP-Gln 20) animals, in comparison to the control animals. However, the incidences were within the range of historical controls, and were comparable in all study groups.

Group Mean Cesarean Malformation and Variation Data in Rabbits

	Group 1 - 7.5 ml/kg/h	Group 2 - 7.5 ml/kg/h	Group 3 - 1.5 ml/kg/h
Total number of litters			
with live fetuses	12	11	14
Total number of live fetuses	104	93	114
Number of fetuses examined externally	104	93	114
Number of fetuses examined viscally	104	92	113
Number of fetuses examined skeletally	104	92	113
Number of fetuses with external/visceral malformations	1	1	3
Number of litters affected	1	1	3
Number of fetuses with skeletal malformations	5	1	1
Number of litters affected	5	1	1
Total number of malformed fetuses	5	2	3
Average per cent malformed fetuses	4.8	2.2	2.6
Total number of litters affected	5	2	3
Percentage of litters examined	41.7	16.2	21.4
Number of fetuses with external/visceral variations	5	5	2
Number of litters affected	4	5	2
Number of fetuses with skeletal variations	103	92	113
Number of litters affected	12	11	14

Group Mean Cesarean Malformation and Variation Data in Rabbits

	Group 4 - 3.0 ml/kg/h	Group 5 - 6.0 ml/kg/h
Total number of litters		
with live fetuses	16	13
Total number of live fetuses	147	113
Number of fetuses examined externally	147 (1)	113
Number of fetuses examined viscally	147	113
Number of fetuses examined skeletally	147	113
Number of fetuses with external/visceral malformations	4	2
Number of litters affected	4	2
Number of fetuses with skeletal malformations	4	7
Number of litters affected	4	4
Total number of malformed fetuses	7	8
Average per cent malformed fetuses	4.8	7.1
Total number of litters affected	7	5
Percentage of litters examined	43.8	38.5
Number of fetuses with external/visceral variations	9	6
Number of litters affected	5	5
Number of fetuses with skeletal variations	145	111
Number of litters affected	16	13

Remark: Number(s) in parenthesis refer to dead fetuses examined additionally

In conclusion, IV infusion of Vamin 18 EF at 540 mg N/kg (or 30 ml/kg/day), or DP-Gln 20 at 135, 270, and 540 mg N/kg (or 6, 12, and 24 ml/kg/day, respectively) for 4 hrs daily, from day 6 to day 18 (13 days) post-coitus did not induce any clinical signs, embryotoxicity or teratogenicity in rabbits.

Study title: Fat Emulsion 73403 (long and medium-chain triglyceride): Teratology Study in the Rabbit.

Study no:	94/0212
Study report location:	Document 21530F, Pages 832 to 975
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 30, 1992
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Fat emulsion 73403, lot # 3561-001; Intralipid 20 %, lot # 64615-51; Ringer acetate, lot # 91J30J59

Key Study Findings

The IV infusion of Fat emulsion 73403 to pregnant rabbits during the period of organogenesis, at doses of 3, and 4.5 g TG/kg/day and Intralipid 20 % at a dose of 4.5 g TG/kg/day had no adverse effect on fetal survival, and fetal growth and development *in utero* were unaffected by the fat emulsions in all treatment groups.

Fat emulsion 73403 administrations was associated with reduced food intake; and increased embryo-fetal loss, seen in Fat emulsion 73403 treatment (4.5 g TG/kg/day dose group) as indicated by abortion and increased post-implantation loss was observed in one female rabbit.

Methods

Doses:	0 (control), 3, 4.5 g TG/kg/day (Fat emulsion 73403); 4.5 g TG/kg/day Intralipid 20 %;
Frequency of dosing:	4 hr/day for 13 days (days 6 to 19 of gestation)
Dose volume:	22.5 ml/kg/day Control, (Intralipid 20 %)15, and 22.5 ml/kg/day (Fat emulsion 73403).
Route of administration:	IV via ear vein
Formulation/Vehicle:	500 ml stock/Ringer acetate vehicle control
Species/Strain:	New Zealand White Rabbits
Number/Sex/Group:	18 pregnant females/group (Control, group 1); 16 and 17 pregnant females/ group (3, 4.5 g Fat emulsion 73403, dose groups 2 and 3); 13 pregnant females/group (Intralipid 20 %, group 4);
Satellite groups:	4 pregnant females from groups 2 and 4 for TK
Study design:	4 groups of pregnant female rabbits, with 3, and 4.5 mg/kg Fat emulsion 73403 (dose groups 2 and 3); and control and Intralipid 20 % (reference article) (dose groups 1 and 4).

Deviation from study protocol:

Observations and Results

Mortality

All the study animals were examined twice daily at the beginning and end of the infusion for mortality and morbidity.

One female rabbit in group 3 (4.5 g TG/kg/day) was found dead, on day 28 post-coitus, without any untoward clinical signs, while 3 females were euthanized; one group 2 female (3 g TG/kg/day) with strangulated abdominal hernia was sacrificed on day 29 post coitus; one group 3 female sacrificed on day 28 was presented with weight loss, reduced food intake, fecal output, bradypnea; one group 3 female was sacrificed on day

10 post-coitus due to the poor ear vein condition, since dosing has to be discontinued. Necropsy of all the unscheduled dead animals revealed only non-specific findings, and was therefore not related to fat emulsion 73403 dosing.

A satellite group female rabbit from the intralipid dose group 4 died on day 12 from accidental overdose of the reference article at an unintended dose of 7.9 ml/kg/hr (31.6 ml/kg/day).

Clinical Signs

All animals were examined at least once daily for clinical signs of ill health or toxicity. The clinical conditions of the Fat emulsion 73403 treated animals and the volume control females were similar, although increased incidence of loose or reduced fecal output was recorded in females dosed with Fat emulsion 73403 or Intralipid 20 %.

Body Weight

All animals were weighed and examined daily throughout the study duration.

Measured body weights of treated and control animals were similar in spite of a slight loss in body weight by all animals at the start of infusion.

Feed Consumption

Food consumption was recorded daily for each of the study animal.

Statistically significant reduction in food intake was observed during the treatment period between the treatment group animals versus the control animals. The decreased food consumption was also dose related in the test article dose groups. The food consumption in all the animals was essentially similar during the post-treatment phase.

Toxicokinetics

Blood samples were obtained from the central auricular artery of each animal prior to, and 2 to 4 hrs after the start of dosing on gestational day 6 and on gestational day 18. Blood was analyzed for β -Hydroxybutyrate (β -HBD), Triglycerides, and lactate levels.

The plasma triglyceride levels increased during the infusion of both the test and reference articles on both gestational day 6 and 18, with a mean triglyceride level of 32 and 26 mmol/L, following infusion of Fat emulsion 73403 for 4 hrs on the respective days. Similarly, the mean triglyceride level for 4 hrs of Intralipid 20 % infusion on gestational day 6 and day 18 was 22 and 16 mmol/L.

Infusion of fat emulsion 73403 resulted in increased plasma β -HBD, which was more pronounced on day 18 (mean of 1.28 mmol/l at 4 hrs) than on day 6 (mean of 0.51 mmol/l at 4 hrs). There were no significant changes in β -HBD with the infusion of Intralipid 20 % on day 6, whereas increased values were observed on day 18 (mean of 1.07 mmol/l at 4 hrs).

The measured plasma free fatty acids (FFA) in rabbits increased during day 6 and day 18 of the test and reference fat emulsions treatment, with higher FFA levels (range 5.3-9.3 mmol/L at 4 hrs) for the test article animals than in the reference group animals (range 1.9-3.8 mmol/L at 4 hrs). The increase was generally less obvious with repeated lipid infusions.

The infusion of Fat emulsion 73403 resulted in the elevation of medium-chain free fatty acids (MCFFA), both octanoic acid (C8:0) and decanoic acid (C10:0), with values ranging from 3.3 to 5.8 mmol/L at 4 hrs after the beginning of infusion.

There were no consistent changes in plasma lactate levels observed with any of the fat emulsion dose groups.

Necropsy

At the end of dosing on day 19 of gestation, or day 29 for satellite animals, rabbits were euthanized by IV injection of Sodium Pentobarbitone prior to macroscopic examination. All animals found dead, moribund, or animals which aborted were also sacrificed and subjected to detailed examination.

Necropsy of the female rabbits on day 29 of gestations did not show any macroscopic changes considered related to fat emulsion 73403 treatment

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The ovaries and uteri of all the animals were dissected out and examined for:

1. Number of corpora lutea in each ovary
2. Number of implantation sites
3. Number of early or late resorption sites
4. Number and distribution of live and dead fetuses in each uterine horn.

A slight increase in post-implantation loss was observed in group 3 Fat emulsion 73403 (4.5 g TG/kg/day) females, in comparison to the volume control and historical control values, with the volume controls at the upper limit of the historical control range.

The number of corpora lutea, implantations, viable fetuses, pre- and post-implantation loss in group 2 (3.0 g TG/kg/day) females were unaffected by dosing.

Group Mean Litter Data in RabbitsGroup mean litter data - females killed on Day 29 of gestation

Group : 1 2 3 4
 Compound : Ringer acetate Fat emulsion 73403 Intralipid^R 20%
 Dosage (ml/kg/day) : 22.5 15 22.5 22.5

Group	Number of pregnant animals		% Abortion and total litter loss ^a	Corpora lutea count	Implantations	Viable young			Resorptions			Implantation loss (%)	
						M	F	Total	Early	Late	Total	Pre-	Post-
1	13	Mean SD	0.0	10.7 2.3	8.7 3.0	3.2 2.3	4.0 2.2	7.2 2.7	1.1 1.0	0.5 0.7	1.5 1.2	18.7	17.7
2	15	Mean SD	6.7	9.9 1.7	7.9 3.2	3.4 2.0	3.7 2.1	7.1 3.0	0.4 0.7	0.3 0.5	0.7 0.8	21.4	9.1
3	15	Mean SD	13.3	10.0 1.6	8.5 2.3	2.9 1.9	3.4 1.9	6.3 2.9	2.1 1.4	0.1 0.3	2.2 1.5	15.4	25.5
4	18	Mean SD	0.0	9.8 2.2	7.9 3.2	4.1 2.2	2.9 2.2	7.1 2.6	0.8 0.9	0.1 0.2	0.9 0.9	18.8	11.2

Background control data (69 studies)

Mean	0.2	10.7	8.8	4.1	4.0	8.0	0.4	0.5	0.9	17.3	8.9
Low	0.0	9.1	6.7	2.9	2.7	5.9	0.1	0.1	0.2	2.2	2.8
High	20.0	12.8	11.1	5.4	6.0	10.3	0.9	1.3	1.9	29.2	19.7

Means are derived from females which survived to term and bore viable young.

^a See Appendix 2.
 SD Standard deviation.

Offspring (Malformations, Variations, etc.)

Fetuses were euthanized by subcutaneous injection of Pentobarbitone sodium, and for each live fetus, and dead fetus, the following data were recorded:

1. Individual fetal weight.
2. Individual placental weight
3. Fetal sex
4. Skeletal or visceral abnormalities (fixed in 95 % alcohol, and examined using Alizarin standing technique)

Reduced fetal weights observed in group 3 and 4 animal dose groups were considered due to isolated litters with exceptionally small fetuses, and not related to treatment.

The fetal anomalies observed after skeletal examinations in all groups show incidences that have been previously seen in historical controls, and bear no consistent relationship to the Fat emulsion treatment.

Group Mean Fetal and Placental Weights (g) in Rabbits

Group : 1 2 3 4
 Compound : Ringer acetate Fat emulsion 73403 Intralipid^R 20%
 Dosage (ml/kg/day) : 22.5 15 22.5 22.5

Group	Foetal weight (g)			Placental weight (g)
		Male	Female	Overall
1	Mean	42.0	41.9	42.5
	SD	3.0	2.9	2.0
	n	11	13	13
2	Mean	43.3	40.0	42.3
	SD	1.9	2.3	1.9
	n	14	13	14
3	Mean	39.0	38.6	38.8
	SD	1.7	2.4	2.0
	n	12	13	13
4	Mean	36.2*	35.5*	36.9*
	SD	3.3	2.8	1.9
	n	17	17	18
Background control (69 studies)				
Mean			40.4	5.5
Low			36.1	4.9
High			45.3	6.5

SD Standard deviation.

n Number of litters.

* Significantly different from Controls; $P < 0.05$ (t-test).

Summary of foetal observations at skeletal examination

Group : 1 2 3 4
 Compound : Ringer acetate Fat emulsion 73403 Intralipid^R 20%
 Dosage (ml/kg/day) : 22.5 15 22.5 22.5

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	93(13)	100(14)	82(13)	127(18)	7608 foetuses	69 studies
Observations: % foetal incidence [‡] (number of litters)					Mean	Study ranges
<u>Vertebrae</u>						
Incomplete ossification of one thoracic vertebral centrum	0.0(0)	0.0(0)	2.4(2)	0.0(0)	0.18	0.0- 2.4
Thoracic vertebra with bipartite centra or agenesis of a hemicentrum	0.0(0)	2.0(1)	1.2(1)	0.0(0)	#	#
Incompletely ossified or asymmetric costal elements of sacral vertebrae	1.1(1)	6.0(6)	9.8(6)	4.7(5)	5.31	0.0-11.8
One caudal vertebra offset, tail tip kinked	0.0(0)	1.0(1)	0.0(0)	1.6(2)	0.53	0.0- 3.1
Caudal vertebrae fused, short and kinky tail	0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.04	0.0- 1.1
26 pre-sacral vertebrae	77.4(13)	84.0(13)	62.2(13)	69.3(18)	80.13	61.4-93.5
27 pre-sacral vertebrae	22.6(8)	16.0(8)	36.6(10)	30.7(13)	16.98	5.2-38.6
Anomalous lumbar vertebrae [¶]	0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.03	0.0- 1.2
Anomalous thoracic vertebrae resulting in scoliosis [¶]	0.0(0)	0.0(0)	0.0(0)	0.8(1)	0.03	0.0- 1.4

‡ One foetus may have more than one observation.

No previous record in background control data.

¶ See key to Appendix B.

Summary of foetal observations at skeletal examination

Group : 1 2 3 4
 Compound : Ringer acetate Fat emulsion 73403 Intralipid^R 20%
 Dosage (ml/kg/day) : 22.5 15 22.5 22.5

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	93(13)	100(14)	82(13)	127(18)	7608 foetuses	69 studies
Observations: % foetal incidence [‡] (number of litters)					Mean	Study ranges
<u>Limbs and girdles</u>						
Incomplete ossification of heads of limb long-bones	33.3(12)	33.0(10)	48.8(11)	56.7(15)	56.65	0.0-78.0
Additional ossification of one or both olecranon processes	3.2(2)	12.0(5)	2.4(2)	2.4(3)	1.63	0.0-10.3
One or both centrales incompletely ossified or unossified	0.0(0)	3.0(2)	8.5(2)	4.7(3)	1.10	0.0- 7.3
Metacarpals and/or phalanges incompletely ossified or unossified	8.6(5)	18.0(5)	20.7(6)	21.3(10)	14.33	2.1-32.7
Acromion processes elongated	0.0(0)	2.0(2)	1.2(1)	0.0(0)	0.26	0.0- 4.0
Pubic bones incompletely ossified or unossified	0.0(0)	3.0(2)	6.1(1)	3.9(2)	1.46	0.0- 7.1
Asymmetric pelvis, ilial bones associated with different sacral vertebrae	1.1(1)	5.0(5)	8.5(6)	3.9(4)	4.38	0.0-11.2
Double association pelvis, ilial bones associated with both sacral vertebrae	2.2(2)	0.0(0)	1.2(1)	1.6(2)	2.10	0.0-15.5

‡ One foetus may have more than one observation.

In conclusion, IV infusion of Fat emulsion 73403 to pregnant rabbits during the period of organogenesis, at doses of 3, and 4.5 g TG/kg/day and Intralipid 20 % at a dose of 4.5 g TG/kg/day had no adverse effect on fetal survival, and fetal growth and development *in utero* were unaffected by the fat emulsion at the administered doses in all treatment groups.

Fat emulsion 73403 administration was associated with reduced food intake and increased embryo-fetal loss seen in Fat emulsion 73403 treatment (4.5 g TG/kg/day dose group) as indicated by abortion and increased post-implantation loss observed in one female rabbit.

10 Special Toxicology Studies

Local Tolerance Test of (b) (4) % in Rabbits after a Single Intravenous, Intra-arterial, Paravenous, Intramuscular and Subcutaneous Administration (RPT # (b) (4))

The objective of this study was to determine the local tolerance of rabbits to (b) (4) % after a single intravenous, intra-arterial (IA), paravenous (PV), Intramuscular (IM), and subcutaneous (SC) injection.

Methods:

Doses: (b) (4) % (lot # (b) (4)) was administered by single dose infusion at 18.8 ml/hr for 4 hrs (75.2 ml/animal) IV, and IA; at 0.5 ml/animal for the PV and SC injection; and 1 ml/animal for the IM injections. 0.9 % NaCl solution was used as the negative vehicle control.

Study design: 12 Male and female (6/sex) Himalayan rabbits, 14-16 weeks old, and weighing 2.7-3.2 kg were infused with (b) (4) %, 75.2 ml IV (via marginal ear vein); 75.2 ml IA (via central ear artery); 0.5 ml PV (beside the *vena saphena parva*); 0.5 ml SC (under the dorsal skin); and 1 ml IM (into the gastrocnemius muscle).

The test solution was administered once on the left side of each animal, and for the IV and IA infusion, two separate animals were used.

As a control, each animal's right side was treated with 0.9 % NaCl solution.

The IV infusion was performed first, followed by the IM, PV, and SC injections at the end of the 4 hr infusion, one after the other, but the IA infusion was done in separate rabbits.

For the IV and IA infusion of 18.8 ml (b) (4) %, a corresponding 18.8 ml of 0.9 % NaCl was also infused. Dosing for the IM, PV, and SC were similarly administered.

In all cases, the dosing started with the test solution on the animal's left side, followed by the 0.9 % NaCl solution on the rabbit's right side.

Clinical signs and mortality were checked and recorded daily, and local reactions were inspected macroscopically 2, 24, 48, 96 hrs, and 14 days after administration in the appropriate rabbit.

Two rabbits/sex/group were sacrificed 48 hrs, 96 hrs, and 14 days after dosing. The injection sites were dissected out, and along with samples of affected tissues and control tissues (approx., 5 mm thickness) fixed in 10 % buffered formalin, stained with

hematoxylin-eosin after paraffin sectioning of 3-5 µm tissue, and examined histologically.

Results

The IV, and IA infusion sites and the PV, IM, and SC injection sites did not show any macroscopically visible changes in both groups of study animals.

Histopathological examinations of the tissues did not show any local changes at the substance-treated and control IV infusion sites in both groups.

Microscopic examination of the IA infusion sites showed a slight thrombosis in the two animals dissected after 48 hrs in the test group. A slight thrombosis was also observed in one of the IA control sites at the 48 hr time point.

Histopathological examination of the PV injection sites revealed minimal or moderate inflammation, mild/moderate mesenchymal activation, mild edema and minimal or mild hemorrhage in both test group and control animals.

Microscopic examination of the IM injection showed mild, moderate or marked necrosis and mild mesenchymal activation in 3 animals from the test group and 2 animals from the control group.

Histopathology of the SC group showed mild or moderate edema in 3 animals, a mild mesenchymal activation in one animal from the test group, and only a mild edema in a 48 hr exposed animal from the control group.

None of the tested dose levels caused any systemic intolerance reactions or body weight changes, and none of the animals died prematurely.

In conclusion, there appeared to be no local intolerance reactions following the IV administration of 75.2 ml (18.8 ml/animal/hr for 4 hrs) of (b) (4) % in rabbits. Moderate but up to 14 days subsiding intolerance reactions were observed following IM injections (administrations made in error) in rabbits

Local Tolerance Test of (b) (4) in Rabbits after a Single Intravenous, Intra-arterial, Intramuscular, Paravenous, and Subcutaneous Administration (RPT # 23837)

The objective of this study was to determine the local tolerance of rabbits to (b) (4) (b) (4) after a single intravenous (IV, the intended route of administration), intra-arterial (IA), paravenous (PV), Intramuscular (IM), and subcutaneous (SC) injection.

Methods:

(b) (4) (lot #10BL8337) was administered by single dose infusion at 60 ml/kg for 6 hrs IV; 10.0 ml/animal IA, 0.5 ml/animal IM, and 1.0 ml/animal PV and SC injections. 0.9 % NaCl solution was used as the negative vehicle control.

Six male Himalayan rabbits, 4-5 months old and weighing 2.1-2.2 kg were infused with (b) (4), 60 ml IV (via marginal ear vein); 10.0 ml IA (via the central artery of the ear); 0.5 ml (into the gastrocnemius muscle); 1.0 ml PV (besides the *vena saphena parva*) and SC (under the dorsal skin). The animals were sacrificed at specified time points after the injections.

Time of sacrifice for each animal:

Test item	Animal number/Sex	Routes of administration (all 5 routes for each animal)	Time point of sacrifice
(b) (4)	1 m	Intravenous infusion over 6 hours; Intraarterial, intramuscular, paravenous and subcutaneous injection	After 48 hours
	2 m		After 96 hours
	3 m		
	4 m		After 14 days
	5 m		
	6 m		

m = male

Clinical signs and mortality were checked and recorded daily, and local reactions were inspected macroscopically 2, 24, 48, 96 hrs, and 14 days after administration in the appropriate rabbit.

The injection sites were dissected out, and along with samples of affected tissues and control tissues (approx., 5 mm thickness) fixed in 10 % buffered formalin, stained with hematoxylin-eosin after paraffin sectioning of 3-5 µm tissue, and examined histologically by a pathologist.

Results

At the test article Intravenous (IV), intra-arterial (IA) infusion sites, and the intramuscular (IM) injection site, no macroscopic changes were observed at the examination time-points 2, 24, 48, 96 hrs and 14 days after administration.

At the subcutaneous injection site of 1.0 ml (b) (4)/animal, reddening was observed in one animal 24 and 48 hrs after administration, and upon 48-hr sacrifice. The change was considered to be caused by the injection procedure and not test item related.

Paravenous injection site of 1.0 ml (b) (4)/animal showed reddening in 4 of 6 animals at 24, 48, 96 hrs and 14 days after administration, as revealed at the 48 and 96 hr, and 14 day sacrifice.

Histopathological examinations of IV and IA infusion sites as well as intramuscular, paravenous, and subcutaneous injection sites did not show any morphological changes considered to be test item related, as all the observed minimal to moderate changes were non-specific reactions caused by the administration procedure.

Intravenous infusion of 60 ml (b) (4)/kg body weight or 60 ml of 0.9 % NaCl solution both resulted in mild to minimal or moderate hemorrhage in animal nos. 1 and 2, as observed after the 48 hr sacrifice, and in animal nos. 3 and 4 as observed after the 96 hr sacrifice. In addition, minimal mixed sub-epithelial cell infiltration was observed in the vehicle injection site in animal no. 2.

Intra-arterial infusion site of 10.0 ml (b) (4)/animal resulted in mild hemorrhage in animal no. 3, but histological examination after sacrifice at 96 hrs did not show any changes.

Intramuscular injection site of 0.5 ml (b) (4)/animal resulted in mild superficial purulent dermatitis in animal no. 4. Histological examination of control sites of IM injection after sacrifice at 96 hrs did not show any changes.

Paravenous injection of 1.0 ml (b) (4)/animal or 1.0 ml 0.9 % NaCl/animal both resulted in minimal superficial purulent dermatitis in animal nos. 4 and 3 respectively after sacrifice at 96 hrs.

Subcutaneous injection site of 1.0 ml (b) (4)/animal resulted in moderate superficial purulent dermatitis in animal no. 3, but histological examination of control site after sacrifice at 96 hrs did not show any changes in this animal.

In conclusion, there appeared to be no local intolerance reactions following the IV administration of 60 ml of (b) (4) for 6 hrs in male Himalayan rabbits.

Functional Activity of the Reticuloendothelial System of the Rat after Total Parenteral Nutrition with Intralipid or Fat Emulsion 73403 (Document No. 92 96 104)

The objective of this study was to determine the effects of total parenteral nutrition (TPN) containing Intralipid (a long chain triglyceride emulsion) or Fat emulsion 73403 (a mixture of medium and long chain triglyceride on a common glycerol backbone) on the activity of the reticuloendothelial system (RES) in the healthy rat.

Methods:

Rats were divided into 3 dose groups, with group 1 receiving total parenteral nutrition (TPN) consisting of amino acids (vamin N) at 1 g nitrogen/kg/day, as well as vitamins, minerals, trace elements, including 25 % glucose and 75 % Intralipid 20 % (20 g fat/kg/day) with a total calorie intake of 313 kcal/kg/day. The group 2 animals received amino acids (vamin N) at 1 g nitrogen/kg/day, as well as vitamins, minerals, trace elements, including 25 % glucose and 75 % Fat emulsion 73403 (20 g fat/kg/day) with a total calorie intake of 305 kcal/kg/day. The group 3 (control) animals received isovolemic infusion of 0.9 % NaCl, including standard diet ad libitum. Infusions were given for 20 hrs each day consecutively for 7 days.

Overnight culture of bacterial *E. coli* (ATCC 8739) was inoculated into a ^{59}Fe -citrate (2 $\mu\text{Ci/ml}$) radiolabeled broth. Animals were anesthetized on the morning of the eighth day, and the catheter flushed with saline prior to the infusion of 1 ml of ^{59}Fe -labeled *E. coli* into each animal, during a 30 sec period. Timing was begun when 50 % of the *E. coli* had been infused, and 25 min later, the rats were sacrificed. Blood samples were obtained from each animal via the tail vein after 2, 5, 8, 12 and 25 min, and total blood volume (estimated at 6.4 % of the body weight) was used to calculate ^{59}Fe remaining in the blood.

Liver lung and spleen of each animal was harvested, dissected out into 200 mg pieces, weighed and processed, and solubilized in a scintillation counter to determine the amount of radioactivity uptake by each organ. The results are expressed as % of infused ^{59}Fe taken up by each tissue.

Results

There were no significant differences in weight gain between the three dose groups over the last 4 days of the study, and there were no observed adverse effects of the IV TPN (Intralipid 20 % or Fat emulsion 73403) nutrition during the study duration.

There was no difference in liver weight (g/kg body weight) between the control group and the TPN groups, but the Fat emulsion 73403 group had significantly elevated liver weight, in comparison to the Intralipid 20 % group.

The lung weight of the TPN groups was significantly increased, when compared with control group lung weight; although the Fat emulsion 73403 animals had significantly lower lung weight than the Intralipid 20 % infused animals.

There were no significant changes in relative spleen weights between the study animals.

The clearance of an IV dose of *E. coli* from the blood was almost identical in all dose groups, with approximately 0.001 % of the infused live *E. coli* remaining in the blood. A stable *E. coli* count of 1×10^3 cfu/ml remained in the blood at the end of the 25 min study period.

The uptake of ^{59}Fe -labeled *E. coli* by the liver of control rats ($62.4 \pm 5.4 \%$) was higher than uptake in Intralipid 20 % rats ($54.8 \pm 3.9 \%$) and in Fat emulsion 73403 rats ($55.7 \pm 5.6 \%$), although not significantly. The uptake of bacteria from the bloodstream by the liver appears to be uniform throughout the liver, from the count of radioactivity obtained from 5 liver samples.

The uptake of *E. coli* from the spleen of the Intralipid 20 % dosed rats ($2.3 \pm 0.9 \%$) and the Fat emulsion 73403 dosed rats ($2.3 \pm 1.1 \%$) was not significantly different from that of the control rats ($3.1 \pm 1.0 \%$).

A considerably significant increase in the *E. coli* uptake by the lungs of the Intralipid 20 % dosed rats ($15.3 \pm 3.7 \%$) and the Fat emulsion 73403 dosed rats ($16.5 \pm 5.2 \%$) was observed, in comparison to uptake by the control animals ($3.0 \pm 0.9 \%$).

The combined capacity of the liver, lungs and spleen to clear bacteria from the blood of rats from the control group (at $68.5 \pm 5.6 \%$), Intralipid 20 % dose group (at $72.4 \pm 5.2 \%$), and the Fat emulsion 73403 dose group (at $74.5 \pm 7.5 \%$) of the infused dose, still leaves about 25 % of the infused dose of bacterial *E. coli* to be accounted for. It may be that uptake by other organs may account for the residue.

In conclusion, TPN infusion with a high dose (20 g fat/kg/day) of Fat emulsion 73403 or Intralipid 20 % concurrently for 7 days in healthy rats sustained normal growth without observable side effects, and did not affect the ability of the animal to clear bacteria from the blood. Lipid emulsion based TPN however, significantly increased bacterial uptake by the lung in comparison to controls.

The Effect of Infusions of Intralipid 20 % and Fat Emulsion 73403 on Reticuloendothelial Function in the Rat (Document No. 92 96 106)

Methods:

Intralipid 20 % at doses of 8 g and 20 g fat/kg body weight (b.w.), and Fat emulsion 73403 or Intralipid 20 % at a dose of 20 g fat/kg/day, and 0.9 % NaCl were infused into rats at 20 hrs each day for 7 days.

In the first of two experiments, two groups of 6 male Sprague-Dawley rats weighing 166-202 g were first anesthetized and a catheter placed into the *vena cava* via the *vena jugularis*, prior to the infusion of each group of animals with 8 g or 20 g of Intralipid 20 %

In the second experiment 3 groups of 8 animals/group were dosed either with 20 g Intralipid 20 %, Fat emulsion 73403, or 0.9 % NaCl (control group).

All rats were infused for 20 hrs each day for 7 consecutive days, and monitored daily for clinical signs of toxicity and body weight changes.

Overnight culture of bacterial *E. coli* (ATCC 8739) was inoculated into a ^{59}Fe -citrate (2.3 $\mu\text{Ci/ml}$) radiolabeled broth.

The animals were anesthetized on the morning of the eighth day, and the catheter flushed with saline prior to the infusion of 1 ml of ^{59}Fe -labeled *E. coli* into each animal, during a 30 sec period. Timing was begun when 50 % of the *E. coli* had been infused, and 25 min later, the rats were sacrificed. Blood samples were obtained from each animal, and total blood volume (estimated at 6.4 % of the body weight) was used to calculate ^{59}Fe remaining in the blood.

Liver lung and spleen of each animal was harvested, dissected out into 200 mg pieces, weighed and processed, and solubilized in a scintillation counter to determine the amount of radioactivity uptake by each organ. The results are expressed as % of infused ^{59}Fe taken up by each tissue.

Results

Three rats in the second experiment were lost from the study, 2 as a result of catheter occlusion, and one rat was septic.

Growth rates of all emulsion treated animals were comparable to the saline-infused rats, but the treated rats had hypophagia as a result of calories infused from the emulsions, and maintained normal body weight during the infusion period.

There were no differences in the relative liver, lung and spleen weights of rats infused with 8 g or 20 g fat/kg/day as Intralipid. However, high fat doses (20 g fat/kg/day) of Intralipid 20 % and Fat emulsion 73403 resulted in significant elevations in relative liver weights compared to saline control animals. Rats infused with Fat emulsion 73403 also showed significantly higher relative spleen weights compared to control animals. Lung weights were unaffected by either 20 g Intralipid 20 % or Fat emulsion 73403.

There were no significant differences in bacterial uptake by the liver, lung, or spleen of rats infused with 8 or 20 g fat/kg/day Intralipid 20 %, and the amount of bacteria remaining in the blood was also similar, in spite of the change in Intralipid dose.

There were no differences in bacterial uptake by the liver, lung, and spleen of animals infused with 20 g fat/kg/day Intralipid or Fat emulsion 73403, when compared to saline infused control animals. However, spleen bacterial uptake was higher in the Fat emulsion 73403 group compared to the Intralipid 20 % group, and the blood bacteria was significantly lower in the fat-infused animals compared to the saline-infused control animals.

The combined total bacterial uptake by the liver, lungs and spleen were significantly higher in the high dose Intralipid 20 % and the Fat emulsion 73403 groups, in comparison to both the control group and the low dose Intralipid 20 % group.

In conclusion, the infusion of Intralipid 20 % or Fat emulsion 73403 at high doses for 7 days does not affect reticular activating system (RES) function in the liver, spleen and lungs of the rat.

Toxicological Evaluation of the 3-CB (Chamber Bag) (b) (4) Packaging System**Introduction****Components/constituents of the 3-CB (b) (4) packaging system**

- A. Primary 3 chamber bag
- B. Overpouch
- C. Oxygen Absorber
- D. Fill-port
- E. Stopper
- F. Cap
- G. Printing Ink ((b) (4))

The primary three chamber bag is made of (b) (4) plastic material designed to contain all the three nutrients (fat emulsion, amino acids and glucose solution). All the manufactured plastic components of the 3-CB (b) (4) consists of (b) (4) which are potential or established toxic migrants that can leach into the drug product over a storage or shelf period of time. The components are shown in tables below.

The Intralipid 20 %, the lipid component of the Kabiven drug product also contain phytosterols, (b) (4). The total concentration of phytosterols in Intralipid 20 % is (b) (4) mg/l. Phytosterols have been suggested to have estrogenic activity, thereby affecting the reproductive system.

In addition, Intralipid 20 % also contain (b) (4) (at a concentration of (b) (4) mg/l) as a (b) (4) (at a concentration of (b) (4) g/l) in Intralipid 20 %. As a (b) (4), ICH guidelines did not specify an adequate permitted daily exposure level (PDE).

Testing Strategy:

To assess the safety of the 3-CB packaging system, the migration of individual components (b) (4) of the bag into extraction mediums and solvents (such as water, saline, 5 % ethanol, dimethyl sulfoxide (DMSO), polyethylene glycol (PEG)) were determined under various storage conditions. The aqueous extracts were then used to conduct biological/toxicological tests in animals, cell cultures, mutagenicity tests, cytotoxicity tests, or local tolerance tests to determine the level and type of toxicity of the extracts.

The highest recommended treatment volume for any of the products stored in the 3-CB container system has been determined to be 40 ml/kg body weight, and is used to estimate exposure to migrants.

Exposure to any migrant is also influenced by the amount of container material that is in contact with the administered volume, so that the largest amount of container material in

contact with 1 ml. of infusion volume comes from the container with the smallest volume (the 1026 ml container for Kabiven).

To estimate the worst case exposure to potential or identified migrants from the 3-CB, the sponsor used the following formula:

Potential migrants-not identified, but analyzed:

Worst case exposure = dose (40 ml/kg) x LOQ (mg/ml)

Where LOQ is the limit of quantitation

Identified migrants-analyzed:

Worst case exposure = dose (40 ml/kg) x concentration in infusion solution

From the formulas above, identified leachables and potential migrants in the 3-CB (b) (4) packaging system has been characterized from toxicity tests and used to determine the safety of the drug product stored in the 3-CB. As shown in the table below. A daily dose estimate of migrants from the 3-CB into infusion solution of the different Kabiven products was also determined, as shown in the table below.

Table 1. Daily dose and amount of (b) (4) in contact with the infusion solution in the different Kabiven (K) and (b) (4) products

	K 1026	K 1540	K 2053	K 2566	(b) (4) 1440	(b) (4) 1920	(b) (4)
Daily dose (mL/kg/d)	(b) (4)						
(b) (4)							
(gram)							
Gram (b) (4)							
mL solution							

Table 3.2.P.7.5-2 Composition of the (b) (4), the fill-port, the cap and the (b) (4)

Constituents	Max amount*	(b) (4)	Fill-port	Cap	(b) (4)
(b) (4)					

3.2.P.7.5 Summary Extraction and Migration Studies (b) (4)**Document-ID: P75** (b) (4) **-Summary-extraction and migration studies (kipdips – 9,697)**

Constituents	Max amount [±]	(b) (4)	Fill-port	Cap	(b) (4)
(b) (4)					

(b) (4) (as an example) is present in the (b) (4), ports and caps in low amounts of less than (b) (4) % by weight ((b) (4)). An estimated worst case migration of (b) (4) % of the total amount of (b) (4) into the 1026 ml Kabiven infusion solution would correspond to a daily dose of (b) (4) % by weight in (b) (4) and fill port; or a recommended daily dose of (b) (4) ml/kg. The total weight of the (b) (4) and fill ports containing (b) (4) g.

So, worst case exposure =

(b) (4)

Established Migrants:

(b) (4) is an established migrant, (b) (4) and is present in amounts equal to or lower than (b) (4) %.

Extraction experiments conducted with the (b) (4) bag film at various pH levels in different extraction media have shown the presence of low levels (b) (4) and its metabolite/degradation product (b) (4).

The highest measured concentration of (b) (4) in a lipid emulsion (intralipid) after storage in a 3-CB container (of 200 ml) under accelerated thermal conditions (up to 6 months, at 40°C) has been (b) (4) µg/ml, whereas the highest concentrations stored under normal conditions (up to 24 months, at 25°C) has been (b) (4) µg/ml. There has not been an increase in concentration of this migrant with storage time, so it has been assumed that (b) (4) leaches into the product during (b) (4) procedures.

Table 2. Estimated release of (b) (4) into the different solutions stored in a 1026 mL 3-CB (b) (4) container system.

	Amino acid	Glucose	Lipid
Conc of (b) (4) in solution (µg/ml)	(b) (4)		
Volume per 1026 mL of product (mL)	(b) (4)		
Total amount of (b) (4) (µg)	(b) (4)		

(b) (4)

Due to the conclusion that (b) (4) is extracted to the product during (b) (4), an upper specification limit for the (b) (4) packaging materials has been set to (b) (4) µg/g, and tests conducted annually to test the product level of (b) (4).

The estimated daily exposure to (b) (4) of (b) (4) µg/g is more than 17,000 times lower than the level where reproductive toxicity effects are seen in rodents, and 4400-fold lower than the lowest NOAEL observed for maternal toxicity in rabbit developmental study (b) (4).

The estimated safety margin above suggests that there are no risks associated with the leaching of minute amounts of (b) (4) into the infusion solutions stored in the 3-CB (b) (4) container system.

The potential migration of the (b) (4) from the 3-CB (b) (4) container into Intralipid from the Kabiven product was studied after a storage period of 24 months at 25 °C and for 6 months at 40 °C. The levels of the (b) (4) were below the limit of detection at (b) (4) and (b) (4) µg/ml for (b) (4) respectively.

(b) (4), another (b) (4) within the 3-CB container system was not followed because it was considered that the amount of this (b) (4) will be in the same range or less than the (b) (4). Thus, based on the low, undetectable levels of migration of the (b) (4) into lipid emulsions stored for up to 6 months at 40°C and for 24 months at 25°C, suggest that there will be minimum exposures to these agents from Kabiven formulations.

Standard Composition of (b) (4) Stopper*, Estimated Weight (% of total weight) of Each Component, and Worst-Case Estimates of Daily Exposure

Chemical name	Content (wt%)	Daily exposure (µg/kg)
(b) (4)		

(b) (4)

The stopper used in the port system of the 3-CB (b) (4) container system contains (b) (4) (b) (4) (b) (4)

Because of the potential toxicity of (b) (4) and possibly the other (b) (4) the stopper was extracted with 6 different extraction media (acetate, water, ethanol, ethanol/chloroform, water/isopropyl alcohol and water/ethanol) for 30 min at 120° C, and the media assayed for contents of the (b) (4)

No (b) (4) was found in any of the extraction media (limit of detection (b) (4) ng/ml). (b) (4) was found at a mean maximal concentration of ≤ (b) (4) ng/ml (water/chloroform or ethanol), and (b) (4) was found at a mean maximal concentration of (b) (4) ng/ml (water isopropyl alcohol).

The exposure estimates shown in the table below were obtained based on the presence of the (b) (4) from the stopper into a volume of 1026 ml, as well as a maximal daily exposure of 40 ml/kg.

Table 4. Estimates of concentration of (b) (4) in infusion solution and daily exposures; (a) worst estimate based on extraction of septum (b) estimate based on extraction of total container (3-CB) with 15% ethanol solution

(b) (4)	Concentration (ng/ml)	Daily exposures ^a (ug/kg b.w.)	Daily exposures ^b (ug/kg b.w.)
(b) (4)			

The exposure estimate of (b) (4) ng/kg ((b) (4) µg/adult/day) for (b) (4) and less than (b) (4) ng/kg ((b) (4) µg/adult/day) for (b) (4) are worst case estimates, and below, or at a threshold value of (b) (4) µg/person/day; a value that poses no risk of toxicity in spite of life long exposure.

(b) (4)

Regulations require that containers used for storage of parenteral solutions are required to have product information printed on the outside of the bags. Since it is evident that components of the plastic containers can leach substances into the infusion solutions, there is a potential for migration of components from print ((b) (4) (b) (4) into the solution.

A worst case scenario of an estimation of potential migration of (b) (4) % of the (b) (4) and other constituents into the infusion solution can be assessed. The estimated daily exposure can be calculated from the following formula:

Worst-case exposure =

(b) (4)

Table 5. Composition of (b) (4) and estimated worst-case exposures for individual components Based on an infusion volume of 40 mL/kg from the 3-CB (b) (4)

Chemical name	Content (wt%)	Daily exposure (ug/kg)
(b) (4)		

(b) (4) and (b) (4) are the (b) (4) of the (b) (4). Toxicity data was found only for the (b) (4) showing positivity in the Ames Salmonella mutagenicity assay in strain TA 100 with or without metabolic activation.

In order to rule out any significant migration occurrence, a worst case model solution of 15 % ethanol was used to stimulate lipid emulsions without negative chromatographic effects. The medium choice of water/ethanol solution was due to the fact that the

(b) (4) (b) (4) has a greater ability to migrate to the water/ethanol solution, rather than to a pure water solution. The amount of (b) (4) (b) (4) in (b) (4) 15 % ethanol solution packed in the (b) (4) Biofine and in a reference solution was below the detection limit of (b) (4) ng/ml.

In other experiments, the amount of (b) (4) in Intralipid 20 % could not be detected above the detection limit of (b) (4) ng/ml after 6 months of storage at 25 °C or 40 °C. In spite of high noise levels in chromatograms, (b) (4) could not be detected above (b) (4) ng/ml in (b) (4) % after storage for up to 24 months at 25 °C or 6 months at 40 °C.

Based on the maximally recommended dose of 40 ml/kg/day, the daily exposure to (b) (4) (b) (4) should be lower than (b) (4) ng/kg or (b) (4) µg/person/day. This value is below the threshold value of (b) (4) µg/person/day. It poses no significant carcinogenicity or other toxic effects, in spite of life long exposure (b) (4).

(b) (4)

With a (b) (4) % amount of (b) (4) in a (b) (4), the estimated worst-case daily exposure to these components, based on a migration of (b) (4) across the (b) (4), is estimated to be (b) (4) µg/kg.

The migration of (b) (4), one of the components of the (b) (4) has been studied in a 10 % amino acid solution. After 30 months of storage at room temperature, (b) (4) was detected at levels below the limit of quantitation (LOQ) of (b) (4) ppb. Based on this analysis, the daily exposure to (b) (4) would be maximally (b) (4) ng/kg (b) (4) µg/person per day), a level slightly above that accepted for life long exposure of a genotoxic substance (b) (4). It is thus determined that there should be no exposures of (b) (4) from the (b) (4) that would be toxic in parenteral nutrients stored in the 3-CB (b) (4) container. There is however, a slight lifelong risk of genotoxicity from (b) (4) in a lifetime use of Kabiven stored in the 3-CB container system.

(b) (4)

The (b) (4) present in the (b) (4) are (b) (4) (b) (4), and the total sum of the estimated worst-case daily exposures for these (b) (4) are no more than (b) (4) µg/kg body weight. As indicated from studies conducted by the sponsor, there is little or no migration of a (b) (4) (b) (4) into a lipophilic solution, it is pertinent to assume that there would be minimal risk for migration of any relevant amounts of (b) (4) across the (b) (4) container.

(b) (4) consists of (b) (4) that are potentially genotoxic and carcinogenic (b) (4). The permissible exposure limit as recommended by (b) (4) is (b) (4) mg/m³ as a (b) (4) hr time weighted average.

Therefore, any low amount of (b) (4) in the infusion solution should not be considered to be toxic.

(b) (4) toxicity review (b) (4) indicate a low order of general toxicity, and devoid of genotoxicity. The compound is widely used in (b) (4) and has very low toxicity.

A study of the (b) (4) shows that as a (b) (4) substance, there is little or no chance of it migrating into a lipophilic solution in the 3-CB container. It is also indicated that it has a low order of toxicity, hence its approval for use (b) (4).

The overall low toxicity of the (b) (4) suggests that exposures similar to the worst-case estimates shown in the table above should not be associated with any risks.

(b) (4)

The (b) (4) consists of (b) (4)

Just as is the case of the (b) (4) should not pose any risk with inadvertent exposure, especially since the (b) (4) are believed to not be able to migrate through the (b) (4) into the parenteral amino acid/glucose solution or lipid emulsion.

The (b) (4) should not be able to migrate through the (b) (4) into the parenteral solutions (amino acid/glucose/lipid emulsion). Although minor amounts of (b) (4) may still be present in the (b) (4), the degree of migration through the (b) (4) and into the parenteral solution should be very restricted. Consequently, there should be no concern for toxicity with the use of the (b) (4).

(b) (4) Including Oxygen Absorber:

The (b) (4) included in the (b) (4) container system (b) (4) from migrating into the solutions of the 3-CB container. It also includes an oxygen absorber placed between the primary and secondary bags before the package is (b) (4).

The oxygen absorber consists of (b) (4)

(b) (4) there is very limited possibility for migration of any of the components of the absorber into the parenteral solution. Hence no further assessment

of this constituent of the packing system was performed because there is no toxicity concern.

However, because it is well established that substances from (b) (4) used in packaging can migrate into emulsions/solutions for IV administration; the issue of biological migration of constituents of the (b) (4) into solutions is addressed below.

(b) (4) composition and worst-case exposure estimates.

The (b) (4) housing the (b) (4) oxygen absorber is composed of (b) (4)

The (b) (4) contents of the (b) (4) are within the stipulated (b) (4) % and other additives in the (b) (4)

also constitute $\leq$ (b) (4) %. Another content of the (b) (4) is (b) (4), which has previously been determined to constitute $<$ (b) (4) weight %.

(b) (4) may have migration potential into the parenteral solution, and exposure to this may be of concern.

(b) (4) have previously been analyzed in water extraction studies, without the detection of any compound in the extract. The analysis methodology involved the detection of the sum of the (b) (4) and the (b) (4) at a detection limit of (b) (4) ng/ml, and the (b) (4) alone at (b) (4) ng/ml.

Based on a daily maximum intake of 40 ml/kg body weight of parenteral fluid, and the threshold limits observed from previous analytical work, the maximum daily exposure to individual (b) (4) (including the (b) (4)) should be less than (b) (4) μ g/kg or (b) (4) μ g/day for a (b) (4) kg person.

Since there is no toxicity data for parenteral exposure to (b) (4) the previous toxicity studies were only about (b) (4)

Genotoxicity studies with (b) (4) have indicated that (b) (4) has no genotoxic activity, whereas older studies show that (b) (4) and (b) (4) were genotoxic, no data was

available on (b) (4) Toxicity of (b) (4) and (b) (4) were suggested to be due to (b) (4) and (b) (4) (their (b) (4)) respectively.

(b) (4)

In risk assessment for genotoxicity or carcinogenicity of exposure levels of (b) (4) (at (b) (4) $\mu\text{g/kg/person}$), the Threshold Limit Value (TLV)/Time Weighted Average Value (TWA) of (b) (4) at (b) (4) $\mu\text{g/kg/day}$ should be considered.

A TLV/TWA value of (b) (4) $\mu\text{g/kg/day}$ is more than 125-fold higher than the estimated worst case exposure value to the (b) (4) at (b) (4) $\mu\text{g/kg/day}$, which is similar to the (b) (4) $\mu\text{g/day}$ (a safe value for a lifelong exposure to a genotoxic/carcinogenic substance (b) (4)). There are therefore no safety concerns for (b) (4) or its (b) (4) migrating from the (b) (4) into parenteral products at a (b) (4) $\mu\text{g/kg/day}$ value, which is well below the established (b) (4) $\mu\text{g/kg/day}$ (b) (4) saline solutions, or Intralipid 20 % stored in a 3-CB made of (b) (4) with the (b) (4) as the printing ink were tested for cytotoxicity. All the tests passed the criteria for cytotoxic activity. In addition, the (b) (4) saline solution also passed the hemolytic activity test.

In conclusion, the non-clinical safety assessment show that there should not be any safety concern with potential migrants from the 3-CB (b) (4) container system into parenteral nutrition products indicated here for use in adults.

11 Integrated Summary and Safety Evaluation

Kabiven (b) (4) and (b) (4) are macronutrients for intravenous nutrition composed of (b) (4) (containing essential and non essential amino acids), Intralipid 20 % (containing (b) (4) of soybean oil), glucose and electrolytes (including sodium glycerophosphate) in a three separable chamber plastic (b) (4) container of different sizes (from 1026 ml to (b) (4) ml). Prior to mixing, the contents of the container have a shelf life of 24 months at room temperature, whereas, the admixture has a shelf life of 24 hours at room temperature.

The Applicant-recommended maximum dose for Kabiven in adults is 40 ml/kg/day; which will provide (b) (4) by intravenous (IV) infusion into a central vein, for an infusion period of (b) (4)-24 hours.

The Applicant has submitted nonclinical studies conducted mainly with the lipid, amino acid, and the sodium glycerophosphate component of Kabiven. The concept adopted for the submission is that, the pharmacological and toxicological properties of the

constituent lipid, amino acid, glucose, or electrolytes (sodium glycerophosphate) in the 3 chamber bag (3-CB) will not change with the admixture of its contents prior to use. Furthermore, no study was submitted for the glucose content of Kabiven because it was identified as a naturally safe macronutrient.

In the non-clinical safety assessment of Kabiven, the pharmacology and toxicology of the lipid and amino acid component was reviewed, along with Sodium glycerophosphate (Na GP), a non referenced electrolyte constituent of Kabiven.

In safety pharmacology studies investigating the effects of rapid infusion of 4 g of lipids for 30 min (Intralipid 20 % and (b) (4) %) on myocardial function, respiration and acid-base equilibrium in the anesthetized dog, there were no observed adverse effects, except for occasional increased myocardial performance in some of the animals. (b) (4) % (5 ml/kg) did not induce a drop in blood pressure in anesthetized cat, and a high dose of 5.4 g TG/kg of Intralipid or Fat emulsion 73403 did not change cardiovascular or respiratory indices in the cat, except for a decrease in blood pH and a slight metabolic acidosis induction in the animals.

Similar safety studies in anesthetized cats with amino acids, Vamin 18 EF, and (b) (4) (a dipeptide containing sodium glycerophosphate) at doses up to 31.7 g/l of nitrogen (216 mg/kg) for 4 hrs did not result in any adverse cardiovascular changes, except a slight but reversible acidosis in the animals.

In the Kabiven formulation, sodium glycerophosphate (a new molecular entity) was substituted as a source of inorganic phosphate in the electrolyte component of the macronutrient. Nonclinical pharmacology and toxicology studies conducted with sodium glycerophosphate were submitted in the NDA application in support of the safety of this compound. In a Pharmacokinetic study to determine the utilization of Na-GP as a substitute for inorganic phosphate in rats, the distribution of Na-GP in rat plasma, along with the generation of inorganic phosphate (Pi) was measured. A good stoichiometric 1:1 increase in glycerol and Pi was observed with glycerol-3-phosphate distribution in the rat plasma, without phosphate precipitation.

In pharmacokinetic studies in dogs, the plasma elimination of 3 g/kg Intralipid 20 % and (b) (4) % infusion in dogs are biphasic, following a zero order elimination rate at low exogenous lipid concentration, and a first order elimination rate at the higher (3 g/kg) lipid concentration. In studies to determine the elimination rate of 100 mg and 200 mg lipids (Intralipid 20 %, fat emulsion 4315, 4501) in rabbits, the half lives and fractional clearance rates were not significantly different for the lipids, with a $t_{1/2}$ of 7.66 min and a K_2 of 9.05 for a 200 mg dose of Intralipid 20 %. However, lipid emulsion 73403 was found to be eliminated significantly faster than Intralipid 20 %, at a similar concentration of 100 mg/kg in male rabbits. There were no differences in the half-lives of Intralipid 10 % in adult dog, cat, rabbit, rat, mouse and human. A mean half life of 10 min was the mean elimination rate of Intralipid 10 % in all species.

Sodium glycerophosphate is relatively nontoxic, with LD₅₀ of 3.4 to 3.8 g/kg in rats, and 2 g/kg in dogs. Previous observations in mice had shown that a single IV dose of 60 ml/kg of 1 mM Na-GP was nontoxic.

In single IV dose administrations of sodium glycerophosphate in mice, rats and dogs, there appears to be good tolerance for the NME compound in these animals.

In repeat-dose IV toxicity studies with individual lipid constituent of Kabiven (Intralipid 20 %, (b) (4) %) at 18 g triglycerides TG/kg in rats (up to 30 days), and 9 g TG/kg in dogs (up to 3 months duration), administered at maximum tolerated doses, tolerance was quite good with minor clinical effects on the animal organs, and little or no histopathological changes. The changes in lipid load of the organs, such as fine lipid droplets in the liver, kidneys and spleen of animals, with acidosis and inflammation were transient and reversible.

In repeat-dose IV toxicity studies with the amino acid constituent of Kabiven ((b) (4) %, (b) (4) %, (b) (4)) either with or without electrolytes or with Na-GP) at 3 g nitrogen (N)/kg in rats (up to 30 days), and 0.9 g N/kg in dogs (up to 13 weeks duration) administered at maximum tolerated doses, the tolerance was good, and observed changes in blood cells (in dogs) were reversible. The histopathological changes of hyperemia observed in the liver, lungs and kidney of two dead male rats dosed with 3 g N/kg for 30 days were due to hyperosmolality of the amino acids at the administered dose. A dose of 3 g N/kg is more than 14X above the maximum daily clinical dose of 0.21 g/kg/day of nitrogen, which is equivalent to 1.3 g/kg/day of amino acid.

In single dose IV toxicity studies in rats, the LD_{50s} for α - and β -glycerophosphates were very high (3800 and 3400 mg/kg, respectively), suggesting very low toxicological profiles, and in intravenous toxicity studies with sodium glycerophosphate in rats and dogs for up to 4 weeks at doses up to 13 times the proposed clinical dose, it was tolerated well without any target organ toxicity.

In long-term oral repeat dose toxicity experiments from published studies (Raheja et al., Toxicology 11:1-4,(1997), sodium glycerophosphate at 20 mg/100 g body weight in squirrel monkeys for 15 months, and oral dosing in rats at 190 mg/100 g body weight in male rats for 33 weeks (Raheja et al., 8:115-119,(1977) were well tolerated in both animals. The observed enlargement of the kidney and an increase in kidney weights were attributed to the high sodium intake in the animals. The increases in biliary and liver phospholipids were observed to be minor changes in the animals because their concentrations were not significantly changed.

In genetic toxicology studies including the *in vitro* reverse mutation assays in bacterial cells, mutagenicity studies in cultured mammalian cells or clastogenic studies in human lymphocytes, with SMOF 20 %, a lipid emulsion, the tests were all negative, indicating the absence of any mutagenic effect with the lipid. SMOF 20 % lipid emulsion at 10 g TG/kg was also negative in tests for chromosomal aberration when administered IV in rat micronucleus tests.

The dipeptide amino acid solution Neoven at 10 mg/ml was also negative in mutagenesis induction in bacterial reverse mutation assay test.

In a battery of genotoxicity tests (bacterial reverse mutation assay, mouse lymphoma cell mutation assay, and mouse micronucleus assay), sodium glycerophosphate was non mutagenic.

In developmental studies performed with IV amino acids (b) (4) (%), at maximum concentrations of 540 mg N/kg/day or lipid (Fat emulsion 73403, Intralipid 20 %) at 4.5 g TG/kg/day in rabbits, there were no treatment induced teratogenicity or embryotoxicity, and no significant toxicity reported in the dams.

There were however, no reproductive toxicity studies submitted for sodium glycerophosphate.

In local tolerance studies with administration of the lipid (b) (4) % (18.8 ml) or (b) (4) (60 ml/kg for 6 hrs), there was good local tolerance after IV dosing and dosing by alternative (paravenous, intra-arterial, intramuscular, subcutaneous) routes of administration in rabbits.

In studies with total parenteral nutrition (TPN) administration of macronutrients, or lipids (Intralipid 20 % or fat emulsion 73403) at concentrations up to 20 g fat/kg/day, to determine their effects on the blood clearance and organ uptake of inoculated bacterial E. coli in healthy rats. Results show that TPN or lipid infusion in rats did not hinder bacterial clearance from blood, or their uptake in the liver, spleen or lungs of rats. The reticular activating system function was not affected in rats, although the bacterial uptake in the lungs was significantly increased in treated animals, when compared to control animals.

In the toxicological evaluation of the safety of the 3 chamber bag packaging system for the Kabiven product, assessments were done of the chemical components of the plastic bag (b) (4) materials (fill-port, stopper, cap, printing ink, oxygen absorber, heavy metal) and the potential migration or leaching of these materials into the kabiven product during storage or (b) (4) of the product before use. Other potential impurities or bioactive compounds (phytosterol) present in the lipid constituent of kabiven were also assessed for toxic effects.

In assessment studies including toxicity experiments conducted with liquid extracts of potential leachables from the 3-CB and constituent impurities, the concentrations and levels of toxic compounds present were determined to be lower than the level of hazardous exposure in the Kabiven product.

In conclusion, based on nonclinical safety information provided on the lipid, amino acid, sodium glycerophosphate constituents of the kabiven product, and the 3-CB packaging system, the proposed use of Kabiven and (b) (4) in humans appears to be safe, and there are no significant safety concerns.

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/s/

BABATUNDE E AKINSHOLA
11/21/2011

SUSHANTA K CHAKDER
11/21/2011

comments on NDA200656 Kabiven
From A. Jacobs, AD
Nov 15, 2011

I concur that there are no outstanding pharm/tox issues for this NDA.
I conveyed to the reviewer and supervisor some comments regarding the writing of the review.

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ABIGAIL C JACOBS
11/15/2011

Pharmacology/Toxicology Supervisory Memorandum

NDA 200-656

Sponsor: APP Pharmaceuticals, LLP

Reviewer: Sushanta Chakder, Ph.D.
Supervisory Pharmacologist

Division name: Division of Gastroenterology and Inborn Errors Products (DGIEP)

Date of Submission: January 28, 2011

Date of Receipt: January 28, 2011

Date of Review: November 8, 2011

Product: Kabiven and (b) (4)

Introduction and Discussion:

Under NDA 200656, the sponsor is seeking approval for two 3-chamber bag (fat, amino acids and dextrose), all-in-one formulations (Kabiven and (b) (4)) for Total Parenteral Nutrition (TPN). The proposed formulations contain dextrose in chamber 1, amino acids with electrolytes in chamber 2, and the fat ((b) (4)) emulsion in chamber 3. The (b) (4) formulation for peripheral administration has lower osmolarity ((b) (4) dextrose), while the Kabiven formulation for central as well as peripheral administration has higher osmolarity ((b) (4) dextrose).

In support of the 505(b)(2) NDA application for the 3-chamber bag formulations, the applicant relied on the Agency's determination of safety and efficacy of the two previously approved products – Intralipid 20% (NDA 18-449 and NDA 20-248) and Novamine 11.4% (NDA 17-957). In addition, the Applicant mentioned that the proposed Kabiven products will also deliver dextrose and electrolytes similar to those in the approved 2-chamber bag products – Aminosyn II with electrolytes in dextrose with calcium (NDA 19-683) and Clinimix E sulfite-free with electrolytes in dextrose with calcium (NDA 20-678).

Both Kabiven formulations contain sodium glycerophosphate as a source of phosphate which is substituted for inorganic phosphates, currently used in approved TPN products. Sodium glycerophosphate is hydrolyzed *in vivo* by nonspecific phosphatases to release

phosphate. Another source of phosphate is the egg phospholipid used as an (b) (4) in the formulation. Sodium glycerophosphate is considered a new molecular entity (NME) in Kabiven and (b) (4) formulations.

The applicant submitted adequate nonclinical information (pharmacology including safety pharmacology, pharmacokinetic, single and repeat dose toxicity and genotoxicity) to support the safety of sodium glycerophosphate. The potential cardiovascular adverse effects of sodium glycerophosphate was evaluated in cats using a dipeptide/amino acid solution (DP-Trauma 20%) containing 20 mmol/L sodium glycerophosphate. Anesthetized cats were administered DP-Trauma 20% solution corresponding to 29.4 mg/kg sodium glycerophosphate for 4 hours without any adverse cardiovascular effects. *In vivo* PK studies in rats and studies with human plasma showed that sodium glycerophosphate is rapidly hydrolyzed to inorganic phosphate and glycerol. Increased blood levels of inorganic phosphate, glycerol and total phosphate, along with increased urinary excretion of these substances following intravenous infusion of DL-glycerol-3-phosphate α -glycerophosphate) in rats suggest an intravascular hydrolysis of this compound.

In single dose IV toxicity studies in rats, the LD₅₀s for α - and β -glycerophosphates were very high, 3800 and 3400 mg/kg, respectively, suggesting very low toxicological profiles. In dogs, short term IV administration (5 days) of sodium glycerophosphate at a dose (648 mg/kg/day) 13 times the maximum recommended daily clinical dose was well-tolerated without any significant adverse effects. Intravenous doses of 500 mg/kg/day of sodium β -glycerophosphate and 1000 mg/kg twice a day of α -glycerophosphate for 14 days were well-tolerated without any signs of toxicity. Intravenous infusion of dipeptide amino acid solution containing sodium glycerophosphate at 20 mg/kg/hour over 20 hours in rats, or 11 mg/kg/hour over 6 hours in dogs for 4 weeks did not show any adverse clinical signs or any morphological evidence of any organ toxicity. In a chronic oral toxicity study, dietary administration of sodium β -glycerophosphate at 1900 mg/kg/day for 33 months was not associated with any remarkable toxicity; only treatment related finding was an increase in kidney weights, without any histopathological changes. Identical results were obtained in a 15 months study in primates (squirrel monkeys) administered sodium β -glycerophosphate in drinking water. The increase in kidney weights could be related to the high sodium content in the formulation. In addition, the sponsor stated that administration of sodium glycerophosphate for 13 weeks in rats and dogs at up to 34 times the maximum recommended daily clinical dose of Kabiven did not reveal any adverse clinical signs or evidence of organ toxicity.

Thus, in the nonclinical toxicity studies, sodium glycerophosphate was well-tolerated following IV administration at doses higher than that to be administered at the maximum recommended dose of Kabiven. Longer-term oral toxicity studies with high doses provide further support for the safety of sodium glycerophosphate.

In a standard battery of genotoxicity studies, sodium glycerophosphate did not show any mutagenic potential. In addition, SMOFlipid 20% (a lipid emulsion containing 60 g/L of soybean oil) Neoven (a dipeptide/amino acid solution) were not found to be genotoxic in standard genotoxicity studies.

Embryofetal developmental studies in rabbits with the amino acid solution (Vamin 18 EF) and the fat emulsion by the IV route did not reveal any embryotoxicity or teratogenicity. Reproductive toxicity studies have not been conducted with sodium glycerophosphate. However, the lack of reproductive toxicity has been addressed in the labeling of Kabiven.

Pregnancy section of the labeling states, “**Pregnancy Category C.** Animal reproduction studies have not been conducted with (b) (4). It is (b) (4) not known whether Kabiven can cause fetal harm when administered to a pregnant woman (b) (4). Kabiven (b) (4) should be given to a pregnant woman only if clearly needed”. Thus, the lack reproductive toxicity data for sodium glycerophosphate is not considered a deficiency.

The applicant conducted an appropriate assessment for each component/constituent of the 3-chamber bag (3-CB) (b) (4) packaging system. Safety evaluation was performed for potential migrants, leachables, extractables and (b) (4) (b) (4) from the primary 3-chamber bag, over-pouch, (b) (4), fill port, stopper, cap, printing ink (b) (4), and Intralipid 20%. The safety assessment for the potential migrants, leachables, extractables and the (b) (4) (b) (4) appears to be adequate and acceptable, and there are no significant safety concerns.

Thus, the nonclinical safety evaluation of Kabiven and (b) (4), based on the safety of individual lipid and amino acid components and of sodium glycerophosphate in the admixture appears to be adequate to support the use of these products in humans at the recommended dosage regimen.

Conclusion:

The safety of sodium glycerophosphate, and the lipid and amino acid components of Kabiven and (b) (4) has been adequately assessed in the nonclinical studies submitted.

However, the Applicant did not provide adequate information on the amount of heavy metals in each compartment of the 3-chamber bag formulations, or did not identify the heavy metals in the formulations. Thus, a safety assessment for the heavy metals was not possible due to a lack of adequate information.

Sushanta Chakder, Ph. D.
Supervisory Pharmacologist, DGIEP

11/08/11

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/s/

SUSHANTA K CHAKDER
11/08/2011

NDA/BLA or Supplement

Applicant: APP Pharmaceuticals Stamp Date: 01/28/2011

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?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
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?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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?			N/A
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		According to ICH guidelines on recommended acceptable amounts of impurities in pharmaceutical solvents
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.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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/s/

BABATUNDE E AKINSHOLA
03/23/2011

SUSHANTA K CHAKDER
03/23/2011