Gamma-Butyrolactone

Health-based recommended occupational exposure limit

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

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Geachte minister,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan gamma-butyrolacton. Het maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over gamma-butyrolacton is opgesteld door de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en aan de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Met vriendelijke groet,

prof. dr. J.A. Knottnerus

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to:

the Minister of Social Affairs and Employment

No. 2008/13OSH, The Hague, December 18, 2008

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie GBBS, een van de vaste commissies van deskundigen, van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in de lucht waaraan mensen blootgesteld kunnen worden tijdens hun beroepsuitoefening. Die vormen vervolgens de basis voor grenswaarden, vast te stellen door de minister, waarmee de gezondheid van werknemers beschermd kan worden. In dit advies bespreekt de commissie de gevolgen van blootstelling aan gamma-butyrolacton (GBL) en stelt een gezondheidskundige advieswaarde vast.

In een eerste stap is de toxiciteit van de stof geëvalueerd. Dat is gedaan in samenwerking met de *Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals*. De resultaten van de evaluatie zijn gepubliceerd in 2004 en in deel 2 van dit advies opgenomen. In het eerste deel van dit advies heeft de Commissie GBBS vervolgens op basis van deze evaluatie en nieuwe relevante informatie een onderbouwing gegeven van haar advies. De conclusies zijn gebaseerd op wetenschappelijke gegevens die voor april 2008 zijn verschenen.

Fysisch-chemische eigenschappen, voorkomen en gebruik

Bij kamertemperatuur is γ-butyrolacton (CAS nr. 96-48-0) een kleurloze olieachtige stof met een relatief lage dampspanning en een milde karamelachtige geur. GBL mengt met zowel water als organische oplosmiddelen, zoals ethanol en benzeen. In een waterige oplossing bestaat er een zuurgraadafhankelijk evenwicht tussen γ-butyrolacton en zijn hydrolyseproduct γ-hydroxyboterzuur (GHB); in zure oplossingen overheerst GBL, in basische oplossingen GHB.

GBL komt van nature voor in een aantal voedselproducten, zoals in vlees, fruit, koffie en alcoholische dranken. In kleine hoeveelheden is het ook te vinden in het menselijk lichaam.

In de arbeidssituatie kent GBL vele toepassingen, waaronder als oplosmiddel voor polymeren en in de elektronica-industrie, als bestanddeel in chemische verfverwijderaars en als intermediair bij de productie van vitaminen, geneesmiddelen en pyrrolidonen. GHB is niet alleen een hydrolyseproduct van GBL, maar wordt los daarvan ook gesynthetiseerd en in de farmaceutische industrie verwerkt tot geneesmiddel. GHB wordt namelijk gebruikt om te kalmeren, bij de behandeling van alcoholverslaving, als ondersteuning bij het staken van drugsmisbruik (opiaten) en bij de behandeling van slaapstoornissen.

GBL en GHB staan verder bekend als partydrug, omdat het euforische en kalmerende effecten kan hebben.

Monitoring en gegevens over blootstelling

De huidige methoden om GBL te kunnen detecteren maken gebruik van een combinatie van gaschromatografie en massaspectrometrie. Verder is het mogelijk om GBL en GHB te scheiden en apart te kwantificeren met behulp van *high performance liquid* chromatografie en *UV-visible* spectrometrie. Er zijn ook verschillende analysemethoden beschreven voor het bepalen van GBL en GHB in bloed en urine. Geen van de genoemde methoden wordt echter algemeen als monitoring toegepast.

Ondanks de brede toepassing zijn er maar weinig gegevens over de hoogte van blootstelling op de werkplek. In één onderzoek wordt melding gemaakt van een blootstelling aan 0,01 tot 1,1 mg GBL/m^3 in de ademzone, tijdens het verwijderen van graffiti.

Huidige grenswaarden

In Nederland noch elders zijn grenswaarden vastgesteld voor GBL. In Denemarken geldt sinds 1994 een voorlopig grenswaarde van 50 ppm (180 mg/m^3).

Opname, verdeling en uitscheiding

Gamma-butyrolacton kan worden opgenomen door het lichaam via inademing, de mond en de huid. Uit onderzoek blijkt dat de doorlaatbaarheid van de huid voor GBL erg hoog is.

In het lichaam wordt GBL vervolgens door het enzym lactonase, dat in het bloed en de lever voorkomt, binnen enkele minuten omgezet in GHB. GBL wordt dan ook voornamelijk als GHB door het lichaam verspreid. GHB komt ook van nature voor in de hersenen van zoogdieren.

Beide stoffen kunnen de bloedhersenbarrière passeren. Na blootstelling worden ze dan ook in hoge concentraties gevonden in bepaalde delen van de hersenen. Hogere concentraties zijn verder ook gemeten in de nieren, hart, spieren en lichaamsvet, in vergelijking met andere lichaamsdelen of organen

GHB kan door het lichaam omgezet en afgebroken worden via verschillende routes. Eén daarvan is via de citroenzuurcyclus, wat resulteert in kooldioxide als eindproduct. Een andere is via de vorming van γ-aminoboterzuur (GABA), een van nature voorkomende neurotransmitter met een remmende werking in de hersenen. De tussen- en eindproducten die door de omzetting van GBL ontstaan verlaten het lichaam bij de mens voornamelijk via uitademing (kooldioxide) en voor een kleiner deel via de urine. De halfwaardetijd voor GHB, dat wil zeggen de tijd die het lichaam nodig heeft om de plasmaconcentratie van GHB te halveren, ligt tussen de 30 en 60 minuten.

Het toxische werkingsmechanisme

Wat betreft de toxiciteit van γ-butyrolacton staan twee effecten op de voorgrond.

Ten eerste zijn er de neurologische effecten. Hoge doses GBL onderdrukken de werking van het centrale zenuwstelsel. Voor deze effecten worden GHB en het daaruit gevormde GABA verantwoordelijk gehouden. Ze binden namelijk aan receptoren in de hersenen, waardoor het vrijkomen van dopamine, een neurotransmitter met een stimulerende werking, wordt geremd. Mogelijk spelen ook andere, nog niet bekende neurotoxische mechanismen een rol.

Ten tweede komt uit dieronderzoek naar voren dat GBL mogelijk de voortplanting remt. Het mechanisme van dit effect is nog niet opgehelderd. Er zijn aanwijzingen dat GBL de hormoonbalans in de hersenen verstoort, maar er zijn ook aanwijzingen gevonden dat GBL de rijping van onbevruchte eicellen direct zou kunnen beïnvloeden.

Effecten

Waarnemingen bij mensen

De meeste gegevens over de toxiciteit van γ-butyrolacton bij mensen zijn afkomstig van acute vergiftigingen na orale inname. Er zijn geen gegevens bekend van inhalatoire of dermale blootstelling op de werkplek of elders.

GBL, maar vooral GHB, wordt onder jongeren als partydrug gebruikt. Dit heeft geleid tot relatief veel rapportages van gevallen van acute GBL- en GHB-vergiftigingen. Zo is er melding gemaakt van verschillende effecten. De belangrijkste is de aan de blootstelling gerelateerde remming van het centrale zenuwstelsel, dat zich uit in slaperigheid en verlies van bewustzijn, toevallen, veranderingen in de pupilreflex, ongecontroleerde bewegingen, verwarring, hallucinatie en euforie. Deze effecten zijn beschreven vanaf 20-30 mg GBL per kilogram lichaamsgewicht (slaperigheid, duizeligheid, euforie) en vanaf zo'n 10 mg GHB per kilogram lichaamsgewicht. Een dosis van ongeveer 60 mg per kilogram lichaamsgewicht leidt tot verlies van het bewustzijn en kan coma veroorzaken. Een bijna fatale afloop is gemeld bij iemand die een drankje met GBL had geconsumeerd, en als gevolg daarvan een interne dosis van zo'n 570 mg GBL per kilogram lichaamsgewicht binnenkreeg. Er zijn ook andere typen effecten door inname beschreven – voor een deel gerelateerd aan de verminderde werking van het centrale zenuwstelsel – zoals verlaagde of verhoogde hartslag en bloeddruk, misselijkheid en verminderde ademhaling.

Herhaald gebruik van GBL kan leiden tot verschillende neurologische effecten, zoals angst, depressie, bevingen en slapeloosheid. Het is echter niet duidelijk bij welke blootstelling precies deze effecten optreden.

Er zijn geen gegevens gevonden over mogelijke huid- en oogirriterende effecten, of effecten op de voortplanting en het nageslacht. Er zijn ook onvoldoende betrouwbare gegevens beschikbaar over mogelijke kankerverwekkendheid bij de mens.

Waarnemingen bij dieren

De weinige gegevens die beschikbaar zijn wijzen op een licht irriterend effect op de huid en op een matige tot ernstige irritatie in de ogen door direct huid- en oogcontact.

De dosis na eenmalige orale inname waarbij de helft van de dieren sterft, ligt bij muizen en ratten in de orde van zo'n 800 tot 1 800 mg γ-butyrolacton per kilogram lichaamsgewicht. Dit duidt op een matige tot lage acute toxiciteit.

Er is één dieronderzoek bekend, waarin ratten gedurende vier uur 5 100 mg GBL per kubieke meter lucht inademden. De dieren vertoonden oppervlakkige ademhaling, neusafscheiding, waren apathisch en bewogen minder. Alle dieren herstelden van deze effecten in de veertiendaagse observatieperiode die volgde na de blootstelling.

In ratten en muizen werden kort na een eenmalige toediening via injecties van GBL in de buikholte een verminderde bewegingsactiviteit, verlaagde lichaamstemperatuur en verstijving van de spieren waargenomen. De effecten werden beschreven vanaf ongeveer 50 mg per kilogram lichaamsgewicht. Enkele uren na de toediening waren deze effecten weer geheel verdwenen.

Er zijn dieronderzoeken uitgevoerd, waarbij ratten en muizen verschillende doses GBL via een maagsonde kregen toegediend. Dit gebeurde gedurende twee weken, dertien weken en twee jaar. De belangrijkste effecten waren afname van het lichaamsgewicht en sterfte bij de hoog gedoseerde dieren, en verminderde activiteit gecombineerd met een onregelmatige ademhaling. Die laatste effecten traden op vanaf 225 mg per kilogram lichaamsgewicht. De onderzoekers meldden dat de verminderde activiteit en onregelmatige ademhaling in de eerste weken van blootstelling binnen enkele minuten na toediening optraden, maar na enkele uren weer volledig waren verdwenen. In de daaropvolgende weken bleven deze effecten zelfs geheel uit, wat adaptatie of tolerantie suggereert. Dieren die een dosis kregen toegediend van 175 mg per kilogram lichaamsgewicht en lager vertoonden geen enkel effect als gevolg van de blootstelling aan GBL.

Uit dieronderzoek is geen duidelijk bewijs voor kankerverwekkendheid gevonden na orale of dermale blootstelling van ratten en muizen aan GBL. In één specifiek onderzoek troffen onderzoekers in de bijnieren van mannelijke muizen in de laagste blootstellingsgroep (262 mg per kilogram lichaamsgewicht) medullaire hyperplasie aan; in de hoogste blootstellingsgroep was de sterfte onder mannelijk muizen zo hoog, dat als gevolg daarvan de gevoeligheid van het onderzoek om nog carcinogene activiteit te vinden was gereduceerd. In hetzelfde

onderzoek werd geen bewijs voor kankerverwekkendheid gevonden voor GBL in groepen vrouwelijke muizen en in ratten van beide seksen.

Er zijn enkele laboratoriumonderzoeken uitgevoerd om te beoordelen of GBL schade kan toebrengen aan het genetische materiaal. De beschikbare gegevens tonen aan dat dit niet het geval lijkt te zijn, hoewel niet helemaal kan worden uitgesloten dat GBL chromosomen kan beschadigen in gekweekte cellen.

Tenslotte zijn er ook nog onderzoeken gepubliceerd waarin de gevolgen van blootstelling aan GBL op de vruchtbaarheid en het nageslacht zijn onderzocht. Er zijn aanwijzingen dat GBL de vruchtbaarheid in vrouwelijke ratten vermindert bij doseringen van 62 mg GBL/kg lichaamsgewicht, na injectie in de buikholte. Er is echter meer onderzoek nodig voor een definitieve conclusie kan worden getrokken voor de mens. Het is verder onvoldoende duidelijk of GBL effecten heeft op het nageslacht, hoewel in één onderzoek is waargenomen dat het foetale gewicht was afgenomen. Betrouwbare gegevens ontbreken echter. De gegevens die er zijn, zijn namelijk van onvoldoende kwaliteit.

Evaluatie en aanbeveling

Uit het voorgaande leidt de commissie af dat effecten op het functioneren van het centrale zenuwstelsel het meest kritisch zijn als gevolg van blootstelling aan γ-butyrolacton (en zijn metaboliet γ-hydroxyboterzuur). Uit de gegevens maakt de commissie verder op dat werknemers al binnen enkele minuten na blootstelling door de effecten op het centrale zenuwstelsel een verminderde alertheid kunnen ondervinden. Om tegen dergelijke snel optredende effecten te kunnen beschermen is een gezondheidskundige advieswaarde nodig voor kortdurende blootstelling. Dat betekent dat de commissie heeft beoordeeld of een advieswaarde als een 15-minuten tijdgewogen gemiddelde concentratie (TGG 15-min) kon worden afgeleid.

Er zijn geen gegevens van de mens bekend over blootstelling via inademing; er zijn wel veel gegevens van inname door de mond. Uit die gegevens valt op te maken dat neurologische effecten nog steeds optreden bij doses van 10 mg GHB per kilogram lichaamsgewicht en 20 mg GBL per kilogram lichaamsgewicht. Gegevens van lagere doses zijn niet bekend. Omdat GBL na opname door het lichaam binnen enkele minuten omgezet wordt in GHB, vindt de commissie het gerechtvaardigd de gegevens van GHB te gebruiken; dat wil zeggen dat zij 10 mg per kilogram lichaamsgewicht als startpunt neemt. Deze dosis is gecorrigeerd met een factor 3 voor de afwezigheid van een duidelijke drempelconcentratie waaronder geen effecten meer zijn waargenomen, en met nog een factor 3 voor

de mogelijke verschillen tussen mensen. Dat levert een dosis op van 1 mg per kilogram lichaamsgewicht.

Omdat de gezondheidskundige advieswaarde blootstelling via de lucht betreft en niet via de mond, is deze dosis vervolgens omgerekend naar een concentratie in de lucht. Daarbij neemt de commissie aan dat via de mond uiteindelijk 27% van het GHB onveranderd in de bloedbaan terecht komt en via inademing 100%; gegevens over GBL zijn niet gevonden. Verder neemt zij aan dat de gemiddelde werkende 70 kilogram weegt en tijdens de werkzaamheden in 15 minuten tijd 0,3 kubieke meter lucht inademt. Op grond van deze aannames komt de commissie uit op een gezondheidskundige advieswaarde voor γ-butyrolacton van 65 milligram per kubieke meter lucht (65 mg/m^3, TGG 15-min).

De commissie merkt op dat in het geval dat elke vijftien minuten de TGG 15-minuten waarde wordt bereikt, er aan het eind van een achturige werkdag accumulatie van de interne dosis kan plaatsvinden.* Dit komt omdat de tijd die het lichaam nodig heeft om GHB uit het lichaam te verwijderen langer is dan 15 minuten (plasma halfwaardetijd van GHB ligt tussen de 30 en 60 minuten). Dit betekent dat de interne dosis hoger kan uitkomen dan de interne dosis bij eenmalige blootstelling aan 65 mg/m^3, en dat tegen het eind van de werkdag werknemers last kunnen hebben van effecten op het centrale zenuwstelsel. Om te voorkomen dat dit gebeurt is ook een gezondheidskundige advieswaarde nodig, als een 8-uurs tijdgewogen gemiddelde concentratie. Deze dient tevens te beschermen tegen de schadelijke effecten op het centrale zenuwstelsel door langdurige blootstelling, zoals is beschreven in mensen.

Het is niet bekend bij welke blootstellingsniveaus mensen die langdurig blootstaan aan GBL of GHB effecten op het centrale zenuwstelsel ervaren. Wel zijn er gegevens beschikbaar uit dieronderzoek. Deze laten nauwelijks effecten na langdurige blootstelling zien; in sommige dieren werd alleen een verminderd lichaamsgewicht geconstateerd. In de eerste weken van blootstelling vertoonden sommige dieren direct na toediening een verminderde activiteit, maar dit fenomeen verdween na enkele weken. Het geheel overwegend vindt de commissie dat de gegevens over langdurige blootstelling te beperkt zijn om een 8-uurs TGG gezondheidskundige advieswaarde te kunnen afleiden. De commissie meent echter dat voor het afleiden van zo'n 8-uurswaarde het mogelijk is de 15-minutenwaarde te gebruiken in combinatie met gegevens over de plasma halfwaardetijden.

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* In Nederland geldt in principe geen wettelijke begrenzing van het aantal keren dat de 15-minuten tijdgewogen gemiddelde grenswaarde mag worden bereikt op een normale werkdag. In ieder geval mag de 8-uurs tijdgewogen gemiddelde grenswaarde niet worden overschreden.

Uitgaande van een halfwaardetijd van 60 minuten, en van de situatie dat *elk* kwartier de 15-minuten TGG advieswaarde wordt bereikt, betekent dit dat aan het eind van een achturige werkdag de interne blootstelling 6,5 x hoger is dan die van een eenmalige blootstelling aan de 15-minuten TGG concentratie. Om te voorkomen dat gedurende de dag een te hoge accumulatie optreedt stelt de commissie voor de 15-minutenwaarde van 65 mg/m^3 te delen door een factor van 6,5. Dit resulteert in een 8-uurs TGG gezondheidskundige advieswaarde voor γ-butyrolacton van 10 milligram per kubieke meter lucht.

Gezondheidskundige advieswaarde

De Commissie GBBS van de Gezondheidsraad beveelt een gezondheidskundige advieswaarde aan voor beroepsmatige blootstelling aan γ-butyrolacton van 65 mg/m^3, gemiddeld over een 15-minuten durende werkperiode (TGG 15-min) en van 10 mg/m^3, gemiddeld over een 8-urige werkperiode (TGG 8-uur).

Vanwege de goede doorlaatbaarheid van de huid van γ-butyrolacton, beveelt de commissie een huidnotatie aan.

Executive summary

Scope

At request of the Minister of Social Affairs and Employment, the Dutch expert Committee on Occupational Exposure Safety (DECOS), one of the permanent committees of experts of the Health Council, proposes health-based recommended occupational exposure limits for chemical substances in the air in the workplace. These recommendations serve as basis in setting legally binding occupational exposure limits by the minister. In this advisory report, the committee evaluates the consequences of exposure to γ-butyrolactone (GBL), and derives a health-based recommended occupational exposure limit (HBR-OEL).

In a first step the toxicity of the compound is evaluated. This is done in cooperation with the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. The results of the evaluation, which are published in 2004, are included in part 2 of this advisory report. In the first part, the DECOS describes the most relevant data, supplied by newly presented data, and advices on an HBR-OEL. The conclusions of the DECOS are based on scientific papers published before April 2008.

Physical and chemical properties

At room temperature γ-butyrolactone (CAS no. 96-48-0) is a colourless oily liquid with a relatively low vapour pressure, and a mild caramel odour. It is soluble

in water and organic solvents, such as ethanol and benzene. In aqueous solutions, there is a pH-dependent equilibrium between γ-butyrolactone (GBL) and its hydrolysis product γ-hydroxybutyrate (GHB), in which the lactone ring is opened; in acidic conditions GBL dominates, in alkaline conditions GHB.

GBL occurs naturally in certain food products, such as in meat, fruit, coffee, and alcoholic beverages. It is also found in small quantities in the human body.

In the workplace, GBL is used for many purposes, such as: a solvent for polymers and in the electronic industry; as component in chemical paint removers; and, as an intermediary in the production of vitamins, medicines and pyrrolidones. GHB is not only an hydrolysis product of GBL, but it is also processed by the pharmaceutical industry as a medicine. It is namely therapeutically used as sedative, in the treatment of alcohol dependency, in opiate withdrawal syndrome, and in the treatment of narcolepsy.

GBL and GHB is furthermore known as a party drugs, due to their euphoric and sedative properties.

Monitoring and data on exposure

The current methods to detect GBL use a combination of gas chromatography and mass spectrometry. It is possible to separate GBL from GHB, and to quantify them separately by high performance liquid chromatography and UV-visible spectrometry. Furthermore, there are several analysis methods described to quantify GBL and GHB in blood and urine. None of these methods are however standardly applied.

Despite the broad use, there are only a few data on the level of exposure in the workplace. In one investigation airborne exposure levels between 0.01 to 1.1 mg GBL/ m^3 have been reported in the breathing zone of workers during removing of graffiti.

Current limit values

In the Netherlands nor elsewhere occupational exposure levels have been set for GBL. In Denmark a provisional exposure level of 50 ppm (180 mg/m^3) is applied since 1994.

Kinetics

Gamma-butyrolactone can be absorbed by the body through inhalation, by oral intake or via the skin. Investigations show that the skin has a high permeability for GBL.

In the body, within minutes GBL is converted to GHB by lactonase, an enzyme that is found in the blood and liver. This means that GBL is distributed in the body mainly in the form of GHB. GHB is also an endogenous compound present in mammalian brain.

Both compounds can cross the blood-brain barrier. It is therefore not surprising that they are found in high concentrations in different parts of the brain. Higher concentrations are also measured in the kidneys, the heart, muscles and body fat compared to other body compartments.

GHB is metabolized and eliminated by various routes. One is by entry into the citric acid cycle, which results in carbon dioxide as end product. Another is by formation of γ-aminobutyric acid (GABA), a natural occurring neurotransmitter with inhibitory properties in the brain. The intermediary and end products of GBL are excreted by the body mainly by exhalation (carbon dioxide) and for a smaller part via the urine. In humans, the plasma half-life of GHB, that is the time for half of the substance in plasma to be converted or disappear, ranges between 30 and 60 minutes.

Mechanism of toxicity

Regarding the toxicity of γ-butyrolactone two types of effects are prominent.

The first are neurological effects in the brain. High doses of GBL depress the functioning of the central nervous system. For these effects its hydrolysis products GHB and GABA are thought to be responsible. These compounds bind to receptors in the brain, by which the release of dopamine is inhibited. Dopamine is a neurotransmitter with stimulating properties. Other yet unknown neurotoxic mechanisms might play a role as well.

Secondly, data from animal research suggest that GBL may adversely affect reproduction, but it is not clear yet what kinds of mechanisms cause reproductive toxicity. However, it is supposed that GBL is able to disturb the hormone balance in the brain, and it is suggested that GBL might arrest directly the maturation of unfertilized ovules.

Effects

Observations in humans

Most data on the toxicity of γ-butyrolactone in humans concern acute poisoning after oral intake. No data are known on inhalatory or dermal exposure at work or elsewhere.

GBL, but mainly GHB, are used among youngsters as party drugs. This has led to many case reports of GBL and GHB poisoning. Different types of adverse health effects have been reported. The primary effect is dose-related depression of the central nervous system (CNS), such as sleepiness and loss of consciousness, epileptic seizures, changes in pupillary reflex, uncontrolled movements, confusion, hallucination and euphoria. These effects have been reported at doses of 20 to 30 mg GBL/kg bw (somnolence, dizziness, euphoria), and of about 10 mg GHB/kg bw. A dose of 60 mg GBL/kg bw can induce anesthesia and coma. A near fatal case has been reported of someone who consumed a GBL-containing drink, leading to a dose of about 570 mg GBL/kg bw. Other effects due to oral intake, which are partly related to CNS effects, were also observed, such as decreased heartbeat and blood pressure, nausea, and decreased respiration.

Repeated use of GBL can result in different neurotoxic effects, such as anxiety, depression, tremor, and sleepiness. It is however not clear at what exposure levels these effects occur.

No data are available on possible irritating effects in the skin and the eyes upon exposure to GBL in humans, nor are there any data reported on reproductive toxicity. In addition, there are insufficient reliable data reported on carcinogenicity in humans.

Observations in animals

The few data that are available indicate that undiluted GBL is slightly irritating to the skin, and moderately to severe irritating to the eyes by direct contact.

The dose after a single oral intake at which half of the exposed animals die, is approximately 800 to 1,800 mg GBL/mg bw in mice and rats. This points to a moderate to low acute toxicity.

In one animal study, rats were exposed to 5,100 mg GBL/m^3 for four hours. The animals showed shallow breathing, nasal discharge, lethargy, and limb disuse. All animals recovered however completely in the following fourteen-day observation period.

Rats and mice given a single intraperitoneal injection of GBL showed inactivity in movement, lowered body temperature and cataleptic effects. These effects started to occur at doses of 50 mg/kg bw and higher. A few hours after administration the effects disappeared completely.

Animal studies have been performed, in which rats and mice received different doses of GBL by gavage during two weeks, thirteen weeks, and two years. The main treatment-related effects observed were lowered body weight and death at the highest dose given, and lowered activity combined with irregular breathing. The latter effects started to occur at 225 mg/kg bw and higher. The investigators reported that in the first two to three weeks the lowered activity and irregular breathing were observed within a few minutes after administration, and then completely disappeared after a few hours. In the following weeks no such effects were observed at all, even not directly after administration, suggesting that some form of adaptation or tolerance has occurred. Animals, which were dosed 175 mg GBL/kg bw or lower did not show treatment-related effects during the whole experimental period.

Animal research did not reveal clear evidence for carcinogenicity after oral and dermal exposure in rats or mice. In one study, a slight increase in medullary hyperplasia in adrenal glands was observed in a low dose group with male mice (262 mg/kg bw); in the highest dose group with male mice (525 mg/kg bw) mortality was increased, as a consequence of which the sensitivity of the study to detect carcinogenic effects was reduced. In that study, no evidence for carcinogenicity was noted in female mice at either of the doses tested, nor in both sexes of rats.

Some studies have been performed to assess whether GBL was able to damage genetic material. The available data were negative, although it cannot be excluded with certainty that GBL is able to damage chromosomes in *in vitro* tests.

Finally, animal studies have been published on the reproductive toxicity of γ-butyrolactone. There are indications that GBL reduces the fertility in female rats given intraperitoneal injections of 62 mg/kg bw. However, more research is needed before the relevance of the finding for humans can be made. It is furthermore unclear whether GBL can affect progeny, because data are lacking, and data which are available are of insufficient quality.

Evaluation and recommendation

From the foregoing, the DECOS considers the adverse effects on the central nervous system (CNS) the most critical effects caused by exposure to γ-butyrolac-

tone (and its metabolite γ-hydroxybutyrate). Furthermore, from the data, the committee deduces that workers can experience CNS effects that can affect negatively the alertness of the worker within a few minutes after inhalation. To protect against such acute effects, a health-based recommended occupational exposure limit (HBR-OEL) is needed for short-term exposure. Therefore, the DECOS has assessed whether an HBR-OEL can be derived, as a 15-minute time weighted average concentration (15-min TWA HBR-OEL).

There are no data on humans exposed by GBL or GHB by inhalation, but there are data on single oral intake of both compounds. From these data, the DECOS concludes that CNS effects still occur at doses of 20 mg GBL/kg bw and 10 mg GHB/kg bw; data on lower doses are not reported. When GBL is absorbed from the gut, it is very rapidly metabolized to GHB. Therefore, the DECOS finds it justified to use data on GHB, and uses the dose of 10 mg/kg bw as starting point in deriving an HBR-OEL. This dose is corrected with a factor of 3 for the absence of a no effect level, and an additional factor of 3 to take into account for inter-individual differences. Applying these factors, a oral dose of 1 mg/kg bw is derived.

Because an HBR-OEL concerns *inhalation* exposure and not oral exposure, the dose should be extrapolated to a concentration of the substance in the air. For that purpose data on oral and inhalation bioavailability are needed. Since data on oral bioavailability of GBL are not available, the committee has used data on GHB. The oral bioavailability of GHB is reported to be 27%. Assuming, furthermore, an inhalation bioavailability of 100%, and that a worker weights on average 70 kg, and inhales a volume of 0.3 m^3 air during a working period of 15 minutes, the DECOS derives an HBR-OEL for γ-butyrolactone of 65 mg/m^3, as a 15-min TWA concentration.

The committee notes that in case of continuous exposure of subsequent 15-minute periods to 65 mg/m^3, at the end of an 8-hour during working day, accumulation of GHB in the body may have occurred. This is due to the fact that the time the body needs to eliminate the substances completely is longer than 15 minutes (plasma half-life values of GHB ranges between 30 and 60 minutes).* This means that the internal dose can rise above the internal dose after a single 15-minute exposure to 65 mg/m^3, and that a worker may experience CNS effects. Therefore, to prevent this from happening, an 8-hour TWA HBR-OEL is required. This 8-hour TWA should also protect against harmful CNS effects found in humans after long-term exposure.

* In the Netherlands, in principle no legally binding limitation applies for the number of times that a 15-minute TWA OEL may be reached during a normal working day, under the condition that the 8-hour TWA OEL is not exceeded.

No exposure data on long-term exposure levels in humans are available, but there are some data from (sub)chronic animal studies. These studies, in which GBL was given orally for up to two years, hardly showed adverse health effects; the only clear observation was a decrease in body weight. At the beginning, the animals showed slight inactivity directly after GBL administration, but this phenomenon disappeared completely after a few weeks in study. Taking all the available data on long-term exposure into account, the DECOS is of the opinion that these are insufficient to derive an 8-hour TWA HBR-OEL. However, the 15-min TWA HBR-OEL in combination with data on plasma-half lifes of GHB allow an assessment of an 8-hour TWA.

Using the plasma half-life of 60 minutes, and assuming that *every* 15 minutes the 15-minute HBR-OEL conditions apply, at the end of an 8-hour working day the internal dose accumulates by a factor 6.5 compared to the internal dose after a single exposure to the 15-minute TWA HBR-OEL. To prevent that accumulation is too high at the end of the day, the 15-minute HBR-OEL is divided by the 'accumulation' factor. This results in a health-based recommended occupational exposure level for γ-butyrolactone of 10 mg/m^3, as an 8-hour time weighted average concentration.

Health-based recommended occupational exposure limit

The DECOS of the Health Council recommends a health-based occupational exposure limit for exposure to γ-butyrolactone of 65 mg/m^3, as a 15-min TWA, and of 10 mg/m^3, as an 8-hour TWA.

Part I

Health Council of the Netherlands: Gamma-butyrolactone (GBL)

Chapter 1

Scope

1.1 Background

At request of the minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations on the toxicity of chemical substances that are used in the workplace (Annex A). The purpose of these evaluations is to recommend health-based occupational exposure limits, which specify levels of exposure to airborne substances, at or below which it may be reasonably be expected that there is no risk of adverse health effects.

In this advisory report, such an evaluation and recommendation is made for γ-butyrolactone (GBL).

1.2 Committees and procedure

The evaluation on the toxicity of GBL is a co-production of the DECOS and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). It is a result of an agreement between both groups to prepare jointly scientific criteria documents, which can be used by the national regulatory authorities in the Netherlands and the Scandinavian countries for establishing exposure limits. The members of the DECOS and NEG are listed in Annex B.

The joint draft evaluation has been prepared by Dr. E. Søderlund from the Norwegian Institute of Public Health, Norway. In addition, the draft was

reviewed first by the NEG and subsequently by the DECOS. In 2004, the final evaluation was published by the Swedish National Institute of Occupational Health. It is included in Part 2 of this advisory report.[13]

On the basis of this evaluation, and additional data published after 2004, the DECOS judged the toxicity of γ-butyrolactone in recommending a health-based occupational exposure limit. This judgment and recommendation are described in Part 1 of this report.

In 2008, the president of the Health Council released a draft of this report for public review. The individuals and organisations that commented on this draft are listed in Annex C. The DECOS has taken these comments into account in deciding on the final version of the report.

1.3 Data

Regarding Part 1, additional literature was retrieved from the on-line databases Medline and Toxline starting from 2003. The final search has been carried out in April 2008. The searches were performed using “CAS 96-48-0” and “butyrolacton?” as search profile.

Chapter 2

Effects

In the sections below, the first part of the sections summarizes the most relevant findings from the joint report. Additional information, which was considered relevant for the purpose of this advisory report, is summarized in the second part of the sections. Since in the body γ-butyrolactone (GBL) is metabolized in γ-hydroxybutyrate (GHB) within minutes, also data on GHB are presented, when considered of additional value by the committee.

2.1 Observations in humans

2.1.1 Irritation and sensitisation

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

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

Additional information

No new data were found.

2.1.2 *Acute and short-term toxicity*

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

No reports were located describing effects following acute occupational and inhalatory exposure.

Data obtained from case-reports on humans intoxicated by oral intake, revealed various neurotoxic effects on the central nervous system and related effects in other organs, such as loss of consciousness and coma, confusion, euphoria and hallucination, combativeness, obtundation, uncontrolled movements, bradycardia and tachycardia, and respiratory depression. First signs of acute adverse health effects in adults were reported to occur at doses of about 20 to 30 mg GBL/kg bw, and 10 mg γ-hydroxybutyrate GHB/kg bw. Reports on the induction of a coma have been published, in which it is suggested that coma can be induced at about 50 to 70 mg GHB/kg bw; a dose of 60 mg GBL/kg bw is considered to induce surgical anaesthesia. In the United Kingdom a near fatal intoxication was described, which corresponded to a dose of about 570 mg GBL/kg bw.

Additional information

New cases of drug abuse by GBL and GHB have been reported and reviewed. [9,12,14] These reports confirm the adverse health effects observed earlier. In addition to neurotoxic effects, also other adverse effects are described, such as nausea and vomiting, diaphoresis, and urinary and fecal incontinence, at unknown dose levels.

2.1.3 *Long-term toxicity and carcinogenicity*

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

Chronic oral use of GBL can lead to several neurotoxic effects, including anxiety, depression, tremor and insomnia.

In two nested case-control studies in workers, who were simultaneously exposed to various kinds of chemicals, including GBL, a few cases of non-Hodgkin's lymphoma and soft tissue sarcoma were found. However, no conclusion on the carcinogenicity of GBL can be drawn, because of the low number of participants, and the simultaneous exposure to many of the compounds examined. No information was found describing genotoxic effects in humans.

No information was found on the reproductive and developmental effects.

Additional information

No new data were found.

2.2 Observations in animals

In a consensus report, the Swedish Criteria Group for Occupational Safety summarized animal data on the toxic effects of GBL in increasing exposure order.[3] This summary is shown in Annex E of this advisory report.

2.2.1 Irritation and sensitisation

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

When applied to the skin, GBL causes weak skin and eye irritation. Data on skin sensitisation are too limited to allow an evaluation on this type of effect.

Additional information

Undiluted γ-butyrolactone caused no or mild irritation to the skin of rabbits.[1] In the same animal species, it, furthermore, caused moderate to severe irritation when applied to the eyes.[1]

2.2.2 Toxicity due to single exposure

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

γ-Butyrolactone has a moderate to low acute toxicity in laboratory animals; the LD_{50} values after a single oral dose are about 800 and 1,800 mg GBL/kg bw in mice and rats, respectively.

In one study, Sprague-Dawley rats were exposed to 5,100 mg/m³ for four hours. Effects observed included prostration, lethargy, breathing problems, limb disuse, and nasal discharge. In the fourteen-day observation period that followed exposure, none of the animals died, and the investigators observed that the effects were clearly reversible.

Intraperitoneal or intravenous administration of GBL in rats and mice induced various acute neurotoxic effects, such as loss of righting and pain reflexes (above 200 mg GBL/kg bw), and complete anaesthesia (above 800 mg GBL/kg bw). Furthermore, respiration was slowed down at 200 mg GBL/kg bw.

Additional information

Groups of seven male Sprague-Dawley rats were given a single intraperitoneal injection of GBL at doses of 0, 18, 32, 56, 100, 178, or 320 mg/kg bw.[2] The compound suppressed simple schedule-controlled behaviour in a dose-dependent manner. Up to 56 mg/kg bw no suppression of response was observed. The response in animals given 178 mg/kg bw was suppressed for one hour and returned to normal by 100 minutes. The authors reported that the dose, which results in a 50% decreased response compared to the control rate, corresponded to 111 mg GBL/kg bw (95% confidence limits 67 – 183 mg/kg bw). The same authors also injected GHB at doses of 0, 100, 178 and 320 mg/kg bw. Responses were suppressed at 178 and 320 mg/kg bw. Within 100 minutes animals returned to normal activity.

Groups of six to eight male Swiss-Webster mice received a single intraperitoneal injection of GBL at doses of 0, 25, 50, 100, or 150 mg/kg bw.[4] In the two hours following treatment, locomotor activity and body temperatures were recorded. No treatment-related effects were observed at a dose of 25 mg/kg bw compared to vehicle controls. A dose of 50 mg/kg bw had a depressing effect on locomotor activity for the first 20 minutes, followed by a stimulatory effect the next 20 minutes, and returning to normal for the rest of the observation time. Doses of 100 and 150 mg/kg bw induced a significant depression in locomotor activity and body temperature shortly after treatment, but within one hour the effects returned to normal levels. The authors considered the effects dose-dependent.

For studying cataleptic effects, groups of eight to ten male C57BL/6J mice were given a single intraperitoneal injection of GBL at doses of 0, 32, 56, 100, 178, or 320 mg/kg bw.[8] Cataleptic effects were observed in mice receiving a dose of 56 mg/kg bw and higher, measured 15 minutes after treatment. More than five hours later the effects diminished. No cataleptic effects were observed at 32 mg/kg bw.

2.2.3 Toxicity due to short-time exposure

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

Groups of five F344/N rats and five B6C3F$_1$ mice, per dose and sex, received daily GBL in corn oil by gavage for 5 days per week for two weeks.[11] The doses applied were 0, 75, 150, 300, 600 or 1,200 mg/kg bw in rats, and 0, 87, 175, 350, 700 or 1,400 mg/kg bw in mice. All animals were killed for

complete necropsy at the end of the experimental period. Rats and mice receiving the highest dose died before the end of the study. In the surviving animals, signs of recumbence or inactivity with irregular and labours respiration were observed soon after dosing 600 mg/kg bw (rats) and 350 mg/kg bw (mice) and above. In female rats given 600 mg/kg bw a decreased body weight gain was observed; in all other animal groups, including mice, mean body weight gains were similar to those of controls.

The same research institute (National Toxicology Program) used a comparable study design, using the same animal species and administration route, for a 90-day study. This time the doses applied ranged between 0, 56, 112, 225, 450 or 900 mg/kg bw in rats, and 0, 65, 131, 262, 525 or 1,050 mg/kg bw in mice. Most animals in the highest dose group died before the end of the experiment. The lowest dose at which significant loss of body weight was observed were 450 mg/kg bw (rats) and 1,050 mg/kg bw (mice). In rats, slight inactivity was observed when 225 and 450 mg/kg bw was administered, and even recumbence was seen at 450 mg/kg bw. However, after a few weeks in study the animals showed adaptation to these anaesthetic effects. Also in mice given 525 mg/kg bw an adaptive response for the same effects was reported. No significant biological differences in organs and microscopic lesions related to GBL-exposure were observed.

Additional information

No new data were found.

2.2.4 *Toxicity due to long-term exposure and carcinogenicity*

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

No evidence of carcinogenicity was found in groups of fifty F344/N rats, which were given GBL in corn oil by gavage, 5 days per week for up to 103 weeks. The doses applied were 0, 112 or 225 mg/kg bw in males, and 0, 225 or 450 mg/kg bw in females.

The same study also included groups of $B6C3F_1$ mice. They received doses of 0, 262 or 525 mg/kg bw (males and females). Decreased mean body weight and depression of the central nervous system were noted shortly after exposure in mice. No evidence was found for carcinogenic activity in female mice. However, in male mice increased incidences of proliferative lesions, primarily hyperplasia, of the adrenal medulla were observed at 262 mg/kg bw. Yet, the sensitivity of the study in male mice to detect a carcinogenic effect is reduced by a low survival of high dose animals; in this group no increase in incidence of tumours was observed compared to the control group. Therefore, the evidence of carcinogenicity in the low dose group is considered equivocal.

No evidence for carcinogenicity of GBL was found in other animal studies performed by other research groups. This included oral administration, repeated dermal application, and subcutaneous injections for at least eighteen months in mice.

Overall, data on the carcinogenicity shows that GBL is not a carcinogen in rats and mice.

Additional information

No new data were found.

2.2.5 *Mutagenicity and genotoxicity*

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

Data on mutagenicity and genotoxicity are presented in Appendix 2 of the Arbete och Hälsa report in Part 2 of this advisory report.

In short, a large number of mutagenicity studies of GBL have been performed. Generally, the vast majority of the *in vitro* mutagenicity experiments, using the standard *S. typhimurium* and *E. Coli* assays in the presence or absence of metabolic activation, were negative. Comparable effects were observed using yeast.

In vitro clastogenicity experiments using mammalian cells have given conflicting results. For instance, in one study increased frequencies of chromosomal aberrations and sister chromatid exchanges were observed, in others not. The studies differed in cell types, exposure concentrations and the presence or absence of an exogenous metabolic system.

GBL did not express mutagenic potential *in vivo* (*i.e.*, micronucleus test of bone marrow cells) at doses up to about 1,000 mg/kg bw in mice, nor did it induce sex-linked recessive lethal mutation and mitotic recombination in *D. melanogaster* flies.

In conclusion, GBL is not mutagenic. However, the possibility that GBL may cause chromosomal aberrations and sister chromatid exchanges *in vitro* cannot be completely ruled out.

Additional information

No new data were found.

2.2.6 *Reproductive and developmental toxicity*

Summary of the data presented in Arbete och Hälsa report (for more details see Part 2)

Regarding fertility, no standard one- or two-generation fertility studies in experimental animals have been found in the literature.

In one study using female rats, results indicate that a single exposure to GBL may interfere with female reproduction. GBL was injected intraperitoneally at doses from 62.5 to 750 mg/kg bw. A reduction in the number of rats ovulating was evident at the lowest dose; a dose of 750 mg/kg bw blocked ovulation completely.

Embryotoxicity and teratogenicity on repeated exposure was investigated in an oral study. Groups of female rats were administered GBL by gavage at doses of 0, 10, 50, 125, 250, and 500 mg/kg bw per day on gestation days 6 through 15. No changes in maternal fertility were observed among the animals. Furthermore, no signs of GBL-related foetal malformations were found. However, the placental weights were significantly reduced in all GBL-exposed groups. Also, the mean foetal weight was increased significantly in groups exposed to 50, 125, and 250 mg/kg bw. The authors could not explain the possible relation between foetal weight increases and GBL exposure.

In a poorly-reported study, GBL, given in tap water at doses of approximately 550 mg/kg bw and higher, reduced gonadal development in young male rats. This resulted in significant reduced testicular weights. However, seminal vesicle weights and serum prolactin levels were similar in the control rats and in the rats treated with GBL. In other repeated dose studies, however, no GBL-related toxic effects in the testes were seen.

Additional information

No new data were found.

Chapter **3**

Hazard assessment

3.1 Hazard identification

No data are available on occupational exposure to γ-butyrolactone (GBL) via inhalation or dermal contact in humans. However, many case reports on GBL and its metabolite γ-hydroxybutyrate (GHB) have been published and reviewed on oral intoxication, because they are used as party drugs. Furthermore, GHB is used as medicine.*

The main adverse health effect reported in humans after single or repeated use, is depression of the central nervous system (CNS). The lowest dose of GBL, resulting in adverse effects of the CNS, was reported to be 20 to 30 mg/kg bw (euphoria); for GHB this was 10 mg/kg bw. No data are presented in the literature on exposure to lower doses. A dose of approximately 60 mg GBL/kg bw induces surgical anaesthesia. In a near fatal case of coma, a dose of 570 mg GBL/kg bw was reported. No clear dose-response data are available, nor are there human data found on adverse effects in other organ systems.

Data obtained from animal studies confirm that γ-butyrolactone affects the CNS. Depression of the CNS was for instance found after single and repeated exposure, via oral intake, intraperitoneal injections, and inhalation. Data were consistent in that the CNS effects (*i.e.*, catalepsy, lowered activity, breathing problems) started to occur within minutes after administration, but disappeared

* For more information: www.emea.europa.eu/humandocs/Humans/EPAR/xyrem/xyrem.htm

within a few hours. The lowest doses, which were reported to induce CNS effects in animals were 50 mg/kg bw (single exposure, rats and mice, intraperitoneal injection), and 225 mg/kg bw (repeated exposure, rats, by gavage). Furthermore, in repeated exposure studies it was observed that after a while the animals did not show any CNS effects anymore, not even after administration. Additionally, no exposure-related gross or microscopic lesions in various organs were found in rats and mice given GBL by gavage for 90 days (doses administered up to 900 mg/kg bw (rats), and up to 1,050 mg/kg bw (mice)). The only significant long-term effect observed in the 90-day study was lowered body weight in rats and mice given the highest dose of GBL.

Regarding carcinogenesis, equivocal evidence of carcinogenicity was found in a long-term animal study using mice, but in other long-term animal studies no signs of carcinogenicity were observed. There is insufficient evidence that GBL can cause cancer in humans. Overall, there is no clear evidence suggesting that γ-butyrolactone is carcinogenic in humans or animals. In 1992, IARC concluded therefore that the compound was not classifiable as to its carcinogenicity to humans (see Annex D). The committee agrees with this conclusion.

Gamma-butyrolactone has been reported to induce adverse reproductive and developmental effects in rats. Reduced ovulation was for instance observed at the lowest-tested single intraperitoneal dose of 62.5 mg/kg bw. In addition, reduced foetal weights were reported at doses of as low as 50 mg/kg bw. However, no signs of GBL-related reproductive effects and developmental malformations have been reported at higher exposure levels, and no standard fertility studies are available. Since the quality of data on reproductive toxicity is insufficient, the committee does not classify the substance as toxic to reproduction.

In conclusion, taking the whole set of available data into account, the committee considers the γ-butyrolactone-induced effects on the central nervous system as the most critical. These include sleepiness and coma, and disturbances in locomotor activity, anxiety and insomnia.

3.2 Quantitative hazard assessment

3.2.1 Recommendation of a health-based recommended OEL, 15-minute TWA

It is evident that most of the CNS effects are observed within minutes after intake. To protect against these acute effects, a health-based recommended occupational exposure limit (HBR-OEL) is needed for short-term exposure. This means that the DECOS has assessed whether an HBR-OEL can be derived, as a 15-minute time weighted average (15-min TWA) concentration.

Table 3.1 Data obtained from clinical studies on healthy volunteers, single administration.[10,13]

Dose administered (mg/kg bw)	Administration route	Observed effects
γ-Butyrolactone (GBL)		
20-30	Oral	Euphoria
60	Oral	Anaesthesia
570	Oral	Coma, near fatal
γ-Hydroxybutyrate (GHB)		
10	Oral, intravenous injection	Short amnesia, hypotonia of skeletal muscles, impairment short-term memory
20-30	Oral, intravenous injection	Promotion REM sleep, sleepiness, dizziness, euphoria
50-70	Oral, intravenous injection	General anaesthesia, coma (usually lasting up to 4 hours), bradycardia, hypotonia, Cheyne-Stokes respiration, nausea

There have been no data reported on humans exposed by inhalation, but there are data on single oral intake. Overall, the results of different clinical studies with oral intake are consistent. For this reason in Table 3.1 a summary of the data is given, instead of data of individual studies. From the table, the DECOS concludes that neurological effects still occur at doses of 10 mg GHB/kg bw and 20 mg GBL/kg bw. Data on lower doses are not reported. When GBL is absorbed from the gut, it is metabolized very rapidly to GHB. Therefore, the DECOS feels that it is justifiable to use data on GHB, that is to use the dose of 10 mg/kg bw as starting point in deriving an HBR-OEL for GBL. This dose is corrected with a factor of 3 for the absence of a no effect level, and an additional factor of 3 to take inter-individual differences into account. Applying these factors an oral dose of 1 mg/kg bw is derived, as a no-observed-adverse-effect-level.

Because an HBR-OEL concerns *inhalation* exposure and not oral exposure, the dose should be extrapolated to a concentration of the substance in the air.

Available data suggest that oral uptake of GBL and GHB from the gut is virtually complete. However, there are no data on the bioavailability of GBL, that is the fraction of the absorbed dose that reaches unchanged the circulation. For GHB it is reported to be poor. Limited human data indicate that about 27% reaches finally the systemic circulation after oral uptake, due to a high first-pass-metabolism.[6] The DECOS uses this bioavailability value also for GBL. Thus a value of 0.27 mg/kg bw is derived as a no-observed-adverse-effect-level for systemically available GBL.

No data are available on inhalation bioavailability on either substance. Therefore, the committee uses the worst case assumption that 100% will be taken up and available after inhalation. In addition, the DECOS assumes that a worker weighs on average 70 kg and inhales during an 8-hour working day 10 m^3 air

(corresponds to 0.3 m^3 of a 15-minute working period).*
Taking the foregoing into account, the DECOS derives an HBR-OEL for γ-butyrolactone of 65 mg/m^3 (= (0.27 mg/kg × 70 kg)/0.3 m^3; rounded off), as a 15-min TWA concentration.

The committee notes that in case of continuous exposure of subsequent 15-minute periods to 65 mg/m^3, at the end of an 8-hour working day, accumulation of GHB in the body may have occurred, because the plasma half-life of the compound is too long to eliminate all GHB within 15 minutes.** This means that the internal dose can rise above the internal dose after a single 15-minute exposure to 65 mg/m^3, and that a worker may experience CNS effects. Therefore, to prevent this from happening, an 8-hour TWA HBR-OEL is required. This 8-hour TWA should also protect against harmful CNS effects found in humans after long-term exposure.

3.2.2 *Recommendation of a health-based recommended OEL, 8-hour TWA*

No exposure data on long-term exposure levels in humans are available, but there are some data from (sub)chronic animal studies. Well-performed (sub)chronic, oral animal studies revealed that on the long-term adverse effects on the CNS disappeared, indicating some ability of adaptation or tolerance.[11] Furthermore, no relevant histopathological changes were observed in any of the exposed animals. However, lowered body weights were recorded in some animal groups; the lowest exposure group were mice to which 262 mg GBL/kg bw was administered for two years. Taking all the available data on long-term exposure into account, the DECOS is of the opinion that these are insufficient to derive an 8-hour TWA HBR-OEL, and thus that no 8-hour TWA HBR-OEL can be determined from epidemiological and (sub)chronic animal studies. However, the 15-min TWA HBR-OEL in combination with data on plasma-half lifes of GHB allow an assessment of an 8-hour TWA.

In the worst-case scenario, serious accumulation of GHB may occur if every fifteen minutes of an 8-hour working day the 15-min TWA HBR-OEL is reached. More specifically, at the end of an 8-hour working, this would mean that the internal exposure is about 3.5x (half-life of 30 minutes) to 6.5x (half-life of 60 minutes) higher at (15-minute average) exposure to 65 mg/m^3 than will be reached after a single (15-minute average) exposure to 65 mg/m^3. As a result, this

* DECOS uses standardly these values as default values for body weight and inhalation volume.
** In the Netherlands, in principle no legally binding limitation applies for the number of times that a 15-minute TWA OEL may be reached during a normal working day, under the condition that the 8-hour TWA OEL is not exceeded.

would imply that at the end of an 8-hour working day, the internal dose could rise above the internal dose that corresponds to the no-observed-adverse-effect-level (NOAEL), and thus that workers may suffer adverse CNS effects. This should be prevented, and, therefore, the DECOS decided to derive an HBR-OEL as an 8-hour TWA concentration by using the 15-minute TWA HBR-OEL as starting point. The HBR-OEL is derived by dividing the 15-minute level (65 mg/m^3) by a factor of 6.5 to adjust for accumulation and assuming a plasma half-life of 60 minutes. Applying this factor, an HBR-OEL for γ-butyrolactone is derived of 10 mg/m^3, as an 8-hour TWA concentration.

3.3 Skin notation

Gamma-butyrolactone is easily taken up by the body through dermal exposure, and, therefore, this route of exposure may add to the systemic effects. To decide whether a skin notation should be recommended to the substance, the DECOS uses the ECETOC criteria for assigning a skin notation.[5] This implies that a skin notation should be applied when the amount by both hands and forearms (2,000 cm^2) in one hour could amount to more than 10% of the amount that can be absorbed via the lungs on exposure to the proposed health-based recommended OEL for 8 hours, provided that this OEL is set on the basis of systemic toxicity.

Regarding γ-butyrolactone, the reported absorption rate for human skin *in vitro* is 110 µg/cm^2 in one hour at steady state.[12,13] Thus, a one-hour absorption by two hands and forearms (2000 cm^2) of GBL results in a total dermal uptake of 220 mg (2000 cm^2×110 µg/cm^2). Assuming that a worker inhales 10 m^3 air during an 8-hour working day, and that the inhalatory uptake is 100%, exposure to the health-based recommended OEL of 10 mg/m^3 results in a total uptake of 100 mg. This means that an one-hour absorption by two hands and forearms contribute to 220% ((220/100)×100%) of the exposure after absorption via the lungs. This highly exceeds the limit of 10%. Therefore, the DECOS recommends applying a skin notation for γ-butyrolactone.

3.4 Groups at risk

No specific groups at risk could be identified.

3.5 Research needs

There is a need of information on γ-butyrolactone toxicity after inhalatory exposure in both humans and animals. Efforts should be made on producing data on

quantitative exposure-response relationships. Furthermore, investigations need to be performed to find out whether, and to what extent, GBL exposure may lead to reproductive and developmental effects.

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as

part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the committee is given in annex B.

Annex **B**

The committees

The Dutch Expert Committee on Occupational Safety (DECOS)

- G.J. Mulder, *chairman*
  emeritus professor of toxicology, Leiden University, Leiden
- R.B. Beems
  toxicologic pathologist, formerly employed at the National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  toxicologist, Shell International BV, The Hague
- J.J.A.M. Brokamp, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
  professor of risk assessment in occupational epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- L.A.L.M. Kiemeney
  professor of cancer epidemiology, University Medical Centre St Radboud, Nijmegen
- H. van Loveren
  professor of immunotoxicology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  occupational physician, Netherlands Center for Occupational Diseases, Amsterdam

- A.H. Piersma
  professor of reproductive toxicology, National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  professor of molecular biology, VU University Amsterdam, Amsterdam
- I.M.C.M. Rietjens
  professor of toxicology, Wageningen University and Research Centre, Wageningen
- H. Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- T. Smid
  occupational hygienist – epidemiologist, KLM Health Services, Schiphol, and professor of working conditions, VU University Amsterdam, Amsterdam
- G.M.H. Swaen
  epidemiologist, Dow Benelux N.V., Terneuzen
- R.A. Woutersen
- toxicologic pathologist, TNO Quality of Life, Zeist
- P.B. Wulp
  occupational physician, Labour Inspectorate, Groningen
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Nordic Expert Group (NEG)

- G. Johanson, *chairman*
  professor of occupational toxicology, Karolinska Institutet, formerly the National Institute for Working Life (Sweden)
- V. Kristjansson
  organic chemist, Administration of Occupational Safety and Health (Iceland)
- K. Savolainen
  professor of toxicology, Finnish Institute of Occupational Health (Finland)
- V. Skaug
  toxicologist, occupational physician, National Institute of Occupational health (Norway)
- K. Sørig Hougaard
  toxicologist, National Research Centre for the Working Environment, formerly the National Institute of Occupational Health (Denmark)
- J. Järnberg, *scientific secretary*
  Swedish Work Environment Authority, formerly the National Institute for Working Life (Sweden)

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments from public review draft

A draft of this advisory report was released in 2008 for public review. The following organisations and persons have commented on the draft:

- Ms Gálvez Pérez, Ministerio de Trabajo e Inmigración, Spain;
- Dr. D. Zumwalde, National Institute for Occupational Safety and Health, the USA.

Annex **D**

IARC summary and evaluation for the purpose of carcinogenic classification

γ-BUTYROLACTONE (Group 3)

VOL.: 71 (1999) (p. 367)[7]
CAS No.: 96-48-0
Chem. Abstr. Name: Dihydro-2(3-*H*)-furanone

Summary of Data Reported and Evaluation

Exposure data

Exposure to γ-butyrolactone may occur in its production and use as an intermediate and as a solvent. It has been detected in alcoholic beverages, tobacco smoke, coffee and several foodstuffs.

Human carcinogenicity data

No adequate data were available to the Working Group.

Animal carcinogenicity data

γ-Butyrolactone was tested for carcinogenicity in two studies in mice and two studies in rats by oral administration. It was also tested in mice by skin application in two studies and by subcutaneous injection in mice and rats in single studies. No carcinogenic effect was observed.

Other relevant data

γ-Butyrolactone rapidly hydrolyses in blood to γ-hydroxybutyric acid. γ-Butyrolactone has been extensively studied in in-vitro genetic toxicity tests in which the overwhelming majority of results did not indicate activity. Positive results were obtained in one study for chromosomal aberrations and sister chromatid exchanges in a Chinese hamster cell line. No mutagenic activity was observed *in vivo* in *Drosophila* or in mouse bone marrow micronucleus tests.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of γ-butyrolactone.

There is *evidence suggesting lack of carcinogenicity* of γ-butyrolactone in experimental animals.

Overall evaluation

γ-Butyrolactone is *not classifiable as to its carcinogenicity to humans* (Group 3).

Previous evaluations: Vol. 11 (1976); Suppl. 7 (1987)

Annex **E**

Summary of animal data on toxic effects of gamma-butyrolactone

Source, Scientific basis for Swedish occupational standards xxv, Arbete och Hälsa report no. 2005:7, page 113.[3]

Table 1. Effects of GBL on mice and rats. (i.p. = intraperitoneal, p.o. = per os)

Exposure	Species	Effects
22 mg/kg bw single dose, i.p.	Mouse	Temporary reduction in mobility and coordination
50 mg/kg bw/day days 6-15 of gestation, p.o.	Rat	Increased fetal weight, somewhat lower placental weight
50 mg/kg bw single dose, i.p.	Rat (juvenile)	Temporary EEG changes and effects on behavior (including inactivity)
55 mg/kg bw single dose, i.p.	Mouse	Temporary declines in activity and coordination
62.5 mg/kg bw single dose, i.p.	Rat	Inhibited ovulation in 22% of animals
125 mg/kg bw single dose, i.p.	Rat	Inhibited ovulation in 20% of animals
150 mg/kg bw single dose, i.p.	Rat	Temporary EEG changes and effects on behavior (including inactivity)
175 mg/kg bw/day 12 days, p.o.	Mouse	NOAEL
225 mg/kg bw/day 5 days/week, 13 weeks, p.o.	Rat	Slight inactivity after dosing during the first few weeks
250 mg/kg bw single dose, i.p.	Rat	Inhibited ovulation in 63% of animals, reduction of LH in serum
262 mg/kg bw/day 5 days/week, 13 weeks, p.o.	Mouse	Moderate inactivity after dosing during the first few weeks
262 mg/kg bw/day 5 days/week 2 years, p.o.	Mouse	Inhibited growth Males: elevated incidence of proliferative damage in the adrenal medulla
300 mg/kg bw/day 12 days, p.o.	Rat	NOAEL
350 mg/kg bw/day 12 days, p.o.	Mouse	Inactivity after dosing, irregular respiration
450 mg/kg bw/day 5 days/week 13 weeks, p.o.	Rat	Slight inactivity after dosing during the first few weeks Males: lower weight gain
450 mg/kg bw/day 5 days/week, 2 years, p.o.	Rat (females)	Inhibited growth
500 mg/kg bw single dose, i.p.	Rat	Anesthetic effect, inhibited ovulation in 71% of animals, reduction of LH and FSH in serum
0.5% in drinking water, (≈550 mg/kg/day) probably 20 days	Rat	40% reduction in testes weight

Part II

Arbete och Hälsa: γ-Butyrolactone

NR 2004:7

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

135. γ-Butyrolactone (GBL)

Erik Søderlund

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.

We acknowledge the Nordic Council for its financial support of this project.

G.J. Mulder
Chairman
DECOS

G. Johanson
Chairman
NEG

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

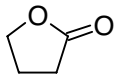
γ-Butyrolactone (GBL) is the cyclic ester of 4-hydroxybutanoic acid. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media γ-hydroxybutyrate (GHB) will predominate while in acid media the lactone form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

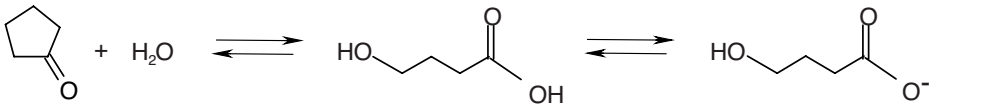
IUPAC name	Dihydro-2(3-H)-furanone
CAS name	γ-Butyrolactone
CAS No	96-48-0
EINECS No	202-509-5
Synonyms	butyric acid lactone; 1, 2-butanolide; 1, 4-butanolide; 4-butyrolactone; 4-hydroxybutanoic acid lactone; γ-hydroxybutyric acid cyclic ester; 4-deoxytetronic acid; tetrahydro-2-furanone

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

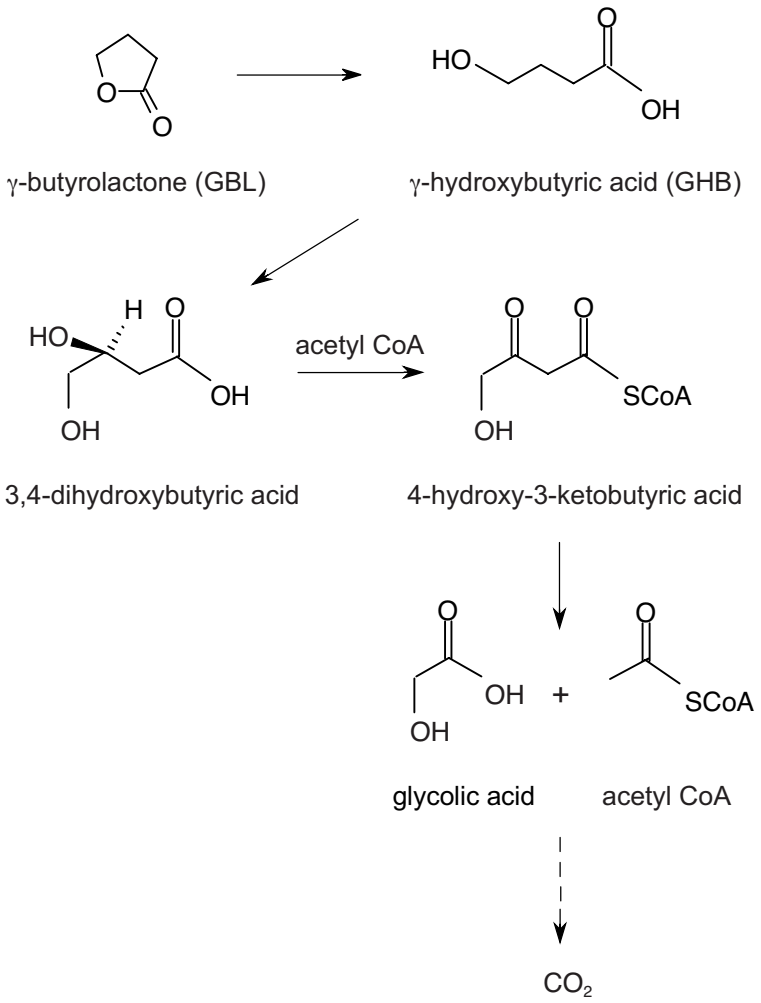


Figure 2. Metabolism of γ-butyrolactone (GBL) in humans. Adapted from (1, 91).

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.