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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and The Dutch Expert Committee on Occupational Standards

135. γ-Butyrolactone (GBL)

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Nordic Council of Ministers

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Preface

An agreement has been signed by the Dutch Expert Committee on Occupational Standards (DECOS) of the Health Council of the Netherlands and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). The purpose of the agreement is to write joint scientific criteria documents, which could be used by the national regulatory authorities in both the Netherlands and in the Nordic Countries.

The document on health effects of γ-Butyrolactone was written by Dr. Eric Søderlund at the Norwegian Institute of Public Health, Oslo, Norway and has been reviewed by DECOS as well as by NEG.

Editorial work and technical editing was performed by Anna-Karin Alexandrie, Ilona Silins, and NEG's scientific secretary, Jill Järnberg, all at the National Institute for Working Life in Sweden.

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Abbreviations

CI	confidence interval
CNS	central nervous system
ED_{50}	effective dose in 50% of population
FDA	US Food and Drug Administration
FSH	follicle stimulating hormone
GABA	γ-aminobutyric acid, *gamma*-aminobutyric acid
GBL	γ-butyrolactone, *gamma*-butyrolactone
GC	gas chromatography
GHB	γ-hydroxybutyrate, *gamma*-hydroxybutyrate, γ-hydroxybutyric acid
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
LD_{50}	lethal dose for 50% of the exposed animals at single administration
LH	luteinizing hormone
MS	mass spectrometry
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
REM	rapid eye movement
SPME	headspace solid-phase microextraction
UV-VIS	ultraviolet-visible

Contents

1. Introduction

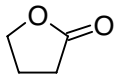
γ-Butyrolactone (GBL) is the cyclic ester of 4-hydroxybutanoic acid. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media γ-hydroxybutyrate (GHB) will predominate while in acid media the lactone form is favoured.

GBL is used in the synthesis of pyrrolidones, as a solvent for polymers, as an intermediate in the preparation of the herbicide 4-(2,4-dichlorophenoxy) butyric acid, as a constituent of paint removers, textile aids, and drilling oil. GBL is also used in electronics, speciality cleaning, and foundry binders. Although GBL is used in several industries, occupational exposure data is limited. Low molecular weight (<C8) lactones occur naturally in berries, fruits, and related alcoholic beverages at concentrations of less than 1 mg/kg. GBL is also used experimentally in medical treatment. Due to its euphoric/hallucinogenic properties the abuse of GBL has increased dramatically the last years.

Most of the toxicity studies with GBL were performed in the 1960s and 1970s and are often not reported in sufficient detail to allow a scientific evaluation of the data. These older studies focused mostly on toxicokinetic parameters, acute and local effects but also to some extent on carcinogenicity. They identified central nervous system (CNS) as a target organ for acute toxic effects but also eye irritation was reported. Furthermore, these earlier studies demonstrated that GBL is extremely rapidly metabolised to GHB in the body. Thus, effects of GHB are of relevance when assessing possible health effects of GBL to humans. An extensive assessment of genotoxic effects was available in 1981, indicating that GBL has a low mutagenic potential (26). A toxicological investigation of GBL focusing on potential carcinogenic effects, was reported by the National Toxicology Program (NTP) in 1992 (118). In addition, the recent abuse of GBL has increased our knowledge of toxic symptoms and their treatment in humans. No reports describing occupational exposure levels and/or occupational health effects in humans were located.

2. Substance identification

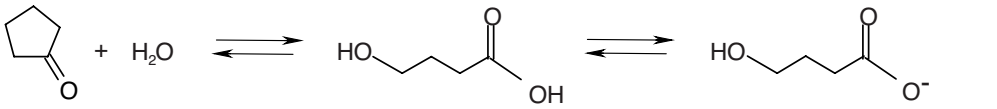
IUPAC name	Dihydro-2(3-H)-furanone
CAS name	γ-Butyrolactone
CAS No	96-48-0
EINECS No	202-509-5
Synonyms	butyric acid lactone; 1, 2-butanolide; 1, 4-butanolide; 4-butyrolactone; 4-hydroxybutanoic acid lactone; γ-hydroxybutyric acid cyclic ester; 4-deoxytetronic acid; tetrahydro-2-furanone

Trade names	BLO; γ-6480; γ-BL; GBL; GHB; Gamma Hydrate; Gamma-OH; Sotomax; Agrisynth BLO; Agsol ExBLO; Blue Nitro; Gamma Ram; ReActive; Renewtrient; Regenerize; Revivarant; Verve
Molecular formula	$C_4H_6O_2$
Molecular weight	86.1
Structural formula	

3. Physical and chemical properties[1]

Description	Colourless oily liquid with a mild caramel odour
Melting point	-44ºC
Boiling point	206ºC
Vapour pressure	0.15 kPa (at 20ºC)
Vapour density (air = 1)	3.0
Flash point	98ºC (open cup)
Autoignition temperature	455ºC
Explosive limits	Upper limit: 16.0 vol %, Lower limit: 3.6 vol %
Density (d_4^{20})	1.1286 g/ml
Refractive index	1.4365 at 20ºC
Solubility in water	Miscible with water (freely soluble)
Solubility in organic solvents	Soluble in methanol, ethanol, acetone, and benzene
Partition coefficient	$\log K_{ow}$ = -0.64
pH	4.51 (10 % aqueous solution)
Odour threshold	–
Surface tension	4.61 x 10^{-2} N/m
Viscosity	1.717 x 10^{-3} m^2/s at 25ºC
Conversion factors in air (20ºC, 101,3 kPa)	1 ppm = 3.57 mg/m^3 1 mg/m^3 = 0.28 ppm

[1]References for data (18, 46, 64, 71, 72, 106, 117, 118, 139, 149).



γ–butyrolactone (GBL) Dominates at low pH

γ–hydroxybutyric acid (GHB)

γ–hydroxybutyrate (GHB) Dominates at high pH

Figure 1. Hydrolysis of γ-butyrolactone (GBL).

GBL undergoes the usual chemical reactions of γ-lactones, namely hydrolytic ring opening to form GHB (Figure 1) and reactions in which oxygen is replaced by another ring heteroatom (e.g. nitrogen or sulphur). GBL is relative rapidly hydrolysed by bases and slowly hydrolysed by acids (1, 106, 118). Under strongly alkaline conditions (pH 12) GBL is completely converted to GHB within minutes. In pure water, GBL forms an equilibrium with GHB of about 2:1 over a period of months. The same equilibrium was reached within days at pH 2. Heat increases and refrigeration decreases the rate of of GBL hydrolysis relative to ambient temperature (20). GHB, when heated, can form GBL. Based on a log octanol-water partition coefficient ($\log K_{ow}$) of -0.64 a bioconcentration factor of 3.2 can be calculated (quoted from (64)).

4. Occurrence, production and use

4.1 Occurrence

Low molecular weight aliphatic lactones (C<9) occur naturally in berries, fruits and related alcoholic beverages at concentrations less than 1 mg/kg (1, 71). GBL has been found in beer (2 mg/l, (148)), apple brandy (5-31 mg/l, (136)), wine (171), vinegar (81), cooked meat (58, 93), roasted filberts (144), coffee (54), and tomatoes (77). It has also been detected in tobacco smoke condensate (113) and in mainstream and sidestream smoke (137).

Data from the Norwegian Product Register (2000) show that GBL occurs in a total of 134 products at a total of approximately 70 tons per year. Forty-nine products account for approximately 80% of the tonnage declared to the product register, with main use as a binder in foundering sand. The total tonnage in products containing GBL seems to have been more or less constant the last years. In Sweden the tonnage of GBL in chemical products was: 1996; 270 tons, 1997; 412 tons, 1998; 379 tons, and 1999; 228 tons (export not included) (Swedish Product Register, 2000).

Small amounts of GBL may be formed in the body and excreted in urine, as noted in rats following intraperitoneal administration of the nitrosamine N-nitro-sopyrrolidine (21). GHB, the hydrolysis product of GBL, occurs naturally in small amounts in mammalian brain. The level in rat brain was approximately 2 nmol/g wet weight tissue (131). The endogenous effects of GHB are not precisely known,

but GHB is believed to play a role in neurotransmission and has an effect similar to that of γ-aminobutyric acid (GABA).

4.2 Production

GBL can be prepared by a variety of methods (83, 106). The method used for commercial production in the USA is the dehydrogenation of 1,4-butanediol over a copper catalyst at 200-250ºC (46, 83, 106). GBL can also be produced by hydrogenation of maleic anhydride (83, 106).

Production of GBL in the USA in 1974 and 1992 was estimated to be 14 million kg and 45 million kg, respectively (72, 118). Information available in 1995 indicated that it was produced in six countries (17).

4.3 Use

GBL is used primarily as a chemical intermediate in the production of all pyrrolidones, as an intermediate for other organic chemicals (pesticides, herbicides and plant growth regulators), and may be formed as an intermediate in the production of vitamins and pharmaceuticals (9, 71, 72). GBL is an intermediate in the preparation of the herbicide 4-(2,4-dichlorophenoxy) butyric acid. GBL is also used as a solvent or in the production of: pesticides, photochemical etching, electrolytes of small batteries and capacitors, viscosity modifiers in polyurethanes, surface etching of metal coated plastics, organic paint disbursement for water soluble inks, pH regulators in the dyeing of wool and polyamide fibres, foundry binders (carrier solvent for the hardener for phenol formaldehyde resins), and curing agents in many coating systems based on urethanes and amides (9, 71, 72, 106).

GBL serves as intermediate in the manufacture of polymers (based on vinylpyrrolidone) used as clarifying agents in beer and wine (1, 106, 118). Low molecular weight lactones (<C8) generally exhibit a sweet herbaceous aroma accompanied by a sweet caramel-like taste and are used as flavouring agents at levels normally less than 50 mg/kg.

GBL and GHB have been used therapeutically in humans as sedatives and in the treatment of alcohol dependency (GHB dose: 0.15 g, three times daily or 50 mg/kg body weight, three times daily for 8 weeks) and the opiate withdrawal syndrome (1, 2, 36). GBL is also being used experimentally in the treatment of narcolepsy. Thus, GBL appears to have been available only as an investigational new drug for specific purposes. GHB has been under investigation in the management of narcolepsy for about two decades (dose: 4 g given twice during the night) (36, 141).

US Food and Drug Administration (FDA) banned GBL and GHB for sale as a food supplement in 1990 due to several cases of intoxication with symptoms like nausea, uncontrolled shaking, coma, respiratory depression, and even death. The FDA also called for its voluntary recall. Since the ban, GBL and GHB have been marketed illegally in the USA to bodybuilders and athletes (108). There are indications that GBL and GHB can induce sleep related growth hormone secretion

in humans (160) and have become popular with bodybuilders for “bulking up” and “building strength”. Furthermore, GBL and GHB have been implicated in an increasing number of sexual insult cases. Due to their euphoric/ hallucinogenic properties, the abuse of GBL and GHB has increased dramatically in the USA and has since 1995 also appeared in Europe, mainly in England but lately also in the Scandinavian countries (36).

The import of GBL to Sweden has been reported to be in the range of 200-300 tons/year (124).

4.4 Purity

GBL are available in different purity grades, depending on production and purification. Specifications for a US grade (not specified) of GBL were as follows: purity; 99.0%, hydroxybutyric acid; max 0.1%, water; max 0.3% (71). Other impurities reported are: 1,4-butanediol and 1-butanol (46). Traces of chlorine, sulphate, nitrate, iron, copper, zinc, lead, sodium, and potassium have been reported in “electronic” grade GBL (99.9% pure) (9). BASF report a standard grade of 99.7% purity containing a maximum of 0.05% water, 0.10% 1,4-butanediol, and 0.03% acid (w/w) (calculated as butyric acid) (9).

5. Occupational exposure data

Occupational exposure is most likely to occur from dermal contact and inhalation during production, formulation, and use. The use of GBL as a solvent in electronic industry and as a chemical intermediate could lead to worker exposure. However, very limited data describing occupational exposure were located in the literature.

Anundi and co-workers have measured the air concentration of GBL in commercial products used during graffiti removal (5). The air concentration in the breathing zone ranged from less than 0.01 to 1.1 mg/m^3 with an arithmetic mean of 0.4-0.53 mg/m^3 depending on the work task. No analyses of GBL levels in urine or plasma were performed.

The US National Institute of Occupational Safety and Health (NIOSH) estimated that 5 200 and 44 000 workers were potentially exposed to GBL in 1974 and 1983, respectively (114, 115). The number of different industries and occupations for these workers increased from 12 and 18 in 1974 to 38 and 42 in 1983. Sixty-five % of the workers were potentially exposed in printing and publishing and in textile mill industries in 1983.

6. Measurements and analysis of workplace exposure

Reports concerning industrial hygiene measurements are limited. The analysis of GBL and GHB is complicated by the small, polar nature of the molecules, which result in short retention times in high performance liquid chromatography (HPLC)

with reversed phase columns. The absence of a strong chromophoric group makes detection by ultraviolet and visible (UV-VIS) spectrometric methods difficult. Current methods use mass spectrometric (MS) methods involving derivatisation followed by gas chromatograpy (GC).

Mesmer and Satzger have reported an HPLC/UV-VIS method for separation and quantification of GBL and GHB (108). The analytical method was developed to detect GBL and GHB in illegal preparations on the black market but should also apply to analysis of work place exposures. The detection limit is 50 ng injected onto the HPLC column. Five μl samples of concentrations of 0.3 mg/ml of GBL and 0.4 mg/ml GHB were easily detected. They have also reported a simple and fast HPLC/thermospray MS method for confirmation. The characteristic mass spectrum can be obtained with as little as a 5 μg of the test chemical.

Couper and Logan have reported a simple liquid-liquid extraction procedure for the analysis of GHB in biological fluids without conversion to GBL (22). Following derivatisation to its di-trimethylsilane derivative, GHB was detected using GC/MS with electron ionisation. The quantification limit in blood was 12 μmol/l (1 mg/l) using 1 ml blood.

A fast, simple, and selective method for determination of GHB in blood and urine by headspace GC/MS has been reported (76). The method is based on the formation of GBL from GHB using headspace sampling. Analysing is done by headspace GC with flame ionisation detector or coupled to a MS. The detection limit is in the low mg/l range.

McCusker and co-workers describe a direct method for analysis of GHB in human urine (104). The method uses solid-phase extraction, liquid extraction, and silyl-derivatisation with trimethylchlorosilane followed by GC/MS using deuterated GHB (d_6-GHB) as the internal standard. The method was linear from 58-5 800 μmol/l (5-500 mg/l) and can discriminate between GHB and GBL. This method, however, is not readily applicable to the analysis of GHB in blood.

GHB has been determined in plasma and urine after it has been converted to GBL and extracted from the biological fluids together with delta-valerolactone as an internal standard (40). Final GC/MS analysis is obtained under electron ionisation, selected ion monitoring conditions. The assay was linear for plasma concentrations of GHB of 23-2 300 μmol/l (2-200 mg/l) and a urine range of 23-1 700 μmol/l (2-150 mg/l) (40).

A very sensitive and specific assay for GHB detection in brain tissue has been reported by Ehrhardt and co-workers (34). GHB was derivatised to give the corresponding pentafluorobenzyl ester of the *N-tert*-butyldimethylsilyl derivative of GHB and analysed using GC/MS with an electron capture detector. The detection limit was about 5 pg per injection. Although the brain is not suitable for biomonitoring, the method as such could possibly be adapted for use in blood, urine, or other tissues more suitable for sampling.

More recently several studies have reported analytical methods to detect GBL or GBL/GHB in body fluids. Frison and co-workers describe the detection of GHB, after conversion to GBL, and subsequent headspace solid-phase micro-extraction (SPME), and detection by gas chromatography/positive ion chemical

ionisation mass spectrometry (GC/PICI-MS) using deuterated GBL (d_6-GBL) as internal standard (47). The limit of detection for GHB (and GBL since GHB is converted to GBL) was 0.05 mg/l in plasma and 0.1 mg/l in urine. Human levels were 0.1-0.5 mg/l in plasma and 0.2-2.0 mg/l in urine.

A similar analytical method as that reported by Frison and co-workers has been published by LeBeau *et al*. (90). The limit of detection in this study was 0.5 mg/l, both in blood and urine. Duer and co-workers have analysed GBL in urine, blood, ocular fluid and brain (32). In order to analyse GBL, existing GHB is first determined, GBL is then converted to GHB under acid conditions (pH 4) followed by a second analysis by GC/MS. In this study γ-valerolactone is used as an internal standard. The limit of detection was 1.5 mg GHB/l and the limit of quantitation was 4.9 mg/l. Similar values are anticipated for GBL since a 100% conversion of GBL to GHB was reported.

Fukui and co-workers have reported a simple GC/MS method for the determination of GBL in human plasma (48). The plasma sample was spiked with deuterated GBL, extracted by dichloromethane in acidic conditions, and analysed by GC/MS.

Nuclear magnetic resonance (NMR) spectroscopy has also been used to identify and directly quantitate GBL and GBH (19), although the sensitivity is low.

Occupational Safety and Health Administration (OSHA) has briefly described an analytical method using GC with flame ionisation detection (119). SPME has been used as a sample concentration technique. SPME combined with GC/MS have been used in the analysis of volatile flavour compounds (including GBL) in kiwi fruits (170). Dahlén and Vriesman have described a method using micellar electrokinetic chromatography (23). The method, however, appears to have a relatively low sensitivity with a detection limit of 340 mg/l.

7. Toxicokinetics

7.1 Uptake

A skin permeability rate of 1.1 g/m^2/hour (0.11 mg/cm^2/hour) was reported by Ursin and co-workers using a Franz diffusion cell and human breast skin with a thickness of 300 to 600 μm (159).

According to Fasset, GBL appears to be readily absorbed through guinea pig skin (39). Dermal absorption has been studied in male Sprague-Dawley rats (49). GBL was applied directly on the shaved abdomen over a 3 x 3 cm area at a dose of 546 mg/kg body weight or after treatment with 4 ml of thioglycolic acid-based depilating agent. The maximum plasma concentration was 1.7 μmol/ml and peaked after 2 hours. The depilating agent increased to some extent the peak plasma concentration and decreased the time to reach the peak concentration. At least 10% of the percutaneous dose was absorbed and the plasma concentration approached the level (4.6 μmol/ml) required for complete hypnosis in rats (49).

GBL is rapidly and completely absorbed over a wide dose range following oral administration (7, 49, 60, 92). The oral/intracardial area under the curve (AUC) ratio in rats dosed with 136 and 546 mg/kg GBL were 0.85 and almost unity, respectively (92). The peak plasma concentration after dosing is proportional to the dose at least up to 500-600 mg/kg body weight. In rats, 136 mg/kg and 546 mg/kg GBL gave a plasma concentration of approximately 4 and 17 μmol/ml, respectively (49, 92). Peak plasma concentrations were reached within 1 hour after exposure.

Hardly any chemical hydrolysis of GBL will take place under acidic conditions. Thus, the lactone form will predominate completely in the stomach following oral administration.

No studies were located describing absorption following inhalation exposure.

7.2 Distribution

GBL is converted to GHB within minutes by enzymatic hydrolysis catalysed by the enzyme lactonase found in blood and in organs such as the liver (42, 60, 130, 133). It must be assumed that GBL is distributed in the body mainly in the form of GHB. GHB also occurs normally in mammalian brain. The highest concentrations of GHB in the human brain are found in the substantia nigra, thalamus and hypothalamus. Ten to fifteen times higher concentrations are found in kidneys, heart, muscles and fat (36).

7.3 Biotransformation

The initial step in the metabolism of GBL is its conversion to GHB. After parenteral or oral administration of GBL to rats the parent compound is rapidly hydrolysed to GHB in blood and liver by lactonase. Other tissues of the rat such as brain, heart, skeletal muscle, intestine, and cerebrospinal fluid were substantially less capable of enzymatic hydrolysis of GBL. In *in vitro* studies the half-time of GBL in rat blood was less than 1 minute (42, 130). For comparison, at pH 7.4 the nonenzymatic hydrolysis half-time of GBL is about 1 000 days (7). *In vivo* absorption studies (see section 7.1 Uptake) have shown that GBL is extensively metabolised to GHB within minutes after absorption. A comparison of human and rat lactonase activity in serum showed a similar V_{max} and a very high K_m (1-3x10^{-2} M) in both species (133). Fishbein and Bessman have reported V_{max} values of 2.18 and 17.2 μmol/10 minutes/mg protein for rat liver and human plasma lactonase, respectively (42). In this study the equilibrium between GBL and GHB in the presence of lactonase (source not described) was also studied. At pH 7.4 only 1.5% existed as lactone. When increasing the pH above the pK_a (i.e. 4.72) more of the acid will be ionised and unavailable for lactonisation.

The metabolism of GBL has recently been reviewed (1, 118). It appears that the pathway for GHB metabolism has not been completely characterised, and may vary either quantitatively or qualitatively depending on plasma levels of GHB and the organ, i.e. whether it is endogenous GHB in the brain or exogenously

administrated and metabolised in the liver (118). Below are reported studies that investigate the further metabolism of GHB. Most of these studies were performed between 1960 and 1975.

Several pathways have been suggested for the metabolism of GHB, such as its conversion into succinic acid and other citric acid cycle intermediates (30, 41), interconversion into GABA (31, 101, 134, 163), and breakdown via β-oxidation (168).

It was originally suggested that GHB is metabolised by entry into the citric acid cycle. Oxidation of GBL to succinate by alcohol dehydrogenase and succinate semialdehyde dehydrogenase occurs primarily in the liver. Succinate then participates in the citric acid cycle (30, 41, 91, 110). However, when incubating rat liver homogenate with ^{14}C-GHB only 6% or less of the radiolabel appeared in succinic acid (130). Furthermore, only a very small proportion of the radiolabel from [1-^{14}C]- and [4-^{14}C]-GHB administrated intravenously or intraperitoneally to rats or cats appeared in succinate (133, 168). It is now accepted that linear aliphatic hydroxycarboxylic acids in general are hydrolysed and rapidly oxidised via the fatty acid pathway. GHB will form acetyl CoA that enters the citric acid cycle and ends up as CO_2.

Brain slices taken from adult male Wistar rats have been shown to metabolise GHB to GABA via a transamination mechanism (γ-aminobutyrate-2-oxoglutarate transaminase) and not through the citric acid cycle (163).

Intermediates of GBL have been detected in human urine after oral administration of GBL(91). Four humans (two males and two females) were given a single oral dose of GBL and urine was collected hourly. (S)-3,4-Dihydroxybutyric acid, glycolic acid and 4-hydroxy-3-oxobutyric acid were present in the urine (91) (Figure 2). The data strongly support that the GBL metabolism, following its hydrolysis to GHB, occurs via β-oxidation in humans.

7.4 Excretion

GBL is eliminated primarily as respiratory CO_2 and urinary metabolites. In humans the rate of urinary excretion of GBL metabolites was 1.2 mg/hour in controls (from dietary and endogenous sources) and 40 mg/hour over a 5-hours period following an oral dose of 1 000 mg of GBL (91). A relatively short terminal half-time of 30 minutes for GHB, due to extensive liver metabolism following oral administration to humans and rats, has been reported (49, 85). The initial half-time in plasma of GBL following an oral dose of 136 mg/kg body weight of GBL to rats is about 1 hour and the terminal half-time about 30 minutes (92). The apparent delayed initial elimination could be due to the rapid oral absorption rate of GBL resulting in nonlinear kinetics.

In rats, [1-^{14}C] or [4-^{14}C]-GHB given intraperitoneally (500 mg/kg) is excreted as $^{14}CO_2$. About two-thirds of the dose was excreted in this manner within 6 hours, and an additional 10-20% over the next 18 hours. The rate of the oxidation to CO_2 suggests a final breakdown via the citric acid cycle. The most likely hypothesis

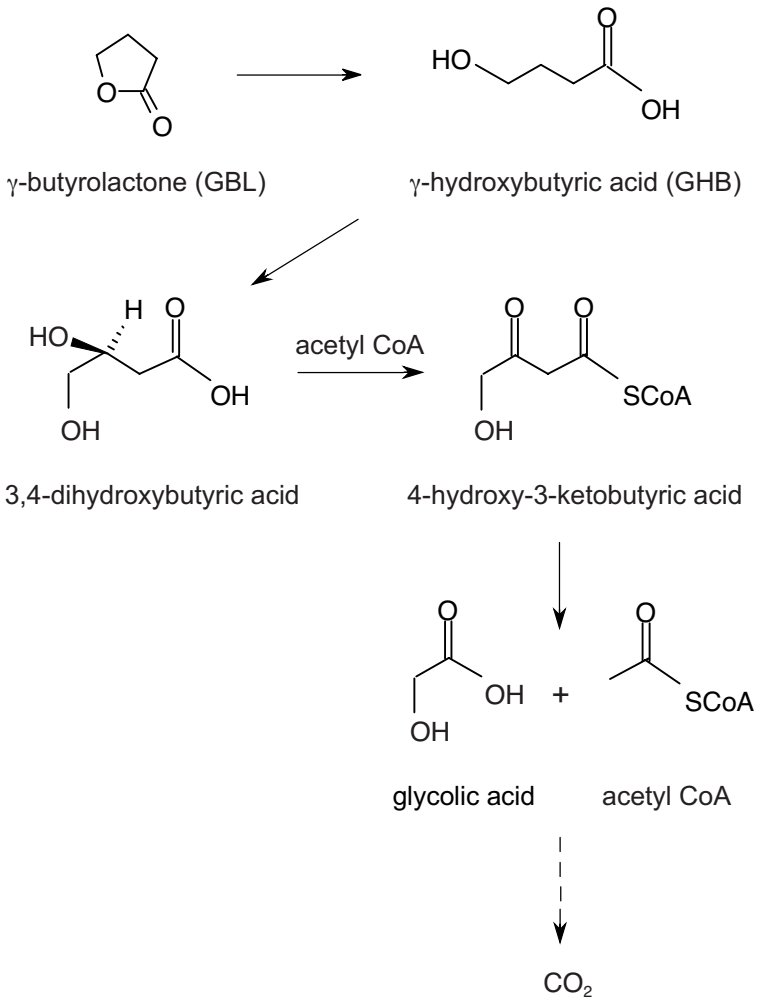


Figure 2. Metabolism of γ-butyrolactone (GBL) in humans. Adapted from (1, 91).

for the biological degradation of GHB is via β-oxidation as the primary step, rather than further oxidation of the terminal hydroxyl group (168).

Following a single intravenous dose of 2 μC ^{14}C-labelled GHB in rats, traces of $^{14}CO_2$ could be detected in respiratory air after less than 4 minutes, and a maximum was reached after 15 minutes. Sixty percent of the total ^{14}C was eliminated as $^{14}CO_2$ within 2.5 hours (130, 133). Similar results were obtained with [1-^{14}C]-GBL. However, the peak of $^{14}CO_2$ was reached in 20 minutes probably reflecting the time needed to convert GBL to GHB (133). From this study the overall half-time of GHB in blood after a 500 mg/kg body weight dose of GBL given intravenously, can be estimated to be about 45 minutes.

Following intraperitoneal administration of 500 mg/kg body weight of GBL to rats the concentration of GBL in brain fell from 170 μg/g tissue at 3 minutes to 29 μg/g tissue at 15 minutes post exposure (60). A study by Möhler and co-workers indicate a half-time of GHB in mouse brain of about 5 minutes following intravenous injection of [1-^{14}C]-GHB (dose was not stated) (110).

8. Methods of biological monitoring

There is no established method for biological monitoring of GBL and GHB. Several methods for determining concentrations of GHB in blood and urine have been reported. Some of the methods used to detect GBL and GHB in biological media are reported in Section 6. Theoretically biological samples will contain both GHB and GBL. Some analytical methods convert GHB back to GBL by heating under acid conditions whereas others are able to discriminate between GBL and GHB. Biological monitoring should ideally take into account both the levels of GHB and GBL in the body. However, since GBL is enzymatically converted to GHB, the levels of GHB measured in the body will within a few minutes after exposure most likely reflect the exposure dose (i.e. GBL). On the other hand, the relatively short half-time of GHB in blood limits its usefulness in monitoring. Intermediates of GBL have been detected in human urine after oral administration of GBL. (S)-3,4-Dihydroxybutyric acid, glycolic acid and 4-hydroxy-3-oxobutyric acid were present in the urine of exposed humans (91). However, the usefulness of these metabolites in biomonitoring remains to be shown.

9. Mechanisms of toxicity

The major concern of GBL is its effect on the CNS. However, GBL also causes eye irritation and may have the potential to induce reproductive toxicity.

GHB induces CNS depression at dose levels that are approximately 100 times those that occur naturally (endogenously) in the brain. The sedation and stupor observed in experimental animals by GBL is likely attributed to its principal metabolite, GHB, or possibly to GABA that can be formed from GHB. GABA seems to be the major precursor of endogenous GHB in the brain although GHB formation represents only a minor route of GABA metabolism (57, 118, 134).

It has been suggested that GHB may be involved in synaptic transmission based on its low and heterogeneous distribution in the brain, extremely rapid turnover rate (57), the immunocytochemical localisation of the GHB synthesising enzyme in the brain (172), and high-affinity binding and release (11, 98-100). GHB has specific binding sites in the brain, where it exerts a GABA-like activity i.e. inhibits dopamine release (14). The affinity to the specific GHB-receptor is approximately 1 000 times that of the $GABA_B$-receptor. At physiological concentrations GHB appears not to have a full agonistic effect on the $GABA_B$-receptor. GHB does not bind to the $GABA_A$-receptor. Thus, most likely the pharmacological effects of GBL are directly mediated by $GABA_B$-receptors (13, 80).

Anaesthetic doses of GBL or GHB produce an acute blockage of cell impulse flow in the nigro-striatal dopaminergic pathway for at least one hour (36, 118, 132, 169). The symptoms of CNS effects of GHB may be explained by an initially inhibited dopamine response followed by an increased dopamine release. It has

been suggested that GBL causes an increase in brain dopamine by antagonising transmitter release from nerve terminals (35). The mechanisms by which GHB exerts its effects in the brain have, however, not been fully elucidated.

The anabolic characteristic of GHB is correlated to, but not associated with, an increased level of prolactin. The anabolic effect of GBL seems, however, to be due to increased sleep-related growth hormone secretion (160). It has been suggested that the decreased alcohol intake observed following GBL exposure to rats with a preference to alcohol, is mediated by an inhibition of firing in dopaminergic neurones (37, 116).

There are indications that GBL may adversely affect reproduction in experimental animals. The inhibitory effect of GBL on ovulation in rats was suggested to be caused by hormonal effects in CNS resulting in reduced levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), and not by a direct effect of GBL or its main metabolite GHB on reproductive organs (10). However, in a resent *in vitro* study by Kubelka and co-workers GBL was shown to arrests meiotic maturation of bovine oocytes probably by inhibiting the activation of p53 kinase or mitogen-activated protein kinase (88). This result provides some evidence for a direct effect on the oocytes.

The mechanism for eye irritation is not known. One could, however, speculate that the rapid biotransformation of GBL to GHB and the resulting equilibrium between the acid and the anion may be a contributing factor.

10. Effects in animals and *in vitro* systems

10.1 Irritation and sensitisation

In a review paper, undiluted GBL is quoted not to cause skin irritation after a 20-hour application to the skin of the back of white rabbits (18). In an older study, GBL induced some skin irritation in the guinea pig (39). The details given in these studies do not allow a grading of the response according to current used grading systems. Altogether it appears that GBL has a weak skin irritation potential.

In an eye irritation test, lesions were observed in the cornea, iris, and conjunctiva after instillation of undiluted GBL in the conjunctival sac. The reported damage to the cornea and iris was reversible (quoted in (18)). GBL was evaluated to be an ocular irritant in an *in vitro* bovine corneal opacity test (52, 53). The effect was completely reversible after 14 days (53). GBL was also positive in the hen's egg test-chorioallantoic membrane (HET-CAM) assay, which can detect potential *in vivo* irritant effects on the conjunctiva (55, 56). The available information with respect to GBL-induced eye irritation is limited and based mostly on older studies supported by new *in vitro* assays. However, these studies show that GBL is an experimental eye irritant that apparently does not lead to permanent eye damage. The eye irritant effect of GBL is likely to be expressed also in humans.

In guinea pigs, no indications of a skin sensitising effect were seen in tests not described in more detail (quoted from (39)). However, the substituted GBLs, α-methyl-γ,γ-dimethyl GBLs and α-methylene-GBL (tulipalin), are skin sensitizers (45, 105). The present toxicological information does not allow an evaluation of GBL skin sensitisation potential.

10.2 Effects of single exposure

Data on the lethal dose for 50% of the exposed animals at single administration (LD_{50}) of GBL is summarised in Table 1. Most of the studies available were performed between 1960 and 1970 and used oral administration. No clinical signs of toxicity following oral administration were reported other than dose-related anaesthetic effects, characterised by loss of righting reflex (62, 89).

From Table 1 it is concluded that GBL has a moderate to low acute toxicity in laboratory animals. The dermal LD_{50} in guinea pigs is considerable higher than the oral LD_{50} (39).

In a study by Monsanto Corporation, Sprague-Dawley rats were exposed by inhalation to 5 100 mg/m^3 of GBL for 4 hours (83% of the particles measured 10 microns or less). No deaths occurred during treatment or the 14-day post-exposure observation (111). Rats exhibited of toxicity prostration, lethargy, shallow breathing, limb disuse, and clear discharge from the nose. The effects were clearly reversible. No treatment-related pathological effects were found at terminal necropsy (111).

Low doses of GBL (intraperitoneally or intravenously: 100 or 200 mg/kg) have a biphasic effect on locomotor activity in the rat (1, 25). The acute toxicity of GBL by intraperitoneal administration was also studied in male white mice (strain R3), and in male Wistar rats. Each dose was injected to 5-8 animals. The LD_{50} for mice was 1 100 mg/kg, and that for rats was 1 000 mg/kg. GBL caused anaesthesia in both species. Doses of GBL above 200 mg/kg almost completely abolished motility in mice and rats. Respiration was markedly slowed and increased in amplitude, reactions to acoustic stimuli were weaker or abolished while reactions to pain stimuli and righting reflex were maintained. Doses of 400 or 500 mg/kg in mice abolished the righting reflex already after 5 minutes without affecting pain reflexes. In rats, the same doses produced deep sleep with loss of righting reflex and pain reflexes. Doses above 800 mg/kg in both species induced

Table 1. Acute toxicity of GBL in different species.

Species	Route of administration	LD_{50} (mg/kg)	Reference
Rat	oral	1 800	(89)
Mouse	oral	1 260	(62)
Mouse	oral	1 245	(140)
Mouse	oral	800-1 600	(39)
Guinea pig	oral	500-700	(46)
Guinea pig	dermal	Approx. 5 600	(39)

deep anaesthesia in which animals died after several hours as a result of respiratory paralysis (146). Intraperitoneal administration of 150-200 mg/kg body weight of GBL to adult Sprague-Dawley rats resulted in immobilisation of the animals and staring behaviour. In infant or young rats, 50 mg/kg body weight (lower doses were not tested) GBL induced behavioural arrest with staring and limb extension (152).

10.3 Effects of short-term exposure

In a study by Nowycky and Roth, the effect of repeated exposure on the CNS was studied in rats (116). Sprague-Dawley rats were given a 1% solution of GBL (about 3 000 mg/kg body weight) in the drinking water for 3 to 4 weeks and then given a single intraperitoneal injection of 350 or 750 mg/kg body weight of GBL. The rats developed a tolerance to the behavioural effects of GBL (measured as duration of loss of righting reflex after a challenge dose of GBL and to elicit increased dopamine synthesis). The four weeks exposure caused only a slight but significant reduction in weight gain in male rats.

F344/N rats and B6C3F$_1$ mice (5 animals per dose and sex) received GBL in corn oil by gavage for 12 consecutive days, excluding weekends (i.e. 16-day study) (118). The daily doses were 0, 75, 150, 300, 600 or 1 200 mg/kg body weight in rats and 0, 87, 175, 350, 700 or 1 400 mg/kg in mice (Table 2). Complete necropsies were performed on all animals. All rats receiving 1 200 mg/kg GBL died within the first 3 days of exposure. One male receiving 600 mg/kg died on day 3. There were no significant differences between the final mean body weights of male rats administered GBL and controls. The mean body weight gain of the female rats given 600 mg/kg was significantly lower than in controls. The mean body weight gains of female rats given 300 mg/kg or less and all male rats were similar to those of the controls. Rats in the 600 and 1 200 mg/kg groups became recumbent or inactive with irregular and laboured respiration soon after dosing (118). All male mice and 4 female mice receiving 1 400 mg/kg died before the end of the study. Mean body weight gains of dosed mice were similar to those of controls. Mice receiving a dose of 350 mg/kg or more became recumbent or inactive shortly after dosing. Some mice also exhibited irregular respiration or dyspnea (118).

10.4 Effects of long-term exposure and carcinogenicity

In connection with the 2-year NTP bioassay F344/N rats and B6C3F$_1$ mice (10 animals per dose and sex) were dosed with GBL by gavage for 90 days (118). The doses were 0, 56, 112, 225, 450, or 900 mg/kg body weight in rats and 0, 65, 131, 262, 525, or 1 050 mg/kg in mice, 5 days/week (Table 2). All animals were observed twice a day and clinical observations were recorded once a week. Necropsy was performed on all animals and the following organs were weighed: brain, heart, right kidney, liver, lung, and thymus. Complete histopathology was carried out on all animals that died or were killed moribund during the study, all

controls, the 900 mg/kg rat group, the 450 mg/kg male rat group and 1 050 mg/kg mice group. The study was conducted in compliance with the FDA Good Laboratory Practice (GLP). All male rats and one female rat given 900 mg/kg GBL died by week 8. The final body weights and body weight gains of males in the 450 mg/kg group were significantly lower than those of the controls but unaffected in males at lower doses and in females at all doses. All rats in the 900 mg/kg rat dose groups became recumbent within several minutes after dosing, but appeared normal later. Rats in the 225 and 450 mg/kg dose groups exhibited slight inactivity after dosing. However, after 2 to 3 weeks an adaptation to this anaesthetic effect occurred. At necropsy no significant biological differences in absolute or relative organ weights between exposed and control rats were noted and no gross lesions related to GBL exposure were reported. Increased incidences of inflammation of nasal mucosa were noted in some dose groups but are likely to be related to the reflux of the gavage solution into nasopharynx after dosing. The significance of the mice study is somewhat reduced due to a relative high number of deaths due to improper gavage technique. Deaths related to GBL administration occurred in three male and one female mice from the 1 050 mg/kg dose group. Except for 11% lower mean body weights of male mice in the 1 050 mg/kg dose group, no reduced final mean body weight was detected in the other dose groups compared to controls. As with rats an adaptive response to the anaesthetic effect of GBL was reported in mice given 525 mg/kg or less. There were no biologically significant differences in absolute and relative organ weights between exposed and control mice. No gross or microscopic lesions related to GBL administration were observed (118).

Among the 12 male weanling albino rats given a total of 4 doses ranging from 200 to 900 mg/kg of GBL by gavage during a 7.5 months period, the ones that received amounts in excess of 700 mg/kg died within a few days from respiratory failure and lung congestion (142). Animals that died showed degenerative lesions and calcifications of the heart and kidneys. However, smaller doses (100-400 mg/kg) were well tolerated and could be given repeatedly. Apparently several rats showed chronic inflammatory lung lesions with bronchiectasis (i.e. abnormal dilatation of bronchi). Also interstitial hyperplasia was present in the testes of two rats. However, similar chronic lung and kidney lesions were found among control rats. Six of the exposed rats survived for more than 12 months after the last dose. Of these, five developed tumours: one of the rats developed an interstitial cell tumour of the testes, two developed squamous cell carcinomas of the jaw, and two developed pituitary tumours. Similar pituitary tumours were found in the control group. Testicular interstitial cell tumours and jaw tumours were reported to occur occasionally in ageing control rats. The GBL used in this study was obtained by distillation from an epoxy resin hardener consisting of 4,4´-diaminodiphenyl-methane (142). The amount of 4,4´-diaminodiphenylmethane occurring together with GBL in the distillate was not reported.

Ninety-five male NMRI mice received 750 mg/kg GBL orally once per week for 18 months. There was no statistically significant difference in the incidences of lymphomas and lung adenomas between exposed and untreated animals (68).

No local tumours were observed in a group of 16 female Swiss-Webster mice given a total of 12 subcutaneous injections of 0.005 mg GBL in 0.1 ml tricaprylin three times a week for 4 weeks and observed for at least 18 months (151).

A 2-year gavage study was carried out according to the NTP protocol for carcinogenicity testing (118). Groups of 50 F344/N rats and $B6C3F_1$ mice of each sex were given GBL in corn oil by gavage 5 days a week for up to 103 weeks (Table 2, 3). Male rats received 0, 112, or 225 mg/kg body weight, female rats received 0, 225, or 450 mg/kg body weight, and mice received 0, 262, or 525 mg/kg body weight. The mean body weight of high dose female rats was lower than that of the controls. There was no evidence of carcinogenic activity of GBL in male or female rats. In the female rat, negative trends were observed in the incidences of cysts and fibroadenomas of the mammary gland, and in cysts of the pituitary pars distalis. Decreased mean body weight and CNS depression were noted shortly after exposure in the mice. There was equivocal evidence of carcinogenic activity in male mice given 262 mg/kg. Increased incidences of proliferative lesions, primarily hyperplasia, of the adrenal medulla in low-dose male mice were associated with GBL exposure (pheochromocytoma, benign or malignant: 2/48 controls, 6/50 low-dose, 1/50 high-dose; hyperplasia: 2/48 controls, 9/50 low-dose, 4/50 high-dose). The incidence of hepatocellular neoplasms in exposed male mice was lower than that in the controls. The sensitivity of the study in male mice to detect a carcinogenic effect was reduced by a low survival of high dose males. There was no evidence of carcinogenic activity in female mice (118).

In mice given repeated skin applications of one drop of a 1% solution of GBL in acetone twice weekly for life, the incidence of lung tumour was 21/30 (70%) compared with 9/17 (53%) in acetone-treated controls. No skin tumours were observed (136). In newborn mice given subcutaneous injections of 1 μg GBL on days 1, 4, and 8, 18/34 (53%) of the animals developed lung tumours whereas 27/44 (61%) of the controls had lung tumours (136).

Mice of both sexes were given 2 mg doses of GBL in 0.1 ml water (about 57 mg/kg) orally twice weekly for life. In treated mice, the average survival was 571 days compared with 595 days in untreated controls. In this case, the incidence of lung tumours was 20/36 (55%) compared with 27/44 (61%) in untreated controls (136).

Mice were painted on the clipped dorsal skin with 0.1 ml of a 10% solution of GBL in benzene (which corresponds to about 330 mg/kg), three times weekly during their total lifespan. Non-carcinogenic effects were not evaluated in this study and no increase in tumour incidence above that observed in benzene-treated controls was found (161). In a second study GBL was dissolved in acetone and administered three times weekly for 495 days. No increase in tumour incidence above controls was observed (162).

Mice of both sexes received a diet containing 1 000 mg GBL/kg of diet for life. No increases in the incidence of mammary tumours in female mice (exposed: 19/30; untreated 43/61) or of hepatomas in male mice (exposed: 5/30; untreated 6/54) were observed (136).

Male Wistar rats received subcutaneous injections of 2 mg GBL in *Arachis* oil twice per week for 61 weeks and were observed up to 100 weeks. All rats survived, and no tumours were observed (29).

Chemical structure combined with short-term genotoxicity and toxicity tests has been used to predict carcinogenicity. Tennant and co-workers have predicted that GBL is not a genotoxic carcinogen and is unlikely to be a non-genotoxic carcinogen (153). King and Srinivasan have predicted that GBL is not a carcinogen based on molecular structure using inductive logic programming (82).

An overall evaluation of the carcinogenicity data shows that GBL in not an experimental carcinogen in rats and mice.

10.5 Mutagenicity and genotoxicity

A large number of mutagenicity studies of GBL have been performed and data thereof is described in several reviews (1, 18, 26, 71, 72, 118). Generally, *in vitro* experiments with and without exogenous metabolism (S9) were performed. The results of the *in vitro* and *in vivo* mutagenicity studies are summarised below. The *in vivo* studies are described in more detail. The individual studies are listed in Tables A1-A5 in Appendix 2.

GBL has been studied in several tests to detect a DNA damaging potential. Such tests include an ADP-ribosyl transferase (ADPRT) mediated decrease in NAD-content in human amnion FL cells without activation (38, 174), a lambda induction assay with activation (154), a modified liquid suspension assay in *E. coli* without activation (129), SOS chromotest in *E. coli* with and without activation (107, 126), several differential toxicity tests in *B. subtilis* with and without activation (59, 74, 78, 158), unscheduled DNA repair synthesis in HeLa S3 cells with and without activation (102), DNA repair in Chinese hamster ovary cells (deficient in nucleotide excision repair) without activation (69), and DNA alkylation of calf thymus DNA without activation (65). The vast majority of these tests were negative. A weak positive response was found in the modified liquid suspension assay in one of the *E. coli* strains (129) and in one of the four differential toxicity tests when using fish S9 (78). Based on the overall results from these tests for primary DNA damage, GBL is considered to be negative.

A large number of studies investigating possible gene mutations (reverse mutation) in bacteria have been reported in the literature. Several test strains of *S. typhimurium,* capable of detecting both base pair substitutions and frame shift mutations (3, 8, 12, 50, 51, 63, 70, 73, 97, 112, 128, 135, 147, 156, 164), but also strains of *E. coli* have been used (51, 103, 164). In all these studies GBL has been tested both in the presence and absence of metabolic activation. GBL was negative in all studies.

GBL has also been tested for cytogenetic and mutagenic effects in yeast. These studies include tests for gene conversion (75, 143, 175), mitotic crossing-over (79), reverse (109) and forward (94) mutations, and aneuploidy (122). Most of the tests were performed with and without metabolic activation. The only positive effect was found using the JD1 strain of *S. cerevisiae* and only when GBL was

dissolved in dimethyl sulfoxide (DMSO) (not with ethanol) in the absence of metabolic activation (143). Thus, GBL is considered not to elicit genotoxic effects in yeast.

Studies on chromosomal damage in mammalian cells *in vitro* have given conflicting results using GBL. In a study by Loveday and co-workers chromosomal aberrations and sister chromatid exchanges were reported in Chinese hamster ovary cells, in the presence but not in the absence of an exogenous metabolism system, at high concentrations (96). Such effects were not found using Chinese hamster ovary cells (sister chromatid exchange) or rat liver RL_1 cells (chromosomal aberrations), at lower concentrations (27, 123). A negative result was also obtained in an *in vitro* gene mutation test using a human fibroblast cell line (HSC172). However, in this test no exogenous metabolism system was included, whereas the other studies were conducted with and without activation. No clear conclusions regarding genotoxic effects of GBL in mammalian cells can be drawn from these experiments.

GBL gave positive results in a baby kidney hamster (BHK-21) cell oncogenic transformation assay (Styles test) using growth in soft agar as the end point. This study is well documented and the test includes auxiliary metabolism (S9) (150). In another study also using BHK-21 cells (with and without metabolic activation), and higher concentrations of GBL than in the Styles study, no increased rate of morphological transformations was noted. However, in the last study positive controls came out negative. In general, the usefulness of the cell transformation assay in predicting carcinogenic effects of chemical is debated. Furthermore, an evaluation of the Styles test has indicated that it is not very useful due to low predictability. Thus, no conclusion can be drawn with respect to the ability of GBL to induce morphological transformation in mammalian cells.

Tests for sex-linked recessive lethal mutations and mitotic recombination in *D. melanogaster* following administration of GBL in feed (up to 2.8% in feed) were negative (44, 118, 166, 167). GBL was also negative in two separate micronucleus tests using bone marrow from mice. In the first study $B6C3F_1$ mice were given two consecutive intraperitoneal injections of 984 mg/kg body weight of GBL (138) and in the second study CD-1 mice were administered two consecutive intraperitoneal injections of 560 mg/kg of GBL (157). GBL was also negative in other *in vivo* tests (mutagenicity and sperm morphology) using mice (121, 155). Taken together these studies show that GBL does not express a mutagenic potential *in vivo* at doses up to about 1 000 mg/kg body weight.

Based on all the above studies it is concluded that GBL is not mutagenic. However, the possibility that GBL may cause chromosomal aberrations and sister chromatid exchanges *in vitro* cannot be completely ruled out.

10.6 Reproductive and developmental effects

10.6.1 Fertility

No standard one- or two-generation fertility studies in experimental animals were located in the open literature, either with GBL or GHB. Most repeated dose

studies have not revealed toxic effects to the testes. However, one study showed a reduced gonadal development resulting in significant reduced testicular weights in rats exposed to GBL (see 10.6.2).

Proestrous serum LH level and ovulation were significantly reduced when GBL in saline was injected intraperitoneally at doses from 62.5 to 750 mg/kg body weight in 4-day cyclic female Sprague-Dawley rats just prior to the proestrous critical period (Table 2) (10). A reduction in FSH was noted at doses of 500 mg/kg and higher. At this dose, increases in uterine wet weight accompanied the increased incidence of uterine ballooning, but only the 750 mg/kg dose showed a significant increase above controls. No change was noted in ovarian weight. The antiovulatory effective dose in 50% of population (ED_{50}) was approximately 250 mg/kg, which is a subanaesthetic dose. A reduction in the number of rats ovulating was evident at 62.5 mg GBL/kg, with a 63% inhibition at 250 mg/kg. A dose of 750 mg/kg blocked ovulation (10). This study indicates that GBL may interfere with female reproduction. Additional studies, preferentially also in an additional species, are needed to address the relevance of these findings to humans.

GBL has been shown to almost totally block reversibly germinal vesicle breakdown (i.e. inhibiting the first meiotic metaphase) in bovine oocytes *in vitro* at a concentration of 100 μM (88). The relevance of this finding to human reproduction is not clear.

10.6.2 Developmental toxicity

The possibility, that GBL might be embryotoxic and/or teratogenic was examined in the rat (86, 87). GBL was administered by gavage on gestation days 6 through 15. The dose levels were 10, 50, 125, 250, 500, and 1 000 mg GBL/kg/day (10 rats per group). On day 21 the females were anaesthetised and the foetuses were removed by Caesarean section. No significant differences were found between the control group and the treated groups with regard to corpora lutea and total implantation sites, alive and dead foetuses, resorptions, preimplantation and post-implantation losses, or male/female ratios. No embryotoxic effects were seen (86, 87). No major soft tissue anomalies or skeletal defects were found. In the 500 mg/kg group a slight decrease in the incidence of bipartite centra of the thoracic vertebrae was found. Furthermore, a slight increase in the frequency of unossified hyoid cartilage was reported at 10 and 125 mg/kg. Foetal weight was, however, significantly increased in rats given 50, 125, and 250 mg/kg compared to controls. Placental weights were significantly reduced for all GBL treated animals. The foetal skeletal alterations were not dose-dependent and were by the authors considered not to be due to GBL exposure. These results indicate that oral administration of GBL up to a dose of 1 000 mg/kg does not cause developmental toxicity in rats.

Male Wistar rats (aged 21 days) were given free access to tap water containing 1% or 2% GBL (Table 2) (28). The corresponding doses of GBL were calculated to be approximately 1 100 and 2 200 mg GBL/kg/day. In a second experiment, animals were given 0.5% or 1.0% GBL. 0.5% (approximately 550 mg/kg) and

higher was shown to reduce gonadal development resulting in significant reduced testicular weights. Body weights were not affected in the rats exposed to 0.5% and 1.0% in the second experiment, but was reduced in the rats exposed to 1% and 2% in the first experiment. The reduction in testicular weight was about 40% at 0.5% GBL and about 50% at 1.0% GBL. The effect of GBL on testicular weight was apparently not due to decreased feeding or to a generally smaller increase in body weight. However, seminal vesicle weights and serum prolactin levels were similar in the control rats and in the rats treated with GBL (28). The study is relatively poorly reported, e.g. the exposure time is not stated. Based on treatment schedules with other substances tested in the same study, an exposure time of 20-21 days is assumed.

10.7 Other studies

No other relevant studies were available.

11. Observations in man

11.1 Acute effects

No reports were located describing effects following acute occupational exposure.

Our earlier knowledge of acute systemic effects in humans is based mainly on poisonings after oral intake of GBL and its use as a drug. GBL and GHB have been tested for therapeutical use in humans as a sedative and in the treatment of alcohol dependency (GHB dose: 0.15 g, three times daily or 50 mg/kg body weight, three times daily for 8 weeks) and the opiate withdrawal syndrome (1, 2, 36). GBL is also being used experimentally in the treatment of narcolepsy. GHB has been under investigation for management of narcolepsy for about 2 decades (dose: 4 g given twice during the night) (36, 141).

GBL is illegally marketed for many claimed purposes, including inducing sleep, releasing growth hormone, enhancing sexual activity and athletic performance, reliving depression, and prolonging life. The recent ever increasing use of GBL and GHB by younger people as a drug and to some extent by athletes to increase muscle mass, have given additional information on dose-effect relationships. The most frequent intoxications with GHB result from its use as a drug, often together with alcohol and other drugs and at peroral doses of 2-3 g (35 mg/kg body weight) (36).

Acute toxic effects based on human cases include bradycardia, hypothermia, CNS depression, prolonged unconsciousness (typically for 1-2 hours), confusion, combativeness, obtundation, and uncontrolled movements, and are similar to those seen in experimental animals (15, 16, 36, 118, 165, 173).

Manifestations of acute GHB toxicity include amnesia and hypotonia at dose levels of 10 mg/kg body weight; a normal sequence of rapid eye movement (REM) and non-REM sleep at 20-30 mg/kg body weight; and anaesthesia at 50

mg/kg body weight. 50-70 mg/kg body weight may induce coma (15, 36, 124). GBL is more potent than GHB and life threatening effects are likely to occur at lower doses than with GHB (85). A lethal peroral dose of GHB has been suggested to be in the order of 500 mg/kg body weight (124). The effects of GHB on the CNS are potentiated by concomitant intake of alcohol and other central stimulants such as amphetamine, ecstasy, and cocaine (36). Surgical anaesthesia is obtained at a dose of approximate 60 mg/kg body weight (142). Euphoria has been reported at dose levels of 20-30 mg/kg body weight (64).

In Scandinavia some cases of poisoning from GBL in children have been reported after ingestion of small amounts (less than 8 ml) of GBL. GBL had a narcotic effect after ingestion and caused unconsciousness rather rapidly (43). A 2-year-old boy was found unresponsive approximately 40 minutes after ingestion of GBL used as a solvent to remove methacrylate glues. The patient was apneic, bradycardic, and flaccid. Six hours after oral intubation, he was alert and breathing spontaneously (67). Some additional case reports from Scandinavia have been published. Two males in their twenties lost consciousness after ingestion of 50 ml nail varnish containing 50% GBL and 50% ethanol. Bradycardia was observed and treated during the first hours, and the patients recovered after a few hours (4). Coma, respiratory depression, and bradycardia were reported in two cases of GBL poisoning following ingestion of a nail polish remover (127).

More than 50 cases of GBL poisonings have been reported in USA (16). The US Centers for Disease Control and Prevention (CDC), has described some cases of GBL intoxication (16): A 24-years-old man vomited and had seizures shortly after drinking 3-4 oz of Revivarant (80-105 mg GBL/kg body weight). A 46-year-old women had a seizure and lost conscious after drinking approximately 2.7 oz (70 mg GBL/kg body weight) of Revivarant in conjunction with ethanol. A 31-year-old man drank approximately 1 oz (26 mg GBL/kg body weight) Revivarant, four beers, and a large sip of wine. Shortly thereafter he gradually lost consciousness. Two men (24- and 26-year-old) drank 10-13 oz (240-340 mg GBL/kg body weight) Revivarant together with alcohol. Both men became unresponsive and altered between somnolence and confusion.

In UK a near fatal GBL intoxication has been reported in a 44-year-old male having ingested several hundred ml of a “health drink”-“Furumax Revitaliser” containing 8 g/100 ml of GBL. An intake of 500 ml would correspond to approximately 570 mg/kg body weight (33). Shortly after, he became unconscious with shaking of the limbs. Respiratory effort was poor and the patient required additional oxygen.

In Italy an alveolar gas exchange impairment has been reported in a 4-year-old child following ingestion, and perhaps also inhalation of chemical product (Destak, paint remover solvent) containing GBL and may be due to a direct toxic effect on the alveolar-capillary membrane (125). The child had a progressive shortness of breath causing cyanosis and eventually respiratory failure. Chest x-ray examination showed diffuse bilateral interstitial oedema and the absence of cardic enlargement.

11.2 Irritation

No published data on skin and eye irritation were found in the scientific literature.

11.3 Effects of repeated exposure on organ systems

No information addressing possible toxic effects on specific organ systems following exposure to GBL was found. However, chronic use of GBL can lead to several neurotoxic effects, including anxiety, depression, tremor, and insomnia (66).

11.4 Genotoxic effects

No information describing genotoxic effects of GBL in humans was found.

11.5 Carcinogenic effects

Kogevinas and co-workers studied the incidence of non-Hodgkin's lymphoma and soft tissue sarcoma in two nested case-control studies in workers exposed to phenoxy herbicides, chlorinated phenols, and dioxins (84). GBL was one of several agents evaluated. The two studies were conducted within an international cohort of workers. Odd ratios are based on cumulative exposure scores grouped in four categories (non-exposed, low, medium and high exposure). One case of soft-tissue sarcoma and one control were classified as exposed (odds ratio, 5.0; 95% confidence interval (CI), 0.3-80). Two cases of non-Hodgkin's lymphoma and three controls were identified as exposed (odds ratio, 3.0; 95% CI, 0.50-18.1). The results are based on few cases and exposure to many of the compounds examined was highly correlated, complicating the identification of the effect of individual chemicals. Thus, no conclusions can be drawn regarding the capability of GBL to cause cancer in humans.

11.6 Reproductive and developmental effects

No human data were available on fertility and developmental toxicity.

12. Dose-effect and dose-response relationships

There are limited and uncertain data concerning dose-effect and dose-response in humans following acute exposure. To our knowledge there are no human repeated dose exposures that can be used to derive dose-effect or dose-response relationships. Furthermore, almost exclusively all repeated dose exposures using experimental animals occur by the oral route (feed, gavage, drinking water).

Manifestations of acute GHB toxicity include amnesia and hypotonia at dose levels of 10 mg/kg body weight; a normal sequence of REM and non-REM sleep at 20-30 mg/kg body weight; and anaesthesia at 50 mg/kg body weight. 50-70

mg/kg body weight may induce coma (16, 36, 124). GBL is more potent than GHB and life threatening effects are likely to occur at lower doses than with GHB (85). A lethal peroral dose of GHB has been suggested to be in the order of 500 mg/kg body weight (124). The effects of GHB on the CNS are potentiated by concomitant intake of alcohol and other central stimulants such as amphetamine, ecstasy, and cocaine (36). Surgical anaesthesia is obtained at a dose of approximately 60 mg/kg body weight (142). Euphoria has been reported at dose levels of 20-30 mg/kg body weight (64).

Effects reported in experimental animals after single or repeated dose exposure are presented in Table 2. Table 3 summarises the results in the carcinogenicity study by NTP (118). No observed adverse effect level (NOAEL) values are given whenever appropriate.

Repeated dose toxicity in rats at various dose levels and exposure durations has been studied by NTP (118). Given daily oral bolus doses of GBL for 12 consecutive days excluding weekends, rats exposed to 1 200 mg/kg body weight and day all died, most likely due respiratory depression caused by effects on the CNS. At 600 mg/kg/day one animal died, and no deaths were noted at lower doses. Increasing the exposure period to 90 days resulted in deaths in all male rats and one female at 900 mg/kg but no deaths at 450 mg/kg. No histopathological lesions were seen in these studies. Besides a decreased body weight in female rats given 450 mg/kg for 2 years, exposed animals showed no signs of toxicity. In a similar study in mice almost all animals died when exposed to 1 400 mg/kg for 12 consecutive days excluding weekends. An acute effect on the CNS was noted in animals exposed to 350 mg/kg or more. In the 90-day study at a dose of 1 050 mg/kg/day, 30% of the males and 10% females died. In the dose-range from 65-525 mg/kg body weight absolute and relative organ weights were not affected. There were no gross or microscopic lesions. In mice exposed by gavage at dose levels of 262 or 525 mg/kg/day for 2 years no other effects besides decreased body weight and CNS depression shortly after exposure were noted. These studies indicate that the CNS is the target organ for GBL. Although reduced weight gain and relative and absolute weights have been reported in some organs, they seem not to be target organs for GBL.

Two studies, both in rats, indicate that exposure to GBL may affect fertility. In the first study, exposure of young male rats (21 days) to 0.5 and 1% in the drinking water for 20 days resulted in 40 and 50% reductions in testicular weight, respectively (28). In the second study, a marked inhibition in ovulation was noted after a single intraperitoneal injection of GBL at a dose level of 250 mg/kg body weight (10). The effect on ovulation was to be dose-dependent with a 22% inhibition at 62.5 mg/kg body weight, 63% at 250 mg/kg body weight, 71% at 500 mg/kg body weight, and 100% at 750 mg/kg body weight. No effects on the testes were reported in the NTP studies at even higher doses (118). Thus it seem that young immature male rats are especially sensitive to gonadal toxicity caused by GBL (e.g. GBL affects hormonal-dependent testicular development) or that differences in kinetics between a bolus dose of GBL and a more steady intake of the same dose from drinking water affects its toxicodynamic potential.

NTP has studied the carcinogenic potential of GBL in rats and mice (118). There is no evidence that GBL is an experimental carcinogen in rats or in female mice. In male mice there was an increased incidence of benign or malignant pheochromocytoma in the low-dose group but not in the high-dose group.

Table 2. Effects of GBL in animals after single or repeated exposure.

Species (no animals per dose-group)	Route of exposure	Exposure data	NOAEL (chronic effects) mg/kg bw/day	Effects	Reference
Rat (5 males+ 5 females)	Oral, gavage	0, 75, 150, 300, 600, or 1 200 mg/kg body weight/day for 12 consecutive days	300	1 200 mg/kg: All rats died within the first 3 days; rats were recumbent or inactive with irregular respiration soon after dosing. 600 mg/kg: One male rat died; Lower weight gain in females; rats were recumbent or inactive with irregular respiration soon after dosing. ≤300 mg/kg: No effects.	(118)
Rat (10 males+ 10 females)	Oral, gavage	0, 56, 112, 225, 450, or 900 mg/kg body weight/day for 90 days	225	900 mg/kg: All male and one female rat died within 8 weeks. All rats became recumbent within several minutes after dosing, but appear normal later. 450 mg/kg: Reduced final body weight and weight gains in males. 225 and 450 mg/kg: Slight inactivity after dosing. No lesions. 56 and 112 mg/kg: No effects.	(118)
Rat (50 males+ 50 females)	Oral, gavage	0, 112, or 225 mg/kg body weight/day for males and 0, 225, or 450 mg/kg body weight/day for females for 2 years	225	450 mg/kg, females: Decreased mean body weight. 112 and 225 mg/kg, males and 225 mg/kg, females: No effects.	(118)
Rat (10-13 males)	Oral, drinking water	0, 0.5 and 1.0%. The duration of exposure was not stated (most likely 20 days)	–[a]	Doses of 0.5% (approx. 550 mg/kg) and higher led to significantly reduced testicular weight in 21 day old rats. 0.5%: 40% reduction in testicular weight. 1.0%: 50 % reduction in testicular weight.	(28)

Table 2. Cont.

Species (no of animals per dose-group)	Route of exposure	Exposure data	NOAEL (chronic effects) mg/kg bw/day	Effects	Reference
Rat (4-6 females)	Intraperi-toneally in saline	Single dose of 0, 62.5, 125, 250, 500, 750 mg/kg body weight	–[a]	The two highest doses caused anaesthetic effects. The inhibition of ovulation was: 62.5 mg/kg: 22% 125 mg/kg: 20% 250 mg/kg: 63% 500 mg/kg: 71% 750 mg/kg: 100%	(10)
Mouse (5 males+ 5 females)	Oral, gavage	0, 87, 175, 350, 700, or 1 400 mg/kg body weight/day for 12 consecutive days	175	1 400 mg/kg: All males and 4/5 females died before the end of the study. No effect on body weight gain. ≥ 350 mg/kg: Mice were recumbent and inactive shortly after dosing. These effects are considered acute effects. A small but significant reduction in final body weight was noted in female mice of the 350 and 700 mg/kg dose-group but not in male mice. 87 and 175 mg/kg: No effects.	(118)
Mouse (10 males+ 10 females)	Oral, gavage	0, 65, 131, 262, 525, or 1 050 mg/kg body weight/day for 90 days	525	1 050 mg/kg: Deaths 3/10 males and 1/10 females; 11% lower mean body weights in males. 65-525 mg/kg: Adaptive anaesthetic effect; mean body weights were not affected; absolute or relative organ weights were not affected; no gross or microscopic lesions.	(118)
Mouse (50 males+ 50 females)	Oral, gavage	0, 262, or 525 mg/kg body weight/day for 2 years	–[a]	262 and 525 mg/kg: Decreased body weight (6% reduction in GBL-exposed males and 17% in low-dose and 14% in high-dose females); sedated or lethargic and inactive shortly after dosing; no non-neoplastic lesions.	(118)

[a] No NOAEL could be identified.

Table 3. Carcinogenesis studies of GBL in experimental animals (118).

Species (no animals per dose-group)	Route of exposure	Exposure data	Effects
Rat (50 males)	Oral, gavage	0, 112, or 225 mg/kg body weight/day for 2 years	Body weight: Dosed groups similar to controls Survival rates: 24/50, 27/50, 32/50 Neoplastic effects: None Uncertain findings: Decreased incidences of mononuclear cell leukemia (16/50, 15/50, 9/50) Level of carcinogenic evidence: No evidence
Rat (50 females)	Oral, gavage	0, 225, or 450 mg/kg body weight/day for 2 years	Body weight: High-dose group lower than controls Survival rates: 28/50, 27/50, 28/50 Neoplastic effects: Decreased incidence of mammary gland fibroadenomas (22/50, 14/50, 6/50) Uncertain findings: None Level of carcinogenic evidence: No evidence
Mouse (50 males)	Oral, gavage	0, 262, or 525 mg/kg body weight/day for 2 years	Body weight: Dosed groups lower than controls Survival rates: 35/50, 30/50, 12/50 Neoplastic effects: Decreased incidence of hepatocellular neoplasms (24/50, 8/50, 9/50) Uncertain findings: Adrenal medulla; benign or malignant pheochromocytoma (2/48, 6/50, 1/50) Level of carcinogenic evidence: Equivocal evidence
Mouse (50 females)	Oral, gavage	0, 262, or 525 mg/kg body weight/day for 2 years	Body weight: Dosed groups lower than controls Survival rates: 38/50, 34/50, 38/50 Neoplastic effects: None Uncertain findings: None Level of carcinogenic evidence: No evidence

13. Previous evaluations by (inter)national bodies

GBL has been evaluated for carcinogenicity by International Agency for Cancer Research (IARC) in 1976 and 1999 (71, 72). In 1999, IARC concluded that there is *inadequate evidence* in human for the carcinogenicity of GBL and there is *evidence suggesting lack of carcinogenicity* of GBL in experimental animals (72). The overall evaluation of GBL is *not classifiable as to its carcinogenicity to humans (group III)*.

14. Evaluation of human health risks

14.1 Groups at extra risk

Since there is no relevant occupational exposure data, no groups at specific risk can be identified. In the general population, people using GBL as a drug and often together with ethanol and other drugs are at high risk.

14.2 Assessment of health risks

The toxicological information on GBL is limited, especially in humans but also in experimental systems. In general, most of the classical toxicological studies with GBL (and GHB) are old and often lack detailed information regarding experimental design and an evaluation of the results. The uptake, distribution, metabolism, and excretion in humans are likely to be similar to that observed in experimental animal. GBL is rapidly and complete transformed to GHB once taken up into the body. Toxicity data for GHB are also relevant in the risk assessment of GBL.

The anaesthetic effects resulting from acute oral GBL exposure are well documented in humans as well as in experimental animals. Acute toxic effects based on human intoxications include bradycardia, hypothermia, CNS depression, prolonged unconsciousness (typically for 1-2 hours), confusion, combativeness, odtundation, and uncontrolled movements. The effects of GHB are dose-dependent: amnesia and hypotonia at dose levels of 10 mg/kg body weight; a normal sequence of REM and non-REM sleep at 20-30 mg/kg body weight; and anaesthesia at 50 mg/kg body weight. 50-70 mg/kg body weight of GHB may induce coma. GBL is more potent than GHB and life threatening effects are likely to occur at lower doses than with GHB. The dose-response curve varies between humans and the dose levels given above for the various effects should be considered as approximate levels. The effects of GHB on the CNS are potentiated by concomitant intake of alcohol and other drugs such as amphetamine, ecstasy, and cocaine.

Eye irritation from GBL exposure has been quoted in several publications, however, the original data was not available for scrutiny. In rabbits, eye irritation has been reported in a study where GBL was instilled into the conjunctival sac.

The damage to the cornea and iris were completely reversible after 14 days. Findings in *in vitro* tests for eye irritation support the eye irritation noted in animals. It is concluded that GBL has the potential to cause eye irritation in humans.

Conflicting results have been obtained in skin irritation studies in animals and humans. The data do not allow a clear conclusion regarding skin irritation in humans. However, there are some indications that GBL may act as a weak skin irritant.

The very limited information on possible skin sensitisation does not allow a conclusion to be drawn at present.

No studies using repeated inhalation or dermal exposure were located. In several oral repeated dose studies of varying length in mice and rats no overt signs of general toxicity, organ toxicity, or histopathological changes were noted. High doses (> 600-900 mg/kg body weight in rats; >1 000 mg/kg body weight in mice) were lethal. The toxic effects noted were those of anaesthesia at doses > 250-300 mg/kg body weight and reduced body weight gain. A NOAEL of 225 mg/kg body weight/day based on reduced weight gain in male rats in the carcinogenicity bioassay is proposed.

No reports on genotoxicity in humans are available. GBL has been extensively tested for genotoxic effects in experimental systems. None of the *in vivo* studies were positive. The only positive results are in one study for gene conversion in yeast, a cell transformation assay and in one test for sister chromatid exchange and chromosomal aberrations. Based on all available genotoxicity studies, GBL should be regarded as non-genotoxic.

The carcinogenic potential of GBL has been studied in several experiments in rats and mice. The most recent study and also that of best scientific quality (118), indicates that GBL is not carcinogenic following oral administration. The NTP study report stated that there were equivocal evidence of carcinogenicity at one dose level in male mice and that the sensitivity of the study to detect an effect in male mice was reduced due to low survival in the high-dose group. Furthermore, the use of different structure-activity models to predict carcinogenicity lends no support for a carcinogenic or mutagenic potential for GBL. An evaluation by IARC in 1999 has concluded that GBL is not classifiable as to its carcinogenicity to humans (72).

No standard fertility studies are available. However, GBL has affected pro-estrous LH and FSH levels and ovulation in rats (ED_{50} approx. 250 mg/kg body weight) and a dose-dependent reduction in the number of rats ovulating were detected from 62.5 mg/kg body weight. 0.5% GBL (approximately 550 mg/kg body weight) in drinking water led to reduced testicular weight in young rats. The effects observed in male and female rats exposed to GBL could result in reduced fertility in adults and in young male rats exposed postnatally. No effects on development were detected when rats were orally exposed to GBL.

14.3 Scientific basis for an occupational exposure limit

The database for deriving occupational limits for GBL is limited. The most relevant human occupational exposure routes are likely to be by inhalation or through dermal contact. Almost all experimental studies have, however, used oral exposure. Furthermore, information on occupational exposure levels is lacking.

The critical effects of GBL are:
- Narcotic and anaesthetic effects: Sedative and hypnotic effects similar to those observed in experimental animals have been seen in humans. Based on human oral exposure, effects on the CNS are evident at dose levels of approximately 10-50 mg GHB/kg body weight.
- Animal studies indicate that GBL exposure could adversely affect fertility. Reduced ovulation was found in GBL-exposed dams at the lowest tested single dose of 62.5 mg/kg body weight.

15. Research needs

Occupational surveys are needed to clarify current levels of GBL exposure in the work place air and identify specific operations that could lead to high exposures or parts of the work force that are at risk.

Data from toxicokinetic studies using inhalation exposure would facilitate an extrapolation of toxicity from the oral route to that of inhalation. Likewise, acute and repeated dose toxicity studies using inhalation exposure would help in evaluating any direct or systemic effects of GBL on the respiratory system and be helpful when setting occupational exposure limits. From a classification point of view, an up to date eye irritation and skin sensitisation study would be helpful and would assist in assessing such risks to humans. The indication that GBL exposure may affect testicular development and ovulation could suggest a possible altered fertility. Accordingly, a two-generation fertility study would be needed.

16. Summary

Søderlund E. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 135. γ-Butyrolactone (GBL)*. Arbete och Hälsa 2004;7:1-49.

γ-Butyrolactone (GBL) is used as an intermediate for the production of other chemicals, as a solvent, and as a binder in foundry. Non-occupational use results from its natural occurrence in fruits and berries, its use as an experimental drug in treatment of alcohol withdrawal symptoms and narcolepsy. Due to its euphoric/hallucinogenic properties the abuse of GBL and γ-hydroxybutyrate (GHB) has increased dramatically in several countries.

GBL is a colourless oily liquid with a mild caramel odour. It has a relatively low vapour pressure, a boiling point of 206ºC, and is miscible with water.

Little information is available regarding occupational exposure. In the USA in 1981-1983 it has been estimated that over 40 000 workers were potentially exposed and of these about 2/3 are exposed in printing and publishing and in textile mill industries.

GBL is easily absorbed after ingestion and to some extent also absorbed through the skin. The enzymatic hydrolysis to GHB in the body is rapid and extensive. GBL is distributed to all organs mainly as GHB. The latter is further metabolised by catabolic enzymes, and finally eliminated as CO_2 and urinary metabolites.

GBL has a low to moderate acute toxicity in experimental animals and causes CNS depression both in humans and animals. GBL causes eye irritation in rabbits, whereas no conclusions can be drawn regarding sensitisation. Repeated oral doses of approximately 1 000 mg/kg body weight/day caused death in mice and rats. No toxic effects, apart from reduced weight gain, were elicited at lower doses. An overall evaluation of an extensive database for genotoxicity indicates that GBL is not genotoxic. There is no support for a carcinogenic effect in experimental animals. An evaluation for carcinogenicity by IARC in 1999 concluded that GBL is not classifiable as to its carcinogenicity in humans. GBL might affect testicular development in young rats and may reduce or block ovulation in adult rats.

There is a need for identifying occupational exposure levels and additional toxicological information regarding acute local and reproductive effects.

Based on available data CNS depression is considered the critical effect from GBL exposure. Reproductive toxicity (reduced ovulation) found in animals cannot be fully assessed with respect to human health at present.

Keywords: carcinogenicity, CNS effects, *gamma*-butyrolactone, genotoxicity, irritation, metabolism, occupational exposure limits, reproductive toxicity.

17. Summary in Norwegian

Søderlund E. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 135. γ-Butyrolactone (GBL).* Arbete och Hälsa 2004;7:1-49.

γ-Butyrolakton (GBL) benyttes som mellomprodukt i produksjon av andre kjemikalier, som løsningsmiddel og som bindemiddel i støpesand. Ikke-yrkesmessig eksponering vil kunne finne sted fordi stoffet forekommer i naturlig i frukt og bær, benyttes i eksperimentell medisinsk behandling av abstinens knyttet til bruk av alkohol og narkolepsi. GBL og γ-hydroxibutyrat (GHB) har i den senere tid hatt en kraftig økning i bruken på det illegale markedet som narkotisk stoffer som gir hallusinasjoner og eufori.

GBL er en fargeløs oljeaktig væske med karamellaktig lukt. Det har et relativt lavt damptrykk, et kokepunkt på 206°C og er blandbart med vann.

Det finnes lite informasjon om yrkeseksponering. I USA er det beregnet at 40 000 arbeidere kan tenkes å være eksponert og 2/3 av disse er eksponert i industrier som driver med trykking og publisering og i tekstilindustrien.

GBL tas lett opp i kroppen etter svelging og i mindre grad gjennom huden. Enzymatisk hydrolyse til GHB foregår raskt og nesten fullstendig i kroppen og stoffet fordeles til alle organer hovedsakelig som GHB. Stoffet metaboliseres videre via kroppens normale nedbrytningsenzymer og utskilles som CO_2 via utåndingsluften og som metabolitter i urin.

GBL har en lav til moderat akutt toksisitet i forsøksdyr og fører til effekter på sentralnervesystemet både hos mennesker og dyr. GBL er øyeirriterende hos kanin. Det er ikke mulig å konkludere om stoffet kan føre til hudallergi. Gjentatt oral eksponering for doser i størrelsesorden 1 000 mg/kg kroppsvekt/dag medførte dødsfall hos mus og rotter. Ingen toksiske effekter, bortsett fra redusert økning i kroppsvekt, er funnet ved lavere doser. En vurdering av alle studier av genitoksisitet tyder på at stoffet ikke gir denne type skader. Det finnes ikke holdepunkter for at stoffet er kreftfremkallende i dyreforsøk. En evaluering foretatt av IARC i 1999 konkluderte med at GBL ikke lar seg klassifisere som mulig humant karsinogen. GBL ser ut til å kunne påvirke utvikling av testikler hos unge rotter og å redusere eggløsning hos voksne rotter.

Det trengs opplysninger om nivåer av GBL ved yrkeseksponering og ytterligere toksikologisk informasjon angående akutte lokale effekter og effekter på reproduksjon.

Basert på tilgjengelig toksikologisk informasjon er den kritiske effekten av GBL en påvirkning av sentralnervesystemet. Det foreligger data fra dyreforsøk som tyder på at GBL kam føre til redusert fertilitet. Det er imidlertid ikke på det nåværende tidspunkt mulig å foreta en endelig vurdering av i hvilken grad GBL kan føre til reproduksjonstoksisitet hos mennesker.

Nøkkelord: CNS effekter, *gamma*-butyrolakton, gentosisitet, irritasjon, karsinogenitet, metabolisme, reproduksjonstoksisitet, yrkeshygieniske grenseverdier.

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19. Data bases used in search of literature

Arbline
Chemical Abstracts
Medline
NIOSHTIC
Toxline
Toxnet
Chemfinder

Last search was performed in November 2003.

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Appendix 1

The Danish Occupational Inspectorate list a tentative limit value of 50 ppm for GBL in their list of organic solvents (6).

In Russia a hygienic standardisation of GBL in the air at populated sites is reported (145). The 24-hour mean limit value is set to 0.1 mg/m^3 (0.028 ppm). This limit value appears to be based on the threshold concentration for GBL in air being 0.51 mg/m^3 following chronic exposure.

Appendix 2

Information on the various mutagenicity studies with GBL is given in Tables A1-A5.

Table A1. DNA damage and repair tests.

Test system	Results		Dose	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ADPRT-mediated decrease of cellular NAD-content in FL cells	–	NT	0.086–8.61 µg/ml (10^{-6}-10^{-4} mol/l)	(174)
ADPRT-mediated decrease of cellular NAD-content in FL cells	–	NT	0.086–86.1 µg/ml (1-1 000 µmol/l)	(38)
Lambda Induction Assay	NT	–	5, and 12.5 mg/ml	(154)
E.coli (pol A^+/A^-*)*, modified liquid suspension assay	(+)	NT		(129)
E. coli (PQ37 strain) SOS Chromotest	–	NT	Not given	(126)
E. coli (PQ37 strain) SOS Chromotest	–	–	Not given	(107)
B. subtilis rec strains, differential toxicity (fish S9)	–	+	max. 22 600 µg/disk (20 µl/disk)	(78)
E. coli rec strains, differential toxicity	–	–	500 µg/plate	(59)
E. coli rec strains, differential toxicity	–	NT	500 µg/plate	(74)
E. coli rec strains, differential toxicity	–	–	250, 500, and1 000 µg/ml	(158)
S. cerevisiae, DNA repair	–	NT	1 000 µg/l	(79)
HeLa cells, unscheduled DNA repair synthesis	–	–	0.1–100 µg/ml	(102)
Chinese hamster ovary cells deficient in nucleotide excision repair or rejoining DNA strand break	–	NT	4 000 µg/ml	(69)
Reactivity towards guanosine, RNA and DNA (alkylation, adduct formation)	–	NT	4 300 µg/ml (40 mM)	(65)

NT, not tested; +, positive effect; (+), weak positive effect; –, no effect.

Table A2. Point mutations in bacteria.

Test system	Results		Dose	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
S. typhimurium TA98 and TA100, reverse mutation	–	–	Not given	(73)
S. typhimurium TA98, TA100, TA1537 and TA1538, reverse mutation	–	–	1 250 µg/plate	(156)
S. typhimurium TA98, TA100, TA1535, TA1535 and TA1538, reverse mutation	–	–	Not given	(147)
S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538, reverse mutation	–	–	0.1-2 000 µg/plate	(135)
S. typhimurium TA98, TA100 and TA1537, reverse mutation	–	–	Not given	(112)
S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538, reverse mutation	–	–	1 000 µg/plate	(8)
S. typhimurium TA92, TA 98, TA100, TA1535, TA1537, TA1538, reverse mutation (with preincubation)	–	–	0.2-2 000 µg/plate	(12)
S. typhimurium TA98, TA100, TA1535 and TA1537, reverse mutation	–	–	Not given	(50)
S. typhimurium TA98, TA100 and TA1537, reverse mutation	–	–	2 000 or 5 000 µg/plate	(97)
S .typhimurium TA98 and TA100, reverse mutation	–	–	500 µg/plate	(70)
S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538, reverse mutation	–	– (TA100 NT)	10 000 µg/plate	(128)
S. typhimurium TA98 and TA100, reverse mutation	–	–	0.5-500 µg/plate	(164)
S. typhimurium TA98, TA100 and TA1535, reverse mutation	–	–	0.1-50 µmol/plate	(95)
S. typhimurium TA98, TA100 and TA102, reverse mutation	–	–	13.0 nmol-1.3 mmol/plate	(3)
S. typhimurium TA98, TA1535 and TA1537, reverse mutation (fluctuation test)	–	–	10-1 000 µg/ml	(51)
S. typhimurium TA98, TA100, 1535 and TA1537, reverse mutation	–	–	10 000 µg/plate	(63)
E. coli WP2urvA, reverse mutation (fluctuation test)	–	–	10-1 000 µg/ml	(51)
E. coli WP2 and WP2urvA, reverse mutation	–	–	0.5-500 µg/plate	(164)
E. coli WP2urvA and WP2urvA/pKM101, reverse mutation	–	–	Not given	(103)

NT, not tested; +, positive effect; (+), weak positive effect; –, no effect.

Table A3. Tests in yeast.

Test system	Results		Dose	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
S. cerevisiae D4, gene conversion	–	–	0.33-333.3 μg/plate	(75)
S. cerevisiae T1 and T2 (*"race XII"),* mitotic crossing-over (homozygosis by mitotic recombination, ade2 locus)	–	–	1 000 μg/ml	(79)
S. cerevisiae JD1, gene conversion	+ with DMSO – with ethanol	NT	500 or 750 μg/ml	(143)
S. cerevisiae DT, mitotic gene conversion	NT	–	2 250 μg/ml	(175)
S. cerevisiae XV185-14C, reverse mutation	–	?	(22.2-222) μl x 10^{-3}/ml	(109)
S. pombe, forward mutation	–	–	5-20 μg/ml	(94)
S. cerevisiae D6, mitotic aneuploid	–	–	1 000 μg/ml	(122)

NT, not tested; +, positive effect; (+), weak positive effect; –, no effect.

Table A4. Tests in mammalian cells.

Test system	Results		Dose	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chinese hamster ovary (CHO) cells, chromosomal aberrations	–	+	2 580 μg/ml	(96)
Chinese hamster ovary (CHO) cells, sister chromatid exchange	–	+	3 010 μg /ml	(96)
Chinese hamster ovary (CHO) cells, sister chromatid exchange	–	–	1 000 μg/ml	(123)
Rat liver RL_1 cells, chromosomal aberrations	–	NT	250 μg/ml	(27)
Human fibroblast HSC172 cell line, gene mutation, diphtheria toxin resistance	–	–	500 μg/ml	(61)
Baby hamster kidney cells (BHK 21 C13/HRC 1), cell transformation	–	–	8 000 (-S9), 1 800 (+S9) μg/ml	(24)
Baby hamster kidney cells (BHK-21), cell transformation	NT	+	25-250 μg/ml	(150)

NT, not tested; +, positive effect; (+), weak positive effect; –, no effect.

Table A5. *In vivo* tests.

Test system	Results	Dose	Reference
Drosophila melanogaster, sex-linked recessive lethal mutations	–	0.2% in feed	(166)
Drosophila melanogaster, sex-linked recessive lethal mutations	–	2.0-2.8% (20 000-28 000 ppm) in feed	(118)
Drosophila melanogaster, interchromosomal mitotic recombination	–	0.43 or 0.86 % (50 or 100 mM) in feed	(167)
B6C3F_1 mouse bone-marrow cells, micronucleus test	–	984 mg/kg ip x 2	(138)
CD-1 mouse bone-marrow cells, micronucleus test	–	560 mg/kg ip x 2	(157)
Mouse testicular cells, flow cytometry, mutagenicity testing, increased portion of diploid sperm	–	100-400 mg/kg body weight	(121)
NMRI-mice, DNA flow cytometric measurements, mutagenicity testing	–	Not given	(120)
(CBA x BALB/c)F1 mice, sperm morphology	–	112–1 120 mg/kg ip x 5	(155)

NT, not tested; +, positive effect; (+), weak positive effect; –, no effect.