

γ -BUTYROLACTONE
CAS # 96-48-0
ORAL RISK ASSESSMENT DOCUMENT



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EXECUTIVE SUMMARY

γ -Butyrolactone – Oral Risk Assessment CAS # 96-48-0			
PARAMETER	LEVEL	UNITS	DERIVED
LOAEL (lowest-observed-adverse-effect level)	12.5	mg/kg	From a single dose human neurotoxicity study
Oral RfD (oral reference dose)	0.1	mg/kg-day	From a single dose human neurotoxicity study
TAC (total allowable concentration)	4	mg/L	For a 70 kg adult drinking 2 L/day, with a data derived Relative Source Contribution of 0.14 mg/day
SPAC (single product allowable concentration)	0.4	mg/L	For a 70 kg adult drinking 2 L/day
STEL (short term exposure level)	4	mg/L	Calculation for a 10 kg child drinking 1 L/day superseded by the TAC
KEY STUDY	Ferrara, S.D., R. Giorgetti, S. Zancaner, R. Orlando, A. Tagliabracci, F. Cavarzeran, P. Palatini. 1999. Effects of Single Dose of Gamma-Hydroxybutyric Acid and Lorazepam on Psychomotor Performance and Subjective Feelings in Healthy Volunteers. Eur J Clin Pharmacol 54: 821-827.		
CRITICAL EFFECTS	Neurotoxic effects in humans, including dizziness, a sense of dullness, uncontentedness, and decreased anxiety.		
UNCERTAINTY FACTORS	Factors applied in calculating the oral RfD include: • 1x for interspecies extrapolation • 10x for intraspecies extrapolation • 3x for extrapolation from a LOAEL to a NOAEL • 1x for study duration • 3x for database deficiencies The total uncertainty factor is therefore 100x.		
TOXICITY SUMMARY	γ-Butyrolactone is metabolized in the body within minutes to γ-hydroxybutyrate; therefore, its toxicity can be assessed based on that of both γ-butyrolactone and γ-hydroxybutyrate. The critical effects in humans associated with γ-hydroxybutyrate administration involve changes in behavior. The critical study evaluated behavioral changes associated with oral administration of 12.5 mg/kg or 25 mg/kg bolus doses of γ-hydroxybutyrate to human volunteers. In the 25 mg/kg dose group, 66% of subjects reported adverse side effects of dizziness and a sense of dullness, while 50% of subjects in the 12.5 mg/kg dose group reported these effects. Also, changes in mood were observed at both doses. Based on this, a LOAEL of 12.5 mg/kg γ-hydroxybutyrate was established. Another human study found that volunteers reported feeling drowsy at doses ranging from 35 to 63 mg/kg γ-hydroxybutyrate. Bolus doses greater than 50 mg/kg led to coma. This second study showed evidence that γ-hydroxybutyrate administration leads to neurophysiological abnormalities, as inconsistent electroencephalographic trends were observed. Based on drowsiness reported at all dose levels, a LOAEL of 35 mg/kg was established. Abnormal electroencephalographic patterns indicative of generalized nonconvulsive seizures, in addition to behavioral and body temperature alterations, were observed in single dose neurotoxicity studies with monkeys and rats. In repeated dose rat and mouse studies, lethality, reduced body weight gain, reduced body weight, and behavioral alterations were observed. Reduced testicular weight was observed in a 3-week reproductive toxicity study with male rats. Several genotoxicity studies demonstrated that γ-butyrolactone is not genotoxic in standard short-term tests. There was equivocal evidence that γ-butyrolactone elicits carcinogenic effects in male mice, with a statistically significant increase in the occurrence of focal hyperplasia of the adrenal medulla in male B6C3F₁ mice orally administered 262 mg/kg-day γ-butyrolactone. However, there was no evidence of carcinogenicity in male or female rats or in female mice. Subsequent review of the mouse data for γ-butyrolactone by the National Toxicology Program concluded that this chemical was unlikely to be carcinogenic.		
CONCLUSIONS	Exposure to γ-butyrolactone elicits neurotoxic effects in humans, monkeys, and rats. The critical acute study for this risk assessment was chosen because it is a human study and because the neurotoxic effects induced by γ-hydroxybutyrate occurred at doses lower than any dose used in any of the repeated-dose animal studies. Based on the uncertainty factors used to account for intraspecies extrapolation, database deficiencies, and extrapolation from a LOAEL to a NOAEL, the drinking water action levels derived in this risk assessment are considered protective of public health.		

1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **γ -butyrolactone** in drinking water, as an extractant from one or more drinking water system components evaluated under NSF/ANSI 61 (2002), or as a contaminant in a drinking water treatment chemical evaluated under NSF/ANSI 60 (2002). Both non-cancer and cancer endpoints have been considered, and risk assessment methodology developed by the U.S. Environmental Protection Agency (U.S. EPA) has been used.

Non-cancer endpoints are evaluated using the reference dose (RfD) approach (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 1993), which assumes that there is a threshold for these endpoints that will not be exceeded if appropriate uncertainty factors (Dourson et al., 1996) are applied to the highest dose showing no significant effects. This highest dose is derived from human exposure data when available but more often is derived from studies in laboratory animals. Either the no-observed-adverse-effect level (NOAEL) taken directly from the dose-response data, or the calculated lower 95% confidence limit on the dose resulting in an estimated 10% increase in response (the LED₁₀ or BMDL from benchmark dose programs) can be used (U.S. EPA, 2001a). The lowest-observed-adverse-effect level (LOAEL) can also be used, with an additional uncertainty factor, although the benchmark dose approach is preferred in this case. The RfD is expressed in mg/kg-day. It is defined by the U.S. EPA as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime” (Barnes and Dourson, 1988; U.S. EPA, 1993; U.S. EPA, 1999a).

NSF uses the RfD to derive three product evaluation criteria for non-cancer endpoints. The total allowable concentration (TAC), generally used to evaluate the results of extraction testing normalized to static at-the-tap conditions, is defined as the RfD multiplied by the 70 kg weight of an average adult assumed to drink two liters of water per day. A relative source contribution (RSC), to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered, is also applied in calculating the TAC. The relative source contribution should be data derived, if possible. Alternately, a 20% default contribution for water can be used (U.S. EPA, 1991a). The TAC calculation is then as follows:

$$\text{TAC (mg/L)} = \frac{[\text{RfD (mg/kg-day)} \times 70 \text{ kg}] - [\text{total contribution of other sources (mg/day)}]}{2\text{L/day}}$$

or

$$\text{TAC (mg/L)} = \frac{\text{RfD (mg/kg-day)} \times 70 \text{ kg}}{2\text{L/day}} \times 0.2 \text{ (RSC)}$$

The single product allowable concentration (SPAC), used for water treatment chemicals and for water contact materials normalized to flowing at-the-tap conditions, is the TAC divided by the estimated total number of sources of the substance in the drinking water treatment and distribution system. In the absence of source data, a default multiple source factor of 10 is used.

This accounts for the possibility that more than one product in the water and/or its distribution system could contribute the contaminant in question to drinking water.

Finally, a short-term-exposure level (STEL), at a higher level than the TAC, may be calculated for contaminants such as solvents expected to extract at higher levels from new product, but also expected to decay rapidly over time. The STEL is calculated from the NOAEL or the LED₁₀ of an animal study of 14- to 90-days duration, with uncertainty factors appropriate to the duration of the study. The contaminant level must decay to the TAC or below under static conditions, or to the SPAC or below under flowing conditions within 90 days, based on the contaminant decay curve generated from over-time laboratory extraction data.

Endpoints related to cancer are evaluated using modeling to fit a curve to the appropriate dose-response data (U.S. EPA, 1996a; U.S. EPA, 1999b). If there is sufficient evidence to use a non-linear model, the LED₁₀ or BMDL, divided by the anticipated exposure, is calculated to give a margin of exposure. If there is insufficient evidence to document non-linearity, a linear model drawing a straight line from the LED₁₀ or BMDL to zero is used as a default. If a linear model (generally reflecting a genotoxic carcinogen) is used, a target risk range of 10^{-6} to 10^{-4} is considered by the U.S. EPA to be safe and protective of public health (U.S. EPA, 1991a). For the purposes of NSF/ANSI 60 (2002) and 61 (2002), the TAC is set at the 10^{-5} risk level, and the SPAC is set at the 10^{-6} risk level. Use of a higher risk level is not ruled out but would generally require documentation of a benefit to balance the additional risk.

The RfD, TAC, SPAC, and STEL values derived in this document are based on available health effects data and are intended for use in determining compliance of products with the requirements of NSF/ANSI 60 (2002) and 61 (2002). Application of these values to other exposure scenarios should be done with care and with a full understanding of the derivation of the values and of the comparative magnitude and duration of the exposures. These values do not have the rigor of regulatory values, as data gaps are generally filled by industry or government studies prior to regulation. Data gaps introduce uncertainty into an evaluation and require the use of additional uncertainty factors to protect public health.

The general guidelines for this risk assessment include those from the National Research Council (1983) and from The Presidential/Congressional Commission on Risk Assessment and Risk Management (1997a, 1997b). Other guidelines used in the development of this assessment may include the following: Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999b), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), and Health Effects Testing Guidelines (U.S. EPA 1996c; U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART/ETIC)
- TOXLINE
- Toxicology Literature from Special Sources (TOXLIT)
- CANCERLIT
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (via PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)

2.0 PHYSICAL AND CHEMICAL PROPERTIES

γ -Butyrolactone is a γ -lactone with synonyms of butyric acid lactone, 4-butyrolactone, 4-hydroxybutyric acid lactone, tetrahydro-2-furanone, and dihydro-2(3H)-furanone. γ -Butyrolactone has been marketed to the general public under names such as RenewTrient, Blue Nitro, and Bullet® (Higgins and Borron, 1996; Ingels et al., 2000; LoVecchio et al., 1998). It has the following structure, and physical and chemical properties listed in Table 1:



Table 1. The physical and chemical properties of γ -butyrolactone

Property	Data	Reference
Empirical Formula	C ₄ H ₆ O ₂	SRC, 2002
CAS#	96-48-0	SRC, 2002
Molecular Weight	86.09	SRC, 2002
Physical State and Color	Liquid Oily, colorless	Budavari, 1996 ChemFinder, 2002
Melting Point	-43.3 °C	SRC, 2002
Boiling Point	204 °C	SRC, 2002
Density	1.1441 g/mL	Budavari, 1996
Vapor Pressure	0.45 mm Hg at 25 °C	SRC, 2002
Water Solubility	≥10 g/100 mL at 13 °C	ChemFinder, 2002
Dissociation Constant (pK _a)	Not located	
n-Octanol/Water Partition Coefficient (log K _{ow})	-0.64 (experimental)	SRC, 2002
Henry's Law Constant (air/water partition)	5.27 E -008 atm-m ³ /mole @ 25 °C	SRC, 2002

2.1 Organoleptic Properties

The odor of γ -butyrolactone in air has been described as pleasant (Lewis, 1993), and as sweet and slightly buttery (Fenaroli's, 1995). According to the Flavor and Extract Manufacturers Association (FEMA), low molecular weight lactones like γ -butyrolactone usually have a sweet, herbaceous aroma and a caramel-like taste (Adams et al., 1998).

3.0 PRODUCTION AND USE

3.1 Production

γ -Butyrolactone is a synthetic chemical produced by the dehydrogenation of 1,4-butanediol, or by the hydrogenation of maleic anhydride to tetrahydrofuran and butyrolactone (Kirk-Othmer, 1985; Budavari, 1996). In the United States, γ -butyrolactone is considered a high production volume (HPV) chemical, meaning it is manufactured in or imported into the United States in amounts equal to or greater than one million pounds per year, and is listed on the "1990 HPV Challenge Program Chemical List" (U.S. EPA, 2001b). In addition, this compound is listed as a high production volume chemical in Europe (OECD, 2000). This indicates its annual production or import by at least one member country of the European Community is greater than 1,000 tons.

3.2 Use

γ -Butyrolactone is used as an intermediate in the synthesis of a number of chemicals, including polyvinylpyrrolidone, DL-methionine, piperidine, phenylbutyric acid, herbicides, and thiobutyric acids. It is also used as a solvent for polymers such as polyacrylonitrile, cellulose acetate, methyl methacrylate, and polystyrene, and can be a constituent of paint removers, textile aids, and drilling oils (Merck, 1996). It may also be used in the production of polymers intended as film formers in hair sprays, as blood plasma extenders, as clarifying agents in beer and wine, and as a solvent for nail polish remover and cyanoacrylate glue (HSDB, 2002).

The Flavor and Extract Manufacturers Association (Adams et al., 1998) lists γ -butyrolactone as a synthetic flavoring ingredient. Foods in which it may be used include baked goods at levels of about 20 ppm, in addition to cereals and other grains, milk and cheese products, fruit ices, meat products, soups, alcoholic and nonalcoholic beverages, gravies, and hard and soft candies, all at levels of about 10 ppm (Fenaroli's, 1995).

γ -Butyrolactone and its hydrolytic product, γ -hydroxybutyric acid, have been used in humans as anesthetic agents or anesthetic adjuvants due to their sedative-hypnotic effects. Both γ -butyrolactone and γ -hydroxybutyrate have been marketed as dietary supplements (U.S. Food and Drug Administration, 1999). In 2000, γ -butyrolactone became a List I chemical, subject to the criminal, civil, and administrative sanctions of the Controlled Substances Act (Federal Register, 2000a). Also in 2000, γ -hydroxybutyrate was made a Schedule I controlled substance (Federal Register, 2000b).

Since NSF is aware of only one water system product incorporating γ -butyrolactone, end use and extractant levels are specific to that application and, as such, are considered confidential.

4.0 ANALYTICAL METHODS

4.1 Analysis in Water

At NSF, γ -butyrolactone is analyzed in water by a method consisting of micro-extraction with methylene chloride followed by gas chromatography with flame ionization detection. The detection limit for this specific analysis is 0.1 mg/L. In addition, γ -butyrolactone can be detected in water by gas chromatography/mass spectrometry using U.S. EPA Method 625 (U.S. EPA, 2001c).

4.2 Analysis in Biological Matrices

Several analytical methods for the determination of γ -hydroxybutyrate have been reported, many of which involve converting γ -hydroxybutyrate to γ -butyrolactone by heating the sample in the presence of a strong mineral acid. Specifically, methods for the analysis of γ -hydroxybutyrate in plasma (van der Pol et al., 1975) and in plasma and urine (Ferrara et al., 1993) have been reported. The method used by van der Pol et al. (1975) involved centrifugation of the plasma with sulfuric acid and chloroform before analysis by gas chromatography with flame ionization detection. The method employed by Ferrara et al. (1993) consisted of heating deproteinized plasma or acidified urine samples to 80 °C for 20 minutes to convert γ -hydroxybutyric acid to γ -butyrolactone. Samples were cooled and their pH values adjusted to 6.5. A small volume of δ -valerolactone solution was added as an internal standard, and the samples were then extracted with benzene. Extracts were centrifuged and concentrated to their final volumes. Aliquots of these solutions were analyzed by gas chromatography/mass spectrometry.

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

γ -Butyrolactone is an ingredient in a variety of consumer products, from dietary supplements to nail polish, and can be accidentally or purposefully ingested in high quantities. Additionally, humans can ingest smaller amounts of γ -butyrolactone in some foods, due to either its natural occurrence or its presence as a food additive. It is possible the production and use of γ -butyrolactone can lead to its contamination in the environment. However, its chemical properties make it unlikely to adsorb to soil or sediment or to bioconcentrate in organisms.

5.1 Sources of Human Exposure

Exposure to γ -butyrolactone is essentially the same as exposure to γ -hydroxybutyrate, since γ -butyrolactone is rapidly converted *in vivo* to γ -hydroxybutyrate by lactonase found in liver and blood, with a half-life of less than one minute (Roth and Giarman, 1965). γ -Hydroxybutyrate is also normally present in small amounts in the human brain (Roth and Giarman, 1969), at levels ranging from 2 to 20 nmol/g of brain tissue, which translates to approximately 0.28 to 2.8 mg of γ -hydroxybutyrate in an average three-pound human brain (Doherty et al., 1978).

Human exposure to γ -butyrolactone and its metabolite γ -hydroxybutyrate can occur in a variety of ways. In the past, γ -hydroxybutyrate was used as an anesthetic. Presently, it has various medicinal purposes in European countries, including its use as a therapeutic agent in the

treatment of alcoholism and opiate addiction (Ferrara et al., 1999; Gallimberti et al., 1992; Gallimberti et al., 1993; Gallimberti et al., 1994). There are no currently approved medical uses for γ -hydroxybutyrate in the United States (Drug Enforcement Administration, 2001). Both γ -hydroxybutyrate and γ -butyrolactone have been marketed in health food stores and via the Internet as dietary supplements under various trade names (Higgins et al., 1996; Ingels et al., 2000; LoVecchio et al., 1998; U.S. FDA, 1999).

Human exposure to γ -butyrolactone may also occur as a result of its natural occurrence in food or its use as a food flavoring (Adams et al., 1998; Fenaroli's, 1995), as a result of the normal use of nail polish remover, cyanoacrylate glue, and other consumer products that contain γ -butyrolactone, or through occupational exposure (HSDB, 2002). It has been estimated that more than 25,000 workers are potentially exposed to γ -butyrolactone in the United States (HSDB, 2002). Occupational exposure to γ -butyrolactone may occur through inhalation and dermal contact.

It has been documented that γ -butyrolactone occurs naturally in more than 10 foods (Adams et al., 1998). Low molecular weight aliphatic lactones, such as γ -butyrolactone, are found naturally in berries, fruits, and alcoholic beverages at levels of less than 1 ppm (Adams et al., 1998). The annual volume of this compound as a flavoring in 1982 and 1987 was 15 kg and 508 kg, respectively, and the most recently reported per capita intake was 2 $\mu\text{g/kg-day}$ (Adams et al., 1998). Popcorn contains 22,000 $\mu\text{g/kg}$ γ -butyrolactone, and it has been reported at 0.4% in raw earth almonds, even after 60 minutes of roasting (HSDB, 2002). γ -Butyrolactone has also been identified in apple brandy (Loquet et al., 1981) and as a volatile constituent of coffee aroma (Gianturco et al., 1966). Drinking water has been reported to be a source of human exposure to γ -butyrolactone, but no quantitative results were given (HSDB, 2002). Drinking water is expected to be a minor source of exposure to γ -butyrolactone, and exposure as a result of NSF certified products (NSF/ANSI 60, 2002; NSF/ANSI 61, 2002) should be negligible, as this compound has only been detected in the leachate from one product.

5.2 Sources of Environmental Exposure

The natural occurrence of γ -butyrolactone is very low. Production of γ -butyrolactone and its industrial use as a chemical intermediate make its release to soil, water, and air possible. With a measured octanol/water partition coefficient ($\log K_{ow}$) value of -0.64 (SRC, 2002) and a high water solubility (ChemFinder, 2002), γ -butyrolactone is a fairly polar molecule. With an estimated soil adsorption coefficient (K_{oc}) of seven (HSDB, 2002), this compound is likely to mobilize quickly through soil. In water, γ -butyrolactone is not likely to adsorb to suspended solids or sediments based upon the low K_{oc} . Because of its hydrophilic properties and an estimated bioconcentration factor of 3.2 (HSDB, 2002), γ -butyrolactone is not expected to bioconcentrate in aquatic organisms. Based on its Henry's Law constant of $5.3 \times 10^{-8} \text{ atm-m}^3/\text{mole}$ (SRC, 2002), volatilization from water and moist soil surfaces is likely. In the ambient atmosphere it is expected to exist as a vapor due to its vapor pressure of 0.45 mm Hg at 25 °C (SRC, 2002). In the vapor state, γ -butyrolactone is likely degraded by reactions with photochemically produced hydroxyl radicals, with a half-life of reaction of approximately seven days (HSDB, 2002).

6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

γ -Butyrolactone is rapidly absorbed in the body, where it is hydrolyzed almost immediately to γ -hydroxybutyrate in blood and in liver. γ -Hydroxybutyrate, which is uniformly distributed in the body, is the biologically active metabolite of γ -butyrolactone. γ -Hydroxybutyrate is converted to succinate, which then enters the citric acid cycle.

6.1 Absorption

Palatini et al. (1993) examined the absorption of γ -hydroxybutyric acid in humans. Eight healthy male volunteers, aged 22 to 26 years, were administered single oral doses of 12.5, 25, or 50 mg/kg γ -hydroxybutyric acid dissolved in a black cherry syrup that was diluted with water, for a total fluid intake of 150 mL for all doses. Blood samples were collected just before dosing and at 10, 15, 20, 30, 45 minutes and at 1, 1.5, 2, 2.5, 3, 4, and 6 hours after dosing. The semilogarithmic plot of the plasma concentration-time data revealed biphasic decay. There was a significant increase in the dose-normalized area under the concentration-time curve as the dose levels increased. Increasing the dose also led to a significant increase in the time of peak plasma levels of γ -hydroxybutyric acid along with a decrease in dose-normalized peak plasma levels. The authors concluded that the absorption of γ -hydroxybutyric acid was capacity limited in this dose range.

Snead (1978) investigated the absorption of γ -hydroxybutyrate in four adult and two prepubescent rhesus monkeys. γ -Hydroxybutyrate was administered through a catheter placed in the saphenous vein, and levels in serum and cerebral spinal fluid were measured. The monkeys were observed for a period lasting from 120 to 240 minutes. Serum concentrations of γ -hydroxybutyrate peaked within the first 15 minutes after administration and ranged from 140 to 2,300 $\mu\text{g/mL}$.

Lettieri and Fung (1978) studied the absorption of γ -butyrolactone relative to γ -hydroxybutyrate. Groups of at least four male Sprague-Dawley rats were fasted for about 15 hours, and then administered 136 mg/kg γ -butyrolactone or 200 mg/kg γ -hydroxybutyrate, representing equimolar 1.58 mmole/kg doses of each compound. Administration to these groups was by both oral and intracardial routes. Other groups received 546 mg/kg γ -butyrolactone or 800 mg/kg γ -hydroxybutyrate, for an equimolar dose of 6.34 mmole/kg, both orally and intravenously. Blood was collected from the tail veins at various time intervals between dosing and eight hours. Each dosing group consisted of at least four animals, and no animal received more than a single dose. The time course of the plasma concentration (total of both compounds) resulting from an oral dose, given by gastric intubation to lightly anesthetized rats, was compared with the time course of the same dose given by intracardial injection.

The oral/intracardial area under the plasma concentration-time curve for γ -butyrolactone was 0.85, compared to 0.59 for γ -hydroxybutyrate, for the animals in the lower dose group. Oral γ -butyrolactone was more rapidly absorbed than oral γ -hydroxybutyrate. The peak plasma level after oral γ -butyrolactone administration was 350 $\mu\text{g/mL}$, whereas the peak plasma level following oral administration of γ -hydroxybutyrate at the same dose never exceeded 200 $\mu\text{g/mL}$.

The oral γ -butyrolactone dose gave results similar to results expected from an intravenous dose because the initial plasma sample exhibited the highest levels of γ -butyrolactone and γ -hydroxybutyrate, indicating rapid absorption. Similar results were seen in the higher dose group. The oral/intracardial area under the plasma concentration-time curve ratio for γ -butyrolactone was 0.96, compared with 0.52 for γ -hydroxybutyrate. Once again, this demonstrated the rapid absorption of γ -butyrolactone compared to the absorption of γ -hydroxybutyrate.

Fung et al. (1979) examined the percutaneous absorption of γ -butyrolactone in 14 male Sprague-Dawley rats. An undiluted dose of 6.34 mmol/kg (546 mg/kg) γ -butyrolactone, equivalent to about 800 mg/kg γ -hydroxybutyric acid, was applied to the shaved intact or depilated skin on a three cm² area of the abdomens of the rats. Plasma concentrations achieved by percutaneous absorption were compared with those achieved after the same dose given by intravenous injection. Peak plasma concentrations of γ -hydroxybutyric acid averaged 200 μ g/mL after the percutaneous dose on both the intact and depilated skin, and approximately 2,000 μ g/mL after the intravenous dose. Overall, approximately 10% of the percutaneous applied dose was absorbed.

6.2 Distribution

Palatini et al. (1993) examined the distribution of γ -hydroxybutyric acid in humans. Eight healthy male volunteers, aged 22 to 26 years, were administered single oral doses of 12.5, 25, or 50 mg/kg γ -hydroxybutyrate dissolved in a black cherry syrup that was diluted with water, for a total fluid intake of 150 mL for all doses. Blood samples were collected just before dosing and at 10, 15, 20, 30, 45 minutes and at 1, 1.5, 2, 2.5, 3, 4, and 6 hours after dosing. The plasma protein binding of γ -hydroxybutyric acid was determined. As the free fraction of γ -hydroxybutyric acid in plasma was almost one, it was concluded by the authors that γ -hydroxybutyric acid did not bind significantly to plasma protein.

Snead (1978) investigated the distribution of γ -hydroxybutyrate in four adult and two prepubescent rhesus monkeys. γ -Hydroxybutyrate was administered at doses ranging from 50 to 1,000 mg/kg through a catheter placed in the saphenous vein. Levels in serum and cerebral spinal fluid were measured. The monkeys were observed for a period lasting from 120 to 240 minutes. γ -Hydroxybutyrate levels in serum fell quickly during the first hour and declined more slowly after that. This pattern was similar in all animals at all dosages. The distribution of γ -hydroxybutyrate into the cerebral spinal fluid peaked at 180 minutes, with levels never exceeding the levels in serum. Peak levels in the cerebral spinal fluid were 20-50% of the serum levels.

Roth and Giarman (1966) examined the distribution of γ -hydroxybutyrate in specific regions of the cat brain. Each of 6 cats was administered 350 mg/kg γ -hydroxybutyrate via intravenous injection and was sacrificed 30 minutes later when observed to be asleep. Certain regions of the brain were dissected, tissue sections were ground and extracted with benzene, and the concentrations of γ -hydroxybutyrate in the extracts were determined by gas chromatography with flame ionization detection. Among the subcortical areas, the concentration of γ -hydroxybutyrate increased as the sections progressed up the brain stem from the cervical cord until a constant level was reached in the thalamus, hypothalamus, and caudate nucleus. The highest concentrations of γ -hydroxybutyrate were found in the cerebellum and the lower temporal lobe.

The authors suggested that exposure of the cerebellum may explain the effects on motor coordination, and effects in the temporal lobe may explain the prolonged amygdaloid and hippocampal seizure activity caused by γ -hydroxybutyrate.

Roth and Giarman (1966) investigated the distribution of γ -butyrolactone and γ -hydroxybutyrate in rats in an attempt to determine why γ -butyrolactone had a longer duration of action than γ -hydroxybutyrate, even though the latter is the biologically active form. They administered γ -butyrolactone and γ -hydroxybutyrate by intravenous injection to adult male rats, of an unspecified strain, in anesthetic doses. Even though the two compounds were administered in equimolar doses, the initial total blood levels of γ -butyrolactone and γ -hydroxybutyrate were about 50% lower after γ -butyrolactone administration than after γ -hydroxybutyrate administration. Additionally, it was noted that the total blood concentration fell more rapidly after γ -hydroxybutyrate administration than after γ -butyrolactone administration. To investigate this finding, the distribution of γ -butyrolactone and γ -hydroxybutyrate in muscle and fat was examined. As was observed in the blood, the total level of γ -butyrolactone and γ -hydroxybutyrate in muscle was higher after the intravenous administration of γ -butyrolactone than of γ -hydroxybutyrate. The total level of γ -butyrolactone and γ -hydroxybutyrate in muscle after γ -butyrolactone administration ranged from about 6×10^3 M immediately after dosing to about 1.2×10^3 M 120 minutes after dosing. The total level of γ -butyrolactone and γ -hydroxybutyrate in muscle after γ -hydroxybutyrate administration ranged from about 3.2×10^3 M immediately after dosing to about 5×10^2 M 120 minutes after dosing. However, there were no differences in the total concentrations in lipid tissue when each compound was administered intravenously. The total level of γ -butyrolactone and γ -hydroxybutyrate in lipid tissue was no greater than 1×10^3 M regardless of which compound was administered. The authors concluded that the lactonase in blood and liver hydrolyzed γ -butyrolactone so quickly that poorly perfused tissues, like lipids, received only small amounts of γ -butyrolactone after its administration.

Van der Pol et al. (1975) examined the distribution of γ -hydroxybutyrate in adult male Swiss mice and in pregnant mice, by the use of whole body autoradiography. Each mouse received an average dose of 0.1 μ Ci labeled γ -hydroxybutyrate per gram of body weight and a total dose of 400 mg/kg injected intravenously in 0.01 mL water per gram of mouse weight. In both experiments, frozen sections of the mice were prepared after sacrifice, and autoradiograms were recorded over a two-to-three week exposure period. Male mice were sacrificed at 1, 2, 5, 20, 30, 45, 60, 90, and 120 minutes and at 4, 8, 12, and 16 hours. The resulting autoradiograms of the male mice were indicative of a uniform distribution pattern, with the exception that, at short periods after dosing, lower levels of radioactivity were evident in fatty tissues, such as thymus, brown fat, and white and gray substances of the brain, than in other tissues. The concentration in the central nervous system was also lower initially. Within 30 minutes after dosing, the level in the brain was equivalent to that in other tissues. After 4 hours, the radioactivity was most concentrated in the liver and intestinal wall. Pregnant mice were sacrificed at 10, 30, and 60 minutes and at 2 and 4 hours. One hour after the radiolabeled γ -hydroxybutyrate was administered to the pregnant mice, comparatively high radioactivity levels were measured in the fetuses. After that, very high levels were observed in the periosteal layers of the bones in the fetuses.

Arena and Fung (1980) examined the qualitative relationship between *in vitro* transport and the previously reported *in vivo* absorption of sodium γ -hydroxybutyrate and γ -butyrolactone. Male Sprague-Dawley rats were sacrificed and an intestinal segment was taken from each and everted. This everted gut was placed in a test tube with the mucosal side exposed to physiological tromethamine buffer incorporating γ -hydroxybutyrate or γ -butyrolactone. γ -Butyrolactone had a higher cumulative mean intestinal transport rate, and a 5-10 times greater increase in intestinal flux with concentration than did γ -hydroxybutyrate. Capacity-limited transport and absorption of γ -butyrolactone was less evident than with γ -hydroxybutyrate. The authors concluded that the increased oral hypnotic activity of γ -butyrolactone over γ -hydroxybutyrate could be the result of its more favorable intestinal transport characteristics.

6.3 Metabolism

The metabolism of γ -butyrolactone has been thoroughly studied and is presented for reference in Figure 1. Early and general studies, with particular focus on effects in the brain, are summarized below. The weight of evidence indicates that γ -butyrolactone is hydrolyzed to γ -hydroxybutyrate, an endogenous component of the mammalian brain. γ -Hydroxybutyrate is further metabolized to succinate, which then enters the citric acid cycle and is eliminated from the body as carbon dioxide.

6.3.1 Evidence for the Hydrolysis of γ -Butyrolactone to γ -Hydroxybutyrate

Roth and Giarman (1965) reported that rat plasma and rat liver homogenate, from rats of unspecified strains, were capable of completely hydrolyzing 0.013 M γ -butyrolactone to γ -hydroxybutyrate *in vitro* within 30 minutes. The half-life of the hydrolysis of γ -butyrolactone to γ -hydroxybutyrate was less than one minute. Hemolyzed erythrocytes and rat brain homogenate did not catalyze the conversion. Kidney, heart, lung, skeletal muscle, intestine, and cerebrospinal fluid were also inactive. Since the hydrolysis was inactivated by heating the plasma to 70 °C, by incubation with 10^{-3} M EDTA, and did not occur in isotonic saline-phosphate buffer at pH 7.4, the presence of a plasma enzyme was suggested. The enzyme was presumed to be a lactonase rather than an esterase, because hydrolysis was not blocked by the acetylcholinesterase inhibitors diisopropylfluorophosphate, prostigmine, or physostigmine. Plasma of rabbits, guinea pigs, cats, and humans were also shown to have lactonase activity. Serum was shown to have higher lactonase hydrolyzing activity than plasma. In further studies of the hydrolysis of γ -butyrolactone by various tissues, Roth and Giarman (1966) found the hydrolysis of γ -butyrolactone in human serum to be 40 meq γ -butyrolactone/min/mL, with a linear reaction rate of about two minutes.

Fishbein and Bessman (1966) isolated γ -lactonase from the microsomal fraction of rat liver, and used selected substrates and enzyme inhibitors to differentiate γ -lactonase, β -esterase purified from rat liver microsomes, and plasma cholinesterase. No lactonase activity was found in homogenates of rat brain, spleen, kidney, heart, or diaphragm, but rat serum, plasma, and liver were shown to contain the enzyme. The γ -lactonase enzymes catalyze lactonization of the corresponding γ -hydroxy acids. In this case, the γ -lactonase can hydrolyze γ -butyrolactone to γ -hydroxybutyrate.

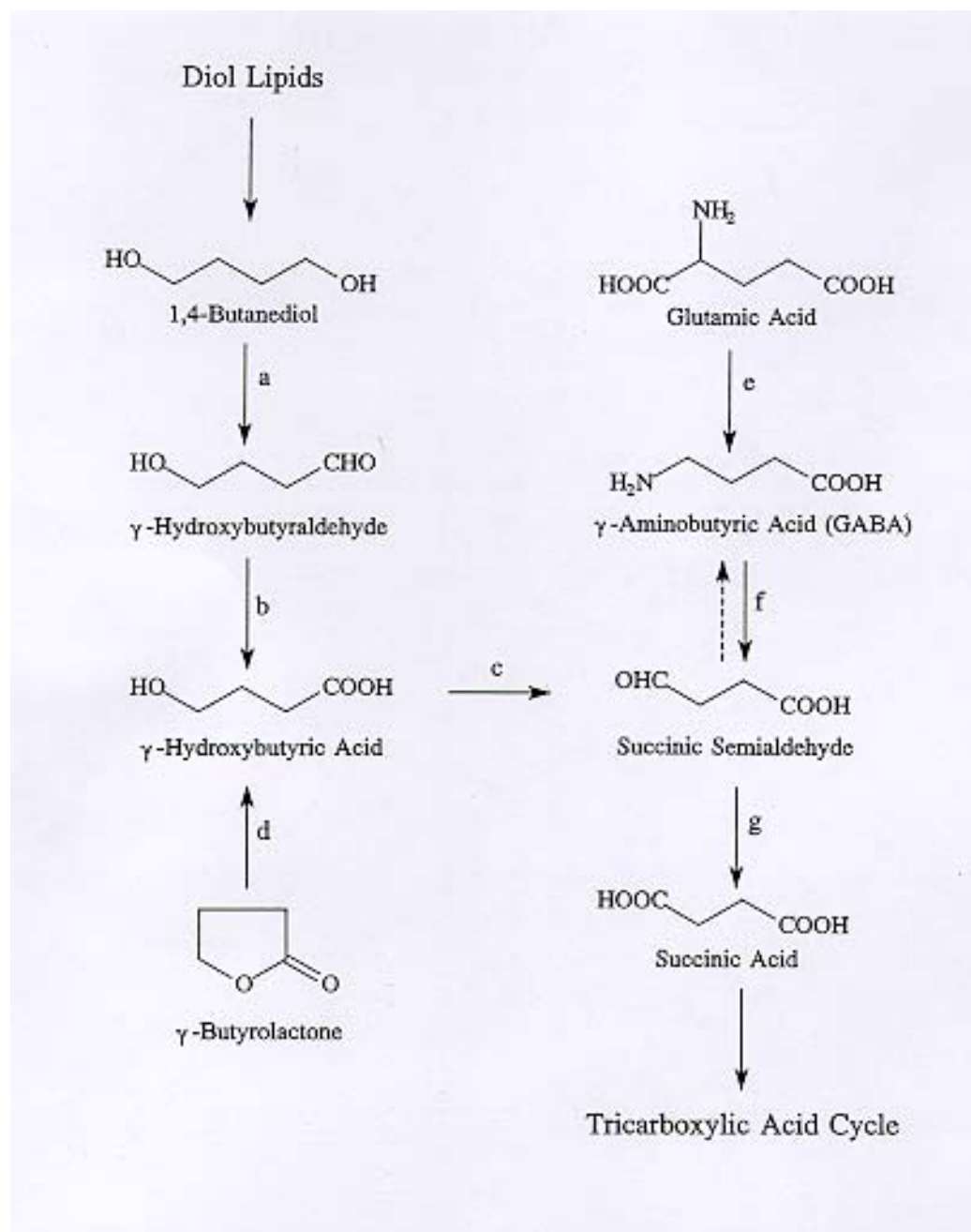


Figure 1. Metabolism of γ -butyrolactone (from NTP, 1996)

Key enzymes: a = alcohol dehydrogenase, b = aldehyde dehydrogenase, c = γ -hydroxybutyric acid dehydrogenase, d = lactonase, e = glutamic acid decarboxylase, f = GABA transaminase, g = succinic semialdehyde dehydrogenase.

6.3.2 Evidence for the Metabolism of γ -Hydroxybutyrate in the Citric Acid Cycle

Roth and Giarman (1966) examined the metabolism of γ -butyrolactone and γ -hydroxybutyrate in rats. Isotopically labeled γ -hydroxybutyrate was used to determine its *in vivo* and *in vitro* metabolism. For the *in vivo* experiments, rats of an unspecified strain were administered intravenous 1- ^{14}C - γ -hydroxybutyrate and 1- ^{14}C - γ -butyrolactone. Following intravenous γ -hydroxybutyrate administration, there were detectable levels of radiolabeled carbon dioxide in expired air within four minutes, with a peak concentration in about 15 minutes. About 60% of the total radioactivity administered was recovered as radiolabeled carbon dioxide in the expired air within 2.5 hours. Comparable results were obtained after intravenous γ -butyrolactone administration, even though the radiolabeled carbon dioxide evolved at a slower rate, with the maximum reached in about 20 minutes. The authors concluded this slower rate of metabolism for γ -butyrolactone versus γ -hydroxybutyrate was due to the added hydrolysis step needed to convert γ -butyrolactone to γ -hydroxybutyrate.

Additionally, Roth and Giarman (1966) conducted *in vitro* tests to determine whether brain and liver slices could metabolize γ -hydroxybutyrate to carbon dioxide. Rat brain and liver slices were incubated with varying levels of radiolabeled γ -hydroxybutyrate. Both the brain and the liver metabolized this compound very quickly to carbon dioxide, with liver metabolizing γ -hydroxybutyrate about twice as fast as the brain.

Fishbein and Bessman (1964) demonstrated that succinic semialdehyde (Figure 1) could be reduced to γ -hydroxybutyrate by lactic dehydrogenase and that γ -hydroxybutyrate could be oxidized to succinic semialdehyde in rat brain tissue. Succinic semialdehyde has been demonstrated to be an intermediate in the metabolism of γ -aminobutyrate in the brain. Succinic semialdehyde is also converted to succinate, which can enter the citric acid cycle. This provided evidence for a metabolic mechanism for both the endogenous formation of γ -hydroxybutyrate and the metabolism of γ -hydroxybutyrate via the citric acid cycle.

Further metabolic studies with γ -hydroxybutyric acid in rat brain were conducted by Doherty et al. (1975). Radiolabeled γ -hydroxybutyric acid and succinic acid were injected into the lateral ventricle of anesthetized male albino rats of an unspecified strain. The rate of disappearance of radiolabeled γ -hydroxybutyric acid was measured. The total radioactivity remaining in the brain after γ -hydroxybutyric acid injection decreased rapidly with time, with half of the radioisotope removed in less than five minutes. Amino acid analysis was conducted to compare the metabolites of γ -hydroxybutyric acid and succinic acid in the brain. Similar patterns of elution of ^{14}C -labeled compounds were observed in the resulting chromatogram, indicating a similar metabolism. All of the peaks were identified with the expected elution pattern of citric acid cycle intermediates. The extent of γ -hydroxybutyric acid metabolism was confirmed by three different analytical methods that indicated that oxidation to succinic acid was the principal pathway for γ -hydroxybutyric acid metabolism in the rat brain. These authors agreed that γ -aminobutyric acid was produced from γ -hydroxybutyric acid primarily through the citric acid cycle.

In a subsequent study, Doherty and Roth (1978) showed that the brains of rats dosed *in vivo* could metabolize [1- ^{14}C]- γ -hydroxybutyric acid to succinate and amino acids having the isotope in the carboxylic acid group adjacent to the α -amino group, suggesting that the label did not enter the citric acid cycle as acetate. The ratio of radiolabeled glutamine to glutamic acid of 4.2 for [1- ^{14}C]- γ -hydroxybutyric acid and of 7.71 for [1- ^{14}C]succinate suggested that the metabolism of γ -hydroxybutyric acid occurred in the compartment associated with glial cells and synaptosomal structures.

6.3.3 Alternate Metabolic Pathways

A β -oxidation metabolic scheme for γ -butyrolactone has been studied by several researchers. While this is not considered the likely metabolic pathway for this compound, a few of these studies were reviewed for this risk assessment. This alternate metabolic pathway was proposed by Walkenstein et al. (1964). Radiorespirometric tests were performed on rats of an unspecified strain dosed with ^{14}C -labeled γ -hydroxybutyric acid. These experiments showed rapid conversion of the labeled γ -hydroxybutyric acid to carbon dioxide. Repeated trials were performed in order to isolate ^{14}C -labeled succinate from the urine of rats dosed with the labeled γ -hydroxybutyric acid. Although succinate was found excreted in the urine, very little was radioactive. Therefore, these researchers proposed an alternate hypothesis for the breakdown of γ -hydroxybutyric acid, with β -oxidation as the first step.

Lee (1977) further examined the possible β -oxidation metabolic pathway of γ -hydroxybutyrate. One of the intermediates of the supposed β -oxidation pathway of γ -hydroxybutyrate is 3,4-dihydroxybutyrate, which is present in human urine and is a potential metabolite of complex carbohydrates. When adult humans ingested one gram of γ -butyrolactone, the concentration of 3,4-dihydroxybutyrate in their urine increased considerably. This 3,4-dihydroxybutyrate was assumed to be from the γ -hydroxybutyrate because it had the same S-configuration. Other excreted compounds included glycolate and the hydroxyepoxide tautomer of 4-hydroxy-3-oxobutyrate. Although these results indicated that γ -hydroxybutyrate was likely to be metabolized by β -oxidation in humans, two other postulated intermediates of the β -oxidation metabolic pathway, trans-4-hydroxy-2,3-butenate and R-3,4-dihydroxybutyrate were not detected.

Doherty et al. (1975) indicated that the β -oxidation pathway described by Walkenstein et al. (1964) was not the primary metabolic pathway of γ -hydroxybutyrate. When the succinate metabolism inhibitor malonate was administered along with succinate and γ -hydroxybutyrate to rat brain tissue, there was a reduction in the overall metabolism of succinate and of γ -hydroxybutyrate, as shown by a decrease in the rate of amino acid formation. The authors concluded that if the β -oxidation pathway were active, then the production of the labeled amino acids would have occurred at their normal rate in the presence of malonate.

Roth and Giarman (1966) investigated the potential for a decrease in the metabolism of uniformly ^{14}C -labeled glucose by rat brain tissue, in the event γ -hydroxybutyrate blocked metabolism in the citric acid cycle through the formation of glyoxylate, as suggested by Walkenstein et al. (1964) in their β -oxidation scheme. The amount of labeled carbon dioxide in

the brain slices was examined in both the presence and absence of γ -hydroxybutyrate. In its presence, there was only a small decrease in the metabolism of the labeled glucose to carbon dioxide. Even with potassium-stimulated cortical brain slices, these experiments were unable to help explain the mechanism of central depression of γ -hydroxybutyrate. Additionally, this study examined the alteration of γ -hydroxybutyrate metabolism by δ -hydroxybutyrate, a structurally similar compound. It appeared that δ -hydroxybutyrate was actually stimulating the metabolism of these chemicals, since levels of γ -hydroxybutyrate in brain and blood decreased approximately two-fold. However, *in vitro* experiments did indicate that δ -hydroxybutyrate might have an inhibitory effect on the metabolism of γ -hydroxybutyrate (Roth and Giarman, 1966).

Mohler et al. (1976) examined the fate of [$1-^{14}\text{C}$] γ -hydroxybutyrate in mouse brain. Male albino CFW strain mice were injected intravenously with a trace amount of [$1-^{14}\text{C}$] γ -hydroxybutyrate. Cerebral uptake of the labeled γ -hydroxybutyrate occurred rapidly, with 91% recovered in the brain within two minutes. It degraded thereafter, with a half-life of approximately five minutes. Degradation of the labeled γ -hydroxybutyrate seemed to take place in the brain, as ^{14}C was incorporated into amino acids associated with the citric acid cycle. Therefore, β -oxidation of γ -hydroxybutyrate did not appear to be the applicable metabolic pathway. Conversion of [^{14}C] γ -hydroxybutyrate into [^{14}C] γ -aminobutyric acid before the oxidation step was minimal, indicating that the pharmacological action of γ -hydroxybutyrate was not likely to be mediated through the formation of γ -aminobutyrate.

6.3.4 Relationship of γ -Hydroxybutyrate to γ -Aminobutyrate

The effects of anesthetic doses of γ -hydroxybutyric acid, alone and in combination with insulin, on the concentrations of γ -aminobutyric acid, glutamic acid, aspartic acid, and glutamine in rat brain, were studied by Margolis (1969). When γ -hydroxybutyric acid was administered intraperitoneally at 1 g/kg, there was no change in brain level of γ -aminobutyric acid, glutamic acid, or glutamine. There was a significant increase in the concentration of aspartic acid. As insulin also leads to an increase in the level of aspartic acid in the brain, the authors speculated that insulin and γ -hydroxybutyric acid shared a common biochemical mechanism affecting aspartic acid metabolism.

Vayer et al. (1985) examined the conversion of γ -hydroxybutyric acid to γ -aminobutyric acid *in vitro* and provided evidence that it was mediated by transamination. The metabolism of radiolabeled γ -hydroxybutyric acid via the citric acid cycle would involve the loss of its radioactivity prior to entry into the γ -aminobutyric acid shunt. In this study, incubation of rat brain tissue with radiolabeled γ -hydroxybutyric acid resulted in a single radioactive compound, identified as γ -aminobutyric acid. Also, when the enzyme succinic dehydrogenase was inhibited, radioactive γ -aminobutyric acid was observed even though radioactive succinate could not be detected. This implied that the formation of γ -aminobutyric acid would not occur through succinate via the citric acid cycle. In addition, when γ -aminobutyric acid transaminase was inhibited, there was a significant decrease in the conversion of radioactive γ -hydroxybutyric acid to γ -aminobutyric acid. This study indicated that the degradation of γ -hydroxybutyric acid occurred through a transamination mechanism to γ -aminobutyric acid.

Roth and Giarman (1969) examined whether γ -aminobutyric acid could act as a precursor of γ -hydroxybutyric acid in the rat brain. Thus, ^3H - γ -aminobutyrate was administered intracisternally into rat brain. Rats were sacrificed 20 minutes later, and γ -butyrolactone was extracted and determined by gas chromatography. About 16% of the administered radioactivity was located in the brain after 20 minutes. However, radioactivity was also found in the brains of the control rats. It was concluded that rat brain is capable of converting ^3H - γ -aminobutyrate to γ -hydroxybutyric acid, even though this pathway does not seem to be very active under normal conditions.

6.4 Elimination/Excretion

Palatini et al. (1993) examined the elimination of γ -hydroxybutyric acid in humans. Eight healthy male volunteers, aged 22 to 26 years, were given single oral doses of 12.5, 25, or 50 mg/kg γ -hydroxybutyrate dissolved in black cherry syrup diluted with water, for a total fluid intake of 150 mL for all doses. Blood samples were collected through an indwelling catheter into heparinized plastic tubes just prior to dosing and at 10, 15, 20, 30, 45 minutes and at 1, 1.5, 2, 2.5, 3, 4, and 6 hours after dosing. The semilogarithmic plot of concentration-time data revealed a biphasic decay phase, concluded by the authors to be typical of chemicals with non-linear elimination kinetics.

Studies by Roth and Giarman (1966) indicated that γ -butyrolactone and γ -hydroxybutyrate were rapidly eliminated in rats. Rats administered intravenous radiolabeled γ -hydroxybutyrate and γ -butyrolactone had detectable levels of radiolabeled carbon dioxide within four minutes, and approximately 60% of the radioactivity had been recovered in expired air as carbon dioxide within 2.5 hours.

Lettieri and Fung (1978) examined the elimination rate of γ -butyrolactone relative to γ -hydroxybutyrate. As described in the absorption study above, male Sprague-Dawley rats were fasted for about 15 hours, and then administered 136 mg/kg γ -butyrolactone or 200 mg/kg γ -hydroxybutyrate, representing an equimolar dose of 1.58 mmole/kg for each compound, via both oral and intracardial administration. Blood was collected from the tail vein at various time intervals, between dosing and eight hours. γ -Butyrolactone had a slower initial rate of elimination than γ -hydroxybutyrate. However, after about an hour, the elimination rates of the two compounds became more similar. Similar results were seen after 6.34 mmole/kg equimolar doses (546 mg/kg γ -butyrolactone or 800 mg/kg γ -hydroxybutyrate) were given orally or by intravenous injection.

Van der Pol et al. (1975) examined the elimination of γ -hydroxybutyrate in three adult male dogs and one adult female dog of different breeds and weights in two sets of experiments. In the first experiment, two dogs were given γ -hydroxybutyrate intravenously using a dual infusion pump for the simultaneous infusion of 18.4 and 26.1 mg sodium γ -hydroxybutyrate per minute in the first and second dogs, respectively, for 118 minutes. In the second experiment, two dogs were dosed with radiolabeled sodium γ -hydroxybutyrate at rates of 44.7 mg/min for 105 minutes and 62.0 mg/min for 120 minutes, through infusion with a pulsating pump into the saphenous vein. Urine was collected and γ -hydroxybutyrate levels were determined in the first experiment. A

steady state of elimination was not reached during the period of infusion, and the erratic data could not be fitted to a curve. Sampling was conducted for up to 332 minutes, when γ -hydroxybutyrate could no longer be detected in the urine. The results of the first experiment indicated that the elimination of γ -hydroxybutyrate did not follow first-order kinetics. The authors concluded that the nonlinear concentration to time course relationship was due to the limited enzyme capacity of the metabolic system. The results of the second experiment confirmed that conclusion.

7.0 EFFECTS ON HUMANS

The available literature on the effects of γ -butyrolactone on humans consists of neurotoxicological studies with γ -hydroxybutyrate, case reports of accidental or intentional ingestion of γ -hydroxybutyrate or γ -butyrolactone, studies involving the therapeutic use of γ -hydroxybutyrate in the treatment of various diseases and disorders, including heroin addiction and alcoholism, and studies regarding the historical use of γ -hydroxybutyrate and γ -butyrolactone in anesthesia. The data indicate that γ -hydroxybutyrate has adverse effects on human neurophysiology and behavior, at single doses as low as 12.5 mg/kg. The case reports summarized below indicate that both γ -butyrolactone and γ -hydroxybutyrate can cause neurotoxicity, with secondary effects of decreased respiratory and heart rates, hypothermia, short-term memory loss, seizures, coma, and death.

7.1 Neurotoxicity Studies

Metcalf et al. (1966) dosed twenty adult male volunteers with single oral doses, in a palatable vehicle, of the sodium salt of γ -hydroxybutyrate in the range of 35 to 63 mg/kg. An eight-channel electroencephalograph with 19 scalp electrodes was used to record the encephalograms. A battery of sensorial tests including orientation and recall, coordination, and examination of eyes for movements at rest, pupillary size, nystagmus on lateral gaze, and corneal reflexes, were conducted to detect alterations of consciousness.

All subjects reported drowsiness during the experimental sessions. Those dosed with γ -hydroxybutyrate showed no deficits in recall or attention span, although there was slurring of speech in one subject who received a 50 mg/kg dose. Minimal pupillary constriction was noted at doses of 40 to 50 mg/kg. Doses greater than 50 mg/kg resulted in unconsciousness in an unstated number of volunteers, consisting of a generalized hypotonia, or decreased muscle tone. In one subject the hypotonia was led by extensor rigidity of the lower extremities as well as an increased tonus of the upper extremities and the neck. The electroencephalographic pattern was consistent at the higher doses and was similar to that observed in slow-wave sleep, with an abrupt transition from an awake alpha wave pattern to theta and delta wave slowing, indicative of drowsiness and slow-wave sleep states. During this time, the subject dosed with greater than 50 mg/kg γ -hydroxybutyrate became unresponsive to sensory stimulation.

Under normal conditions, no alpha rhythms would be observed in unconscious subjects and theta and delta waves would not be observed in fully awake subjects. In fact, theta and delta slowing during wakefulness represents a sign of brain dysfunction. This study showed that humans administered 40 to 50 mg/kg γ -hydroxybutyrate exhibited the abnormal electroencephalographic

patterns of alpha rhythms when unconscious and brief theta and delta slowing when awake. Therefore, this study demonstrated abnormal neurophysiological effects in humans administered γ -hydroxybutyrate. The authors did not report a LOAEL or a NOAEL.

A double-blind, four-way cross-over behavioral study was conducted by Ferrara, et al. (1999). The primary purpose of this study was to understand the potential effects of γ -hydroxybutyrate on human psychomotor performance and on subjective feelings that are necessary for skilled activity. Twelve volunteers, six males and six females, aged 22 to 36 years, were administered γ -hydroxybutyrate, lorazepam, and placebo according to a single-dose, double-blind, balanced, four-way, cross-over design. Single oral doses of placebo, 0.03 mg/kg lorazepam (a reference sedative), and γ -hydroxybutyrate at 12.5 mg/kg or 25 mg/kg were administered, separated by a wash-out period of one week. These dose levels were chosen based on the therapeutic doses of γ -hydroxybutyrate in the treatment of alcohol dependence, which range from 12.5 to 50 mg/kg. The psychomotor performance of the subjects was measured prior to treatment and at 15, 60, 120, and 180 minutes after treatment, while their mood was assessed before treatment and 120 minutes post-treatment.

The following tests were conducted on the volunteers. The Critical Fusion Frequency test is an objective test that measures a novel combination of the psychophysical Methods of Limits and Successive Approximations in a computer-controlled system (Ramaekers et al., 1992). The subject is seated looking into a visual tunnel that displays a white light source. The computer alternatively increases and decreases the source flicker frequency, and the subject responds by pressing separate buttons whenever perception changes from one state to another. After three cycles, the approximate location of the subject's critical fusion frequency is defined. The program then identifies three frequencies in 1 Hz steps above and below the supposed threshold. Each of these six stimuli is displayed five times in separate, randomized presentations. The subject is supposed to give one of two responses indicating the perception of flicker or fusion, after the presentation period. The proportions of each type of response are used to calculate intersecting linear functions in the frequency domain, and the equal probability point where the functions intersect defines the Critical Fusion Frequency.

The Critical Tracking Test measures the person's ability to control a displayed error signal with a joystick in a first-order compensatory tracking test (Ramaekers et al., 1992). Error is shown as the horizontal deviation of a cursor from the midpoint of a linear scale. The cursor's deviations increase in velocity as the task proceeds, increasing the frequency of the subject's compensatory responses with an increasing phase lag. The subject loses control when the compensatory response lags the last movement of the cursor by 180°, at which point the response frequency is defined as the "critical frequency."

The Response Competition Task (Eriksen and Eriksen, 1974; Ramaekers et al., 1992) measures the speed at which the subject reacts to the appearance of any one of four possible target letters (C, S, K, and H) on the screen of a personal computer. Two of the letters required the subject to press a red button, and the other two letters required the subject to press a green button. The target letter was either alone or flanked on each side by pairs of other letters on separate trials. The delay in responding is caused by the added requirement to detect the difference of the

flanking letters and inhibit the competing response. The dependent variable in this task was the number of errors.

The Choice Reaction Time test is based on the memory scanning processes of Sternberg (1969). In this test, the subject is shown a set of four letters on a computer screen and memorizes them. Series of 60 separate letters are presented after each memory set at intervals of one second. After each letter, the subject determines whether or not the presented letter was in the memory set and responds using the suitable buttons. The dependent variables in this task were the mean reaction times for “yes” and “no” responses and frequencies of incorrect responses after each memory set.

The Visual Vigilance Task (Nuechterlein, 1983) is a fast paced visual discrimination test in which a digit appears on the screen of a computer for 35 ms every half second. Stimuli are letters, with the letter “O” being the target letter. The dependent variable in this task takes into account values of both correct and false detections.

The subjective assessment included the use of an “Adverse Events Form” in which the subjects reported the incidence and severity of possible side effects, including drowsiness, weakness, headache, fatigue, nervousness, nausea, dizziness, memory disturbances, and dullness. Additionally, the subject’s mood was assessed according to a mood scale developed by Bond and Lader (1974). In this assessment, subjects were given 16 scales of 100 mm, onto which they were to rate their moods. The scales were scored by measuring from the end of the line to the subject’s mark, and the 16 scores for each subject were punched onto cards. The frequency distribution of each scale was computed and plotted. From this, three statistically independent scores for measuring mood and respective alertness, calmness, and contentedness scales were created.

Doses of 12.5 or 25 mg/kg γ -hydroxybutyrate had no affect on psychomotor activity when compared to placebo results. No changes were observed in attention, alertness, or short-term memory when compared to placebo results. However, changes in mood were observed at both doses of γ -hydroxybutyrate as contentedness decreased significantly at both doses ($P < 0.05$) while significant anxiety-reducing effects were observed at the lower dose ($P < 0.05$). Further, within 20 minutes, dizziness or a sense of dullness were reported by 66% of subjects treated with 25 mg/kg γ -hydroxybutyrate and 50% of subjects treated with 12.5 mg/kg γ -hydroxybutyrate. These symptoms faded within 30 to 60 minutes. The authors did not report a LOAEL or a NOAEL.

Results of human neurotoxicity studies are summarized in Table 2.

Table 2. Summary of the neurotoxic effects of γ -hydroxybutyrate on humans

Study	Oral Dose, mg/kg γ -hydroxybutyrate	Primary Adverse Effects
Ferrara et al., 1999	12.5	Decrease in contentedness, calmness, and dizziness or a sense of dullness in 50% of subjects
	25	Decrease in contentedness, and dizziness or a sense of dullness in 66% of subjects
Metcalf et al., 1966	35	Drowsiness
	40 - 50	Minimal pupillary constriction
	50	Slurring of speech
	> 50 - 63	Unconsciousness with a generalized hypotonia

7.2 Case Reports

Descriptions of human consumption of γ -butyrolactone have been reported. This compound was formerly available to the general population and was available via the Internet and in many health food stores. As discussed above, γ -butyrolactone is readily metabolized in the body to γ -hydroxybutyrate, which was controlled some time ago, resulting in increased popularity of γ -butyrolactone in products until they were also controlled (Federal Register, 2000a; Federal Register, 2000b). Table 3 summarizes a number of case reports involving human exposure to γ -butyrolactone and γ -hydroxybutyrate.

Table 3. Summary of case studies for γ -butyrolactone (GBL) and γ -hydroxybutyrate (GHB)

Subject	Dose	Key Effect	Treatment	Reference
21-year-old male	Ingested substance containing GBL in addition to ethanol; blood GHB concentration was 291 mg/L and blood ethanol level was 0.1%.	Death, mechanism of which appeared to be severe respiratory depression.	None reported	Davis, 1999
26-year-old female	Ingested substance containing GBL in addition to ethanol; blood GHB concentration was 721 mg/L and blood ethanol level was 0.17%.	Death, mechanism of which appeared to be severe respiratory depression.	None reported	Davis, 1999
2-year-old male	Ingested about one ounce of a solvent composed of 100% GBL; dose $\cong$ 3,000 mg/kg based on a 10 kg child	Became unconscious with a Glasgow Coma Scale score of 3. His breathing was agonal, and he became apneic in a short time. His body temperature dropped to 36.3 °C (97.3 °F) and pupils were 2-3 mm, equal, and sluggishly reactive. He recovered about 6 hours after admission to the hospital.	He was intubated and given 0.2 mg intravenous atropine. He was also given 2 mg of intravenous dexamethasone every 6 hours for 24 hours and was released the next day.	Higgins and Borron, 1996
27-year-old male	Ingested 100 mL of a nail polish remover that contained 71% GBL; dose was $\cong$ 1,000 mg/kg based on a 70 kg adult	About 45 minutes after ingestion, experienced respiratory depression and became comatose with a Glasgow Coma Scale score of 3. His pupils were not reactive to light. He awoke confused and agitated five hours after ingestion. He recovered nine hours after ingestion, with no memory of what occurred the previous day.	He was intubated and mechanical breathing was started without sedative drugs. He was given 50 g activated charcoal, which he vomited. He was extubated five hours later, upon waking, and given droperidol. Two hours later he had another episode of agitation and was given sultopride as a sedative.	Rambourg-Schepens et al., 1997

Table 3. Summary of case studies for γ -butyrolactone (GBL) and γ -hydroxybutyrate (GHB)

Subject	Dose	Key Effect	Treatment	Reference
14-month-old male	Ingested a small amount of a liquid, possibly a nail polish remover that contained 71% GBL	Quickly became cyanotic and hypertonic, then comatose, with a Glasgow Coma Scale score of 7. He was limp and responsive to painful stimuli, and his pupils were contracted and unreactive. He awoke five hours after ingestion and showed signs of confusion. He completely recovered eight hours after ingestion.	Vomiting was induced before being admitted to the hospital. He was intubated and was intravenously administered 2 mg dexamethasone.	Rambourg-Schepens et al., 1997
36-year-old male	Ingested 2 ounces or 59 mL of a dietary supplement containing GBL	Lethargy, perspiring, vomiting, driving erratically when found 30 minutes after ingestion. His mental state returned to normal about 1.5 hours after ingestion.	None reported	LoVecchio et al., 1998
24-year-old male	Ingested 3-4 ounces of a substance that contained 1.82 g GBL per fluid ounce; dose was $\cong$ 100 mg/kg GBL based on a 70 kg adult consuming 4 ounces	Shortly after ingestion, he vomited and had seizures. His behavior alternated between extreme agitation and calm. He soon became comatose, with a Glasgow Coma Scale score of 7. His skin was warm, flushed, and perspiring; he had bradycardia; and his body temperature was 34.9 °C (94.8 °F). He recovered after about eight hours, with no memory of what occurred after he ingested the solution.	Intubation	CDC, 1999
46-year-old female	Ingested about 2.7 ounces of a substance containing 1.82 g GBL per fluid ounce, in addition to ethanol; dose was $\cong$ 70 mg/kg GBL based on a 70 kg adult	She had a seizure and became unconscious with severe respiratory depression. She had bradycardia, a body temperature of 35.6 °C (96.1 °F), and contracted pupils. She had recovered by the next day with no memory of what had occurred.	Mechanical ventilation	CDC, 1999