# CENTER FOR DRUG EVALUATION AND RESEARCH

## APPLICATION NUMBER: 020772

## PHARMACOLOGY REVIEW(S)

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Pharmacologist, HFD-180

Review #1

**Sponsor**: Orphan Medical, Inc.
Minnetonka, MN

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## REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

### ORIGINAL SUMMARY



**Drug**: Sucraid™ (Sacrosidase); oral solution

Chemical Name: beta-D-fructofuranoside fructohydrolase.

Average Molecular weight: 97,000 Daltons

**Formulation**: Each mL of Sucraid contains 8,500 International Units (I.U.) of the enzyme, sacrosidase (sucrase). Sucraid also contains 50% glycerin (glycerol) in an aqueous solution. Sucraid is a pale, yellow, clear solution with a pleasant sweet taste. Sucraid is available in 118 mL (4 fluid ounce) translucent plastic bottles, packaged two per box.

**Category**: Enzyme Replacement.

**Related Drugs/INDs/NDAs/MFs**:

**Proposed Marketing Indication**: Sucraid is an oral enzyme replacement therapy indicated for the treatment of confirmed or suspected congenital sucrase-isomaltase deficiency (CSID) and the prevention of the associated symptoms of sucrose malabsorption such as frequent watery stools, gas, bloating, abdominal cramping, explosive diarrhea, and growth retardation.

**Dose**: The recommended dosage is as follows: 1 mL (one full measuring scoop or 32 drops) per meal or snack for patients up to 15 kg in body weight, or 2 mL (two full measuring scoops of 44 drops) per meal or snack for patients over 15 kg in body weight.

Preclinical Studies and Testing Laboratories:

In a pre-NDA meeting held in October 1996 between the FDA and Orphan Medical, Inc., it was agreed that no additional preclinical toxicity studies of the Sucraid product were warranted based upon the following: (1) Yeast-derived sacrosidase is a Generally Recognized as Safe (GRAS) food material under FDA provision 21 CFR § 170.30; (2) because sacrosidase is a large macromolecule, it cannot be transported across the gastrointestinal mucosa into the systemic circulation following oral ingestion; and (3) because sacrosidase is a naturally occurring glycoprotein, it will be digested to peptides and eventually amino acids within the small intestine, which are absorbed into the circulation and utilized as nutrients.

The major excipient in the sacrosidase solution is glycerin (glycerol), which is present at a 50% (wt/v) concentration and contributes to the stability of the enzyme within the drug product. At the request of the FDA, the sponsor conducted a literature search on glycerol toxicity.

The review below is focused in large part on an analysis of preclinical toxicology studies with glycerin and aluminum.

I. SUCRASE:

| Study Type | Reference # | Review Page # |
|---|---|---|
| PHARMACOLOGY: | 1,2 | 6 |

II. GLYCEROL:

| Study Type | Reference # | Review Page # |
|---|---|---|
| **ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:** | | |
| Absorption, Distribution, Metabolism, and Excretion of Glycerol in Mammals. | 4,7,10,12 | 7 - 9 |
| **ACUTE TOXICITY:** | | |
| **Mouse, Rat, Guinea Pig, and Dog:** | | |
| Acute oral toxicity of glycerin in mouse, rat, guinea pig, and dog. | 7,9 | 12 - 13 |
| **SUBACUTE TOXICITY:** | | |
| **Rats:** | | |
| 96 day and 3 month subacute oral toxicity studies of glycerin in rats. | 7 | 13 |
| **CHRONIC TOXICITY:** | | |
| **Rats:** | | |
| 20-week to 2-year toxicity studies in rats. | 7 | 13 - 14 |
| 2-year glycerin feeding study in rats. | 11 | 14 - 16 |
| **Dogs:** | | |
| 2-year glycerin feeding study in dogs. | 7 | 16 - 17 |
| **REPRODUCTIVE TOXICOLOGY:** | | |
| Segment I fertility and reproductive performance studies in rats with glycerin. | 7 | 17 - 18 |
| Segment II tetratogenicity studies in mice and rats with glycerin and a multi-generational study with glycerin. | 3 | 18 |
| **GENOTOXICITY:** | | |
| Ames Test with glycerin. | 5,6,8 | 18 - 19 |
| Rat hepatocyte unscheduled DNA synthesis assay with glycerin. | 5 | 19 |
| Chinese hamster ovary chromosome aberration assay with glycerin. | 5 | 19 - 20 |
| Chinese hamster fibroblast chromosome aberration assay with glycerin. | 8 | 20 - 21 |
| Chinese hamster ovary sister chromatid exchange assay with glycerin. | 5 | 21 |
| Chinese hamster HGPRT with glycerin. | 5 | 21 - 22 |

1. Disaccharide Digestion and Maldigestion. Scand. J. Gastroenterol. 31 Suppl. 216: 111-121, 1996.

2. Congenital Sucrase-Isomaltase Deficiency: Identification of a Glutamine to Proline Substitution that Leads to a Transport Block of Sucrase-Isomaltase in a Pre-Golgi Compartment. J. Clin. Invest. 97:633-641, 1996.

3. Glycerol: Toxicity Profile, BIBRA Toxicology International, 1993.

4. In Vivo Glycerol Metabolism in the Pregnant Rat. Biol. Neonate 37:172-179, 1980.

5. The Genotoxic Activity of Glycerol in an In Vitro Test Battery. Food and Chemical Toxicology 26: 631-635, 1988.

6. Salmonella Mutagenicity Test Results for 250 Chemicals. Environmental Mutagen 5(Suppl. 1): 3-142, 1983.

7. Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227 prepared by Informatics, Inc. 1973.

8. Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Food and Chemical Toxicology 22: 623-636, 1984.

9. Sequential Studies on the Pathophysiology of Glycerol-Induced Acute Renal Failure. J. Lab. Clin. Med. 96:356-363, 1980.

10. Comparative Utilization In Vivo if [U-$^{14}$C] glycerol, [2-$^{3}$H] Glycerol, [U-$^{14}$C] Glucose and [1-C$^{14}$] Palmitate in the Rat (Archives Internationales de Physiologie et de Biochimie 88:255-263, 1980).

11. Comparative Toxicity of Synthetic and Natural Glycerin. Archives of Industrial Hygiene and Occupational Medicine 7: 282-291, 1953.

12. Glycerol Utilization and Its Regulation in Mammals. Annual Review of Biochemistry 46: 765-795, 1977.

**PHARMACOLOGY:**

Congenital Sucrase-Isomaltase Deficiency (CSID) is a chronic malabsorption disease characterized by an autosomal recessive inheritable disease of sucrase and isomaltase deficiency. CSID patients generally have no endogenous sucrase activity, markedly reduced isomaltase activity, and moderately reduced maltase activity. Sucrase metabolizes the disaccharide, sucrose, into its two component monosaccharides, glucose and fructose. In the absence of this enzyme, sucrose is not metabolized or absorbed by the intestines. The presence of intact disaccharides, such as sucrose, in the intestinal lumen leads to osmotic retention of water, resulting in loose stools. Further, unabsorbed sucrose can be metabolized by intestinal bacteria leading to the production of gases, such as hydrogen, methane, and carbon dioxide. These gases can cause gastrointestinal discomfort due to flatulence, bloating, abdominal cramps, watery diarrhea, nausea, and vomiting. Treatment has usually consisted of a diet completely free of sucrose. The sponsor has developed Sucraid™ as an enzyme replacement therapy for the treatment of CSID. A purified form of sucrase, prepared from Saccharomyces cerevisiae (yeast), was commercially available from Red Star Yeast & Products. The food form, often called invertase, has been used in the food and candy industry for decades. The major excipient in the sacrosidase solution is glycerin (glycerol), which is present at a 50% (wt/v) concentration and contributes to the stability of the enzyme within the drug product. Aluminum is present in the final drug product at a concentration of <20 ppm. The review below is focused in large part on an analysis of preclinical toxicology studies with glycerin and aluminum.



**Primary Pharmacology**



Food carbohydrates are hydrolyzed to monosaccharides prior to transport across the microvillus membrane of the small intestine. Digestion of the disaccharide, sucrose, is performed by the brush border enzyme, sucrase-isomaltase. Sucrase-isomaltase activity is distributed along the whole length of the small intestine (Scand. J. Gastroenterol. 31(Suppl. 216): 111-121, 1996). Sucrase-isomaltase is composed of two polypeptide chains linked by non-covalent bonds (J. Clin. Invest. 97: 633-641, 1996). Deficiencies of sucrase-isomaltase are extremely rare; however, sucrose maldigestion is frequently found among the Inuits in Greenland and possibly, the Eskimos in Alaska and Canada and Indians in Canada (Scand. J. Gastroenterol. 31(Suppl. 216): 111-121, 1996). In CSID patients, the small intestinal epithelium lacks sucrase activity, while isomaltase activity can vary from absent to practically normal (J. Clin. Invest. 97: 633-641, 1996).

**ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:**



Glycerol

Glycerol plays an important role as a precursor of sn-glycerol 3-phosphate, a compound that provides the carbon skeleton for gluconeogenesis, carries reducing equivalents from the cytosol to mitochondria for oxidative phosphorylation, and acts as the backbone for glyceride lipids (Annual Review of Biochemistry 46: 765-795, 1977). Glycerol is rapidly absorbed from the intestines. It is also absorbed from the stomach, but at a slower rate. The serum glycerol levels for rats and humans have been determined to be 0.1 and 0.05-0.1 mM, respectively. The rate of utilization of serum glycerol by tissues, principally the liver and kidney, is proportional to the serum concentration at ≤1 mM. Glycerol is rapidly converted to glucose by most mammals. In studies with rats, the liver was found to be responsible for approximately 75% of the total capacity for converting glycerol to glucose. Glycerol is normally completely reabsorbed by the kidney; however, with concentration exceeding 1 mM, this substance can appear in the urine.



The absorption, distribution, and metabolism of glycerin was examined in rats maintained on drinking water containing 15% glycerin (estimated dose was 18.75 g/kg/day) for 1 to 2 weeks (Life Sciences 3:1021-3; a summary of this publication was provided in Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227 prepared by Informatics, Inc. 1973). Rats were found to have elevated plasma levels of glycerol and phospholipid, and an increased liver triglyceride content.



The incorporation of glycerol into lipid was examined in female rats following intravenous administration of [U-$^{14}$C]glycerol (Archives Internationales de Physiologie et de Biochimie 88:255-263, 1980). The greatest levels of radioactivity were found in the carcass. In the liver, the largest incorporation of [U-$^{14}$C] glycerol into lipid was found. In the heart, spleen, and kidney, [U-$^{14}$C]glycerol was present primarily in the form of glycerol.

The <u>in vivo</u> synthesis of glucose from U-$^{14}$C-glycerol was examined in pregnant, female rats at days 12 and 21 of gestation (Biol. Neonate 37:172-179, 1980). Female rats, both pregnant and non-pregnant, received an intravenous injection of 10 μCi U-$^{14}$C-glycerol and conversion into glucose was determined at periods up to 10 min later. Endogenous plasma glycerol levels for pregnant females on day 21 of gestation were 144% of control, non-pregnant females (63.77 μmole/L); however, there was no difference at day 12. Plasma glucose levels for pregnant females on days 12 and 21 of gestation were decreased to 85.5 and 77% of the control (111.60 mg/100 mL). The disappearance of radiolabeled glycerol in

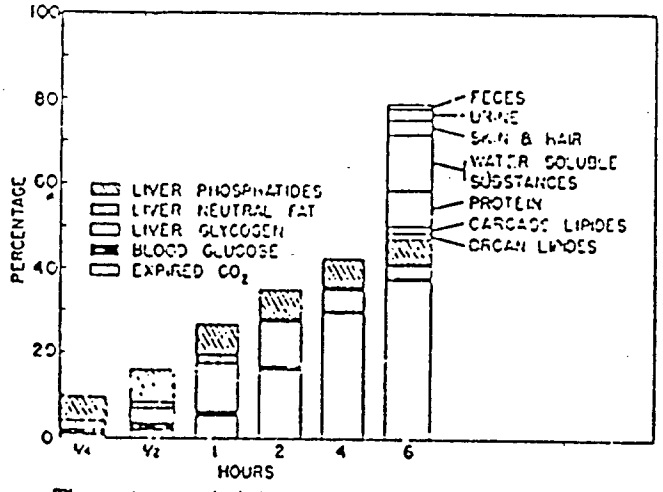
plasma was rapid in control rats and pregnant rats at day 12 with <7% remaining at 1 min after injection; however, disappearance of label was slower for pregnant rats at day 21. This slower disappearance of radiolabeled glycerol in pregnant rats on day 21 was due to dilution with higher circulating levels of endogenous glycerol. The production of glucose from glycerol was rapid with 20-30% conversion by 1 min after injection in both pregnant and non-pregnant rats. The rate of conversion was higher in pregnant females at day 21 due to augmented lipogenesis and gluconeogenesis by the liver. Total $^{14}$C-glycogen and $^{14}$C glycogen specific activity were higher in pregnant rats at day 21. For pregnant rats at day 21, the slower disappearance of $^{14}$C-glycerol from plasma and its increased conversion to glucose suggested decreased utilization of glycerol by other tissues. Radioactivity in the carcass hydrosoluble fraction for pregnant rats at day 21 was reduced to 65% of the control

Glucose, formed as a result of glycerol metabolism by the mother's liver on day 21 of gestation, is rapidly utilized by the growing fetus. In contrast, on day 12 of gestation, the fetus is significantly smaller and hepatic metabolism of glycerol by the mother was unchanged as compared to non-pregnant rats.

**The Fate of $^{14}$C-Glycerol in the Rat Following Administration of up to 238 mg** (J. Biol. Chem. 206:229-242, 1954).

The fate of $^{14}$C-glycerol in the rat, following administration of up to 238 mg, was determined by Gidez and Kornovsky to be as follows (526):






The percentage of administered counts in different metabolites at various time intervals. The $CO_2$ values and the counts of organ lipides (other than liver), urine, feces, protein, "water-soluble substances," skin, and hair, and carcass lipides are from one typical animal. All other data are the means of two to six animals.

The absorption, distribution, and metabolism of glycerol was examined in rabbits following daily intragastric administration of 20 mL of a 50% glycerol solution (Vopr. Med. Khim. 6: 544-546, 1960; a summary of this report was provided in Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227 prepared by Informatics, Inc. 1973). After 2 weeks of treatment, a 100% increase in the blood content of cholesterol was found. Atherosclerotic plaques, in early stages of development, were found in the acrtic arch.

The absorption, distribution, and metabolism of glycerol were determined in rabbits following administration of glycerol at a dose of 50 mg/kg/day for 5-6 months (Tr. Kuilyshevsk Med. Inst. 29: 10-18; a summary of this report was provided in Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227 prepared by Informatics, Inc. 1973). The route of administration was not specified. Glycerin levels in the blood, liver, skeletal and cardiac muscle, and intestines were found to be increased.

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TOXICOLOGY OF GLYCEROL:

Acute Toxicity of Glycerol in Mice, Rats, Guinea Pigs, and Dogs.

Acute toxicity of glycerin in mice, rats, guinea pigs, and dog ("Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227" prepared by Informatics, Inc. 1973).

| Species | Route | Doses | $LD_{50}$ g/kg | Maximum nonlethal dose, g/kg | Minimum lethal dose, g/kg |
|---|---|---|---|---|---|
| Mice | Oral | | 20.7, 23.0, 26.0, 27.2, 31.5 | | |
| Rats | Oral | 16.2, 18.7, 21.2, 23.7, 26.2, & 28.7 | 27.4 | 18.7 | 21.2 |
| Rats | Oral | | 27.50 | | |
| Guinea pig | Oral | | 7.75, 10.0, 11.5 | | |
| Dog | | | | >11 | |

Substances with an $LD_{50}$ value >15 g/kg are classified as practically nontoxic. Glycerol is practically nontoxic for mice and rats as shown in the table above. For mice, rats and guinea pigs, the principal effect was stimulation of the central nervous system. Muscle spasms and generalized clonic convulsions preceded death. Hyperemia of the small intestine were found in mice, rats, and guinea pigs. Glycerin produces a strong laxative effect in rats, guinea pigs, and dogs. For dogs, a dose >11 g/kg caused intestinal disturbances and vomiting; however, no toxic signs were reported with doses ≤8 g/kg.

The acute toxicity of glycerin by the oral route to mice and rats is low as $LD_{50}$ values for both species exceed 20 g/kg. With lethal doses, the principal toxic effect was stimulation of the central nervous system with resultant muscle spasms and convulsions.

Subacute Toxicity of Glycerol

Rats

Subacute Toxicity of Glycerol in Rats **("Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227" prepared by Informatics, Inc. 1973).**

Rats received glycerol in drinking water at levels ranging from 0 to 20% for periods of either 96 days (Arzneimittel-Forsch. 4:643-646, 1955) or 3 months (Arch. Exptl. Pathol. Pharmakol. 221:14-33, 1954). In the 96 day study, for rat receiving ≤10 g/kg/day, growth was found to be normal; however, at 20 g/kg/day, growth was temporarily impaired. In the 3 month study, two (16.7%) of 12 rats that received glycerol at 20 g/kg/day died during week 6. Other animals receiving glycerol at 20 g/kg/day displayed decreased growth and altered development; however, as the study progressed, these animals recovered and by the end of treatment, there was no indication of injury attributable to glycerin. Organ weights were unchanged. A target organ of toxicity was not found.

For rats that received glycerol in drinking water at levels for period of 96 days or 3 months, the no effect dose appeared to be 10 g/kg/day. A target organ of toxicity was not found.

Chronic Toxicity of Glycerol



Rats

Chronic Toxicity of Glycerol in Rats **("Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227" prepared by Informatics, Inc. 1973).**

The chronic toxicity of glycerol was evaluated with weanling and adult rats in studies ranging (Bulletin of the National Formulary Commission 15:205-229, 1947; American Journal of Physiology 103:517-534, 1933; Journal of the American Pharmaceutical Association 39:583-585; Archives of Experimental Pathology and Pharmacology 221:14-33, 1954; Anonymous Food Additive Petition Number 925

Glycerol was administered either in the diet or in the drinking water. Glycerol levels in the diet ranged Levels in drinking water ranged

The estimated glycerol dose ranged for adult animals and for weanling animals.

In a 20 week study, doses of ≥6 g/kg/day for males and ≥5 g/kg/day for females produced dose-related hydropic and fatty degeneration of liver parenchymatous cells; however, these findings were not reported in other studies of equal or longer duration. Doses of ≤3.6 g/kg/day for males and ≤3.0 g/kg/day for females had no effect on the liver. In a second 20 week study, for rats that received 45.75 g/kg/day, growth was impaired, activity was decreased, and coats lacked the smooth gloss of controls; however, no effects were observed at 30.75 g/kg/day. For females that received glycerol in drinking water at 5 g/kg/day for 6 months, calcified masses were found in the tubules near the junction of the kidney cortex and medulla in 6 of 10 rats; however, this finding was not replicated in other studies. In 1 and 2 year studies with rats at starting doses of up to 60 g/kg/day for weanling animals and adjusting to 15 g/kg/day for an adult animal, there were no significant findings and a target organ of toxicity was not identified. In a second 2 year study with rats at doses up to 10 g/kg/day, there were no significant findings and a target organ of toxicity was not found.



The chronic toxicity of glycerol was evaluated in rats at doses ranging from 0 to 46 g/kg/day in studies with a duration ranging from 20 weeks to 2 years. For 2 year studies, the no effect dose was approximately 10 g/kg/day. A target organ of toxicity was not found.



**Two Year Feeding Study in Rats (Archives of Industrial Hygiene and Occupational Medicine 7: 282-291, 1953).**

**Methods**: Rats of the Long-Evans strain were maintained on diets containing 5, 10, or 20% natural or synthetic glycerin for 1 year. In a continuation of the study, a diet containing 5 or 10% natural or synthetic glycerin was administered to separate groups of rats for 2 years. Estimated doses for males receiving 5, 10, or 20% glycerin in the diet were 3, 6, or 12 g/kg/day, respectively. Estimated doses for females receiving 5, 10, or 20% glycerin in the diet were 2.5, 5, or 10 g/kg/day, respectively. There were 22 rats/treatment group. Twenty-six animals served as controls. The diet consisted of a standard dog food meal, into which, the glycerin was added and thoroughly mixed. Animals were monitored daily for mortality and signs of toxicity. At weekly intervals, body weight was measured and animals were given a physical examination. At 3, 6, 12, 18, and 24 months after the start of treatment, five rats/group were placed into metabolism cages for 24-48 hr for collection of urine specimens. Blood was collected for determination of hematological parameters. Food consumption was measured during the first year of the study. For animals that died or were killed during the study, a gross necropsy examination was performed. Any gross abnormalities were recorded. Organ weights for liver, lung, heart, kidneys, and spleen were determined. Microscopic analysis of the liver, spleen, adrenal gland, kidney, small intestine, bladder, and reproductive organs were performed. Liver glycogen and lipid content was determined.

Results:

1. Observed Effects: None reported.



2. Mortality: The authors did not report the incidences of mortality; although, they stated there were no differences in the incidences between control and treatment groups.

3. Body Weight: There were no significant treatment-related changes of body weight gain for male treatment groups after 52 and 105 weeks of treatment. Body weight gains for female treatment groups were lower than corresponding gains for controls at 52 and 105 weeks; although, the degree of impairment did not correlate with the glycerol dose. Body weights for male control rats at the initiation of treatment, 52 weeks, and 105 weeks were 101.4, 345.7, and 385.3 g, respectively. Body weights for female control rats at the initiation of treatment, 52 weeks, and 105 weeks were 100.7, 206.3, and 226.6 g, respectively.



Percent body weight gains for rats receiving 5, 10, or 20% natural or synthetic glycerin in the diet for 1 year and 5 or 10% natural or synthetic glycerin in the diet for 2 years. Estimated doses for males receiving 5, 10, or 20% glycerin in the diet were 3, 6, or 12 g/kg/day, respectively. Estimated doses for females receiving 5, 10, or 20% glycerin in the diet were 2.5, 5, or 10 g/kg/day, respectively.

| Sex | 1 Year (52 weeks) | | | | | | 2 Years (105 weeks) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5% | | 10% | | 20% | | 5% | | 10% | |
| | Nat. | Syn. | Nat. | Syn. | Nat. | Syn. | Nat. | Syn. | Nat. | Syn. |
| Males | 84.5 | 93.8 | 91.5 | 92.8 | 89.7 | 88.8 | 69.9 | 87.1 | 71.2 | 99.4 |
| Fe-males | 49.8 | 66.9 | 60.6 | 85.3 | 74.0 | 99.0 | 62.7 | 52.4 | 54.4 | 84.3 |

Nat. = Natural and Syn. = Synthetic

4. Hematology: There were no treatment-related effects on hemoglobin levels.



5. Blood Chemistry and Urinalysis: For animals that received 20% glycerin in the diet for 1 year, there were no significant effects on liver glycogen and lipid content as compared to controls. Albuminuria and glycouria were observed in both control and treatment groups, and there was no relationship to glycerin treatment.



6. Physical Examination: There were no treatment-related differences found with physical examinations.

7. Organ Weights: Relative liver weight for females receiving 20% glycerin for 1 year was increased to 111% of the control (3.78%).

8. Gross Pathology: There were no treatment-related gross pathological lesions.



9. Histopathology: There were no treatment-related histopathological changes.



Male rats received glycerin in the diet for 3 or 6 g/kg/day for 2 years or 12 g/kg/day for 1 year. Female rats received glycerin in the diet for 2.5 or 5 g/kg/day for 2 years or 10 g/kg/day for 1 year. The no effect dose for the 2 year study was 6 g/kg/day for males and 5 g/kg/day for females. Body weight gains were unaffected for male treatment groups. Body weight gains for female treatment groups were generally impaired by >10% as compared to the control; although, observed changes did not occur in a dose-related manner. There was no evidence of systemic toxicity. A target organ of toxicity was not observed.



Dogs

**Chronic Toxicity of Glycerol in Dogs ("Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227" prepared by Informatics, Inc. 1973).**

Dogs were maintained on diets containing 0, 5, 10, or 20% synthetic or natural glycerin for 2 years. Estimated doses were 0, 1.25, 2.50, and 5 g/kg/day. There were 4 dogs/sex/group. Untreated groups served as controls. General appearance and behavior were monitored. Body weight, food consumption, water intake, and urine output were measured. Hematology and blood chemistry parameters and liver function tests were determined. One animal/sex/group was sacrificed at 12 weeks. At 2 years, all surviving animals were sacrificed and subjected to gross examination. Liver, kidney, spleen, heart, and gonad weights were determined. Major tissues and organs were evaluated histologically. No deleterious or toxicological effects attributable to glycerin ingestion were reported. A target organ of toxicity was not found.

For dogs maintained on diets containing 1.25, 2.50, and 5 g/kg/day glycerin for 2 years, the no effect dose was 5 g/kg/day. No target organ of toxicity was identified.

Reproductive Toxicology of Glycerol



Rats

Segment I Studies in Rats: Effects on Fertility and Reproductive Performance ("Generally Recognized as Safe Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB 221 227" prepared by Informatics, Inc. 1973).

The effects of glycerol on fertility and reproductive performance in rats were evaluated in two Segment I studies. In the first study, rats received 10 mL of a 20% aqueous glycerin solution per kg body weight on a daily basis (estimated dose of 2 g/kg/day) for 8 weeks (Archives of Experimental Pathology and Pharmacology 220:414-417, 1953). For one-half of the females, treatment continued until birth of offspring, while for the other half, treatment continued until weaning. The fertility index was 100% for the glycerin-treated group. Litter size and growth and development of the $F_1$ generation were similar between the control and treatment groups. Selected rats of the $F_1$ generation were sacrificed at 100 days of age and histological evaluation revealed no treatment-related changes of "inner-secretory organs". Fertility and reproductive performance of the $F_1$ generation as well as growth and development of the $F_2$ generation were unaffected. Histological evaluation of the "inner secretory organs" of the $F_2$ generation revealed no abnormalities. In a second Segment I study, rats were maintained on diets containing 0, 41, or 61% glycerol (estimated doses of 0, 30.75, or 45.75 g/kg/day); however, the treatment period was not specified (American Journal of Physiology 103:517-534, 1933). No adverse effects on reproduction were observed for rats receiving glycerin at 30.75 g/kg/day; however, female rats that received 45.75 g/kg/day failed to become pregnant.

In a Segment I study with rats, glycerin administered in drinking water at 2 g/kg/day had no effects on fertility or reproductive performance. Growth and maturity of the $F_1$ and $F_2$ generations were unaffected by treatment of $F_0$ generation with glycerin. In a second Segment I, glycerin at a dose of 30.75 g/kg/day had no effect on fertility or reproductive performance; however, the treatment period was not specified.

Mice and Rats



Segment II Studies in Mice and Rats and a Multigenerational Study in Rats (Summary provided in a report entitled "Glycerol: Toxicity Profile" by BIBRA Toxicology International, 1993).

Pregnant female mice or rats received glycerin at 1.28 or 1.31 g/kg/day through the diet, respectively, from day 6 through 15 of gestation (FDRL 1973). There were no clearly discernable

effects on nidation or maternal and fetal survival. The incidences of malformations or variations observed in either soft or skeletal tissues of glycerol-treated groups did not differ from controls. In a multigenerational study, glycerol administered at 15 g/kg/day to 7 successive generations of rats led to a reduced pup weight; however, no similar effects were observed with glycerol at 5 g/kg/day administered over 6 successive generations (Bulletin of the National Formulary Commission 15:205-229, 1947).

In Segment II studies with mice and rats, glycerin was not teratogenic. In a multigenerational study, glycerin at 5 g/kg/day had no significant effects when administered to 6 successive generations of rats.



Genotoxicity of Glycerol

The Genotoxic Activity of Glycerol in the Ames Salmonella Typhimurium Mutagenesis Assay.

**Methods**: The mutagenic potential of glycerol was assessed using the Ames Salmonella typhimurium mutagenesis assay by three different laboratories (The Genotoxic Activity of Glycerol in an In Vitro Test Battery In: Food and Chemical Toxicology 26: 631-635, 1988; Salmonella Mutagenicity Test Results for 250 Chemicals In: Environmental Mutagenesis Supplement 1:3-142, 1983; and Primary Mutagenicity Screening of Food Additives Currently Used in Japan In: Food and Chemical Toxicology 22: 623-636, 1984). Tester strains included TA98, TA100, TA1535, TA1537, TA1538, TA92, and TA94. The pre-incubation technique was used in all studies. Assays were performed with glycerol at concentrations of 200-1000, 10-10,000, and 0-50,000 µg/plate in the presence and absence of metabolic activation. The liver S-9 fraction was prepared from rats treated with Aroclor 1254 in all studies; although, one lab also included results obtained using the S-9 fraction prepared from livers of Syrian hamsters treated with Aroclor 1254. Assays included standard positive controls in the presence or absence of metabolic activation. For two laboratories, a positive response was a reproducible, dose-related increase in the number of histidine revertants per plate in one or more tester strains, while for the third laboratory, a two-fold increase in the number of revertants was required.



**Results**: For the three different studies, glycerol was not mutagenic in any tester strain either in the presence or absence of metabolic activation.

Glycerol was not mutagenic in the Ames Salmonella typhimurium mutagenesis assay either in the presence or absence of metabolic activation.

Rat Hepatocyte Unscheduled DNA Synthesis Assay (The Genotoxic Activity of Glycerol in an In Vitro Test Battery In: Food and Chemical Toxicology 26: 631-635, 1988).

Methods: The autoradiographic unscheduled DNA synthesis assay was performed using standard procedures described in Single Cell Mutation Monitoring Systems (Edited by A.A. Ansari and F.J. de Serres) published by Plenum Press in New York, NY). Hepatocytes were prepared from male CD rats, and viability was >90% by trypan blue dye exclusion. 2-acetylaminofluorene (0.5 and 3.0 μg/mL) was used as a positive control. A test result was considered positive if there was a reproducible, dose-dependent increase ($p < 0.01$) in net nuclear grain counts compared with the solvent control.

Results: Glycerol was negative in the unscheduled DNA synthesis assay with rat hepatocytes. The positive control produced a characteristic increase in net nuclear grains counts, while counts for glycerol were negative.



Glycerol was negative in the unscheduled DNA synthesis assay with rat hepatocytes.



Chinese Hamster Ovary Chromosome Aberration Assay (The Genotoxic Activity of Glycerol in an In Vitro Test Battery In: Food and Chemical Toxicology 26: 631-635, 1988).

Methods: The chromosome aberration assay was performed in the presence and absence of metabolic activation (Aroclor-1254-induced rat liver S-9 preparation) using the Chinese hamster ovary (CHO) cell line WBL. Glycerol was tested at concentrations of 100, 200, 400, 600, 800, and 1000 μg/mL with or without metabolic activation. For assays without metabolic activation, treatment time with glycerol was for 10 or 14 hr with no recovery time. For assays with metabolic activation, treatment time with glycerol was 2 hr, followed by 10 or 14 hr recovery times. Multiple treatment and harvest times were used to reduce the chance of false results due to possible glycerol related alterations of the cell cycle. A test result was considered positive if there was a reproducible dose-dependent increase ($p < 0.01$) in the frequency of cells with structural chromosome aberrations compared with the solvent control cells.



Results: The CHO chromosome aberration assay was negative with glycerol. An isolated, statistically significant increase in aberrations was found with 200 μg/mL in the presence of metabolic activation following a 10 hr recovery period; however, this result was considered spurious due to the lack of change at other concentrations and this finding was not reproduced in a second experiment. Studies published from another laboratory found that glycerol was negative for the production of chromosomal aberrations with CHO cells (Food and Chemical Toxicology 22: 623, 1984).

The Chinese hamster ovary chromosome aberration assay was negative with glycerol.

**Chinese Hamster Fibroblast Chromosome Aberration Assay (Primary Mutagenicity Screening of Food Additives Currently Used in Japan In: Food and Chemical Toxicology 22: 623-636, 1984).**

**Methods**: Chromosomal aberration tests with glycerol were performed with glycerol using a Chinese hamster fibroblast cell line, CHL. The modal chromosome number was 25 and the doubling time was 15 hr. Cells were exposed to glycerol at three concentrations for 24 or 48 hr. No metabolic activation system was used in these studies. The maximum dose was selected by a preliminary test in which the dose that inhibited cell growth by 50% was determined, unless restrictions due to osmotic pressure limited this dose. The maximum concentration of glycerol tested was 1 mg/mL. Cultures were treated with colcemid 2 hr before harvesting. Cells were trypsinized, fixed, and stained with Giemsa solution. One hundred well-spread metaphases were observed and the incidence of polyploid cells as well as cells with structural chromosomal aberrations (i.e., chromatid or chromosome gaps, breaks, exchanges, ring formations, fragmentations) was determined. Untreated or solvent-treated cells served as negative controls in which the incidence of aberrations was generally <3%. Results were considered negative if the incidence was <5%, equivocal and positive with ≥10%.



**Results**: Glycerin was negative in the chromosomal aberration assay using Chinese hamster fibroblasts. The incidence of structural aberrations and polyploid cells with glycerol was 1 and 2%, respectively, which is indicative of a negative finding.

Glycerin was negative in the chromosomal aberration assay using Chinese hamster fibroblasts.

**Chinese Hamster Ovary Sister Chromatid Exchange Assay (The Genotoxic Activity of Glycerol in an In Vitro Test Battery In: Food and Chemical Toxicology 26: 631-635, 1988).**

**Methods**: The Sister Chromatid Exchange assay was performed with and without metabolic activation (Aroclor-1254-induced rat liver S-9 preparation) using the Chinese Hamster cell line WBL. Treatment duration with glycerol at concentrations of 200, 400, 600, 800, and 1000 μg/mL was 2 hr with metabolic activation and 25.5 hr without metabolic activation. At least 50 well-spread metaphases were scored from each dose. A test result was considered positive if there was a reproducible dose-dependent increase ($p < 0.01$) in the mean frequency of sister chromatid exchanges/cell compared with the solvent control.

**Results**: Glycerol did not produce an increase in the sister chromatid exchange frequency in the presence or absence of metabolic activation.

Glycerol was negative in the sister chromatid exchange assay with or without metabolic activation.

**Chinese Hamster Ovary HGPRT Gene Mutation Assay (The Genotoxic Activity of Glycerol in an In Vitro Test Battery In: Food and Chemical Toxicology 26: 631-635, 1988).**

**Methods**: The hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene mutation assay was performed in the presence and absence of metabolic activation (Aroclor-1254-induced rat liver S-9 preparation) using the CHO-K1-BH4 cell line. To reduce the spontaneous background mutation rate, cells were grown in medium containing hypoxanthine, aminopterin, glycine, and thymidine for 48 hr, and then grown in normal culture medium for 3 days prior to use. Cells were exposed to glycerol at concentrations of 100, 200, 400, 600, 800, and 1000 μg/mL for 5 hr with or without metabolic activation. A test result was considered positive if there was a three-fold increase in the mutation frequency above the solvent control that displayed dose-dependency.

**Results**: Glycerol at 800 and 1000 μg/mL without metabolic activation increased the mutation frequency to 24 x $10^{-6}$ and 9 x $10^{-6}$, respectively, as compared to 2 x $10^{-6}$ for the solvent control; however, there was a lack of a dose response relationship. Toxicity of glycerol at 1000 μg/mL with the Chinese hamster ovary cell line was not stated. Glycerol at 800 and 1000 μg/mL with metabolic activation increased the mutation frequency to 9 x $10^{-6}$ and 15 x $10^{-6}$, respectively, as compared to 7 x $10^{-6}$ for the solvent control; however, these changes were not significant. The effect of glycerol in this Chinese hamster ovary HGPRT gene mutation assay was somewhat unclear; although, there was no evidence of dose relationship at 800 and 1000 μg/mL, where an increased mutation frequency was observed.

The effect of glycerol in this Chinese hamster ovary HGPRT gene mutation assay was somewhat unclear; although, there was no evidence of dose relationship at 800 and 1000 μg/mL, where an increased mutation frequency was observed. It is possible that the high osmolality of glycerol at 800 and 1000 μg/mL may have contributed to these results.

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SUMMARY AND EVALUATION

Food carbohydrates are hydrolyzed to monosaccharides prior to transport across the microvillus membrane of the small intestine. Digestion of the disaccharide, sucrose, is performed by the brush border enzyme, sucrase-isomaltase (SI). Congenital Sucrase-Isomaltase Deficiency (CSID) is a chronic malabsorption disease characterized by an autosomal recessive inheritable disease of sucrase and isomaltase deficiency. CSID patients generally have no endogenous sucrase activity, markedly reduced isomaltase activity, and moderately reduced maltase activity. In the absence of SI, sucrose is not metabolized or absorbed by the intestines. The presence of intact disaccharides, such as sucrose, in the intestinal lumen leads to osmotic retention of water, resulting in loose stools. Further, the unabsorbed sucrose can be metabolized by intestinal bacteria leading to the production of gases, such as hydrogen, methane, and carbon dioxide. These gases can cause gastrointestinal discomfort due to flatulence, bloating, abdominal cramps, watery diarrhea, nausea, and vomiting. Treatment has usually consisted of instituting a diet completely free of sucrose. The sponsor has developed Sucraid™ as an enzyme replacement therapy for the treatment of CSID. A purified form of sucrase, prepared from Saccharomyces cerevisiae (yeast), was commercially available from Red Star Yeast & Products. The food form, often called invertase, has been used in the food and candy industry for decades. The major excipient in the sacrosidase solution is glycerin (glycerol), which is present at a 50% (wt/v) concentration and contributes to the stability of the enzyme within the drug product. Aluminum is present in the Sucraid product at a final concentration <20 ppm. This review is focused in large part on an analysis of toxicity studies with glycerin and aluminum.

The marketing indication for Sucraid™ will be as an oral enzyme replacement therapy indicated for the treatment of confirmed or suspected congenital sucrase-isomaltase deficiency (CSID) and the prevention of the associated symptoms of sucrose malabsorption such as frequent watery stools, gas, bloating, abdominal cramping, explosive diarrhea, and growth retardation. The recommended dosage is as follows: 1 mL per meal or snack for patients up to 15 kg in body weight, or 2 mL per meal or snack for patients over 15 kg in body weight. Glycerin is present in the sacrosidase solution at a concentration of 50% (wt/v). For an infant, weighing 5 kg, the daily consumption of glycerol would be approximately 0.625 g/kg/day [(2.5 mL/day x 1.249 g/mL)/5 kg]. For a child, weighing 15 kg, the daily consumption of glycerol would be approximately 0.416 g/kg/day [(10 mL/day x 1.249 g/mL)/15 kg].

For this new drug application, the pharmacological and toxicological properties of sucrase, glycerol were evaluated in several reports and publications as outlined at the beginning of this review.

No studies have been published that examine the toxicity of the enzyme sacrosidase (sucrase, invertase), either endogenous human sacrosidase or exogenous yeast-derived sacrosidase. Yeast-derived sacrosidase has been widely utilized within the human food industry for decades. It is a Generally Recognized as Safe (GRAS) food material under FDA provision 21 CFR § 170.30. Because sacrosidase is a large macromolecule, it will not be transported across the gastrointestinal mucosa and into the systemic circulation following oral ingestion. Thus, no systemic toxicity directly from the sacrosidase molecule is likely. Because sacrosidase is a naturally occurring glycoprotein, it will be digested to peptides and eventually amino acids within the small intestine. These amino acids and peptides will be absorbed into the circulation and utilized as nutrients.

The major excipient in the sacrosidase solution is glycerin (glycerol), which is present at a 50% (wt/v) concentration and contributes to the stability of the enzyme within the drug product. Glycerin is a polyhydric alcohol (1,2,3-propanetriol). It is a clear, colorless, sweet tasting liquid. A search of the Physicians Desk Reference and the Merck Manual identified 306 products in which glycerin was either the active ingredient or the part of the formulation. Glycerin is the active ingredient for Osmoglyn®, an oral osmotic agent used for short term reduction of intraocular pressure. The usual dosage is 1.256-1.884 g glycerol/kg, which is 3-4 times higher than Sucraid. It is also used in foods. Animal and vegetable fats contain about 10% by weight of glycerin. It is present in animal tissues to the extent of about 1% of the body weight. A National Research Council subcommittee estimated daily human intake of glycerol by age group as follows: 0-5 months, 0.1 g/kg; 6-11 months, 0.8 g/kg; 12-23 months, 0.6 g/kg; and 2-65 + yr, 0.1 g/kg. The daily consumption of glycerol in Sucraid™ for an infant and a child could represent increase in the daily consumption of glycerol.

The acute oral toxicity of glycerin has been evaluated in mice, rats, guinea pigs, and dogs. Oral $LD_{50}$ values for mice, rats, and guinea pig were
respectively. For mice, rats, and guinea pigs, the principal effect was stimulation of the central nervous. At lethal doses, muscle spasms and generalized clonic convulsions preceded death. Hyperemia (i.e., an abnormally large blood supply) of the small intestine were found in mice, rats, and guinea pigs. For dogs, a dose of 11 g/kg caused intestinal disturbances and vomiting; however, no toxic signs were reported with doses ≤8 g/kg. Glycerin produces a strong laxative effect in rats, guinea pigs, and dogs.

The subacute toxicity of glycerol has been evaluated in rats at doses for periods of 96 days and 3 months. In the 96 day study, the no effect dose was 10 g/kg/day. For rats that received 20 g/kg/day, growth was temporarily impaired. In a 3 month study, the no effect dose was 6.25 g/kg/day. Mortality occurred at 12.5 g/kg/day. For animals that received 12.5 g/kg/day and survived to the end of treatment, decreased growth and altered development were observed initially in the study; however, recovery had occurred by the end of the study. A target organ of toxicity was not identified in either study.

The chronic toxicity of glycerol has been evaluated in rats and dogs in studies ranging up to 2 years in duration.

The chronic toxicity of glycerol has been evaluated with weanling and adult rats in studies ranging Glycerol was administered either in the diet or in the drinking water . The estimated glycerol dose for adult animals and for weanling animals. For 2 year studies with adult rats, the no effect dose was approximately 10 g/kg/day. There was no significant systemic toxicity. A target organ of toxicity was not identified.

In a 2 year study, dogs received glycerol in the diet at doses equivalent to 1.25, 2.5, or 5.0 g/kg/day. The no effect dose was 5.0 g/kg/day. A target organ of toxicity was not identified.

Glycerol was evaluated as a potential tumor promoter with several different strains of mice. Inayama and associates (Japanese Journal of Cancer Research 77:103-105, 1986 and 77:345-350, 1986) reported that glycerol administered by the oral route in drinking water at 10 g/kg/day for a period of 25 weeks enhanced 4-nitroquinoline 1-oxide (4NQO)-induced pulmonary tumorigenesis in male ddY mice. Witschi and associates (Fundamentals of Applied Toxicology 13:174-180, 1989) repeated these studies using several

different strains of mice and known carcinogens including 4NQO, 3-methylcholanthrene, and urethane, and found that glycerol did not act as a tumor promoter. Further, Witschi and associates found that glycerol administered to C3H mice by the oral route in drinking water at 10 g/kg/day for 1 year did not increase tumor incidence in the lung or liver as compared with controls. It must be noted that these study do not conform to FDA guidelines with regard to the dose selection, treatment period for a mouse carcinogenicity study, or use of good laboratory practice procedures.



In a Segment I study with rats, glycerol administered by oral gavage at 2 g/kg/day had no effects on fertility or reproductive performance. Further, growth and development of the $F_1$ and $F_2$ generations were unaffected by treatment of the $F_0$ generation with glycerol. In a second Segment I study, glycerin administered in the diet at 30.75 g/kg/day had no effects on fertility or reproductive performance; however, a dose of 45.75 g/kg/day blocked fertility.



In Segment II studies with mice and rats, glycerin administered by the oral route at doses of 1.28 or 1.31 g/kg/day, respectively, from day 6 through 15 of gestation, had no teratogenic effects. In a Segment II study with rats, aluminum was administered by oral gavage at doses of 13, 26, or 52 mg aluminum/kg/day from day 6 through 14 of gestation (Teratology 38: 253-257, 1988). Decreased fetal body weight and an increased incidence of several skeletal variations were observed at all dose levels.

In a multi-generational study with rats, glycerin administered by the oral route at 5 g/kg/day to six successive generations had no significant toxicological effects.

Glycerol was found to have no mutagenic potential <u>in vitro</u> using the Salmonella typhimurium gene reverse mutation test (Ames test), rat hepatocyte unscheduled DNA synthesis assay, the Chinese hamster fibroblast chromosome aberration assay, and the Chinese hamster ovary sister chromatid exchange assay. Aluminum (as $AlCl_3$) was found to have no mutagenic potential using the Ames test.

In humans, Sucraid™ will be administered daily by the oral route on chronic basis exceeding 6 months and presumed to span the patient's entire life. There are no toxicology studies for the enzyme, sucrase; however, its toxicity potential is expected to be negligible as the enzyme is being administered as a replacement therapy and the enzyme should not enter the systemic circulation. Glycerin, the major excipient in the sacrosidase solution, is present at a 50% concentration and contributes to the stability of the enzyme within the drug product. The pharmacological and toxicological properties of glycerin administered by the oral route have been extensively characterized over the past 60 years. The chronic toxicity of glycerin administered by the oral route has been extensively studied in rodent with treatment periods ranging from 6 months to two years. Further, the chronic toxicity of glycerin has been examined in dogs with at least one 2 year study. For chronic studies in both rodent and nonrodent species, a target organ of toxicity was not found and there were no significant adverse effects.

From a preclinical standpoint, the application is approvable.

The label is according to 21 CFR, 201.50 Subpart B (April 1, 1996); however, minor changes in text as outlined in the review portion are needed.

**RECOMMENDATION:** From a preclinical standpoint, the application is approvable. The sponsor should be asked to change the labeling as outlined in the review portion.

APPEARS THIS WAY
ON ORIGINAL

/S/

Timothy W. Robison, Ph.D.

8-20-97
Date

cc:
Orig NDA 20,772
HFD-180
Orig IND 53,372 (Initial Submission dated 5/23/97)
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Robison
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9/5/97

R/D Init.: J. Choudary 8/8/97

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