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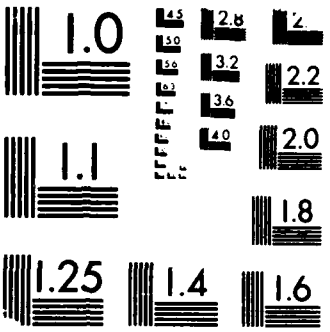

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THE SUBCHRONIC TOXICITY OF DIETHYLENE GLYCOL MONOBUTYL ETHER ADMINISTERED ORALLY TO RATS

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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Diethylene glycol monobutyl ether (DGBE), a primary component of aqueous film forming foams (AFFF) used by the U.S. Navy in shipboard firefighting systems, was assayed for acute and subchronic toxicity in male and female Fischer-344 rats. The estimated LD_{50} (oral) was 6.53 g/kg and 5.08 g/kg for male and female rats, respectively. Rats (16/group) were treated five days/wk for 13 weeks by gavage with: DGBE, in amounts equal to 1, 5, or 25% of the LD_{50}; EGBE (positive control) or water (negative control). All animals received equivalent dose volumes. Male rats receiving DGBE (high dose) showed a significant and persistent reduction in body weight after one week exposure; food consumption for these animals was lower during the first three weeks of exposure. Female body weights were unchanged from controls. After 13 weeks exposure, mortality for male and female rats receiving water, EGBE, and low, medium, or high dose DGBE was 0, 30, 0, 60, 83, and 0, 10, 30, and 92 percent, respectively. All rats exposed to EGBE for 13 weeks had elevated liver and spleen weights and exhibited lowered red blood cell counts, lymphocyte counts, and mean

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corpuscular hemoglobin concentration (MCHC). A dose-related decrease in MCHC was observed in female rats exposed to DGBE. Generally, dose-related gross and microscopic lesions were restricted to the thoracic cavity and respiratory tract where pulmonary congestion and edema were common findings in rats which failed to survive the entire dosing schedule. Lesions compatible with gavage trauma were common in several dose groups exhibiting increased mortality.

PREFACE

THE SUBCHRONIC TOXICITY OF DIETHYLENE GLYCOL MONOBUTYL ETHER ADMINISTERED ORALLY TO RATS

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This work was supported by the Naval Medical Research and Development Command, Research Task No. MR04122010006. The opinions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The experiments described were conducted in accordance with the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council. This information was presented in part at the 25th Annual Meeting of the Society of Toxicology, New Orleans, Louisiana, March 3-7, 1986. The current address of Dr. Hobson is Battelle Laboratories, Columbus, OH 43201-2693.

SUBCHRONIC EGBE/DGBE STUDY

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INTRODUCTION

Diethylene glycol monobutyl ether (DGBE) is a principal component of Aqueous Film Forming Foam (AFFF), a liquid concentrate used in U.S. Navy shipboard fire protection proportioning systems. DGBE also has been used in some household cleaning products. For firefighting purposes, AFFF is added to seawater by the shipboard proportioning systems to produce concentrations from 6 to 50%. Firefighters may come into contact with AFFF during application or may be forced to wade in the foam produced by AFFF for prolonged periods.

Characterization of the toxicity of DGBE has been limited to a few reports in the literature. Like most other diethylene glycol ethers, DGBE has a low acute toxicity with reported oral LD_{50} values of 6.56 g/kg in rats and 2.0 g/kg in guinea pigs (Browning, 1965) Repeated exposures for 5-35 days at doses of 650 mg/kg is reported to have produced hydropic degeneration of the renal tubules in rats (Kesten et al., 1939).

Recent reproductive toxicity studies have shown that rats orally dosed with DGBE at doses as high as 1.0 g/kg/day exhibited no adverse affects on male or female fertility or on embryos, fetuses, or neonates, except a slight reduction in mean pup weight during the latter stages of lactation. In the same study, rabbits demonstrated no adverse effects on intrauterine survival or on the incidence of fetal malformations at DGBE exposures as high as 1.0 g/kg/day (Nolen et al., 1985).

A homologue analog of DGBE, ethylene glycol monobutyl ether (EGBE) has been shown to produce toxicologic changes in the

kidneys and erythrocytes of subchronically exposed rats (Dodd et al., 1983; Krasavage, 1986). It has been shown that the toxicity of the glycol ethers generally decreases with branching or increased length of the alkyl side chain (Hardin et al., 1983). Based on structural similarity to DGBE, EGBE was selected for use as a comparison control chemical for the present study.

The purpose of this study was; (1) to determine if rats exposed to DGBE via the oral route for 13 weeks, exhibit significant toxicologic effects and; (2) to contrast these effects with the effects of EGBE when administered to rats in a similar fashion.

Although the anticipated route of DGBE exposure for firefighters is the dermal route, the oral route was selected for use in this study because; (1) the route of administration appears to have little effect on the expression of toxicity of the glycol ethers; (2) glycol ethers are generally absorbed more rapidly when administered via the oral route than via the dermal route; (3) systemic toxicity should be enhanced following oral administration relative to dermal application of the same dose; (4) and because oral dosing allows the comparison of the results obtained from this study to those of other glycol ethers where the oral route was also selected.

MATERIALS AND METHODS

Test Materials: EGBE and DGBE were purchased from Aldrich Chemical Company, Milwaukee, WI; the stated purity of each

chemical was 99% (the respective lot numbers were #CM0925EK and #AM3914AM).

Animals: Male and female Fischer-344 rats (approx. age=8 weeks,) were obtained from Charles River Breeding Laboratories and both groups were sampled for quality control purposes two weeks prior to initiating the study. Throughout the study, water was provided ad libitum and food was provided ad libitum except during urine collection periods when the animals were fasted.

Body weight gain and food consumption were monitored on a weekly basis.

Experimental Design: For each sex, the experiment consisted of three DGBE treatments (1, 5 and 25% of the oral LD_{50} value per day); one EGBE treatment (25% of the oral LD_{50} value per day) and a water control. Each treatment group consisted of 16 male and female rats each. A computerized randomization procedure was used to assign rats to their respective groups.

The rats were dosed via oral gavage 5 days per week for 13 weeks. The dose volume administered was constant relative to body weight (0.2% of body wt.). Deionized water was used as the diluent.

An interim sacrifice was conducted at 6 weeks. Following this sacrifice, all groups consisted of 10 rats each, except the high dose DGBE group, which consisted of 4 males and 4 females due to an increased rate of mortality at this dose level.

At 6 weeks and at 13 weeks, all rats scheduled for necropsy were placed in metabolism cages to obtain 24 hour urine

collections. Thereafter, the animals were killed via anesthetic overdose (Halothane, Halocarbon Laboratories, Inc., Hackensack, NJ), whole blood and serum samples were obtained from the posterior vena cava, necropsies were performed, and organ weights were recorded.

Clinical Measurements: Serum and urine chemistries were performed using commercially available kits and reagents (Sigma Diagnostics, St. Louis, MO) adapted for use with a Cobas-Bio centrifugal analyzer (Roche Analytical Instruments, Inc., Nutley, NJ). Serum clinical chemistry parameters measured included alanine aminotransferase (ALT), albumin, alkaline phosphatase (AP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatine phosphokinase (CPK), creatinine, calcium, glucose, lactate dehydrogenase (LDH), and total protein. Isoenzyme analysis was not performed. Commercially prepared sera (Decision, Beckman Instruments, Inc., Brea, CA) were used to monitor the quality of clinical chemical assays. Urine parameters measured were calcium, creatinine, AST, and n-acetyl-glucosaminidase (NAG). AST and NAG were used as enzyme markers to detect damage to the renal tubular epithelium. Prior to the determination of urinary calcium, urine samples were acidified to pH 1.0 with hydrochloric acid to release oxalate and phosphate bound calcium. Urine volume, specific gravity (Hand Protometer, National Instrument Co., Inc., Baltimore, MD) and pH (Model 801A Digital Ionalyzer, Orion Research, Inc., Cambridge, MA) were also recorded. Additional, semi-quantitative, measures of urinary protein, blood, urobilinogen, bilirubin, ketones,

glucose and pH were performed using urine chemistry test strips (Chemstrip 7, Bio-Dynamics, Indianapolis, IN).

Hematological measurements of red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and white blood cell count (WBC) were made using a Coulter Model S Plus counter (Coulter Electronics, Hialeah, FL). Differential white cell counts were obtained from whole blood smears stained with Wright's stain.

Pathology: Gross pathological changes were recorded at necropsy and the following tissues were submitted for histopathologic examination: bone, bone marrow, nose, thyroid, parathyroid, lungs, heart, liver, spleen, thymus, mandibular and mesenteric lymph nodes, kidneys, adrenal gland, salivary gland, urinary bladder, testes, prostate, seminal vesicles, ovaries, uterus, ileum, duodenum, jejunum, pancreas, brain, colon, nerve, muscle, esophagus and trachea.

Statistics: LD_{50} values were determined using a method which utilizes a small number of animals (Bruce et al., 1985). The LD_{50} values obtained for DGBE were 6.53 g/kg for male rats and 5.08 g/kg for female rats. A literature LD_{50} value of 0.47 g/kg was used as a basis for determining the EGBE dose to be administered to both male and female rats (Browning, 1965).

Significant differences ($p \leq 0.05$) between control and treatment means for serum and urinary clinical chemistry values, terminal body and organ weights, and hematological parameters

were determined using one-way analysis of variance and an *a posteriori* Duncan's multiple range test (Duncan, 1955).

RESULTS

Six Week Observations:

Following 6 weeks of exposure, increased mortality was observed in both sexes of rats exposed to the highest dose of DGBE. Although there had been observations of bloody urine, labored respiration and abnormal behavior in male rats exposed to 5.75 g/kg acute doses of DGBE or greater during LD_{50} determinations, no outward indication of similar effects was noted for rats in the high dose DGBE group in the subchronic study. Clinical chemistry parameters determined at this time were generally unremarkable except for those associated with the high dose DGBE rats.

Serum chemistry parameters for the high dose DGBE female rats indicated significant increases in AP, AST and ALT over controls. Male rats exposed to the highest DGBE dose showed a significant elevation in BUN. Serum protein was significantly decreased in high dose DGBE males and females relative to controls. Serum parameters were otherwise unremarkable in the other treatment groups.

Urine chemistries indicated a significant elevation in NAG excretion in high dose DGBE males and females relative to controls. AST was also elevated in high dose DGBE males and females versus controls, but this increase was statistically significant only in females. High dose males, but not females,

exhibited significant increases in urinary volume and calcium excretion relative to controls. Urinary test strip measurements indicated marked hemoglobinuria in both sexes exposed to DGBE at the high dose level, and mild hemoglobinuria in both sexes exposed to either medium dose DGBE or EGBE.

Significant hematological changes noted after six weeks of exposure consisted of decreased RBC counts and Hgb levels in both sexes exposed to EGBE and high dose DGBE. Absolute lymphocyte counts were significantly decreased in high dose DGBE males. Medium and high dose DGBE males exhibited significant increases in their absolute segmented neutrophil counts.

Both sexes exposed to high dose DGBE had significantly increased liver and kidney weights; however, other than mild congestion, no consistent microscopic changes were observed which would explain organ weight increases. A significant decrease in body weight was recorded only in high dose DGBE males. Rats exposed to EGBE exhibited increased splenic weights without remarkable histopathologic alterations.

Histopathologic findings in 7 male and 9 female rats from the high dose group which died during the first 6 weeks of exposure were somewhat inconsistent, and in some cases, the precise cause of death could not be established. Mild to moderate pulmonary congestion (with or without edema) was present in 6 early death animals from both sex groups. Of these early deaths, 3 males and 5 females also exhibited lesions which were compatible with gavage trauma or foreign body pneumonia. In several other subjects, however, acute pulmonary congestion and

edema were present without distinct evidence of a dosing accident, and it could not be determined if death resulted from the tracheal instillation of DGBE or from systemic effects following gastrointestinal absorption. In addition to respiratory lesions in early deaths, mild liver congestion was noted in 3 males and 4 females and was believed to be secondary to passive pulmonary congestion.

A final microscopic observation which deserves comment was the presence of increased numbers of "laminated bodies" in the kidneys of exposed rats. These laminated structures represented focal proliferations of the tubular basal lamina and they have been common findings in control F-344 rats in our laboratory. Although their pathogenesis is poorly understood, they are not thought to represent a nephrotoxic effect of DGBE exposure. Urinary tract lesions which might explain the hemoglobinuria noted in several dose groups were not observed microscopically, and distinct, dose-related changes were not recorded in other tissues.

Thirteen Week Observations:

Tables 1 through 6 summarize the terminal body and organ weights, terminal serum chemistries, urine clinical chemistries, body weight data, hematological data and food consumption data for rats orally exposed to EGBE or DGBE for 90 days. Cumulative mortality, as a percent of the total number of animals in each exposure group for each phase of the study, is presented in Figure 1 for males and in Figure 2 for females. There was no

mortality in the controls of either sex, in the low dose DGBE males, or in the EGBE exposed females. Following the first week of dosing and continuing for the remainder of the study, a significant increase in the mortality rate was observed in both sexes exposed to high dose DGBE, relative to their respective controls. Increased mortality was also observed in the medium dose DGBE rats beginning at week eight for males and at week four for females and continued throughout the study. EGBE males exhibited increased mortality from week 9 to the end of the study. Only 1 death was recorded in the low dose DGBE females.

The high incidence of mortality seen in the high dose DGBE group (82.5% for males and 91.9% for females, at study termination) resulted in insufficient numbers of animals for statistical comparisions involving all terminal parameters.

Significant changes noted in terminal parameters were: increased spleen weights in EGBE rats, decreased spleen weights in medium dose DGBE males and increased liver weights in all EGBE treated rats. Low dose DGBE males also showed a significant increase in mean liver weight over their respective controls. This observation has marginal biologic significance since this group exhibited a slightly greater mean body weight at study termination, and the mean body-weight-corrected liver weight was not found to be significantly different from the controls. Medium dose DGBE males exhibited a significant increase in mean body-weight-corrected liver weight but not in their respective uncorrected liver weight mean value. Increased serum BUN, AP, and CPK values as well as urinary NAG values were observed for

medium dose DGBE male rats (although little evidence of nephrotoxicity was indicated by the histopathologic findings for this group). Urinary hemoglobin concentrations were elevated in 6 of the 9 terminal urine collections obtained from EGBE dosed females relative to their controls.

Body weight and food consumption data revealed a loss in body weight in all high dose DGBE animals during the first 4 weeks of the study. After 6 weeks, loss of body weight ceased in the high dose DGBE females, but continued in the high dose male rats. There was some evidence that this loss in body weight was associated, initially, with decreased food consumption.

Hematological changes noted in rats exposed to EGBE consisted of mild anemia and leukopenia (with decreased lymphocytes) in both sexes. leukopenia with significant lymphopenia, was also evident in low and medium dose DGBE females.

The most important histopathologic findings in rats which died or were killed following the 6 week interim sacrifice were restricted to the respiratory tract and thoracic cavity. Acute rhinitis, laryngitis and tracheitis, along with mild to moderate pulmonary congestion and edema were commonly observed in both sexes assigned to the medium and high dose DGBE groups and the positive EGBE controls. These lesions, combined with sporadic observations of foreign body pneumonia and acute pleuritis suggested that gavage accidents were a possible cause of morbidity and mortality in EGBE and medium and high dose DGBE groups. In considering all animals which died or were killed

during the entire study, 4 males and 5 females assigned to the DGBE high dose group and 3 males and 6 females included in the DGBE medium dose group exhibited lesions consistent with gavage trauma or dosing misadventure. It should be emphasized, however, that the precise cause of death could not be established in a modest number of "early death" animals, and technicians only reported 3 probable gavage accidents during all dosing procedures.

In addition to inflammatory lesions of the respiratory tract and thoracic cavity, mild squamous metaplasia of nasal epichelium was present in five high dose and two medium dose DGBE-exposed males and one high dose female. This nasal change was not observed in controls or any other dose group. Furthermore, hyaline droplet formation was noted in the renal tubular epithelium of three DGBE high dose females and four EGBE-exposed females. In females, hyaline droplets were regarded as evidence of hemoglobinuria and these observations were supported by clinical evidence of mild anemia. Renal hyaline droplets were also common in males of all dose groups and controls, and they were regarded as being within normal physiologic limits.

DISCUSSION

These findings indicate that oral doses of DGBE administered, 5 days per week for 6 weeks produce minimal or no toxicologic effects in male or female rats at a dose level of 1 or 5 percent of their respective LD_{50} values, whereas moderate

effects were noted in male and female rats exposed to 25% of their respective LD_{50} values.

Oral doses of DGBE administered, 5 days per week for 13 weeks produced minimal or no toxicologic effects in male or female rats at dose levels of 1% the respective LD_{50} values; Mild effects were noted in male and female rats exposed to 5% of the respective LD_{50} values for DGBE, and significant mortality was observed in DGBE exposed male and female rats at 25% of their respective LD_{50} values.

Many of the rats which died or were sacrificed in the medium and high dose DGBE groups exhibited microscopic lesions which suggested gavage injury. While it is plausible that some of the deaths recorded in this study may have resulted from gavage accidents, a definitive relationship between gavage procedure and spontaneous deaths was not completely supported by histopathologic findings, the dose-related distribution of mortality or the experimental observations by dosing technicians. In many "early deaths" where precise lethal factors escaped detection, mild to moderate pulmonary congestion and edema along with acute catarrhal rhinitis were observed. These findings suggested that small amounts of DGBE deposited in the oropharynx or upper airways may have proved to be highly irritating with subsequent inflammation and restricted ventilation. Because the rat is an obligate nose breather, compromise of nasal or pharyngeal air passages may have caused asphyxiation with only minor histopathologic changes in lower airways or the lung itself. Also, the powerful surfactant properties of DGBE may

have contributed to the development of pulmonary congestion and edema by a mechanism not yet understood. In this connection, pulmonary effects may have followed aspiration of small amounts of DGBE or absorption from the gastrointestinal tract. Additional studies are indicated to more fully explain the toxic effects of DGBE on the respiratory system.

Indications of systemic toxicity in surviving female rats exposed to both DGBE and EGBE for thirteen weeks included decreased WBC counts, lymphocyte counts, and MCHC in the low and medium dose groups. The pathogenesis of these blood cell changes remains obscure. It is noteworthy, however, that microscopic sections of lymphoreticular tissues were essentially unremarkable. Also, studies of the lymphoreticular toxicity of the glycol ethers, in general, have been inconclusive to date (House et al., 1985).

The only remarkable indications of systemic DGBE toxicity in surviving male rats were limited to the medium dose group where some experimental and clinical chemical parameters indicated mild nephrotoxicity, and increased liver and spleen weights. The biologic significance of these findings is unclear. Histopathologic studies failed to identify distinct liver, kidney, or splenic changes. It was postulated that liver and spleen weights may have been increased due to passive congestion associated with pulmonary lesions.

It is concluded that an oral dose of 0.07 g/kg/day (1% of the acute LD_{50}) is an apparent no-effect-level for male rats exposed to DGBE for 13 weeks. Although findings of slightly

altered hematogical parameters somewhat cloud the issue in the case of DGBE exposed female rats. An oral dose of 0.05 g/kg/day (1% of the acute LD_{50}) appears to be sufficiently close to a true no-observable-effect level to be considered as such for practical purposes. If only the histopathological findings from this study were to be considered, the 13 week, no-observable-effect levels would be 5% of the respective acute LD_{50} values for each sex, 0.33 g/kg/day for male rats, and 0.25 g/kg/day for female rats.

Dugard et al., (1984) has previously shown that DGBE is only very slowly absorbed through human skin (0.035 $mg/cm^2/hr$). This rate is only 17 percent that reported for EGBE and only 1.2 percent tha- reported for ethylene glycol monomethyl ether (EGME), which are two of the more toxic glycol ethers. Thus it can be assumed, based on the rate of dermal penetration alone, that the dermal DGBE dose required to produce significant systemic toxicity (if one exists) in the rat will be very much larger, probably greater than 5 times assuming equivalent toxicity for EGBE and DGBE, than a similar dose for EGBE. In previous dermal studies, Hobson et al., (1986), with EGME and diethylene glycol monomethyl ether (DGME) using male guinea pigs, we observed the longer chain glycol ether (DGME) to exhibit only mild, reversible toxicologic changes at a dermal dose as high as 1.00 g/kg/day. Hobson et al., (1986). If a similar relationship holds for EGBE relative to DGBE, it would be reasonable to assume that a dermal dose at least as high as 1.00 g/kg/day or greater would be required in the rat before significant systemic toxicity

would occur, given the relative differences in the rates of dermal penetration for EGME, EGBE, DGME and DGBE.

Based on the above values for DGBE, and assuming an equal dermal penetration rate for all regions of the body, in order for an average human adult male (surface area = 1.73 m^2, weight = 70 kg) to absorb a dose equivalent to 0.07 g/kg/day of DGBE at the rate of 0.035 $mg/cm^2/hr$, he would have to have approximately 33 percent of his skin surface areas continuously in contact with DGBE daily (24 hr/day). Since such a situation would not be expected to occur, especially for prolonged periods, during operations involving the use of AFFF, it is concluded that contact with AFFF during shipboard firefighting operatings poses a negligible threat of toxic injury to personnel due to its DGBE content.

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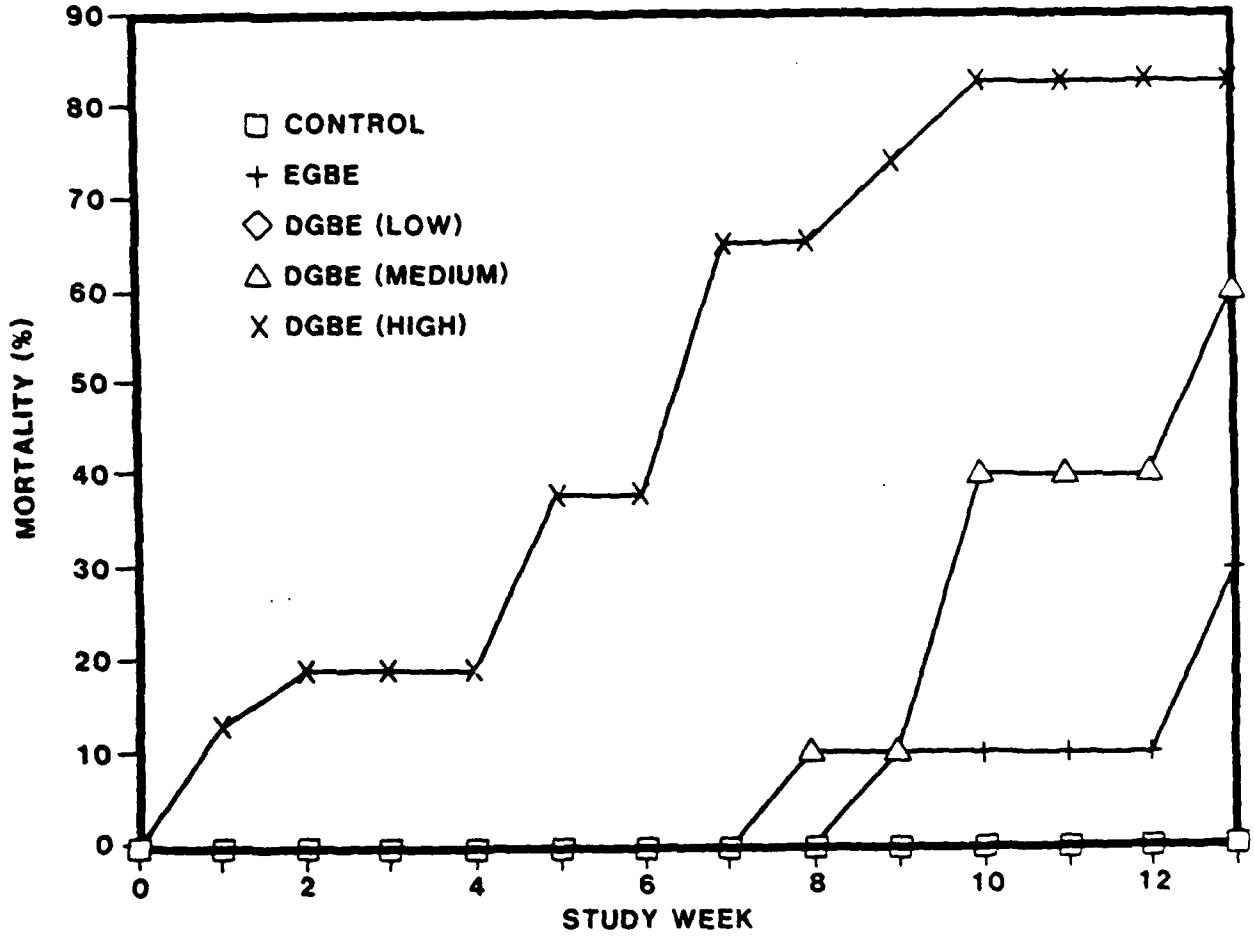

Figure 1. Cumulative mortality plot for male F-344 rats exposed to DGBE or EGBE for 13 weeks. Study design and findings are presented in more complete detail in the text. Symbols for DGBE (low) do not appear, as their values were identical to the controls.

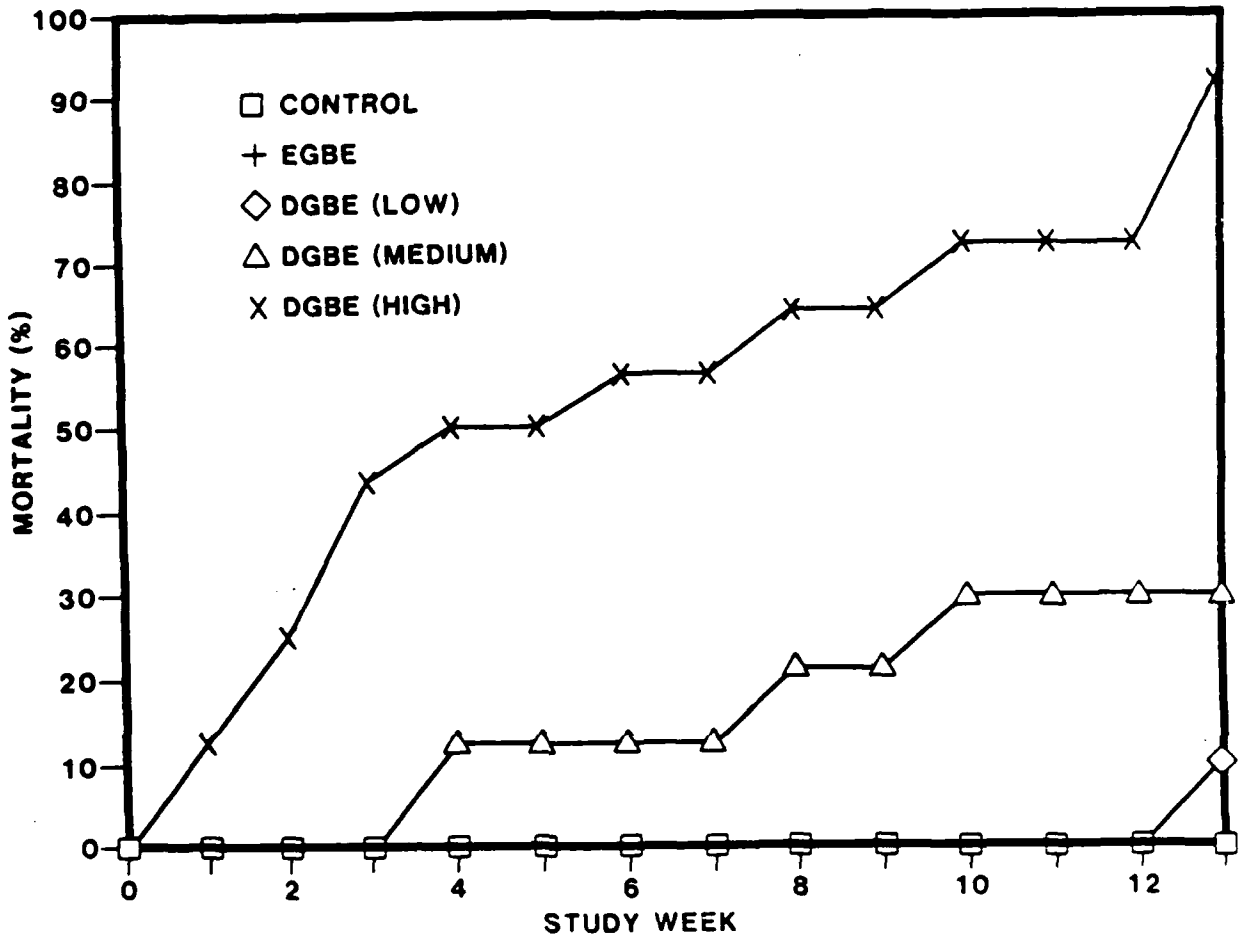

Figure 2. Cumulative mortality plot for female F-344 rats exposed to DGBE or EGBE for 13 weeks. The findings are described in more detail in text. Symbols for EGBE, except for week 13, and DGBE (low) do not appear, as their values were identical to control values.

Table 1. Summary of Terminal Body & Organ Weights data for Rats orally exposed to EGBE or DGBE for 13 weeks.

Weight parameters measured	Experimental Group Values (a,b) Control H_2O	EGBE (25% LD_{50})	DGBE (1% LD_{50})	DGBE (5% LD_{50})	DGBE[c] (25% LD_{50})
MALE RATS					
Dose (g/kg/day)	1.00	0.12	0.07	0.33	1.63
N	10	7	10	4	2
Body (g)	289.00(15.17)	292.00(14.36)	303.8(15.56)	275.75(15.09)	227.5
Thymus (g)	0.40(0.06)	0.36(0.08)	0.35(0.08)	0.33(0.09)	0.28
Thymus (g/100)	0.14(0.02)	0.12(0.02)	0.11(0.03)	0.12(0.04)	0.12
Liver (g)	7.43(0.45)	8.51(0.70)*	8.38(0.57)*	7.72(0.78)	8.84
Liver (g/100)	2.57(0.12)	2.91(0.14)*	2.76(0.17)	2.80(0.17)*	3.88
Spleen (g)	0.62(0.06)	0.84(0.10)*	0.62(0.04)	0.51(0.11)*	0.61
Spleen (g/100)	0.22(0.02)	0.29(0.03)*	0.20(0.01)	0.18(0.04)*	0.27
Kidneys (g)	2.04(0.12)	2.15(0.12)	2.13(0.11)	2.05(0.20)	2.04
Kidneys (g/100)	0.70(0.03)	0.74(0.03)	0.70(0.04)	0.74(0.03)	0.90
Testes (g)	2.93(0.18)	3.11(0.36)	2.89(0.32)	2.88(0.14)	2.90
Testes (g/100)	1.01(0.05)	1.07(0.10)	0.95(0.12)	1.04(0.03)	1.27
FEMALE RATS					
Dose (g/kg/day)	1.00	0.12	0.05	0.25	1.27
N	10	9	9	8	1
Body (g)	161.20(10.3)	158.00(16.19)	170.89(11.34)	160.00(9.91)	155.00
Thymus(g)	0.24(0.04)	0.23(0.05)	0.25(0.05)	0.26(0.06)	0.14
Thymus (g/100)	0.15(0.03)	0.14(0.03)	0.15(0.03)	0.16(0.03)	0.09
Liver (g)	4.60(0.38)	5.23(0.74)	4.78(0.37)	4.81(0.55)	5.44
Liver (g/100)	2.85(0.20)	3.30(0.21)*	2.81(0.23)	3.00(0.18)	3.51
Spleen (g)	0.42(0.03)	0.53(0.07)*	0.42(0.04)	0.41(0.03)	0.49
Spleen (g/100)	0.26(0.01)	0.34(0.03)*	0.25(0.03)	0.26(0.03)	0.32
Kidneys (g)	1.22(0.10)	1.23(0.12)	1.26(0.07)	1.29(0.10)	1.25
Kidneys (g/100)	0.76(0.06)	0.78(0.04)	0.74(0.04)	0.81(0.05)	0.81

a. Values are mean (standard deviation)
b. g/100 = gram of tissue per 100g of body weight
c. Statistics were not performed due to insufficient N.
* Statistically different from control (p 0.05)

Table 2. Summary of Terminal Serum Clinical Chemistry data for Rats orally exposed to EGBE or DGBE for 13 weeks.

Serum Parameters measured	Experimental group values (a,b) Control: H_2O	EGBE (25%LD_{50})	DGBE (1%LD_{50})	DGBE (5%LD_{50})	DGBE[c] (25%LD_{50})
MALE RATS					
Dose (g/kg/day)	1.00	0.12	0.07	0.33	1.63
N	10	7	10	4	2
Glucose(mg/dl)	149.72(26.4)	166.19(40.43)	214.73(56.34)*	201.96(18.2)	257.85
Creatinine(mg/dl)	0.80(0.05)	0.90(0.14)	1.08(0.11)*	1.25(0.23)*	1.38
BUN (mg/dl)	13.05(1.57)	13.86(2.32)	14.41(3.31)	34.73(25.75)*	16.51
Alk. Phos (U/L)	61.99(10.08)	64.97(6.24)	64.91(4.94)	84.11(19.12)*	97.77
CPK (U/L)	71.99(14.85)	58.52(10.88)	49.29(7.82)	112.49(110.69)	55.02
AST (U/L)	53.35(3.54)	66.25(9.84)	80.23(69.25)	90.99(42.51)	61.35
ALT (U/L)	43.51(3.84)	56.48(9.71)	47.95(23.27)	62.64(39.38)	39.14
Albumin(g/dl)	3.83(0.36)	3.91(0.23)	3.73(0.76)	3.80(0.35)	4.15
Total Protein(g/dl)	4.23(0.26)	4.18(0.21)	4.34(0.10)	4.33(0.45)	4.79
Calcium (mg/dl)	8.32(1.84)	9.11(0.52)	9.11(0.81)	7.57(1.08)	8.65
LDH (U/L)	62.92(16.12)	62.17(20.69)	144.86(298.53)	70.92(38.96)	44.95
FEMALE RATS					
Dose(g/kg/day)	1.00	0.12	0.05	0.25	1.27
N	10	9	9	8	1
Glucose(mg/dl)	130.09(12.58)	151.91(42.21)	131.17(18.55)	139.13(43.84	127.69
Creatinine(mg/dl)	0.82(0.10)	0.86(0.15)	0.87(0.20)	0.97(0.24)	0.63
BUN (mg/dl)	14.49(1.24)	14.12(1.84)	15.23(1.12)	16.49(2.24)	18.08
Alk. Phos. (U/L)	51.11(10.08)	69.72(18.52)	47.57(6.26)	61.67(13.38)	60.31
CPK (U/L)	66.23(14.21)	85.29(41.84)	60.68(9.03)	70.41(37.06)	46.21
AST (U/L)	52.49(4.69)	81.62(29.77)*	55.03(7.21)	57.34(8.45)	49.72
ALT (U/L)	26.98(3.49)	39.31(7.56)*	30.27(6.97)	31.43(4.65)	36.19
Albumin(g/dl)	4.25(0.20)	4.09(0.27)	4.13(0.48)	3.51(1.12)	4.27
Total Protein(g/dl)	4.11(0.21)	3.83(0.40)	4.36(0.23)	4.06(0.19)	4.07
Calcium (mg/dl)	9.17(0.52)	8.40(2.32)	8.60(0.74)	9.10(0.93)	9.82
LDH (U/L)	63.10(29.82)	116.84(69.71)*	58.16(19.80)	65.57(43.87)	24.88

a. Values are given as means (standard deviation)
b. BUN = Urea Nitrogen ALT = Alanine Aminopeptidase
CPK = Creatine phosphokinase LDH = Lactate dehydrogenase
AST = Aspartate Aminotransferase
c. Statistics were not performed on this treatment group due to insufficient N.
* Statistically different from controls (p 0.05)

Table 3. Summary of Urine Clinical Chemistry data for Rats orally exposed to EGBE or DGBE for 13 weeks.

Urine parameters measured	Experimental Group Values (a,b) Control:H_2O	EGBE (25%LD_{50})	DGBE (1%LD_{50})	DGBE (5%LD_{50})	DGBE[c] (25%LD_{50})
MALE RATS					
Dose(g/kg/day)	1.00	0.12	0.07	0.33	1.63
N	10	8	10	4	2
24 Hr.Volume(ml)	9.37(7.89)	11.26(6.14)	7.63(3.33)	8.06(2.44)	14.13
pH	7.95(1.03)	8.43(0.63)	8.30(0.88)	6.96(0.78)	6.65
Specific Gravity(g/ml)	1.036(0.013)	1.031(0.010)	1.037(0.008)	1.040(0.006)	1.034
Creatinine(mg/day)	16.27(20.93)	8.75(2.97)	10.15(1.78)	9.25(0.81)	8.58
NAG(U/day)	195.46(98.05)	191.41(165.47)	125.85(46.91)	459.89(210.51)*	448.6
AST (U/day)	0.25(0.23)	0.18(0.14)	0.24(0.21)	0.69(1.18)	0.18
Calcium (mg/day)	0.62(0.22)	0.79(0.38)	0.69(0.24)	0.38(0.14)	1.87
FEMALE RATS					
Dose(g/kg/day)	1.00	0.12	0.05	0.25	1.27
N	10	9	9	8	2
24 Hr Volume (ml)	10.84(7.35)	14.09(10.26)	6.01(3.57)	5.04(3.31)	3.68
pH	8.80(0.59)	8.37(0.61)	8.23(1.14)	7.20(0.69)*	6.85
Specific Gravity(g/ml)	1.021(0.009)	1.021(0.01)	1.033(0.014)	1.038(0.010)*	1.038
Creatinine (mg/day)	5.05(1.26)	5.58(1.18)	5.12(0.83)	5.00(1.85)	6.76
NAG (U/day)	105.03(55.81)	158.80(90.63)	100.17(74.52)	155.82(89.29)	255.49
AST (U/day)	0.06(0.06)	0.11(0.08)	0.02(0.01)	0.025(0.013)	--
Calcium (mg/day)	0.65(0.36)	1.12(0.51)	0.76(0.27)	1.08(0.25)	1.93

a. Values are given as mean (standard deviation)
b. NAG = N-Acetyl-Beta-Glucasaminidase
AST = Aspartate Aminotransferase
c. Statistics were not performed on this treatment group due to insufficient N.
* Statistically different from controls (p 0.05)

Table 4. Summary of Body Weight[a] data for Rats orally exposed to EGBE or DGBE for 13 weeks.

Study week	Control:H_2O	N	EGBE (25%LD_{50})	N	DGBE (1%LD_{50})	N	DGBE (5%LD_{50})	N	DGBE[b] (25%LD_{50})	N
MALE RATS										
0	173.93(10.59)	16	181.31(13.08)	16	180.81(9.57)	16	174.44(15.83)	16	173.38(11.6)	16
1	195.88(11.11)	16	201.25(12.80)	16	203.19(9.17)	16	195.69(19.21)	16	185.38(13.01)*	16
2	214.88(10.55)	16	219.56(12.66)	16	222.75(8.46)	16	212.25(18.28)	16	193.00(16.15)*	14
3	231.31(10.99)	16	236.75(13.80)	16	239.75(10.96)	16	230.25(17.89)	16	209.62(11.73)*	13
4	245.25(12.06)	16	250.63(15.16)	16	253.00(11.45)	16	243.38(17.74)	16	219.15(12.77)*	13
5	257.13(12.54)	16	260.06(15.74)	16	265.38(13.65)	16	255.19(19.1)	16	221.70(19.6) *	10
6[c]	263.92(13.94)	13	265.08(16.16)	13	275.54(13.78)	13	264.15(19.07)	13	229.25(13.31)*	8
7	271.5 (16.65)	10	275.10(32.95)	10	282.80(12.63)	10	272.70(19.52)	10	228.75(16.88)*	4
8	276.40(17.35)	10	276.10(26.43)	10	289.30(15.34)	10	277.33(21.45)	9	229.50(6.76) *	4
9	282.60(16.88)	10	286.22(21.41)	9	295.70(15.00)	10	283.44(24.52)	9	232.67(1.53) *	3
10	289.50(16.31)	10	290.00(21.51)	9	302.90(16.33)	10	281.67(22.17)	6	244.50	2
11	289.70(19.72)	10	301.00(18.19)	9	309.00(19.23)	10	287.33(20.61)	6	246.50	2
12	299.90(17.72)	10	305.56(15.40)	9	321.80(17.00)	10	292.67(23.48)	6	248.00	2
FEMALE RATS										
0	126.00(6.27)	16	123.63(10.04)	16	128.56(8.90)	16	123.88(10.56)	16	127.69(13.15)	16
1	136.00(7.63)	16	131.31(10.83	16	138.31(9.13)	16	133.63(9.05)	16	128.43(9.4) *	14
2	141.63(8.94)	16	138.94(11.93)	16	145.69(9.28)	16	140.25(8.68)	16	138.58(5.62)	12
3	148.63(8.41)	16	144.00(11.92)	16	152.19(9.75)	16	146.19(7.84)	16	140.44(9.86) *	9
4	152.94(8.21)	16	147.56(10.37)	16	158.31(10.2)	16	150.29(8.84)	14	141.75(8.73) *	8
5	156.63(8.16)	16	153.44(10.57)	16	162.06(10.17)	16	155.93(7.82)	14	150.75(5.5)	8
6[c]	157.46(9.41)	13	154.85(12.51)	13	164.00(10.00)	13	157.54(9.31)	13	152.20(7.33)	5
7	159.80(10.28)	10	160.10(15.2)	10	168.30(11.10)	10	160.20(9.61)	10	159.25(6.4)	4
8	163.20(8.98)	10	160.40(16.26)	10	170.89(11.86)	9	163.78(7.93)	9	163.33(6.11)	3
9	165.80(8.7)	10	163.50(16.19)	10	173.22(11.94)	9	166.33(8.77)	9	166.33(5.51)	3
10	169.60(9.4)	10	164.50(15.85)	10	176.22(12.77)	9	167.50(9.4)	8	166.33(11.24)	3
11	172.80(9.54)	10	173.20(24.06)	10	179.00(12.77)	9	170.75(8.61)	8	175.00	2
12	176.10(9.64)	10	171.90(15.84)	10	182.67(13.64)	9	174.75(8.46)	8	178.50	2

a.Grams: values are given as mean (standard deviation)
b.Statistics were not performed on values where N=2.
c.At six weeks an interim sacrifice was performed.
* Statistically different from Control (p 0.05).

Table 5.a. Summary of Terminal Hematological data for Male Rats orally exposed to EGBE or DGBE for 13 weeks.

Hematological parameters measured	Experimental Group Values (a,b) Control:H_2O	EGBE (25%LD_{50})	DGBE (1%LD_{50})	DGBE (5%LD_{50})	DGBE[c] (25%LD_{50})
MALE RATS					
Dose(g/kg/day)	1.00	0.12	0.07	0.33	1.63
N	9	7	9	4	2
WBC($x10_3/mm^3$)	4.54(1.55)	2.91(0.38)	3.84(1.22)	4.58(1.05)	4.90
RBC($x10^6/mm^3$)	8.04(0.51)	6.99(0.23)*	8.62(0.27)	9.16(1.21)*	7.99
Hgb(g/dl)	15.68(1.13)	13.84(0.50)*	16.29(0.57)	17.40(2.19)*	15.85
Hct(%)	42.32(3.20)	38.33(1.68)	43.54(1.53)	46.30(6.47)	44.35
MCV(m^3)	52.62(2.48)	54.80(1.39)*	50.44(0.33)	50.45(0.39)	55.45
MCH(pg)	19.53(0.77)	19.81(0.35)	18.90(0.23)	19.03(0.21)	19.90
MCHC(g/dl)	37.09(0.41)	36.17(0.33)*	37.41(0.40)	37.65(0.62)	35.80
PLT($x10^3/mm^3$)	688.44(34.28)	751.00(57.10)	729.00(43.33)	713.75(41.76)	785.00
MPV(m^3)	7.11(0.30)	6.57(0.22)*	6.96(0.34)	6.75(0.25)	6.50
Neutrophils (d)	0.85(0.29)	0.71(0.15)	0.92(0.48)	1.21(0.37)	0.96
Lymphocytes (d)	3.51(1.31)	2.10(0.39)*	2.74(0.75)	3.18(0.90)	3.76
Monocytes (d)	0.13(0.09)	0.09(0.07)	0.16(0.07)	0.16(0.09)	0.18
Eosinophils (d)	0.09(0.04)	0.03(0.01)*	0.07(0.03)	0.04(0.01)*	0

a. Values are given as Mean (Standard Deviation)

b. WBC = White Blood Cells
RBC = Red Blood Cells
Hgb = Hemoglobin
Hct = Hematocrit
MCV = Mean Corpuscular Volume
MCHC = Mean Corpuscular Hemoglobin Concentration
PLT = Platelet
MPV = Mean Platelet Volume
MCH = Mean Corpuscular Hemoglobin

c. Statistics were not performed on this treatment group due to insufficient N.

d. Values given are absolute counts ($x10^3/mm^3$)

* Statistically different from Controls (p 0.05)

Table 5.b. Summary of Hematological data for Female Rats orally exposed to EGBE or DGBE for 13 weeks.

Hematological parameters measured	Experimental Group Values (a,b) Control:H_2O	EGBE (25%LD_{50})	DGBE (1%LD_{50})	DGBE (5%LD_{50})	DGBE[c] (25%LD_{50})
FEMALE RATS					
Dose(g/kg/day)	1.00	0.12	0.05	0.25	1.27
N	7	5	9	7	1
WBC($x10^3/mm^3$)	4.46(0.73)	2.94(0.57)*	3.03(0.59)*	2.56(0.31)*	5.40
RBC($x10^6/mm^3$)	7.66(0.30)	6.31(0.31)*	7.74(0.52)	8.01(0.45)	7.51
Hgb (g/dl)	15.67(0.69)	13.38(0.68)*	15.48(1.12)	15.87(0.93)	14.80
Hct (%)	40.29(1.74)	36.48(2.04)	41.22(2.68)	43.14(2.68)	43.50
MCV (m^3)	52.53(0.35)	57.72(0.69)*	53.19(0.38)	53.77(0.41)*	57.90
MCH (pg)	20.47(0.36)	21.20(0.17)*	19.99(0.42)	19.81(0.16)*	19.70
MCHC (g/dl)	38.90(0.72)	36.70(0.52)*	37.52(0.85)*	36.79(0.24)*	33.90
PLT($x10^3/mm^3$)	805.14(40.75)	754.80(108.15)	766.38(41.60)	852.14(74.10)	837.00
MPV (m^3)	6.51(0.13)	6.44(0.29)	6.50(0.20)	6.54(0.22)	6.70
Neutrophils (d)	0.75(0.25)	0.54(0.18)	0.62(0.10)	0.59(0.24)	1.08
Lymphocytes (d)	3.55(0.60)	2.24(0.48)*	2.31(0.42)*	1.85(0.31)*	4.32
Monocytes (d)	0.16(0.07)	0.14(0.07)	0.11(0.08)	0.09(0.04)	
Eosinophils (d)	0.07(0.03)	0.04(0.02)	0.05(0.03)	0.03(0.01)	

a. Values are given as Mean (Standard Deviation)

b. WBC = White Blood Cells
RBC = Red Blood Cells
Hgb = Hemoglobin
Hct = Hematocrit
MCV = Mean Corpuscular Volume
MCHC = Mean Corpuscular Hemoglobin Concentration
MCH = Mean Corpuscular Hemoglobin
PLT = Platelet
MPV = Mean Platelet Volume

c. Statistics were not performed on this treatment group due to insufficient N.

d. Values given are absolute counts($x10^3/mm^3$)

* Statistically different from Controls (p 0.05)

Table 6. Summary of Food Consumption[a] data for Rats orally exposed to EGBE or DGBE for 13 weeks.

Study week	Control:H_2O	N	EGBE (25%LD_{50})	N	DGBE (1%LD_{50})	N	DGBE (5%LD_{50})	N	DGBE (25%LD_{50})	N
MALE RATS										
1	100.56(3.10)	16	100.36(3.76)	16	102.83(2.49)	16	101.47(7.36)	16	86.19(8.03)*	16
2	100.33(3.17)	16	101.11(4.29)	16	103.63(2.32)	16	99.64(4.65)	16	74.63(20.38)*	16
3	103.67(4.80)	16	105.72(4.14)	16	104.94(3.51)	16	102.44(3.56)	16	94.02(14.14)*	14
4	104.61(4.66)	16	104.81(6.83)	16	108.00(1.64)	16	104.89(4.56)	16	93.67(23.52)	13
5	102.58(4.10)	16	106.30(6.94)	16	106.02(4.47)	16	102.22(4.84)	16	109.72(37.88)	13
6[c]	96.67(2.20)	16	93.75(9.44)	16	102.02(5.23)	16	96.31(10.67)	16	90.83(29.50)	10
7	101.04(2.67)	13	94.63(21.91)	13	105.42(1.48)	13	101.67(9.10)	13	100.00(13.11)	8
8	100.33(2.80)	10	96.42(9.90)	10	102.13(2.78)	10	96.42(9.80)	10	124.67(65.77)	4
9	101.17(4.56)	10	107.92(3.52)	10	107.46(6.09)	10	97.13(17.23)	9	119.00(28.28)	4
10	100.17(5.81)	10	104.13(8.46)	9	100.29(5.63)	10	91.54(5.02)	9	141.00(49.50)*	3
11	94.29(8.45)	10	103.21(8.58)	9	100.08(6.37)	10	96.50(12.40)	6	107.00	2
12	101.25(5.00)	10	91.29(11.95)	9	99.38(16.61)	10	105.25(46.86)	6	63.50	2
13	86.54(2.13)	10	96.88(16.09)	9	91.83(7.74)	10	73.63(32.18)	6	89.50	2
FEMALE RATS										
1	70.03(3.57)	16	62.08(3.56)	16	61.75(24.77)	16	72.08(11.69)	16	56.51(10.32)*	16
2	68.97(5.94)	16	68.86(4.90)	16	72.69(3.56)	16	68.81(3.53)	16	66.89(3.86)	14
3	70.92(4.75)	16	67.14(5.40)	16	70.97(2.68)	16	69.67(2.05)	16	52.28(19.33)	12
4	72.78(4.59)	16	67.33(3.68)	16	73.31(3.87)	16	70.19(11.64)	16	58.33(10.09)*	9
5	69.14(4.05)	16	69.97(3.77)	16	70.33(2.62)	16	67.81(3.08)	14	69.83(5.27)	8
6[c]	62.03(5.76)	16	62.53(3.82)	16	63.75(5.17)	16	62.11(5.81)	14	59.86(8.02)	8
7	68.46(8.70)	13	67.58(4.95)	13	68.08(3.46)	13	64.88(5.00)	13	74.50(3.42)	5
8	71.08(6.28)	10	67.33(5.66)	10	64.33(13.99)	10	75.75(11.79)	10	59.00(28.62)	4
9	75.75(5.78)	10	72.33(4.74)	10	71.96(3.64)	9	72.00(4.06)	9	79.67(4.04)	3
10	70.92(4.30)	10	68.33(4.44)	10	68.33(3.78)	9	62.67(3.86)	9	66.67(5.77)	3
11	69.29(6.60)	10	69.96(14.17)	10	64.92(3.03)	9	65.13(6.79)	8	74.00	3
12	70.83(17.43)	10	63.50(14.83)	10	60.71(11.43)	9	76.67(20.92)	8	94.00	2
13	69.08(6.33)	10	63.10(6.64)	10	69.13(5.92)	9	72.29(3.47)	8	76.50	2

a. grams/week/rat = values are given as mean (standard deviation)
b. Statistics not performed where N = 2 animals.
c. An interim sacrifice was conducted at 6 weeks. The interim sacrifice was accomplished in a staggered fashion so that 3 animals were sacrificed before the weekly weighing and 3 after the weekly weighing. The number of animals is 13 for that time period only. The body weights measured at necropsy were not included since they followed a 24 hour fast in metabolic cages.
* Statistically different from Control ($p \leq 0.05$).

END

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