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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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γ-Butyrolactone No. 7

CAS No. 96-48-0

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γ-Butyrolactone

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1 Summary and assessment

In rat studies, γ-butyrolactone is absorbed rapidly and completely from the gastrointestinal tract upon oral administration, with peak plasma levels being reached within a few minutes. Following dermal application to the skin of rats, absorption rates of 7 and 11% have been ascertained, depending on the source of information. The elimination half-life from plasma is 0.3 hours in the rat. Catalysed by lactonases, γ-butyrolactone is rapidly metabolised to γ-hydroxybutyric acid, which is further catabolised to carbon dioxide. The citric acid cycle and β-oxidation are suggested as mechanisms of γ-hydroxybutyric acid catabolism to CO_2. Following intraperitoneal administration of ^{14}C-labelled γ-butyrolactone, rats were found to eliminate approx. 60% of the administered radioactivity as CO_2 in the exhaled air within 2,5 hours.

On single oral administration, γ-butyrolactone has proved harmful in the rat, mouse and guinea pig, with LD_{50} values being in the range from 500 to 1800 mg/kg body weight. Acute toxicity is low following single dermal application and inhalation exposure, the dermal LD_{50} for guinea pigs being > 5640 mg/kg body weight and the LC_{50} values found in rats after 4-hours' inhalation being > 2680 and > 5100 mg/m³, depending on the source of information. On intraperitoneal administration, LD_{50} values determined in rats were 200 to 1580 mg/kg body weight, while in mice they were 200 to 1470 mg/kg body weight. Following intravenous injection, an LD_{50} value of 880 mg/kg body weight was reported. The clinical signs of toxicity seen after oral and intraperitoneal administration include, inter alia, salivation, lying on the abdomen or side, apathy, atonia and dyspnoea, and independently of the route of administration, a narcotic effect is observed. When rats received 5 or 12 oral doses of γ-butyrolactone, observations at and above a dose of 600 mg/kg body weight included deaths, sedative and narcotic effects as well as retarded body weight development. In mice, 12 oral administra-

tions of up to 1400 mg/kg body weight resulted in death at the high dose level. Starting at a dose level of 350 mg/kg body weight, sedative effects and breathing difficulties were noted. In exploratory studies, 5 exposures to γ-butyrolactone at a concentration level of approx. 2668 mg/m³ were tolerated without signs of toxicity by rats, rabbits and guinea pigs, whereas cats exhibited panting respiration, reeling and vomiting, and mice showed reeling and lying on the side.

γ-Butyrolactone causes no or only mild irritation to the skin of the rabbit but is moderately to severely irritating to the rabbit eye.

It has been reported that γ-butyrolactone has no sensitising effect on guinea pig skin; however, there are no further details of the study.

Following subchronic oral administration of γ-butyrolactone at dose levels of 56 to 900 mg/kg body weight for a period of 3 months, rats showed transient sedative effects and increased mortality at the high doses. From 450 mg/kg body weight, body weight retardation was noted (in the males). Histopathologically, dose levels of and above 56 mg/kg body weight were observed to be associated with inflammation of the nasal mucosa, a finding which was attributed to possible aspiration of the test substance. In mice, 90-day oral administration of doses in the range from 65 to 1050 mg/kg body weight was also found to cause transient sedative effects from 262 mg/kg body weight, and at the highest dose of 1050 mg/kg body weight, decreased body weights (males) as well as increased mortality were observed. Dogs tolerated 90-day oral administration of γ-butyrolactone doses of 70 to 280 mg/kg body weight in their food without harm.

The genotoxic potential of γ-butyrolactone has been comprehensively studied in numerous in-vitro and in-vivo test systems. Results are available from, inter alia, in-vitro studies of the chemical with regard to genotoxicity in Salmonella/microsome assay systems, in *Escherichia coli*, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* as well as in the HPRT test in V79 cells; with respect to the chemical's clastogenic potential in chromosome aberration tests in CHO and RL1 cells; regarding its DNA-damaging effects in DNA repair test systems in *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and CHO cells, in the SCE test in CHO cells and the UDS test in HeLa cells as well as with regard to the induction of recombinations and genomic mutations in *Saccharomyces cerevisiae*. In vivo, the genotoxic effects of

γ-butyrolactone have been investigated in micronucleus tests conducted in B6C3F1 and CD1 mice, in the sex-linked recessive lethal test and the eye mosaic assay in *Drosophila melanogaster* as well as with regard to induction of diploid spermatozoa in the NMRI mouse. No findings have been reported which would indicate that γ-butyrolactone has a relevant genotoxic potential.

In a 2-year chronic toxicity study with oral administration of γ-butyrolactone at dose levels of up to 450 mg/kg body weight (rats) and 525 mg/kg body weight (mice), there were no indications in male and female rats or in female mice that the chemical had a carcinogenic potential. The male mice were found to have, in the adrenal medulla, a marginally increased incidence of pheochromocytoma, which was also higher than the frequency seen in the historical controls. Further carcinogenicity studies in which mice were treated with oral doses of γ-butyrolactone by gavage or in the feed revealed no increase in the incidence of tumours. Similarly, no increased tumour frequencies were observed in mice after chronic dermal application. An in-vitro cell transformation test using BHK cells is reported to have given a positive result. From the data of the long-term studies, it appears that γ-butyrolactone has no relevant carcinogenic potential.

In rats and rabbits, oral γ-butyrolactone doses of up to 500 mg/kg body weight and inhalation exposure up to the highest test concentration of 5000 mg/m³, respectively, caused no maternal toxicity, embryotoxicity, fetotoxicity or teratogenicity. Repeated administration of γ-butyrolactone in the drinking water (1 and 2%) to sexually immature male rats resulted in decreased testicular weights. In male mice, 5 intraperitoneal injections of γ-butyrolactone at dose levels of up to 1128 µg/kg body weight did not produce morphologically abnormal sperm heads.

γ-Butyrolactone is classed with the neuroactive substances which act on the catecholamine receptors in the brain. The chemical's sedative and narcotic actions, which are seen at high doses and have been investigated in numerous neuropharmacological studies, is attributed to its metabolite, γ-hydroxybutyric acid.

In addition, further studies have demonstrated analgesic, anticonvulsive and motility-reducing effects, inhibition of the transmission of nerve impulses as well as actions on the dopaminergic system.

Upon oral administration of γ-butyrolactone to humans, urinary excretion of S-3,4-dihydroxybutyrate, γ-hydroxybutyrate and glycolic acid is increased. Cases of accidental poisoning in humans, particularly children, have repeatedly been reported in connection with consumer products which contained γ-butyrolactone. The signs of intoxication have been reported to include unconsciousness and coma, which did not, however, result in permanent damage. γ-Butyrolactone induces sleep in healthy volunteers. In humans, γ-butyrolactone causes no skin irritation or sensitisation.

2 Name of substance

2.1	Usual name	γ-Butyrolactone
2.2	IUPAC name	Dihydro-2(3H)-furanone
2.3	CAS No.	96-48-0
2.4	EINECS No.	202-509-5

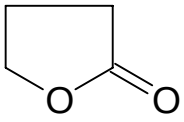
3 Synonyms, common and trade names

Agrisynth BLO
gamma-BL
γ-BL
BLO
BLON
4-Butanolid
1,4-Butanolide
Butyric acid, 4-Butyro-, gamma-lactone
Butyric acid lactone
Butyrolactone
gamma-Butyrolactone
γ-Butyrolacton
4-Butyrolactone
γ-Butyryl lactone
Butyryl lactone
4-Deoxytetronic acid
2(3H)-Furanone, dihydro-
GBL

4-Hydroxybutanoic acid gamma lactone
4-Hydroxybutanoic acid lactone
4-Hydroxybuttersäurelacton
gamma-Hydroxybutyric acid cyclic ester
4-Hydroxybutyric acid, gamma lactone
gamma-Hydroxybutyric acid lactone
3-Hydroxybutyric acid lactone
4-Hydroxybutyric acid, gamma-lactone
gamma-Hydroxybutyrolactone
gamma-Lactone, 4-hydroxy-butanoic acid
2-Oxolanone
1-Oxacyclopentan-2-one
Tetrahydro-2-furanon
Tetrahydro-2-furanone

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_4H_6O_2$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	86.09	
5.2	Melting point, °C	–44	(Falbe and Regitz, 1996; Hort and Taylor, 1991)
		–43.53	(Mercker and Kieczka, 1985)
		–43.5	(EC, 1996)
		–43.3	(Lide and Frederikse, 1996)
5.3	Boiling point, °C	206	(Falbe and Regitz, 1996; Mercker and Kieczka, 1985)
		204	(Hort and Taylor, 1991; Lide and Frederikse, 1996)
		204 to 206	(EC, 1996)

5.4	Vapour pressure, hPa	0.42 (at 20 °C)	(BASF, not dated)
		0.344 (at 20 °C)	(EC, 1996)
		1 (at 35 °C)	(EC, 1996)
		3.0 (at 50 °C)	
		300 (at 160 °C)	(BASF, not dated)
5.5	Density, g/cm³	1.129 (at 20 °C)	(Falbe and Regitz, 1996; Hort and Taylor, 1991)
		1.128	(EC, 1996)
		1.1284 (at 16 °C)	(Lide and Frederikse, 1996)
5.6	Solubility in water	Miscible in all proportions with water, volatile with steam	(Falbe and Regitz, 1996; Mercker and Kieczka, 1985)
		Miscible with water	(EC, 1996)
5.7	Solubility in organic solvents	Soluble in ethanol, ether, acetone, benzene, methanol, tetrachloromethane, chlorobenzene, propylene glycol, polyethylene glycol 400	(GAF, 1971; Windholz et al., 1983)
		Dissolves well in acetone, benzene, diethyl ether and ethanol	(Lide and Frederikse, 1996)
		Low solubility in aliphatic hydrocarbons	(Hort and Taylor, 1991; Mercker and Kieczka, 1985)
5.8	Solubility in fat	No information available	
5.9	pH value	4.5 (10% aqueous solution)	(GAF, 1971)
		4 (at 100 g/l and 20 °C)	(EC, 1996)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 3.51 mg/m³ 1 mg/m³ ≙ 0.28 ml/m³ (ppm) (at 1013 hPa and 25 °C)	

6 Uses

Used as a solvent for polyacrylonitrile, cellulose acetate, polystyrene, shellac and resins; as an additive in drilling-oils, organic coating strippers and textile auxiliaries; as a reagent in organic synthesis in acylation, condensation, Friedel-Crafts and Wittig reactions; as a starting material in the manu-

facture of pyrrolidone, N-methylpyrrolidone and polyvinylpyrrolidone, plasticisers, synthetic resins, piperidine and methionine (Mercker and Kieczka, 1985; IARC, 1976; Falbe and Regitz, 1996). The chemical is also used as an intermediate in the manufacture of the herbicide γ-2-methyl-4-(chlorophenoxy)butyric acid and of α-acetobutyrolactone (a vitamin B_1 intermediate; Mercker and Kieczka, 1985); in hairwave compositions, sun lotions and pharmaceuticals; as a nematocide; in printing inks, as an extractant in the petroleum industry and as a stabiliser for chlorohydrocarbons and phosphorus-based pesticides (Mercker and Kieczka, 1985).

The chemical is used as a selective solvent for petroleum components (acetylene), agricultural chemicals; as a starting material for the synthesis of 2-amino-4-(methylthio)butyric acid, DL-homoserine, 2-amino-4-hydroxybutyric acid, glutamine, glutamic acid and various vitamins; in photography, dyes and pigments, detergents, etc. (GAF, 1971).

7 Experimental results

7.1 Toxicokinetics and metabolism

Absorption and distribution

γ-Butyrolactone underwent rapid and complete absorption from the gastrointestinal tract in the rat studies discussed in the following. When applied dermally, the fraction of the γ-butyrolactone dose absorbed was 7 and 11%, depending on the method used for pretreatment of the rat skin (mechanical depilation or mechanical and subsequent chemical depilation, respectively; Fung et al., 1979; Guidotti and Ballotti, 1970; Lettieri and Fung, 1978 a, b).

Male Sprague-Dawley rats (weighing 200 to 250 g, 6 rats/experiment) received a single γ-butyrolactone dose of 500 mg/kg body weight by the oral, intraperitoneal or intravenous route. The investigators analysed the blood and brain for γ-butyrolactone and γ-hydroxybutyric acid, the chemical's primary metabolite, for a period of 60 minutes after administration. Blood levels of γ-butyrolactone after oral administration were below the limit of detection of 18 µg/ml in 3 and 5 out of 6 animals, independently of the time of measurement, whereas the remaining animals had time-independent levels

between 22.5 and 82.5 µg/ml. The levels observed for the primary metabolite, γ-hydroxybutyric acid, reached a peak value of 611 µg/ml at 15 minutes and were still 466 µg/ml at 60 minutes. Following intraperitoneal and intravenous administration, there was also very rapid hydrolysis of γ-butyrolactone to γ-hydroxybutyric acid. The blood γ-butyrolactone level was 63 µg/ml 5 minutes after intraperitoneal injection, while it was 22.5 µg/ml at 60 minutes; however, only 2 out of 6 animals had levels above the limit of detection at 60 minutes. Upon intravenous injection, the 5-minute value was 85 µg/ml, whereas γ-butyrolactone was no longer detectable after 15 minutes. The concentration levels of γ-hydroxybutyric acid seen after intraperitoneal dosing dropped from a maximum of 694 µg/ml (5-minute value) 521 µg/ml at 60 minutes, whereas intravenous injection led to a maximum level of 550 µg/ml (15-minute value) which dropped to 430 µg/ml at 60 minutes. In brain, oral dosing resulted in maximum γ-butyrolactone levels of 37 µg/g at 30 minutes and maximum γ-hydroxybutyric acid levels of 98.9 µg/g at 60 minutes. On intraperitoneal administration, cerebral γ-butyrolactone levels of 170 µg/g were found after 3 minutes, and time-independent γ-butyrolactone levels of 14.8 to 29.1 µg/g were detected 15 to 60 minutes after dosing; the concentration of γ-hydroxybutyric acid reached its peak of 191.6 µg/g after 30 minutes and fell to 129.1 µg/g at 60 minutes (no brain levels were measured after intravenous administration; Guidotti and Ballotti, 1970). The data permit the conclusion that γ-butyrolactone is very rapidly and apparently completely absorbed from the gastrointestinal tract and undergoes very rapid metabolism to γ-hydroxybutyric acid.

Further studies on the absorption of γ-butyrolactone were carried out in at least 3, usually 4, male Sprague-Dawley rats (weighing 260 to 340 g; no precise details of the number of rats used)/dose and type of treatment. γ-Butyrolactone (purity not specified) was administered orally by gastric intubation at dose levels of 1.58 and 6.34 mmol (approx. 136 and 546 mg)/kg body weight, intracardially at 1.58 mmol or intravenously at 6.34 mmol/kg body weight. Starting immediately after administration, the plasma was analysed for γ-butyrolactone over a total period of 3 to 8 hours. The analytical procedure did not distinguish between γ-butyrolactone and γ-hydroxybutyric acid, the primary metabolite of γ-butyrolactone, as earlier studies by the investigators had detected only γ-hydroxybutyric acid in rat blood after administration of γ-butyrolactone. When γ-butyrolactone was given intracardially at 136 mg/kg body weight, the plasma level was 500 µg/ml immedia-

tely upon dosing, about 400 µg/ml after 30 minutes and slightly more than 100 µg/ml after 2 hours but then dropped to below 50 µg/ml after 2.5 hours. After oral administration of 136 mg/kg body weight, plasma levels reached their peak of approx. 350 µg/ml at 15 to 30 minutes before declining with an elimination half-life of 0.3 hours to approx. 25 µg/ml within 3 hours. Plasma levels after oral administration of γ-butyrolactone at 546 mg/kg body weight remained at ≥ 1000 µg/ml for approx. 3 hours, as they did after intravenous administration of the same dose. Four hours after oral dosing, the plasma concentration was about 800 µg/ml, while it was approx. 450 µg/ml after 6 hours and slightly above 100 µg/ml after 8 hours. Subsequent to intravenous injection, plasma levels dropped somewhat more rapidly, reaching approx. 55 µg/ml after 8 hours (the graphical representation does not permit a more accurate reading). In percutaneous absorption studies, male Sprague-Dawley rats (weighing 275 to 525 g) had undiluted γ-butyrolactone applied to the mechanically or mechanically and chemically depilated skin at 6.34 mmol (approx. 546 mg)/kg body weight for 4 hours. The rate of percutaneous absorption depended on the method employed to pretreat the rat skin. When the skin was only shaved, the plasma concentrations rose slowly, reaching maximum levels of approx. 150 µg/ml after 1.5 to 2 hours; when the skin was shaved and subsequently treated with a commercial depilating agent (a thioglycolic acid-based formulation), the maximum plasma concentration of approx. 175 µg/ml was reached only approx. 10 minutes after γ-butyrolactone application. Based on the areas under the plasma concentration-time curves (AUC values) it was found that, relative to the value obtained after intravenous administration, the fractions of dose absorbed were 85 and 98% upon oral administration, and 7 and 11% upon dermal application onto the mechanically or mechanically and chemically depilated skin, respectively (Fung et al., 1979; Lettieri and Fung, 1978 a, b).

A further study also investigated the blood concentration of total γ-butyrolactone and γ-hydroxybutyric acid following administration of γ-butyrolactone. Following intravenous administration of γ-butyrolactone to 5 male rats at 500 mg/kg body weight, the blood concentration dropped from 13 µmol (approx. 1119 µg)/ml (1-minute value) to approx. 6.5 µmol (approx. 560 µg)/ml after 45 minutes, reaching a level as low as approx. 1 µmol (approx. 86 µg)/ml after 3 hours. The concentration levels in muscle were markedly

higher than those in fat (more precise details can not be given due to unclear units of measurement; Roth and Giarman, 1966).

The brains of untreated rats were found to contain total γ-butyrolactone and γ-hydroxybutyric acid at concentration levels of about 1 to 2 mmol/kg (approx. 86 to 172 mg/kg when expressed as γ-butyrolactone equivalents). Brain tissue obtained 3 hours post mortem from a patient (who died of multiple myeloma, renal failure and pneumonia) was found to contain 0.2 to 0.3 mmol/kg (approx. 17 to 26 mg/kg when expressed as γ-butyrolactone equivalents; Fishbein and Bessman, 1964).

In vitro, the absorption of γ-butyrolactone was studied in isolated everted gut segments from Sprague-Dawley rats. Gut segments, 12 cm long, were incubated at 37 °C with the mucosa everted and exposed to concentrations of 0.4, 0.8 and 1.2 M (approx. 34, 69 and 103 mg/ml, respectively), and transport of the chemical from the mucosal side to the serosal side of the gut segments was measured for a period of 25 minutes. The respective γ-butyrolactone fluxes were approx. 30, 60 and 70 µmol/min (approx. 2.6, 5.2 and 6 mg/min). At the 0.4 M concentration level, amounts of approx. 780 µmol (approx. 67 mg) were detected at constant fluxes on the serosal side within 25 minutes (Arena and Fung, 1980).

Specimens of human skin obtained from healthy females during plastic surgery of the breast were studied in a Franz Diffusion Cell regarding their permeability to various solvents. The limit of detection being 0.2 ppm, the skin-specimen permeability rates found for γ-butyrolactone were 1.1 ± 0.1 g/m² x hour (Ursin et al., 1995).

Biotransformation and excretion

γ-Butyrolactone undergoes rapid and quantitative conversion by lactonases, yielding γ-hydroxybutyric acid. Lactonases have been identified in human blood and in the blood and liver of rats, but not in the rat brain, spleen, kidney, heart, diaphragm, lung, skeletal muscle or gastrointestinal tract. The enzyme isolated from human plasma had a markedly higher activity than that isolated from rat liver microsomes. Moreover, conversion in human serum had a K_m value of 2.7×10^{-2} M and thus was more efficient than in rat serum (K_m value 1.6×10^{-2} M; Fishbein and Bessman, 1966 a, b; Roth and Giarman, 1965, 1966). In vitro, rat blood studies gave a half-life

of less than one minute for the conversion of γ-butyrolactone to γ-hydroxybutyric acid. Hydrolysis took place in serum and plasma, haemolysed erythrocytes being inactive. In rat plasma, hydrolysis was 92% after 15 minutes and 100% after 30 minutes, whereas in haemolysed erythrocytes it was only 3% after 30 minutes. The percentage hydrolysed in rat liver homogenate was 87% after 15 minutes and 94% after 60 minutes. In cat blood, hydrolysis of γ-butyrolactone was slower than in rat blood. Samples of rabbit and guinea pig serum also showed hydrolysis of γ-butyrolactone (no precise details). The various media were incubated with 1.3 x 10^{-2} M γ-butyrolactone at 37 °C (Roth and Giarman, 1965, 1966).

It was observed following oral administration of γ-butyrolactone that γ-hydroxybutyric acid can also be formed in the intestinal tract nonenzymatically by hydrolysis (no further details; NTP, 1992).

The results of several studies from the 1960s measuring the conversion of γ-hydroxybutyric acid to γ-butyrolactone and/or selectively assaying γ-butyrolactone in blood and brain (e. g. Bessman and Skolnik, 1964; Giarman and Roth, 1964), upon scrutinisation of the analytical procedures by another group of investigators, can not be considered relevant as they demonstrated that the earlier procedures were of questionable validity (Lettieri and Fung, 1978 c).

It was postulated that γ-hydroxybutyric acid undergoes oxidation to butanedioic acid (also referred to as succinic acid) prior to further metabolism to CO_2 in the citric acid cycle and/or undergoes degradation to CO_2 via β-oxidation (Fishbein and Bessman, 1964; Roth and Giarman, 1966).

In vivo, ^{14}C-labelled γ-butyrolactone and ^{14}C-labelled γ-hydroxybutyric acid are rapidly metabolised to $^{14}CO_2$. Following single intravenous administration of a 2 µCi dose of ^{14}C-γ-hydroxybutyric acid (specific activity 5.478 mCi/mmol), rats showed the first detectable traces of $^{14}CO_2$ in the exhaled air within only 4 minutes. Respiratory $^{14}CO_2$ reached a peak after 15 minutes. Sixty percent of the total amount of ^{14}C radioactivity was recovered as exhaled $^{14}CO_2$ within 2.5 hours. When the same dose of ^{14}C-γ-butyrolactone (2 µCi) was administered, peak respiratory ^{14}C concentration occurred at just under 20 minutes. Expiration of $^{14}CO_2$ followed a course similar to that seen with ^{14}C-γ-hydroxybutyric acid. The difference in time seen between the occurrence of the $^{14}CO_2$ peaks after γ-hydroxybutyric acid and

γ-butyrolactone administration was considered by the investigators to be likely to result from the time required by the body to metabolise γ-butyrolactone to γ-hydroxybutyric acid. Data on ^{14}C excretion in urine and/or faeces and residual activity in the body are lacking (Roth and Giarman, 1965, 1966).

7.2 Acute and subacute toxicity

Acute toxicity

The acute toxicity data for γ-butyrolactone are shown in Table 1. On single oral administration, the chemical proved harmful in the rat, mouse and guinea pig, with LD_{50} values ranging from 500 to 1800 mg/kg body weight. Acute toxicity is low following single dermal application and inhalation exposure, the dermal LD_{50} for guinea pigs being > 5640 mg/kg body weight (Fassett, 1967), and the LC_{50} values found in rats after 4-hour inhalation being > 2680 (HRC, 1991) and > 5100 mg/m³ (Monsanto, 1986). On intraperitoneal administration of γ-butyrolactone, LD_{50} values determined in rats were 200 to 1580 mg/kg body weight, while in mice they were 200 to 1470 mg/kg body weight. Following intravenous injection, an LD_{50} value of 880 mg/kg body weight was reported (Hampel and Hapke, 1968). The clinical signs of toxicity seen after oral and intraperitoneal administration include, inter alia, salivation, lying on the abdomen or side, apathy, atonia and dyspnoea (BASF, 1960, 1961), and independently of the route of administration, a narcotic effect was reported. Necropsy of rats was without abnormal findings upon oral, inhalation and intraperitoneal administration (BASF, 1960, 1961; HRC, 1991).

Beginning of Table 1

Table 1. Acute toxicity of γ-butyrolactone

Species, strain, sex*	Route of administration	Dose (mg/kg body weight or mg/m³)	Effects	Observation period	Reference
Rat	oral	ca. 1580	approximate LD_{50}; reeling, lying on the abdomen and side, narcosis; necropsy without findings	7 days	BASF, 1960
Rat	oral	1540	LD_{50}	no data	RTECS, 1997
Rat	oral	1800	LD_{50}	no data	Kvasov, 1974
Rat	oral	ca. 1690	LD_{50}	no data	ITL, 1952
Rat	oral	800–1600	LD_{50}	no data	Fassett, 1967

Table 1. Acute toxicity of γ-butyrolactone					
Species, strain, sex*	Route of administration	Dose (mg/kg body weight or mg/m³)	Effects	Observation period	Reference
Mouse, NMRI/Han	oral	1260	LD_{50}	no data	Hampel and Hapke, 1968
Mouse	oral	1720	LD_{50}	no data	RTECS, 1997
Mouse	oral	800–1600	LD_{50}	no data	Fassett, 1967
Guinea pig	oral	ca. 1690	LD_{50}	no data	ITL, 1952
Guinea pig	oral	500–700	LD_{50}	no data	Freifeld and Hort, 1967
Rabbit	oral	ca. 1800 ca. 900 ca. 450	animal died 6 days after treatment; lying on the abdomen and side, apathy, atonia, convulsions, dyspnoea, salivation; necropsy: mild pneumonia survived by 2 animals; apathy, atonia, lying on the side and narcosis, reversible after 24 hours tolerated without signs of toxicity by 2 animals	no data	BASF, 1960
Cat	oral	ca. 900 ca. 450	animal died one day after treatment; apathy, atonia, lying on the abdomen, laboured breathing, salivation and narcosis; necropsy: emphysematous lung, fatty degeneration of the liver and kidneys, bladder dilatation survived by 2 animals; reeling, apathy, atonia, lying on the side, salivation and narcosis, reversible after 24 hours	no data	BASF, 1960
Guinea pig	dermal	5640	LD_{50}	no data	Fassett, 1967
Rat, Sprague-Dawley, male, female	inhalation (4 hours)	> 2680	LC_{50}; reduced activity, and exophthalmus, temporarily reduced food consumption in males; necropsy without findings	14 days	HRC, 1991
Rat, Sprague-Dawley, male, female	inhalation (4 hours)	> 5100	LC_{50}; shallow breathing immediately upon exposure, hypoactivity, prostration, nasal discharge; necropsy without findings	14 days	Monsanto, 1986
Rat	inhalation (8 hours)	atmosphere saturated at 20 °C	inhalation hazard test; tolerated without signs of toxicity by 6 animals	7 days	BASF, 1960
Rat, Sprague-Dawley, Holtzmann, male	intraperitoneal	800	LD_{50}	14 days	Benda and Perlès, 1960; Sprince et al., 1966
Rat	intraperitoneal	ca. 1580	approximate LD_{50}; deep narcosis; necropsy of survivors without findings	8 days	BASF, 1961
Rat, Wistar, male	intraperitoneal	1000	LD_{50}; deep sleep and loss of pain and righting reflexes after 400 or 500 mg/kg body weight	no data	Sieroslawska, 1965
Rat	intraperitoneal	200–400	LD_{50}; deaths delayed as long as 2 weeks	no data	Fassett, 1967

Table 1. Acute toxicity of γ-butyrolactone					
Species, strain, sex*	Route of administration	Dose (mg/kg body weight or mg/m³)	Effects	Observation period	Reference
Rat, albino, male	intraperitoneal	200 400 700–800	spontaneous activity abolished for 87 minutes, body temperature decreased by 0.7 °C spontaneous activity abolished for 3 hours, body temperature decreased by 0.5 °C immediately after injection, then hyperthermia spontaneous activity depressed for 5 to 8 hours, body temperature decreased, then hyperthermia	no data	Borbély and Huston, 1972
Rat	intraperitoneal	350–700	sleep with loss of reflexes within 10 minutes of injection	no data	Benda and Perlès, 1960
Rat, Wistar, male	intraperitoneal	200 300 400 500	no effect loss of co-ordination after 11 minutes lying on the side after 10.5 minutes lying on the side after 7.3 minutes	no data	Jouany et al., 1960
Mouse	intraperitoneal	ca. 1470	approximate LD_{50}; reeling, lying on the abdomen or side and dyspnoea; necropsy without findings	7 days	BASF, 1960
Mouse, R3, male	intraperitoneal	1100	LD_{50}; loss of righting reflex and reduction of pain reflex after 400 or 500 mg/kg body weight; respiratory paralysis at doses > 800 mg/kg body weight	no data	Sieroslawska, 1965
Mouse, NMRI/Han	intraperitoneal	880	LD_{50}	no data	Hampel and Hapke, 1968
Mouse	intraperitoneal	200–400	LD_{50}; deaths delayed as long as 2 weeks	no data	Fassett, 1967
Mouse, B6C3F1, male	intraperitoneal	ca. 990	LD_{50}	7 days	Salamone, 1981
Mouse, NMRI/Han	intraperitoneal	100 and 200 500	sedation 5 to 8 minutes after injection, lasting 1 to 1.5 hours tachypnoea, lying on the abdomen, clonic convulsions, recovery after 4 hours	no data	Hampel and Hapke, 1968
Guinea pig	intraperitoneal	300 500 700 and 1000	lying on the side with poor tolerance for pain stimuli starting 10 minutes after injection complete tolerance for pain stimuli lasting 3 hours complete tolerance for pain stimuli, the effect lasting 7 and > 10 hours, respectively	no data	Hampel and Hapke, 1968
Cat	intraperitoneal	500 700 1000	deep sleep starting 15 minutes after injection; vomiting, corneal reflex abolished, pupillary reflex maintained lying on the side, increasing respiratory depression 5 minutes after injection, death after one hour respiratory depression, bradycardia, death after 30 minutes	no data	Hampel and Hapke, 1968

Table 1. Acute toxicity of γ-butyrolactone					
Species, strain, sex*	Route of administration	Dose (mg/kg body weight or mg/m³)	Effects	Observation period	Reference
Rat	intracerebral	4–64 mg/animal	no effect	no data	Gessa et al., 1967
Mouse, NMRI/Han	intravenous	880	LD_{50}	no data	Hampel and Hapke, 1968
Mouse, NMRI/Han	intravenous	100 300	sedation 3 to 5 minutes after injection, lasting 1 hour absolute immobility, lying on the abdomen, breathing difficulties	no data	Hampel and Hapke, 1968
Rabbit	intravenous	500	minimum lethal dose	no data	Sieroslawska, 1965
Rabbit	intravenous	500	sleep several minutes after injection	no data	Benda and Perlès, 1960
Dog	intravenous, subcutaneous or intramuscular	100 300 500 700	no effect ataxia, mild sedation, sleepiness, vomiting, salivation, defecation lying on the side 3 to 5 minutes after injection, marked salivation, defecation, vocalisation, deep sleep after 15 minutes, no tolerance for pain stimuli defecation, retching movements, severe vocalisation, tolerance for pain stimuli after 15 minutes	no data	Hampel and Hapke, 1968
Mouse	subcutaneous	2500 1000 500 100	lethal; necropsy: hyperaemia of the lungs, haemorrhages in the subcutaneous tissue at the injection site 1/3 animals died; lying on the abdomen, laboured breathing; necropsy revealed small haemorrhages in the lung survived; reeling, lying on the abdomen, laboured breathing tolerated	no data	I.G. Farbenindustrie, 1940
* where indicated					

End of Table 1

A cat, a rabbit, a guinea pig and a rat underwent a single 3-hour exposure to a γ-butyrolactone concentration of 2000 or 2100 mg/m³ which was followed by a second, 8-hour exposure 12 days later (dynamic study). No changes were observed in the animals' state of health (no further details; I.G. Farbenindustrie, 1940).

Subacute toxicity

Groups of 5 to 10 rats received a total of 5 oral doses of γ-butyrolactone at 452, 904 or 1130 mg/kg body weight within 14 days, three doses one week

and two the next. Following administration of 1130 mg/kg body weight, 2 out of 10 rats died within 14 days. There were no deaths in the two lower dose groups. As a clinical sign of toxicity, narcosis was observed at and above 904 mg/kg body weight, setting in approx. 20 minutes after administration and lasting 5 hours in most cases (no further details; BASF, 1961).

In a preliminary study for a 90-day study, groups of 5 male and 5 female rats (F344/N) received a total of 12 doses of γ-butyrolactone (> 97% pure) in corn oil by oral gavage at dose levels of 0 (controls), 75, 150, 300, 600 or 1200 mg/kg body weight 5 days/week. All high-dose males and females died within the first 3 days, and one male from the 600 mg/kg group died on day 3. Mean body weight gains in female rats of the 600 mg/kg group was significantly lower than those of the controls. The rats receiving 600 or 1200 mg/kg body weight became inactive or recumbent with irregular and laboured respiration shortly after dosing. γ-Butyrolactone caused no noteworthy macroscopic changes up to the lethal dose range (no further details; SRI, 1981; NTP, 1992).

Groups of 5 male and 5 female B6C3F1 mice in a preliminary study for a 90-day study received a total of 12 doses of γ-butyrolactone (> 97% pure; dissolved in corn oil) by oral gavage at dose levels of 0 (controls), 87, 175, 350, 700 or 1400 mg/kg body weight 5 days/week. Following administration of 1400 mg/kg body weight, all males and 4 out of 5 females had died by the end of the study. Mean body weight gains in all dose groups were similar to those of the controls. At and above 350 mg/kg body weight, clinical signs of toxicity set in shortly after dosing and consisted in recumbence or inactivity and irregular respiration or dyspnoea. γ-Butyrolactone caused no noteworthy macroscopic changes up to the lethal dose range (no further details; SRI, 1981; NTP, 1992).

The toxicity of γ-butyrolactone (approx. 99.5% pure) after repeated inhalation was investigated in a preliminary study involving 2 cats, 2 rabbits, 2 guinea pigs, 4 rats and 10 mice. The animals were exposed to a nominal concentration of approx. 760 ppm (equivalent to approx. 2668 mg/m³) for 6 hours/day on 5 consecutive days. One of the two cats exhibited panting respiration, reeling and vomiting on the second day of the study. The two first-mentioned signs were also observed on the third and the fifth day of the study. The rabbits, guinea pigs and rats tolerated exposure without showing signs of intoxication. At 760 ppm, γ-butyrolactone caused reeling

and transient lying on the side in some mice. Two of the mice died after 4 or 5 exposures, and another 2 mice died during the 14-day post-exposure observation period. As mortality was 4 out of 10 in an untreated control group, the deaths observed after γ-butyrolactone exposure could not be attributed to the chemical with certainty, according to the investigator. Necropsy of the deceased mice was without findings (no further details; BASF, 1960).

Groups of 10 to 15 rats received γ-butyrolactone intraperitoneally at dose levels of 452, 904 or 1130 mg/kg body weight 3 times weekly, 5 times in total. By day 14 of the study, mortality was 2 out of 10 in the top dose group and 2 out of 15 in the mid dose group. As a clinical sign of toxicity, narcosis was observed after every administration, setting in approx. 20 minutes after administration and lasting 5 hours in most cases (no further details; BASF, 1961).

7.3 Skin and mucous membrane effects

The skin irritancy of undiluted γ-butyrolactone (approx. 99.5% pure) was tested on the dorsal skin of white rabbits. Exposure (semiocclusive) lasted 1, 5 and 15 minutes and 20 hours. When tested on the rabbit ear, the chemical was applied for 20 hours. γ-Butyrolactone proved to be nonirritating in these tests even when applied for 20 hours (BASF, 1960).

In a further skin irritation study, 6 albino rabbits were patch-tested with 1 ml γ-butyrolactone. The shaved skin was assessed for reactions after 24 to 72 hours. Very mild, hardly perceptible erythema and moderate erythema and very slight, hardly perceptible oedema occurred (no further details, particularly as regards the reversibility of the reactions; Szirmai et al., 1989). γ-Butyrolactone thus proved to be mildly irritating to the skin in this study.

Four rabbits each had 0.5 ml undiluted technical-grade γ-butyrolactone applied to the skin. At 72 hours, 3 of the animals exhibited moderate to severe erythema with oedema, while one animal had clearly circumscribed erythema without oedema formation. On the basis of these results, the substance was evaluated as moderately to severely irritating to the skin (no further details, particularly as regards reversibility of the reactions; Bio/dynamics, 1987).

Undiluted γ-butyrolactone caused no irritation to the skin of the rabbit ear after 24-hour exposure (no further details; I.G. Farbenindustrie, 1940).

In order to assess the eye irritancy of undiluted γ-butyrolactone (approx. 99.5% pure), white rabbits had 50 µl of the chemical instilled into the conjunctival sac of one eye. This resulted in inflammatory irritation and slight clouding of the cornea, both of which did not subside within 8 days (BASF, 1960). γ-Butyrolactone thus proved to be irritating to the eye in this study.

Another eye irritation study of γ-butyrolactone was conducted in 6 albino rabbits in accordance with the U.S. Code of Federal Regulations, 1500.42. The eyes were not washed following instillation. The findings were scored after 24, 48 and 72 hours and 7 days. All 6 rabbits developed conjunctival, iridial and corneal irritation. The effects cleared up during the 7-day observation period in 5 animals. On the basis of the observed data, undiluted γ-butyrolactone was evaluated as a severe irritant to the eye (FDRL, 1977; GAF, 1985).

In an eye irritation study conducted in accordance with EEC Directive 67/548, the eyes of 3 rabbits were scored up to 14 days after instillation of undiluted γ-butyrolactone. A mean maximum score of 39.3 resulted, on the basis of which the investigators evaluated γ-butyrolactone as moderately irritating to the eye (no further details; Gautheron et al., 1994 a).

Four rabbits each had 0.1 ml undiluted technical-grade γ-butyrolactone instilled into one eye. All animals exhibited severe irritation of the conjunctivae (redness, chemosis, discharge and necrosis), iridial damage or changes, corneal opacity and ulceration at 24 hours after instillation. Ocular irritation, including pannus (neovascularisation of the corneal surface) in 3 animals, continued until the end of the observation period on day 7. On the basis of these results, the substance was evaluated as corrosive to the eye (Bio/dynamics, 1987).

An in-vitro eye irritation test also led to γ-butyrolactone being evaluated as an irritant. The test was carried out on bovine corneas which were mounted in a special two-chamber corneal holder and incubated for one hour at 32 °C. The epithelial side of the cornea was then exposed to 0.5 ml γ-butyrolactone at concentration levels of 20, 40, 60, 80 or 100% at room temperature for 30 minutes (6 corneas/concentration). Subsequently, the epithelial side was washed and incubated for another 2 hours. After a recovery

period, corneal opacity was measured. It was found to be 16.3, 46.0, 48.4, 39.2 and 35.8 at concentrations of 20%, 40%, 60%, 80% and 100%, respectively. The investigators considered readings of 0 to 20 opacity units as mild irritation, 21 to 40 as mild to moderate irritation, 41 to 70 as moderate irritation and from 71 as severe irritation (Gautheron et al., 1992).

Further in-vitro studies employing the experimental technique described above, which were carried out in parallel at 12 different laboratories, gave a mean opacity value of 45.6 for undiluted γ-butyrolactone, on the basis of which the chemical was evaluated as moderately irritating to the eye. The interlaboratory variation in the opacity score ranged from 30 to 80. Per experiment, 12 corneas were treated with the test substance and 3 corneas served as controls (Gautheron et al., 1994 a).

7.4 Sensitisation

It has been reported that γ-butyrolactone had no sensitising effect on guinea pig skin (no further details; Fassett, 1967; GAF, 1986).

7.5 Subchronic and chronic toxicity

Groups of 20 male and 20 female rats (Food and Drug Research Laboratories strain, 28 to 30 days old) received γ-butyrolactone in the feed at daily dose levels of 0 (controls), 100, 200 or 400 mg/kg body weight for 90 days. There were no deaths. Treatment was tolerated without clinical signs of toxicity or changes in haematological, biochemical or urine analytical parameters (5 animals/group examined) and without substance-related organ weight changes or histopathological findings (FDRL, 1970).

In a subchronic toxicity study of γ-butyrolactone (> 97% pure), groups of 10 male and 10 female F344/N rats (51 days old) were given doses of 0 (controls), 56, 112, 225, 450 or 900 mg/kg body weight (dissolved in corn oil) by oral gavage 5 times/week for 13 weeks. All males and one female rat of the 900 mg/kg dose group died by study week 8. The 450 mg/kg dose caused significant depression of body weight gain in males. The males in the lower dose groups and all females showed no difference in body weight gain compared with the controls. The top-dose animals became recumbent within a few minutes after dosing as a clinical sign of toxicity but appeared nor-

mal again several hours later. The rats in the 225 and 450 mg/kg groups became inactive after dosing. After 2 to 3 weeks, the animals had apparently adapted to the sedative effect of γ-butyrolactone. None of the dose groups exhibited any differences in organ weights of the brain, heart, kidney, liver, lung or thymus relative to controls. No macroscopic changes were noted. Comprehensive histopathological examination revealed that most of the dosed rats had inflammation of the nasal mucosa. The lesions were focal or multifocal and consisted of small accumulations of neutrophils or macrophages in the lumen or mucosa. According to the investigators, similar lesions were also observed in other gavage studies and may have been related to the reflux of the gavage solution into the nasopharynx after dosing. There were no other histopathological changes. No haematology or clinical chemistry studies were carried out (SRI, 1981; NTP, 1992).

The same experimental conditions were employed in a study in which groups of 10 male and 10 female B6C3F1 mice were treated with γ-butyrolactone (> 97% pure) at 0 (controls), 65, 131, 262, 525 or 1050 mg/kg body weight. In this study, 3 male mice and one female mouse died in the 1050 mg/kg group. Body weights of the males in the highest dose group was approx. 11% lower than that of the controls at the end of the study, whereas it remained unaffected in the other dose groups. When 1050 or 525 mg/kg body weight was given, the animals became recumbent several minutes after dosing but the effect was reversible after several hours. Administration of 262 mg/kg body weight was observed to cause reduced activity. At dose levels of 525 mg/kg body weight or below, the signs disappeared after 3 to 4 weeks. Organ weights in all dose groups were similar to those of the controls. Macroscopic and histopathological examination revealed no substance-related findings. Haematology and clinical chemistry parameters were not studied (SRI, 1981; NTP, 1992).

Groups of 3 male and 3 female beagle dogs were administered γ-butyrolactone in their diets at concentration levels of 0 (controls), 0.2, 0.4 or 0.8% one hour/day, 6 days/week for 90 days. Based on a mean food intake of approx. 370 g/day and a mean body weight of approx. 11 kg, this corresponded to dose levels of 0, approx. 70, 140 and 280 mg/kg body weight/day. Body weight gain was not affected. The determination of organ weights, haematological, clinical-chemical and urine analytical parameters, and the gross and comprehensive histopathological examinations at the end of the study revealed no changes (FDRL, 1970).

The chronic toxicity studies of γ-butyrolactone are discussed in Section 7.7.

7.6 Genotoxicity

The numerous data on the genotoxic effects of γ-butyrolactone in vitro and in vivo are summarised in the following Tables 2 to 5 (in-vitro genotoxicity tests with γ-butyrolactone on bacteria, fungi and mammalian cells, and in-vivo genotoxicity tests with γ-butyrolactone). On the basis of the overall result of the available in-vitro and in-vivo genotoxicity studies it can be concluded that γ-butyrolactone has no relevant genotoxic potential.

7.6.1 In vitro

Tests for mutagenic activity

Consistently negative results were obtained when γ-butyrolactone was studied for its potential to cause gene mutations in numerous Salmonella/microsome assays, which were carried out as standard plate incorporation tests, preincubation tests or fluctuation tests, with and without metabolic activation. Metabolic activation was achieved with S9 mix from rat or hamster liver induced with Aroclor 1254, Kanechlor 500 or phenobarbital, or S9 mix from uninduced rat liver or uninduced rat hepatocytes. In one preincubation test, norharman was used in addition to S9 mix from Kanechlor 500-induced rat liver (Nagao and Takahashi, 1981). Results are available for *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537 and TA 1538 (see Table 2). Further negative results were obtained for the mutagenic potential of γ-butyrolactone in the 8-azaguanine resistance assay conducted in *Salmonella typhimurium* TM677 with and without metabolic activation, in several tryptophan reversion tests in *Escherichia coli* WP2 strains, in the forward mutation assay in *Schizosaccharomyces pombe* P1 and in the HPRT test on Chinese hamster lung fibroblasts (V79 cells, see Table 2; Loprieno, 1981; Knaap et al., 1981). The reversion assay in *Saccharomyces cerevisiae* XV185-14C gave a negative result in the absence of metabolic activation. The result obtained in the presence of metabolic activation was considered equivocal by the investigators as a slight increase in reversion rate was noted in the low test concentrations (Mehta and von Borstel, 1981).

Tests for chromosome-damaging activity

Chromosome-damaging effects of γ-butyrolactone were seen in the chromosome aberration test in Chinese hamster ovary (CHO) cells with metabolic activation (S9 mix from Aroclor 1254-induced rat liver) only at very high test concentrations of ≥ 2580 µg/ml (see also NTP, 1992). Without metabolic activation, the test was negative (Loveday et al., 1989). A chromosome aberration test performed in metabolically competent rat liver epithelial cells (RL1) was negative (Dean, 1981).

Tests for DNA-damaging activity

γ-Butyrolactone exhibited no DNA-damaging potential, be it with or without metabolic activation, in the majority of DNA repair tests carried out in polymerase- (polA) and/or recombination-proficient/deficient (recA) *Escherichia coli* strains, in the studies in *Escherichia coli*, in which induction of an SOS response served as a parameter of DNA-damaging activity (prophage lambda induction test in strains 58-161 envA and C600, and SOS chromotest in strain PQ37), or in the studies in repair-proficient/deficient *Saccharomyces cerevisiae* strains (see Tables 2 and 3). The result of a DNA repair test which was carried out in *Escherichia coli* as a suspension test, but only without metabolic activation, was evaluated as equivocal, because although inhibition was stronger in the $polA^-$ strain than in the $polA^+$ strains, it did not reach the level considered a clearly positive result by the investigators (Rosenkranz et al., 1981). A DNA repair test conducted in *Bacillus subtilis* rec strains was negative both in the absence of metabolic activation and in the presence of an uncommon metabolic activation system, S9 mix from Japanese clams, whereas a positive result was obtained with an equally uncommon metabolic activation system, S9 mix from yellowtail fish liver (Kada, 1981). Studies conducted in mammalian cells also gave no relevant indications of a DNA-damaging potential for γ-butyrolactone. A UDS test in HeLa S3 cells and a DNA repair test in repair-proficient/deficient Chinese hamster ovary cells gave negative results both with and without metabolic activation (S9 mix from phenobarbital- or methylcholanthrene-induced rat liver, or Aroclor 1254-induced hamster liver). A sister chromatid exchange (SCE) test conducted in Chinese hamster ovary (CHO) cells was negative in the absence of metabolic activation, but a positive result was obtained in the presence of metabolic activation, though only at unusually

high test concentrations of ≥ 3010 µg/ml (Martin and McDermid, 1981; Hoy et al., 1984; Loveday et al., 1989; see also NTP, 1992). γ-Butyrolactone tested negative in an assay system based on a permanent cell line isolated from human amniotic fluid (FL cells). In that assay, an increase in adenosine diphosphate ribosyl transferase (ADPRT) activity and the associated reduction in nicotinamide-adenine dinucleotide (NAD) was considered to indicate DNA-damaging potential (Fang et al., 1990; Yu et al., 1990).

Other tests

γ-Butyrolactone, when tested with and without metabolic activation, did not induce resistance to diphtheria toxin in human lung fibroblasts (HSC 172) or mitotic aneuploidy in *Saccharomyces cerevisiae* D6 (Gupta and Goldstein, 1981; Parry and Sharp, 1981).

In addition, in-vitro studies were conducted on the reactivity of β-propiolactone, β-butyrolactone and γ-butyrolactone with guanosine, ribonucleic acid, deoxyribonucleic acid, denatured deoxyribonucleic acid and 4-(p-nitrobenzyl)pyridine. A 3-hour incubation was carried out at 37 °C in a reaction volume of 2 ml which contained the respective lactone at 50 mM. β-Propiolactone was found to be 50 to 100 times more active than β-butyrolactone with respect to the aforementioned chemicals, whilst γ-butyrolactone was completely inactive. The investigator discussed the observation that this correlated with the carcinogenic potential of the lactones (Hemminki, 1981).

Beginning of Table 2

Table 2. In-vitro genotoxicity tests with γ-butyrolactone in bacteria

Test system	Concentration range tested (µg/plate)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
1. Gene mutation					
1.1 Salmonella/microsome assay systems					
1.1.1 Salmonella/microsome assay as standard plate incorporation test					
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	up to 1000 in DMSO, toxicity tested	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Baker and Bonin, 1981
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537	probably up to 500 in DMSO	S9 mix from phenobarbital- and 3-methylcholanthrene-induced rat liver	negative	negative	Garner et al., 1981; Bridges et al., 1981

Table 2. In-vitro genotoxicity tests with γ-butyrolactone in bacteria					
Test system	Concentration range tested (µg/plate)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
Salmonella typhimurium TA 98, TA 100, TA 1537	2–5000 in DMSO, toxicity tested	S9 mix from Aroclor 1254-induced rat liver	negative	negative	MacDonald, 1981
Salmonella typhimurium TA 98, TA 100	up to the toxic concentration range, in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Ichinotsubo et al., 1981 a; Bridges et al., 1981
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	10–10000 in DMSO, toxicity tested	S9 mix from Aroclor 1254-induced rat liver	negative[1)]	negative[1)]	Richold and Jones, 1981; Bridges et al., 1981
[1)] positive results obtained in a first experiment could not be reproduced in two independent retests					
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	0.1–2000 in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Rowland and Severn, 1981
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	up to the toxic concentration range, in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Simmon and Shepherd, 1981
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	4–2500 probably in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Trueman, 1981
Salmonella typhimurium TA 98, TA 100	0.5–500 in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Venitt and Crofton-Sleigh, 1981
Salmonella typhimurium TA 98, TA 100, TA 1535	8.6–4305 (0.1–50 µmol) in DMSO or ethanol	S9 mix from phenobarbital-induced rat liver	negative	negative	Loquet et al., 1981
Salmonella typhimurium TA 98, TA 100, TA 1537	no details, in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Martire et al., 1981
1.1.2 Salmonella/microsome assay as preincubation test					
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537	100–10000 in distilled water	S9 mix from Aroclor 1254-induced rat and hamster liver	negative	negative	Haworth et al., 1983
Salmonella typhimurium TA 98, TA 100, TA 1537	no precise details, 7 concentrations in DMSO	S9 mix from KC 500[1)]-induced rat liver, ± norharman	negative	negative	Nagao and Takahashi, 1981
[1)] Kanechlor 500, a PCB mixture comparable to Aroclor 1254					
Salmonella typhimurium TA 92, TA 98, TA 100, TA 1535, TA 1537, TA 1538	0.2–2000 in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Brooks and Dean, 1981
Salmonella typhimurium TA 98, TA 100, TA 102	1.12–111917 (13 nmol–1.3 mmol) in methanol, toxicity tested	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Aeschbacher et al., 1989
A modified test for volatile chemicals was carried out.					

Table 2. In-vitro genotoxicity tests with γ-butyrolactone in bacteria					
Test system	Concentration range tested (µg/plate)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
1.1.3 Salmonella/microsome assay as fluctuation test					
Salmonella typhimurium TA 98, TA 100	1–500 µg/ml in DMSO, toxicity tested	S9 mix from uninduced rat liver or uninduced rat hepatocytes	negative	negative	Hubbard et al., 1981
Salmonella typhimurium TA 98, TA 1535, TA 1537	10–1000 µg/ml, toxicity tested	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Gatehouse, 1981
The test was carried out as a microtiter fluctuation test with preincubation.					
1.2 Further gene mutation tests in bacteria					
Salmonella typhimurium TM677, 8-azaguanine resistance test with preincubation	up to 1000 µg/ml (solubility limit) in DMSO	S9 mix from phenobarbital- or Aroclor 1254-induced rat liver	negative	not tested	Skopek et al., 1981
Escherichia coli WP2uvrA, tryptophan reversion test	10–1000 µg/ml, toxicity tested	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Gatehouse, 1981
The test was carried out as a microtiter fluctuation test with preincubation.					
Escherichia coli WP2uvrA, WP2uvrA/pKm101, tryptophan reversion test with preincubation	no details, in DMSO	S9 mix from KC 500[1)]-induced rat liver	negative	negative	Matsushima et al., 1981
[1)] Kanechlor 500, a PCB mixture comparable to Aroclor 1254					
Escherichia coli WP2, WP2 B/r, tryptophan reversion test, as a plate incorporation test	up to 3000	–	not tested	negative	Kuroda et al., 1986
Escherichia coli WP2(p), WP2uvrA(p), tryptophan reversion test, as a plate incorporation test	0.5–500 in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Venitt and Crofton-Sleigh, 1981
2. DNA damage					
2.1 DNA repair test systems					
Escherichia coli WP2, WP67(uvrA polA), CM871 (uvrA recA lexA), DNA repair test, as a preincubation spot test	probably up to 2500 µg/ml in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Green, 1981
Escherichia coli recA$^-$ strains JC 2921, JC 9239, JC 8471, JC 5519, JC 7623, JC 7689, DNA repair test, as a cylinder plate diffusion spot test	up to the toxic concentration range, in DMSO	S9 mix (no further details)	negative	negative	Ichinotsubo et al., 1981 b
Escherichia coli WP2, WP67 (uvrA polA), CM871 (uvrA lexA recA), DNA repair test, as a preincubation spot test	up to 1000 µg/ml in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Tweats, 1981

Table 2. In-vitro genotoxicity tests with γ-butyrolactone in bacteria					
Test system	Concentration range tested (µg/plate)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
Escherichia coli WP3110 ($polA^+$), P3478 ($polA1^-$), DNA repair test, as a suspension test	250 µg/test in DMSO	–	not tested	equivocal[1)]	Rosenkranz et al., 1981
[1)] inhibition was stronger in the $polA^-$ strain than in the $polA^+$ strain but did not reach the level considered a definitely positive result by the investigator					
Bacillus subtilis spores H17 (rec^+), M45 (rec^-), DNA repair test, as a disc-plate diffusion test	20 µl/disc	S9 mix from Aroclor 1254-induced rat liver, yellowtail fish liver or Japanese clams	toxic without DNA damage (rat liver), positive (yellowtail fish liver), negative (Japanese clam)	negative	Kada, 1981
2.2 Tests to study induction of an SOS response					
Escherichia coli 58-161 envA lambda, C600 prophage lambda induction test	5000 and 12500 µg/ml in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Thomson, 1981
Escherichia coli PQ37, SOS chromotest	no details, in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Quillardet et al., 1985

End of Table 2

Beginning of Table 3

Table 3. In-vitro genotoxicity tests with γ-butyrolactone in fungi					
Test system	Concentration range tested (µg/ml)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
1. Gene mutation					
Schizosaccharomyces pombe P1, forward mutation test	5–20, cell survival rate 100–69%	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Loprieno, 1981
Saccharomyces cerevisiae XV185-14C, reversion test	22.2 and 222 in DMSO	S9 mix from Aroclor 1254-induced rat liver	equivocal[1)]	negative	Mehta and von Borstel, 1981
[1)] concentration-independent increase, but no doubling of reversion rates at the low test concentration					
2. DNA damage					
Saccharomyces cerevisiae 197/2d(wt), rad3, rad18, rad52, trp2, DNA repair test	100–500 in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Sharp and Parry, 1981 b
This test in repair-proficient and repair-deficient strains of *Saccharomyces cerevisiae* was discussed by the investigators as an analogous test to the rec assay in bacteria, though the test was not yet sufficiently validated in the investigators' opinion.					
Saccharomyces cerevisiae T4, T5, rep assay	no details, in DMSO	–	not tested	negative	Kassinova et al., 1981
This test was discussed by the investigators as an analogous test to the rec assay in *Bacillus subtilis*.					

Table 3. In-vitro genotoxicity tests with γ-butyrolactone in fungi					
Test system	Concentration range tested (µg/ml)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
3. Recombination					
Saccharomyces cerevisiae D4, mitotic gene conversion, preincubation test	0.33–333.33 µg/plate in DMSO	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Jagannath et al., 1981
Saccharomyces cerevisiae D7, mitotic gene conversion	up to 2, cell survival rate 100%	S9 mix (no further details)	negative	negative	Zimmermann and Scheel, 1981
Saccharomyces cerevisiae JD1, mitotic gene conversion	up to 750 in ethanol or DMSO, cell survival rate 100–43.5%	S9 mix from Aroclor 1254-induced rat liver	negative[1)]	negative[1)]	Sharp and Parry, 1981 a
[1)] negative when ethanol was used as a solvent. With DMSO, the test result was evaluated by the investigators as being false positive (not specified whether with or without S9 mix)					
Saccharomyces cerevisiae T1, T2, mitotic crossing-over assay	1000 in DMSO, cell survival rate 38.9–107.5% –S9, 42.4–121.8% +S9	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Kassinova et al., 1981
4. Genome mutation					
Saccharomyces cerevisiae D6, induction of mitotic aneuploidy	up to 1000 µg/ml	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Sharp and Parry, 1981

End of Table 3

Beginning of Table 4

Table 4. In-vitro genotoxicity tests with γ-butyrolactone in mammalian cells					
Test system	Concentration range tested (µg/ml)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
1. Gene mutation					
Chinese hamster lung fibroblasts (V79), HPRT test	0.5 and 1.25[1)] +S9, 0.5-2.5[1)] –S9	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Knaap et al., 1981
[1)] relative cell survival rate at the highest test concentration was 83% with S9 and 113% without S9					
2. Chromosome damage					
Chinese hamster ovary (CHO) cells, chromosome aberration test	500–4990 –S9, 400–3990 +S9, cytotoxicity tested	S9 mix from Aroclor 1254-induced rat liver	positive (from 2580 µg/ml[1)])	negative	Loveday et al., 1989
[1)] no data given as to the cytotoxicy caused by these concentrations					
Rat liver epithelial cells (RL1), chromosome aberration test	62.5–250 in DMSO, cytotoxicity tested	test using metabolically active hepatocytes	negative	not tested	Dean, 1981

Table 4. In-vitro genotoxicity tests with γ-butyrolactone in mammalian cells					
Test system	Concentration range tested (µg/ml)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
3. DNA damage					
Chinese hamster ovary (CHO) cells, sister chromatid exchange assay	148–1480 –S9, 494–5010 +S9 in water, cytotoxicity tested	S9 mix from Aroclor 1254-induced rat liver	positive (from 3010 µg/ml[1)])	negative	Loveday et al., 1989
[1)] no data given as to the cytotoxicy caused by these concentrations					
HeLa S3 cells, UDS test	0.1–100 in DMSO	S9 mix from phenobarbital- or methylcholanthrene-induced rat liver	negative	negative	Martin and McDermid, 1981
Chinese hamster ovary cells (AA8, EM9, UV4, UV5), DNA repair test[1)]	up to 4 x 10^3	S9 mix from Aroclor 1254-induced hamster liver	negative	negative	Hoy et al., 1984
[1)] test in repair-proficient wild-type cells (AA8) and repair-deficient cells (EM9, UV5, UV4); reduced growth (higher cytotoxicity) of the repair-deficient strains compared with the repair-proficient strain was interpreted as indicating induction of repairable DNA damage					
Human amniotic fluid-derived permanent cell line (FL), ADPRT test[1)]	ca. 0.09–86.09 (1–1000 µmol/l), no cytotoxicity data	–	not tested	negative	Fang et al., 1990; Yu et al., 1990
[1)] the investigators considered an increase in adenosine diphosphate ribosyl transferase (ADPRT) activity and the associated reduction in nicotinamide-adenine dinucleotide (NAD) content in the cells to indicate a DNA-damaging effect; γ-butyrolactone was tested as part of a screening test which was carried out to validate the test system and which compared the results of the ADPRT and UDS tests; 15 out of 16 compounds gave identical results in both test systems					
4. Other tests					
Human lung fibroblasts (HSC 172), induction of diphtheria toxin resistance	100 and 500, 100% relative cell survival rate	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Gupta and Goldstein, 1981
According to the investigators, the test system was still under development and not yet sufficiently well validated.					

End of Table 4

7.6.2 In vivo

None of the in-vivo studies were able to demonstrate a genotoxic potential for γ-butyrolactone. Studies included micronucleus tests with single and repeated intraperitoneal administration of maximum tolerated doses to B6C3F1 and CD1 mice, the sex-linked recessive lethal test and the eye mosaic assay in *Drosophila melanogaster*, and a study of diploid spermatozoa induction in the NMRI mouse (see Table 5).

Table 5. In-vitro genotoxicity tests with γ-butyrolactone				
Test system	Dose, regimen	Toxicity	Results	Reference
Tests in mammals				
Micronucleus test, mouse (B6C3F1), 5 mice/time point of investigation; 500 polychromatic erythrocytes/mouse (bone marrow) were examined	2 doses of 80% of the LD_{50} in DMSO, given i.p. at an interval of 24 hours; examination at 48, 72 and 96 hours after last dosing	80% of the LD_{50} (7-day observation period) tested (two administrations)	negative	Salamone et al., 1981; Katz et al., 1981
Micronucleus test, mouse (B6C3F1), 4 or 5 mice/dose and time point of investigation; 500 polychromatic erythrocytes/mouse (bone marrow) were examined	100 or 50% of the LD_{50} in DMSO given i.p.; examination at 36[1)], 48 and 72 hours after dosing	80% of the LD_{50} (7-day observation period) tested	negative	Salamone et al., 1981; Katz et al., 1981
[1)] according to the detailed report by Salamone et al., 1981; 30 hours according to Katz et al., 1981				
Micronucleus test, mouse (B6C3F1), 2 male and 2 female mice/dose; 1500 polychromatic erythrocytes/mouse (bone marrow) were examined	2 doses of 0.11, 0.22 or 0.44 ml/kg b.w. (124, 248 and 496 mg/kg b.w.) in DMSO, given i.p. at an interval of 24 hours; examination at 6, 72 and 96 hours after last dosing	12.5, 25 or 50% of the LD_{50} (7-day observation period) tested (2 administrations)	negative	Tsuchimoto and Matter, 1981
Cytogenetic study of sperm from NMRI mice, test for diploidy	100, 200 or 400 mg/kg b.w. as a single intraperitoneal dose, determination of diploid spermatozoa by flow cytometry after 14 days	no information	negative	Otto and Oldiges, 1986
Tests in *Drosophila melanogaster*				
Drosophila melanogaster, sex-linked recessive lethal test	0.1 or 0.2% in 5% sucrose solution was fed to adult males for 3 days; subsequent mating to virgin females for 48 or 72 hours	no information	negative	Vogel et al., 1981
Drosophila melanogaster, sex-linked recessive lethal test	20000 or 28000 ppm in 5% sucrose solution was fed to adult males for 72 hours; males were then mated to 3 fresh virgin females/male every 2 to 3 days to make a total of 3 broods	Mortality was 20 and 38%, respectively, at the end of exposure	negative	Foureman et al., 1994
Drosophila melanogaster, sex-linked recessive lethal test	15000 ppm in 0.75% NaCl solution was injected into adult males; males were then mated after 24 hours to 3 fresh virgin females/male every 2 to 3 days to make a total of 3 broods	Mortality was 26% 72 hours after dosing	negative	Foureman et al., 1994
Drosophila melanogaster, eye mosaic assay, somatic interchromosomal mitotic recombination	4300 or 8600 µg/ml (50 or 100 mM) in 15% sucrose solution was fed to 58–72 hour-old larvae for 30 minutes	no information	negative	Vogel and Nivard, 1993
i.p. intraperitoneal b.w. body weight				

7.7 Carcinogenicity

Groups of 50 male and 50 female F344/N rats (mean initial weight approx. 190 and 140 g, respectively) received 5 weekly doses of γ-butyrolactone (> 97%) as a solution in corn oil by oral gavage for a period of 2 years. Based on the results of a preliminary study (see Section 7.5; NTP, 1992), doses were set at 0 (controls), 112 or 225 mg/kg body weight for male rats and 0 (controls), 225 or 450 mg/kg body weight for female rats. At the end of the study, mean survival in the male rats of the control group was 662 days, whilst it was 668 and 688 days in the low and the top dose group, respectively. The corresponding values for the female rats were 678, 669 and 644 days. Body weight development in the male rats was similar to that seen in the control group whilst it was retarded in the females of the top dose group. By the end of the study, body weights were 20% lower than in the control group. No treatment-related clinical signs of toxicity were observed. Macroscopic and comprehensive histopathological examination revealed no substance-related non-neoplastic lesions or increased incidences of neoplasms. Hence, it was concluded that γ-butyrolactone showed “no evidence of carcinogenic activity” in rats (NTP, 1992).

A total of 10 male rats (initially weighing 45 to 60 g, strain not specified) received γ-butyrolactone 3 to 6 times by oral gavage at varying dose levels (100 to 600 mg/kg body weight; total doses from 450 to 1700 mg/kg body weight) over a period of 7.5 months. Only 6 rats survived longer than a year after the last dose. Of these animals, 5 had tumours (2 pituitary tumours, 2 squamous carcinomas of the jaw, 1 testicular interstitial tumour and 1 pulmonary adenoma). Another animal had degenerative lesions and calcifications of the heart. No control group was included in the study. Several animals showed chronic inflammatory lung lesions, and in two, interstitial hyperplasia was present in the testes. The investigator noted that similar lung lesions and pituitary tumours, and more rarely testicular interstitial tumours and jaw tumours, were seen spontaneously in ageing control rats (no further details; Schoental, 1968). The study was based on a small number of animals, lacked controls and used γ-butyrolactone which was distilled from a mixture of 4,4'-diaminodiphenylmethane and was only characterised by the documented boiling point. For these reasons, the study is not suitable for the evaluation of the chemical’s carcinogenic potential.

Groups of 50 male and 50 female B6C3F1 mice (mean initial weight approx. 25 g) received 5 weekly doses of γ-butyrolactone (> 97% pure) as a solution in corn oil at dose levels of 0 (controls), 262 or 525 mg by oral gavage for a period of 2 years. Body weight development was initially affected in male mice of both dose groups. By the end of the study, body weights were 6% lower than in the control group. Similar body weight development was seen in the female mice of both dose groups. By the end of the study, body weights in the high and low dose groups were lower than in the controls by 14 and 17%, respectively. The high-dose males and females were observed to be partially sedated and inactive shortly after gavage dosing. Survival was reduced in the high-dose males (controls: 674 days; 525 mg/kg body weight: 481 days). At the end of the study, survival was 70% in the male mice of the control group, while it was 60% in the low dose group and 25% in the high dose group. The corresponding values for the female rats were 76, 68 and 76%. Low survival in the male high-dose mice was attributed to fighting and bite wounds during the first year of the study. Thereafter, the animals were housed individually. Macroscopic and comprehensive histopathological examination revealed no substance-related changes in the female mice. In the male low-dose mice, the incidence of benign or malignant phaeochromocytomas in the adrenal medulla was found to be marginally increased but nevertheless higher than the incidence seen in historical controls (3.1%, range: 0 to 6%), and there was a statistically significant increase in the incidence of focal hyperplasia (see Table 6).

Table 6. Adrenal medullary lesions in male B6C3F1 mice following chronic administration of γ-butyrolactone

	Controls	262 mg/kg b.w.	525 mg/kg b.w.
Hyperplasia	2/48 (4%)	9/50 (18%)*	4/50 (8%)
Benign phaeochromocytomas	1/48 (2%)	5/50 (10%)	1/50 (2%)
first incidence (days)	582	729	640
Malignant phaeochromocytomas	1/48 (2%)	1/50 (2%)	0/50 (0%)
Benign or malignant phaeochromocytomas, overall rates	2/48 (2%)	6/50 (12%)	1/50 (2%)

* significantly different from the controls
b.w. body weight

The investigators attributed the lack of dose-dependency to reduced survival seen in the high dose group. Thus, for female mice, there were no indi-

cations of a carcinogenic potential of γ-butyrolactone. The findings noted in male mice were evaluated as “equivocal evidence of carcinogenic activity” (NTP, 1992).

In a further study, 95 male NMRI mice, 8 weeks old, received a total of 78 doses of γ-butyrolactone (≥ 99.5% pure) at 750 mg/kg body weight in soy bean oil by oral gavage once weekly for a period of 18 months. A control group of 45 male mice was included. After 26 weeks, 17 animals receiving the test substance and 10 controls were sacrificed and histopathologically examined, the procedure being repeated with 14 treated animals and 10 controls after 52 weeks. The remaining animals were placed under observation up to study week 123. Body weight gain in the mice receiving γ-butyrolactone was similar to that of the controls up to study week 48. After this, decreased mean body weights were observed. Survival was not affected. Histopathological examination revealed no increase in tumour incidences compared with the controls (Holmberg et al., 1983).

A group of 36 male and female XVII/G mice – a mouse strain with a spontaneous pulmonary tumour rate of 50% – received 2 mg γ-butyrolactone in 0.1 ml water/mouse by oral gavage twice weekly throughout their lifetime. A control group of 33 animals was treated with water. Out of the 36 mice treated, 20 (55.5%) developed pulmonary tumours, mean survival being 571 days. The control group showed a pulmonary tumour incidence of 23 out of 33 (63.6%), mean survival being 542 days. The pulmonary tumour rate was not increased by the administration of γ-butyrolactone. No increased tumour incidences were observed in other organs or tissues (no further details; Rudali et al., 1976).

A group of 30 male and 30 female C3H mice – a mouse strain with spontaneous mammary and hepatic tumour rates of 60 and 15%, respectively – received γ-butyrolactone at 1000 mg/kg feed (1000 ppm, equivalent to approx. 143 mg/kg body weight) from day 24 to the end of their life. Fifty-four male and 61 female mice served as controls. In the female mice, 19 out of 30 treated female mice (63.3%) and 43 out of 61 untreated controls (70.5%) developed mammary tumours. Mean latency of tumorigenesis was 315 ± 63 days in treated mice while it was 327 ± 59 days in controls. In the male mice, 5 out of 30 (10.9%) treated mice and 6 out of 54 mice of the control group (16.6%) were observed to develop hepatomas. However, the difference was not statistically significant. Hence, spontaneous tumour ra-

tes were not increased by γ-butyrolactone administration (Rudali et al., 1976).

A group of 30 XXVII/G mice (sex not specified) had one drop of a 1-percent acetone solution of γ-butyrolactone applied to the skin of the neck twice weekly throughout their lifetime. Seventeen controls were treated with the solvent. Mean survival was 601 days in treated mice, with 21 out of 30 mice (70%) developing pulmonary tumours, whilst in the control group (acetone) mean survival was 499 days, with 9 out of 17 mice (52.9%) developing pulmonary tumours. No increased tumour incidences were observed in other organs or tissues (Rudali et al., 1976).

In another study, 30 male Swiss-Millerton mice, approx. 8 weeks old, received lifetime skin applications of 100 mg of a 10-percent γ-butyrolactone solution in benzene onto the clipped dorsal skin (10 mg/mouse) three times weekly. Two animals (approx. 7%) developed skin tumours, including one carcinoma. Out of the 150 controls treated with benzene, 11 (approx. 7%) showed skin tumours, as did 13 out of 267 untreated controls (approx. 5%), each group exhibiting one carcinoma (van Duuren et al., 1963).

In addition, 30 female Swiss-Millerton mice had 100 mg of a 10-percent solution of γ-butyrolactone in acetone painted onto the clipped dorsal skin three times weekly. Median survival time was 495 days, and there were no skin tumours or signs of local irritation. Median survival time in acetone-treated controls (60 animals) was 447 days. Skin tumours did not occur in the controls (van Duuren et al., 1965).

Five male Wistar rats, 8 weeks old, received subcutaneous injections of an oily solution of 2 mg γ-butyrolactone twice weekly for a period of 61 weeks. Following an observation period of 100 weeks in total, no tumours were observed (Dickens and Jones, 1961).

Thirty-four XXVII/G mice (sex not specified) were injected subcutaneously with 1 µg γ-butyrolactone on days 1, 4 and 8 of life. Mean survival was 590 days, 18 out of 34 mice (52.9%) developing pulmonary tumours. Untreated controls had a mean survival of 595 days and a pulmonary tumour rate of 61.3% (Rudali et al., 1976).

Sixteen female Swiss-Webster mice were injected subcutaneously with 0.005 mg γ-butyrolactone in 0.1 ml tricaprylin three times weekly for a pe-

riod of 4 weeks (12 injections). Eleven mice survived for 18 months. No local or other tumours were found (Swern et al., 1970).

γ-Butyrolactone was investigated in vitro in a cell transformation test carried out in BHK cells with metabolic activation (S9 mix, no further details). Concentrations of 0.025, 0.25, 2.5 and 25 µg/ml were used. At and above 25 µg/ml, there was an increase in transformation rate/10^6 surviving cells which was concentration-dependent. γ-Butyrolactone was evaluated as positive in this test (Styles, 1981).

7.8 Reproductive toxicity

Groups of 10 female Sprague-Dawley rats received γ-butyrolactone at dose levels of 10, 50, 125, 250 or 500 mg/kg body weight by oral gavage from days 6 to 15 of gestation (the day of sperm-positive vaginal smear was considered to be day 0). Nine animals treated with the vehicle served as controls. Soybean oil was used as the vehicle, but because γ-butyrolactone was not sufficiently soluble at 500 mg/kg body weight, no higher doses were tested. The treated dams exhibited no change in body weight gain or food and water consumption compared with the controls. At 125 and 500 mg/kg, one animal per dose group died. Necropsy revealed oedema, hyperaemia and of the lungs (probably due to gavage errors). The foetuses were removed by Caesarean section on day 21 of gestation. Placental weights were non-dose-dependently reduced in all dose groups. No differences relative to controls were observed in the numbers of corpora lutea, total implantations, live and dead foetuses, resorptions and preimplantation and postimplantation losses. Mean foetal weights were significantly increased in the dose groups receiving 50, 125 or 250 mg/kg body weight. No substance-related visceral or skeletal abnormalities were observed (Kronevi et al., 1988).

In a range-finding study for a embryotoxicity/teratogenicity study, groups of 5 pregnant Himalayan rabbits (weighing 2 to 3.5 kg) were exposed to γ-butyrolactone (99.7%) at analytical concentrations of 0 (controls), 510, 1410 or 5030 mg/m³ for 6 hours/day (head-nose exposure) from days 7 to 19 after insemination. The high concentration yielded a considerable amount of aerosol (mass median aerodynamic particle diameter 3.4 µm). No clinical signs of toxicity were observed. Body weight development in the groups

that were exposed to the test substance was similar to that seen in the controls. Haematology and clinical chemistry parameters were normal. Placental weights were unaltered in all concentration groups. The findings for the other study parameters (numbers of corpora lutea, implantation sites, preimplantation and postimplantation losses, resorptions and viable foetuses) were also similar to those observed in the controls. The reduced mean placental weight and marginally reduced foetal weights noted in the high concentration group were possibly related to the test substance. External examination of the foetuses revealed no appreciable findings (BASF, 1993).

In the subsequent embryotoxicity/teratogenicity study conducted in accordance with OECD guideline No. 414, groups of 15 Himalayan rabbits (average initial weight 2.36 kg) underwent daily 6-hour exposure to γ-butyrolactone (99.7%) on days 7 to 19 after insemination (insemination on day 0), the chemical being administered at 0 (controls), 500, 1400 or 5000 mg/m³ as a vapour or vapour/aerosol mixture (head-nose exposure). The mass median aerodynamic diameter of the particles was 2.4 µm with a geometric standard deviation of 3.3, and the respirable fraction was 92%. Even at the highest test concentration of 5000 mg/m³, no effects on the dams were noted with regard to clinical signs, body weights, body weight changes, uterine weights or macroscopic findings. Compared with the controls, no biologically relevant differences were observed in the conception rate, the numbers of corpora lutea, total implantations, resorptions, live foetuses, the sex ratio or the numbers of preimplantation and postimplantation losses. The exposed animals were not found to have differences in placental or foetal weights relative to the controls. No substance-related external, visceral or skeletal abnormalities were observed in the foetuses. In this study, γ-butyrolactone showed no maternal toxicity, embryo-/foetotoxicity or teratogenic effects up to the highest test concentration of 5000 mg/m³ (BASF, 1993).

The inhibitory effect of γ-butyrolactone on ovulation was studied in adult female Sprague-Dawley rats (weighing 225 to 300 g). Groups of 5 to 11 animals were given the test substance in physiological saline solution by single intraperitoneal injection of 0 (controls), 62.5, 125, 250, 500 or 750 mg/kg body weight on pro-oestrus. In order to determine the levels of luteinising hormone and follicle-stimulating hormone, hourly blood samples were taken for 4 hours after dosing. The animals were sacrificed on the morning of the expected oestrus, and the degree of ovulation was assessed. At and above 250 mg/kg body weight, dose-dependent inhibition of ovulation

(by 63 to 100%) and increases in uterine weight were noted in association with an increased incidence of uterine ballooning. No change was noted in ovarian weight. Administration of γ-butyrolactone doses of and above 250 mg/kg body weight produced a significant, dose-related decrease in serum luteinising hormone levels over the entire 4-hour period sampled. Furthermore, follicle-stimulating hormone levels were significantly reduced 3 hours after intraperitoneal injection of 500 mg/kg body weight or higher doses. The investigators suggested that the effects on the hormones investigated could be due to the actions of γ-butyrolactone on central dopaminergic nerve impulse flow (see also Section 7.11; Beattie et al., 1976).

Repeated oral administration of γ-butyrolactone (treatment period not specified) at concentration levels of 1 or 2% in the drinking water of prepubertal 21-day-old male rats (10 to 14 rats/group) resulted in reduced body and testicular weights relative to controls. Serum prolactin levels in the treated animals were not different from those seen in the controls. As there was a significant reduction in body weight in this study, the experiment was repeated, the difference being that food consumption was carefully controlled. In the repeat study, body weights were similar to those observed in the control group that was included, but again, testicular weights were lower. Seminal vesicle weights and serum prolactin levels did not differ from those seen in the controls (no further details; Debeljuk et al., 1983).

Groups of 5 male (CBAxBALB/c)F1 mice were given intraperitoneal injections of γ-butyrolactone at 0.1, 0.25, 0.5 or 1.0 ml/kg body weight (equivalent to approx. 113, 282, 564 and 1128 µg/kg body weight, respectively) in physiological saline solution on 5 consecutive days. Five weeks after the last dose, the sperm from the cauda epididymis (250 sperm/mouse) were scored for sperm with morphologically abnormal heads. Part of the animals died after receiving the highest dose (no further details). No morphologically abnormal sperm heads were observed (Topham, 1981).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

γ-Butyrolactone is classed with the neuroactive substances which act onthe catecholamine receptors in the brain. The chemical's sedative and narcotic actions, which are seen at high doses and have been investigated in numerous neuropharmacological studies, is attributed to its metabolite, γ-hydroxybutyric acid. The studies are discussed in detail in Section 7.11.

7.11 Other effects

γ-Butyrolactone undergoes rapid metabolism, which is catalysed by lactonases, to γ-hydroxybutyric acid, the compound to which the anaesthetic effect is attributed (Roth et al., 1966; see also Section 7.1).

Sedative/narcotic and analgesic effects

Upon single intraperitoneal injection of γ-butyrolactone at 480 mg/kg body weight, mean sleep induction time in rats was 10 ± 2 minutes, and mean sleeping time was 202 ± 32 minutes. This was associated with characteristic changes in the electroencephalogram (EEG) tracings. In comparison, equimolar doses of γ-hydroxybutyric acid resulted in a greater sleep induction time of 17 ± 4 minutes and a shorter sleeping time of 133 ± 32 minutes (Sprince et al., 1966).

A similar study in male Sprague-Dawley rats compared the effects of γ-butyrolactone and γ-hydroxybutyrate by means of EEG recordings. Administration of equimolar doses (4.8 to 6.8 m-equiv./kg body weight given intraperitoneally) in this study also resulted in a slightly shorter sleep induction time with γ-butyrolactone than with γ-hydroxybutyrate, whereas there was no difference with respect to duration of action. Intraperitoneal injection of lower doses (0.8 to 1.8 m-equiv./kg body weight) had no effect on orthodox or paradoxical sleep (Marcus et al., 1967).

The effects of γ-butyrolactone on orthodox and paradoxical sleep were also investigated in cats. When injected intraperitoneally into normal animals, 200 to 500 mg/kg body weight caused deep narcosis with the characteristic electroencephalographic patterns after a latency of 2 to 6 minutes. When cats that had no orthodox sleep due to implanted electrodes were given a

single intraperitoneal injection of 50 mg/kg body weight, this caused paradoxical sleep, but not narcosis, for approx. 10 minutes, a second and a third injection inducing approx. 15 and approx. 20 minutes' paradoxical sleep, respectively. In cats without paradoxical sleep (due to coagulation of the caudal pontine reticular nucleus), 50 mg γ-butyrolactone/kg body weight caused narcosis with a cortical pattern, and hypertension. Paradoxical sleep did not occur. Fifteen days later, following a γ-butyrolactone dose of 50 mg/kg body weight, patterns of paradoxical sleep were recorded again (Jouvet et al., 1961).

The effect on hexobarbital sleeping time (intravenous sodium hexobarbital at 60 mg/kg body weight) was investigated at 30, 60, 120 and 240 minutes after pretreatment with γ-butyrolactone (100 to 400 mg/kg body weight given intraperitoneally). Sleeping time was increased in a dose-dependent manner (controls 6.3 minutes, 400 mg/kg 50.8 minutes, 30 minutes after pretreatment; Hampel and Hapke, 1968).

When γ-butyrolactone was intraperitoneally administered to mice at a dose of 55 mg/kg body weight, it potentiated the effect of a subanaesthetic dose of pentothal (25 mg/kg body weight given intraperitoneally). The righting reflex was abolished in 6 out of 10 animals studied. In 9 out of 10 animals treated with 25 mg pentothal/kg body weight, γ-butyrolactone induced sleep at 110 mg/kg body weight. The righting reflex was not affected when the control group (50 animals) was treated with pentothal at 25 mg/kg body weight. Even at 5.5 mg/kg body weight, intraperitoneally administered γ-butyrolactone prolonged pentothal sleeping time by 100%, whilst at 55 mg/kg body weight prolongation by 240% was seen. A γ-butyrolactone dose of 110 mg/kg body weight prolonged the duration of sleep induced by evipan (70 mg/kg body weight given intraperitoneally) by 90%, 55 mg/kg body weight resulting in prolongation by 34%. Doses lower than 55 mg/kg body weight produced no effect. The duration of sleep induced by chloral hydrate (250 mg/kg body weight given intraperitoneally) was prolonged by 140% after 55 mg γ-butyrolactone/kg body weight and by 300% after 110 mg/kg body weight. The duration of anaesthesia induced by ethanol (3.2 mg/kg body weight) was also increased by γ-butyrolactone. The lowest effective dose was 55 mg/kg body weight (Sieroslawska, 1965).

A sedative-hypnotic effect of γ-butyrolactone was demonstrated in mice by means of motility assessments involving a photographic technique that

establishes relative activity scores between controls and treatment groups, or a photoelectric-barrier box. Photographic measurement of motility demonstrated that in the animals treated with γ-butyrolactone (200 and 400 mg/kg body weight given intraperitoneally) there was a dose-dependent effect as regards intensity and duration of sedation. Motility measurements in the photoelectric-barrier box also indicated a dose-dependent effect with regard to intensity and duration of sedation (test doses of 100, 200 or 400 mg/kg body weight were given intraperitoneally; Hampel and Hapke, 1968).

The effect of γ-butyrolactone on unconditioned and conditioned avoidance and escape reactions was investigated in male Wistar rats (weighing 160 to 200 g, 5 rats/group). Upon intraperitoneal administration of γ-butyrolactone at dose levels of 50 and 100 mg/kg body weight, no effects were observed on the animals' behaviour. The 200 mg/kg dose diminished the number of conditioned reactions by 25%, 250 mg/kg body weight inhibited conditioned and unconditioned reactions after 60 and 120 minutes, and 500 mg/kg body weight exerted a narcotic effect and completely abolished conditioned and unconditioned reactions (Sieroslawska, 1965).

In unanaesthetised cats, intracerebral administration of γ-butyrolactone (7 to 9 µg) caused changes in the electroencephalogram (EEG) resembling the pattern of psychomotor epilepsy. Nevertheless, the behaviour of the animals was tranquil or, less often, somnolent. Acoustic stimuli elicited a strong arousal syndrome in the EEG. After 60 minutes the tracings returned to the normal appearance (Sieroslawska, 1965).

Rats (weighing 305 to 400 g) were lightly anaesthetised with ether and had a needle inserted into the cisterna magna for withdrawal of cerebrospinal fluid. Intracerebral administration of 115 to 230 µmol γ-butyrolactone did not depress central nervous activity, whereas administration of the product of hydrolysis, γ-hydroxybutyric acid, resulted in marked and persistent depression of the central nervous system. On administration of the highest dose of γ-hydroxybutyric acid (230 µmol) some animals died of respiratory paralysis. The investigators explained the lack of activity of γ-butyrolactone by the fact that neither brain nor cerebrospinal fluid contained any appreciable amounts of lactonase, so that hardly any γ-butyrolactone could be hydrolysed to the pharmacologically active metabolite, γ-hydroxybutyric acid (Roth et al., 1966).

Direct infusion via a permanently implanted micro-cannula of γ-butyrolactone into the brain (thalamus) of 2 unanaesthetised monkeys (*Macaca mulatta*) caused no changes in the electroencephalogram at 1, 15 and 60 minutes after infusion (Roth et al., 1966).

Male mice were housed individually for 3 weeks, and the effect of γ-butyrolactone (100 to 400 mg/kg body weight given intraperitoneally) on their aggressiveness was then studied in the aggression test. The aggressiveness of the mice was reduced in a dose-dependent manner (Hampel and Hapke, 1968).

In the writhing test in mice of both sexes, γ-butyrolactone produced no analgesic effect at a dose of 500 mg/kg body weight (mean number of writhings: 14.5 ± 3, controls: 14.2 ± 2). At 800 to 1000 mg/kg body weight, no anti-inflammatory effect was observed in the carrageenin test in rats (Szirmai et al., 1989).

The analgesic effect of γ-butyrolactone was assessed in mice by means of applying electrical stimuli or a heat beam to the snout. Absence of vocalisation after a maximum of 4 individual stimuli and prolongation of the reaction time to withdrawal response were evaluated as indicating an analgesic effect. When electrical stimulation was applied to the mouse tail, γ-butyrolactone inhibited the occurrence of vocalisations at dose levels of 200 and 400 mg/kg body weight. The duration of the effect was dose-dependent. Intraperitoneal administration of γ-butyrolactone at doses of 100 and 200 mg/kg body weight significantly prolonged the reaction time following the application of a heat beam. At 50 mg/kg body weight, there was no effect (Hampel and Hapke, 1968).

When γ-butyrolactone was administered to albino rabbits at doses of 20 to 100 mg/kg body weight, it slightly potentiated the effect of a subanalgesic dose of dolantine (25 mg/kg body weight given intravenously). The study investigated pain induced by faradic irritation of the dental pulp (no further details; Sieroslawska, 1965).

The local anaesthetic effect of γ-butyrolactone was studied by mechanical irritation of the ensuing weal up to 90 minutes after intracutaneous injection into the shorn skin of the guinea pig. Test solutions of 0.5 to 2% were used (0.05 ml). γ-Butyrolactone produced positive effects in a dose-dependent manner (Hampel and Hapke, 1968).

The ability of γ-butyrolactone to produce conduction anaesthesia was investigated in the guinea pig by measuring the appearance and duration of hindlimb paralysis following epidural injection in the region of the last lumbar vertebra. A 0.05 ml dose of a 1-percent γ-butyrolactone solution was without effect. Solutions containing 2 or 4 percent produced mean durations of action of 3 or 5.7 minutes, respectively. Longer-lasting conduction anaesthesia could not be achieved with γ-butyrolactone (Hampel and Hapke, 1968).

Effects on the dopaminergic system

When male Long-Evans rats were given γ-butyrolactone at 250 or 500 mg/kg body weight by intraperitoneal injection, brain dopamine levels were increased after 2 hours and were similar, on an equimolar basis, to those seen after γ-hydroxybutyrate (no further details; Gessa et al., 1966).

Male Sprague-Dawley rats (weighing approx. 100 g) received a single γ-butyrolactone dose of 500 mg/kg body weight by the oral, intraperitoneal or intravenous route. The animals were sacrificed 1.5 hours later, and the brain levels of dopamine, norepinephrine, homovanillic acid – a dopamine metabolite – and 5-hydroxytryptamine were determined. There was a significant increase in the level of dopamine from 664 (controls) to 1354 ng/g, whereas the level of norepinephrine was unchanged (329 ng/g, controls: 323 ng/g). The increase in dopamine was accompanied by a decrease in homovanillic acid to 0.199 µg/g (controls: 0.370 µg/g). No significant increase in 5-hydroxytryptamine was observed. The synthesis of dopamine and norepinephrine from ^{14}C-labelled tyrosine was also investigated. Rats received γ-butyrolactone intraperitoneally at 750 mg/kg body weight followed by intravenous injection of 25 µCi ^{14}C-tyrosine 10 minutes later. After one hour, the incorporation of ^{14}C-labelled tyrosine into brain dopamine and norepinephrine was studied. In addition to an increase in brain dopamine by 94%, a 90-percent increase in the specific activity of dopamine was noted, while the specific activity of norepinephrine was only slightly increased. Studies employing α-methyl-p-tyrosine methyl ester, an inhibitor of catecholamine biosynthesis, indicated that γ-butyrolactone antagonised the inhibitor-induced disappearance of dopamine while having little or no effect on norepinephrine depletion. The duration of sleep induced in rats by 350 mg γ-butyrolactone/kg body weight was not altered by concurrent admini-

stration of p-chlorophenylalanine, which causes depletion of brain 5-hydroxytryptamine, whereas concurrent administration of α-methyl-p-tyrosine increased sleeping time by approx. 100%. Pretreatment with dihydroxyphenylalanine, the immediate precursor of dopamine, did not antagonise γ-butyrolactone-induced sleep. Treatment with D-amphetamine, a substance known to release dopamine, did however partly antagonise the effect. On the basis of these findings, the investigators suggested that γ-butyrolactone increases brain dopamine levels primarily by selectively blocking its release from the dopamine-containing neurons (Roth and Suhr, 1970).

In more sophisticated studies, the accumulation of dopamine in different brain regions of male Sprague-Dawley rats was investigated upon administration of γ-butyrolactone. Intraperitoneal injection of γ-butyrolactone at 750 mg/kg body weight resulted in significant increases in dopamine levels relative to controls by 50 to 180% in the corpus striatum, nucleus accumbens, olfactory tubercle, limbic cortex, rostral cortex, cortex of the corpus caudatum, and hypothalamus after 60 minutes. The highest level was found in the rostral cortex. Only the cerebellum showed no increase in dopamine concentration. Concurrent administration of the selective dopamine receptor antagonist B-HT 920 (1 mg/kg body weight given intraperitoneally) or of B-HT 920 plus haloperidol (2 mg/kg body weight given intraperitoneally), a dopamine receptor antagonist, did not reduce the concentration of dopamine in the various brain regions. The investigators therefore suggested that the accumulation of dopamine caused by γ-butyrolactone was probably the result of decreased release rather than increased synthesis (Andén et al., 1983).

In male Sprague-Dawley rats, intraperitoneal injection of γ-butyrolactone at 750 mg/kg body weight caused a transient increase in dopamine synthesis in the corpus striatum, after which synthesis dropped to between 40 and 50% of the control levels for 2 to 12 hours. Dopamine synthesis was determined as DOPA accumulation following inhibition of DOPA decarboxylase. γ-Butyrolactone-induced inhibition of dopamine synthesis was abolished by haloperidol (2 to 5 mg/kg body weight given intraperitoneally). According to the investigators, these results suggested that delayed inhibition of dopamine synthesis following administration of γ-butyrolactone was due to stimulation of supersensitive dopamine autoreceptors (Argiolas et al., 1982).

Increased dopamine concentration was visualised by means of a fluorescence histochemical method. To this aim, male Charles-River rats were given γ-butyrolactone by the intraperitoneal route at 750 mg/kg body weight. The animals were killed 1.5 hours after the injection, and dopamine fluorescence in 2 µm-thick brain sections was studied under the fluorescence microscope. In contrast to the specimens obtained from control animals, which exhibited only diffuse fluorescence, those from γ-butyrolactone-treated animals were found to have intensely green fluorescent dots, 0.5 to 1.0 µm in diameter, in the caudate nucleus, accumbens nucleus and olfactory tubercle. Other brain areas were not affected. Biochemical analysis conducted in parallel revealed that dopamine was increased by 68% relative to the controls. The norepinephrine concentration was not affected (Aghajanian and Roth, 1970).

In a study in male Sprague-Dawley rats, the concentration and distribution of dopamine in the neostriatum was investigated following intraperitoneal administration of γ-butyrolactone at 750 mg/kg body weight. By 20 minutes after the injection, dopamine levels had doubled compared with the controls. γ-Butyrolactone did not affect the concentration of norepinephrine in the cortex. Administration of α-methyl-p-tyrosine, an inhibitor of tyrosine hydrolase, shortly after injection of γ-butyrolactone prevented the increase in dopamine levels. Fluorescence studies conducted in 4 µm brain sections from animals that were killed 1.5 hours after γ-butyrolactone administration revealed bright varicosities in the neostriatum, which the investigators presumed to represent dopaminergic nerve terminals (Walters et al., 1973).

Male ICR mice received a single dose of γ-butyrolactone at 40, 80 or 160 mg/kg body weight by intraperitoneal injection. This resulted in dose-dependent reductions of locomotor activity. Repeated injection 3 times a day (160 mg/kg body weight on day 1, 320 mg/kg on days 2 to 5, 480 mg/kg on days 6 to 9 and 640 mg/kg on days 10 to 13), followed by a single subcutaneous injection of γ-butyrolactone at 40, 80 or 160 mg/kg body weight 24 hours after the last intraperitoneal administration, resulted in reduced locomotor depression. Upon single intraperitoneal injection of γ-butyrolactone doses of 320 or 640 mg/kg body weight, the dopamine levels in the brains of mice were dose-dependently elevated after 60 or 30 minutes, respectively. When the same doses were injected subcutaneously 24 hours on completion of the repeated administrations, the dopamine-elevating action was reduced. When α-methyltyrosine methyl ester (250 mg/kg body

weight), an inhibitor of dopamine synthesis, was administered intraperitoneally 24 hours on completion of the repeated treatment with γ-butyrolactone, it reduced the dopamine concentrations in the corpus striatum and olfactory tubercle. Concurrent administration of γ-butyrolactone reduced the decline of dopamine concentrations in both brain regions, whereas administration of γ-butyrolactone to controls treated with α-methyltyrosine methyl ester completely inhibited the decline of dopamine. According to the investigators, the development of tolerance to the effects on the animals' behaviour and the neurochemical parameters was not the result of metabolic tolerance but rather possibly that of altered characteristics of the dopaminergic neurons (Gianutsos and Moore, 1978).

Male Charles-River rats and Swiss mice received a single γ-butyrolactone dose of 725 mg/kg body weight by the intraperitoneal route. When central nervous depression was deepest (after 15 to 30 minutes), the brain levels of acetylcholine, 5-hydroxytryptamine (rats) and γ-aminobutyric acid (mice) were determined. Acetylcholine brain levels in mice were elevated by 44%, whilst γ-aminobutyric acid levels remained unchanged. Levels of 5-hydroxytryptamine in rats were unaltered. The elevated acetylcholine levels in rats were sharply localised in the cortex and in the regions of the brain which contain the corpora quadrigemina (Giarman and Schmidt, 1963).

Male Sprague-Dawley rats (weighing 150 to 200 g) received γ-butyrolactone in their drinking water for a period of 4 weeks. The dose level was 1% during the first week of the study and 2% thereafter until the end of administration, the latter concentration corresponding to a daily intake of 3000 mg/kg body weight. At the end of the treatment period, the animals treated with γ-butyrolactone weighed significantly less than the controls. Animals underwent various neurochemical and neurophysiological investigations. On the one hand, the effect of 4-week γ-butyrolactone treatment was studied on the duration of sleep induced by acute administration of γ-butyrolactone (350 or 750 mg/kg body weight given intraperitoneally). Compared with the controls, sleeping time was reduced by 58% and 11% on administration of 350 and 750 mg/kg body weight, respectively. Other animals were given ordinary tap water for 2 days. Subsequently, apomorphine was administered intraperitoneally at 0.25 mg/kg body weight, and the animals' motor activity was then scored for one hour. In the γ-butyrolactone group, animals were more active than the controls during the first 45 minutes, after which time there were no differences. Determination of dopamine levels in

the striata revealed no differences between animals treated with γ-butyrolactone, and controls. Thirty minutes after injection of a DOPA decarboxylase inhibitor, Ro4-4602 (800 mg/kg body weight given intraperitoneally), the two study groups showed no difference in DOPA levels. In addition, the levels of dihydroxyphenylacetic acid, a major metabolite of dopamine, were measured in the striata 45 minutes after injection of chloral hydrate. The levels measured in the γ-butyrolactone group were only 75% of those in the controls. In order to determine whether 4-week γ-butyrolactone treatment caused a persistent depression of dopaminergic neurophysiological activity, the firing patterns of dopaminergic cells in the substantia nigra were studied with the aid of electrodes in chloral hydrate-anaesthetised rats. The firing rates of A9 dopaminergic cells were significantly lower than in controls. The effect of electrical stimulation on the nigro-neostriatal pathway was also investigated in chloral hydrate-anaesthetised rats. On 20-minute electrical stimulation, the animals were injected with Ro4-4602, and striatal DOPA levels were determined as an index of the post-stimulation activation of tyrosine hydroxylase. Neither the γ-butyrolactone-treated animals nor the controls exhibited any differences between the stimulated and non-stimulated striata. In addition, the effect of electrothermic lesion of the nigro-neostriatal tract was investigated in anaesthetised rats. On injection of Ro4-4602, no difference in DOPA accumulation was noted between the two groups. Furthermore, presynaptic dopamine receptor activity was estimated by determining the effect of a submaximally effective dose of apomorphine on tyrosine hydroxylase activity after elimination of impulse flow in the dopaminergic axons. Apomorphine caused significantly more depression of the lesion-induced DOPA accumulation in γ-butyrolactone-treated animals than in controls (Nowycky and Roth, 1979).

Motility studies

Female Wistar rats (weighing 200 ± 10 g) received γ-butyrolactone at 0 (controls), 50, 100, 200 or 400 mg/kg body weight by the intraperitoneal route. Subsequently, their locomotor activity was recorded every 10 minutes for a period of 2 hours. The 50 mg/kg dose did not affect locomotor activity, whereas 100 and 200 mg/kg body weight had a biphasic effect on activity. Initial dose-dependent reduction in activity was followed by a phase of hyperactivity. The 400 mg/kg dose produced a prolonged inactive pe-

riod. In addition, animals were pretreated with α-flupenthixol, atropine, benztropine, protriptyline and clomipramine 90 minutes prior to γ-butyrolactone administration (200 mg/kg body weight) in order to study the effect of these drugs on the observed biphasic actions of the test chemical. α-Flupenthixol significantly reduced hyperactivity. Benztropine prolonged the inactive phase and potentiated hyperactivity significantly. Atropine significantly prolonged the inactive phase but, similarly to protriptylin and clomipramine, had no effect on hyperactivity. These results led the investigator to conclude that the increase in activity was probably due to the release of dopamine (Davies, 1978).

The effects of γ-butyrolactone on the spontaneous motility of male R3 mice (weighing approx. 20 g) after intraperitoneal administration were investigated in the actographic test and the climbing test at dose levels ranging from 5.5 to 220 mg/kg body weight. In the actographic test, the mice were placed in a special cage with an impulse counter, their movements being recorded 3 times at intervals of 10 minutes. The climbing test assessed their ability to climb in a cylinder made of wire netting. In the actographic test, doses of 5.5 to 55 mg/kg body weight produced no effect or did not significantly reduce spontaneous motility in the first hour after administration. The dose of 110 mg/kg body weight reduced motility by 55 to 67% in the first hour after dosing, while 220 mg/kg body weight completely abolished motility for more than 2 hours. In the climbing test, doses of 5.5 and 11 mg/kg body weight were without effect, while 22, 55 and 110 mg/kg body weight prolonged climbing time by 37, 53 and 130%, respectively, and 220 mg/kg body weight abolished climbing ability completely. The latter dose group showed unco-ordinated movements and marked muscular flaccidity (Sieroslawska, 1965).

The co-ordinating ability of mice (Porton, weighing approx. 20 g, 10 and 20 mice/group) was assessed in the rotating rod (rota rod) test and the roller cage test after intraperitoneal administration of γ-butyrolactone. In the rota rod test, the animals had to maintain position on the rotating rod for at least 120 seconds, whereas in the roller cage, a cylindrical cage made of wire netting which rotated around its long axis at 10 revolutions per minute, they had to maintain position during 3 30-minute periods within 3 hours. The rota rod test showed an effect even at 22 mg/kg body weight after 15 minutes because 3 out of 10 animals dropped off the rotating rod. The effects were reversible after 30 minutes. After 110 and 220 mg/kg body weight, 3 out of

20 mice and 4 to 7 out of 10 mice, respectively, dropped off the rod during the first hour after injection. In the roller cage test, the number of mice falling out of the cage was increased by 76% and 160% relative to the control group after doses of 55 and 110 mg/kg body weight, respectively (Sieroslawska, 1965).

Mice (R3 or Porton) were studied with respect to the effect of γ-butyrolactone on their motility after stimulation with caffeine (20 mg/kg body weight given intraperitoneally), amphetamine (5 mg/kg body weight given intraperitoneally), iminodipropionitrile (1500 mg/kg body weight given intraperitoneally for 4 to 5 days) or harmine-rigidyl (8 mg/kg body weight given subcutaneously, or 45 mg/kg body weight given intraperitoneally). Doses of less than 55 mg/kg body weight produced no effect on caffeine- or amphetamine-stimulated motility. At 110 mg/kg body weight, motility stimulated by caffeine or amphetamine was diminished by 49 and 36%, respectively; at 220 mg/kg, decreases were 32 and 69%, respectively. At and above 55 mg/kg body weight, γ-butyrolactone inhibited all iminodipropionitrile-induced motor excitation. In psychomotor stereotypia induced by harmine and rigidyl, γ-butyrolactone prevented convulsions and inhibited jerking movements of the paws and heads at doses from 110 mg/kg body weight (Sieroslawska, 1965).

Effects on the transmission of nerve impulses

Ether-anaesthetised cats of either sex (weighing approx. 2 kg) were used in in-situ experiments on the nictitating membrane and the right superior cervical ganglion. Upon intra-arterial injection of γ-butyrolactone (1 to 10 mg), there was no observable effect of submaximal electrical stimulation on the response of the nictitating membrane or of acetylcholine on the cervical ganglion. When γ-butyrolactone was intravenously administered in an anaesthetic dose of 345 mg/kg body weight, transmission (submaximal electrical stimulation of the preganglionic nerve trunk) in the ganglion was depressed (Roth et al., 1966).

The effects of γ-butyrolactone on neuromuscular conduction were investigated in vivo in the gastrocnemius muscle of cats under urethane anaesthesia (1400 mg/kg body weight) and in vitro in the isolated rat diaphragm. The cats were injected with γ-butyrolactone into the contralateral

femoral vein or iliac artery. After 367 and 500 mg/kg body weight, a dose that caused respiratory paralysis, there was no effect on the amplitude of gastrocnemius muscle contractions. In vitro, the phrenic nerve (4 preparations/concentration) was stimulated at intervals of 2 minutes. γ-Butyrolactone concentrations from 2 x 10^{-6} to 10^{-3} M caused no change in the contractions of the diaphragm (Sieroslawska, 1965).

In order to study the effect on interneurons, the linguomandibular reflex of the rat was studied, stimulation being achieved by means of a tongue electrode at intervals of 5 minutes. The reaction to the stimulus consisted in the spontaneous opening of the mouth. γ-Butyrolactone was noted to produce a dose-dependent inhibitory and blocking effect on interneurons. Upon intraperitoneal administration of 100 mg/kg body weight or intravenous injection of 200 mg/kg body weight, the response to stimulation was observed to be inhibited by 50% for a period of 50 to 60 minutes. A complete block of stimulation lasting more than one hour was achieved with both modes of administration at a dose level of 400 mg/kg body weight (Hampel and Hapke, 1968).

Experiments were carried out in cats anaesthetised with chloralose (80 mg/kg body weight given subcutaneously) to investigate the effect of intravenously administered γ-butyrolactone on spinal reflexes such as the flexor reflex and the patellar tendon reflex. γ-Butyrolactone inhibited the flexor reflex even at a dose of 25 mg/kg body weight. This dose temporarily diminished the amplitude of the contractions of the gastrocnemius muscle by 21 to 45% but had no effect on the patellar reflex. The dose of 50 mg/kg body weight induced, in all the cats, a rapid fall in the amplitude and complete disappearance of reactions to electrical stimulation of the contralateral sciatic nerve for 2 hours, but either had no effect on the patellar reflex, or slightly increased it. The dose of 100 mg/kg body weight inhibited the flexor reflex, and after initially increasing the patellar reflex, diminished it by 16 to 27% (Sieroslawska, 1965).

Anticonvulsive effects

Thirty minutes after intraperitoneal pretreatment with γ-butyrolactone (100 to 400 mg/kg body weight), the effect of the chemical on the toxicity of strychnine (1.1 mg/kg body weight given subcutaneously) and nicotine (1

mg/kg body weight given intravenously) was assessed. There was no detectable effect on the acute toxicity of strychnine. In contrast, there was a dose-dependent effect on the acute toxicity of nicotine to the degree of complete reversal (no indication of the species used in the study; Hampel and Hapke, 1968).

In addition, the effect of intraperitoneal γ-butyrolactone (100 to 400 mg/kg body weight) on the toxicity of amphetamine (9 mg/kg body weight given subcutaneously) was investigated 30, 60 and 120 minutes after dosing. There was no detectable effect (no indication of the species used in the study; Hampel and Hapke, 1968).

In a further study, the effect of γ-butyrolactone on amphetamine toxicity was investigated in groups of 10 R3 mice given an intraperitoneal injection of amphetamine (35 mg/kg body weight) 15 minutes prior to intraperitoneal γ-butyrolactone doses of 0 (controls), 11, 22 or 110 mg/kg body weight. After 24 hours, there were 3 out of 10 survivors in the control group, while there were 5 out of 10 in the 11 and 22 mg/kg groups and 8 out of 10 in the 110 mg/kg group (Sieroslawska, 1965).

The anticonvulsive action of γ-butyrolactone (100 to 400 mg/kg body weight given intraperitoneally) was studied in mice by means of maximum electroshocks, the absence of the tonic phase of convulsion serving as the relevant criterion, or by subcutaneous administration of the convulsant drug pentetrazol at 50, 70 or 100 mg/kg body weight. γ-Butyrolactone suppressed the electroshock-induced appearance of tonic convulsions, the duration and intensity of the effect being dose-dependent. In contrast, the pentetrazol convulsion test gave no indications as to a protective effect of γ-butyrolactone against the occurrence of the clonic phase of convulsion with loss of the body righting reflex (Hampel and Hapke, 1968).

Other pharmacological effects

Within 10 minutes after single intraperitoneal injection of γ-butyrolactone at 100 mg/kg body weight, the electroencephalograms of male rats exhibited bilateral and synchronised spike-and-wave discharges, which lasted 90 minutes. The animals showed clonus of the vibrissae and occasionally of the head. Upon administration of 200 mg γ-butyrolactone/kg body weight, similar discharges were observed within 5 minutes. They became almost conti-

nuous for 60 minutes and were observed for up to 120 minutes post-injection. Bilateral injection into the substantia nigra of 2 mg muscimol, an agonist of γ-aminobutyric acid, significantly reduced the cumulated duration of the discharges without modifying their latency of onset (Depaulis et al., 1989).

In male Sprague-Dawley rats, intraperitoneal injection of γ-butyrolactone at doses of ≤ 400 mg/kg body weight produced hypothermia associated with bilaterally synchronous spike-and-wave discharges in the electroencephalograms. Higher doses, however, led to an increase in core temperature by a maximum of 2 °C. This lasted approx. 60 to 90 minutes and was followed by a hypothermic phase. These doses produced burst suppression in the electroencephalogram. The threshold dose levels for the hypothermia and spike-and-wave discharges were the same as with γ-hydroxybutyric acid, but temperature changes occurred later and lasted longer than those induced by γ-hydroxybutyric acid. Young rats (less than 28 days old) showed less hypothermia when treated with comparable doses of γ-butyrolactone. Ethosuximide, an antiepileptic drug that attenuates the spike-and-wave discharges produced by γ-hydroxybutyric acid, had no effect on the hyperthermia induced by γ-butyrolactone doses ≤ 400 mg/kg body weight. Hypothermia induced by γ-butyrolactone doses ≤ 400 mg/kg body weight was blocked by raising the ambient temperature from 26 to 32 °C. When the ambient temperature was increased to 35 °C, the hyperthermic response was enhanced and there was marked lethality. According to the investigator, the results suggest that the spike-and-wave discharges and the hypothermia are produced via different mechanisms (Snead, 1990).

The effects of γ-butyrolactone on glucose utilisation in 29 structural components of the brain were studied in male Sprague-Dawley rats by means of the ^{14}C-deoxyglucose technique. Doses of 0 (controls), 75, 150, 300 or 600 mg/kg body weight were administered intravenously in divided doses within 30 minutes, and 50 µCi ^{14}C-deoxyglucose was injected intravenously 15 minutes after the final γ-butyrolactone injection. At γ-butyrolactone doses of 75 and 150 mg/kg body weight, there were no changes in the behavioural and clinical states of the animals, but there was clear evidence of slowing of the electroencephalogram. The 300 and 600 mg/kg doses resulted in clinical signs, including loss of spontaneous activity, unresponsiveness and suppression of electroencephalographic activity. Dose-dependent depression of cerebral glucose utilisation was observed. It was more pronounced in the grey structures than in the white structures, reaching a maximum of

32 and 56%, respectively, of the control values. There was no evidence of selectivity in the grey structures of the brain. All grey structures studied exhibited similar dose-response relationships (Wolfson et al., 1977).

Male Wistar rats were used to study the effect of γ-butyrolactone (300 mg/kg body weight given intravenously) upon the energy metabolism of the brain under normoxic and hypoxic conditions. Under normoxic conditions, all treated animals survived. When exposed to hypoxia, 45% of the controls and the animals receiving γ-butyrolactone intravenously at 300 mg/kg body weight suffered cardiovascular collapse and died in the course of the 30-minute observation period. Biochemical studies conducted in blood, cerebrospinal fluid and brain tissue under normoxic conditions showed that γ-butyrolactone-treated rats, compared with controls, had marked increases of glycogen and glucose brain levels without a simultaneous increase in serum glucose level, and marked decreases of lactate and pyruvate contents. The levels of adenylates were unaltered, whereas phosphocreatine levels were increased. Hypoxic γ-butyrolactone-treated rats, compared with the relevant control group, exhibited higher glycogen and glucose, decreased lactate, normal adenylates and increased phosphocreatine. According to the investigator, γ-butyrolactone given under normoxic conditions produced alterations of the energy metabolism of the brain which were similar to those documented for barbiturate anaesthesia. During hypoxic exposure, the chemical maintained the cerebral energy state with lesser accumulation of tissue lactate (MacMillan, 1978).

Furthermore, the neuroleptic effect of γ-butyrolactone on catatonia induced by chlorpromazine (3 mg/kg body weight given intraperitoneally) was investigated in R3 mice. When γ-butyrolactone was administered at doses of 55 and 110 mg/kg body weight simultaneously with or 15 minutes after chlorpromazine, a statistically nonsignificant increase in the symptoms of catatonia was observed. Smaller doses had no effect. When γ-butyrolactone was given 40 minutes after chlorpromazine, doses of 55 and 110 mg/kg body weight increased catatonia significantly by 106 and 104%, respectively (Sieroslawska, 1965).

The γ-butyrolactone-induced signs of excitation in dogs were blocked slightly by atropine (1 mg/kg body weight given intravenously), markedly by chlorpromazine (2 mg/kg body weight given intravenously) and completely by diazepam (2 mg/kg body weight given intravenously). With a diazepam

dose of 2 mg/kg body weight and γ-butyrolactone at 600 mg/kg body weight it was possible to achieve complete tolerance without excitation. Adrenergic reactions were depressed at this stage, while rectal temperature was unchanged compared with waking animals (Hampel and Hapke, 1968).

Administered to anaesthetised dogs at 100 mg/kg body weight, γ-butyrolactone produced rises in blood pressure, stimulation of respiratory activity and increased intestinal tone. Pulse rate and electrocardiogram were unchanged. Anaesthesia was not deepened. In anaesthetised cats, intravenous administration of γ-butyrolactone doses of 20 or 40 mg/kg body weight had no effect on blood pressure, electrocardiogram or respiratory activity. Administered intravenously, 500 mg/kg body weight led to a decrease in blood pressure which lasted 3 hours. Five minutes after dosing, bradycardia and breathing difficulties set in (Hampel and Hapke, 1968).

A γ-butyrolactone dose of 220 mg/kg body weight decreased the frequency of respiration in mice. The amplitude was very slightly decreased. Rabbits and cats anaesthetised with urethane (1400 mg/kg body weight) or chloralose (90 mg/kg body weight) exhibited accelerated and deepened respiratory movements after γ-butyrolactone only when blood pressure was distinctly lowered. In rats anaesthetised with urethane, the chemical failed to stimulate respiration even during the fall in blood pressure. Large doses inhibited respiration and caused respiratory death (no further details; Sieroslawska, 1965).

In rats (5 per group), intraperitoneal injection of γ-butyrolactone at 10 mg/kg body weight caused a drop in body temperature by 0.7 °C and 0.9 °C after 30 minutes and 2 hours, respectively. At a dose of 100 mg/kg body weight, a drop in body temperature of up to 1.9 °C was observed after 2 hours (Sieroslawska, 1965).

Isolated rat ileum was exposed to γ-butyrolactone concentrations ranging from 1 to 100 µg/ml and studied for intrinsic cholinolytic and contractive effects on smooth-muscle structures. No peripheral parasympathomimetic or parasympatholytic effects were noted (Hampel and Hapke, 1968).

In vitro, γ-butyrolactone concentrations of 94.8 mg/ml and 68.9 mg/ml increased the permeability of Madin-Darby canine kidney cells (MDCK) to fluorescein by 50% and 20%, respectively (Gautheron et al., 1994 b).

Antineoplastic effects

γ-Butyrolactone was studied in the Walker carcinosarcoma 256 of the rat (10 rats/group) for its antineoplastic potential. No significant inhibition of tumour growth was observed after 5 intraperitoneal injections of 452 or 904 mg/kg body weight or 5 oral administrations of up to 1130 mg/kg body weight (BASF, 1961).

γ-Butyrolactone was investigated in rats with respect to its effect on gastric carcinomas induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), which are inhibited by baclofen, an antagonist of γ-aminobutyric acid. A total of 100 male Wistar rats were treated with MNNG at 50 µg/ml in their drinking water for 25 weeks. Subsequently, they were divided up into groups of 25 animals and given ordinary tap water. Group 1 received the vehicle, olive oil, group 2 baclofen (8 mg/kg body weight), group 3 baclofen (8 mg/kg body weight) plus γ-butyrolactone (200 mg/kg body weight) and group 4 γ-butyrolactone (200 mg/kg body weight) by subcutaneous injection every other day. After 52 weeks, the survivors were histopathologically examined. The incidence of gastric cancer was 16 out of 20 in the olive oil controls, 8 out of 20 in baclofen-treated rats, 8 out of 19 in rats treated baclofen plus γ-butyrolactone and 13 out of 20 in rats treated with γ-butyrolactone alone. Thus, γ-butyrolactone had no effect on gastric cancer or the inhibitory effect of baclofen on gastric carcinogenicity (Tatsuta et al., 1992).

8 Experience in humans

Four healthy subjects (2 male, 2 female) each drank 1 g γ-butyrolactone (dissolved in water; 11:00 a.m.). Subsequently, hourly urine samples were collected for 4 hours and analysed. Increases were observed in the excretion of S-3,4-dihydroxybutyrate, glycolic acid and γ-hydroxybutyrate. A further metabolite was probably the tautomeric hydroxyepoxide of 4-hydroxy-3-oxobutyrate. According to the investigator, these results suggested that 4-hydroxybutyrate is metabolised by β-oxidation (Lee, 1977).

There have been various reports from Scandinavia of cases of accidental poisoning with γ-butyrolactone or consumer products containing γ-butyrolactone, such as nail polish remover, including the cases described in the following.

Two young men (23 and 24 years old) drank about 50 ml of a nail polish remover containing 50% γ-butyrolactone. Both of them fell into deep coma and developed bradycardia. They were treated with atropine in an intensive care unit and recovered after a few hours (Blomgren Andersen and Netterstrøm, 1992; ARCO, 1992).

A total of 5 other cases of poisoning in children aged 1½ to 2½ years were reported. Case 1: A boy, 2½ years old, drank a maximum of 2 ml of a glue remover consisting of γ-butyrolactone. About 15 minutes later he became very restless, fell asleep after 30 minutes and became cyanotic on the way to the hospital. On admittance to hospital 45 minutes after the ingestion he was comatose with a pulse of 60. The electrocardiogram showed signs of sinus arrhythmia. Blood gases, electrolytes and urine were normal. Alanine aminotransferase activity and creatinine levels were low. The patient was comatose during the first hours, but he gradually woke up after 3 to 4 hours and recovered not long after. Case 2: A girl, 2 years old, drank about 5 ml γ-butyrolactone (glue remover), spitting out some of it so that the amount swallowed was probably 3 to 4 ml. Shortly afterwards, the child became unconscious and was comatose after 5 to 6 minutes. Oxygen ventilation was administered soon afterwards. On the way to hospital respiration became depressed. Intubation was performed and assisted ventilation given. About 5 hours after the ingestion, the respiration improved, but after 7 hours the child was still soporose with mydriasis, reacting to stimuli. Upon extubation, 10 hours after ingestion, the child slept normally during the night and seemed quite recovered. Case 3: A girl, nearly 2 years of age, removed the cap of a bottle with γ-butyrolactone (glue remover) and drank no more than 2 ml of the content. After about 10 minutes, the child was comatose and was referred to hospital after 45 minutes. Upon arrival, she was still comatose, but reacted slightly to pain and showed normal respiration and a body temperature of 34.4 °C. The electrocardiogram showed nodal rhythm, whereas the electroencephalogram and computer tomography scan were normal. Following treatment with oxygen, 0.1 mg atropine and intravenous fluid, the child woke up after 3 hours and soon showed complete recovery. Case 4: A 1½-year-old girl ingested an unknown quantity of nail polish remover containing about 30% γ-butyrolactone. Within half an hour, the child became comatose and was referred to hospital. Intubation was performed and sodium bicarbonate given by intravenous infusion, a few hours after which the child woke up. A chest x-ray revealed signs of aspiration. Body

temperature was slightly increased. The girl was discharged and allowed home after one day. Case 5: A 2½-year-old boy was found with an empty bottle of a glue solution originally containing 8 ml γ-butyrolactone. He vomited and fell asleep within 15 minutes. On admittance to hospital, he was agitated and screamed, but then relapsed into central nervous depression. The next morning, he was fully awake and was discharged and allowed home. Blood gases and chest x-ray were normal (Nordic Poison Centers, 1992).

Undiluted γ-butyrolactone caused no irritation to the skin of the human upper arm after 24-hour exposure (I.G. Farbenindustrie, 1940).

A study was conducted in 100 male and 100 female volunteers to investigate the skin-sensitising potential of γ-butyrolactone in patch tests. A patch saturated with undiluted γ-butyrolactone remained in contact with the skin under occlusive cover for 5 days. The skin was then scored for erythema and oedema. Three weeks later, the procedure was repeated with a 48-hour contact period. There were no skin reactions after the initial or challenge applications. γ-Butyrolactone showed no dermal irritation or skin-sensitising potential in these tests (ITL, 1950; GAF, 1985).

Amounts of 1 to 2 ml of a 10-percent lotion of γ-butyrolactone in ethanol/water were applied daily to parts of the body (no further details) of 5 subjects for 4 weeks. The lotion was well tolerated. No reddening, itching, allergic skin reactions or hypersensitivity reactions occurred (Szirmai et al., 1989).

When γ-butyrolactone was tested for mucous membrane effects in a self-experiment in which 0.1 ml was applied to the tongue, there was no irritation, only a short burning sensation lasting 5 to 10 seconds, then an intense bitter taste (no further details; Szirmai et al., 1989).

Five healthy subjects received 2.5 g γ-butyrolactone orally. After about 20 minutes, this produced sleep (behaviour and electroencephalogram), lasting one hour. The changes in the electroencephalogram resembled those obtained with pentobarbital, but were of shorter duration (no further details; Jenney et al., 1962).

γ-Butyrolactone was administered to more than 50 patients by the intravenous (15 to 20 mg/kg body weight), intramuscular (20 to 30 mg/kg body weight) or oral (dose unspecified) routes. No details were given about the patients' diseases. Adverse effects seen particularly often after intravenous

injection included nausea and vomiting. The narcotic effect of γ-butyrolactone lasted between half an hour and 3 hours. The pattern of sleep, which was studied in 25 patients, showed differences in the electroencephalogram in comparison with physiological or barbiturate-induced sleep (Benda et al., 1960).

In a double-blind study, γ-butyrolactone doses of 66 mg/kg body weight caused profound sleep in 34% of children, compared with 17% in children receiving chloral hydrate (44 mg/kg body weight). Respiratory depression and prolonged postoperative sleep were rare (Root, 1965).

A 10-percent lotion of γ-butyrolactone in ethanol/water was tested for its effect on sports injuries (distortions, sprains, strains and/or contusions of the extremities, spine or thorax) in 6 subjects (35 to 67 years old) by having them rub 2 to 5 ml of the lotion into the skin covering the painful sites. The pain decreased noticeably within 10 to 20 minutes, disappearing completely within 1 to 2 hours. The effect on rheumatic disorders (self-experiments with 14 subjects) was assessed by rubbing in 2 to 5 ml 2 or 3 times daily. Alleviation of pain set in within 1 to 3 hours in all patients. After 5 days of treatment, pain due to pressure or movement, resting pain and limited mobility were markedly less pronounced than when no treatment was given or known commercial liniments were used for treatment. Migraine headache pain (4 subjects) disappeared within 40 minutes without reappearing after 3 to 5 ml of the lotion was rubbed onto the forehead and temples within 10 minutes. Following application of 5 to 10 ml daily, the subjects felt neither tired nor sleepy nor did they experience any other unpleasant effects (Szirmai et al., 1989).

When working in the laboratory, one individual poured hot γ-butyrolactone on himself while cooling a reaction flask. In contrast to water, which would have scalded the hand, there was no such injury. No blisters formed, and the pain was not as severe as expected and lasted only 1 to 2 hours. The ability to work with the hand was in no way limited. It was concluded from this observation that γ-butyrolactone has an analgesic effect (Szirmai et al., 1989).

9 Classifications and threshold limit values

No information available.

References

Aeschbacher, H.U., Wolleb, U., Löliger, J., Spadone, J.C., Liardon, R.
Contribution of coffee aroma constituents to the mutagenicity of coffee
Food Chem. Toxicol., 27, 227–232 (1989)

Aghajanian, G.K., Roth, R.H.
γ-Hydroxybutyrate-induced increase in brain dopamine: localization by fluorescence microscopy
J. Pharmacol. Exp. Ther., 175 (1), 131–138 (1970)

Andén, N.E., Grabowska-Andén, M., Liljenberg, B.
Demonstration of autoreceptors on dopamine neurons in different brain regions of rats treated with gammabutyrolactone
J. Neural Transmission, 58, 143–152 (1983)

ARCO Chemical Company, Pennsylvania
Written communication to the U.S. Environmental Protection Agency (1992)
NTIS/OTS 0538281

Arena, C., Fung, H.L.
Absorption of sodium γ-hydroxybutyrate and its prodrug γ-butyrolactone: relationship between in vitro transport and in vivo absorption
J. Pharm. Sci., 69, 356–358 (1980)

Argiolas, A., Fadda, F., Melis, M.R., Marcou, M., Porceddu, M.L., Gessa, G.L.
Delayed inhibition of dopamine synthesis by γ-butyrolactone and baclofen: dopamine autoreceptor supersensitivity?
Eur. J. Pharmacol., 85, 23–27 (1982)

Baker, R.S.U., Bonin, A.M.
Study of 42 coded compounds with the Salmonella/mammalian microsome assay
In: de Serres and Ashby, p. 249–260 (1981)

BASF AG, Gewerbehygienisch-Pharmakologisches Institut
Bericht über die toxikologische Prüfung von γ-Butyrolacton
Unpublished report No. X/90 (1960)

BASF AG, Gewerbehygienisch-Pharmakologisches Institut
Bericht über die Prüfung von γ-Butyrolacton auf cytotoxische Wirkung
Unpublished report No. X/446 (1961)

BASF AG, Department of Toxicology
Prenatal toxicity of γ-butyrolactone as a vapor resp. vapor/aerosol mixture in rabbits after inhalation
Unpublished report, Project No. 41R0468/91065 (1993)
On behalf of BG Chemie

BASF AG
Zwischenprodukte – gamma-Butyrolacton (not dated)

Beattie, C.W., Gluckman, M.I., Corbin, A.
A comparison of γ-butyrolactone and pimozide on serum gonadotrophins and ovulation in the rat
Proc. Soc. Exp. Biol. Med., 153, 147–150 (1976)

Benda, P., Perlès, R.
Étude expérimentale de l'abaissement de la vigilance par la γ-butyrolactone
C.R. Acad. Sci., 251, 1312–1313 (1960)

Benda, P., Deshaies, G., Perlès, R., Deshaies-Pellier, S., Rosenberger-Debiesse, J.
Premier essai de la butyrolactone en clinique
Ann. Med. Psychol., 118, 770–776 (1960)

Bessman, S.P., Skolnik, S.J.
Gamma hydroxybutyrate and gamma butyrolactone: concentration in the rat tissues during anesthesia
Science, 143, 1045–1047 (1964)

Bio/dynamics Inc.
Acute irritation studies – C-1070
Report, Project Nos. 6907-86 and 6908-86 (1987)
On behalf of Hoechst-Celanese Corporation, New Jersey
NTIS/OTS 0536954

Blomgren Andersen, M., Netterstrøm, B.
Loss of consciousness after ingestion of nail varnish remover
Ugeskr. Laeger., 154, 3064 (1992)

Borbély, A.A., Huston, J.P.
γ-Butyrolactone: an anesthetic with hyperthermic action in the rat
Experientia, 28, 1455 (1972)

Bridges, B.A., MacGregor, D., Zeiger, E.
Summary report on the performance of bacterial mutation assays
In: de Serres and Ashby, p. 49–67 (1981)

Brooks, T.M., Dean, B.J.
Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay with preincubation
In: de Serres and Ashby, p. 261–270 (1981)

Davies, J.A.
The effect of gamma-butyrolactone on locomotor activity in the rat
Psychopharmacology, 60, 67–72 (1978)

Dean, B.J.
Activity of 27 coded compounds in the RL_1 chromosome assay
In: de Serres and Ashby, p. 570–579 (1981)

Debeljuk, L., Diaz, M.D.C., Maines, V.M., Seilicovich, A.
Prolonged treatment with γ-aminobutyric acid (GABA)-mimetic substances in prepubertal rats
Arch. Androl., 10, 239–243 (1983)

Depaulis, A., Snead III, O.C., Marescaux, C., Vergnes, M.
Suppressive effects of intranigral injection of muscimol in three models of generalized non-convulsive epilepsy induced by chemical agents
Brain Res., 498, 64–72 (1989)

Dickens, F., Jones, H.E.H.
Carcinogenic activity of a series of reactive lactones and related substances
Br. J. Cancer, 15, 85–100 (1961)

Duuren, van, B.L., Nelson, N., Orris, L., Palmes, E.D., Schmitt, F.L.
Carcinogenicity of epoxides, lactones, and peroxy compounds
J. Natl. Cancer Inst., 31, 41–55 (1963)

Duuren, van, B.L., Orris, L., Nelson, N.
Carcinogenicity of epoxides, lactones, and peroxy compounds. Part II
J. Natl. Cancer Inst., 35, 707–717 (1965)

EC (European Commission)
European Chemicals Bureau, Joint Research Centre, Ispra, Italy
IUCLID data set gamma-butyrolactone
CD-ROM, ed. I (1996)

Falbe, J., Regitz, M. (eds.)
Römpp Lexikon Chemie
10th ed., p. 560
Georg Thieme Verlag, Stuttgart, New York (1996)

Fang, M., Yu, Y.N., Chen, X.R.
DNA damaging agents and the ADPRT mediated decrease of cellular NAD content
Chin. J. Pharmacol. Toxicol., 4, 216–220 (1990)

Fassett, D.W.
γ-Butyrolactone
In: Patty, F.A. (ed.)
Industrial hygiene and toxicology
2nd ed., vol. 2, p. 1824–1825
Interscience Publishers, New York (1967)

FDRL (Food and Drug Research Laboratories, Inc.)
Subacute feeding studies with butyrolactone in rats and dogs
Report (1970)
On behalf of GAF Corporation

FDRL (Food and Drug Research Laboratories, Inc.)
Rabbit eye irritation study
Report (1977)
On behalf of GAF Corporation

Fishbein, W.N., Bessman, S.P.
γ-Hydroxybutyrate in mammalian brain. Reversible oxidation by lactic dehydrogenase
J. Biol. Chem., 239, 357–361 (1964)

Fishbein, W.N., Bessman, S.P.
Purification and properties of an enzyme in human blood and rat liver microsomes catalyzing the formation and hydrolysis of γ-lactones. I. Tissue localization, stoichiometry, specificity, distinction form esterase
J. Biol. Chem., 241, 4835–4841 (1966 a)

Fishbein, W.N., Bessman, S.P.
Purification and properties of an enzyme in human blood and rat liver microsomes catalyzing the formation and hydrolysis of γ-lactones. II. Metal ion effects, kinetics, and equilibria
J. Biol. Chem., 241, 4842–4847 (1966 b)

Foureman, P., Mason, J.M., Valencia, R., Zimmering, S.
Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program
Environ. Mol. Mutagen., 23, 208–227 (1994)

Freifeld, M., Hort, E.V.
1,4-Butylene glycol and gamma-butyrolactone
In: Kirk, R.E., Othmer, D.F. (eds.)
Encyclopedia of chemical technology
2nd ed., p. 667–676
John Wiley & Sons, New York (1967)
Cited in: IARC (1976)

Fung, H.L., Lettieri, J.T., Bochner, R.
Percutaneous butyrolactone absorption in rats
J. Pharm. Sci., 68, 1198–1200 (1979)

GAF Corporation, Wayne, NJ, USA
BLO, butyrolactone – solvent – intermediate (1971)

GAF Corporation, Wayne, NJ, USA
Butyrolactone – summary of toxicity screening tests
Unpublished report (1985)

GAF Corporation, Wayne, NJ, USA
Material safety data sheet (1986)

GAF Chemicals Corporation
Toxicology summary
Written communication to BG Chemie (1988)

Garner, R.C., Welch, A., Pickering, C.
Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay
In: de Serres and Ashby, p. 280–284 (1981)

Gatehouse, D.
Mutagenic activity of 42 coded compounds in the “microtiter” fluctuation test
In: de Serres and Ashby, p. 376–386 (1981)

Gautheron, P., Dukic, M., Alix, D., Sina, J.F.
Bovine corneal opacity and permeability test: an in vitro assay of ocular irritancy
Fundam. Appl. Toxicol., 18, 442–449 (1992)

Gautheron, P., Giroux, J., Cottin, M., Audegond, L., Morilla, A., Mayordomo-Blanco, L., Tortajada, A., Haynes, G., Vericat, J.A., Pirovano, R., Gillio Tos, E., Hagemann, C., Vanparys, P., Deknudt, G., Jacobs, G., Prinsen, M., Kalweit, S., Spielmann, H.
Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay
Toxicol. in Vitro, 8 (3), 381–392 (1994 a)

Gautheron, P., Duprat, P., Hollander, C.F.
Investigations of the MDCK permeability assay as an in vitro test of ocular irritancy
In Vitro Toxicol., 7 (1), 33–43 (1994 b)

Gessa, G.L., Vargiu, L., Crabai, F., Boero, G.C., Caboni, F., Camba, R.
Selective increase of brain dopamine induced by gamma-hydroxybutyrate
Life Sci., 5, 1921–1930 (1966)

Gessa, G.L., Vargiu, L., Crabai, F., Adamo, F., Boero, G.C., Camba, R.
Effetti dell'iniezione de γ-idrossibutirrato de Na e di γ-butirrolattone in differenti zone del cervello e nel sistema liquorale – I) Effetti comportamentali ed elettrocorticografici
Boll. Soc. Ital. Biol. Sper., 43, 283–286 (1967)

Gianutsos, G., Moore, K.E.
Tolerance to the effects of baclofen and γ-butyrolactone on locomotor activity and dopaminergic neurons in the mouse
J. Pharmacol. Exp. Ther., 207, 859–869 (1978)

Giarman, N.J., Roth, R.H.
Differential estimation of gamma-butyrolactone and gamma-hydroxybutyric acid in rat blood and brain
Science, 145, 583–584 (1964)

Giarman, N.J., Schmidt, K.F.
Some neurochemical aspects of the depressant action of γ-butyrolactone on the central nervous system
Br. J. Pharmacol., 20, 563–568 (1963)

Green, M.H.L.
A differential killing test using an improved repair-deficient strain of Escherichia coli
In: de Serres and Ashby, p. 183–194 (1981)

Guidotti, A., Ballotti, P.L.
Relationship between pharmacological effects and blood and brain levels of gamma-butyrolactone and gamma-hydroxybutyrate
Biochem. Pharmacol., 19, 883–894 (1970)

Gupta, R.S., Goldstein, S.
Mutagen testing in the human fibroblast diphtheria toxin resistance (HF Dip^r) system
In: de Serres and Ashby, p. 614–625 (1981)

Hampel, H., Hapke, H.J.
Ein Beitrag zur Pharmakologie des gamma-Butyrolacton
Arch. Int. Pharmacodyn. Ther., 171, 306–322 (1968)

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., Zeiger, E.
Salmonella mutagenicity test results for 250 chemicals
Environ. Mutagen., Suppl. 1, 3–142 (1983)

Hemminki, K.
Reactions of β-propiolactone, β-butyrolactone and γ-butyrolactone with nucleic acids
Chem. Biol. Interact., 34, 323–331 (1981)

Holmberg, B., Kronevi, T., Ackevi, S., Ekner, A.
The testing of carcinogenic activity in diphenylamine and gamma-butyrolactone by peroral administration in male mice (English translation of the Swedish)
Arbete och Hälsa, 34, 1–35 (1983)

Hort, E.V., Taylor, P.
Acetylene-derived chemicals
In: Kroschwitz, J.I., Howe-Grant, M. (eds.)
In: Kirk-Othmer encyclopedia of chemical technology
4th ed., vol. 1, p. 195–231
John Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore (1991)

Hoy, C.A., Salazar, E.P., Thompson, L.H.
Rapid detection of DNA-damaging agents using repair-deficient CHO cells
Mutat. Res., 130, 321–332 (1984)

HRC (Huntingdon Research Centre Limited), England
gamma-Butyrolactone, BG No. 7, CAS No. 96-48-0, acute inhalation toxicity study in rats, 4 hour exposure
Unpublished report BGH 19/91325 (1991)
On behalf of BG Chemie

Hubbard, S.A., Green, M.H.L., Bridges, B.A., Wain, A.J., Bridges, J.W.
Fluctuation test with S9 and hepatocyte activation
In: de Serres and Ashby, p. 361–370 (1981)

IARC (International Agency for Research on Cancer)
IARC monographs on the evaluation of carcinogenic risk of chemicals to man, vol. 11, p. 231–239 (1976)

Ichinotsubo, D., Mower, H., Mandel, M.
Mutagen testing of a series of paired compounds with the Ames Salmonella testing system
In: de Serres and Ashby, p. 298–301 (1981 a)

Ichinotsubo, D., Mower, H., Mandel, M.
Testing of a series of paired compounds (carcinogen and noncarcinogenic structural analog) by DNA repair-deficient E. coli strains
In: de Serres and Ashby, p. 195–198 (1981 b)

I.G. Farbenindustrie AG, Gewerbehygienisches I.G. Laboratorium, Wuppertal-Elberfeld
Bericht über eine toxikologische Prüfung von γ-Butyrolacton
Unpublished report (1940)

ITL (Industrial Toxicology Laboratories)
Human repeated insult patch test: gamma-butyrolactone (1950)
Cited in: GAF (1988)

ITL (Industrial Toxicology Laboratories)
Acute oral toxicity in the rat and guinea pig: gamma-butyrolactone (1952)
Cited in: GAF (1988)

Jagannath, D.R., Vultaggio, D.M., Brusick, D.J.
Genetic activity of 42 coded compounds in the mitotic gene conversion assay using Saccharomyces cerevisiae strain D4
In: de Serres and Ashby, p. 456–467 (1981)

Jenney, E.H., Murphree, H.B., Goldstein, L., Pfeiffer, C.C.
Behavioral and EEG effects of γ-butyrolactone and γ-hydroxybutyric acid in man
Pharmacologist, 4, 166 (1962)

Jouany, J.M., Gérard, J., Laborit, H., Carlo, P.
Action de la butyrolactone en association avec des composés ammoniogénétiques
C.R. Soc. Biol., 154, 2039–1042 (1960)

Jouvet, M., Cier, A., Mounier, D., Valatx, J.L.
Effets du 4-butyrolactone et du 4-hydroxybutyrate de sodium sur l'E.E.G. et le comportement du chat
C.R. Soc. Biol., 155, 1313–1316 (1961)

Kada, T.
The DNA-damaging activity of 42 coded compounds in the rec-assay
In: de Serres and Ashby, p. 176–182 (1981)

Kassinova, G.V., Kovaltsova, S.V., Marfin, S.V., Zakharov, I.A.
Activity of 40 coded compounds in differential inhibition and mitotic crossing-over assays in yeast
In: de Serres and Ashby, p. 434–455 (1981)

Katz, M., Heddle, J.A., Salamone, M.F.
Mutagenic activity of polycyclic aromatic hydrocarbons and other environmental pollutants
Chem. Anal. Biol. Fate: Polynucl. Aromat. Hydrocarbons, Int. Symp. 5th, 519–528 (1981)

Knaap, A.G.A.C., Goze, C., Simons, J.W.I.M.
Mutagenic activity of seven coded samples in V79 Chinese hamster cells
In: de Serres and Ashby, p. 608–613 (1981)

Kronevi, T., Holmberg, B., Arvidsson, S.
Teratogenicity test of γ-butyrolactone in the Sprague-Dawley rat
Short Commun. Pharmacol. Toxicol., 62, 57–58 (1988)

Kuroda, M., Yoshida, D., Mizusaki, S.
Bio-antimutagenic effect of lactones on chemical mutagenesis in Escherichia coli
Agric. Biol. Chem., 50, 243–245 (1986)

Kvasov, A.R.
Toxicological characteristics of γ-butyrolactone and 2-pyrrolidone as industrial poisons (English translation of the Russian)
Sb. Nauchn. Tr. Rostov.-na-Donu Gos. Med. Inst., 17, 84–87 (1974)

Lee, C.R.
Evidence for the β-oxidation of orally administered 4-hydroxybutyrate in humans
Biochem. Med., 17, 284–291 (1977)

Lettieri, J., Fung, H.L.
Improved pharmacological activity via pro-drug modification: comparative pharmacokinetics of sodium γ-hydroxybutyrate and γ-butyrolactone
Res. Commun. Chem. Pathol. Pharmacol., 22, 107–118 (1978 a)

Lettieri, J.T., Fung, H.L.
Absorption kinetics of γ-hydroxybutyrate and its pro-drug γ-butyrolactone
Abstract of Papers presented before the APHA Academy of Pharmaceutical Sciences, 8 (1), 134 (1978 b)

Lettieri, J.T., Fung, H.L.
Evaluation and development of gas chromatographic procedures for the determination of γ-hydroxybutyric acid and γ-butyrolactone in plasma
Biochem. Med., 20, 70–80 (1978 c)

Lide, D.R., Frederikse, H.P.R. (eds.)
CRC handbook of chemistry and physics
77th ed., p. 3-170
CRC Press, Boca Raton, New York, London, Tokyo (1996)

Loprieno, N.
Screening of coded carcinogenic/noncarcinogenic chemicals by a forward-mutation system with the yeast Schizosaccharomyces pombe
In: de Serres and Ashby, p. 424–433 (1981)

Loquet, C., Toussaint, G., LeTalaer, J.Y.
Studies on mutagenic constituents of apple brandy and various alcoholic beverages collected in western France, a high incidence area for oesophageal cancer
Mutat. Res., 88, 155–164 (1981)

Loveday, K.S., Lugo, M.H., Resnick, M.A., Anderson, B.E., Zeiger, E.
Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro: II. Results with 20 chemicals
Environ. Mol. Mutagen., 13, 60–94 (1989)

MacDonald, D.J.
Salmonella/microsome tests on 42 coded chemicals
In: de Serres and Ashby, p. 285–297 (1981)

MacMillan, V.
The effects of gamma-hydroxybutyrate and gamma-butyrolactone upon the energy metabolism of the normoxic and hypoxic rat brain
Brain Res., 146, 177–187 (1978)

Marcus, R.J., Winters, W.D., Mori, K., Spooner, C.E.
EEG and behavioral comparison of the effects of gamma-hydroxybutyrate, gamma-butyrolactone and short chain fatty acids in the rat
Int. J. Neuropharmacol., 6, 175–185 (1967)

Martin, C.N., McDermid, A.C.
Testing of 42 coded compounds for their ability to induce unscheduled DNA repair synthesis in HeLa cells
In: de Serres and Ashby, p. 533–537 (1981)

Martire, G., Vricella, G., Perfumo, A.M., De Lorenzo, F.
Evaluation of the mutagenic activity of coded compounds in the Salmonella test
In: de Serres and Ashby, p. 271–279 (1981)

Matsushima, T., Takamoto, Y., Shirai, A., Sawamura, M., Sugimura, T.
Reserve mutation test on 42 coded compounds with the E. coli WP2 system
In: de Serres and Ashby, p. 387–395 (1981)

Mehta, R.D., von Borstel, R.C.
Mutagenic activity of 42 encoded compounds in the haploid yeast reversion assay, strain XV185-14C
In: de Serres and Ashby, p. 414–423 (1981)

Mercker, H.J., Kieczka, H.
Butyrolactone
In: Ullmann's encyclopedia of industrial chemistry
5th ed., vol. A4, p. 495–498
VCH Verlagsgesellschaft mbH, Weinheim (1985)

Monsanto Company, Environmental Health Laboratory
Acute toxicity of gamma-butyrolactone administered by inhalation to Sprague-Dawley male and female rats
Report, Study Number 85061 (1986)
NTIS/OTS 0534527

Nagao, M., Takahashi, Y.
Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay
In: de Serres and Ashby, p. 302–313 (1981)

Nordic Poison Centers Copenhagen, Stockholm, Oslo
Written communication from ARCO Chemical Company to the U.S. Environmental Protection Agency (1992)
NTIS/OTS 0538281

Nowycky, M.C., Roth, R.H.
Chronic gamma-butyrolactone (GBL) treatment: a potential model of dopamine hypoactivity
Naunyn Schmiedebergs Arch. Pharmacol., 309, 247–254 (1979)

NTP (National Toxicology Program)
Toxicology and carcinogenesis studies of gamma-butyrolactone (CAS No. 96-48-0) in F344/N rats and B6C3F1 mice (gavage studies)
NTP Technical Report 406, PB92-189323, U.S. Department of Health and Human Services (1992)

Otto, F.J., Oldiges, H.
Entwicklung einer durchflußcytophotometrischen Methode für Mutagenitätsprüfungen an Keimzellen der Maus
Wissenschaft und Umwelt, 1, 15–30 (1986)

Parry, J.M., Sharp, D.C.
Induction of mitotic aneuploidy in the yeast strain D6 by 42 coded compounds
In: de Serres and Ashby, p. 468–480 (1981)

Quillardet, P., de Bellecombe, C., Hofnung, M.
The SOS chromotest, a colorimetric bacterial assay for genotoxins: validation study with 83 compounds
Mutat. Res., 147, 79–95 (1985)

Richold, M., Jones, E.
Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay
In: de Serres and Ashby, p. 314–322 (1981)

Root, B.
Oral premedication of children with 4-hydroxybutyrate
Anesthesiology, 26 (2), 259–260 (1965)

Rosenkranz, H.S., Hyman, J., Leifer, Z.
DNA polymerase deficient assay
In: de Serres and Ashby, p. 210–218 (1981)

Roth, R.H., Giarman, N.J.
Preliminary report on the metabolism of γ-butyrolactone and γ-hydroxybutyric acid
Biochem. Pharmacol., 14, 177–178 (1965)

Roth, R.H., Giarman, N.J.
γ-Butyrolactone and γ-hydroxybutyric acid – I. Distribution and metabolism
Biochem. Pharmacol., 15, 1333–1348 (1966)

Roth, R.H., Suhr, Y.
Mechanism of the γ-hydroxybutyrate-induced increase in brain dopamine and its relationship to “sleep”
Biochem. Pharmacol., 19, 3001–3012 (1970)

Roth, R.H., Delgado, J.M.R., Giarman, N.J.
γ-Butyrolactone and γ-hydroxybutyric acid – II. The pharmacologically active form
Int. J. Neuropharmacol., 5, 421–428 (1966)

Rowland, I., Severn, B.
Mutagenicity of carcinogens and noncarcinogens in the Salmonella/microsome test
in: de Serres and Ashby, p. 323–332 (1981)

RTECS (Registry of Toxic Effects of Chemical Substances)
2(3H)-Furanone, dihydro-, RTECS Number LU3500000
produced by NIOSH (National Institute for Occupational Safety and Health) (1997)

Rudali, G., Apiou, F., Boyland, E., Castegnaro, M.
Carcinogenic activity of γ-butyrolactone in the mouse
C. R. Acad. Sci. Paris, Série D, 282, 799–802 (1976)

Salamone, M.F.
Toxicity of 41 carcinogens and noncarcinogenic analogs
In: de Serres and Ashby, p. 682–685 (1981)

Salamone, M.F., Heddle, J.A., Katz, M.
Mutagenic activity of 41 compounds in the in vivo micronucleus assay
In: de Serres and Ashby, p. 686–697 (1981)

Schoental, R.
Pathological lesions, including tumors, in rats after 4,4'-diaminodiphenylmethane and γ-butyrolactone
Isr. J. Med. Sci., 4, 1146–1158 (1968)

de Serres, F.J., Ashby, J. (eds.)
Evaluation of short-term tests for carcinogen. Progress in mutation research, vol. I
Elsevier/North Holland, New York, Amsterdam, Oxford (1981)

Sharp, D.C., Parry, J.M.
Induction of mitotic gene conversion by 41 coded compounds using the yeast culture JD1
In: de Serres and Ashby, p. 491–501 (1981 a)

Sharp, D.C., Parry, J.M.
Use of repair-deficient strains of yeast to assay the activity of 40 coded compounds
In: de Serres and Ashby, p. 502–516 (1981 b)

Sieroslawska, J.
Pharmacologic properties of γ-aminobutyric acid and its derivatives
Arch. Immun. Ther. Exp., 13, 70–126 (1965)

Simmon, V.F., Shepherd, G.F.
Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay
In: de Serres and Ashby, p. 333–342 (1981)

Skopek, T.R., Andon, B.M., Kaden, D.A., Thilly, W.G.
Mutagenic activity of 42 coded compounds using 8-azaguanine resistance as a genetic marker in Salmonella typhimurium
In: de Serres and Ashby, p. 371–375 (1981)

Snead III, O.C.
γ-Hydroxybutyric acid-induced seizures bear no relation to core temperature
Epilepsia, 31 (3), 253–258 (1990)

Sprince, H., Josephs, J.A., jr., Wilpizeski, C.R.
Neuropharmacological effects of 1,4-butanediol and related congeners compared with those of gamma-hydroxybutyrate and gamma-butyrolactone
Life Sci., 5, 2041–2052 (1966)

SRI (Southern Research Institute)
gamma-Butyrolactone C55878 – subchronic toxicity report in Fischer 344 rats and B6C3F1 mice
Report (1981)
On behalf of the U.S. NTP

Styles, J.A.
Activity of 42 coded compounds in the BKH-21 cell transformation test
In: de Serres and Ashby, p. 638–646 (1981)

Swern, D., Wieder, R., McDonough, M., Meranze, D.R., Shimkin, M.B.
Investigation of fatty acids and derivatives for carcinogenic activity
Cancer Res., 30, 1037–1046 (1970)

Szirmai, E., Klosa, J., Gonsior, B., Srebro, Z., McGuigan, J.F.
Über eine ungewöhnliche schmerzstillende Wirkung einer vielverwendeten Substanz (γ-Butyrolacton)
Pharmazie, 44 (8), 570–571 (1989)

Tatsuta, M., Iishi, H., Baba, M., Nakaizumi, A., Uehara, H., Taniguchi, H.
Effect of gamma-butyrolactone on baclofen inhibition of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats
Oncology, 49, 123–126 (1992)

Thomson, J.A.
Mutagenic activity of 42 coded compounds in the lambda induction assay
In: de Serres and Ashby, p. 224–235 (1981)

Topham, J.C.
Evaluation of some chemicals by the sperm morphology assay
In: de Serres and Ashby, p. 718–720 (1981)

Trueman, R.W.
Activity of 42 coded compounds in the Salmonella reverse mutation test
In: de Serres and Ashby, p. 343–350 (1981)

Tsuchimoto, T., Matter, B.E.
Activity of coded compounds in the micronucleus test
In: de Serres and Ashby, p. 705–711 (1981)

Tweats, D.J.
Activity of 42 coded compounds in a differential killing test using Escherichia coli strains WP2, WP67 (uvrA polA), and CM871 (uvrA lexA recA)
In: de Serres and Ashby, p. 199–209 (1981)

Ursin, C., Hansen, C.M., Van Dyk, J.W., Jensen, P.O., Christensen, I.J., Ebbehoej, J.
Permeability of commercial solvents through living human skin
Am. Ind. Hyg. Assoc. J., 56, 651–660 (1995)

Venitt, S., Crofton-Sleigh, C.
Mutagenicity of 42 coded compounds in a bacterial assay using Escherichia coli and Salmonella typhimurium
In: de Serres and Ashby, p. 351–360 (1981)

Vogel, E.W., Nivard, M.J.M.
Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination
Mutagenesis, 8 (1), 57–81 (1993)

Vogel, E., Blijleven, W.G.H., Kortselius, M.J.H., Zijlstra, J.A.
Mutagenic activity of 17 coded compounds in the sex-linked recessive lethal test in Drosophila melanogaster
In: de Serres and Ashby, p. 660–665 (1981)

Walters, J.R., Roth, R.H., Aghajanian, G.K.
Dopaminergic neurons: similar biochemical and histochemical effects of γ-hydroxybutyrate and acute lesions of the nigro-neostriatal pathway
J. Pharmacol. Exp. Ther., 186 (3), 630–639 (1973)

Windholz, M., Budavari, S., Blumetti, R.F., Otterbein, E.S. (eds.)
The Merck index
10th ed., p. 220–221
Merck & Co., Inc., Rahway, N.J., USA (1983)

Wolfson, L.I., Sakurada, O., Sokoloff, L.
Effects of γ-butyrolactone on local cerebral glucose utilization in the rat
J. Neurochem., 29, 777–783 (1977)

Yu, Y., Dai, Y., Fang, M., Chen, X.
ADPRT-mediated decrease of cellular NAD content and the detection of chemically induced DNA damage – development of a new short-term screening test for mutagens
Proc. CAMS and PUMS, 5, 19–24 (1990)

Zimmermann, F.K., Scheel, I.
Induction of mitotic gene conversion in strain D7 of Saccharomyces cerevisiae by 42 coded chemicals
In: de Serres and Ashby, p. 481–490 (1981)