(b) (4)

Butyrolactone

Toxicity monograph

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Prepared by:

(b) (4)

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TABLE OF CONTENTS

Butyrolactone

Toxicity monograph

INTRODUCTION

(b) (4) was asked to produce a toxicity monograph of butyrolactone (CAS RN[1] 96-48-0), focussing on the inhalation route of exposure. Data on the inhalation of tobacco smoke containing the ingredient (if available) have not been included in this monograph.

EXPERTISE

(b) (4)

TOXICITY DATA SEARCH CRITERIA

Searches of the (b) (4) (see Appendix for details) identified several recent and relevant expert group reports that formed the basis for this assessment. The most recent is EFSA, 2012. A subsequent search of the primary literature was restricted to (b) (4) and Toxline (the toxicity subset of Medline, via TOXNET) in an attempt to identify more recent data since the 2012 review. The remainder of the TOXNET system (which includes HSDB, GENETOX, DART, CCRIS, IRIS, ITER and CPDB) was also consulted. A large number of potentially-relevant references were identified in the searches, but only those published from 2015 onwards were considered for inclusion in the monograph at this stage. Since the key review could not necessarily be relied upon specifically to identify cardiopulmonary data, and to ensure all critical local and systemic inhalation data were identified, no date restriction was placed on searches in PubMed tailored to identify such information. A very limited amount of additional searching was undertaken in an attempt to fill data gaps.

All searches were conducted in August 2016 using the CAS RNs and (in PubMed only) name identified below, as appropriate.

The data summarised in this report refers to the unheated form unless otherwise stated.

[1] Chemical Abstracts Service Registry Number.

(b) (4)

IDENTIFICATION, REACH STATUS AND EU CLASSIFICATION

Identifier / status	
Name	Butyrolactone
Synonym(s)	4-Hydroxybutanoic acid lactone gamma-Butyrolactone Butyro-1,4-lactone Oxolan-2-one GBL
CAS RN	96-48-0
Additional CAS RN	187997-16-6
REACH registration number[3]	01-2119471839-21-xxxx
Classification, according to EU CLP (EC 1272/2008)	**Harmonised classification** None available

TOXICOLOGY

LOCAL EFFECTS

Respiratory tract irritation

No substance-specific data were identified.

Skin irritation

Human

No skin irritation was observed when undiluted gamma-butyrolactone was applied to the skin of 200 volunteers [no further details in citing source] (Larsen and Sderlund, 1993). [See also Skin sensitisation section.]

Non-human

Undiluted gamma-butyrolactone was applied, semi-occluded, to the dorsal skin of white rabbits for 1, 5 or 15 minutes or 20 hours. No skin irritation was observed (BG Chemie, 2000).

When six albino rabbits were patch-tested with 1 mL of [presumably neat] gamma-butyrolactone, very mild to moderate erythema and very slight oedema occurred after 72 hours (BG Chemie, 2000).

gamma-Butyrolactone (0.5 mL; 100%) was applied to the skin of four rabbits. After 72 hours, three of the animals exhibited moderate to severe erythema with oedema and one animal exhibited erythema without oedema. The investigators considered gamma-butyrolactone to be moderately to severely irritating to the skin on the basis of this study (BG Chemie, 2000).

[3] REACH registration numbers are substance and company specific. Therefore, the substance-specific part of the registration number is included here, from data disseminated on the ECHA 'registered substance' website. The REACH dossier has not been consulted for toxicity data.

Eye irritation

Human

No substance-specific data were identified.

Non-human

A single, 0.1 mL dose of gamma-butyrolactone was instilled into one eye of each of an unspecified number of New Zealand White rabbits. Eyes were rinsed with water after 24 hours, and examined at 1, 24, 48 and 72 hours after application then once per week for 4 weeks. Observations included slight to severe discharge, slight to moderate corneal opacity, moderate to severe swelling and moderate redness of the conjunctiva, and moderate to severe inflammation of the iris. Furthermore, contracted pupil, discharge of blood and increased formation of blood vessels were observed. The average scores were 1.3 (corneal opacity), 1.0 (iris), 2.0 (conjunctival redness) and 2.3 (swelling). The effects were not reversible after 28 days, therefore the investigators considered gamma-butyrolactone to be a severe eye irritant (BASF, 2005).

Other local effects

No substance-specific data were identified.

SENSITISATION AND INTOLERANCE

Respiratory tract sensitisation

No substance-specific data were identified.

Skin sensitisation

Human

No skin sensitisation reactions were observed when undiluted gamma-butyrolactone was applied to the skin of 200 volunteers [no further details in citing source] (Larsen and Sderlund, 1993). [See also Skin irritation section.]

Non-human

In a LLNA[4] conducted according to OECD guideline 429[5] and to GLP[6], groups of five female CBA mice received dermal applications (to the ear) of 0, 30, 60 or 100% *gamma*-butyrolactone on 3 consecutive days. No statistically significant changes in SI[7] were calculated for any dose group, therefore the investigators considered *gamma*-butyrolactone to be non-sensitising (Anon., 2010).

Oral allergy/intolerance

No substance-specific data were identified.

[4] Local lymph node assay.
[5] Test Guideline No. 429: Skin Sensitisation.
[6] Good laboratory practice.
[7] OECD test guideline 429 defines stimulation index (SI) as the ratio of lymphocyte proliferation in a treated group to that in the vehicle control group. An SI of ≥3 is considered a sensitising material.

INHALATION TOXICITY STUDIES

Single dose toxicity

Human

No substance-specific data were identified.

Non-human

No mortality was observed when rats inhaled gamma-butyrolactone vapour for 8 hours at concentrations of up to 1056 mg/m³ (saturation). The inhalation LC_{50} value[8] was therefore >1056 mg/m³ [no further details specified in citing source] (BPPD Consortium, 2003).

Repeated dose toxicity

Human

No substance-specific data were identified.

Non-human

Pregnant rabbits were exposed to *gamma*-butyrolactone vapour at up to 5000 mg/m³ for 6 hours/day for 8 days. No adverse effects were reported. The study NOAEC[9] is therefore 5000 mg/m³ (the highest concentration tested) [no further details specified in citing source] (BPPD Consortium, 2003). [See Reproductive and developmental toxicity section.]

TOXICITY STUDIES – OTHER EXPOSURE ROUTES

Single dose toxicity

Human

The accidental ingestion of a single 20-30 mg/kg bw dose of gamma-butyrolactone resulted in shallow respiration, bradycardia [slow heart-rate], and nausea, as well as central nervous system effects including seizure-like activity [uncontrolled movements], changes in the pupil reflex, euphoria, loss of inhibitions, and increases in libido, dizziness, sleepiness, REM[10] sleep and entactogenic [empathy/sympathy] and sensory perception (Persson *et al.*, 2001; Soderlund, 2004).

A single, oral 60 mg/kg bw dose of gamma-butyrolactone has been reported to produce anaesthetic effects in humans [no further details specified in citing source] (Soderlund, 2004).

When 50-70 mg/kg bw gamma-butyrolactone was ingested on a single occasion, coma was induced (but the exposure was seldom fatal) (Persson *et al.*, 2001; Soderlund, 2004).

A 45-year-old man drank 50 mL [approximately 800 mg/kg bw][11] of an "unknown clear liquid" that was suspected to be gamma-butyrolactone, and a 25-year-old man drank an unspecified quantity of gamma-butyrolactone liquid. Signs and symptoms of toxicity included a comatose state, respiratory depression, hypotension, and metabolic and respiratory acidosis. The

[8] Lethal Concentration 50, i.e. the concentration that is lethal to 50% of the exposed group.
[9] No-observed-adverse-effect concentration.
[10] Rapid eye movement.
[11] Conversion based on a density of 1.13 g/mL and a body-weight of 70 kg.

individuals regained consciousness and recovered completely several hours after arrival at hospital (van Vugt and Hofhuizen, 2012).

"When abused, GBL is administered orally in a liquid form… Doses are measured in the single milliliter range[12], either taken all at once or sipped over the course of a night." "There is a steep dose-effect curve between doses producing desired and excessive effects, and there have been numerous published reports of adverse reactions to GBL including fatalities. Signs and symptoms can include: euphoria, relaxation, reduced inhibition and sedation progressing to vomiting, urinary and fecal incontinence, agitation, convulsions, bradycardia, respiratory depression, coma and death" (WHO, 2014).

Non-human

In two separate studies, mice were administered single oral doses of butyro-1,4-lactone and observed for mortality. The LD_{50} values[13] were subsequently calculated as 1245 mg/kg bw (Schafer and Bowles, 1985) and 1260 mg/kg bw [no further details specified in citing sources] (Hampel and Hapke, 1968).

Oral LD_{50} values in the rat have been reported as 1580-1920 mg/kg bw (BPPD Consortium, 2003) and 1800 mg/kg bw (Kvasov AR, 1974) [no further details specified in citing sources].

After fasting for 12-15 hours, groups of 4-5 or 10 male rats were administered *gamma*-butyrolactone at 165, 497 or 1240 mg/kg bw by oral gavage. Respiration was monitored using a whole-body plethysmograph, both before the administration of *gamma*-butyrolactone (as a baseline) and every 15 min afterwards, for up to 12 hours (after an initial 15-min acclimation period). Death due to respiratory arrest occurred in 7 of the 10 rats given the highest dose of *gamma*-butyrolactone. The respiratory rate was also decreased significantly at 497 mg/kg bw (Morse and Morris, 2013). [See also Cardiopulmonary effects section.]

The LD_{50} values for the oral and dermal routes in guinea pigs were reported as 500-700 and 5640 mg/kg bw, respectively [no further details specified in citing source] (BPPD Consortium, 2003).

Repeated dose toxicity

Human

No substance-specific data were identified.

Non-human

B6C3F1 mice (5/sex/group) received *gamma*-butyrolactone by gavage at doses of 0, 87, 175, 350, 700 or 1400 mg/kg bw/day on 5 days/week for 16 days. Observations were made twice daily, body-weights were measured and complete necropsies were performed on all animals. Nine of the ten animals receiving 1400 mg/kg bw died before the end of the study, and further deaths were reported amongst the other dose groups due to "improper gavage technique". Mice receiving 350 mg/kg bw or more became recumbent or inactive shortly

[12] As a "party-drug", about 1.5 mL of the liquid substance is drunk to bring on the euphoria.

[13] Lethal Dose 50, i.e. the dose that is lethal to 50% of the exposed group.

after dosing, and some mice also exhibited irregular respiration or dyspnoea [shortness of breath] (NTP, 1992). JECFA considered the study NOAEL[14] to be 175 mg/kg bw/day (JECFA, 1998). [See also Cardiopulmonary effects section.]

B6C3F1 mice (10/sex/group) were gavaged with *gamma*-butyrolactone at 0, 65, 131, 262, 525 or 1050 mg/kg bw/day, 5/days/week for 13 weeks. Surviving animals were killed at the end of the study, and necropsies were performed on all animals. The brain, heart, kidney, liver, lungs and thymus of survivors were weighed at necropsy. Complete histopathology[15] was performed on all animals killed or dying during the study, all controls, and mice receiving 1050 mg/kg bw/day. The body-weight gain and final body weights were lower for the high-dose male mice than for controls, therefore the NOAEL was considered to be 525 mg/kg bw/day (NTP, 1992). [See also Cardiopulmonary effects section.]

In a good-quality chronic toxicity study, B6C3F1 mice (50/sex/group) were administered *gamma*-butyrolactone by oral gavage at 0, 262, 525 or 1050 mg/kg bw/day, 5/days week for 2 years. Observations were made twice daily, body-weights were measured and necropsy was performed on all animals. Complete histopathological examinations[16] were performed on all controls, low-dose mice and high-dose males as well as any gross lesions in all dose groups. No histopathological abnormalities occurred at any dose level, but the mean body-weights of the treated males and females were 6% and 14-17% lower than those of controls, respectively. High-dose males also displayed signs of lethargy and inactivity, which led to increases in fighting-related trauma and reduced body-weights and survival (NTP, 1992). EFSA considered the study NOAEL to be 262 mg/kg bw/day; however JECFA reported a NOAEL of 525 mg/kg bw/day (EFSA, 2012; JECFA, 1998). [See also Carcinogenicity and Cardiopulmonary effects sections.]

F344/N rats (5/sex/group) received *gamma*-butyrolactone by gavage at doses of 0, 75, 150, 300, 600 or 1200 mg/kg bw/day on 5 days/week for 16 days. Observations were made twice daily, body-weights were measured and complete necropsies were performed on all animals. Rats in the 600 or 1200 mg/kg bw dose groups became recumbent or inactive with irregular and laboured breathing shortly after dosing (NTP, 1992). JECFA therefore considered the study NOAEL to be 300 mg/kg bw/day (JECFA, 1998). [See also Cardiopulmonary effects section.]

F344/N rats (10/sex/group) were gavaged with *gamma*-butyrolactone at 0, 56, 112, 225, 450 or 900 mg/kg bw/day, 5/days/week for 13 weeks. Surviving animals were killed at the end of the study, and necropsied were performed on all animals. The brain, heart, right kidney, liver,

[14] No-observed-adverse-effect level.

[15] Tissues examined included: adrenal gland, bone and marrow (femur), brain, gallbladder, heart, kidney, large intestine, liver, lung with main-stem bronchi, lymph nodes, mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skeletal muscle (thigh), skin, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes).

[16] Tissues examined included: adrenal gland, bone and marrow (femur), brain, gallbladder, epididymis, oesophagus, harderian gland (low-dose males), heart, kidney, large intestine, liver, lung with main-stem bronchi, lymph nodes, mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes).

lungs and thymus of survivors were weighed at necropsy. Complete histopathology[17] was performed on all animals killed or dying during the study, all controls, all rats receiving 900 mg/kg bw/day and males receiving 450 mg/kg bw/day. The liver and nose (nasal cavity and turbinates) were examined from rats in the 56, 112 and 225 mg/kg bw dose groups, and from females at 450 mg/kg bw/day. All males and one female administered 900 mg/kg bw/day died by week 8. At 450 mg/kg bw/day, the body-weight gain and final body-weights of the males were lower than those of the controls. Signs of sedation were observed in rats given over 225 mg/kg bw/day for the first few weeks only. An increased incidence of focal inflammation of the nasal mucosa was also observed, but this was considered to be a non-specific effect caused by the gavage procedure. The NOAELs were therefore 225 and 450 mg/kg bw/day for males and females, respectively (NTP, 1992). [See also Cardiopulmonary effects section.]

In a good-quality chronic toxicity study, male and female F344/N rats (50/sex/group) were administered *gamma*-butyrolactone by oral gavage at 0, 112 or 225 mg/kg bw and 0, 225 or 450 mg/kg bw/day, respectively, 5/days week for 2 years. Observations were made twice daily, body-weights were measured and necropsy was performed on all animals. Complete histopathological examinations[18] were performed on all rats dying or killed prior to day 637, all controls and all high-dose rats. No histopathological abnormalities occurred at any dose level, but the mean body-weights of the treated females were 10-20% lower than those of the controls in the second year. A reduced survival of males from 225 mg/kg bw/day was observed, therefore the study NOAEL is 112 mg/kg bw/day for males. For females the NOAEL is considered 450 mg/kg bw/day according to JECFA (1998) (NTP, 1992). [See also Carcinogenicity and Cardiopulmonary effects section.]

Neurotoxicity has been reported [in various animal studies] following repeated treatment with high doses of gamma-butyrolactone [no further details specified] (NSF, 2003; van Amsterdam *et al.*, 2014).

GENOTOXICITY

Expert-group opinion

An EFSA expert panel[19] noted that although genotoxicity data were limited for a group of lactones (for which *gamma*-butyrolactone was identified as a supporting substance), there were no indications of concerns for genotoxicity (EFSA, 2012).

[17] Tissues examined included: adrenal gland, bone and marrow (femur), brain, clitoral glands, epididymis, heart, kidney, large intestine, liver, lung with main-stem bronchi, lymph nodes, mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skeletal muscle (thigh), skin, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes). Tissues examined from rats in the 56, 112 and 225 mg/kg bw/day groups and the 450 mg/kg bw/day female dose group included liver (males only), nasal cavity and turbinates, and gross lesions.

[18] Tissues examined included: adrenal gland, bone and marrow (femur), brain, clitoral or preputial glands, epididymis, oesophagus, heart, kidney, large intestine, liver, lung with main-stem bronchi, lymph nodes, mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes). Tissues examined in low-dose rats dying or killed moribund after day 636, or killed at study end: liver, mammary gland, spleen, testes and gross lesions.

[19] EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel).

According to Adams *et al.* (1998), the FEMA expert panel concluded that, "on the basis of weight of evidence, that γ-butyrolactone… is not mutagenic and that isolated positive results… [obtained] in non-standard assays at high solution concentrations are not compelling evidence of genotoxic potential".

Mammals (*in vivo*)

In a micronucleus assay, B6C3F1 mice were intraperitoneally injected with a single dose of butyro-1,4-lactone (calculated to be 80% of the LD_{50} value). No increase in micronuclei induction was observed [no further details specified in the citing source] (Salamone *et al.*, 1981).

CD-1 mice were given a single intraperitoneal dose of butyro-1,4-lactone at 0.11-0.44 mg/kg bw in another bone-marrow micronucleus assay. No evidence of chromosomal damage was observed (Tsuchimoto and Matter, 1981).

No increase in micronuclei induction was observed after B6C3F1/BR hybrid mice were administered an intraperitoneal dose of 4-hydroxybutyric acid lactone at 0.7 mg/kg bw/day on two consecutive days (Katz *et al.*, 1981).

In a sperm-head abnormality assay, CBA X Balb/c F1 mice were administered an intraperitoneal dose of butyro-1,4-lactone at 0.1-1 mg/kg bw/day, daily for 5 days. No abnormalities were observed (Topham, 1980).

Mammalian cells (*in vitro*)

No unscheduled DNA synthesis was observed when HeLa S3 cells[20] were treated with butyro-1,4-lactone at 0.0001-0.1 mg/mL, in the absence and presence of S9[21] (Martin and McDermid, 1981).

No evidence of DNA damage was shown when butyro-1,4-lactone failed to stimulate ADP-ribosyl transferase activity in human amnion FL cells when tested, with S9, at concentrations of 0.0086-86 µg/mL (Yingnian *et al.*, 1990).

In a limited polyploidy assay, no changes in chromosome number were observed after human leukocytes were exposed to butyro-1,4-lactone at 0.06 mg/mL (Withers, 1966).

No clastogenic activity was reported in rat liver RL1 cells treated with butyro-1,4-lactone at 0.25 mg/mL in a limited assay (Dean, 1981). [No further details specified in citing source.] Chinese hamster ovary cells were treated with *gamma*-butyrolactone using three dose ranges and assessed for sister chromatid exchanges (SCEs) and chromosome aberrations. In the SCE assay, concentrations of 0.148-1.48 mg/mL were tested without S9, and 0.494-4.94 mg/mL and 3.01-5.01 mg/mL with S9, in trial 1 and trial 2, respectively. In the chromosome aberration assay, concentrations of 0.5-4.99 mg/mL (without S9), 0.4-3.99 (with S9) and 2.21-2.95 (with S9) were tested. Both assays showed signs of genotoxicity when the cells were treated in the presence of S9 (NTP, 1992).

[20] A human cervical cancer cell line.

[21] Induced mammalian liver post-mitochondrial fraction used for metabolic activation.

Micro-organisms

A number of bacterial mutation (Ames) studies[22] are cited by the EFSA CEF Panel[23], which demonstrate that butyro-1,4-lactone lacks mutagenic potential in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537, TA1538 in the absence or presence of S9 (cited in EFSA, 2012). Furthermore, no evidence of mutagenicity was observed in studies using *Escherichia coli* strains WP2 and SA500. Several of these studies have limitations including low test concentrations, few bacterial strains used (when at least five strains are recommended in current OECD guidelines) and sometimes only one test concentration used (cited in EFSA, 2012).

Positive indications of DNA damage were observed in a limited Rec assay[24] when *Bacillus subtilis*, strains H17 and M45 were treated with a high dose (about 20 mg) of butyro-1,4-lactone (Kada, 1981).

No evidence of DNA damage was reported in two limited differential killing tests in which *E. coli* strains WP2 pol A, WP2 uvrA, WP67 uvrA, WP67 pol A, CM871 uvrA recA and LexA were treated with 2.5 mg/plate, with and without S9 (Green, 1981) or at 1 mg/mL without S9 (Tweats, 1981).

When *E. coli* strains W3110 and P3478 were treated with about 10 mg of butyro-1,4-lactone, with and without metabolic activation in a DNA polymerase I inhibition test, evidence of DNA damage was observed, but only in the presence of S9 (Rosenkranz *et al.*, 1981).

Other

In a sex-linked recessive lethal mutation assay conducted according to OECD test guideline 477[25], *Drosophila melanogaster* were treated with butyro-1,4-lactone at 20,000 and 28,000 ppm in the diet. No evidence of mutagenicity was observed (Foureman *et al.*, 1994).

CARCINOGENICITY

Expert-group opinion

IARC classified *gamma*-butyrolactone as "not classifiable as to its carcinogenicity to humans" (Group 3), based on "inadequate evidence" for carcinogenicity in humans and "evidence suggesting lack of carcinogenicity" in experimental animals (IARC, 1999).

Human

No substance-specific data were identified.

[22] Several of these studies have limitations including low test concentrations, few bacterial strains used (when at least five strains are recommended in current OECD guidelines) and sometimes only one test concentration used.
[23] EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel).
[24] Indicative test.
[25] Test Guideline No. 477. Genetic Toxicology: Sex-Linked Recessive Lethal Test in Drosophila melanogaster.

Non-human

In a good-quality chronic toxicity and carcinogenicity study, B6C3F1 mice (50/sex/group) were administered *gamma*-butyrolactone by oral gavage at 0, 262, 525 or 1050 mg/kg bw/day, 5/days week for 2 years. Necropsy was performed on all animals and complete histopathological examinations[26] were conducted on all controls, high-dose mice and low-dose males as well as on any gross lesions in all dose groups. No evidence of carcinogenicity was observed in females, however the investigators concluded that equivocal evidence was seen in male mice, based on increased incidences of adrenal medulla pheochromocytomas and hyperplasia [proliferative lesions] at 262 mg/kg bw/day (but not 525 mg/kg bw/day). It was noted that "the sensitivity of the study in male mice to detect a carcinogenic effect was reduced by the low survival of the high-dose group associated with fighting" (NTP, 1992). Nevertheless, in its review of the carcinogenicity of γ-butyrolactone, IARC concluded for "animal carcinogenicity data" that "no carcinogenic effect was observed" (IARC, 1999). [See also Repeated dose toxicity section.]

In another good-quality chronic toxicity study, male and female F344/N rats (50/sex/group) were administered *gamma*-butyrolactone by oral gavage at 0, 112 or 225 mg/kg bw and 0, 225 or 450 mg/kg bw/day, respectively, 5/days week for 2 years. Necropsy was performed on all animals and complete histopathological examinations[27] were performed on all rats dying or killed prior to day 637, all controls and all high-dose rats. No evidence of carcinogenicity was observed (NTP, 1992). [See also Repeated dose toxicity section.]

No evidence of carcinogenicity was observed when six rats were administered 2 mg *gamma*-butyrolactone by subcutaneous injection twice/week [averaged daily dose approximately 130 mg/kg bw/day][28]for 104 weeks [no further details given in citing source] (Dickens and Jones, 1965).

Cell transformation (a potential sign of carcinogenicity) was observed in hamster kidney BHK-21 cells treated with butyro-1,4-lactone at 0.25 mg/mL, with and without S9, in an *in vitro* mammalian cell transformation assay (Styles, 1981).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Human

No substance-specific data were identified.

[26] Tissues examined included: adrenal gland, bone and marrow (femur), brain, gallbladder, epididymis, oesophagus, harderian gland (low-dose males), heart, kidney, large intestine, liver, lung with main-stem bronchi, lymph nodes, mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes).

[27] Tissues examined included: adrenal gland, bone and marrow (femur), brain, clitoral or preputial glands, epididymis, oesophagus, heart, kidney, large intestine, liver, lung with main-stem bronchi, lymph nodes, mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes). Tissues examined in low-dose rats dying or killed moribund after day 636, or killed at study end: liver, mammary gland, spleen, testes and gross lesions.

[28] Conversion based on a body weight of 30 g.

Non-human

Rabbits were exposed to *gamma*-butyrolactone vapour at up to 5000 mg/m³ for 6 hours/day on gestation days 9-17. Maternal toxicity, reproductive indices and foetal toxicity were examined; however no adverse effects were reported. The study NOAEC is therefore 5000 mg/m³ (the highest concentration tested) [no further details specified in citing source] (BPPD Consortium, 2003). [See Inhalation toxicity studies section.]

Female Sprague-Dawley rats (10/group) were administered butyro-1,4-lactone at 10, 50, 125, 250 or 500 mg/kg bw/day by oral gavage on gestation days 6-15. No evidence of foetal malformations or embryotoxicity was observed, however, placental weights were significantly lower in all treated animals, and foetal weights were significantly increased in all but the top and bottom dose levels (Kronevi *et al.*, 1988). The study NOAEL was still considered 500 mg/kg bw/day (the highest tested dose) by an EFSA CEF expert panel (EFSA, 2012).

Testicular weights were significantly reduced when male rats were administered oral doses of *gamma*-butyrolactone at 500-1000 mg/kg bw [no further details specified in citing source] (Larsen and Sderlund, 1993).

CARDIOPULMONARY EFFECTS[29]

The ingestion of a single 20-30 mg/kg bw dose of gamma-butyrolactone was reported to result in shallow respiration, bradycardia [slow heart-rate] and nausea, as well as central nervous system effects (Persson *et al.*, 2001; Soderlund, 2004). Similarly, when a 45-year-old man drank approximately 800 mg/kg bw of an "unknown clear liquid" that was suspected to be gamma-butyrolactone, and a 25-year-old man drank an unspecified quantity of gamma-butyrolactone liquid, respiratory depression, hypotension, and metabolic and respiratory acidosis were reported (van Vugt and Hofhuizen, 2012). [See also Single dose toxicity section.]

After fasting for 12–15 hours, groups of 4–5 or 10 male rats were administered *gamma*-butyrolactone at 165, 497 or 1240 mg/kg bw by oral gavage. Respiration was monitored using a whole-body plethysmograph, both before the administration of *gamma*-butyrolactone (as a baseline) and every 15 min afterwards, for up to 12 hours (after an initial 15-min acclimatisation period). Death due to respiratory arrest occurred in 7 of the 10 rats given the highest dose of *gamma*-butyrolactone. The respiratory rate was also decreased significantly at 497 mg/kg bw (Morse and Morris, 2013). [See also Single dose toxicity section.]

No histopathological abnormalities of the heart or lungs were reported in good-quality subchronic and chronic toxicity studies with rats and mice when treated with doses of up to 1050 mg/kg bw/day by oral gavage. However, in 16-day studies, some rats and mice that received *gamma*-butyrolactone at doses of 350 or 600 mg/kg bw /day and above,

[29] Potential effects on the heart, blood vessels and/or respiratory tract.

respectively, exhibited irregular respiration or dyspnoea (NTP, 1992). [See Repeated dose toxicity section for further details.]

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APPENDIX: The (b) (4) database and databank

(b) (4)

(b) (4) includes information from peer-reviewed toxicology and nutrition journals as well as secondary sources and websites. In addition to primary literature on the health effects of chemicals, (b) (4) covers official publications and evaluations issued by authoritative groups including:

- WHO/IPCS reports and evaluations (including CICADs and EHCs, and IARC, JECFA and JMPR monographs), and the WHO Air Quality and Drinking-Water Quality Guidelines
- OECD SIDS dossiers/SIARS
- IUCLID data sets
- EU Risk Assessment Reports
- EU expert committee opinions (including EU scientific committees, and EFSA scientific panels) and other reports from EU agencies and institutes etc (including ECHA, ECVAM, EMA and CPS&Q)
- ECETOC, HERA, Council of Europe and other pan-European programmes
- UK government agency (including Defra, EA, FSA, DoH, HSE, HPA, PSD and VMD) and advisory committee (e.g. COT, COM, COC, ACNFP, SACN, ACP, ACAF, VPC, VRC and ACRE) reports and evaluations
- Opinions from other UK organisations such as the Royal Society
- US agency reports and evaluations (EPA, ATSDR, FDA, NTP, OSHA, NCEA, CFSAN, CERHR, NIEHS, CDC, OEHHA and ACGIH)
- Health Canada evaluations
- BUA, DFG, BG Chemie and BfR reports and monographs
- Gezondheidsraad opinions, including those from its various committees such as DECOS
- RIVM reports
- Danish EPA reviews
- Reports and other information provided by Swedish governmental organisations, including the National Food Administration and the Swedish Chemicals Agency
- Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals
- Australian agency reviews including NICNAS Priority Existing Chemical Assessments, APMVA reports and (jointly with New Zealand) FSANZ assessments
- Japanese Chemical Industry Ecology-Toxicology & Information Center reports
- CIR, RIFM and other specialist industry groups
- (b) (4)