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Acute and Subchronic (28-Day) Oral Toxicity Study in Rats Fed with Novel Surfactants

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Ranjit Madhukar Bidhe[1] and Sangita Ghosh[2]

[1]Indian Institute of Toxicology, KIM 2057, Sadashiv Peth, Vijaynagar Colony, Pune, India 411 030
[2]BioZone Laboratories Inc, Pittsburg, CA 94565

ABSTRACT

The toxicity of 2 new synthetic lipids, 1,2-dioleoyl-*rac*-glycerol-3-dodecaethylene glycol, GDO-12 (lipid **1**) and 1,2-distearoyl-*rac*-glycerol-3-dodecaethylene glycol, GDS-12 (lipid **2**) has been evaluated in acute and subchronic toxicity studies. Acute oral toxicity studies in male and female rats documented no deaths or treatment-related signs at high doses. The lipids were individually administered (by gavage) to male and female Sprague-Dawley rats at concentrations of 250, 500, and 1000 mg/Kg bodyweight for 28 days. All animals survived the duration of the study, with no significant changes in clinical signs, hematological parameters, organ weights, ophthalmology evaluations, or histopathological findings. These studies establish that both GDO-12 (lipid **1**) and GDS-12 (lipid **2**) are nontoxic in rats following oral administration. The no-observed-adverse-effect level ranged between 250 mg/Kg and 1000 mg/Kg following oral administration.

KEYWORDS: oral toxicity, rats, polyoxyethylene glycol (PEG), liposomes

INTRODUCTION

Liposomes have received much attention for their usefulness in reducing toxicity and improving therapeutic effectiveness.[1] Although liposomes are a very promising, broadly applicable, and highly researched novel delivery system, they suffer from serious stability problems.[2] Phenomena associated with the aqueous suspension of liposomes such as aggregation, fusion, and phospholipid hydrolysis limit their shelf life. Polyoxyethylene glycol-lipid (PEG-lipid) conjugates have been reported, although in injectable formulations[3-7] that address the biologic stability problems associated with the conventional liposomes in vivo. To our knowledge, no such modified PEG-lipid has been reported for oral use. The oral route of drug administration is generally preferred because of its versatility, safety, and relative patient comfort. Hence, there is an outstanding need for lipids that form liposomes, are stable for prolonged shelf life, and yet have no toxicity when administered orally.

We have developed 2 synthetic PEG-lipid conjugates for use as a drug carrier and solubilizer. These lipids have the general structure shown in Figure 1 and have a PEG-12 head group and long hydrocarbon chains (oleoyl for 1 and stearoyl for 2). Liposomes formed spontaneously upon hydration of 2 new lipids.

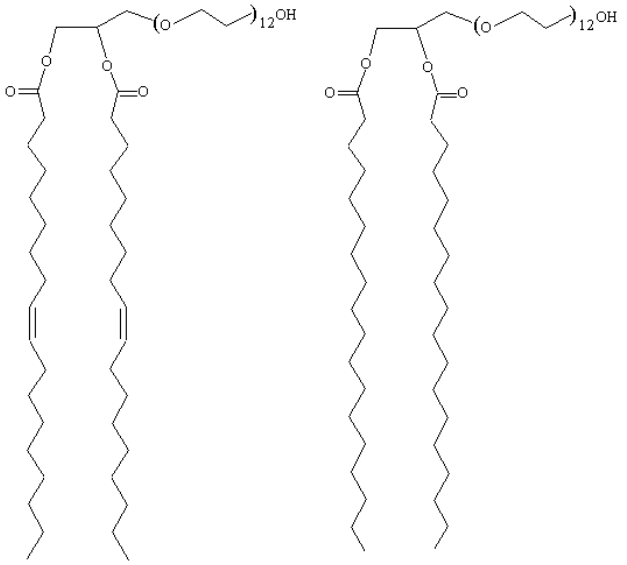

Figure 1. Chemical structure of 2 synthetic lipids: (A) PEG-12 glyceryl dioleate, GDO-12 (lipid **1**), and (B) PEG-12 glyceryl distearate, GDS-12 (lipid **2**).

However, no evaluation has been made so far regarding the oral toxicity of any PEGylated lipid conjugate. Safety evaluation would be more pertinent in view of the differences of the PEG head group of these types of lipids with respect to conventional phospholipids.

Corresponding Author: Sangita Ghosh, BioZone Laboratories Inc, Pittsburg, CA 94565.
Tel: (925) 473-1000. Fax: (925) 473-1001.
Email: sghosh@biozonelabs.com.

The joint expert committee on food additive's[8] typical criteria for safety evaluation include physical appearance and behavior, growth and body weight gain, food consumption, and absorption. In addition, the recommended criteria include the evaluation of utilization of other nutrients such as nitrogen, fat, calcium, and phosphorus. Digestibility and bioassay procedures for evaluation of feed utilization, plasma hematology and blood chemistry, gross pathological examination on necroscopy, organ weight, and histopathology are also recommended.

A rigorous safety evaluation of these lipids, GDO-12 (lipid **1**) and GDS-12 (lipid **2**) would enable their use as liposomal carriers of drugs and nutritional supplements in oral formulations. Therefore, we conducted a short-term subchronic toxicity study in rats by feeding both lipids individually at various levels for a period of 28 days to assess the oral safety aspect of these PEGylated lipids.

MATERIALS AND METHODS

Test Material

The lipids used in this work were 1,2-dioleoyl-*rac*-glycerol-3-dodecaethylene glycol (1) and 1,2-distearoyl-*rac*-glycerol-3-dodecaethylene glycol (2), synthesized by BioZone Laboratories (Pittsburg, CA). They have been abbreviated as GDO-12 (lipid 1) and GDS-12 (lipid 2) respectively. GDO-12 (lipid 1) was a liquid at room temperature (RT) while GDS-12 (lipid 2) was a waxy solid at RT. One mole of glycerol was reacted with 2 moles of oleic acid at 150°C under nitrogen to give glyceryl dioleate. Complete consumption of starting material as followed by liquid chromatography-mass spectrometry (LC-MS) indicated quantitative conversion. Glyceryl dioleate was then alkylated by passing ethylene oxide gas for 8 hours using sodium methylate as catalyst under nitrogen at 150°C until LC-MS analysis showed complete consumption of starting material. Completion of reaction was also monitored by measuring acid value and saponification value to give final product GDO-12 (lipid 1). For GDO-12 (lipid 1), saponification value was 105.7 and acid value was 0.001. Synthesis of GDS-12 (lipid 2) was similar to that of GDO-12 and stearic acid was used instead of oleic acid. For GDS-12 (lipid 2), saponification value was 104.7 and acid value was 1.0. The yields of the 2 lipids were quantitative.

Animals

Sprague-Dawley rats (aged 6-8 weeks) were in polycarbonate cages. Each cage contained 5 rats of the same sex with a bedding of husk, and 12-hour light/dark cycles were provided. Feed and water were given ad lib. Environmental conditions were maintained at a temperature of 22°C ± 2°C and a relative humidity of 60% ± 10%.

Preparation of Test Diets

GDO-12 (lipid 1) and GDS-12 (lipid 2) were diluted with corn oil and administered to the rats at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg, in the volume of 5 mL/kg. These test diets were freshly prepared everyday for 28 days. The control animals were administered vehicle only.

Experimental Design and Conduct

Forty healthy rats, 20 male and 20 female, were acclimatized to laboratory conditions for 7 days prior to initiation of dosing. They were randomly assigned to cages and the individual animal was fur marked with picric acid. The females were nulliparous and nonpregnant. Rats were assigned to treatment groups of 5 males and 5 females. The rats were deprived of feed for 16 hours before and 3 hours after administration of the test substance. The test substance, diluted with corn oil, was administered by gavage to rats of both sexes using a ball-tipped intubation needle fitted onto a syringe.

Observations of pharmacotoxic signs were made at 10, 30, 60, and 120 minutes and at 4 and 6 hours after dosing during the first day and daily thereafter for 28 days. The time of onset, intensity, and duration of these symptoms, if any, were recorded. All animals were observed twice daily for mortality during the 28-day period of study. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. The group mean body weights were calculated. The quantity of food consumed by groups consisting of 5 rats each was recorded weekly, and the food consumption per rat was calculated for control and dose groups. The eyes of control and animals from different groups were examined prior to the initiation of the dosing in week 4 of the study period on day 28. Eye examination was carried out using a hand slit lamp after the induction of mydriasis with 0.5% solution of tropic amide sulfate.

All reagents used were of analytical grade. At the end of the 28-day period the animals were fasted overnight. The following morning, each animal was heparinized and blood samples were collected from the orbital sinus. The hematological parameters hemoglobin concentration (Hb),[9] mean corpuscular volume (MCV), total erythrocyte count (RBC), reticulocyte concentration (Rt), mean corpuscular hemoglobin (MCH), hematocrit

(HCT), and total and differential leucocyte count were determined by standard methods using Serono 9110 automated hematology analyzer (Serono-Baker Diagnostics Inc, Allentown, PA. Plasma concentrations of glucose, total protein, albumin and blood urea nitrogen, sodium, and potassium were analyzed using a Boehringer Knoll autoanalyzer 4010 system (Boehringer GmbH, Mannheim, Germany). The plasma activities of serum alkaline phosphatase (SAP), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) were also assayed using Boehringer Knoll autoanalyzer 4010 system. Urine samples were also collected at the end of the study period and analyzed for pH, glucose, proteins, ketones, and occult blood. The rats were humanely killed on day 29 using CO_2 asphyxiation technique. Necroscopy of all animals was carried out and the weights of liver, kidneys, adrenals, and testes were recorded. The organ weights were recorded as absolute values and their relative values (ie, percentage of the body weight) were calculated.

Feed conversion efficiency percentage was calculated as follows:

$$Feed\ Conversion\ \ Efficiency\ \ (\%) = \frac{Weekly\ \ Body\ Weight\ \ Gain\ (g)}{Weekly\ \ Food\ \ Consumptio\ \ n\ (g)} \times 100 \qquad (1)$$

Histopathological observations were carried out in control and animals treated at the highest dose level of 1000 mg/kg. Tissue samples were preserved in 10% neutral buffered formalin.

Statistical analysis was done using Bartlett's homogeneity test, analysis of variance (ANOVA) and Dunnett test. The Student t test was employed to compare the statistical significance between control and experimental groups.

RESULTS

It was observed that the animals fed with the novel lipids GDO-12 (lipid **1**) and GDS-12 (lipid **2**) were healthy. No unusual changes in behavior or in locomotor activity, no ataxia, and no signs of intoxication were observed during the 28-day period. No differences were found in growth between the control group and the animals fed with different levels of the alkoxylated diesters GDO-12 (lipid **1**) and GDS-12 (lipid **2**) (Figures 2, Figure 3, Figure 4 & Figure 5). The food consumption (and therefore the feed conversion efficiency) of male and female rats of control and experimental groups was similar, indicating that the feed intake and utilization was not affected (Tables 1 and 2).

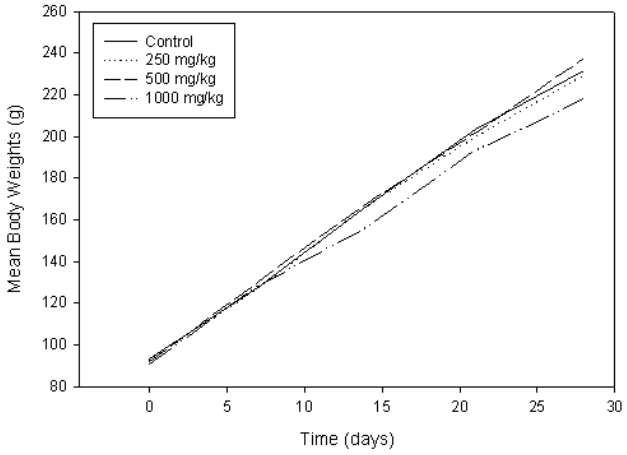

Figure 2. Growth curve of male rats fed GDO-12 (lipid **1**).

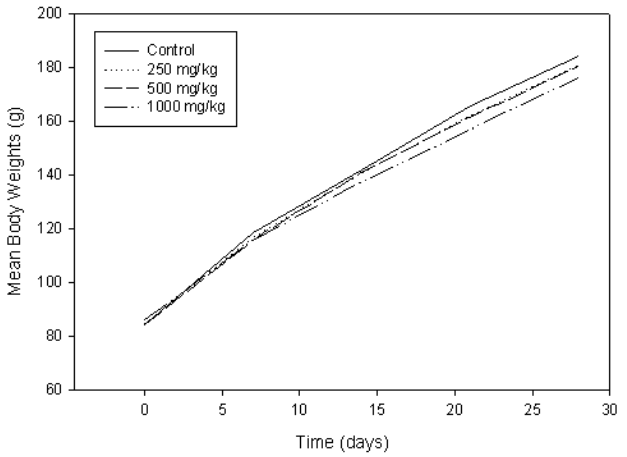

Figure 3. Growth curve of female rats fed GDO-12 (lipid **1**).

Hematological parameters, hemoglobin concentration, total and differential erythrocyte count, total and differential leucocyte count, hematocrit, and mean cell hemoglobin concentration, in both control and experimental rats, were not significantly different ($P > .05$) for both lipids **1** and **2** (Tables 3 and 4). All values were found to be within the normal range for rats,[10] and there were no differences between the groups.

The levels of plasma analytes, such as total protein, blood urea nitrogen (BUN), glucose, bilirubin, albumin, creatine, cholesterol, chloride, calcium, phosphorus, sodium, and potassium ions were not significantly different between the control and the experimental groups of rats ($P > .05$) when fed with lipids **1** and **2** (Tables 5 and 6). Analysis of the urinary metabolite levels (glucose, proteins, hemoglobin, and ketones) showed trace or no

Table 1. Mean Food Consumption of the Animals During the Study Period (g/Animal) With GDO-12 (lipid **1**)

Male	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	10.42	12.60	14.80	16.14	17.76
250 mg/kg	10.74	13.12	15.16	15.92	18.08
500 mg/kg	10.04	11.92	14.22	15.08	17.00
1000 mg/kg	10.58	12.16	14.54	15.48	17.58
Female	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	8.76	11.26	13.66	14.62	16.76
250 mg/kg	8.68	12.16	13.92	14.80	17.20
500 mg/kg	7.72	10.68	13.02	14.18	15.98
1000 mg/kg	9.30	10.92	13.38	14.30	16.70

Table 2. Mean Food Consumption of the Animals During the Study Period (g/Animal) With GDS-12 (lipid **2**)

Male	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	10.42	12.60	14.80	16.14	17.76
250 mg/kg	10.48	12.90	15.02	15.96	17.84
500 mg/kg	9.72	12.28	14.36	15.36	17.04
1000 mg/kg	10.20	12.44	14.64	15.60	17.56
Female	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	8.76	11.26	13.66	14.62	16.76
250 mg/kg	8.78	11.78	13.82	14.82	16.80
500 mg/kg	8.12	11.12	13.26	14.58	16.24
1000 mg/kg	9.00	11.24	13.52	14.76	16.58

presence of these in both control and experimental animals fed with lipids **1** and **2**.

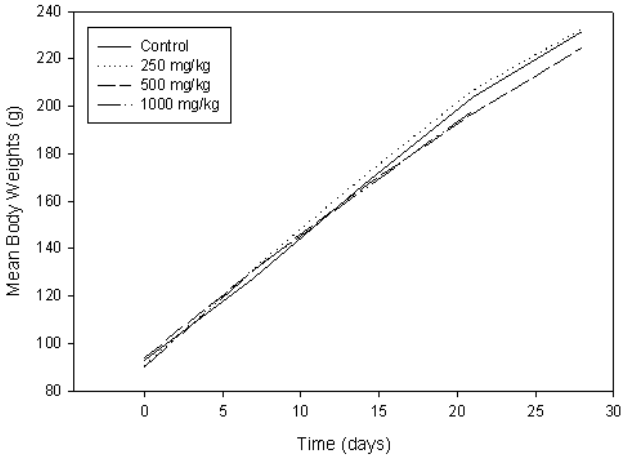

Figure 4. Growth curve of male rats fed GDS-12 (lipid **2**). Note: Possible changes (slight reduction) in locomotor activity were observed in 2 of the 5 male rats at doses of 1000 mg/kg after 20 days. Hence, the growth curve at concentration of 1000 mg/kg has been plotted to day 20.

Tables 7 and 8 detail the activities of enzymes analyzed in plasma. No significant differences were observed in enzyme activities between the control and lipid-fed animals.

Figures 6 and 7 depict the organ-to-body mass ratios of animals at the end of 28 days' feeding. No abnormal changes were observed in organ mass with respect to body mass of GDO-12 (lipid **1**) and GDS-12 (lipid **2**) fed rats in comparison with controls. Observations of gross pathology immediately after dissection, on rats of all groups were found to be uniformly healthy, lacking in any apparent pathological abnormalities.

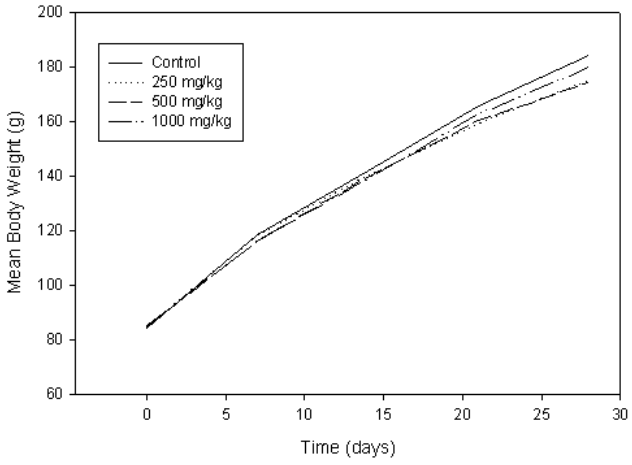

Figure 5. Growth curve of female rats fed GDS-12 (lipid **2**).

Histopathological examination of the liver and kidneys in the control and the GDO-12 (lipid **1**) and GDS-12

Table 3. Hematological Data of Rats Fed with GDO-12 (lipid **1**) for 28 Days*

Sex	Dose mg/kg	Hb (g%)	RBC (10^6/mm^3)	Rt (%)	HCT (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelets (10^5/mm^3)	Total Leucocytes (10^3/mm^3)	N %	L %	E %	M %
Male	Control	14.5 ± 0.5	7.55 ± 0.6	1.34 ± 0.4	44.06 ± 1.7	58.46 ± 2.4	19.22 ± .85	32.92 ± 0.2	3.4 ± 0.2	9.06 ± 1.2	21.8	74.8	1	2.4
	250	14.46 ± 0.3	7.51 ± 0.3	1.5 ± 0.4	43.9 ± 0.9	58.52 ± 1.3	19.22 ± 0.4	32.94 ± 0.2	3.42 ± 0.5	9.2 ± 1.9	21.2	75.6	1.2	2.4
	500	14.44 ± 0.4	7.52 ± 0.45	1.5 ± 0.3	43.62 ± 0.8	58.08 ± 2.4	19.18 ± .63	33.06 ± 0.4	3.5 ± 0.2	9.9 ± 2.1	22.6	74.6	0.6	2.2
	1000	14.54 ± 0.3	7.57 ± 0.3	1.52 ± 0.3	44.02 ± 1	58.18 ± 1.6	19.22± 0.37	33.02 ± 0.3	3.4 ± 0.3	10.0 ± 1.9	21.8	75.2	1.2	1.8
Female	Control	14.4 ± 0.3	7.44 ± 0.3	1.36 ± 0.4	43.68 ± 0.8	58.78 ± 1.6	19.38 ± 0.4	32.96 ± 0.3	3.35 ± 0.3	9.32 ± 1.8	21.2	75.4	1	2.4
	250	14.5 ± 0.3	7.56 ± 0.4	1.42 ± 0.4	44.08 ± 1.0	58.34 ± 1.6	19.18 ± 0.5	32.86 ± 0.2	3.38 ± 0.2	9.36 ± 1.9	22.4	75.8	0.8	2.2
	500	14.48 ± 0.3	7.52 ± 0.3	1.4 ± 0.3	43.92 ± 0.8	58.38 ± 1.1	19.14 ± 0.5	32.96 ± 0.2	3.5 ± 0.3	9.76 ± 1.9	21.4	75.4	1	2.2
	1000	14.42 ± 0.3	7.58 ± 0.3	1.48 ± 0.3	43.78 ± 0.9	58.58 ± 1.2	19.38 ± 0.5	32.94 ± 0.2	3.37 ± 0.3	9.42 ± 2.4	21.0	76.0	0.6	2.4

*Hb indicates hemoglobin; RBC, red blood cells; Rt, reticulocyte; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; N, neutrophils; L, lymphocytes; E, eosinophils; and M, monocytes. All values are expressed as mean ± SD.

Table 4. Hematological Data of Rats Fed With GDS-12 (lipid **2**) for 28 Days*

Sex	Dose mg/kg	Hb (g%)	RBC (10^6/mm^3)	Rt (%)	HCT' (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelets (10^5/mm^3)	Total Leucocytes (10^3/ mm^3)	N %	L %	E %	M %
Male	Control	14.5 ± 0.5	7.55 ± 0.6	1.34 ± 0.4	44.0 ± 1.7	58.7 ± 2.4	19.22 ± 0.85	32.9 ± 0.2	3.4 ± 0.2	9.06 ± 1.2	21.8	74.8	1	2.4
	250	14.4 ± 0.3	7.42 ± 0.4	1.4 ± 0.3	43.54 ± 0.8	58.7 ± 1.6	19.38 ± 0.4	33.04 ± 0.3	3.4 ± 025	9.32 ± 1.7	21.2	75.8	0.8	2.2
	500	14.46 ± 0.4	7.5 ± 0.3	1.46 ± 0.3	43.82 ± 0.8	58.4 ± 1.3	19.26 ± 0.3	33.02 ± 0.4	3.3 ± 0.2	9.86 ± 2.2	21.8	75.0	0.8	2.4
	1000	14.48 ± 0.2	7.6 ± 0.3	1.44 ± 0.3	43.9 ± 0.8	57.8 ± 1.4	19.04 ± 0.5	32.98 ± 0.2	3.48 ± 0.2	9.08 ± 1.7	21.4	75.4	1	2.2
Female	Control	14.4 ± 0.3	7.4 ± 0.3	1.4 ± 0.4	43.7 ± 0.8	58.8 ± 1.6	19.4 ± 0.4	32.9 ± 0.3	3.4 ± 0.3	9.3 ± 1.8	21	75	1	2.4
	250	14.5 ± 0.3	7.6 ± 0.4	1.4 ± 0.4	44.1 ± 1.0	58.3 ± 1.6	19.2 ± 0.5	32.9 ± 0.2	3.4 ± 0.2	9.4 ± 1.9	22	76	0.8	2.2
	500	14.5 ± 0.3	7.5 ± 0.3	1.4 ± 0.3	43.9 ± 0.8	58.4 ± 1.1	19.1 ± 0.5	32.9 ± 0.1	3.5 ± 0.3	9.8 ± 1.9	21	75	1	2.0
	1000	14.4 ± 0.3	7.6 ± 0.3	1.5 ± 0.4	43.7 ± 0.9	58.6 ± 1.2	19.4 ± 0.5	32.9 ± 0.2	3.4 ± 0.3	9.4 ± 2.4	21	76	0.6	2.2

*Hb indicates hemoglobin; RBC, red blood cells; Rt, reticulocyte; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; N, neutrophils; L, lymphocytes; E, eosinophils; and M, monocytes. All values are expressed as mean ± SD.

Table 5. Plasma Biochemical, Na^+, K^+, Ca^{2+}, and Cl^- Profile of the Control and Experimental Groups Fed With GDO-12 (lipid **1**) for 28 Days*

Sex	Dose mg/kg	Total Serum Protein (g%)	BUN (mg%)	Blood Sugar (mg%)	Calcium (mg%)	Phosphorus (mg%)	Bilirubin (mg%)	Albumin (mg%)	Creatine (mg%)	Sodium mmol/L
Male	Control	6.37 ± 0.2	11.52 ± 0.9	78.78 ± 3.8	9.45 ± 0.2	4.04 ± 0.3	0.59 ± 0.09	3.41 ± 0.2	0.95 ± 0.04	139.38 ± 1.4
	250	6.35 ± 0.2	11.17 ± 1.4	80.8 ± 5.4	9.4 ± 0.3	4.0 ± 0.1	0.63 ± 0.1	3.42 ± 0.2	0.95 ± 0.03	138.94 ± 2.2
	500	6.42 ± 0.3	10.68 ± 1.3	79.86 ± 1.9	9.5 ± 0.3	3.98 ± 0.3	0.65 ± 0.1	3.38 ± 0.2	0.94 ± 0.02	138.16 ± 2.1
	1000	6.44 ± 0.3	11.55 ± 1.5	78.78 ± 2.9	9.47 ± 0.3	3.92 ± 0.1	0.62 ± 0.07	3.31 ± 0.2	0.95 ± 0.04	136.54 ± 5.3
Female	Control	6.31 ± 0.2	11.24 ± 1.2	79.38 ± 2.5	9.47 ± 0.2	4.02 ± 0.3	0.65 ± 0.07	3.41 ± 0.2	0.95 ± 0.02	139.2 ± 1.8
	250	6.48 ± 0.2	11.1 ± 1.3	79.58 ± 3.9	9.34 ± 0.4	3.98 ± 0.2	0.58 ± 0.08	3.43 ± 0.19	0.95 ± 0.04	138.2 ± 1.8
	500	6.32 ± 0.2	11.3 ± 1.3	80.46 ± 3.9	9.45 ± 0.3	4.01 ± 0.2	0.62 ± 0.12	3.36 ± 0.29	0.95 ± 0.03	138.68 ± 1.45
	1000	6.58 ± 0.2	11.15 ± 0.9	79.72 ± 2.5	9.46 ± 0.2	3.95 ± 0.1	0.66 ± 0.08	3.37 ± 0.23	0.96 ± 0.03	138.22 ± 3.9

Sex	Dose mg/kg	Potassium mmol/L	Chloride mmol/L	Cholesterol (mg%)
Male	Control	3.84 ± 0.1	102.66 ± 1.7	62.68 ± 7.7
	250	3.64 ± 0.5	103.5 ± 1.5	61.62 ± 3.2
	500	3.48 ± 0.4	103.08 ± 1.4	60.0 ± 5.4
	1000	3.7 ± 0.5	102.34 ± 2.9	61.5 ± 5.6
Female	Control	3.58 ± 0.3	103.28 ± 1.2	61.08 ± 2.4
	250	3.57 ± 0.5	103.36 ± 1.3	63.3 ± 4.7
	500	3.61 ± 0.4	103.98 ± 1.4	61.12 ± 3.0
	1000	3.71 ± 0.4	103.1 ± 2.3	64.02 ± 7.7

*BUN indicates blood urea nitrogen.

Table 6. Plasma Biochemical, Na^+, K^+, Ca^{2+}, and Cl^- Profile of the Control and Experimental Groups Fed With GDS-12 (lipid **2**) for 28 Days*

Sex	Dose mg/kg	Total Serum Protein (g%)	BUN (mg%)	Blood Sugar (mg%)	Calcium (mg%)	Phosphorus (mg%)	Bilirubin (mg%)	Albumin (mg%)	Creatine (mg%)	Sodium mmol/L
Male	Control	6.37 ± 0.2	11.52 ± 0.9	78.78 ± 3.8	9.45 ± 0.2	4.04 ± 0.3	0.59 ± 0.09	3.41 ± 0.2	0.95 ± 0.04	139.38 ± 1.4
	250	6.44 ± 0.3	11.17 ± 1.8	81.12 ± 2.9	9.3 ± 0.2	4.07 ± 0.2	0.64 ± 0.08	3.39 ± 0.2	0.94 ± 0.02	139.14 ± 3.5
	500	6.45 ± 0.3	11.35 ± 1.3	80.46 ± 5.5	9.32 ± 0.3	4.08 ± 0.2	0.62 ± 0.1	3.58 ± 0.1	0.95 ± 0.03	138.3 ± 2.9
	1000	6.43 ± 0.3	11.79 ± 1.7	79.77 ± 3.2	9.36 ± 0.2	3.91 ± 0.1	0.65 ± 0.1	3.31 ± 0.2	0.95 ± 0.03	137.44 ± 3.3
Female	Control	6.31 ± 0.2	11.24 ± 1.2	79.38 ± 2.5	9.47 ± 0.2	4.02 ± 0.3	0.615 ± 0.07	3.41 ± 0.2	0.95 ± 0.02	139.2 ± 1.8
	250	6.32 ± 0.3	11.6 ± 1.2	79.8 ± 4.2	9.43 ± 0.3	4.0 ± 0.3	0.61 ± 0.07	3.55 ± 0.2	0.95 ± 0.03	137.4 ± 4.7
	500	6.34 ± 0.2	11.48 ± 1.4	80.24 ± 3.6	9.52 ± 0.3	3.93 ± 0.1	0.61 ± 0.09	3.32 ± 0.3	0.94 ± 0.03	138.74 ± 2.4
	1000	6.37 ± 0.2	11.67 ± 1.2	79.08 ± 3.4	9.34 ± 0.3	3.98 ± 0.3	0.61 ± 0.11	3.36 ± 0.3	0.94 ± 0.03	139.22 ± 3.9

Sex	Dose mg/kg	Potassium mmol/L	Chloride mmol/L	Cholesterol (mg%)
Male	Control	3.84 ± 0.1	102.66 ± 1.7	62.68 ± 7.7
	250	3.87 ± 0.2	102.72 ± 1.3	60.54 ± 5.5
	500	3.63 ± 0.3	102.66 ± 1.7	61.76 ± 3.0
	1000	3.63 ± 0.4	102.84 ± 1.9	62.84 ± 2.0
Female	Control	3.58 ± 0.3	103.28 ± 1.2	61.08 ± 2.4
	250	3.75 ± 0.4	102.94 ± 2.0	60.4 ± 7.9
	500	3.66 ± 0.4	102.6 ± 1.6	61.34 ± 12.0
	1000	3.52 ± 0.4	103.4 ± 2.0	60.9 ± 5.8

*BUN indicates blood urea nitrogen.

Table 7. Enzyme Activities in Plasma of the Rats Fed With GDO-12 (lipid **1**) for 28 Days*

Sex	Dose mg/kg	SGPT (IU/L)	SGOT (IU/L)	SAP (IU/L)	ϒ GT (U/L)
Male	Control	19.10 ± 1.5	21.04 ± 1.5	23.64 ± 1.6	14.8 ± 3.9
	250	19.98 ± 1.7	20.74 ± 2.05	23.66 ± 2.03	14.8 ± 3.56
	500	19.64 ± 1.5	20.42 ± 1.4	24.94 ± 1.6	15.2 ± 4.09
	1000	19.86 ± 1.1	20.84 ± 1.3	24.22 ± 2.6	15.4 ± 3.36
Female	Control	18.54 ± 1.3	20.44 ± 1.8	23.58 ± 2.4	14.04 ± 3.05
	250	19.32 ± 2.2	20.28 ± 2.4	24.2 ± 1.5	13.4 ± 2.88
	500	19.38 ± 1.5	20.84 ± 2.0	24.5 ± 2.2	15.00 ± 3.54
	1000	19.92 ± 2.4	21.02 ± 2.00	24.32 ± 2.04	14.6 ± 3.8

*SGPT indicates serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; SAP, serum alkaline phosphatase; and ϒ GT, gamma Glutamyl Transferase.

Table 8. Enzyme Activities in Plasma of the Rats Fed With GDS-12 (lipid **2**) for 28 Days*

Sex	Dose mg/kg	SGPT (IU/L)	SGOT (IU/L)	SAP (IU/L)	ϒ GT (U/L)
Male	Control	19.10 ± 1.5	21.04 ± 1.5	23.64 ± 1.6	14.8 ± 3.9
	250	19.32 ± 1.5	20.7 ± 1.3	24.68 ± 2.4	13.4 ± 2.9
	500	19.92 ± 1.5	20.94 ± 1.75	24.08 ± 2.0	13.6 ± 3.21
	1000	19.6 ± 2.1	21.14 ± 2.1	25.16 ± 1.9	13.2 ± 2.86
Female	Control	18.54 ± 1.3	20.44 ± 1.8	20.44 ± 1.8	14.04 ± 3.05
	250	19.92 ± 2.1	20.62 ± 1.96	20.62 ± 1.96	14.8 ± 3.7
	500	19.7 ± 2.4	20.8 ± 2.3	20.8 ± 2.3	14.8 ± 3.7
	1000	19.54 ± 1.8	21.08 ± 1.5	21.08 ± 1.5	15.0 ± 3.5

*SGPT indicates serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; SAP, serum alkaline phosphatase; and ϒ GT, gamma Glutamyl Transferase.

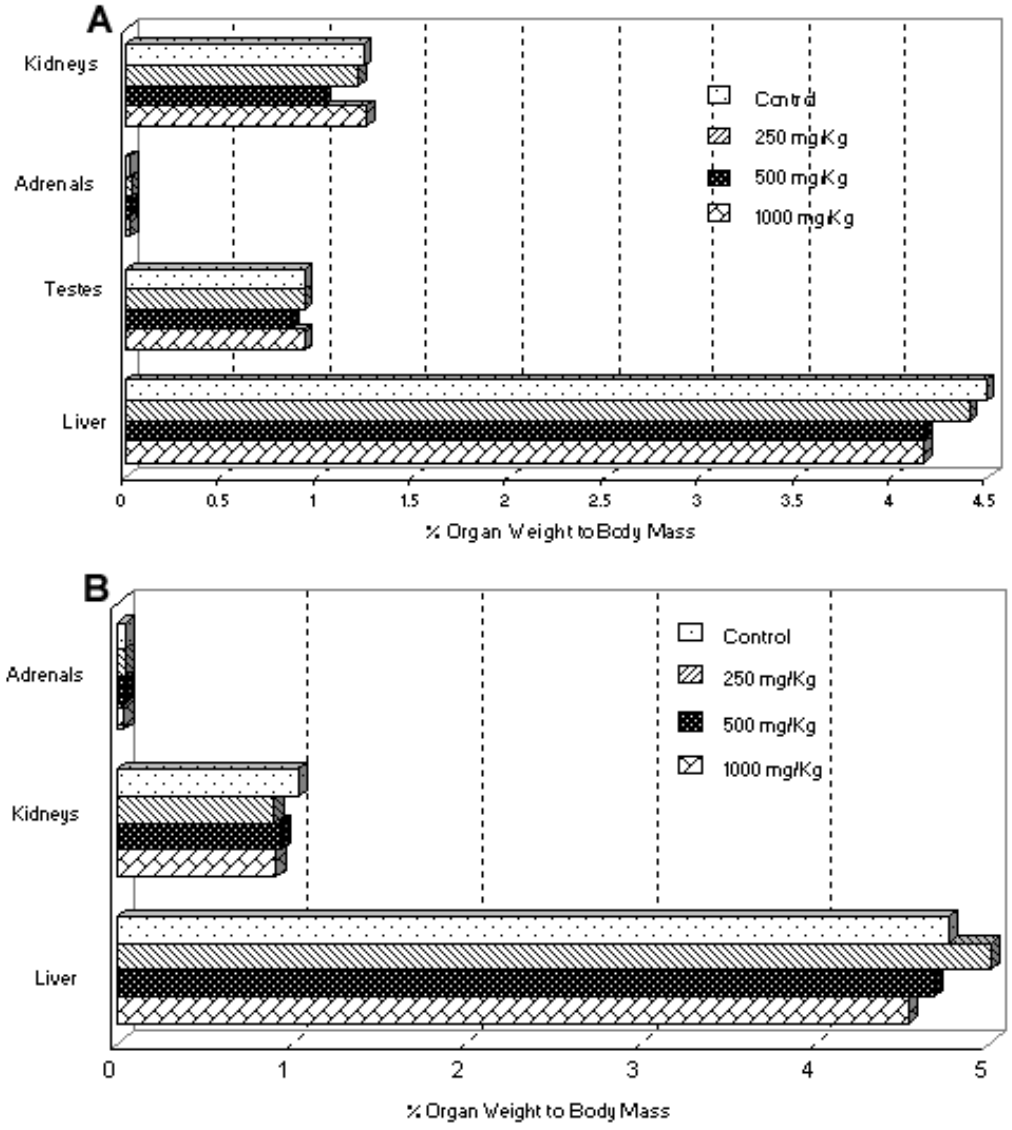

Figure 6. (A) Percentage organ weight to body mass of male rats fed GDO-12 (lipid **1**). (B) Percentage organ weight to body mass of female rats fed GDO-12 (lipid **1**).

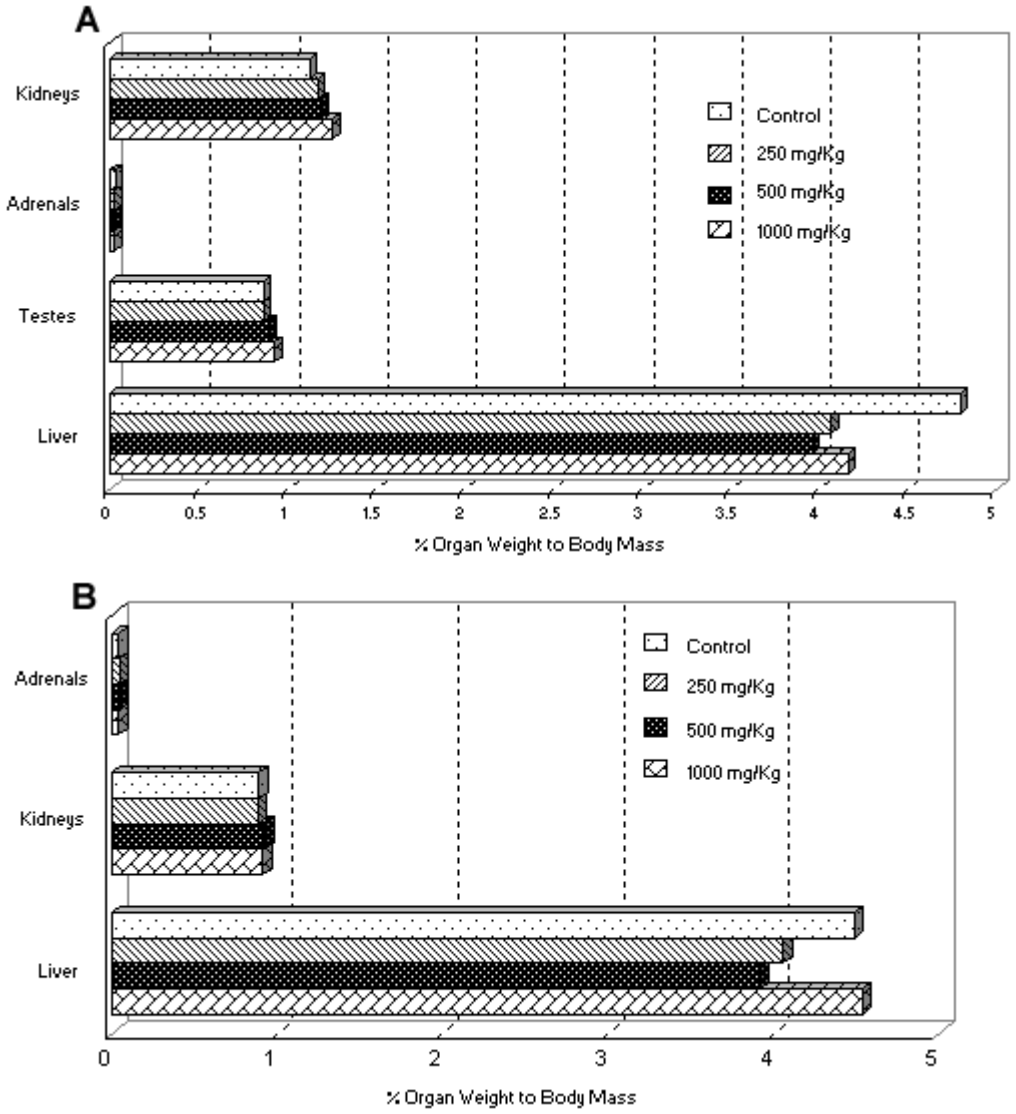

Figure 7. (A) Percentage organ weight to body mass of male rats fed GDS-12 (lipid **2**). (B) Percentage organ weight to body mass of female rats fed GDS-12 (lipid **2**).

(lipid **2**) fed groups showed no differences, indicating that feeding these synthetic lipids at these levels to the rats did not result in any adverse toxicological effect on these organs.

DISCUSSION

The novel synthetic lipids used in this study are unique and different from conventional phospholipids. Unlike phospholipids, GDO-12 (lipid **1**) and GDS-12 (lipid **2**) lack a phosphate ester head group, and they are uncharged. In addition, these lipids have a polyoxyethylene glycol head group. A unique feature of these lipids is that they form vesicles spontaneously when hydrated. Liposome encapsulation of drug with GDO-12 (lipid **1**) and GDS-12 (lipid **2**) does not require organic solvents and sonication methods. This characteristic increases the scope of using this drug delivery system for pharmaceutical use.

In structure, these lipids are similar to their phospholipid analogs, which have been explored extensively as drug delivery vehicles.[11] However, for the first time, application of GDO-12 (lipid **1**) and GDS-12 (lipid **2**) as drug delivery vehicles is being sought. (Formulations of drugs incorporated in GDO-12 (lipid **1**) or GDS-12 (lipid **2**) were made. A few examples follow. Example 1: Methotrexate (5% wt, Sigma, St Louis, MO) was added to GDO-12 (lipid **1**) (10% wt) and heated to 55°C and gen-

tly mixed until dissolved. The resultant mixture was a clear solution. Deionized water (85% wt) was slowly added to the solution and gently mixed for 5 minutes. The resultant was an opaque yellow solution. The preparation was examined under a phase contrast microscope and showed multilamellar liposomes. Example 2: Weighed amounts of GDO-12 (lipid **1**) (18 g), betamethasone dipropionate (50 mg), and cholesterol (100 mg) were combined and heated to 50°C while mixing. Uniphen-23 (LipoChemicals, Paterson, NJ) (1.5 mg) and deionized water (81.85 g) were separately combined and the mixture heated to 50°C. The 2 mixtures were commingled at 50°C while stirring gently. The final mixture was cooled to room temperature. Examination by phase contrast optical microscopy showed multilamellar liposomes.)

The purpose of this study was to look at the toxicity profile of the synthetic lipids. A 28-day study is considered a subchronic study, which is well accepted for eliciting any toxicity on long-term feeding. These lipids at various levels did not appear to retard growth or affect food consumption and utilization (Figures 2, Figure 3, Figure 4 & Figure 5). The feed conversion efficiency of the different groups followed a similar pattern indicating a normal metabolism of the animals (Table 1).

The mean food consumption of the animals in the control and experimental groups was similar (Tables 1 and 2). This finding indicates that the feed intake and utilization of protein and other nutrients were not affected by the intake of the synthetic lipid. Moreover, there were no differences between the sexes with respect to feed conversion efficiency.

There were no significant changes in the hematological parameters between the control and the experimental groups (Tables 3 and 4), suggesting that the lipids GDO-12 (lipid **1**) and GDS-12 (lipid **2**) may not be toxic as they do not affect the circulating red cells, nor the hematopoiesis and leucopoiesis that could otherwise have caused a megaloblastic anemia, nor changes in packed cell volume (PCV) and eosinophils. Plasma levels of glucose, total proteins, albumin, blood urea nitrogen, sodium, and potassium ions were not affected by feeding GDO-12 (lipid **1**) and GDS-12 (lipid **2**) (Tables 5 and 6). This finding also indicates that the normal metabolism of the animals was not affected.

It is clear that the liver and kidneys play significant roles in various metabolic processes. Therefore, emphasis was placed on the effect these lipids might have on the function of these organs. In addition, the liver plays an important role in xenobiotic function, while kidneys are sites for reabsorption. Feeding either GDO-12 (lipid **1**) or GDS-12 (lipid **2**) did not alter the urinary levels of glucose, protein, hemoglobin, bilirubin, or creatinine indicating normal hepatocellular and nephrotic function.

A review of the literature on the safety of PEGylated lipids and other polymers suggests that they are safe for use in parenterally administered pharmaceuticals.[12] GDO-12 (lipid **1**) and GDS-12 (lipid **2**) are both PEGylated lipids that form spontaneous liposomes and have successfully solubilized a variety of hydrophobic compounds like tetracaine, lidocaine, cholesterol, tretenoin, bethamethasone propionate etc.[13] In our efforts to determine the possible use of these lipids in oral formulations, we have evaluated the acute and subchronic toxicity of these 2 lipids. Our findings indicate that these lipids are nontoxic and well tolerated for the 28-day study period and therefore have potential for safe use in oral formulations. Further work toward developing formulations of different drugs using these 2 delivery vehicles and evaluating their efficacy as oral drug delivery systems is in progress and shall be reported elsewhere.

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