National Toxicology Program
Toxicity Report Series
Number 54

NTP Summary Report
on the Metabolism, Disposition,
and Toxicity of

1,4-Butanediol

(CAS No. 110-63-4)

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Post Office Box 12233
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NIH Publication 96-3932
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United States Department of Health and Human Services
Public Health Service
National Institutes of Health

NOTE TO THE READER

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

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CONTRIBUTORS

This summary NTP report on the metabolism, disposition, and toxicity studies of 1,4-butanediol is based partially on studies that took place from December 1988 through February 1989.

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PEER REVIEW

The draft summary report on the metabolism, disposition, and toxicity of 1,4-butanediol was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that this summary report of the metabolism, disposition, and toxicity studies presents the experimental results and conclusions fully and clearly. The comments of the reviewers were received and reviewed prior to the finalization of this document. Changes have been made such that the concerns of the reviewers have been addressed to the extent possible.

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$HO\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}OH$

1,4-BUTANEDIOL

CAS No. 110-63-4

Chemical Formula: $C_4H_{10}O_2$ Molecular Weight: 90.14

Synonyms: Butanediol, butane-1,4-diol, 1,4-butylene glycol, 1,4-dihydroxybutane, 1,4-tetramethylene glycol, butylene glycol, tetramethylene 1,4-diol
Trade names: Diol 14B, Sucol B

ABSTRACT

1,4-Butanediol is an industrial chemical used in the manufacture of other organic chemicals. It was nominated by the National Cancer Institute and selected for evaluation by the NTP because of high production volume, the potential for worker exposure, the lack of adequate toxicological characterization, and the lack of evaluation for carcinogenic potential.

As documented in the scientific literature, 1,4-butanediol is rapidly absorbed and metabolized to γ-hydroxybutyric acid in animals and humans. A metabolism and disposition study conducted in F344/N rats by the NTP confirmed the rapid and extensive conversion of 1-[^{14}C]-1,4-butanediol to $^{14}CO_2$. Because of this rapid and extensive conversion, the toxicological profile of 1,4-butanediol reflects that of γ-hydroxybutyric acid. γ-Hydroxybutyric acid is a naturally occurring chemical found in the brain and peripheral tissues which is converted to succinate and processed through the tricarboxylic acid cycle. Although the function of γ-hydroxybutyric acid in peripheral tissues is unknown, in the brain and neuronal tissue it is thought to function as a neuromodulator. γ-Hydroxybutyric acid readily crosses the blood-brain barrier, and oral, intraperitoneal, or intravenous administration elicits characteristic neuropharmacologic responses. These same responses are observed after administration of 1,4-butanediol.

The lactone of γ-hydroxybutyric acid, γ-butyrolactone, is also rapidly converted to γ-hydroxybutyric acid by enzymes in the blood and liver of animals and humans. γ-Butyrolactone was previously evaluated by the NTP in 14-day and 13-week toxicology studies and 2-year toxicology and carcinogenesis studies in F344/N rats and B6C3F$_1$ mice. No organ-specific toxicity occurred in the toxicology studies. In the carcinogenesis studies, an equivocal response occurred in male mice, based on a marginal increase in the

incidence of pheochromocytomas of the renal medulla. Because of the rapid and extensive conversion of γ-butyrolactone to γ-hydroxybutyric acid, the evaluation of γ-butyrolactone was in fact an evaluation of γ- hydroxybutyric acid.

This summary report presents a review of the current literature which documents that both 1,4-butanediol and γ-butyrolactone are rapidly metabolized to γ-hydroxybutyric acid, and the pharmacologic and toxicologic responses to these chemicals are due to their metabolic conversion to γ-hydroxybutyric acid. Because the toxicity and carcinogenicity of γ-hydroxybutyric acid was fully evaluated in the NTP studies of γ-butyrolactone, and a lack of organ-specific toxicity or carcinogenic potential was demonstrated, it is concluded that there is a high likelihood that 1,4-butanediol would be negative in a similar set of studies. For these reasons, it is the opinion of the NTP that 1,4-butanediol should be considered not carcinogenic in animals and no further evaluation of 1,4-butanediol is needed at this time.

CHEMICAL AND PHYSICAL PROPERTIES

1,4-Butanediol is a colorless, viscous liquid with a molecular weight of 90.14, a density of 1.0154 at 25 ° C, and a boiling point of 228 ° C (Lewis, 1991). It is soluble in water, dimethyl sulfoxide, acetone, and 95% ethanol.

PRODUCTION, USE, AND HUMAN EXPOSURE

Industrial synthesis of 1,4-butanediol is accomplished in three steps. In the first step, formaldehyde and acetylene are condensed in the presence of a catalyst to produce 1,4-butynediol. This acetylenediol intermediate is then catalytically reduced to 1,4-butanediol in a reactor containing a fixed bed catalyst. In the last step, 1,4-butanediol is refined by column distillation (*Kirk-Othmer*, 1978; HSDB, 1994).

$$2\ H_2C{=}O + HC{\equiv}CH \rightarrow HO\text{-}CH_2\text{-}C{\equiv}C\text{-}CH_2\text{-}OH \rightarrow HO\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}OH$$

1,4-Butanediol is available in grades ranging from greater than 96% pure to 99% pure with a water content of less than 0.1%.

From 1978 to 1985, domestic production of 1,4-butanediol ranged from 138.2 to 353.5 million pounds annually (USITC, 1982a,b, 1983a,b, 1984a,b, 1985, 1986; *Chemical Economics Handbook* , 1993), while the quantity imported for the same period ranged from 2.0 to 27.7 million pounds annually.

1,4-Butanediol is primarily used in the manufacture of tetrahydrofuran, γ-butyrolactone, and polyvinylpyrrolidinone. 1,4-Butanediol is also used as a chain extender for polyurethanes and in the manufacture of poly-(butylene terephthalate) (*Kirk-Othmer*, 1978; HSDB, 1994).

Occupational exposure to 1,4-butanediol was examined in a National Occupational Hazard Survey conducted by the National Institute of Occupational Safety and Health (NIOSH) covering the years from 1972 to 1974. During this time, an estimated 21,169 workers in 369 facilities were potentially exposed to 1,4-butanediol (NIOSH, 1976). These estimates were based on direct observations of actual use of 1,4-butanediol, on observations of the use of trade-name products containing 1,4-butanediol, and on observations of the use of generic products suspected to contain the chemical. In a second survey of workplace exposure (National Occupational Exposure Survey) covering the years from 1981 to 1983, and based only on direct observation of the actual use of 1,4-butanediol, 16,809 workers were potentially exposed (NIOSH, 1995). Thirteen 1,4-butanediol-containing products with industrial applications are listed in the NIOSH Tradename Ingredient Data Base (NIOSH, 1976).

No information about the presence of 1,4-butanediol in consumer products was found in the literature. The database maintained by the U.S. Consumer Product Safety Commission contained no listings for 1,4-butanediol at the time of publication; however, these data have not been updated since 1988 (USCPSC, 1988).

NOMINATION AND RECOMMENDATIONS

1,4-Butanediol was nominated by the National Cancer Institute and selected for evaluation by the NTP because of high production volume, the potential for worker exposure, the lack of adequate toxicological characterization, and the lack of evaluation for carcinogenic potential.

PHARMACOLOGY

Early investigations of 1,4-butanediol (Sprince *et al.*, 1966) indicated a pronounced pharmacologic effect on the central nervous system (CNS). Administration of 496 mg/kg 1,4-butanediol to male Sprague-Dawley or Holtzman rats caused CNS depression and induced a state resembling sleep or anesthesia characterized by loss of righting reflex, struggle response, and voluntary motor activity, but retention of the ability to respond to pain and tactile stimuli (Sprince *et al.*, 1966). Very similar neuropharmacologic responses were observed after administration of γ-hydroxybutyric acid, except that sleep induction time and sleeping time were longer after administration of 1,4-butanediol than after administration of γ-hydroxybutyric acid (Sprince *et al.*,

1966). After administration of γ-butyrolactone, the γ-lactone of γ-hydroxybutyric acid, sleep induction was similar to that observed with γ-hydroxybutyric acid but sleeping time was more similar to that observed with 1,4-butanediol. Since previous work (Giarman and Roth, 1964; Roth and Giarman, 1966; Roth *et al.*, 1966) had indicated that the CNS depressant effects of γ-butyrolactone were due to its metabolism to γ-hydroxybutyric acid, it was suggested that the CNS depressant activity of 1,4-butanediol was the result of biotransformation to γ-hydroxybutyric acid (Sprince *et al.*, 1966; Menon *et al.*, 1973; Snead *et al.*, 1982).

γ-Hydroxybutyric acid is a naturally occurring chemical found in the brain and peripheral tissue (Roth, 1970; Roth and Giarman, 1970). In the brain, γ-hydroxybutyric acid is present in micromolar concentrations. In peripheral tissues (liver, heart, kidney), γ-hydroxybutyric acid concentrations are 5 to 10 times higher than in the brain; however, neither the source (precursors) nor physiological function of γ-hydroxybutyric acid in the peripheral tissues is known with certainty (Mamelak, 1989; Cash, 1994). γ-Hydroxybutyric acid readily crosses the blood-brain barrier, and oral, intraperitoneal, or intravenous administration elicits characteristic neuropharmacologic responses. Current evidence suggests it may function as a neuromodulator (Mandel *et al*., 1987; Vayer *et al*., 1987). γ-Hydroxybutyric acid is synthesized and released in specific neuronal pathways (Rumigny *et al*., 1981; Maitre *et al*., 1983) and its actions are mediated by a set of specific, high-affinity receptors which are heterogeneously distributed within the cerebral cortex and hippocampus (Hechler *et al*., 1992). Administration of exogenous γ-hydroxybutyric acid induces a state described as behavioral arrest characterized by specific dose-dependent changes in the electroencephalogram which have been well characterized in the rat, cat, and monkey (Snead, 1992). Administration of low doses (12.5 mg/kg) of γ-hydroxybutyric acid to male Wistar rats had no effect on behavior or on the electroencephalogram (Godschalk *et al*., 1977). At doses of 150 mg/kg or greater, γ-hydroxybutyric acid induces a state characterized by behavioral arrest, facial myoclonus, vibrissal twitching, and loss of righting reflex. The behavioral changes are accompanied by bilaterally synchronous electroencephalogram patterns characterized by spike-wave discharge similar to that observed during seizures in humans with nonconvulsive or absent (petit mal) epilepsy (Snead, 1992). Administration of anti-petit mal drugs such as ethosuximide, trimethadione, or *n*-propylacetate to male Wistar rats prior to administration of γ-hydroxybutyric acid antagonizes the induction of the hypersynchronous electroencephalogram pattern and eliminates the loss of the righting reflex (Godschalk *et al*., 1977). Essentially the same results have been observed in cats (Winters and Spooner, 1965), chicks (Osuide, 1972), and humans (Yamada *et al.*, 1967). Because of these findings, the γ-hydroxybutyric acid-treated animal has been proposed as an animal model for petit mal epilepsy (Snead, 1988, 1992).

More direct evidence that γ-hydroxybutyric acid is responsible for the CNS action of 1,4-butanediol was obtained by Roth and Giarman (1968), who found that the length of sleeping time in rats administered

1,4-butanediol was proportional to the concentration of γ-hydroxybutyric acid in brain tissue. Within 15 minutes after intravenous administration of 520 mg 1,4-butanediol per kilogram body weight to Sprague-Dawley rats, blood and brain concentrations of γ-hydroxybutyric acid (determined using gas chromatography) were significantly increased, and these concentrations continued to increase to a maximum that occurred approximately 60 minutes (blood) or 90 minutes (brain) after administration. This increase in γ-hydroxybutyric acid blood and brain concentrations was accompanied by sleep onset 30 minutes after administration, and sleep continued until the γ-hydroxybutyric acid concentration returned to normal (approximately 150 minutes after administration). Concomitant administration of 1,4-butanediol and 2 g β-hydroxybutyric acid/kg, a compound which antagonizes γ-hydroxybutyric acid sleep induction, resulted in antagonized sleep induction, shortened sleeping time, and lower brain γ-hydroxybutyric acid concentration than that observed in rats after administration of 1,4-butanediol alone. In addition, both blood and brain γ-hydroxybutyric acid concentrations were lower in animals that received β-hydroxybutyric acid than in those given only 1,4-butanediol.

Zabic *et al.* (1974) examined the dose response for behavioral effects of 1,4-butanediol in male Sprague-Dawley rats. Spontaneous motor activity was reduced at doses as low as 50 mg/kg with 100% cessation of motor activity at 300 mg/kg. Rotorod performance was unaffected at 100 mg/kg, but was significantly impaired at 200 mg/kg, while loss of righting reflex occurred at 300 mg/kg.

γ-Butyrolactone, in the form of an unhydrolyzed, cyclic ester, does not produce behavioral arrest or spike-wave discharge in the electroencephalogram of male Sprague-Dawley rats (Snead, 1991, 1992). However, the pharmacologic activity of γ-butyrolactone is essentially identical to that of 1,4-butanediol and γ-hydroxybutyric acid (Giarman and Roth, 1964; Sprince *et al*., 1966; Snead, 1992) after metabolic conversion to γ-hydroxybutyric acid. γ-Butyrolactone is rapidly hydrolyzed by an enzyme found in the blood and liver to γ-hydroxybutyric acid. γ-Butyrolactone has a half-life ($t_{1/2}$) of less than 1 minute in this conversion (Roth and Giarman, 1965; Fishbein and Bessman, 1966). γ-Butyrolactone is less polar and therefore better absorbed after oral administration than its hydrolyzed, free acid form, γ-hydroxybutyric acid. γ-Butyrolactone is converted to γ-hydroxybutyric acid so rapidly after absorption that γ-hydroxybutyric acid bioavailability is actually greater after administration of γ-butyrolactone than after administration of an equivalent dose of γ-hydroxybutyric acid (Lettieri and Fung, 1978; Vree *et al*., 1978).

Maxwell and Roth (1972) examined the ability of various rat tissues to metabolize [^{14}C]-1,4-butanediol *in vitro*. The brain, liver, kidney, and heart are able to convert [^{14}C]-1,4-butanediol to [^{14}C]-γ-hydroxybutyric acid, with the liver exhibiting the greatest conversion capacity per gram of tissue. Intravenous administration of 520 mg 1,4-butanediol/kg containing [^{14}C]-labeled material to male

Sprague-Dawley rats resulted in the onset of sleep (behavioral arrest) within 23 minutes. Blood and brain [^{14}C]-γ-hydroxybutyric acid concentrations increased rapidly and peaked within 60 minutes. Blood levels of 1,4-butanediol decreased rapidly after injection while brain levels increased for the first 15 minutes after injection but then decreased at approximately the same rate as the blood concentration. A brain concentration of 0.76 millimolar γ-hydroxybutyric acid was found to correlate with the onset of sleep. Giarman and Roth (1964) calculated a similar brain γ-hydroxybutyric acid concentration for sleep induction after administration of γ-hydroxybutyric acid or γ-butyrolactone. Partial hepatectomy caused a significant reduction in the formation of [^{14}C]-γ-hydroxybutyric acid derived from radiolabeled 1,4-butanediol and reduced the length of 1,4-butanediol-induced sleep time, indicating that the liver is the major site where exogenously administered 1,4-butanediol is converted to γ-hydroxybutyric acid (Maxwell and Roth, 1972).

Vree *et al*. (1978) determined the concentration of γ-hydroxybutyric acid in the blood of dogs, monkeys, and humans after intravenous administration of sodium γ-hydroxybutyric acid, γ-hydroxybutyric acid ethyl ester, or 1,4-butanediol. Conversion of 1,4-butanediol to γ-hydroxybutyric acid was rapid in all species, but was most rapid in humans. γ-Hydroxybutyric acid blood levels peaked and began to decay within 2 minutes of intravenous administration of 15 or 30 mg/kg 1,4-butanediol to humans. Equal doses of 1,4-butanediol or γ-hydroxybutyric acid yielded nearly superimposable decay curves in humans indicating essentially 100% conversion of 1,4-butanediol to γ-hydroxybutyric acid. The nonlinear decay kinetics of γ-hydroxybutyric acid as a metabolite were nearly identical to the kinetics of γ-hydroxybutyric acid after administration of γ-hydroxybutyric acid ethyl ester or sodium γ-hydroxybutyric acid, and indicated a capacity-limited elimination in humans as well as in the dog and monkey. However, no pharmacokinetic parameters were calculated from the data. Van der Pol *et al*. (1975) also observed capacity-limited elimination kinetics in dogs after intravenous infusion of doses ranging from 160 to 402 mg/kg and infusion times ranging from 105 to 118 minutes. Analyzing the decay data (obtained at the end of the infusion period) with an open one-compartment pharmacokinetic model yielded a $t_{1/2}$ of 72 to 84 minutes, indicating rapid clearance. Absorption and elimination of γ-hydroxybutyric acid have been observed to be capacity-limited processes in rats (Lettieri and Fung, 1976, 1978, 1979; Arena and Fung, 1980).

Ferrara *et al*. (1992) examined the pharmacokinetics of γ-hydroxybutyric acid in alcohol-dependent patients after single (50 mg/kg) or repeated (25 mg/kg every 12 hours for 7 days) oral doses. Maximum blood concentrations occurred with a mean time to maximum blood concentration (t_{max}) (n=10) of 30 minutes after oral administration and decayed with a mean $t_{1/2}$ of 27 minutes. First-order linear elimination kinetics were observed in five patients, all of whom had normal liver-function tests. Nonlinear-elimination kinetics indicative of capacity-limited elimination were observed in five other patients, three of whom exhibited abnormal liver function. Administration of 50 mg/kg to five patients on the last day of dosing resulted in

nonlinear-elimination kinetics indicative of a capacity-limited process. However, no accumulation of γ-hydroxybutyric acid was observed in these studies, and even after administration of 50 mg/kg on the last day of dosing, complete elimination was achieved within 4 to 6 hours.

Sodium γ-hydroxybutyric acid has been widely used as an intravenous anesthetic in certain obstetric and pediatric procedures (Hunter *et al*., 1971) and for the treatment of sleep disorders (Mamelak *et al*., 1986). The demonstration that γ-hydroxybutyric acid inhibits voluntary ethanol consumption and suppresses the ethanol withdrawal syndrome in physically dependent rats (Fadda *et al*., 1989) has prompted its use in humans to treat the effects of alcohol withdrawal. Since 1990, sodium γ-hydroxybutyric acid has been marketed by health food stores and mail-order firms as a diet aid for body builders, with claims that it stimulates the release of growth hormone. As a result of misuse, numerous cases of adverse reactions including coma and tonic-clonic seizures have been reported (Dyer, 1991; Adornato and Tse, 1992; Mack, 1993).

METABOLISM AND DISPOSITION

The major biotransformation pathways of 1,4-butanediol are illustrated in Figure 1. 1,4-Butanediol has been identified as a component of a class of neutral lipids called diol lipids, in which fatty acids are esterified to dihydric alcohols (diols) such as 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, and 1,4-butanediol, instead of glycerol. These neutral fats are widespread in nature, being identified in plants, animals, and microorganisms (Bergleson *et al*., 1966; Smith *et al*., 1983). Unesterified 1,4-butanediol has been detected in rat brain and liver (Barker *et al.*, 1985) and presumably represents free diol released during the metabolic processing of diol lipids. Because of its presence in diol lipids, some unesterified material must be reused for lipid biosynthesis. However, the incorporation of 1,4-butanediol into lipids has not been investigated directly, and based on current information this appears to be a quantitatively minor pathway, at least for exogenously administered material.

The major biotransformation route of 1,4-butanediol in the brain, liver, kidney, and heart (the only tissues examined) is oxidation to γ-hydroxybutyric acid (Roth and Giarman, 1966, 1968; Maxwell and Roth, 1972; Vree *et al*., 1978; Snead *et al*., 1989). This conversion occurs rapidly; following intravenous injection of 1,4-butanediol in humans, the plasma concentration-time profile of γ-hydroxybutyric acid as a metabolite is nearly superimposable over that obtained after intravenous injection of γ-hydroxybutyric acid as the parent (Vree *et al*., 1978). Current evidence is consistent with 1,4-butanediol first being oxidized to γ-hydroxybutyraldehyde by alcohol dehydrogenase. The intermediate aldehyde is oxidized by aldehyde

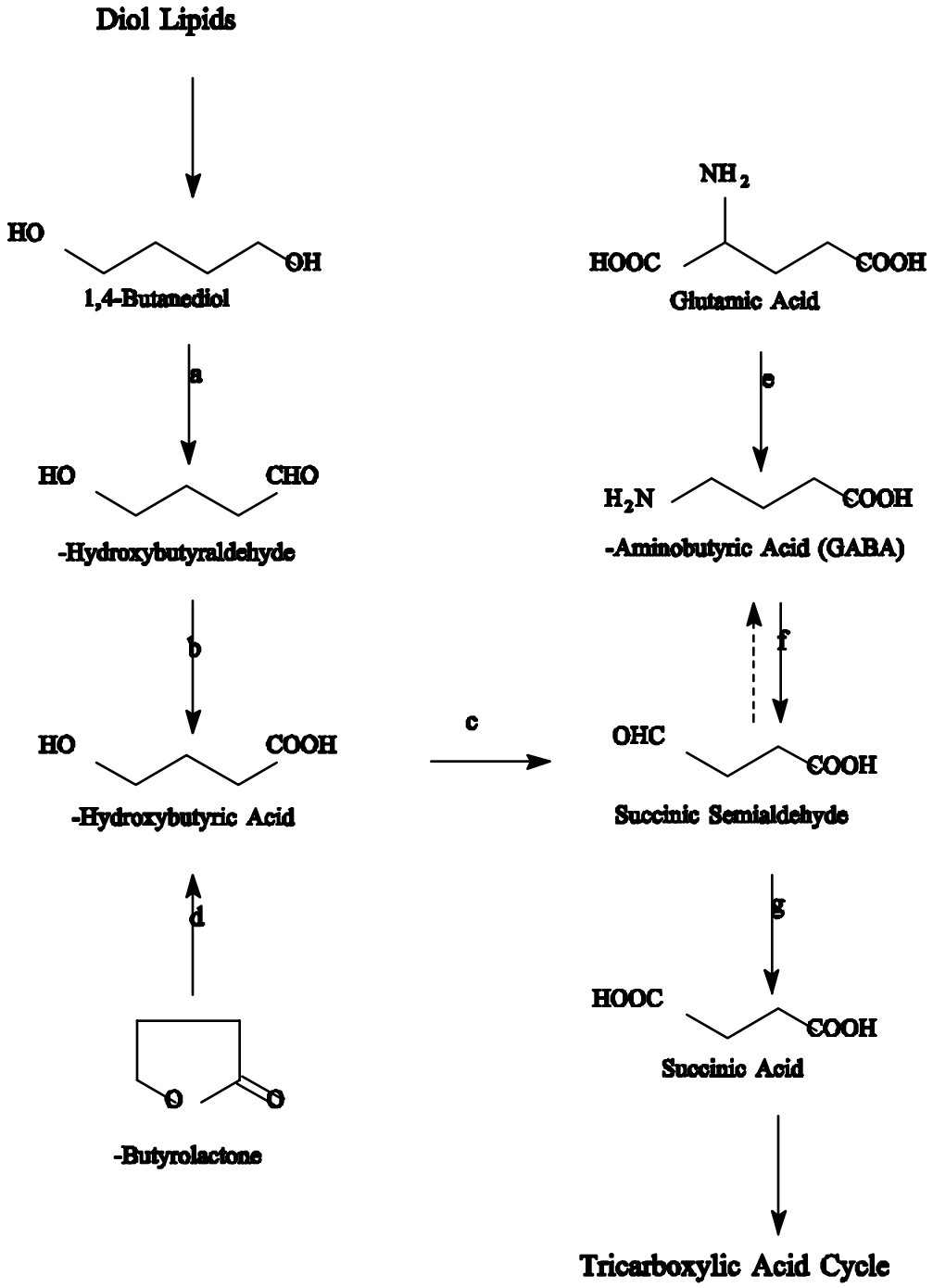

FIGURE 1
Metabolism of 1,4-butanediol. Key enzymes: a = alcohol dehydrogenase, b = aldehyde dehydrogenase, c = γ-hydroxybutyric acid dehydrogenase, d = lactonase, e = glutamic acid decarboxylase, f = GABA transaminase, g = succinic semialdehyde dehydrogenase.

dehydrogenase to γ-hydroxybutyric acid. 1,4-Butanediol is oxidized with concomitant reduction of NAD by pure, crystalline horse liver alcohol dehydrogenase as well as alcohol dehydrogenase preparations from rat liver (Bessman and McCabe, 1972; Poldrugo and Snead, 1986) or mouse liver (Taberner and Pearce, 1974). Pyrazole, an alcohol dehydrogenase inhibitor, blocks the reaction catalyzed by liver enzymes *in vitro* and antagonizes the induction of sleep or sedation in rats and mice administered 1,4-butanediol (Bessman and McCabe, 1972; Taberner and Pearce, 1974; Poldrugo and Snead, 1986). In brain or liver alcohol dehydrogenase preparations derived from male Sprague-Dawley rats, ethanol is a competitive inhibitor of 1,4-butanediol oxidation as assayed by measuring γ-hydroxybutyric acid formation. Disulfiram, an aldehyde dehydrogenase inhibitor, and pyrazole inhibited the conversion of 1,4-butanediol to γ-hydroxybutyric acid in the liver, but did not inhibit the reaction in the brain at the concentrations used (Poldrugo and Snead, 1986).

Once formed, γ-hydroxybutyric acid is oxidized to succinic semialdehyde by cytosolic and mitochondrial γ-hydroxybutyric acid dehydrogenases. The cytosolic enzyme is an NADP-dependent oxidoreductase which couples the oxidation of γ-hydroxybutyric acid to the reduction of D-glucuronate to L-gulonate (Kaufman and Nelson, 1981, 1987, 1991). The mitochondrial enzyme oxidizes γ-hydroxybutyric acid to succinic semialdehyde concomitant with the reduction of α-ketoglutarate to α-hydroxyglutarate. This is a reversible reaction in which the transfer of hydrogen occurs without the intervention of a cofactor. Both cytosolic and mitochondrial enzymes are present in brain and peripheral tissue (Bessman and McCabe, 1972; Erwin and Deitrich, 1972). Succinic semialdehyde dehydrogenase catalyzes the conversion of succinic semialdehyde to succinic acid, which is processed through the tricarboxylic acid cycle.

In the brain and neuronal tissue, succinic semialdehyde is also formed from γ-aminobutyric acid (GABA) in a reaction catalyzed by GABA-transaminase. The reversibility of the GABA-transaminase and γ-hydroxybutyric acid dehydrogenase reactions provides a metabolic path between γ-hydroxybutyric acid and GABA, and the conversion of γ-hydroxybutyric acid to GABA *in vitro* has been reported (van Bemmelen *et al.*, 1985; Vayer *et al.*, 1985). It is unclear if this occurs to any extent *in vivo* (Mandel *et al.*, 1987; Kaufman and Nelson, 1991; Cash, 1994).

β-Oxidation is also a potential route of γ-hydroxybutyric acid degradation (Walkenstein *et al.*, 1964); however, this does not appear to be a major pathway. Individuals with γ-hydroxybutyric acid aciduria caused by genetic deficiency of succinic semialdehyde dehydrogenase exhibit significantly elevated γ-hydroxybutyric acid concentrations in the blood and spinal fluid and of γ-hydroxybutyric acid and succinic semialdehyde in the urine. 3,4-Dihydroxybutyric acid, a product of β-oxidation, is also present in the urine; however, the concentration is not elevated in proportion to the increase in plasma concentration of γ-hydroxybutyric acid

and succinic semialdehyde, as would be expected if β-oxidation were a major pathway of γ-hydroxybutyric acid degradation (Jakobs *et al*., 1981; Gibson *et al*., 1983).

γ-Butyrolactone may also serve as a source of γ-hydroxybutyric acid. Snead *et al*. (1989) reported the presence of endogenous γ-butyrolactone in the brains of male Sprague-Dawley rats which apparently was not formed from either GABA, 1,4-butanediol, or by cyclization of γ-hydroxybutyric acid. However, γ-butyrolactone does not appear to be converted to γ-hydroxybutyric acid in the brain. Roth *et al.* (1966) injected γ-hydroxybutyric acid or γ-butyrolactone directly into the brain of rats and monkeys and found that γ-hydroxybutyric acid produced the expected electroencephalogram alterations, whereas γ-butyrolactone had no effect. This observation has been confirmed by more recent work (Snead, 1991, 1992).

After exogenous administration, γ-butyrolactone is rapidly hydrolyzed to γ-hydroxybutyric acid by a lactonase that utilizes four to eight carbon γ-lactones as substrates and hydrolyzes them to their respective hydroxy acids. The $t_{1/2}$ for this conversion in rat blood is less than 1 minute. $^{14}CO_2$ was detectable in the breath of rats within 4 minutes after intravenous administration of 1-[^{14}C]-γ-butyrolactone and followed a concentration-time profile similar to that observed after administration of 1-[^{14}C]-γ-hydroxybutyric acid (Roth and Giarman, 1966). The activity of this lactonase is highest in the plasma and liver; in the liver, activity is concentrated in the microsomal fraction (Fishbein and Bessman, 1966; Roth and Giarman, 1966).

As part of the NTP evaluation of the disposition of 1,4-butanediol, 1-[^{14}C]-1,4-butanediol was administered at doses of 4, 40, 120, or 400 mg/kg to male F344/N rats (Appendix A). Within the first 2 hours after administration of 4, 40, or 120 mg/kg, approximately 50% of the administered radiolabel was eliminated as $^{14}CO_2$. After 4 hours, approximately 80% of the administered radiolabel had been eliminated as $^{14}CO_2$, and at the end of the 72-hour recording period a total of 85% to 86% of the administered 1-[^{14}C]-1,4-butanediol had been eliminated as $^{14}CO_2$ (Tables A1 and A2).

$^{14}CO_2$ accounted for 94% of the radiolabel recovered in excreta. Approximately 4% of the administered radioactivity was excreted in the urine and 0.6% in the feces over the 72-hour collection period, with the majority of material excreted within the first 4 hours after administration. At 400 mg/kg, slight saturation of elimination was apparent at the early time points, as evidenced by somewhat slower formation of $^{14}CO_2$; however, at later time points, the total production of $^{14}CO_2$ was close to that observed at the lower doses (Tables A1 and A2).

The distribution of radioactivity was also studied (Table A3). Seventy-two hours after administration, a total of 2.28% of the dose remained in the carcass, with the largest amounts present in liver, muscle, and skin. The

greatest concentration of test article per gram of tissue was in liver and skin. There was no evidence of bioaccumulation in any tissue. The results indicate that 1,4-butanediol is rapidly metabolized and excreted, primarily as CO_2, clearly showing that conversion to γ-hydroxybutyric acid and succinic acid and processing through the tricarboxylic acid cycle is the major route of degradation.

TOXICITY

The results of acute and short-term studies involving oral and intraperitoneal administration to 1,4-butanediol are shown in Table 1. In dermal irritancy studies, 1,4-butanediol applied neat to the intact or abraded back skin of New Zealand white rabbits produced no indications of primary irritancy after 72 hours (GAF Corporation; Jedrychowski *et al*., 1990a). Intraocular administration of 0.1 mL of 1,4-butanediol was considered nonirritating (GAF Corporation) or slightly irritating (Jedrychowski *et al*., 1990a) to the eyes of New Zealand white rabbits. Hartley guinea pigs sensitized with 1,4-butanediol exhibited no contact dermatitis upon rechallenge (Jedrychowski *et al*., 1990a).

Repeated-dose studies have been conducted using gavage administration (Jedrychowski *et al*., 1990b) and inhalation exposure (Kinney *et al*., 1985). Groups of eight male and eight female Wistar rats were administered 1,4-butanediol by gavage at doses of 5, 50, or 500 mg/kg for 28 days. There were no deaths during the study. Mean body weights, organ weights, and feed consumption of dosed groups were similar to those of the controls. Hematology parameters determined from blood samples obtained at necropsy indicated some potential differences between dosed and control groups; however, the differences were small and not indicative of chemical-related toxicity and thus were considered of questionable toxicologic significance. Mild to moderate inflammation of the liver was observed in some dosed animals, primarily from the 500 mg/kg groups, but the increased severity over that in the controls was not statistically significant in males or females (Jedrychowski *et al*., 1990b).

Groups of 10 male Crl:CD rats were exposed nose only to aerosols containing 0.2, 1, or 5 mg 1,4-butanediol/L for 6 hours per day, 5 days per week for 2 weeks. After the ninth exposure, overnight urine samples were collected from all animals. Five rats per exposure group were killed after the tenth exposure, and the five remaining rats in each group were killed at the end of a 14-day recovery period, which immediately followed the 14-day exposure period. Prior to necropsy, blood samples were collected from all rats for hematology and clinical chemistry evaluations. No effects associated with chemical exposure were observed in rats exposed to 0.2 or 1 mg 1,4-butanediol/L. Rats exposed to 5 mg/L exhibited lower (7% to 9%) mean body weights than air-exposed controls. Rats receiving

TABLE 1

Acute Toxicity Values for 1,4-Butanediol

Species/Strain	Number	Route	LD_{50}[a]	Reference
Rat/Albino	25 Male 25 Female	Oral	1,550 mg/kg	(GAF Corporation)
Rat/Wistar	30 Male 30 Female	Oral	1,830 mg/kg 2,000 mg/kg	(Jedrychowski *et al.*, 1990a)
Rat/—[b]	—	Oral	1,525 mg/kg	(MSDS, 1985)
Rat/Wistar	18 Male 18 Female	Intraperitoneal	1,070 mg/kg	(Taberner and Pearce, 1974)
Rat/Albino	88 Male (Sprince *et al.*, 1966)	Intraperitoneal	1,000 mg/kg	
Rat/Sprague-Dawley	— (Zabic *et al.*, 1974)	Intraperitoneal	1,328 mg/kg	
Mouse/—	— (Kinney *et al.*, 1985)	Oral	2,180 mg/kg	
Guinea Pig	90 (MSDS, 1985)	Oral	2,000 mg/kg	

[a] LD_{50} = median lethal dose
[b] Data not provided

10 exposures had slight atrophy of the lymphoid cells of the thymus. After 14 days of recovery, mean body weights returned to control values and no indication of thymic atrophy was present (Kinney *et al*., 1985).

In an NTP-sponsored study, the developmental toxicity of 1,4-butanediol was evaluated by administering 1, 100, 300, or 600 mg/kg by gavage in water to timed-pregnant Swiss albino mice on gestation days 6 through 15 (Price *et al*., 1993). No maternal deaths occurred during the study; however, signs of acute CNS intoxication including hypoactivity, immobility, and loss of righting reflex occurred after dosing in the 300 and 600 mg/kg groups, but usually resolved within 4 hours after dosing. No apparent tolerance was noted during the 10-day dosing period. Other indications of maternal toxicity included body and liver weights and feed consumption that were lower than those of the controls in the 300 and 600 mg/kg groups and kidney weight lower than that of controls in the 600 mg/kg group.

Significant reductions in live fetal weight occurred in the 300 and 600 mg/kg groups. The incidence of resorptions was not increased by chemical exposure, and the percentage of litters with one or more late fetal deaths was actually lower in the 300 and 600 mg/kg groups than in the control or 100 mg/kg groups. The incidence of fetuses with external or visceral malformations were similar in all groups; however, there was an increasing trend in skeletal malformations (missing or branched ribs and fused thoracic vertebrae), primarily in the 600 mg/kg group (Price *et al*., 1993).

CARCINOGENICITY

1,4-Butanediol has not been evaluated for chronic toxicity or carcinogenicity; however, γ-butyrolactone was evaluated in 2-year studies by the NTP (NTP, 1992). During the 2-year studies, γ-butyrolactone was administered by gavage in corn oil 5 days per week for 102 weeks. Males rats received 0, 112, or 225 mg/kg, female rats received 0, 225, or 450 mg/kg, and male and female mice received 0, 262, or 525 mg/kg. Exposure to γ-butyrolactone caused no adverse effects in rats or female mice. Focal hyperplasia of the adrenal medulla was increased in male mice in the 262 mg/kg group but not in 525 mg/kg males, and pheochromocytomas were present in the adrenal medulla of two control, six 262 mg/kg males, and one 525 mg/kg male. Although the incidence of pheochromocytomas in the 262 mg/kg group was not significantly increased over that in the control group, focal hyperplasia and pheochromocytoma of the adrenal medulla are considered a morphologic and biologic continuum, and the increased incidence in the 262 mg/kg group is suggestive of a proliferative response associated with exposure to γ-butyrolactone. The incidence of proliferative lesions was not increased in the 525 mg/kg group of males; however, reduced survival as a result of deaths that occurred during the first year of the study may have reduced the sensitivity of this group for detecting a carcinogenic response. Therefore an association between γ-butyrolactone exposure and hyperplasia and pheochromocytomas of the adrenal medulla on male mice in the 262 mg/kg group was considered uncertain. A structural isomer, 1,3-butanediol, has also been evaluated in a chronic study (Scala and Paynter, 1967). In this study, Sprague-Dawley rats (30 males and 30 females per group) received 1%, 3%, or 10% in feed, and dogs (four males and four females per group) received 0.5%, 1%, or 3% 1,3-butanediol in feed for 2 years. Blood and urine samples were collected from rats at six time points and from dogs at eight time points during the study. Blood samples were evaluated for hematology parameters, and urine was analyzed for specific gravity, pH, glucose, protein, and porphyrins. After 1 year of chemical exposure, 10 rats and 2 dogs from each group were evaluated; after 2 years of chemical exposure, the surviving animals were evaluated. At necropsy, organ weights were taken and all major tissues were fixed and prepared for histopathologic examination. No adverse effects were observed during the study, and there were no gross or microscopic lesions attributable to chemical exposure (Scala and Paynter, 1967).

GENETIC TOXICITY

No genetic toxicology studies of 1,4-butanediol were identified in the literature. The NTP has not evaluated the genetic toxicity of 1,4-butanediol.

DISCUSSION

The preceding sections document the rapid conversion of exogenously administered 1,4-butanediol to γ-hydroxybutyric acid and its subsequent catabolism through the tricarboxylic acid cycle. It is also well documented that like γ-butyrolactone, the pharmacologic action of 1,4-butanediol is due to its conversion to γ-hydroxybutyric acid. For exogenously administered 1,4-butanediol, catabolism occurs primarily in the liver, although γ-hydroxybutyric acid readily crosses the blood-brain barrier and is catabolized in the brain. Because of rapid and extensive metabolism, the toxicologic profile of 1,4-butanediol reflects that of its metabolite, γ-hydroxybutyric acid.

γ-Hydroxybutyraldehyde, the aldehyde intermediate formed by the initial oxidation of 1,4-butanediol and which is subsequently oxidized to γ-hydroxybutyric acid, is a potentially toxic metabolite. However, the rapid conversion of 1,4-butanediol to γ-hydroxybutyric acid makes it unlikely that the steady-state concentration of the aldehyde would reach toxic levels. None of the toxicology studies of 1,4-butanediol have identified organ-specific toxicity or other significant effects associated with exposure to this chemical, except for behavioral changes (GAF Corporation; Taberner and Pearce, 1974; Zabic *et al.*, 1974; Kinney *et al.*, 1985; Jedrychowski *et al*., 1990a,b). Zabic *et al.* (1974) demonstrated reduction of spontaneous motor activity in rats with intraperitoneal doses as low as 50 mg/kg, impaired rotorod performance at 200 mg/kg, and complete cessation of spontaneous motor activity and loss of righting reflex at 300 mg/kg. During the NTP-sponsored developmental toxicity study, hypoactivity, immobility, loss of righting reflex, and prone posture were observed soon after dosing in pregnant Swiss mice administered 300 or 600 mg/kg (Price *et al.*, 1993). As indicated by the NTP metabolism and disposition studies (Appendix A), 1,4-butanediol is rapidly metabolized in this dose range. Therefore, over the dose range practical for toxicologic investigation, responses observed for 1,4-butanediol reflect its conversion to γ-hydroxybutyric acid rather than responses associated with the parent compound or another, more toxic metabolite.

The rapid conversion of γ-butyrolactone to γ-hydroxybutyric acid is also well documented in the literature presented in the preceding sections. γ-Butyrolactone is converted to γ-hydroxybutyric acid very rapidly, and because of the more rapid absorption of the less polar lactone compared to the open chain, free acid form, bioavailability of γ-hydroxybutyric acid as a metabolite of γ-butyrolactone is greater than that observed after administration of sodium γ-hydroxybutyric acid. Therefore, the NTP evaluation of γ-butyrolactone was in fact an evaluation of γ-hydroxybutyric acid. γ-Butyrolactone has undergone 16-day, 13-week, and 2-year evaluations by the NTP in rats and mice (NTP, 1992). No organ-specific toxicity was observed in the 16-day or 13-week studies even though chemical-related mortality occurred at the highest doses administered. The only toxic response was behavioral arrest, and this response served as a basis for selecting doses for the 2-

year rat and mouse studies. During these studies, γ-butyrolactone exhibited no toxic or carcinogenic potential; therefore it is unlikely that 1,4-butanediol would be carcinogenic in animals.

CONCLUSION

Because the chronic toxicity and carcinogenicity of γ-hydroxybutyric acid was in effect fully evaluated in NTP prechronic and chronic studies of γ-butyrolactone, with a lack of toxic or carcinogenic potential being demonstrated, it is concluded that there is a high likelihood that 1,4-butanediol would be negative in a similar study. For these reasons it is the opinion of the NTP that 1,4-butanediol is unlikely to be carcinogenic in animals, and no further evaluation of 1,4-butanediol is needed at this time.

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APPENDIX A
METABOLISM AND DISPOSITION STUDIES OF 1,4-BUTANEDIOL IN MALE F344/N RATS

METABOLISM AND DISPOSITION STUDIES OF 1,4-BUTANEDIOL IN MALE F344/N RATS

INTRODUCTION

1,4-Butanediol is a high production chemical used primarily as an intermediate in the manufacture of tetrahydrofuran, γ-butyrolactone, and polyvinylpyrrolidinone. 1,4-Butanediol is also used as a chain extender for polyurethanes and in the manufacture of poly-(butylene terephthalate) (*Kirk-Othmer*, 1978; HSDB, 1994). An estimated 21,169 workers in 369 facilities were potentially exposed during the years from 1972 to 1974 (NIOSH, 1976). These estimates were based on direct observations of actual use of 1,4-butanediol, on observations of the use of trade-name products containing 1,4-butanediol, and on observations of the use of generic products suspected to contain the chemical. From 1981 to 1983, an estimated 16,809 workers were potentially exposed to 1,4-butanediol (NIOSH, 1995). These estimates were based only on direct observation of the actual use of 1,4-butanediol. Thirteen 1,4-butanediol-containing products with industrial applications are listed in the NIOSH Tradename Ingredient Data Base (NIOSH, 1976).

The metabolism of 1,4-butanediol proceeds through the oxidation of one of the hydroxyl groups first to γ-butyraldehyde and then to γ-hydroxybutyric acid. The liver is the major site of these biotransformations (Bessman and McCabe, 1972; Erwin and Deitrich, 1972; Weiner, 1980). γ-Hydroxybutyric acid can either cyclize to γ-butyrolactone, or the remaining hydroxyl group can be further oxidized to succinic acid semialdehyde and ultimately succinic acid, where it enters the tricarboxylic acid cycle.

The objectives of this series of studies was to determine the absorption, distribution, and clearance of 1,4-butanediol after gavage administration to male F344/N rats.

MATERIALS AND METHODS

Chemical Analyses and Dose Formulations

[^{14}C]-1,4-Butanediol (C-1 and C-4 carbons labeled, lot CFQ.5344) was obtained from Amersham (Buckinghamshire, England). It was supplied as a neat liquid with a specific activity of 17.6 millicuries per millimole. The radiochemical purity of lot CFQ.5344 was established with high-performance liquid chromatography (HPLC) and liquid scintillation spectroscopy, and determined to be greater than 97%. Impurities were not identified. Unlabeled 1,4-butanediol (lot 02019PP) was obtained from Aldrich Chemical

Company, Inc. (Milwaukee, WI). The identity of unlabeled 1,4-butanediol was confirmed by mass spectroscopy and proton nuclear magnetic resonance spectroscopy.

Dose formulations were formulated to contain approximately 20 microcuries radiolabeled 1,4-butanediol, an appropriate amount of unlabeled 1,4-butanediol, and sufficient distilled water to provide for a single dose of 5 mL per kilogram test-animal body weight. The radiochemical purity of each dose formulation was determined the day of dosing by HPLC after all animals had been dosed.

Metabolism and Disposition Studies

Groups of four male F344/N rats were given a single target dose of 4, 40, 120, or 400 mg [^{14}C]-1,4-butanediol per kilogram body weight by gavage. During the studies, animals were housed individually in Roth-type glass metabolism chambers (Jencons Scientific, Ltd.). These chambers provided for separate collection of urine, feces, and exhaled breath.

Urine and feces were collected separately from each animal at 8, 24, 48, and 72 hours in round-bottomed flasks cooled over dry ice. The weight of the urine and feces collected at each time point was determined and the samples stored at -20 ° C until analyzed.

Radiolabeled compounds in breath were collected by passing air from the each metabolism cage (flow rate = 200 to 500 mL/minute) through a series of four traps. The first two traps, intended to remove volatile compounds, each contained 50 to 80 mL ethanol. The first trap was maintained at 0 ° C by an ice-water bath; the second was maintained at -60 ° C by an isopropanol and dry ice bath. The second two traps each contained 500 mL of 1 N sodium hydroxide for the collection of carbon dioxide. The traps were changed at 2, 4, 8, 12, 24, 32, 48, 56, and 72 hours and the weight of the solutions in the traps was measured.

At the end of each experiment (72 hours), the rats were anesthetized with an intraperitoneal injection of ketamine and xylazine, and blood was withdrawn into a heparinized syringe by cardiac puncture. Rats were sacrificed by an intracardiac injection of sodium pentobarbital.

Adipose tissue, muscle, and skin (three samples of each) and the entire liver and brain were removed from animals administered [^{14}C]-1,4-butanediol 40 mg/kg and the samples were assayed for ^{14}C content. All carcasses were stored in the dark at -20 ° C. Two rats each from the 4 and 400 mg/kg groups were selected for analysis of carcass ^{14}C content. Each carcass was dissolved in approximately 400 mL of 2 N ethanolic sodium hydroxide.

Aliquots of ethanol from the breath traps were added directly to vials containing scintillation cocktail. Aliquots of urine, plasma, dissolved carcass, and sodium hydroxide trap solutions were added to empty scintillation vials and counted after the addition of scintillation cocktail. Samples of tissue, feces, and blood were digested in Soluene-350. After digestion, samples requiring bleaching were decolorized with perchloric acid and hydrogen peroxide prior to the addition of scintillation cocktail. Alkaline samples (containing sodium hydroxide or Soluene-350) and scintillate were stored in the dark overnight before they were assayed.

Analysis of Data

Radioactivity was expressed in microcuries. The percentage of the administered dose recovered in excreta was expressed as the quotient of the mean amount of radioactivity recovered divided by the mean amount of radioactivity of the dose administered multiplied by 100. Tissue-to-blood ratios were expressed as the ratio of the radioactivity in the tissue (expressed as nanogram-equivalents of parent compound per gram of tissue) to the radioactivity in the blood (expressed as nanogram-equivalents of parent compound per gram of blood).

RESULTS AND DISCUSSION

Greater than 75% of oral doses of 1,4-butanediol were excreted as carbon dioxide within 24 hours after administration. There were only slight differences in the profiles of radioactivity excretion between the 4, 40, and 120 mg/kg dose levels (Table A1). There was an indication of capacity-limited metabolism at the 400 mg/kg, as evidenced by lower carbon dioxide production. This difference was apparent 2 hours after dosing where carbon dioxide production was 50% to 54% of the administered dose for the 4, 40, and 120 mg/kg groups, but only 24% for the 400 mg/kg group (Table A2). These differences decreased with time, and total [^{14}C]-carbon dioxide production was 79% at the highest dose, approaching that of the lower doses (85% to 86%). Excretion of radiolabel in the urine and feces as a percent of the administered dose was low, accounting for approximately 3% to 6% in urine and 0.04% to 0.6% in feces. Since less than 1% of the administered radioactivity was recovered as volatile compounds present in breath after oral doses of 4 or 400 mg/kg, volatile compounds were not collected for the 40 or 120 mg/kg groups.

Persistence of [^{14}C]-1,4-butanediol equivalents in tissues collected 72 hours after a single dose of 40 mg/kg was low (2% of administered dose), with the highest accumulation in muscle (0.9% of administered dose, tissue/blood ratio of 0.9) and liver (0.5% of administered dose, tissue/blood ratio of 5.2) (Table A3). The percent of dose remaining in blood 72 hours after dosing was approximately 0.1% for each dose concentration (Table A4). No effects on the central nervous system were obvious, and only 0.01% of the 40 mg/kg dose was recovered in the brain 72 hours after dosing. The residual ^{14}C in the carcasses analyzed

was found to be approximately 2.2% of the administered dose for the 4 mg/kg dose level and 2.8% of the administered dose for the 400 mg/kg dose level.

In conclusion, the disposition of oral doses of 1,4-butanediol is characterized by rapid absorption and metabolism to carbon dioxide.

TABLE A1
Cumulative Excretion of Radioactivity after Oral Administration of [^{14}C]-1,4-Butanediol to Male F344/N Rats[a]

End of Interval (Hours)	Cumulative Percent of Dose				
	Volatiles	CO_2	Urine	Feces	Total Excreta
4 mg/kg[b]					
2	0.32 ± 0.14	50.3 ± 12.1			50.7 ± 12.0
4	0.47 ± 0.20	65.5 ± 9.6			66.0 ± 9.5
8	0.50 ± 0.22	78.9 ± 3.9	2.83 ± 0.23	0.16 ± 0.04	82.4 ± 3.7
12	0.52 ± 0.23	82.1 ± 1.9			85.6 ± 1.6
24	0.52 ± 0.23	83.9 ± 1.8	3.67 ± 0.28	0.46 ± 0.06	88.5 ± 1.4
32		84.4 ± 1.8			89.0 ± 1.4
48	0.53 ± 0.23	85.0 ± 1.8	3.82 ± 0.26	0.60 ± 0.04	89.9 ± 1.4
56		85.2 ± 1.8			90.2 ± 1.4
72	0.53 ± 0.23	85.6 ± 1.8	3.89 ± 0.25	0.64 ± 0.04	90.6 ± 1.4
40 mg/kg[c]					
2		54.4 ± 8.0			54.4 ± 8.0
4		70.1 ± 6.1			70.1 ± 6.1
8		79.3 ± 2.1	2.99 ± 0.29	0.12 ± 0.10	82.4 ± 1.8
12		81.7 ± 1.0			84.8 ± 0.8
24		83.6 ± 0.6	4.19 ± 0.15	0.47 ± 0.12	88.2 ± 0.6
32		84.0 ± 0.5			88.7 ± 0.6
48		84.7 ± 0.4	4.37 ± 0.15	0.60 ± 0.09	89.7 ± 0.4
56		85.0 ± 0.4			89.9 ± 0.4
72		85.3 ± 0.3	4.43 ± 0.15	0.63 ± 0.09	90.4 ± 0.3
120 mg/kg[c]					
2		51.5 ± 3.7			51.5 ± 3.7
4		69.0 ± 3.3			69.0 ± 3.3
8		80.9 ± 1.2	2.99 ± 0.12	0.09 ± 0.09	84.0 ± 1.1
12		83.1 ± 1.1			86.6 ± 1.0
24		84.7 ± 1.0	4.35 ± 0.26	0.42 ± 0.18	89.4 ± 1.2
32		85.1 ± 1.0			89.9 ± 1.2
48		85.7 ± 1.0	4.51 ± 0.27	0.60 ± 0.09	90.8 ± 1.2
56		85.9 ± 1.0			91.0 ± 1.2
72		86.2 ± 1.0	4.57 ± 0.26	0.64 ± 0.09	91.4 ± 1.1
400 mg/kg					
2	0.60 ± 0.48	23.9 ± 2.6			24.5 ± 2.2
4	0.92 ± 0.55	51.0 ± 2.5			52.0 ± 2.1
8	0.99 ± 0.59	71.6 ± 3.0	4.84 ± 0.51	0.07 ± 0.03	77.5 ± 2.3
12	1.00 ± 0.59	75.2 ± 2.7			81.1 ± 2.1
24	1.01 ± 0.60	77.1 ± 2.7	6.19 ± 0.65	0.40 ± 0.04	84.8 ± 2.3
32		77.5 ± 2.7			85.1 ± 2.2
48	1.01 ± 0.59	78.2 ± 2.7	6.36 ± 0.68	0.56 ± 0.04	86.2 ± 2.2
56		78.5 ± 2.6			86.4 ± 2.2
72	1.01 ± 0.59	78.8 ± 2.6	6.45 ± 0.69	0.61 ± 0.06	86.9 ± 2.1

[a] Data are represented as mean percentage of administered radioactivity ± standard deviations for groups of four rats.
[b] Target doses
[c] Volatile compounds were not collected in this dose group.

TABLE A2
Cumulative Excretion of $^{14}CO_2$ after Oral Administration of [^{14}C]-1,4-Butanediol to Male F344/N Rats[a]

Time at End of Interval (Hours)	Dose (mg/kg)[b]			
	4	40	120	400
2	50.3 ± 12.1	54.4 ± 8.0	51.5 ± 3.7	23.9 ± 2.6
4	65.5 ± 9.6	70.1 ± 6.1	69.0 ± 3.3	51.0 ± 2.5
8	78.9 ± 3.9	79.3 ± 2.1	80.9 ± 1.2	71.6 ± 3.0
12	82.1 ± 1.9	81.7 ± 1.0	83.1 ± 1.1	75.2 ± 2.7
24	83.9 ± 1.8	83.6 ± 0.6	84.7 ± 1.0	77.1 ± 2.7
32	84.4 ± 1.8	84.0 ± 0.5	85.1 ± 1.0	77.5 ± 2.7
48	85.0 ± 1.8	84.7 ± 0.4	85.7 ± 1.0	78.2 ± 2.7
56	85.2 ± 1.8	85.0 ± 0.4	85.9 ± 1.0	78.5 ± 2.6
72	85.6 ± 1.8	85.3 ± 0.3	86.2 ± 1.0	78.8 ± 2.6

[a] Data are represented as mean percentage of administered radioactivity ± standard deviation for groups of four rats.
[b] Target doses

TABLE A3
Tissue Distribution of Radioactivity 72 Hours after Oral Administration of 40 mg/kg [^{14}C]-1,4-Butanediol to Male F344/N Rats[a]

Tissue	ng-Eq 1,4-Butanediol per g of tissue	Tissue-to-Blood Ratio[b]	Percent of Dose in Total Tissue
Adipose	891 ± 129	0.95 ± 0.14	0.15 ± 0.02
Blood	941 ± 41	1.00 ± 0.00	0.12 ± 0.01
Brain	805 ± 147	0.85 ± 0.12	0.01 ± 0.00
Liver	4,930 ± 1,230	5.24 ± 1.32	0.52 ± 0.18
Muscle	803 ± 164	0.85 ± 0.16	0.93 ± 0.21
Skin	1,350 ± 251	1.44 ± 0.25	0.55 ± 0.12
Total			2.28 ± 0.44

[a] Target dose. Data are represented as mean results ± standard deviation for four rats.
[b] Mean ratio of radioactivity in tissue (ng-Eq) to radioactivity in blood (ng-Eq)

TABLE A4
^{14}C-Blood Levels 72 Hours after Oral Administration of [^{14}C]-1,4-Butanediol to Male F344/N Rats[a]

Dose (mg/kg)[b]	Percent of Dose in Blood	ng-Eq 1,4-Butanediol per g Blood
4	0.09 ± 0.02	72 ± 11
40	0.12 ± 0.01	941 ± 41
120	0.12 ± 0.00	2,780 ± 151
400	0.11 ± 0.00	8,580 ± 295

[a] Data are represented as mean percentage of administered radioactivity ± standard deviation for groups of four rats.
[b] Target doses

APPENDIX B
SURVIVAL AND MEAN BODY WEIGHT RESULTS FOR F344/N RATS AND B6C3F$_1$ MICE IN THE 16-DAY AND 13-WEEK GAVAGE STUDIES OF γ-BUTYROLACTONE

National Toxicology Program (NTP) (1992). Toxicology and Carcinogenesis Studies of γ-Butyrolactone (CAS No. 96-48-0) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 406. NIH Publication No. 92-3137. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

TABLE B1
Survival and Mean Body Weights of Rats in the 16-Day Gavage Study of γ-Butyrolactone

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	134 ± 4	219 ± 4	85 ± 3	
75	5/5	128 ± 2	214 ± 4	85 ± 3	97
150	5/5	132 ± 2	211 ± 4	79 ± 2	96
300	5/5	124 ± 1*	206 ± 4	82 ± 4	94
600	4/5[c]	132 ± 2	213 ± 1	80 ± 1	97
1,200	0/5[d]	133 ± 3	—	—	—
Female					
0	5/5	112 ± 5	154 ± 4	42 ± 3	
75	5/5	109 ± 3	154 ± 5	44 ± 3	100
150	5/5	118 ± 2	162 ± 2	43 ± 1	105
300	5/5	105 ± 2	143 ± 3	38 ± 2	93
600	5/5	114 ± 2	146 ± 2	32 ± 1**	95
1,200	0/5[e]	107 ± 3	—	—	—

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No data were calculated for groups with 100% mortality.
[c] Day of death: 3
[d] Day of death: 1, 3, 3, 3, 3
[e] Day of death: 1, 2, 3, 3, 3

TABLE B2
Survival and Mean Body Weights of Rats in the 13-Week Gavage Study of γ-Butyrolactone

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	9/10[c]	148 ± 2	370 ± 7	223 ± 6	
56	10/10	146 ± 4	375 ± 8	229 ± 5	101
112	10/10	147 ± 3	379 ± 4	232 ± 5	102
225	10/10	147 ± 3	363 ± 4	216 ± 4	98
450	10/10	149 ± 4	345 ± 7**	196 ± 6**	93
900	0/10[d]	149 ± 3	—	—	—
Female					
0	10/10	119 ± 2	203 ± 3	84 ± 2	
56	10/10	115 ± 2	203 ± 3	87 ± 3	100
112	9/10[c]	117 ± 2	209 ± 2	90 ± 3	103
225	10/10	118 ± 2	208 ± 3	90 ± 4	103
450	10/10	116 ± 2	202 ± 4	86 ± 3	100
900	9/10[e]	115 ± 2	198 ± 3	82 ± 3	98

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No data were calculated for groups with 100% mortality.
[c] Accidental death
[d] Week of death: 1, 1, 1, 1, 1, 1, 1, 5, 5, 5
[e] Week of death: 8

TABLE B3
Survival and Mean Body Weights of Mice in the 16-Day Gavage Study of γ-Butyrolactone

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	4/5[c]	24.6 ± 0.6	28.0 ± 0.6	3.3 ± 0.3	
87	5/5	24.4 ± 0.2	27.8 ± 0.4	3.4 ± 0.2	99
175	4/5[c]	24.8 ± 0.2	28.3 ± 0.5	3.5 ± 0.5	101
350	5/5	23.4 ± 0.5	26.4 ± 0.9	3.0 ± 0.5	94
700	5/5	24.8 ± 0.4	27.2 ± 0.4	2.4 ± 0.4	97
1,400	0/5[d]	24.0 ± 0.7	—	—	—
Female					
0	5/5	19.8 ± 0.5	22.4 ± 0.9	2.6 ± 0.6	
87	5/5	20.4 ± 0.2	21.4 ± 0.2	1.0 ± 0.3	96
175	3/5[c]	19.6 ± 0.4	21.7 ± 0.9	1.7 ± 0.3	96
350	5/5	18.2 ± 0.4*	19.8 ± 0.4*	1.6 ± 0.5	88
700	4/5[c]	19.4 ± 0.2	20.8 ± 0.3*	1.5 ± 0.3	93
1,400	1/5[e]	19.0 ± 0.6	20.0	2.0	89

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No data were calculated for groups with 100% mortality. No standard error was calculated for groups with high mortality.
[c] Accidental deaths
[d] Day of death: 2, 3, 8, 8, 8
[e] Day of death: 2, 9, 10, 10

TABLE B4
Survival and Mean Body Weights of Mice in the 13-Week Gavage Study of γ-Butyrolactone

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	8/10[c]	25.3 ± 0.4	37.3 ± 0.8	11.8 ± 0.8	
65	6/10[c]	24.7 ± 0.5	35.2 ± 0.8	10.2 ± 0.8	94
131	8/10[c]	24.7 ± 0.5	38.1 ± 0.5	13.4 ± 0.7	102
262	9/10[c]	24.7 ± 0.5	35.7 ± 0.9	11.0 ± 0.8	96
525	10/10	24.6 ± 0.5	34.9 ± 0.8	10.3 ± 0.5	94
1,050	7/10[d]	24.5 ± 0.5	33.3 ± 1.4**	9.3 ± 1.0*	89
Female					
0	7/10[c]	18.6 ± 0.3	25.9 ± 0.7	7.0 ± 0.5	
65	7/10[c]	18.1 ± 0.4	25.3 ± 0.6	7.3 ± 0.5	98
131	7/10[c]	18.7 ± 0.3	26.0 ± 0.6	7.1 ± 0.7	101
262	10/10	19.0 ± 0.3	26.3 ± 0.4	7.3 ± 0.3	102
525	8/10[c]	18.8 ± 0.3	26.5 ± 0.7	7.8 ± 0.7	103
1,050	7/10[c,e]	18.2 ± 0.3	25.9 ± 1.0	7.9 ± 0.8	100

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Accidental deaths
[d] Week of death: 1, 1, 12
[e] One chemical-related death week 1