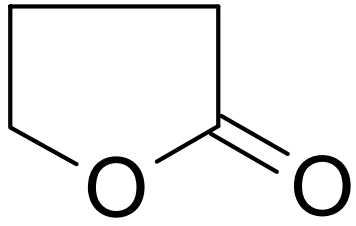
201-14672A

γ-Butyrolactone



CAS Number 96-48-0

U.S. EPA HPV Challenge Program
Revised Submission

15 August 2003

Submitted by:

BPPB Consortium

Prepared by:
Toxicology and Regulatory Affairs
1201 Anise Court
Freeburg IL 62243
618-539-5280

RECEIVED
OPPT CBIC

2003 AUG 15 PM 3:50

Table of Contents

Executive Overview

γ-Butyrolactone is a water-soluble oily liquid, with a boiling point of 206° C. It has a vapor pressure of 0.344 hPa at 20˚ C and a log $K_{o/w}$ of -0.64. It is used as a chemical intermediate, as a solvent for polymers, in some paint removers, and in some printing inks.

γ-Butyrolactone was found to be readily biodegradable and has an estimated indirect photolysis half-life in the atmosphere of 5.5 days. It is labile to hydrolysis by water, especially at high pH levels, giving γ-Hydroxybutyrate as a product. In water at low pH, it can exist as an equilibrium mixture of γ-Butyrolactone and γ-Hydroxybutyrate. γ-Butyrolactone has a low order of toxicity for fish, daphnids and green algae, and its hydrolysis product is predicted to have even lower toxicity.

γ-Butyrolactone is readily absorbed by the oral or inhalation routes and can penetrate the skin. It is rapidly converted to γ-Hydroxybutyrate in the body and excreted quickly, primarily as carbon dioxide, which shows that it undergoes extensive metabolism. Due to its rapid metabolic conversion to γ-Hydroxybutyrate, which has an effect on the central nervous system, γ-Butyrolactone has a weak narcotic effect. It displays low acute-oral toxicity with an oral LD_{50} for rats in the range of 1500 to 2000 mg/kg-bw. Adverse effects are limited to clinical signs of weakness, unconsciousness, and increased depth of respiration. Other than narcotic action on the CNS, no target organs have been identified. In rats, inhalation of a saturated atmosphere (8 hours at 20°C) did not cause any adverse effects and indicates a low acute-toxic effect of γ-Butyrolactone by inhalation. This observation has been confirmed and extended by mixed vapor/aerosol studies.

Repeated exposure causes no specific effects other than sedation, which animals develop a tolerance to within a few weeks. In a 13-week oral gavage study in rats, the NOAEL was 225 mg/kg for males (based on body weight gain) and 450 mg/kg for females (based on one death in the 900-mg/kg group). No specific target organs were identified. In the companion study in mice, except for minor sedation during the first few weeks of study, the NOAEL was 525 mg/kg for males (based on body weights and mortality), and 525 mg/kg for females (based on one death in the 1050-mg/kg group). No specific target organs were identified.

Two-year carcinogenicity studies were essentially negative and the NAOEL for male or female rats was 225 mg/kg-day. In mice, the NOAEL for males was 262 mg/kg (survival) and the NOAEL for females was < 262 mg/kg (body weight gain). No specific target organs were identified.

Extensive genotoxicity testing has yielded primarily negative results but high concentrations, in the presence of an exogenous metabolizing system caused a positive response in the *in vitro* sister chromatid exchange and chromosome aberration assays. *In vivo* studies were negative.

Fetal weight was significant increased in pregnant female rats treated by gavage on days 6 to 15 of pregnancy. No differences from unexposed animals in the corpora lutea, total implantations, ratio of dead to live fetuses, resorptions, and pre- and post-implantation losses were noted at doses up to 1000 mg/kg. In addition, there were no visceral or skeletal malformations due to γ-Butyrolactone exposure. One-time intraperitoneal dosing during proestrus interfered with FSH and LH production and inhibited ovulation in Sprague-Dawley rats. The relevance of this finding to fertility is unknown. Examination (gross and histopathological) of reproductive organs in rats and mice after 90-days of administration to rats and mice of each sex indicates that effects of γ-Butyrolactone on fertility are unlikely. This was also confirmed by lack of reproductive organ effects in the chronic studies or rats and mice.

The critical effect for γ-Butyrolactone appears to be a weak narcotic effect on the CNS, which is an effect also know to occur in humans due to the rapid metabolism of γ-Butyrolactone to the neurologically active γ-Hydroxybutyrate.

It is concluded that the available information adequately fills all the data elements of the U.S. EPA HPV program. Although the available studies do not meet all requirements of the current OECD guidelines in all cases, conduct of additional similar studies would not add significantly to our understanding of this material's hazard and are not recommended.

Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 96-48-0 γ-Butyrolactone	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	Y	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	N	N	Y	N	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	N	N	Y	N	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	N	?	Y	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	N	N	Y	N	Y	N	
48-Hour Invertebrate	Y	Y	N	Y	N	Y	N	
96-Hour Algae	Y	Y	N	Y	N	Y	N	
Toxicity								
Acute	Y	N	N	Y	N	Y	N	
Repeated Dose	Y	N	Y	Y	N	Y	N	
Genetic Toxicology *in vitro*	Y	N	Y	Y	N	Y	N	
Genetic Toxicology *in vivo*	Y	N	Y	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	N	N	N	N	Y	N	

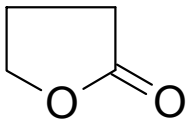
Introduction

γ-Butyrolactone, CAS no. 96-48-0, is a cyclic ester (lactone) most commonly prepared from 1,4-Butanediol by a Reppe process. The reaction is carried out in the gas phase between 180 and 240° C, over a copper catalyst and provides approximately 95% yield. The γ-Butyrolactone is separated from impurities (1,4-Butanediol, Butyric acid and high-boiling oligomers) by distillation giving a commercial product of about 99.7% purity. γ-Butyrolactone is also commercially prepared by the hydrogenation of Maleic anhydride using a nickel catalyst (1). It can also by synthesized from acetylene and formaldehyde under high pressure (2).

γ-Butyrolactone is a clear-oily liquid (3) with a pleasant odor (2). It has low volatility and is miscible with water and most organic solvents (3).

This material has numerous industrial applications due to its chemical structure and solvent properties. The bulk of γ-Butyrolactone production is used as an intermediate in the synthesis of N-Methylpyrrolidone (NMP) and 2-Pyrrolidone. It is also used to manufacture herbicides, growth regulators, vitamin B1, and the rubber additive thiodibutyric acid.

Several diverse solvent applications have been reported including as a solvent for polymers, in hairwave compositions and sun lotions, as a cosolvent for capacitor electrolytes and as a cosolvent for electronic photoresists. It is also used in printing inks, (for example ink-jet printer inks), as an extracting solvent in the petroleum industry. γ-Butyrolactone has also found application as a nematocide (1).



The structure of γ-Butyrolactone is shown above. This material is also known as:

- Dihydro-2(3H)-furanone
- Dihydro-2-furanone
- Butyrolactone,
- 4-Butyrolactone
- 4-Hydroxybutyric acid cyclic ester
- 4-Hydroxybutanoic acid lactone

- Tetrahydro-2-furanone
- 1,2-Butanolide
- 1,4-Butanolide
- 4-Deoxytetronic acid

Exposure in industrial applications is limited by process controls, protective equipment, low vapor pressure and by warning properties due to its odor. No occupational exposure level set by any governmental agency was located. Use as a co-solvent in digital inks may result in a very low-level of inhalation exposure by consumers; however, this potential exposure is limited by the very low quantities of ink used by consumer digital printing devices.

Several physicochemical, fate and toxicity studies have been conducted with γ-Butyrolactone. These studies are briefly reviewed in this testing rationale document, which also describes how these studies meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (U.S. EPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the U. S. EPA HPV Program. The majority of data elements are filled by high-reliability studies on γ-Butyrolactone, where direct data are not available or data are sparse, surrogates and acceptable estimation methods are used to fill or supplement the data element, as encouraged by the U.S. EPA and other regulatory authorities to avoid unnecessary testing cost and animal usage.

Physicochemical Data

Physicochemical data for γ-Butyrolactone are available from the literature and manufacturer's information.

Melting Point	-43.5˚ C (4)
Boiling Point	204° C @ 1013 hPa (5)
Vapor Pressure	0.344 hPa @ 20˚ C (6)
Partition Coefficient	Log $K_{o/w}$ = -0.64 (7)
Water Solubility	Soluble in all proportions (5)

Table 1: Physicochemical data for γ-Butyrolactone

These properties indicate that γ-Butyrolactone is a slightly volatile liquid with high water solubility. The value of the partition coefficient suggests that γ-Butyrolactone will partition preferentially into water and,

therefore, has little potential for bioaccumulation. The determination of an accurate and representative $K_{o/w}$ of γ-Butyrolactone is complicated by the fact that, in aqueous solution, γ-Butyrolactone can be an equilibrium mixture of γ-Butyrolactone, γ-hydroxybutyric acid, and γ-hydroxybutyrate. The equilibrium is pH and temperature dependent with sub-dependencies of alkalinity and dilution (see water stability discussion). The above value is supported by a value of –0.566 from BASF (8) and is considered representative of the conditions that would be encountered in the environment as it was derived by a "shake-flask" method.

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

Biodegradation potential has been determined using the MITI test and a BOD test. In the MITI test, a degradation of 60-92% was reported in 14 days (9), indicating that this material is considered readily biodegradable. In the BOD test with non-acclimated sludge, a removal of >95 % was recorded after 8 days (10). This ready biodegradation is anticipated based on the structure and its hydrolysis product γ-hydroxybutyrate, which is quickly metabolized to carbon dioxide in mammals. The biochemical pathways are well known and the structure is linear.

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant of 2.31 E-12 cm^3/molecule-sec. Using the default atmospheric hydroxyl radical concentration in APOWIN and the estimated rate constant for reaction of γ-Butyrolactone with hydroxyl radical, the estimated half-life of γ-Butyrolactone vapor in air is approximately 56 hours (see accompanying robust summary) (11).

Water stability has been thoroughly investigated for this material. In *Ullmann's Encyclopedia of Industrial Chemistry* it is stated that, in water γ-Butyrolactone is an equilibrium mixture of the lactone (closed ring) and hydroxybutyric acid. The equilibrium is pH and temperature dependent. At neutral pH and 0° C, the equilibrium lies 100% on the lactone side; at 100° C, the equilibrium mixture is 80% lactone. In the presence of base, the equilibrium shifts to the acid form, and the equilibrium mixture is 100% acid form in the presence of 1 equivalent of base. These observations have been quantitatively refined in a recent study published it the *Journal of Forensic Science* in which the equilibrium was investigated in pure water and in buffered water at several pH values (12). The results show that γ-Butyrolactone is relatively stable in pure unbuffered water. A 0.5% solution takes about 120 days to reach a stable pH of approximately 3.3 and an equilibrium of 67% lactone to 33% acid form. This mixture was

stable for at least 100 days after reaching steady state. In neutral buffered solutions, the hydrolysis is more rapid with a 15-30 day half-life and proceeds to completion (97% measured after 202 days). Additional studies showed that at higher pH values the hydrolysis is more rapid and at pH 12 , it proceeds to completion in about 10 minutes. At more acidic pH levels, it is slower and a 0.5% solution at pH 2 reaches an equilibrium of about 2:1 lactone to acid form. In addition, if the initial 0.5% solution at pH 2 starts as 100% acid form, it still reaches the same equilibrium ratio in about the same time frame. Studies were not conducted to determine the effect of concentration on hydrolysis, but it can be predicted from the hydrolysis equation that dilution will increase the proportion of acid form by dilution of the hydrogen ion concentration. It is concluded that the water stability is well characterized.

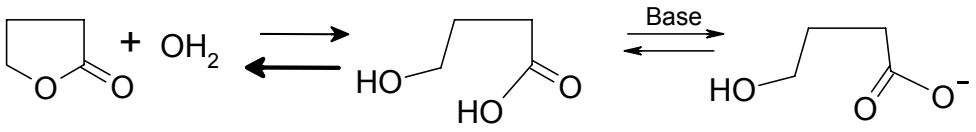


Figure 1: Hydrolysis of γ-Butyrolactone

Theoretical Distribution (Fugacity) of γ-Butyrolactone in the environment was estimated using the MacKay EQC level III model with standard defaults in EPIWIN v 3.05 but using the measured vapor pressure of 0.259 mm Hg, the measured log Ko/w, and data-adjusted estimates for half-life in water, soil and sediment. (13). The results for distribution using a model calculated Ko/c (adsorption coefficient based on organic carbon content) of 0.0939 and equal initial distribution to air, water and soil are:

o Air	3.2 %
o Water	34.3 %
o Soil	62.4 %
o Sediment	0.02 %

Table 2: Theoretical Distribution (Fugacity) of γ-Butyrolactone in the environment

Recommendation: No additional fate and pathway studies are recommended. The available data fill the HPV required data elements.

Ecotoxicity

An unpublished study of the acute toxicity of γ-Butyrolactone to the freshwater fish *Leuciscus idus* showing a 96-hour LC_{50} of 316 mg/L (14) indicates that this material presents little acute hazard to

freshwater fish. Another older study, with more concentration levels was conducted that shows the 48-hour LC_{50} for *Leuciscus idus* is in the range of 275-302 mg/L (15). The similarity of the 48-hour and 96-hour LC_{50} values is expected for this highly water-soluble compound. A 48-hour daphnia study indicates an EC_{50} greater than 500 mg/L (16). Green algae tests indicate an IC_{50} of 79 mg/L (17). These values, with references, are shown in the table along with results of ECOSAR modeling using the "Esters" model based on the measured $K_{o/w}$ of –0.64. The measured data appear to fit the ECOSAR "Esters" model well.

Aquatic Toxicity of γ-Butyrolactone		
	Reported Values	ECOSAR Prediction
Fish, 96-hour LC_{50}	316 mg/L (14)	334 mg/L*
Daphnia, 48 hour EC_{50}	> 500 mg/L (16)	17300 mg/L*
Algae, 96 hour EC_{50}	79 mg/L (17)	24 mg/L*

* Estimated using ECOSAR with measured $K_{o/w}$ (18)

Table 3: Aquatic Toxicity of γ-Butyrolactone.

Two issues are potential confounders in these aquatic studies. The first is volatility, however, based on the vapor pressure and water solubility (Henry's Law Constant) of the test material, this is not considered to be a significant concern in these short-term studies.

A more important consideration for γ-Butyrolactone is the water stability of the test material. Data show that this material converts to γ-Hydroxybutyrate in neutral and basic solution. The kinetics of this hydrolysis are well established (12) and at pH 7 it is known there is only about 15% hydrolysis in 3 days. Based on the known pH dependency, it can be extrapolated that the 48-hour loss in the pH 7-8 range for the fish and daphnid studies was less than around 20%. The impact of this conversion to γ-Hydroxybutyrate can be evaluated by comparing the aquatic toxicity of γ-Butyrolactone with that of γ-Hydroxybutyrate. No measured aquatic toxicity values could be located for γ-Hydroxybutyrate; however, its toxicity was estimated with the ECOSAR modeling program. The estimated fish 96-hour LC_{50} is 13,900 mg/L and the estimated Daphnia EC_{50} is 12,600 mg/L (19). Thus, the impact of the hydrolysis product γ-Hydroxybutyrate on the aquatic toxicity results for fish and daphnids is considered minor.

For the longer-term algae studies, however, where the pH of inoculated flasks containing the lower concentrations of test material exceeded 10.0 at the 96-hour interval, the loss of test material may be in excess of 50%. In this case, the solution by the end of the algae studies could have contained 50% or more of the test material as γ-Hydroxybutyrate. No measured algal inhibition values could be located for γ-Hydroxybutyrate to determine its potential impact on the result; however, its algal toxicity was estimated with the ECOSAR modeling program. The estimated 96-hour IC_{50} for green algae is 68,800 mg/L. The impact, therefore, would be to reduce the apparent inhibition of the test material. This happens to be in agreement with the observed IC_{50} of 79 mg/L as compared to the model-calculated, or "expected", value of 24 mg/L. The value of 24 mg/L is considered more representative of the 96-hour green algae IC_{50} for

γ-Butyrolactone. On the other hand, since the realistic situation is that hydrolysis will be occurring and as no "flow through" system is available for algae studies and as the hydrolysis kinetics of γ-Butyrolactone are well understood; it can be concluded that the value is know with sufficient accuracy for the purposes of the HPV assessment.

Recommendation: No additional ecotoxicity studies are recommended. The available data fill the HPV required data elements. The data are also consistent with the ECOSAR model and with available hydrolysis data.

Health Effects

Several studies have been conducted to estimate the potential health effects of γ-Butyrolactone to man. These span from acute studies (by all potential routes of absorption) to lifetime studies in rats and mice. In addition several studies have carefully examined the reproductive organs after various exposure durations and found no effects. Developmental toxicity using the recommended maximum dosage for experimental animals has been conducted. Since γ-Butyrolactone was part of an inter-laboratory collaborative program to improve genotoxicity testing, it has a wealth of high-quality genotoxicity studies available. ADME studies are also available defining its toxicokinetic characteristics. Few systemic adverse effects have been associated with the administration of γ-Butyrolactone to experimental animals. The most important effect involves its rapid metabolism to γ-Hydroxybutyrate, which is active in the CNS producing sedation and other higher-level effects on dopaminergic and GABAnergic neural pathways. This section summarizes the available data and assesses potential health effects from exposure to γ-Butyrolactone.

Metabolism

Adsorption, distribution, metabolism and excretion are important components in developing an understanding of the potential health effects of a material and of extrapolating data between studies, between routes of administration and between compounds. In the case of γ-Butyrolactone a considerable amount of information has been generated.

Absorption by the oral route has been determined to be both rapid and complete with a peak plasma concentration after dosing proportional to the dose. The completeness of absorption by the oral route is supported by the observation that after oral administration, if total plasma concentration of the compound and its principal metabolite, γ-Hydroxybutyrate, is plotted against time, the area under the curve is nearly identical to that following intravenous administration of γ-Butyrolactone (20, 21). Dermal dosing has been estimated to result in about ten percent absorption of γ-Butyrolactone (22).

Butyrolactone is metabolized rapidly with elimination primarily via respiratory CO_2 and urinary metabolites. Roth and Giarman reported that after a single intravenous dose of ^{14}C labeled γ-Butyrolactone to rats, traces of $^{14}CO_2$ were detectable in respiratory air after only 4 minutes, and reached a maximum in 15 minutes. Sixty percent of the total radioactivity was eliminated as carbon dioxide in less than 2.5 hours (23, 24). The plasma half-life of intravenously administered γ-Butyrolactone is less than one minute in rats. It has been reported that γ-Butyrolactone is converted to γ-Hydroxybutyrate by a "lactonase" enzyme present mainly in the plasma and liver; enzymatic activity was not detected in other tissues including brain, kidney, heart, skeletal muscle, and intestine. A γ-lactonase catalyzing the formation and hydrolysis of four- to eight-carbon lactones has been purified from human blood and has similar kinetic properties to that isolated from rat liver microsomes (25).

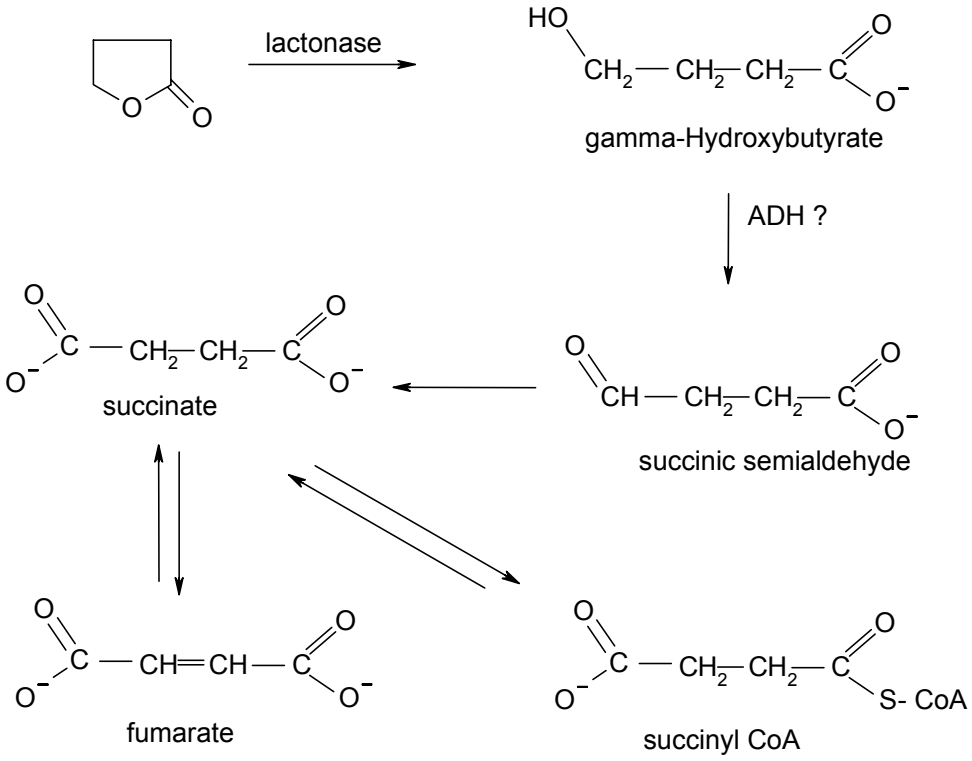


Figure 2: Proposed Metabolic Pathway of γ-Butyrolactone

The pathway from γ-hydroxybutyrate to carbon dioxide is controversial (26) with early work suggesting that the tricarboxylic-acid cycle was primarily involved via succinate (27). Later investigators obtained substantial labeling of succinate and its amino acid metabolites in the brain of rats after intraventricular administration of [1-^{14}C]-labeled γ-Hydroxybutyrate (28). In addition, demonstration that the labeling pattern in the mouse brain after an intravenous injection of [1-^{14}C]-labeled γ-Hydroxybutyrate can be explained by oxidation via succinate, but not by ß-oxidation, eliminates beta-oxidation as a probable pathway (29). Data showing that γ-Hydroxybutyric acid is metabolized to γ-aminobutyric acid in incubated brain slices and that specific inhibitors of γ-aminobutyrate-2-oxoglutarate transaminase blocked

the production of labeled γ-aminobutyric acid from labeled γ-Hydroxybutyric acid and of labeled 2-oxoglutarate from labeled glutamate, suggested that the catabolism of γ-Hydroxybutyric acid to γ-aminobutyric acid occurs via a transamination mechanism and not through the Krebs cycle (30). In spite of the transaminase pathway possibly having importance in the neurologic effects of γ-Butyrolactone, the rapidity of excretion and low degree of toxicity argue that a more generalized mechanism (such as intermediary metabolism and/or Krebs cycle) is prevalent. More recent work by Gibson and Nyhan (31) has shown that homogenates of liver and kidney mitochondria, but not heart, readily converted [U-^{14}C]-γ-Butyrolactone to ^{14}C organic acids via a pathway of conversion to ^{14}C-succinic acid, followed by further metabolism through the tricarboxylic acid cycle. Furthermore, this conversion was facilitated by exogenous NAD^+ and $NADP^+$. No evidence for the beta-oxidation of γ-Butyrolactone was obtained in any of the mitochondrial sonicates. Studies with exogenous non-labeled succinic semialdehyde indicated that this compound is an intermediate in the conversion of γ-Butyrolactone to succinic acid. Further evidence that succinate is involved in the metabolism of γ-Butyrolactone comes from the finding that patients with the rare genetic defect leading to succinic semialdehyde dehydrogenase deficiency (SSADH), accumulate 4-hydroxybutyric acid in physiologic fluids (32). Based on these findings and considerations, the figure above presents the proposed primary initial metabolic pathways for γ-Butyrolactone. After conversion to succinate and fumarate, it enters intermediary metabolism where it is driven to carbon dioxide by dose-dependent mass balance. This rapid conversion into labile components of intermediary metabolism and the Krebs cycle is considered to be responsible for the low systemic toxicity of γ-Butyrolactone.

This proposed pathway is also in accord with the known low systemic toxicity of succinic acid and offers a logical explanation for the low degree of γ-Butyrolactone toxicity to mammals. This metabolic understanding adds to our confidence in hazard and risk assessment for γ-Butyrolactone.

Acute Toxicity

Oral Exposure

Multiple determinations of the oral LD_{50} of γ-Butyrolactone have been reported in the rat, mouse, rabbit, cat and guinea pig (33); the studies universally indicate a low order of acute oral toxicity for this material. Two robust summaries have been prepared from representative studies in rats. One that used seven dose groups provides an oral LD_{50} in the rat of 1920 mg/kg (34). A supporting study has also been summarized that found an oral rat LD_{50} of 1580 mg/kg (35). An overview of all the acute studies indicates that a consistent narcotic or sedative effect at dose levels of approximately 500 mg/kg and above has been reported. Depending on the dose and the species, narcosis lasts from a few minutes to several hours but surviving animals show no other adverse effects or specific target organ effects.

Inhalation Exposure

It has been reported that there were no deaths when rats were exposed to saturated vapor of γ-Butyrolactone for 8 hours (35). The actual concentration was not measured but based on the vapor pressure the vapor concentration at saturation is calculated to be in the range of 300 ppm. Other investigations have reported LC_{50} values of > 2680 mg/m^3 (36) and > 5100 mg/m^3 (37); clinical signs were exophthalmus, difficulty breathing, hypoactivity and temporary reduction in food intake but no adverse effects were found at necropsy.

Dermal Exposure

One study in guinea pigs was found in the literature that indicated the dermal LD_{50} of γ-Butyrolactone is 5640 mg/kg (38).

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, the weight of evidence shows that the oral and inhalation toxicity is very low. Likewise, the limited study of dermal toxicity provides support for low hazard by this route. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted.

Repeat Dose Toxicity

Oral Exposure

Definitive oral subchronic gavage studies have been conducted in both rats and mice by the U.S. National Toxicology Program (26). In the rat studies, groups of 10 rats of each sex received test material by gavage at doses of 0, 56, 112, 225, 450, or 900 mg/kg of body weight, 5 days a week for 13 weeks. All high-dose males and one high-dose female died. Males receiving 450 mg/kg gained less body weight. There was no body-weight effect in females at any dose level. Other than inflammation of the nasal mucosa in all groups of dosed rats, there were no specific organ effects. The nasal mucosa irritation was considered to be a non-specific effect of gavage with a volatile agent. Rats at the higher dose levels (225 mg/kg and above) showed signs of sedation after dosing during the first 2-3 weeks of study that diminished in intensity with continued dosing, and dosed rats showed no visible signs of sedation after three weeks of dosing. The NOAEL was 225 mg/kg for males (based on body weights), and 450 mg/kg for females (based on one death in the 900-mg/kg group). No specific target organs were identified.

In the mouse studies, groups of 10 mice of each sex received test material by corn-oil gavage at doses of 0, 65, 131, 262, 525, or 1,050 mg/kg five days a week for 13 weeks. Groups of 10 mice received test

material by corn-oil gavage at doses of 0, 65, 131, 262, 525, or 1,050 mg/kg five days a week for 13 weeks.

In addition to these subchronic exposures, 2-year studies have also been conducted by oral gavage. In the rat studies, groups of 50 rats of each sex were administered γ-Butyrolactone in corn oil by gavage five days a week for up to 103 weeks. Male rats received 0, 112, or 225 mg/kg, female rats received 0, 225, or 450 mg/kg of body weight. In male rats there was no body weight change associated with administration of 112 or 225 mg/kg-day test material. Likewise, there was no apparent adverse effect of the test substance on survival as there was a marginal increase in survival of high-dose males. This was attributed to a marginal decrease in mononuclear cell leukemia in the high-dose males. There were no non-neoplastic toxic lesions or increased incidences in neoplasms in dosed male rats that were attributed to the administration of γ-Butyrolactone.

In the 2-year mouse studies, groups of 50 mice of each sex were administered γ-Butyrolactone in corn oil by gavage 5 days a week for up to 103 weeks. Both male and female mice received 0, 262, or 525 mg/kg-day test substance. Mean body weight and survival of high-dose male mice were significantly lower than controls. High-dose mice were partially sedated or lethargic and inactive shortly after dosing; however, administration seemed to contribute to an increase in fighting related trauma in dosed males, resulting in lower body weights and excess mortality. After the male mice were individually housed (week 67), the difference between mean body weights of dosed and control groups decreased. Body weights of low- and high-dose female mice were lower than that of the controls throughout much of the study, but there was no improvement following the change to individual housing. Survival of dosed female mice was similar to controls.

Administration of γ-Butyrolactone to mice for 2 years was associated with a statistically significant increased incidence of focal hyperplasia of the adrenal medulla in low-dose but not high-dose males. There were no non-neoplastic degenerative lesions associated with the administration of γ-Butyrolactone to male or female mice.

Recommendation: No additional repeated-dose studies are recommended. The available studies conducted by the NTP Statement if Work Guideline under GLP adequately fills the HPV required data element for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points, one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate tests have been conducted that cover both of these endpoints.

Genetic Toxicology in vitro

Several adequate *in vitro* tests of genetic toxicity for γ-Butyrolactone are available. A Salmonella typhimurium reverse mutation assay conducted by the NTP is representative and has been prepared as a robust summary (see attached robust summaries)(39). There are several supporting studies that have been published in the literature (40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56). Similarly, there are supporting reverse mutation tests in *E. coli* that show either clear negative results or results that cannot be interpreted (57, 58, 59, 48). Other various DNA damage tests using *E. coli* also support lack of genotoxic activity in bacterial systems (60, 61, 62, 63, 64). A chromosome aberration study in CHO cells, conducted for the National Toxicology Program (and prepared as a robust summary for this HPV document) gave a reproducible positive result in the presence of high concentration of test material and metabolic activation but not in the absence of the metabolic activation system (26). In a similar study investigating the induction of sister chromatid exchanges by γ-Butyrolactone, it was found that high concentrations (above 2,500 mcg/mL) of γ-Butyrolactone induced a significant increase of SCE's in Chinese hamster ovary cells in the presence but not the absence of metabolic activation. In this report (65), both endpoints (the Loveday publication also covers the CHO chromosome aberration study) were significantly increased only in the presence of induced *S9* and the authors speculated that the addition of S9 enzymes coupled with 10-fold higher concentration of γ-Butyrolactone allowed detection of cytogenetic effects which were not observed in the earlier negative study with a rat liver cell line (66).

Tests in yeast for mitotic gene conversion and aneuploidy induction were also negative (67). Negative results were obtained with γ-Butyrolactone in tests for chromosome aberration induction using a rat liver epithelial cell line without supplemental *S9* (68) and in tests for unscheduled DNA repair in HeLa cells with and without S9 (69). γ-Butyrolactone was also negative for induction of gene mutations in Chinese hamster *V79* cells (70) and human fibroblasts (71) with and without S9.

Genetic Toxicology in vivo

Mammalian genotoxicity was assessed *in vivo* using the Mouse Micronucleus Test as reported by multiple investigators. One representative test result had been included in the robust summaries (72) and the others are supporting (73, 74, 75). In this study, two i.p. doses of γ-Butyrolactone given at 80% of the LD_{50} with intervals between dosing and sacrifice of 24, 48, 72 or 96 hours, failed to produce an increase in polychromatic erythrocytes containing micronuclei. It was concluded that the test material did not show genotoxic activity in this system (72). Additional negative *in vivo* studies have been reported in *Drosophila melanogaster* (76).

Recommendation: The SIDS requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using acceptable protocols. No additional testing is recommended.

Reproductive Toxicity

Reproductive effects have been adequately assessed through the combination of the negative developmental toxicity study (83) and the subchronic (and chronic) studies in which reproductive organs were evaluated and found to be unaffected by treatment with γ-Butyrolactone (26). As the description of reproductive organ examination and evaluation in the published NTP Technical Report 406 is rather brief, the individual animal histopathology tables for the 90-day rat and mouse studies were obtained from NTP and lesions of the reproductive organs summarized in a separate robust summary. The list of organs examined was also obtained from the NTP archives and added to the new reproductive-toxicity robust summary. Results of the reproductive organ examination of dosed rats and mice did not indicate any compound related adverse effects or suggest any impairment of fertility or other reproductive function. Conduct of additional studies would not add significantly to our understanding of this material's reproductive toxicity.

Potentially relevant to reproductive toxicity, one published study described the inhibition of ovulation in rats by γ-Butyrolactone (77). In this study, the investigators examined the effect of γ-Hydroxybutyrate when administered in proestrous on ovulation in rats. They had postulated that γ-Hydroxybutyrate, which had been reported to produce a significant increase in dopamine without affecting other brain neurotransmitters, might result in reduction in the production of FSH and/or LH by the pituitary. This reduction in FSH and LH surge has the potential to interfere with ovulation in the rat. Their results showed that after a single i.p. injection of γ-Butyrolactone in proestrus, serum LH and especially FSH levels were reduced, in a dose-dependent manner, from one to four hours after the injection. In addition, the number of rats ovulating was reduced in a dose dependent manner. The ED_{50} for antiovulatory activity in the Sprague-Dawley rat was determined to be approximately 250 mg/kg, which was below the anesthetic dose. This finding is biologically plausible as it is known that ovulation is under control of a neuroendocrine cascade that involves receptors in the hypothalamus that are sensitive to dopamine or GABA and that cause the release of GnRH into the pituitary with subsequent release of LH and FSH, which are required for ovulation.

The relevance of this finding in rats for humans is unknown. Some considerations are that humans have a menstrual cycle rather than an estrous cycle; however, the same neuroendocrinal substances are involved (78). Another factor is that rats quickly develop a tolerance to the sedative action of γ-Hydroxybutyrate (79) and it has been proposed that this is associated with a down-regulation of the γ-Hydroxybutyrate receptors (80). If it is also the case that the neuronal systems regulating GnRH are subject to the rapid development of tolerance, then repeated dosing of animals with γ-Butyrolactone might not affect ovulation. It is also controversial if and under what conditions of administration γ-Hydroxybutyrate elevates or lowers CNS dopamine levels (81, 82). These investigators have also presented evidence that the effect of γ-Hydroxybutyrate on CNS dopamine levels can be reversed by varying the route of

administration from i.p. to s.c. injection, implicating the route of administration as a critical variable. In conclusion, not enough is known about the mechanisms involved to make an informed decision concerning the potential of γ-Butyrolactone to act as an ovulation inhibiter in humans. In addition, due to the significant differences in ovulatory cycles between humans and rodents, it is questionable if additional studies of reproductive toxicity in rodents that focus on antiovulatory effects will add any information of value to human hazard or risk assessment.

Recommendation: No additional reproductive testing is recommended, as the available data are sufficient to assess the reproductive toxicity of this material according to the HPV guidelines.

Developmental Toxicity

A modern OECD 414 Guideline study has been conducted with γ-Butyrolactone. The results of this investigation conducted in rabbits using inhalation at 0.5, 1.4 and 5.0 mg/L for 6 hours per day on days 9 to 17 of gestation did not produce either maternal or fetal toxicity. None of the reproductive, embryonic, or fetal parameters were affected by this treatment at the highest recommended inhalation dose. The high dose level also exceeded the saturation vapor concentration of γ-Butyrolactone in air and was conducted as a mixed vapor-aerosol exposure. Both the developmental and maternal NOAEL was 5.0 mg/L (83).

This result is supported by a study in which groups of 10 pregnant Sprague-Dawley-rats were given 0, 10, 50, 125, 250, or 500 mg/kg γ-Butyrolactone by gavage on days six through 15 of gestation (84). Dams were observed for signs of intoxication, body weights were measured daily from days zero through 21 of gestation, and food and water consumption were monitored at 3-day intervals. Dams were killed on gestation-day 21, and the uteri were removed. Fetal data were recorded and fetuses were examined for malformations. Placental weights were significantly reduced in treated animals at all doses. Mean fetal weights were significantly increased in the 50, 125, and 250-mg/kg groups. No other treatment related changes of significance were seen either in the dams or fetuses. The maximum dose was controlled by the solubility of the test substance in the vehicle and as there was no maternal toxicity produced, a higher dose-level might have been achieved with an alternate vehicle. This study indicates no developmental hazard up to a dosage level of 500-mg/kg body weight but does not define the maternal or developmental LOAEL. As this dose level is half of the OECD-recommended maximum of 1000 mg/kg in the current OECD 414 test and as no adverse effects were produced, this study supports low developmental hazard.

Taken together, the two rat developmental toxicity studies indicate a low developmental toxicity hazard for γ-Butyrolactone by the inhalation or oral routes

Recommendation: No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental toxicity of this material.

Conclusions

With regard to the parameters specified in the U. S. EPA HPV Challenge program, it is concluded that the available information on 2-Pyrrolidone fills all of the requirements for physicochemical parameters, fate information, aquatic toxicity and mammalian toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, taken together the information provided a reliable hazard assessment. Conduct of additional studies would not add significantly to our understanding of this material's toxicity.

References

1 *Ullmann's Encyclopedia of Industrial Chemistry* ©2002 Wiley-VCH Verlag GmbII, Weinheim, Germany

2 Lewis, RJ. *Hawley's Condensed Chemical Dictionary*, Fourteenth Edition, J Wiley and Sons, New York, 2001, p184.

3 O'Neil, MJ (ed.). *The Merck Index* - An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001 p268/

4 Lide, D.R. (ed.). *CRC Handbook of Chemistry and Physics*. 79th ed. Boca Raton, FL: CRC Press Inc., 1998-1999.,p. 3-170

5 O'Neil, M. (ed.). *The Merck Index* - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., Thirteenth Edition 2001. p 268 entry # 1569.

6 BASF AG, Analytisches Labor; unveroeffentlichte Untersuchung (BRU 86.267)

7 Hansch C et al; *Exploring QSAR*. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 9 (1995)

8 BASF AG, Analytisches Labor; unveroeffentlichte Untersuchung (J.Nr. 119046/02 vom 25.09.1987)

9 *Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan*, edited by Chemicals Inspection & Testing Institute Japan, published by Japan Chemical Industry Ecology-Toxicology & Information Center, October 1992

10 BASF AG, Unpublished result of a BOD study. dated 18.8.77 (1977)

11 EPIWIN 3.05, Syracuse Research Corporation, AOP Program v1.90, Toxicology and Regulatory Affairs Calculation -2002

12 Ciolino LA, Mesmer MZ, Satzger RD, Machal AC, McCauley HA, Mohrhaus AS. The chemical interconversion of GHB and GBL: forensic issues and implications. J Forensic Sci.46:1315–1323 (2001).

13 EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

14 BASF AG, Unpublished result of a fish acute toxicity study of Butyrolactone on the Golden Orfe, dated 14.05.90 (1990)

15 BASF AG, Unpublished result of a fish toxicity study. dated 20.06.77 (1977)

16 BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0009/88)

17 BASF AG, Unpublished results of two algae studies. 1. dated 3.06.1988 ref 2/00w9/88/t96; 2. dated 28.05.1988 ref 2/0009/87/t96

18 ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

19 ECOSAR program (ver 0.99f) run with the "Neutral Organics-acid" model and the KoWin calculated Ko/w of -0.40.

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

21 Arena, C., and 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).

22 Fung, H.-L., Lettieri, J.T., and Bochner, R. Percutaneous butyrolactone absorption in rats. J. Pharmacol Sci. 68: 1198-1200 (1979).

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

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

25 Fishbein, W.N., and Bessman, S.P. Purification and properties of an enzyme in human blood and rat liver microsomes catalyzing the formation and hydrolysis of γ-lactones. J. Biol. Chem. 241: 4842-4847 (1966).

26 NTP Technical Report 406. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Gamma-Butyrolactone (CAS No. 96-48-0) In F344/N Rats and B6C3Fl Mice (Gavage Studies) March 1992 pp 12-13.

27 Walkenstein, S.S., Wiser, R., Gudmundsen, C., and Kimmel, H. Metabolism of γ-hydroxybutyric acid. Biochem. Biophys Acta 86, 640-642 (1964).

28 Doherty, J.D., and Roth, R.H. Metabolism of -y-hydroxy[1-14C]butyrate by rat brain: relationship to Krebs cycle and metabolic compartmentation of amino acids. J. Neurochem. 30, 1305-1309 (1978).

29 Mähler, H., Patel, A.J., and Balazs, R. Gamma-hydroxybutyrate degradation in the brain in vivo: negligible direct conversion to GABA. J. Neurochem. 27: 253-258 (1976).

30 Vayer, P., Mandel, P., and Maitre, M. Conversion of γ-hydroxybutyrate to γ-aminobutyrate in vitro. J. Neurochem. 45: 810-814(1985).

31 Gibson KM, Nyhan WL. Metabolism of [U-14C]-4-hydroxybutyric acid to intermediates of the tricarboxylic acid cycle in extracts of rat liver and kidney mitochondria. Eur J Drug Metab Pharmacokinet 14:61-70 (1989).

32 Gibson KM, Hoffmann GF, Hodson AK, Bottiglieri T, Jakobs C. 4-Hydroxybutyric acid and the clinical phenotype of succinic semialdehyde dehydrogenase deficiency, an inborn error of GABA metabolism. Neuropediatrics 29:14-22 (1998).

33 Toxikologische Bewertung. Heidelberg, Berufsgenossenschaft der chemischen Industrie PG:76 p YR:2000 IP: VI:7 p 17.

34 Industrial Toxicology Laboratories, Laboratory report on acute-oral toxicity of gamma-butyrolactone in rats and guinea pigs. Dated may 17, 1952, sponsored by General Aniline and Film Corp.

35 BASF AG: Abt. Toxikologie, unveroeffentlichte Untersuchungen, (X/90), 02.11.1960

36 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 unveröffentlichter Bericht BGH 19/91325 (1991) im Auftrag der Berufsgenossenschaft der chemischen Industrie. As cited in :Toxikologische Bewertung. Heidelberg, Berufsgenossenschaft der chemischen Industrie PG:76 p YR:2000 IP: VI:7

37 Monsanto Company, Environmental Health Laboratory Acute toxicity of gamma-butyrolactone administered by inhalation to Sprague-Dawley male and female rats Bericht, Study Number 85061 NTIS/OTS 0534527 (1986). As cited in :Toxikologische Bewertung. Heidelberg, Berufsgenossenschaft der chemischen Industrie PG:76 p YR:2000 IP: VI:7

38 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).

39 Haworth S. et al.:Salmonella Mutagenicity Test Results for 250 Chemicals. Environ. Mutagen. 5 (Suppl.1), 3-142, (1983)

40 Garner, R.C., Welch, A., Pickering, C. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay in*: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 280-284 (1981)

41 Baker, R.S.U., Bonin, A.M. Study of 42 coded compounds with the Salmonella/mammalian microsome assay in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 249-260 (1981)

42 Ichinotsubo, D., Mower, H., Mandel, M. Mutagen testing of a series of paired compounds with the Ames Salmonella testing system in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 298-301 (1981)

43 Bridges, B.A., MacGregor, D., Zeiger, E. Summary report on the performance of bacterial mutation assays in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 49-67 (1981)

44 Richold, M., Jones, E. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 314-322 (1981)

45 Rowland, I., Severn, B. Mutagenicity of carcinogens and noncarcinogens in the Salmonella/microsome test in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 323-332 (1981)

46 Simmon, V.F., Shepherd, G.F. Mutagenic activity of 42 coded compounds in the Salmonella/microsomeassay in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens:* Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 333-342 (1981)

47 Trueman, R.W. Activity of 42 coded compounds in the Salmonella reverse mutation test in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 343-350 (1981)

48 Venitt, S., Crofton-Sleigh, C. Mutagenicity of 42 coded compounds in a bacterial assay using Escherichia coli and Salmonella typhimuriumin*: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 351-360 (1981)

49 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)

50 Martire. G., Vricella, G., Perfumo, A.M., De Lorenzo, F. Evaluation of the mutagenic activity of coded compounds in the Salmonella test in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 271-279 (1981)

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

52 Brooks, T.M., Dean, B.J. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay with Preincubation in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens:* Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 261-270 (1981)

53 Nagao, M., Takahashi, Y. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 302-313 (1981)

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

55 Hubbard, S.A., Green, M.H.L., Bridges, B.A., Wain, A.J., Bridges, J.W. Fluctuation test with S9 and hepatocyte activation in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 361-370 (1981)

56 Gatehouse, D. Mutagenic activity of 42 coded compounds in the ‚microtiter" fluctuation test in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 376-386 (1981)

57 Skopek, T.R., Andon, B.M., Kaden, D.A., Thilly, W.G. Mutagenic activity of 42 coded compounds using 8-azaguanine resistance as a marker genetic in Salmonella typhimurium in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 371-375 (1981)

58 Matsushima, T., Takamoto, Y., Shirai, A., Sawamura, M., Sugimura, T. Reserve mutation test on 42 coded compounds with the E. coli WP2 system in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 387-395 (1981)

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

60 Tweats, D.J. Activity of 42 coded compounds in a differential killing test using Escherichia coli strains WP2, WP67 (uvrA polA), and CM871 (uvrA IexA recA) in: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 199-209 (1981)

61 Rosenkranz, H.S., Hyman, J., Leifer, Z. DNA polymerase deficient assay in*: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 210-218 (1981)

62 Kada, T. The DNA-damaging activity of 42 coded compounds in the rec-assay in*: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 176-182 (1981)

63 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: *Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 195-198 (1981)

64 Green, M.H.L. A differential killing test using an improved repair-deficient strain of Escherichia coli in*: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 183-194 (1981)

65 Loveday, KS., Lugo, M.H., Resnick, M.A., Anderson, B.E., and 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).

66 Dean, B.J. Activity of 27 coded compounds in the RLl chromosome assay. In *Progress In Mutation Research: Evaluation of Short-Term Tests for Carcinogens* (F.J. de Serres and J. Ashby, Eds.), Vol. I, pp. 570-579. Elsevier North Holland, New York. (1981)

67 Vogel, E., Blijleven, W.G.H., Kortselius, M.J.H., and Zijlstra, J.A. (1981). Mutagenic activity of 17 coded compounds in the sex-linked recessive lethal test in *Drosophila melanogaster.* In *Progress In Mutation Research: Evaluation of Short-Term Tests for Carcinogens* (R.J. de Serres and J. Ashby, Eds.), Vol. I, pp. 660-665. Elsevier North Holland, New York.

68 Dean, B.J. (1981). Activity of 27 coded compounds in the RL$_l$ chromosome assay**.** In *Progress In Mutation Research: Evaluation of Short-Term Tests for Carcinogens (F.J.* de Serres and J. Ashby, Eds.), Vol. I, pp. 570-579. Elsevier North Holland, New York

69 Martin, C.N., and McDermid, A.C. (1981). Testing of 42 coded compounds for their ability to induce unscheduled DNA repair synthesis in HeLa cells. In *Progress In Mutation Research: Evaluation of Short Term Tests for Carcinogens* (F.J. de Serres and J. Ashby, Eds.), Vol. 1, pp. 533-537. Elsevier North Holland, New York.

70 Knaap, A.G.A.C., Goze, C., and Simons, J.W.I.M. (1981). Mutagenic activity of seven coded samples in V79 Chinese hamster cells. In *Progress In Mutation Research: Evaluation of Short-Term Tests for Carcinogens* (F.J. de Serres and J. Ashby, Eds.), Vol. I, pp. 608-613. Elsevier North Holland, New York.

71 Gupta, R.S., and Goldstein, S. (1981). Mutagen testing in the human fibroblast Diptheria toxin resistance (HF Dip`s system). In Progress In Mutation Research*: Progress In Mutation Research:Evaluation of Short-Term Tests for Carcinogens* (F.J. de Serres and J. Ashby, Eds.), Vol. I, pp. 614-625. Elsevier North Holland, New York.

72 Salamone, M.F., Heddle, J.A., Katz, M. Mutagenic activity of 41 compounds in the *in vivo* micronucleus assay. In Mutation Research, Volume 1, *Progress In Mutation Research: Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 686-697 (1981)

73 Tsuchimoto, T., Matter, B.E. Activity of coded compounds in the micronucleus test. in: Mutation Research, Volume 1, *Progress In Mutation Research: Evaluation of Short-Term Tests for Carcinogens*: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 705 - 711 (1981)

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

75 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).

76 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), and references therin.

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

78 Hardman, JG and LE Limbird. *Goodman and Gillmans's The Pharmacological Basis of Therapeutics 10th Edition*, McGraw Hill 2001.

79 Itzhak Y, Ali SF. Repeated administration of gamma-hydroxybutyric acid (GHB) to mice: assessment of the sedative and rewarding effects of GHB. Ann N Y Acad Sci 965:451-60 (2002)

80 Maitre M. The gamma-hydroxybutyrate signaling system in brain: organization and functional implications. Prog Neurobiol 51:337-61 (1997)

81 Feigenbaum JJ, Howard SG. Does gamma-hydroxybutyrate inhibit or stimulate central DA release? Int J Neurosci 88:53-69 (1996)

82 Howard SG, Feigenbaum JJ. Effect of gamma-hydroxybutyrate on central dopamine release in vivo. A microdialysis study in awake and anesthetized animals. Biochem Pharmacol 53:103-10 (1997)

83 BASF AG, Prenatal Toxicity of gamma-Butyrolactone as a vapor resp. vapor/aerosol mixture in rabbits after inhalation. Project No. 41R0468/91065, sponsored by the BG Chemie. 20 October 1993.

84 Kronevi, T. Holmberg, B. Arvidsson. S. Teratogenicity Test of gamma-Butyrolactone in the Sprague-Dawley Rat. Pharmacology and Toxicology, 62: 57-58 (1988)