FOREWORD INTRODUCTION

DL-LACTONE

CAS N°:79-50-5

SIDS Initial Assessment Report

For

SIAM 21

Washington, D.C., 18-20 October 2005

1.	**Chemical Name:**	DL-Lactone
2.	**CAS Number:**	79-50-5
3.	**Sponsor Country:**	Switzerland, Dr Georg Karlaganis Federal Office for the Environment, Forests and Landscape CH-3003 Berne
4.	**Shared Partnership with:**	F. Hoffmann-La Roche Ltd
5.	**Roles/Responsibilities of the Partners:**	Dr Louis Schnurrenberger Corporate Safety & Environmental Protection, CSE 49/2.046 CH-4070 Basle Switzerland +41 616 886 638 +41 616 881 920 www.roche.com
•	Name of industry sponsor /consortium	F. Hoffmann-La Roche Ltd DSM Nutritional Products Limited
•	Process used	This document was prepared by NOTOX BV and peer-reviewed by all partners involved
6.	**Sponsorship History**	
•	How was the chemical or category brought into the OECD HPV Chemicals Programme ?	This substance is sponsored by Switzerland in phase 6 of the OECD HPVC Programme and is submitted for first discussion at SIAM 21
7.	**Review Process Prior to the SIAM:**	The industry consortium collected new data and prepared the updated IUCLIDs for DL-lactone, draft versions of the SIAR and SIAP. Swiss authorities peer-reviewed the documents.
8.	**Quality check process:**	By industry before submission to the sponsor country: Internal cross-checking by two people involved; late last

literature search in public databases for confirmation.

Jointly by industry and government:

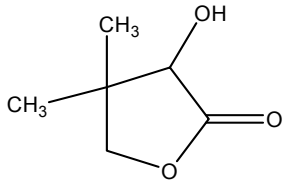
Independent checking by two different government agencies (health and environment), discussion with industry

9. Date of Submission:

10. Date of last Update: 18 January 2006

11. Comments:

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	79-50-5
Chemical Name	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl (DL-lactone)
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

There is no information on the toxicokinetics of DL-lactone available.

The acute oral LD_{50} of DL-lactone in rats and mice is above 2000 mg/kg bw.

In a test with rabbits (OECD 404) DL-Lactone was not irritating to the skin. However, based on occupational exposure experience in humans, DL-lactone is expected to be irritating to the eyes and upon prolonged and intensive exposure also to the skin. No sensitisation potential is found in the guinea-pig maximisation test (OECD 406).

In a combined repeated dose reproduction/developmental toxicity screening study (OECD 422) female rats treated at an oral dose of 1000 mg/kg bw/day showed aggression and restlessness during part of the study period. Findings on body weight, food consumption, haematology, clinical chemistry, organ weights, macroscopy and histopathology were within normal ranges. The NOAEL for repeated dose toxicity was set at 200 mg/kg bw/day.

DL-lactone was negative in an Ames test (OECD 471) and an *in vivo* micronucleus test (OECD 474). There are no indications that DL-lactone possesses mutagenic properties.

In an OECD 422 repeated dose reproduction/developmental toxicity screening study with rats exposed to DL-lactone, no effects on reproductive performance, stage of spermatogenesis, pup mortality, weight, sex and viability were reported up to oral doses of 1000 mg/kg bw/day. Animals were dosed prior to and during mating, gestation and following gestation until lactation day 4. Based on the available data, DL-lactone does not show evidence of reproductive or developmental toxicity. The NOAEL for reproductive toxicity is ≥1000 mg/kg bw/day.

Environment

DL-lactone is a white crystalline powder with a melting point of 78°C, boiling point of 247°C and a vapour pressure of about 0.1 hPa at 25°C (calculated from experimental vapour pressure at 60°C). The substance is very soluble in water (> 500 g/l) and has a log Kow of -0.69 (OECD 107). Based on its pKa (>13) DL-lactone is most likely present in the unionised form under environmental conditions. The substance is readily biodegradable. Hydrolysis half-live for DL-lactone is expected to be one year at pH 4, 30 days at pH 7 and approximately 12 days at pH 9 (25°C).

Various model calculations (based on log Kow) indicate that DL-Lactone does not bioaccumulate in fish and/or worms.

DL-lactone has an LC_{50} of >140 mg/L in fish, an EC_{50} of >130 mg/L in daphnia and an EC_{50} for biomass and growth rate of >78 mg/L (nominal 100 mg/L) in algae. Data on the toxicity towards micro-organisms of the d-

isomer are indicative of an EC_{50} for micro-organisms above 100 mg/L.

Exposure

For the year 2004 the global market for DL-lactone was estimated to be 1000-5000 tonnes. DL-lactone is used in the synthesis of cosmetics and pharmaceuticals. At the production site of the main producer in UK DL-lactone is further processed on-site in closed systems in the synthesis of Calcium D-Pantothenate. Only a small amount (<0.5%) is isolated and sold to a third party. According to the product registers in Nordic Countries (Norway, Sweden and Denmark) and in Switzerland DL-lactone is not used in industrial and consumer products.

Occupational exposure may occur during synthesis, mainly through completion of process sampling and potentially during drumming-off operations.

Based on a production mass balance at the manufacturing plant of the main producer in UK for the year 2004, a maximum of 0.4 % of the total produced DL-lactone is lost to the waste water and a maximum of 0.75 % to the distillation residues which are incinerated. Waste water is treated in an on-site wastewater treatment plant. Since DL-lactone is ready biodegrable releases to surface water with effluents will be low.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for human health (skin and eye irritation). These hazards do not warrant further work as they are related to reversible effects. They should nevertheless be noted by chemical safety professionals and users.

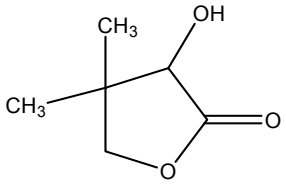
Environment: The chemical is currently of low priority for further work because of its low hazard profile.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 79-50-5
IUPAC Name: 2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl
Molecular Formula: C_6-H_{10}-O_3
Structural Formula:



Molecular Weight: 130.14
Synonyms: DL-Lactone, dl-alpha-Hydroxy-beta,beta-dimethyl-gamma-butyrolactone, alpha-Oxy-beta,beta-dimethyl-gamma-butyrolactone, Dihydro-3-hydroxy-4H-dimethyl-2(3H)-furanone, RS-Dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, (RS)-Pantolactone, (±)-Pantolactone, Pantothenic lactone, DL-Pantolactone, dl-Pantoyllactone

DL-Lactone is a racemic mixture of D-Pantolactone [(3R)-dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, CAS 599-04-2] and L-Pantolactone [(3S)-dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, CAS 5405-40-3].

1.2 Purity/Impurities/Additives

The product specification indicates that the test substance contains a minimum of 98% DL-lactone, 0-2% 2,4-dihydroxy-3,3-dimethyl butyric acid and 0-0.5% water.

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	Solid (powder)	Conduit, 2002
Melting point	78ºC	F. Hoffmann-La Roche
Boiling point	247ºC	F. Hoffmann-La Roche
Density (20ºC)	1.17 g/cm^3	F. Hoffmann-La Roche
Vapour pressure	0.7038 hPa (60ºC) 0.0844 hPa (25ºC)	F. Hoffmann-La Roche Calculation, Uses 4.02, 2004
Water solubility	>500 g/L	F. Hoffmann-La Roche
Partition coefficient n-octanol/water (log value)	-0.69	OECD 107, Willems, 1999
Henry's law constant	<0.0001 atm.m^3/mol	Calculation, Uses 4.02, 2004; EPISuite 3.11, 2003; SPARC
Partition coefficient organic carbon/water	<10	Calculation, Uses 4.02, 2004; EPISuite 3.11, 2003;
Partition coefficient in activated sludge/water	Ca. 220 l/kg	Desmares-Koopmans, 2004
Auto ignition temperature	Ca.400ºC	Schildknecht, 1981
Flash point	122ºC	Schildknecht, 1981
Explosive properties	At ≥ 500ºC moderate dust explosion hazard	F. Hoffmann-La Roche
pKa (acidic group)	≥13.1	Calculation, Willems, 1999; SciFinder, 2004; SPARC

2 GENERAL INFORMATION ON EXPOSURE

DL-Lactone is used in the synthesis of cosmetics and pharmaceuticals. It is an intermediate in the production of Calcium D-Pantothenates. A small amount (0.17-0.53%) is isolated and sold to a third party.

2.1 Production Volumes and Use Pattern

The yearly production volume of DL-lactone is estimated by industry at 1000-5000 tonnes worldwide (see IUCLID, 2004) with a slightly decreasing tendency.

In the Nordic Countries (Norway Sweden and Denmark) no use of DL-lactone was identified (SPIN database 12-05-05).

DL-lactone is not listed in the Swiss Toxic Products Register (Giftliste online database, 13-JUN-2005).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

During use of DL-lactone some losses to the environment may occur. Based on a production mass balance at the manufacturing plant of the main producer in UK, a maximum of 30 kg/day DL-lactone is lost to the waste water and approximately 50 kg/day to the distillation residues. The wastewater enters an on-site chemical and biological wastewater treatment plant with a hydraulic flow of approximately 7'000 m^3/day. The water treated on-site subsequently enters a municipal sewage works (STP) where the industrial water is mixed up with approximately the seven-fold amount of municipal wastewater (total influent ~ 54'000 m^3). The effluent of the plant is discharged to coastal waters.

In wastewater treatment plants, 87.3–91.5% of DL-lactone will be removed through biodegradation while 8.5–12.7% will be released to receiving waters, 0.0–0.4% will partition to sludge and <1E–4% to air (Simple Treat Model v3.1; STP Model v1.50, both configured for ready biodegradability). The concentration of DL-lactone in the influent of the on-site wastewater treatment plant is approximately 4.3 mg/l, in the effluent ~ 0.43 mg/l. The concentration of DL-lactone in the influent of the municipal wasterwater treatment plant is estimated at about 0.056 mg/l. The predicted concentration in the effluent is 0.006 mg/l.

Dewatered sludge of the industrial plant is sent to landfill. The liquid waste stream is incinerated together with other wastes during the process. It is filled directly into a dedicated bulk tanker, will be treated as special waste and is used as CEMfuel® (i.e., hazardous compounds will not reach the environment).

2.2.2 Photodegradation

The calculated half-life for the photo-oxidation (reaction with hydroxyl radicals) of DL-lactone in air is 39 hours (EPISuite 3.11, 2003).

2.2.3 Stability in Water

A hydrolysis study at 50 °C, resulted in half-lives for DL-lactone of 144, 3.7 and 1 day(s) at pH 4, 7 and 9, respectively (based on nominal concentrations). After extrapolation from nominal and measured concentrations at 50 °C to values at 25 °C, DL-lactone is expected to have a half-life of 1-3 years at pH 4, of approximately 30 days at pH 7 and of approximately 6-20 days at pH 9 (Brekelmans, 1999). No degradation products were identified by analytical method, but, assuming hydrolysis of the cyclic lactone ester, 2,4-dihydroxy-3,3-dimethyl-butanoic acid (pantoic acid) is the probable hydrolysis product of DL-lactone.
In conclusion, DL-lactone is expected to be hydrolytically stable at low pH values and to be prone to moderate hydrolysis at higher pH values.

2.2.4 Transport between Environmental Compartments

Level III fugacity modelling shows that after release to surface water approximately 99.9% of DL-lactone ends up in water. Negligible amounts will be distributed towards air (5E-05%), soil (5E-0.3%) and sediment (0.14%). In the model physico-chemical properties were withdrawn from table 1 (Level III Model v2.7, 2004).

2.2.5 Biodegradation

Several tests for ready and inherent biodegradation indicate that DL-lactone is biodegradable (see table 2). In a Modified Sturm test the relative degradation values calculated in the 28-day test period were 82% and 76% degradation, for the duplicate bottles tested. Furthermore, more than 60% degradation of DL-Lactone was reached within a 10-day window. No inhibition of microbial activity, adsorption nor abiotic degradation occurred (Desmares-Koopmans, 2004). DL-Lactone was readily biodegradable under the conditions of the test.

Table 2 Results from biodegradation studies

Type	OECD Guideline	Degradation	Reliability code	Reference
Modified Sturm Test	301B	76-82%	1	Desmares-Koopmans, 2004
Zahn-Wellens test (14 days)	302B	≥98%	2	Gröner, 1983
Zahn-Wellens test (21 days)	302B	≥80%	2	Gröner, 1983
Zahn-Wellens test (21 days)	302B	≥97%	2	Gröner, 1985
MITI test (13 days)	302C	Ca. 82%	2	Gröner, 1983
MITI test (21 days)	302C	>98%	2	Gröner, 1983
COD test (7 days)	-	>95%	2	Blechschmitt, 1995

2.2.6 Bioaccumulation

The potential of DL-lactone to bioaccumulate in fish and worms was investigated using various QSARs. Based on modelled bioconcentration factors, 0-3.2 for fish and 3.23 for worms, the substance is not expected to bioaccumulate (Uses 4.02, 2004; EPISuite 3.11, 2003; SciFinder, 2004; ChemSCORER; Veith, 1979).

2.2.7 Other information on Environmental Fate

Exposure of DL-lactone to the environment is expected to occur through sewage works into surface waters. In the specific case of the Dalry plant, the effluent from the plant sewage works goes into the municipal sewagw works of the town of Dalry, where further removal is expected (O'Leary 2001), before release into receiving waters. Based on the ready biodegradability attained in a test, the EU Technical Guidance Document (2003) suggests a surface-water half-life for biodegradation of 15 days.

In conclusion, based on partitioning properties, ready biodegradability, extrapolated hydrolysis and suggested surface-water degradation rate in water as well as calculated photodegradation in the atmosphere, DL-lactone is not considered a persistent compound in the environment.

2.3 Human Exposure

2.3.1 Occupational Exposure

Occupational exposure to DL-lactone is expected to be low, as the substance is used as an intermediate in a closed process. Exposure mainly occurs through completion of process sampling and potentially during drumming-off operations (COSHH assessment).

2.3.2 Consumer Exposure

In view of the use of the substance consumer exposure is not anticipated.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

No data available.

3.1.2 Acute Toxicity

The acute toxicity of DL-lactone was investigated in rats and mice.

Studies in Animals

The oral LD_{50} in rats and mice was 9700 and 4380 mg/kg bw, respectively. No information on clinical symptoms, body weight or macroscopy was available from these studies. Animals were observed for a total of 10 days (Bächtold, 1976).

In the range finding test for a micronucleus test in mice (Meerts, 2002) 2000 mg/kg bw dosed orally caused 4 of 8 animals to die within 1.5 hours. At 1500 mg/kg bw no mortality was found. No LD_{50} can be drawn from this study. Clinical signs were reported for all animals treated with 2000 mg/kg bw. Within 1.5 hours, all animals showed lethargy or convulsions, one animal had tremors. Lethargy and rough coat was also observed within 1.5 hours after treatment with 1500 mg/kg bw. At both doses the survivors showed no abnormalities anymore after 2 and 3 days.

Studies in Humans

No data available.

Conclusion

The LD_{50} of DL-lactone via the oral route is > 2000 mg/kg bw.

3.1.3 Irritation

Skin Irritation

Studies in Animals

DL-lactone was not irritating to the skin of rabbits after 4 hours exposure under semi-occlusion (according to OECD 404). No symptoms of systemic toxicity were observed during the 72-hours treatment period (Teunissen, 2005a).

Studies in Humans

Single instances of dermal irritation in production workers after prolonged and intensive exposure have been noted; but there are no studies available (R. Strobel and A. Flückiger, 1995).

Eye Irritation

Studies in Humans

DL lactone may cause irritation upon direct contact in occupational settings (unpublished data).

Conclusion

Based on occupational exposure experience in humans DL-lactone is expected to be irritating to the eyes and upon prolonged and intensive exposure also to the skin.

3.1.4 Sensitisation

Studies in Animals

Skin

In a guinea-pig maximisation test performed according to OECD 406, no sensitising potential of DL-lactone was found. Animals were induced with a 5% solution (intracutaneous) and a 50% solution (epicutaneous in presence of SDS to elicit irritation) and challenged with a 50% solution. The concentrations applied were selected based on the outcome of a preliminary irritation study. No skin reactions were seen after the challenge exposure in both experimental and control animals (Teunissen, 2005b).

Studies in Humans

No data available

Conclusion

Based on the guinea-pig maximisation test it can be concluded that DL-lactone is not sensitising to the skin.

3.1.5 Repeated Dose Toxicity

For repeated dose toxicity a combined 28-day reproduction/developmental toxicity screening study was available, performed according to OECD 422.

Studies in Animals

Oral

Rats (10/sex/treatment) were treated by gavage with 0, 40, 200 and 1000 mg/kg bw DL-lactone during 14 days prior to mating (1:1) and 14 days (males) or maximum 56 days (females) thereafter. Females were allowed to litter and to nurse their pups until day 4 of lactation. No mortality occurred. Females at 1000 mg/kg bw were aggressive and restless during day 5-15 of treatment. There were no treatment related effects on body weight, food consumption, haematology, clinical chemistry, organ weights, macroscopy and histopathology. Based on the behavioural effects in females the NOAEL was set at 200 mg/kg bw (Beekhuijzen, 2003).

Studies in Humans

No data available.

Conclusion

The NOAEL for repeated dose toxicity is 200 mg/kg bw based on aggression and restlessness of females treated at 1000 mg/kg bw during part of the study period.

3.1.6 Mutagenicity

Studies in Animals

In vitro Studies

An Ames test in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102 and TA1535 (OECD 471) gave negative results with and without metabolic activation. No toxicity was observed at the highest concentration tested (5000 µg/plate) (Gocke, 1999).

In vivo Studies

DL-Lactone at an oral dose of 1500 mg/kg bw did not induce any increase in the incidence of micronucleated polychromatic erythrocytes in an *in vivo* micronucleus test in mice performed according to OECD 474. Animals were treated with 0, 375, 750 and 1500 mg/kg bw (5/sex/treatment, single dose). The test groups treated with DL-lactone did not show any decrease in the ratio of normochromatic to polychromatic erythrocytes, which reflects a lack of toxic effects of DL-lactone on erythropoiesis (Meerts, 2002).

Studies in Humans

No data available.

Conclusion

Based on the outcome of the tests available, it can be concluded that DL-lactone does not possess mutagenic properties.

3.1.7 Carcinogenicity

No data available.

3.1.8 Toxicity for Reproduction

For reproduction toxicity a combined 28-day reproduction/developmental toxicity screening study was available, performed according to OECD 422.

Studies in Animals

Effects on Fertility/Developmental Toxicity

Rats (10/sex/treatment) were treated by gavage with 0, 40, 200 and 1000 mg/kg bw DL-lactone during 14 days prior to mating (1:1) and 14 days (males) or maximum 56 days (females) thereafter. Females were allowed to litter and to nurse their pups until day 4 of lactation[1].

[1] Day 1 of lactation was identified as the day when a litter was found completed (i.e. membranes, placentas cleaned up, nest build up and/or feeding of pups started)

Male spermatogenesis seemed to be unaffected: there were no observed changes in the testes (e.g. weight, and macroscopic lesions). At 40 mg/kg bw one female did not mate. Pregnancy rate was 8/10, 9/10, 8/10 and 9/10 at 0, 40, 200 and 100 mg/kg bw, respectively. Duration of gestation, fertility performance and number of live pups were similar for the control and treated groups. Pup mortality, weight, sex and viability did not differ between treatment groups and controls. No effects on reproductive organs and no effects on clinical signs, body weights and macroscopic examination of the pups during their lactation period were observed. The NOAEL for reproductive and developmental effects was ≥1000 mg/kg bw (Beekhuijzen, 2003).

Studies in Humans

No data available

Conclusion

Based on the outcome of the combined 28-day reproduction/developmental toxicity screening study, the NOAEL for reproductive and developmental effects is ≥1000 mg/kg bw.

3.2 Initial Assessment for Human Health

Animal studies on the acute toxicity of DL-lactone by the oral route of exposure are available. The acute oral LD_{50} was above 2000 mg/kg bw. DL-Lactone is irritating to the skin and the eyes in humans. No sensitisation potential is found in the guinea-pig maximisation test.

In a combined repeated dose reproduction/developmental toxicity screening study (OECD 422) female rats showed aggression and restlessness during part of the study period. In absence of other findings, the NOAEL for repeated dose toxicity was set at 200 mg/kg bw/day.

DL-Lactone does not induce gene mutations *in vitro*. The *in vivo* micronucleus test was negative.

In an OECD TG 422 repeated dose reproduction/developmental toxicity screening study with rats exposed to DL-lactone, no effects on reproductive performance, stage of spermatogenesis, pup mortality, weight, sex and viability were reported up to oral doses of 1000 mg/kg bw/day. Animals were dosed prior to and during mating, gestation and following gestation until lactation day 4. Based on the available data, DL-lactone does not show evidence of reproductive or developmental toxicity. The NOAEL for reproductive toxicity is ≥1000 mg/kg bw/day.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute toxicity tests on three trophic levels are available. All available tests are summarised in table 3.

Acute Toxicity Test Results

DL-Lactone was tested in a semi-static limit test in carps (96 hours exposure). At a measured concentration of 140 mg/L no mortality or other visible effects were observed. Therefore it was concluded that the LC_{50} for DL-lactone is >140 mg/L (Bogers, 1999a). No immobilised daphnids were observed after 48 hours exposure to a mean measured concentration of 130 mg/L in a semi-static limit test. The EC_{50} is >130 mg/L (Migchielsen, 1999). In a static test in algae (*Selenastrum capricornutum*) with DL-lactone no effects on algal biomass and growth rate were observed at the concentration tested (100 mg/L nominal). During the 72-hours test period the measured

concentration decreased from 105 mg/L (t=0) to 45 mg/L (t=72). The EC_{50} for both algal growth and growth rate is >78 mg/L (time weight average concentration) (Bogers, 1999b).

Table 3 Results from aquatic toxicity studies

Organism	Exposure time	Guideline	LC/EC_{50}	Reliability code	Reference
Cyprinus carpio	96 hours	OECD 203	>140 mg/L	1	Bogers, 1999a
Daphnia magna	48 hours	OECD 202	>130 mg/L	1	Migchielsen, 1999
Selenastrum capricornutum	72 hours	OECD 201	>78 mg/L	1	Bogers, 1999b

Toxicity to Micro-organisms

The toxicity towards micro-organisms was investigated in a study conducted according to OECD 209. The EC_{50} for D-pantolactone (one of the two isomers in DL-lactone) was > 100 mg/L (BASF, 2003). This finding was confirmed by the findings in the biodegradation studies, where no toxicity toward micro-organisms was observed.

4.2 Terrestrial Effects

No data available.

4.3 Other Environmental Effects

No data available.

4.4 Initial Assessment for the Environment

DL-Lactone is soluble in water and has a logKow of -0.69. Based on its pKa (>13) DL-lactone is most likely present in the unionised form under environmental conditions. The substance is expected to disappear from the aqueous environment via biodegradation (readily) and hydrolysis ($t_{1/2}$ 1 day to 1 year depending on the pH).

DL-Lactone does not bioaccumulate in fish and worms. The substance has an LC_{50} of >140 mg/L in fish, an EC_{50} >130 mg/L in daphnia's and an EC_{50} >78 mg/L (nominal 100 mg/L) in algae. Based on data on the toxicity towards micro-organisms of the d-isomer, the EC_{50} for micro-organisms is >100 mg/L.

5 RECOMMENDATIONS

DL-lactone is currently of low priority for further work

Human Health: The chemical possesses properties indicating a hazard for human health (skin and eye irritation). These hazards do not warrant further work as they are related to reversible effects. They should nevertheless be noted by chemical safety professionals and users.

Environment: The chemical is currently of low priority for further work because of its low hazard profile.

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SPARC On-Line Calculator: University of Georgia, GA, USA, online at http://ibmlc2.chem.uga.edu/sparc/index.cfm

Teunissen MS (2005a): Primary skin irritation/corrosion study with dl-lactone in the rabbit (4-hour semi-occlusive application). NOTOX 420975, 02.02.2005, on behalf of F. Hoffmann-La Roche Ltd.

Teunissen MS (2005b): Assessment of contact hypersensitivity to dl-lactone in the albino guinea pig (maximisation test). NOTOX 420986, 02.02.2005, on behalf of F. Hoffmann-La Roche Ltd.

TNO BIBRA (1995): Toxicity Profile Pantolactone. 1st ed, 1995. TNO BIBRA International Ltd, Carshalton, Surrey SM5 4DS, UK.

Unpublished data F. Hoffmann-La Roche Basel

USES 4.02 (2004) Uniform System for the Evaluation of Substances, version 4.02. © Rijksinsitut voor Volksgezondheid en Milieuhygiëne (RIVM), Bilthoven, The Netherlands.

Veith GD, DeFoe DL, Bergstedt BV (1979): Measuring and estimating the bioconcentration factor of chemicals in fish. J Fish Res Board Can 36: 1040-1048.

Willems H (1999): Determination of the partition coefficient (n-octanol/water) of dl-lactone. NOTOX Project 257603, on behalf of F. Hoffmann-La Roche Ltd, Basle, Switzerland, 19.08.1999.

S I D S

Dossier

Existing Chemical ID: 79-50-5
CAS No. 79-50-5
EINECS Name (±)-dihydro-3-hydroxy-4,4-dimethylfuran-2(3H)-one
EC No. 201-210-7
TSCA Name 2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-
Molecular Formula C6H10O3

Producer Related Part
Company: F.Hoffmann-La Roche Ltd
Creation date: 30-JUN-1995

Substance Related Part
Company: F.Hoffmann-La Roche Ltd
Creation date: 30-JUN-1995

Memo: "EU Existing Chemicals Program" Phase 3 (Anmeldung)

Printing date: 18-JAN-2006
Revision date: 30-JUN-1995
Date of last Update: **18-JAN-2006**

Number of Pages: **113**

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: sponsor country
Name: Switzerland
Contact Person: Dr Georg Karlaganis **Date:** 16-AUG-2004
Street: Federal Office for the Environment, Forests and Landscape
Town: CH-3003 Berne
Country: Switzerland
Email: georg.karlaganis@buwal.admin.ch
Homepage: http://www.umwelt-schweiz.ch/buwal/eng/index.html

16-AUG-2004

Type: lead organisation
Name: F. Hoffmann-La Roche Ltd
Contact Person: Dr Louis Schnurrenberger **Date:** 16-AUG-2004
Street: Corporate Safety & Environmental Protection, CSE 49/2.046
Town: CH-4070 Basle
Country: Switzerland
Phone: +41 616 886 638
Telefax: +41 616 881 920
Email: louis.schnurrenberger@roche.com
Homepage: www.roche.com

Remark: Several years ago, F. Hoffmann-La Roche committed itself to preparing the SIDS for dl-Lactone, CAS 79-50-5, within the OECD HPVC Programme. In the year 2003, the former Roche Vitamins & Fine Chemicals Division was taken over by DSM Company, the Netherlands, as DSM Nutritional Products. This is why the substance data are still referenced by Roche specifications and safety data sheets, however, the whole responsibility for production of dl-Lactone rests with the new owners, DSM.

17-AUG-2004

Type: manufacturer
Name: DSM Nutritional Products Limited
Contact Person: Dr Michael Matthes **Date:** 16-AUG-2004
Street: Wurmisweg 576; Health, Safety and Environment
Town: CH-4303 Kaiseraugst
Country: Switzerland
Phone: +41 616 883 333
Telefax: +41 616 883 330
Email: michael.matthes@dsm.com
Homepage: www.dsm.com

31-MAR-2005

1.0.2 Location of Production Site, Importer or Formulator

Type: manufacturer
Name of Plant: DSM Nutritional Products (UK) Limited
Town: KA24 5JJ Dalry, Ayrshire, Scotland
Country: United Kingdom
Phone: +44 1294 832345
Telefax: +44 1294 832700
Homepage: http://www.dsm.com/en_US/html/dnp/sites_dalry.htm

Remark: DSM Nutritional Products corresponds to the former Vitamins and Fine Chemicals Division of F. Hoffmann-La Roche Ltd.
16-AUG-2004

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

IUPAC Name: 2(3H)-Furanone, dihydro-3-dihydroxy-4,4-dimethyl
Smiles Code: O1C(=O)C(O)C(C)(C)C1
Mol. Formula: C6-H10-O3
Mol. Weight: 130.14

Remark: dl-Lactone (CAS 79-50-5, deleted CAS 52126-90-6) is a racemic mixture of D-Pantolactone [(3R)-dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, CAS 599-04-2] and L-Pantolactone [(3S)-dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, CAS 5405-40-3].
Reliability: (1) valid without restriction
source is maintained by the American Chemical Society respectively the Chemical Abstracts Services.
Flag: Critical study for SIDS endpoint
16-AUG-2004 (44)

1.1.1 General Substance Information

Purity type: measured for specific batch
Substance type: organic
Physical status: solid
Purity: 99.8 - % w/w
Colour: white
Odour: charcteristic

Result:

Product	RS Pantolactone
Description	DL-Lactone
Item No.	0421758 ((former Roche code))
Lot No.	Bx 226
Manufacturing Date	31-05-2002
Best use before	end 05-2003

Characteristic	Result	Specification
Appearance	crystalline mass	crystalline mass
Colour	white	slightly yellow, white
Odour	characteristic	characteristic, faint
Spec Rotation (589 nm, 20 °C, C=3 water)	0.0°	-0.3 to 0.3°
Water	0.04%	0 to 0.5%
Free acid	0.24%	0 to 2.0%
Assay (as dry)	99.8%	98.0 to 100.5%

Reliability: (1) valid without restriction

Quality assurance SQS Certificate ISO 9001:2000, hence reliability undoubted.
17-AUG-2004 (16)

Purity type: other: specifications
Substance type: organic
Physical status: solid
Purity: 98 - 100.5 % w/w
Colour: white to slightly yellow
Odour: faint, characteristic, butyric acid smell

Remark: crystalline powder or mass
specific rotation -0.3 to 0.3 (589 nm, 20 °C, C=3 water)
water content 0 to 0.5%
free acid 0 to 2.0%
assay (dry) 98.0 to 100.5%
17-AUG-2004 (41) (47)

1.1.2 Spectra

Type of spectra: IR

Result: An Infrared Spectrum for "Dl-pentoyllactone", CAS 79-50-5, is available from the NIST Webbook (EPA-IR Vapor Phase Library).
21-SEP-2004 (38)

Type of spectra: mass spectrum

Result: A Mass Spectrum for "Dl-pentoyllactone", CAS 79-50-5, is available from the NIST Webbook (EPA MS number 134315).
21-SEP-2004 (38)

1.2 Synonyms and Tradenames

dl-.alpha.-Hydroxy-.beta.,.beta.-dimethyl-.gamma.-butyrolactone

18-AUG-2004 (44)

.alpha.-Oxy-.beta.,.beta.-dimethyl-.gamma.-butyrolactone

18-AUG-2004 (44)

Dihydro-3-hydroxy-4H-dimethyl-2(3H)-furanone

18-AUG-2004 (44)

RS-Dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone

18-AUG-2004

(RS)-Pantolactone

18-AUG-2004 (44)

(±)-Pantolactone

18-AUG-2004 (44)

Pantothenic lactone

18-AUG-2004 (56)

DL-Pantolactone

18-AUG-2004 (44)

dl-Pantoyllactone

18-AUG-2004 (44)

1.3 Impurities

Purity type: other: Specifications
CAS-No: 470-29-1
EINECS-Name: 2,4-Dihydroxy,3,3-dimethyl butyric acid
Mol. Formula: C6-H12-O4
Contents: 0 - 2 % w/w

17-AUG-2004

Purity type: other: Specifications
CAS-No: 7732-18-5
EC-No: 231-791-2
EINECS-Name: water
Mol. Formula: H2-0
Contents: 0 - .5 % w/w

17-AUG-2004

1.4 Additives

1.5 Total Quantity

Quantity: ca. 1000 - 5000 tonnes produced in 2004

Remark: Industry estimate of worldwide production, based on extrapolation of own share of global total.
Reliability: (4) not assignable
Estimate based on known own production and extrapolation according to estimated market share, fraught with some uncertainty.
13-JUN-2005 (45)

1.6.1 Labelling

Labelling: no labelling required (no dangerous properties)

Remark: based on available physico-chemical, toxicological and environmentally relevant substance data
16-AUG-2004 (41)

1.6.2 Classification

Classified: no classification required (no dangerous properties)

Remark: based on available physico-chemical, toxicological and environmentally relevant substance data

16-AUG-2004 (41)

1.6.3 Packaging

1.7 Use Pattern

Type: industrial
Category: Chemical industry: used in synthesis

21-JUL-1998

1.7.1 Detailed Use Pattern

Industry category: 3 Chemical industry: chemicals used in synthesis
Use category: 15 Cosmetics
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available
Processing: no

16-AUG-2004

Industry category: 3 Chemical industry: chemicals used in synthesis
Use category: 41 Pharmaceuticals
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available

16-AUG-2004

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Method: Isobutyraldehyde is reacted with formaldehyde in the presence of a catalyst to produce an aldol (the catalyst is recovered and partially recycled). The aldol is then converted to a cyanohydrin by reacting it with hydrogen cyanide, which is produced in situ by reacting sodium cyanide with sulphuric acid under pH control. Any cyanides remaining at the end of the reaction are removed by boiling and nitrogen purging the reaction. The cyanohydrin is reacted with excess sulphuric acid to produce DL-lactone, which is extracted from the aqueous reaction mixture into dichloromethane (DCM). The DCM/DL-lactone mixture is distilled to recover the DCM and isolate crude DL-lactone.

Crude DL-lactone is purified by distillation, with the resulting residue being sent off site for disposal. The purified DL-lactone is either transferred to [the next production step] for the production of R-pantolactone or transferred to [the next production step] for the production of RS-pantothenyl ether or RS-panthenol.

Reliability: (2) valid with restrictions
13-JUN-2005 (45)

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: other: Internal Occupational Exposure Limit
Limit value: 10 mg/m3

Result: No IOEL established based on toxicological data, the limit value for inert dust was adopted.
17-AUG-2004 (41)

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: own classification according to German VwVwS dated 17.05.1999
Class of danger: 1 (weakly water polluting)

16-AUG-2004

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

Type: NDSL
Additional Info: Canada Gazette, Part I, January 31, 1998

Reliability: (1) valid without restriction
16-AUG-2004 (44)

Type: TSCA
Additional Info: May 2004 Inventory tape

Reliability: (1) valid without restriction
16-AUG-2004 (44)

Type: other: SPIN database (substances used in preparations in Nordic countries: N, S, DK, SF)
Additional Info: Not listed, no use identified.

Reliability: (1) valid without restriction
Database maintained by government agencies, considered fully reliable.
13-JUN-2005 (48)

Type: Poisonous Chemicals List (Switzerland)
Additional Info: Not listed.

Reliability: (1) valid without restriction
Database maintained by government agency, considered fully reliable.
13-JUN-2005 (53)

Type: EINECS
Additional Info: no. 201 210 7

Reliability: (1) valid without restriction
16-AUG-2004 (15) (44)

Type: ENCS
Additional Info: no. 9-997X

Reliability: (1) valid without restriction
16-AUG-2004 (44)

Type: other: ASIA-PAC

16-AUG-2004 (44)

1.9.1 Degradation/Transformation Products

Type: degradation product in water
CAS-No: 470-29-1
EINECS-Name: Butanoic acid, 2,4-dihydroxy-3,3-dimethyl-
IUCLID Chapter: 3.1.2 Stability in Water

Remark: 2,4-Dihydroxy-3,3-dimehtyl-butanoic acid (=pantoic acid) is the probable hydrolysis product of dl-lactone, through opening of the butyrolactone cyclic ester in the hydrolysis pretest. However, this product was not confirmed analytically.
Reliability: (2) valid with restrictions
Highly probable from a chemist's expectation but not confirmed through analytics, hence reliability 2.
26-AUG-2004

1.9.2 Components

1.10 Source of Exposure

Source of exposure: Environment: exposure from production
Exposure to the: Substance

Result: Based on a production mass balance at the then Roche manufacturing plant at Dalry for the year 2004, subtracting measured yields from measured educts and incorporating information on measured concentrations in aqueous effluents,

a maximum of 0.4% of the total produced DL-lactone was estimated to be lost to aqueous effluent treated in the on-site sewage works and a maximum of 0.75% of the total produced DL-lactone was estimated to be lost with the distillation residues which are used (incinerated) as CEMfuel; losses to the atmosphere were estimated to be 0%, due to the closed production system.

Reliability: (2) valid with restrictions
Communication from production site production manager.

05-JAN-2006 (45)

Source of exposure: Human: exposure by production
Exposure to the: Substance

Result: DSM Nutritional Products Dalry has conducted a risk assessment programme for all chemicals on its site, called COSHH (Control of Substances Hazardous to Health) in accordance with the applicable UK legislation. The results of the COSHH assessment programme for the DL-lactone production process indicate that occupational exposure to DL-lactone is very low, due to the semi-liquid nature of this intermediate and the closed system in which it is to be found.
Exposure to DL-lactone mainly occurs through completion of process sampling and potentially during drumming-off operations. The in-process sampling schedule for DL-lactone at sampling port A is 6 samples per month with a potential exposure duration of 1 minute/sample, for DL-lactone 90% in water at sampling port B is 1 sample per day with a potential exposure of 1 minute/day and for DL-lactone 90% in water at sampling port C is 1 sample per day with a potential exposure of 1 minute/day.

Reliability: (2) valid with restrictions
Communication from production site production manager.

13-JUN-2005 (45)

1.11 Additional Remarks

Memo: Natural occurrence and formation?

Result: The Merck Index (1996) describes Pantolactone as a "degradation product of pantothenic acid from liver: Williams, Major, Science 91, 246 (1940)". Based on this statement, the TNO (1995) Toxicity Profile on Pantolactone states that "Pantolactone [presumably the D- form] is a degradation product of D-pantothenic acid. The latter is a member of the B-complex vitamins and occurs widely in animal and plant tissue, the animal liver being the richest common source (Merck 1989)".
Both statements are clear but not quite unambiguous. The tempting conclusion that Pantolactone, either D- or L- or dl-lactone, is a natural degradation product that is present in the liver, is not substantiated by the original source. Both Williams and Major (1940) and Wolley et al. (1939) did not find Pantolactone in the liver but degraded a substance from liver extracts that was later identified as pantothenic acid; one cleavage product was beta-alanine, the other was identified as a hydroxy acid (later shown to correspond to pantoic acid), which "easily forms a lactone" (Wolley et al,

1939, p 679), viz. Pantolactone.

Reliability: (4) not assignable

21-SEP-2004 (34) (63) (64)

1.12 Last Literature Search

Type of Search: Internal and External
Date of Search: 02-DEC-2004

15-MAR-2005

1.13 Reviews

Memo: TNO BIBRA Toxicity Profile Pantolactone (1995)

Reliability: (2) valid with restrictions
Professional précis of toxicity information from a serious, highly regarded company.

18-AUG-2004 (56)

2.1 Melting Point

Value: = 78 degree C

Method: other: not stated
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
25-AUG-2004 (22)

2.2 Boiling Point

Value: = 247 degree C

Method: other: not stated
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
16-AUG-2004 (22)

2.3 Density

Type: density
Value: ca. 1.17 g/cm³ at 20 degree C

Method: other: not stated
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
16-AUG-2004 (22)

Type: bulk density
Value: .59 g/cm³

21-JUL-1998 (57)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .7038 hPa at 60 degree C

Method: other (measured): exact method not stated
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
16-AUG-2004 (22)

Value: = .0844 hPa at 25 degree C

Method: other (calculated)
Year: 2004
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Substance basic data for dl-Lactone were entered into USES 4.02, including the experimental vapour pressure at 60 °C, USES provides an extrapolation to the vapour pressure at 25 °C.
Reliability: (2) valid with restrictions
Computerised application of the EU Technical Guidance Document, prepared by professional Dutch state institution, with high quality assurance.
Flag: Critical study for SIDS endpoint
19-AUG-2004 (60)

Value: = 5.106 hPa at 100 degree C

Method: other (measured): exact method not stated
GLP: no

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
19-AUG-2004 (22)

Value: = 1366 hPa at 260 degree C

Method: other (measured): exact method not stated
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
19-AUG-2004 (22)

Value: ca. .102 hPa at 20 degree C

Method: other (calculated): extrapolated from measured values at 60, 100 and 260 °C
Year: 2004
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The three measured values were entered into a SigmaPlot spreadsheet, based on which a log-log graph of temperature (K) vs pressure (hPa) was drawn. The SigmaPlot regression line has the equation: f(x) = x*b[1] - b[0], where
b[0] = -40.2665002287
b[1] = 15.9193396988, with
r^2 = 0.9997113091.
Result: Based on the equation parameters described in Methods, a vapour pressure of approximately 0.102 hPa was extrapolated for a temperature of 20 °C.
Reliability: (2) valid with restrictions
Rational but coarse extrapolation, fraught with some uncertainty.
19-AUG-2004

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = -.69 at 21 degree C
PH prec: = 6.3 - 6.5

Method: OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year: 1999
GLP: yes

Method: Based on a preliminary calculation of the n-octanol/water partition coefficient by the Rekker method, which resulted in a logKow of -0.02, the flask-shaking method OECD 107 was selected for the experimental determination of the logKow. The (acidic) pKa for dl-lactone was modelled by NOTOX to be 13.2, hence the water phase was not buffered for the test.
According to the OECD 107 method, a stock solution was prepared by dissolving 64.6 mg dl-lactone in 25.0 ml double-distilled water that had been saturated with n-octanol (99% HPLC Grade, Sigma-Aldrich, USA). In order to dissolve the test substance, the solution was sonicated for for 5 min. Three tests were carried out at 21±0.5°C using different volumes of n-octanol and double-distilled water (mutually saturated). The volumetric ratios of water and octanol were 1:2, 1:1 and 2:1, respectively. For each test, duplicate vessels containing the required, accurately measured amounts of the two solvents and the stock solution were prepared and shaken by hand during 5 min. Phase separation was achieved by centrifugation for 5 min at 3500 g and 20°C. The pH of each aqueous phase was determined.
For the preparation of a blank, a vessel containing equal amounts of octanol saturated with water and water saturated with octanolwas shaken, centrifuged and analysed similar to the test substance vessels.

Samples from all vessels were taken with a syringe for the aqeous phase and a pipette for the octanol phase. Aqueous phase samples were diluted with mobile phase (see below) prior to analysis, octanolphase samples were diluted first with acetonitrile (HPLC grade, Labscan Co, Dublin, Ireland) and then with mobile phase prior to analysis. dilution factors were chosen in such a way that the final test substance concentrations fell well within the calibration range.
The concentration of dl-lactone in the two phases was determined using an HPLC method:

Column	LiChrospher 100RP-18, 250*4 (i.d.) mm, d(rho)= 5 µm (Merck, Germany)
Mobile phase	20/80/0.1 (v/v/v) acetonitrile/Milli-Q water/formic acid
Flow	1 ml/min
Detection	SCIEX MSMS system API-300 mass spectrometer (Perkin Elmer, USA)
Interface	ion-spray, positive mode
Monit masses	MRM m/z 131.3 --> 113.0 (test substance) MRM m/z 127.2 --> 98.9 (internal standard)
Inject volume	100 µl
Int standard	4-hydroxy-6-methyl-2-pyrone (98%, Sigma-Aldrich, USA)

Calibration solutions were made up from stock solutions on each day of analysis.

Result: Partition coefficients of the single vessels

n-Octanol/water ratio	Kow	logKow	pH(aq)
1:1 (blank)	-	-	
1:1	0.219	-0.66	6.3
1:1	0.223	-0.65	6.3
1:2	0.203	-0.69	6.5
1:2	0.198	-0.70	6.5
2:1	0.190	-0.72	6.4
2:1	0.179	-0.75	6.4
Artihmetic average	0.202±0.017	-0.69	

Test substance: dl-Lactone from Roche Dalry, batch no. 805046, purity 100.0% as per the CoA.

Conclusion: Based on an experimental n-octanol/water partition coefficient, dl-lactone is not expected to partition significantly to organic phases or tissues, but rather to remain in aqueous phases.

Reliability: (1) valid without restriction
OECD 107 test under GLP.

Flag: Critical study for SIDS endpoint

17-AUG-2004 (62)

Partition Coeff.: water - air

Method: other (calculated): QSAR modelling and approximation
Year: 2004
GLP: no

Result:

Henry's Law Constant KH, atm*m3/mol	Source
	EPISuite v3.11 (Henry v3.10):
2.82E-5	bond estimate;
2.05E-10	VP estimate/Wsol estimate;
-	no group estimate
4.31E-9	SPARC On-Line (25 °C)

	1.31E-8	0.102 hPa extrapolated VP divided by 1000 g/l WS, units converted to atm*m3/mol
	1.02E-8	USES 4.02, 1.03E-3 Pa*m3/mol converted to atm*m3/mol

Conclusion: Based on accepted QSAR programs, the Henry's Law Constant KH for dl-lactone is expected to be smaller than 1E-4 atm-m3/mol. In view of the highest value, there may still be some partitioning into the air from aqueous solutions.
Reliability: (2) valid with restrictions
Accepted QSAR programmes respectively accepted approximation.
Flag: Critical study for SIDS endpoint
10-MAR-2005 (20) (46) (60)

Partition Coeff.: soil-water
log Pow: < 10

Method: other (calculated): QSAR modelling
Year: 2004
GLP: no

Result:

QSAR Organic-carbon/water partition coefficient, Koc	Source
1	EPISuite v3.11 (PCKOCWIN v1.66)
8.74 (pH 1-8)	SCiFinder (ACD Solaris V4.67)
8.73 (pH 10)	SCiFinder (ACD Solaris V4.67)
0.348	USES 4.02

Conclusion: Based on accepted QSAR programs, the organic-carbon/water partition coefficient Koc for dl-lactone is expected to be smaller than 10. Hence, dl-lactone is not expected to adsorb significantly to organic carbon in activated sludge, soil or sediment.
Reliability: (2) valid with restrictions
Accepted QSAR programmes.
Flag: Critical study for SIDS endpoint
10-MAR-2005 (20) (44) (60)

Result: Activated-sludge to water partition coefficient (Kd) was determined in parallel with the OECD301B GLP ready biodegradation test at 3 hours from adding substrate to activated sludge at two concentrations:

dl-lactone added	activated sludge	Kd
22 mg/l	30 mg/l sterilised	no significant adsorption
100 mg/l	1000 mg/l not sterilised	~220 l/kg (3h)

Adsorption in the 100 mg dl-lactone/1000 mg/l activated sludge remained constant for the first day, then DOC measurements declined in parallel with the biodegradation test kinetics, which is interpreted to show biodegradation of dl-lactone.
Conclusion: At comparatively high concentrations of both activated sludge (1000 mg/l) and dl-lactone (100 mg/l), there is some short-term adsorption with a 3-hour Kd of ~220 l/kg, but at lower concentrations (30 mg activated sludge/l, 22 mg dl-lactone/l) there was no significant adsorption as determined by DOC.
Reliability: (1) valid without restriction

Parallel tests to OEDC biodegradation test, performed under GLP.
10-MAR-2005 (18)

2.6.1 Solubility in different media

Solubility in: Water
Descr.: other: of very high solubility, > 500000 mg/l

Method: other: exact method not stated
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Aqueous solubility is extremely high for dl-lactone and was never a limiting factor in production. Besides, due to a tendency for hydrolysis (see chapter 3.1.2), no prolonged stirring or sonication could be used for exact results.
Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
17-AUG-2004 (22)

Solubility in: Water
Value: 260 - 2518 g/l

Method: other: various broadly accepted QSAR programmes
Year: 2004
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result:

QSAR water solubility	Source
260000 mg/l	IA logS
289320 mg/l	EPISuite v3.11 (WATERNT v1.01)
340000 mg/l	ALOGPS 2.1
336900 mg/l	EPISuite v3.11 (ECOSAR v0.99g)
994400 mg/l	EPISuite v3.11 (WSKOW v1.41)
2518147 mg/l	SPARC On-Line Calculator
very soluble (pH 1-10)	SCiFinder (ACD Solaris V4.67)

(less than 1 part of solvent required for 1 part of solute, i.e. >1000000 mg/l)
Conclusion: Based on accepted QSAR programs, the aqueous solubility for dl-lactone is expected to be greater than 260 g/l.
Reliability: (2) valid with restrictions
Accepted QSAR programmes.
Flag: Critical study for SIDS endpoint
17-AUG-2004 (2) (20) (27) (44) (46)

2.6.2 Surface Tension

2.7 Flash Point

Value: = 122 degree C

Type: open cup

Method: other: DIN guideline 51 794
Year: 1981
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test substance: "DL-Lacton destilliert" (DL-Lactone distilled), no actual purity given.
Conclusion: Pure, distilled dl-lactone has a flash point of 122 °C.
Reliability: (2) valid with restrictions
Internal data, more than 20 years old, acquired by company-internal physico-chemical properties laboratory. Only a brief reference to the DIN method used is available, but data from this database are used and trusted within the company.
16-NOV-2005 (43)

2.8 Auto Flammability

Value: ca. 400 degree C

Method: other: not stated
Year: 1981
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Pure, distilled dl-lactone has a high auto-ignition temperature at 400 °C.
Reliability: (2) valid with restrictions
Internal data, more than 20 years old, acquired by company-internal physico-chemical properties laboratory. Only a brief reference to the DIN method used is available, but data from this database are used and trusted within the company.
16-NOV-2005 (43)

2.9 Flammability

Result: other: low flammability

Method: other: according to Abel-Pensky, DIN guidelines 53 169, 51 755 and 53 213
Year: 1981
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Flammability temperature = 122 °C.
Test substance: "DL-Lacton destilliert" (DL-Lactone distilled), no actual purity given.
Conclusion: Pure, distilled dl-lactone has a flammability temperature of 122 °C and is therefore not a highly flammable substance.
Reliability: (2) valid with restrictions
Internal data, more than 20 years old, acquired by company-internal physico-chemical properties laboratory. Only a brief reference to the DIN method used is available, but data from this database are used and trusted within the company.

05-JAN-2006 (43)

2.10 Explosive Properties

Result: other: moderate dust explosion hazard

Method: other: Hartmann tube dust explosion test
Year: 1979
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Exactly measured amounts of test substance were placed in the Hartmann apparatus, a dust aerosol was produced with an air blast and a high-voltage electrical discharge produced a spark in the aerosolised dust. The occurrence, rapidity and strength of a dust explosion was determined by measuring the opening (or not) and its rate of the cover of the Hartmann tube.
Result: dl-Lactone produced a moderate dust explosion at dust concentrations of 500 g/m3 and higher.
Test substance: dl-Lactone technical, purity 98.9% according to
Conclusion: Crystalline dl-lactone has a certain dust explosion hazard. Dry dl-lactone must therefore only be handled with technical and organisational precautions, eg, closed systems, if possibly under inert gas atmosphere, with grounding of all installations and handling precautions to avoid and minimise dust formation.
Reliability: (2) valid with restrictions
Professional industry safety laboratory standard test.
25-AUG-2004 (57)

2.11 Oxidizing Properties

2.12 Dissociation Constant

Acid-base Const.: acidic group pKa >= 13.1

Method: other: QSAR calculation
Year: 2004
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result:

QSAR pKa for acidic group	Source
13.11±0.20	SciFinder (ACD Solaris V4.67)
13.2	Willems (1999): PKalc 2.0
>14	SPARC On-Line

Conclusion: Based on accepted QSAR programs, the acidic dissociation constant pKa for dl-lactone is expected to be greater than 13. Therefore, dl-lactone is not expected to be present in ionised, charged for at environmentally relevant pH ranges.
Reliability: (2) valid with restrictions
Accepted QSAR programmes.
Flag: Critical study for SIDS endpoint
17-AUG-2004 (44) (46) (62)

2.13 Viscosity

2.14 Additional Remarks

Memo: hygroscopic, up to deliquescence when exposed to high relative humidity for longer time

25-AUG-2004 (6)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH

Method: other (calculated)
Year: 1997
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Rorije and colleagues computed QSAR atmospheric half-lives for 1073 high production volume chemicals using the AOP v1.83 program (nowadays integrated in EPISuite, please see there) and the MOOH method described by Klamt [Klamt A (1993): Estimation of gas-phase hydroxyl radical rate constants of organic compounds from molecular orbital calculations. Chemosphere 26(7): 1273-1289; Klamt A (1996): Estimation of gas-phase hydroxyl radical rate constants of oxygenated compounds based on molecular orbital calculations. Chemosphere 32(4): 717-726].

Result:

QSAR half-life, h	Mediator	Source
6.27	OH radicals	AOP v1.83
- (none predicted)	O3	AOP v1.83
38.801	OH radicals	EPISuite v3.11 (AOP v1.91)
21.88	OH radicals	MOOH model

-
22.3 average value, will be used for further modelling.

Assumed OH radical concentration in all models:
1.5*10E6 molecules/(cm3 * 12-hour day)

Reliability: (2) valid with restrictions
The authors used rational QSAR methods to predict degradation rates in a project sponsored and quality-controlled by the Dutch governmant.
Flag: Critical study for SIDS endpoint
20-AUG-2004 (20) (40)

3.1.2 Stability in Water

Type: abiotic
t1/2 pH4: > 1 year at 25 degree C
t1/2 pH7: 1 - 365 day(s) at 25 degree C
t1/2 pH9: 1 - 365 day(s) at 25 degree C
t1/2 pH 7 **:** ca. 30.1 day(s) at 25 degree C
Degradation: = 57 % after 5 day(s)
at pH 7 and 50 degree C
Deg. products: not measured

Method: Directive 92/69/EEC, C.7
Year: 1999
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Sterile buffer solutions were prepared for target pH 4, 7 and 9 as follows:
pH 4 acetate buffer: sodium acetate, acetic acid (both p.a., Merck) and Milli-Q water;

pH 7 phosphate buffer: potassium dihydrogen phosphate, sodium hydroxide(both p.a., Merck) and Milli-Q water;
pH 9 borate buffer: boric acid, potassium chloride, sodium hydroxide (all p.a., Merck) and Milli-Q water.
Test solutions were prepared at concentrations of 0.05 M for pH 4,7 and 9 and additionally 0.5 M for pH 9. Amounts of between 63 and 66 mg test substance were accurately weighed into 50.0 ml buffer solutions. After sonication for 5 min, the solutions were filter-sterilised through a 0.2-µm membrane filter (FP 030/3, Schleicher & Schuell, The Netherlands) and transferred into sterile glass vessels. To exclude oxidation from dissolved oxygen, nitrogen gas was bubbled through each solution for 5 min. Then, each vessel was tightly sealed with a septum-crimpcap. After preparation, the test vessels were plced in a thermostatically controlled waterbath at 50±0.5 °C in the dark.
The concentration of the test substance was determined immediately after preparation (t = 0), after 2.4 h and after 5 d by HPLC (see below). For each test solution, the pH value at room temperature was determined for each sample taken.
2.ml samples were taken at the predetermined time and cooled to room temperature. Prior to analysis, the test solutions in 0.05 M buffer were diluted by a factor of 100 with mobile phase to obtain concentrations within the calibration range, the 0.5-M solution at pH 9 was diluted 25 times with mobile phase. As an internal standard, 4.hydroxy-6-methyl-2-pyrone was added to a final concentration of 2.04 mg/l. On the first day of the test, blank buffer solutions were diluted with mobile phase by the same factor as the corresponding test solutions and 4-hydroxy-6-methyl-2-pyrone was added to a final concentration of 2.04 mg/l.
The concentration of dl-lactone in the dilued samples was determined using an HPLC method:

Column	LiChrospher 100RP-18, 250*4 (i.d.) mm, d(rho)= 5 µm (Merck, Germany)
Mobile phase	20/80/0.1 (v/v/v) acetonitrile/Milli-Q water/formic acid
Flow	1 ml/min
Detection	SCIEX MSMS system API-300 mass spectrometer (Perkin Elmer, USA)
Interface	ion-spray, positive mode
Monit masses	MRM m/z 131.3 --> 113.0 (test substance) MRM m/z 127.2 --> 98.9 (internal standard)
Inject volume	100 µl
Int standard	4-hydroxy-6-methyl-2-pyrone (98%, Sigma-Aldrich, USA)

Remark: The low recovery of dl-lactone in both pH 9 test solutions at the start of the test (t = 0) suggests rapid hydrolysis in water at elevated pH values. Therefore, hydrolysis rates and half-lives were also computed relative to the nominal substance concentration.
No degradation product was identified by analytical method, but, assuming hydrolysis of the cyclic lactone ester, the main degradation product to be expected would be 2,4-dihydroxy-3,3-dimethyl-butanoic acid (= pantoic acid, CAS 470-29-1 for the dl form, CAS 1112-32-9 for the d form and CAS 1112-33-0 for the l form).

Result:

pH		dl-lactone, mg/l, at 50 °C, after		
target	measured(1)	t = 0 h	t = 2.4 h	t = 5 d

4(0.05M) 4.0/4.0/4.0	1361	1278	1269
7(0.05M) 7.0/7.0/6.7	1202	1231	514
9(0.05M) 8.4/8.4/8.3	339	226	42.7
9(0.5M) 9.0/8.9/8.9	81.4	81.1	21.3

(1): measured pH at t=0 h/t=2.4 h/t=5 d

Half-lives and rate constants at 50 °C relative to measured concentrations at t = 0

pH	t½, h	t½, d	k
4(0.05M) avg pH 4.0	1184.4	49.5	0.000583
7(0.05M) avg pH 6.9	97.9	4.1	0.007079
9(0.05M) avg pH 8.37	40.1	1.7	0.017265
9(0.5M) avg pH 8.93	62.0	2.6	0.011172

Half-lives and rate constants at 50 °C relative to nominal concentrations at preparation of test solutions

pH	t½, h	t½, d	k
4(0.05M) avg pH 4.0	3446.3	143.6	0.000201
7(0.05M)	89.6	3.7	0.007732
9(0.05M)	24.3	1.0	0.028466
9(0.5M)	20.2	0.8	0.034300

Extrapolated half-lives and rate constants at 25 °C measured concentrations at t = 0 and EU TGD formula (2)

pH	t½, h	t½, d	k
4(0.05M) avg pH 4.0	8781.3	365.9	7.894E-5
7(0.05M) avg pH 6.9	725.5	30.1	0.000958
9(0.05M) avg pH 8.37	296.7	12.4	0.002337
9(0.5M) avg pH 8.93	458.4	19.1	0.001512

Extrapolated half-lives and rate constants at 25 °C relative to nominal concentrations at preparation of test solutions and EU TGD formula (2)

pH	t½, h	t½, d	k
4(0.05M) avg pH 4.0	25465.2	1061.0	2.722E-5
7(0.05M) avg pH 6.9	662.4	27.6	0.001046
9(0.05M) avg pH 8.37	179.9	7.5	0.003852
9(0.5M) avg pH 8.93	149.3	6.2	0.004642

Extrapolated half-lives and rate constants at 12 °C relative to measured concentrations at t = 0 and EU TGD formula (2)

pH	t½, h	t½, d	k
4(0.05M) avg pH 4.0	24844.1	1035.2	2.79E-5
7(0.05M) avg pH 6.9	2046.9	85.3	0.000339
9(0.05M) avg pH 8.37	839.3	35.0	0.000826
9(0.5M)	1297.0	54.0	0.000534

Extrapolated half-lives and rate constants at 12 °C relative to nominal concentrations at preparation of test solutions and EU TGD formula (2)

pH	t½, h	t½, d	k
4(0.05M) avg pH 4.0	72046.6	3001.9	9.621E-6
7(0.05M) avg pH 6.9	1874.0	78.1	0.000370
9(0.05M) avg pH 8.37	509.0	21.2	0.001361
9(0.5M) avg pH 8.93	422.5	17.6	0.001641

---

(2) $t_{½}(x\ °C) = t_{½}(50\ °C) * e\ exp[0.08*(50-x)]$,
EU TGD (2003), Part II, p. 49, equation (25).

Test substance: dl-Lactone from Roche Dalry, batch 805046, purity 100.0% according to certificate of analysis.

Conclusion: Based on an EU hydrolysis pretest at 50 °C, at a standard temperature of 25 °C dl-lactone is broadly expected to be stable (= hydrolysis half-life greater than 1 year) at pH 4 and to have a hydrolysis half-life between 1 day and 1 year at pH 7 and 9.
Extrapolating half-lives using a EU TGD formula, at 25 °C dl-lactone is expected to have a hydrolysis half-life of approximately 1-3 years at pH 4, of approximately 30 days at pH 7 and of approximately 6-20 days at pH 9.
Extrapolating half-lives using a EU TGD formula, at an environmentally relevant temperature for middle to northwestern Europe of 12 °C, dl-lactone is expected to have a hydrolysis half-life of approximately 3-8 years at pH 4, of approximately 78-85 days at pH 7 and of approximately 18-54 days at pH9.
In conclusion, dl-lactone is expected to be hydrolytically stable at low pH values and to be prone to moderate hydrolysis with higher pH values.

Reliability: (1) valid without restriction
Study according to international protocol under GLP.

Flag: Critical study for SIDS endpoint

16-NOV-2005 (10) (21)

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: other: distribution and fate in a sewage works model
Media: other: wastewater - activated sludge - air - effluent
Method: other: SimpleTreat Model v3.1
Year: 2005

Method: Physico-chemical basic data for DL-lactone were entered into the SimpleTreat 3.1 spreadsheet as follows:
Molecular weight = 130.14 g/mol, Kow = 2.04E-1 (based on logPow of -0.69), vapour pressure = 8.44 Pa, aqueous solubility = 1E6 mg/l, Ka = 7.94E-14 (based on pKa >= 13.1); the emission rate was set at 30 kg/d and the biodegradation rate constant set at 1/h as per the default value of the EU Technical Guidance Document for readily biodegradable substances.

Result: SimpleTreat 3.1 computed the following output values ion per cent of influent for DL-lactone in two types of sewage works (SW)

Compartments/ Removal	SW with primary sedimentation	SW without primary sedimentation
to air	0.0%	0.0%
to water	12.7%	8.5%
via sludge(s)	0.0%	0.0%

degraded	87.3%	91.5%
total	100.0%	100.0%

Conclusion: SimpleTreat 3.1 predicts 87.3-91.5% of DL-lactone to be biodegraded in activated-sludge type sewage works (with and without primary sedimentation) and the remaining 12.7% respectively 8.5% to be discharged with the effluent. No significant amounts are expected in the sludge or in the air.

Reliability: (2) valid with restrictions
Accepted QSAR models and guidance document.

05-JAN-2006 (51)

Type: other: distrbution and fate in a sewage works model
Method: other: computer model.
Year: 2005

Method: The following basic data for DL-lactone were entered into STP v1.50:
Molar mass = 130.14 g/mol, data temperature = 12 °C, water solubility = 1E6 g/m3, vapour pressure = 8.44 Pa, logKow = 0 (as STP v1.50 does not accept negative logKow values), half-life in 2000 mg MLSS/l 0.693 h for ready biodegradability in both primary, aeration and settling tanks. STP v1.50 Default values were used for the sewage treatment plant, chemical concentration in the influent was set at 4.3 g/m3.

Result: STP v1.50 predicts the following mass balance for DL-lactone:

Compartments	% of influent
to air	2.6E-5%
to sludge	0.467%
biodegraded	88.6%
final effluent	10.9%
total	99.97%

Conclusion: STP v1.50 predicts 88.6% of DL-lactone to be biodegraded in activated-sludge type sewage works (with primary sedimentation) and 10.9% to be discharged with the effluent. Just below 0.5% is expected in the sludge, no significant amounts are expected in the air.

Reliability: (2) valid with restrictions
Accepted QSAR model

05-JAN-2006 (49)

3.3.2 Distribution

Media: other: air - sediment - soil - water
Method: Calculation according Mackay, Level I
Year: 2004

Result: Level III Model v2.70, Level I static distribution without reaction, total mass in system = 10000 kg

	Air	Water	Soil	Sediment
Mass	23.2 kg	99958 kg	18.1 kg	0.402 kg
Percentage	0.0232%	99.958%	0.018%	0.0004%

Conclusion: In an infinite-time equilibrium distribution model without any reaction or advection, virtually all dl-lactone will partition

to the aquatic compartment.

Reliability: (2) valid with restrictions
Accepted QSAR model.

16-NOV-2005 (32)

Media: other: air - sediment - soil - water - suspended particles - biota

Method: Calculation according Mackay, Level II

Year: 2004

Result: Level III Model v2.70, Level II dynamic distribution with reaction and advection (substance flux out of system), constant emission rate into system (compartment not specified, ie, complete distribution) 1000 kg/h:

Compartment	Mass, kg	Percentage	Reaction, kg/h	Advection, kg//h
Air	70.1	0.023%	1.51	0.848
Water	3.03E5	>99.9%	695	303
Soil	54.7	0.018%	0.053	0
Sediment	1.22	4.02E-4%	0.001	2.43E-5
Susp. particles	0.038	1.26E-5	-	-
Fish	3.09E-3	1.02E-6	-	-

Losses from system:

Compartment	Advection, % total losses	Reaction, % total losses	Reaction half-life, h
Air	0.0701	0.218	22.3 (1)
Water	30.3	69.4	302 (2)
Soil	-	5.27E-3	720 (3)
Sediment	2.43E-6	1.17E-4	720 (3)
Total losses, %	30.37	69.62	

Overall residence time (system half-life), h	303
Overall reaction time, h	435
Overall advection time, h	998
Total mass in system, kg	30300

Reaction half-life inputs:
(1) QSAR atmospheric half-life from chapter 3.1.1.
(2) Aquatic half-life derived by adding rate constants for surface water biodegradation ($t_{½}$ = 15 d for readily biodegradable substances, TGD 2003, Part II, p. 54) and hydrolysis (see chapter 3.1.2) by using TGD 2003, Part II, equation 31, p. 57.
(3) Soil half-life for readily biodegradable substances according to TGD 2003, Part II, p. 56.

Conclusion: In a Level II dynamic distribution model with a constant emission rate into the system of 1000 kg/h, assuming complete fugacity-driven distribution, with reaction within and advection out of the system, dl-Lactone is predicted to partition virtually completely to the aquatic compartment, while partitioning into other compartments, specifically into biota, is very low. Water is also the most important compartment for substance losses, both through advection (accounting for 30.3% of total losses) and reaction by biodegradation and hydrolysis (69.4%). Losses in water add up to 99.7% while losses in the atmosphere through OH-radical-mediated degradation account for 0.2%.
The predicted overall average residence time in the system of

303 hours (12.6 d) suggests that dl-lactone is not a persistent substance.

Reliability: (2) valid with restrictions
Accepted QSAR models and guidance document.

24-AUG-2004 (20) (21) (32)

Media: other: air - sediment - soil - water - suspended particles - biota

Method: Calculation according Mackay, Level III

Year: 2004

Result: Level III Model v2.70, Level III dynamic distribution with reaction and advection (substance flux out of system), with a constant emission rate only into water of 1 kg/h, based on actual production of dl-lactone:

Compartment	Mass, kg	Percentage, %	Reaction, kg/h	Advection, kg/h
Air	1.36E-4	4.49E-5	4.24E-6	1.36E-6
Water	303	99.9	0.696	0.303
Soil	0.0148	4.88E-3	1.43E-5	-
Sediment	0.436	0.144	4.20E-4	8.83E-6
Susp. particles	3.81E-5	1.25E-5	-	-
Fish	3.10E-6	1.02E-6	-	-

Losses from system:

Compartment	Advection, % total losses	Reaction, % total losses	Reaction half-life, h
Air	1.36E-4	4.24E-4	22.3 (1)
Water	30.3	69.6	302 (2)
Soil	-	1.43E-3	720 (3)
Sediment	8.73E-4	0.042	720 (3)
Total losses, %	30.3	69.7	

Overall residence time (system half-life), h	304
Overall reaction time, h	436
Overall advection time, h	1001
Total mass in system, kg	304

Reaction half-life inputs:
(1) QSAR atmospheric half-life from chapter 3.1.1.
(2) Aquatic half-life derived by adding rate constants for surface water biodegradation (15 d for readily biodegradable substances, TGD 2003, Part II, p. 54) and hydrolysis (see chapter 3.1.2) by using TGD 2003, Part II, equation 31, p. 57.
(3) Soil half-life for readily biodegradable substances according to TGD 2003, Part II, p. 56.

Conclusion: In a Level III dynamic distribution model with a constant emission rate into the system of 1 kg/h exclusively into the aquatic compartment, based on production information, with reaction within and advection out of the system, dl-Lactone is predicted to partition virtually completely to the aquatic compartment, while partitioning into other compartments, specifically into biota, is very low. Water is also the most important compartment for substance losses, both through advection (accounting for 30.3% of total losses) and reaction by biodegradation and hydrolysis (69.6%). Losses in water add up to 99.9% while losses in all other compartments account for less than 0.1%.
The predicted overall average residence time in the system of

304 hours (12.7 d) suggests that dl-lactone is not a persistent substance.

Reliability: (2) valid with restrictions
Accepted QSAR models and guidance document.

20-AUG-2004 (20) (21) (32)

3.4 Mode of Degradation in Actual Use

Memo: Aqueous extracts from the dl-lactone synthesis in the Dalry production plant

Remark: Aqueous extracts from the dl-lactone synthesis step in the Dalry production plant enter the on-site wastewater treatment plant; based on a mass balance, amounts are estimated at approximately 30 kg dl-lactone/7000 m3 total site wastewater/day, corresponding to an influent concentrations of approximately 4.3 mg/l. Due to ready biodegradability, approximately 90% is estimated to be biodegraded and the remainder (~10%, ~0.43 mg/l) to be discharged with the effluent.
The effluent from this industrial plant subsequently enters the Dalry municipal sewage works serving approximately 70,000 inhabitants, with an average total influent of 54,000 m3/day, where first dilution (~0.056 mg/l) and then further biodegradation takes place. The final effluent containing approximately 0.006 mg DL-lactone/l is then discharged into the sea.
Biological degradation in two serial wastewater treatment plants, btoh with secondary (biological) treatment, is the main mechanism of degradation for residual dl-lactone (and by-products).

Reliability: (2) valid with restrictions
Communication from own production plant Safety & Environment Officer, high reliability.

05-JAN-2006 (39)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Concentration: 22 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 76 - 82 % after 28 day(s)
Result: readily biodegradable
Kinetic:
3 day(s) = 0 - 1 %
6 day(s) = 1 - 4 %
8 day(s) = 10 - 12 %
15 day(s) = 50 - 57 %
20 day(s) = 68 - 71 %
Control Subst.: Acetic acid, sodium salt
Kinetic:
3 day(s) = 27 %
15 day(s) = 68 %
Deg. product: not measured

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year: 2004
GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Method: Test substance concentrations
dl-Lactone was tested in duplicate at 43 mg per 2 litres, corresponding to 12 mg TOC/l. The organic carbon content was based on the molecular formula. Furthermore, an additional adsorption control bottle was prepared at 100 mg dl-Lactone/l. Since dl-Lactone was easily soluble in water the test media were prepared using a stock solution of 1 g/l in milli-RO water (tap water purified by reverse osmosis; Millipore Corp., Bedford, Mass., USA). A weighed amount of 1006.7 mg of dl-Lactone was dissolved in milli-RO water and made up to 1000 ml. The stock was a clear and colourless solution. Aliquots of 43 ml of the stock solution were added to the test medium, containing the microbial organisms, of test substance bottles A and B, toxicity control, the abiotic control and the adsorption control. An aliquot of 200 ml of the stock solution was added to the test medium of the additional adsorption control, resulting in a final dl-Lactone concentration of 100 mg/l. All test solutions were continuously stirred during the test, to ensure optimal contact between the test substance and the test organisms.

Reference substance: A solution of sodium acetate (>=99%, Merck) was prepared by dissolving 202.4 mg in Milli-RO water and making this up to a total volume of 50 ml. Volumes of 20 ml from this stock solution were added to 2 litres of the test medium of the positive control bottle and the toxicity control bottle, resulting in a final concentration of 40 mg/l sodium acetate (12 mg TOC/l).
Test system
Source: The source of test organisms was activated sludge freshly obtained from a municipal sewage treatment plant: 'Waterschap de Maaskant', 's-Hertogenbosch, the Netherlands, receiving predominantly domestic sewage.
Treatment: The sludge was kept under continuous aeration until further treatment. The concentration of suspended solids was 4.9 g/l in the concentrated sludge (information obtained from the municipal sewage treatment plant). The sludge was coarsely sieved and washed twice with tap-water. After washing the sludge was made up to the original volume. A small amount of the sludge was weighed and dried at approximately 105 °C to determine the amount of suspended solids. An amount of the sludge corresponding to approximately 30 mg/l suspended solids was added to the mineral medium, except for one bottle, an additional adsorption control, in which the amount of the sludge corresponded to approximately 1000 mg/l suspended solids.
On the day the sludge was sampled, a rough indication on the concentration of suspended solids was made (drying for 4 hours). This indication was used for the addition of the sludge to the test bottles. The exact amount of suspended solids was determined after drying overnight.
Colony count: From the supernatant of the sludge the heterotrophic microbial colony count was determinedon agar plates (diameter 9 cm), which contained purified agar (Oxoid Ltd, UK, 18 g/l) and nutrient broth (Oxoid Ltd, UK, 25 g/l).
Test procedure and conditions
Test duration: 28 days (last CO2-measurement on the 29th day).

During the test period the test media were aerated and stirred continuously.
Test vessels: 2 litre all-glass brown coloured bottles.
Milli-RO/Milli-Q water: Tap-water purified by reverse osmosis (Milli-RO) and subsequently passed over activated carbon and ion exchange cartridges (Milli-Q; Millipore Corp., Bedford, Mass., USA).
Stock solutions for reconsituting water from Milli-Q or Milli-RO water were as per the OECD guideline. Such water was stored in a sealed vessel to prevent absorption of CO2 from the air.
Synthetic air (CO2 < 1 ppm): A mixture of oxygen (21%) and nitrogen (79%) was passed through a bottle, containing 0.5-1 l 0.0125 M Ba(OH)2 solution to trap CO2 which might be present in small amounts. The synthetic air was sparged through the scrubbing solutions at a rate of approximately 1-2 bubbles per second (ca. 30-100 ml/min).
Preparation of bottles
Pre-incubation medium: Mineral components, Milli-RO water (ca. 80% total volume) and inoculum (1% final volume) were added to each bottle. This mixture was aerated with synthetic air overnight to purge the system of CO2.
Type and number of Test suspension: containing test substance bottles and inoculum (2 bottles).
Inoculum blank: containing only inoculum (2 bottles).
Procedure control: containing reference substance and inoculum (1 bottle).
Toxicity control: containing test substance, reference substance and inoculum (1 bottle).
Abiotic control: containing test substance and sterilising agent (1 ml/l of a solution containing 10 g/l of HgCl2; 1 bottle).
Adsorption control: containing test substance, inoculum and sterilising agent (1 ml/l of a solution containing 10 g/l of HgCl2; 1 bottle).
Additional adsorption bottle: Additional adsorption control (only used for DOC analyses): containing test substance at 100 mg/l and inoculum at 1000 mg/l (1 bottle). This bottle was not in series with the other bottles and not connected to CO2-absorbers.
Preparation: The test substance and positive control were added to the bottles containing the microbial organisms and mineral components (ca. 80% of total volume). The volumes of suspensions were made up to 2 litres with Milli-RO water, resulting in the mineral medium described before.
Three CO2-absorbers (bottles filled with 100 ml 0.0125 M Ba(OH)2) were connected in series to the exit air line of each test bottle.
Determination of CO2
Test bottles: All test bottles, except the additional adsorption bottle.
Experimental CO2 production: The CO2 produced in each test bottle reacted with the barium hydroxide in the gas scrubbing bottle and precipitated out as barium carbonate. The amount of CO2 produced was determined by titrating the remaining Ba(OH)2 with 0.05 M standardized HCl (1:20 dilution from 1 M HCl (Titrisol ampul; Merck KGaA, Germany).
Measurements: Titrations were made every second or third day during the first 10 days and thereafter at least every fifth day until the 28th day. Each time the CO2-absorber nearest to

the test bottle was removed for titration; each of the remaining two absorbers was moved one position in the direction of the test bottle and a new CO2-absorber was placed at the far end of the series.
Phenolphthalein (1% solution in ethanol, Merck KGaA, Germany) was used as pH-indicator. On the 28th day, the pH of the test suspensions was measured and 1 ml of concentrated HCl (37%, Merck KGaA, Germany) was added to each bottle. The bottles were aerated overnight to drive off CO2 present in the test suspension. The final titration was made on day 29.
Theoretical CO2 production: The theoretical CO2 production was calculated from the molecular formula.
Sampling and DOC analyses
Test bottles: Samples were taken from the bottles containing the abiotic control, adsorption control (both containing sterilizing agent) and additional adsorption control (no sterilizing agent).
Sampling procedure: A 30 ml sample of the test solutions was passed through a rough paper filter, and thereafter through a 0.45 µm filter. At least, the first 5 ml was discarded.
Frequency of sampling: At the start of the test (0 h) and 3 h after addition of the test substance and on days 1, 7, 14, 16, 21, 28 and 29; in order to estimate any adsorption of dl-Lactone by the activated sludge and/or degradation of dl-Lactone. Furthermore TOC analyses were performed on the stock solution of dl-Lactone.
DOC analyses: Analyses of the samples were performed using a Shimadzu TOC-VCPH total organic carbon analyzer combined with a Shimadzu ASI-V autosampler (Shimadzu.Benelux, The Netherlands).
Measurements and recording
pH: At the start of the test and on the 28th day.
Temperature of medium: Continuously in a vessel with Milli-RO water in the same room.

Interpretation
Degradation: Relative degradation values were calculated from the cumulative CO2 production relative to the total expected CO2 production based on the total carbon content of the amount of test material present in the test bottles. They were plotted versus time together with the relative degradation of the positive control. A figure of more than 10% degradation was considered as significant.
Toxicity control: if less than 25% degradation (based on ThCO2) occurred within 14 days, the test substance was assumed to be inhibitory. The total CO2 evolution in the inoculum blank was determined by the cumulative difference (in ml of titrant) between the blank Ba(OH)2 traps and fresh Ba(OH)2.

Acceptability of the test
1. The positive control substance was degraded by at least 60% (67%) within 14 days.
2. The difference of duplicate values for %-degradation of dl-Lactone was always less than 20.
3. The total CO2 release in the blank at the end of the test exceeded 40 mg/l, but did not exceeded 70 mg/l (84 mg CO2 per 2 litres of medium, corresponding to 42 mg/l).
Since all criteria for acceptability of the test were met the study was considered acceptable.

Result: Theoretical CO2 production
The Theoretical CO2 production (ThCO2) of dl-Lactone was calculated to be 2.03 mg CO2/mg. The concentration of dl-Lactone was 43.3 mg in 2 litres test medium. Hence, the theoretical CO2 production following complete degradation was 87.9 mg per 2 litres for the duplicate bottles A and B, the abiotic control and the adsorption control.
The positive control contained 81.0 mg sodium acetate (ThCO2= 1.07 mg CO2/mg) resulting in a theoretical CO2 production following complete degradation of 86.7 mg per 2 litres. The toxicity control contained 81.0 mg sodium acetate and 43.3 mg dl-Lactone in 2 litres of test medium. Hence, the theoretical CO2 production following complete degradation of dl-Lactone plus sodium acetate was 174.6 mg per 2 litres.

Biodegradation (based on CO2 determinations)
The relative degradation values calculated from the measurements performed during the test period revealed 82 and 76% degradation of dl-Lactone, for the duplicate bottles tested. Furthermore, more than 60% degradation of dl-Lactone was reached within a 10-day window.
In the toxicity control more than 25% degradation occurred within 14 days (56%, based on ThCO2). Thus, dl-Lactone was not inhibitory to microbial activity.
The relative degradation values calculated from the measurements performed during the test period revealed no significant degradation of dl-Lactone in the abiotic control and the adsorption control.

DOC determinations
No significant DOC removal was observed in the abiotic control and the adsorption control (both containing sterilizing agent). The degradation values calculated from the DOC measurements in the abiotic control and in the adsorption control, revealed no degradation of dl-Lactone (both containing sterilizing agent). In the additional adsorption control (dl-Lactone 100 mg/l, inoculum 1000 mg/l ss, no sterilising agent), 18% DOC removal was observed after 3 hours. This was the result of adsorption of dl-Lactone by the activated sludge. Since the DOC oncentration of day 1 was approximately the same as after 3 hours, no more adsorption of dl-Lactone by the activated sludge was observed.
The degradation values calculated from the DOC measurements in the additional adsorption control (dl-Lactone 100 mg/l, inoculum 1000 mg/l ss, no sterilising agent) revealed more than 60% biodegradation (79%) within 14 days. This was in agreement with the biodegradation pattern obtained after CO2 determinations.
Monitoring of temperature and pH
The temperature recorded in a vessel with water in the same room varied between 21.1 and 22.9°C. The pH values in all vessels at time 0 and 29 days were all within the range of 7.5 to 7.8.

Test substance: dl-Lactone from DSM (until 2003 Roche) Dalry, sample no. 06085776, purity 99.6%, dated 24-Sep-2004.

Conclusion: dl-Lactone was readily biodegradable under the conditions of the modified Sturm test presently performed.
Furthermore, no significant elimination of dl-Lactone (22 mg/l), by abiotic degradation or adsorption by the activated sludge (30 mg/l ss), was observed.

At a higher concentration of dl-Lactone (100 mg/l) and activated sludge (1000 mg/l ss) 18% adsorption by the activated sludge was observed within 3 hours. Thereafter, no more adsorption of dl-Lactone by the activated sludge was observed. The DOC measurements in the additional adsorption control, revealed more than 60% degradation (79%) within 14 days, which was in agreement with the biodegradation pattern obtained after CO2 determinations.

Reliability: (1) valid without restriction
Test according to OECD guideline under GLP.

Flag: Critical study for SIDS endpoint

15-MAR-2005 (18)

Type: aerobic
Concentration: 100 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 100 % after 28 day(s)
Result: readily biodegradable
Deg. product: not measured

Method: other: corresponding to OECD guideline 301 C
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions

10-MAR-2005 (58)

Type: aerobic
Inoculum: other: activated sludge, mixed domestic and industrial, non-adapted
Concentration: 533 mg/l related to Test substance
295 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 14 day(s)
Degradation: >= 98 % after 14 day(s)
Result: inherently biodegradable
Kinetic:

1 day(s)	ca. 97 %
2 day(s)	ca. 95 %
7 day(s)	ca. 59 %
9 day(s)	ca. 33 %
13 day(s)	ca. 3 %

Control Subst.: other: none
Deg. product: not measured

Method: other: Zahn-Wellens test, corresponding to OECD 302B
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The standardised in-house Zahn-Wellens test for inherent biodegradability was run as follows (not explicitly described in the reference, standard lab procedure). Activated sludge sources were a small municipal sewage works at Therwil (Switzerland, near Basle), serving approximately 5000 inhabitants and having no chemical or pharmaceutical industry input, and activated sludge from the Roche in-house pilot sewage treatment plant, which is continuously fed a flow-proportional sample of the actual Roche chemical effluent to the industrial wastewater treatment plant in Basle. Sludge samples were rinsed, mixed in equal proportions and

standardised to 2000 mg sludge (dry mass)/l as stated on the lab form. 533 mg/l dl-lactone was added, resulting in (an analysed) 295 mg DOC/l. 2-l batches were set up in large Erlenmeyer flasks and stood on a magnetic stirrer. The vessels were continuously aerated and kept at room temperature in the lab (generally not in the dark). Samples were taken at indicated intervals, filtered and DOC-analysed. Percentage of elimination (biodegradation combined with adsorption) was expressed as residual DOC at time t divided by initial DOC at time 0.

Result: Degradation of pure dl-lactone in an inherent Zahn-Wellens test at 533 mg/l test substance and 2000 mg/l mixed activated sludge started rapidly, followed a sigmoid curve and attained 98% elimination as measured by DOC at 14 days, when the test was terminated.

Test substance: dl-Lactone from Roche, "pure", not otherwise characterised.

Conclusion: dl-Lactone is rapidly inherently biodegradable even at higher concentrations. As dl-lactone is highly water-soluble and has a low n-octanol/water partition coefficient, the observed elimination is attributed wholly to biodegradation and not to adsorption to sludge. Therefore, a high rate of biodegradation is also predicted for dl-lactone in sewage works.

Reliability: (2) valid with restrictions
Biodegradability and inhibition assessment from the in-house wastewater lab, not GLP but highly standardised test in a professional laboratory with many years of experience.

Flag: Critical study for SIDS endpoint

10-MAR-2005 (24) (25)

Type: aerobic
Inoculum: other: activated sludge, mixed domestic and industrial, non-adapted
Concentration: 100 mg/l related to Test substance
1000 mg/l related to Test substance
Contact time: 21 day(s)
Degradation: >= 80 % after 21 day(s)
Result: inherently biodegradable
Deg. product: not measured

Method: other: comparable to OECD Guide-line 302 C
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: A standard inherent degradation test was performed with pure dl-lactone from lab production in a Sapromat respirometer in a test setup corresponding to OECD 302C, except that the sludge used as an inoculum was a 1:1 (dry weight) mixture of non-adapted activated sludge from one single small municipal sewage works and of activated sludge from the Roche in-house pilot sewage plant, which receives a flow-proprtional sample of the industrial wastewater. Test concentrations were 100 and 1000 mg dl-lactone/l. Activated sludge concentration is not explicitly stated but was 200 mg (dry weight)/l in other comparable tests. Degradation was followed by oxygen consumption in test substance vessels minus the oxygen consumption of a blank sludge vessel, compared to the COD for dl-lactone and calculated as percent degradation.

Result: Results of this particular test are only available as a paper copy of the oxygen demand recording with handwritten final

degradation percentages achieved. Therefore, only a qualitative and semi-quantitative description can be given. The BOD in both 100 and 1000 mg/l test vessels remained below the blank sludge BOD for 6 respectively 8 days. Then net biodegradation (test substance BOD minus blank BOD) took off in both concentrations.
In the 100-mg/l vessel, the net BOD attained an estimated rate as measured on the printout of three-quarters of the final net BOD within 6 days from the start of net biodegradation, ie, on total day 12. At the end of the test at 21 days, the biodegradability of the 100-mg/l vessel is marked in handwriting as "91%".
In the 1000-mg/l vessel, the net BOD attained an estimated rate as measured on the printout of three-quarters of the final net BOD within 9 days from the start of net biodegradation, ie, on total day 17. At the end of the test at 21 days, the biodegradability of the 1000-mg/l vessel is marked in handwriting as "80%". The BOD curve for this vessel was still going upwards steeply.

Test substance: dl-Lactone from Roche, "pure", not otherwise characterised.

Conclusion: Pure dl-lactone was well inherently biodegradable in a respirometric test at both 100 and 1000 mg/l starting concentration. In contrast to the DOC Zahn-Wellens test, an initial lag phase was seen with a reduced BOD compared to the activated sludge blank that lasted 6 days for the lower and 8 days for the higher test concentration.

Reliability: (2) valid with restrictions
Biodegradability assessment from the in-house wastewater lab, not GLP but highly standardised test in a professional laboratory with many years of experience.

Flag: Critical study for SIDS endpoint

10-MAR-2005 (24) (25)

Type: aerobic
Inoculum: other: activated sludge, mixed domestic and industrial, non-adapted
Concentration: 100 mg/l related to Test substance
Contact time: 15 day(s)
Degradation: ca. 82 % after 13 day(s)
Result: inherently biodegradable
Deg. product: not measured

Method: other: comparable to OECD Guide-line 302 C
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: A standard inherent degradation test was performed with pure dl-lactone from lab production in a Sapromat respirometer in a test setup corresponding to OECD 302C, except that the sludge used as an inoculum was a 1:1 (dry weight) mixture of non-adapted activated sludge from one single small municipal sewage works and of activated sludge from the Roche in-house pilot sewage plant, which receives a flow-proprtional sample of the industrial wastewater. The only test concentration was 100 mg dl-lactone/l. Activated sludge concentration is explicitly stated on the net degradation printout as 200 mg (dry weight)/l. Degradation was determined by oxygen consumption in test substance vessels minus the oxygen consumption of a blank sludge vessel divided by the COD for

dl-lactone and calculated as percent degradation.

Remark: The default Kinetic of test substance array in IUCLID 3.5, Biodegradation does not allow negative degradation values, therefore the kinetics had to be presented in a Results free text entry.

Result: The result from this test is only given as a printout of the net degradation curve over time (BOD in test vessel minus BOD in sludge blank divided by COD, given as percent).
Degradation kinetics, [BOD(dl-lactone)-BOD(blank)]/COD, %

Time, d	Degradation, %	
0	0	
1	0	
2	0	
3	-4	
4	-8	
5	-4	
6	8	
7	32	
8	62	
9	68	
10	73	
11	78	
12	80	
13	82	
14	82	plateau
15	82	

The test was stopped after degradation had reached a plateau and remained there for two days.

Test substance: dl-Lactone from Roche, "pure", not otherwise characterised.

Conclusion: dl-Lactone was well inherently biodegradable in this respirometric test. There was an initial lag phase consisting of two days of 0% relative degradation (test substance BOD minus sludge blank BOD), then just under four days of negative degradation until the degradation curve starts rising steeply, attaining 80% within 7 days from crossing the zero line. The lag phase suggests adaptation of the (non-adapted) sludge.

Reliability: (2) valid with restrictions
Biodegradability assessment from the in-house wastewater lab, not GLP but highly standardised test in a professional laboratory with many years of experience.

10-MAR-2005 (25)

Type: aerobic
Inoculum: other: activated sludge, mixed domestic and industrial, non-adapted
Concentration: 1000 mg/l related to Test substance
Contact time: 21 day(s)
Degradation: > 98 % after 21 day(s)
Result: inherently biodegradable
Kinetic:

3 day(s)	ca. 97 %
7 day(s)	ca. 90 %
14 day(s)	ca. 31 %
21 day(s)	< 1 %

Control Subst.: other: peptone/yeast extract as substrate for co-metabolic degradation
Kinetic: 21 day(s) < 1 %
Deg. product: not measured

Method: other: comparable to OECD Guide-line 302 C
Year: 1983

GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: A standard inherent degradation test was performed with pure dl-lactone from lab production in a Sapromat respirometer in a test setup corresponding to OECD 302C, except that the sludge used as an inoculum was a 1:1 (dry weight) mixture of non-adapted activated sludge from one single small municipal sewage works and of activated sludge from the Roche in-house pilot sewage plant, which receives a flow-proprtional sample of the industrial wastewater. Test concentrations were 10, 100 and 1000 mg dl-lactone/l. Peptone/yeast extract was added to test cometabolic degradation. Degradation was followed by oxygen consumption in test substance vessels minus the oxygen consumption of a blank sludge vessel and compared to the COD for dl-lactone respectively the COD for dl-lactone plus peptone/yeast extract and calculated as percent degradation.
Result: Degradation for both 10, 100 and 1000 mg dl-lactone/l reached 99% in 21 days. There was no inhibition of peptone/yeast extract degradation in the presence of 10 or 100 mg dl-lactone/l; there was a slight inhibition (14%, borderline significance) of peptone/yeast extract co-metabolic degradation in the presence of 1000 mg dl-lactone/l.
Test substance: dl-Lactone from Roche, "pure", not otherwise characterised.
Conclusion: dl-Lactone was well inherently biodegradable by non-adapted municipal sewage sludge at 10, 100 and 1000 mg/l. Further, it was not inhibitory on the biodegradation of a well degradable substrate, peptone/yeast extract, at 10 and 100 mg/l and it was only slightly inhibitory (14%, borderline significance) at 1000 mg/l.
Reliability: (2) valid with restrictions
Biodegradability and inhibition assessment from the in-house wastewater lab, not GLP but highly standardised test in a professional laboratory with many years of experience.
Flag: Critical study for SIDS endpoint
10-MAR-2005 (24)

Type: aerobic
Inoculum: activated sludge
Concentration: 600 mg/l related to Test substance
905 mg/l related to COD (Chemical Oxygen Demand)
Contact time: 7 day(s)
Degradation: > 95 % after 7 day(s)
Result: inherently biodegradable
Control Subst.: other: no data
Deg. product: not measured

Method: other: in-house biodegradation test with COD determination
Year: 1995
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: In a modification of a Zahn-Wellens test protocol, 1.2 g of test substance were dissolved in tap water; the solution was not neutralised. Then, mineral salts as per the Zahn-Wellens test method and rinsed activated sludge were added and the batch was supplemented with tap water to a total volume of 2 l. The final activated sludge concentration was approximately 1 g dry substance/l.
The test flask or flasks (not stated whether one or two

parallel flasks) were areated and continuously stirred with a magnetic stirrer.
The chemical oxygen demand in the test flask(s) was determined regularly to follow degradation through the decrease in COD. The technicque for COD determination is not given in the short protocol.
No mention is made of a possible control substance.

Result:

Time	Residual COD mg/l	Degradation relative to COD
0 (start)	905	-
1 d	905	0%
2 d	860	5%
5 d	845	7%
7 d	<20	>95%

(end of test)
In an in-house inherent biodegradability test following COD decrease, dl-lactone showed an initial lag phase of 5 days until very rapid biodegradation set in, attaining a total degradation above 95% within a further 48 hours, ie, by day 7 from start.

Test substance: Described as "DL-Lakton from Roche Basel", not otherwise specified.

Conclusion: After a comparatively long lag phase of 5 days, aerobic biodegradation of dl-lactone at 600 mg/l with activated sludge at approximately 1 g/l was very rapid and attained over 95% by day 7. In conclusion, dl-Lactone was well inherently biodegradable but did need adaptation of the activated sludge bacteria.

Reliability: (2) valid with restrictions
Short report from Roche Grenzach (Germany) QC Laboratory, Wastewater Section, describing a standard in-house inherent biodegradability test. While there are no details in the report these tests were performed on a regular basis according to a highly standardised protocol and are used as a basis for Roche-internal discharge permits. Reliability 2.

10-MAR-2005 (7)

Type: aerobic
Inoculum: other: activated sludge, not otherwise specified
Degradation: > 70 % after 21 day(s)
Result: other: "not readily biodegradable (according to OECD criteria)", hence probably missed 10-d-window criterion

Method: other: DIN/EN/ISO 7827 (aerobic)
GLP: no data
Test substance: other TS

Remark: No further data on method, year or GLP due to the characteristics of the source, a safety data sheet.

Test substance: Crystalline D-pantolactone, CAS 599-04-2, purity 99% (communication from BASF, 26-Aug-2004).

Conclusion: In a safety data sheet for D-pantolactone (one of the two isomers in dl-lactone) from BASF Company in Germany, D-pantolactone was well degradable in a ready biodegradability test but is stated not to be readily biodegradable according to OECD criteria, meaning that it probably missed the 10-day-window criterion.

Reliability: (4) not assignable
The safety data sheet received from BASF is only a condensed source without any experimental details, as is typical for

safety data sheets.
30-JUN-2005 (3)

Type: aerobic
Inoculum: other: activated sludge, mixed domestic and industrial, non-adapted
Concentration: 30 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 21 day(s)
Degradation: = 97 % after 21 day(s)
Result: inherently biodegradable
Kinetic:
5 day(s) = 34 %
7 day(s) = 47 %
14 day(s) = 90 %
21 day(s) = 97 %
Control Subst.: other: none
Deg. product: not measured

Method: other: Zahn-Wellens Test, corresponding to OECD guideline 302 B
Year: 1985
GLP: no
Test substance: other TS

Method: In order to characterise the aqueous phase of the dl-lactone step in sythesis, a Zahn-Wellens test with combined samples from lab batches was performed in the in-house wastewater lab. The standadised in-house Zahn-Wellens test for inherent biodegradability was run as follows (not explicitly described in the reference, but standard lab procedure). Activated sludge sources were a small municipal sewage works at Therwil (Switzerland, near Basle), serving approximately 5000 inhabitants and having no chemical or pharmaceutical industry input, and activated sludge from the Roche in-house pilot sewage treatment plant, which is continuously fed a flow-proportional sample of the actual Roche chemical effluent to the industrial wastewater treatment plant in Basle. Sludge samples were rinsed, mixed in equal proportions and standardised to 100 mg sludge (dry mass)/l. The concentrated composite extract was diluted at 9 ml/l with the 100 mg/l activated sludge, resulting in 130 mg DOC/l. 2-l batches were set up in large Erlenmeyer flasks and stood on a magnetic stirrer. The vessels were continuously aerated and kept at room temperature in the lab (generally not in the dark). Samples were taken at indicated intervals, filtered and DOC-analysed. Percentage of elimination (biodegradation combined with adsorption) was expressed as residual DOC at time t divided by initial DOC at time 0.
Result: While the whole extract was certainly not readily biodegradable (BOD5 = 0, see methods), a diluted extract at 130 mg DOC/l was well biodegradable in this Zahn-Wellens test, achieving 90% elimination as measured by DOC in 14 days and 97% in 21 days.
Test substance: Test substance was a composite sample of aqueous extraction phases from laboratory batches of the dl-lactone production step. This was characterised by summary parameters as follows:

Acid value (pH 7)	6460 mval/l
COD	40000 mg O2/l
BOD5	0 mg O2/l
TOC	14000 mg C/l

Ash contents 247300 mg/l
Evaporation residue 739000 mg/l

Conclusion: A representative wastewater from the dl-lactone synthesis step was well inherently biodegradable in a lab test. Based on this test the Dalry plant received the permission for draining this particular aqueous production waste.

Reliability: (2) valid with restrictions
Wastewater assessment from the in-house wastewater lab, not GLP but highly standardised test in a professional laboratory with many years of experience.

Flag: Critical study for SIDS endpoint

10-MAR-2005 (26)

3.6 BOD5, COD or BOD5/COD Ratio

Method:

C O D

Method: other: chromate titration
Year: 1983
GLP: no
COD: = 1680 mg/g substance
Method: An aqueous solution containing 100 mg dl-lactone/l was titrated with chromate as a strong oxidiser until full oxidation. The amount of chromate-oxygen was related to the amount of test substance.
Result: Stoichiometric calculation for the complete oxidation of dl-lactone (C6-H10-O3, 130.14 g/mol):

Stoichiometric reaction	Oxygen requirement
C6 -> 6 CO2	12 O
H10 -> 5 H2O	5 O
O3 -> (CO2 or H2O)	-3 O

Oxygen demand per molecule	14 O or 7 O2

Molecular mass of oxygen (O): 15.999 Da (1)

Theoretical oxygen demand (ThOD) = 7*2*15.999/130.14
= 1.721 g O2/g dl-Lactone
= 1721 mg O2/g
==========

(1) Source: Coleman & Dewar (1997).

Titrated COD = 1680 mg O2/g

Titrated COD = 98% of ThOD

Reliability: (4) not assignable
Experimental result given as the bare number on a lab form.

26-AUG-2004 (14) (25)

3.7 Bioaccumulation

Species: other: fish model
BCF: ca. 0 - 3.2

Method: other: QSAR calculated
Year: 2004

GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result:

Bioconcentration factor	Source
BCF fish	
1 (pH 1-10)	SciFinder (ACD Solaris V4.67)
3.162	EPISuite v3.11 (BCF v2.15)
0	ChemSCORER beta100
1.41	USES 4.02
0.05	calculated, Veith et al. (1979): logBCF = 0.85*logPow - 0.70
Bioaccumulation factor (sum of BCF and biomagnification factor), fish	
0	ChemSCORER beta100

Conclusion: Based on four modelled bioconcentration factors, which are consistently below 5, dl-lactone is not expected to bioaccumulate to a significant degree.
An additional modelled bioaccumulation factor, that incorporated both bioconcentration from the medium and biomagnification from the food chain, also suggests nonsignificant bioaccumulation.
Reliability: (2) valid with restrictions
Accepted QSAR programmes and regression equation.
Flag: Critical study for SIDS endpoint
11-MAR-2005 (13) (20) (44) (60) (61)

Species: other: earthworm model
BCF: ca. .84 - 3.23

Method: other: QSAR calculated
Year: 2004
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: USES 4.02. Basic physicochemical data for dl-lactone were entered into the respective USES fields and the model was run.

EU TGD, Connell & Markwell: The Kow of dl-lactone (0.204) is outside the range based on which the regression is based, hence the minimum of logKow = 1 was used as a default value.

EU TDG, Jager:
BCF(earthworm) = (0.84 + 0.012*Kow)/RHO(earthworm)
= (0.81 + 0.012*0.204)/1
[RHO(earthworm) default value = 1]
Remark: Connell & Markwell derived the empirical equation thorugh regression on experimental data for pesticides with a logKow ranging from 1.0 to 6.5. Since the logKow of dl-lactone isoutside this range (-0.69 <=> Kow = 0.204) the minimum logKow of 1 was used as a default.
Result:

Bioconcentration factor for earthworms	Source
3.23 kg/kg	USES 4.02
1 kg/kg	EU TGD; based on Connell & Markwell (1990)
0.84 kg/kg	EU TGD; based on Jager (1998)

Conclusion: Based on three modelled bioconcentration factors, dl-lactone is not expected to bioaccumulate in earthworms from soil or

soil pore water to a significant degree.
Reliability: (2) valid with restrictions
Accepted QSAR programme and regressions.
16-NOV-2005 (17) (28) (60)

3.8 Additional Remarks

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: semistatic
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: ca. 140 - measured/nominal
LC0: ca. 140 - measured/nominal
LC50: > 140 - calculated
Limit Test: yes

Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1999
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The study was performed according to OECD Guideline 203 under GLP.
Fish:
Juvenile carp from Zodiac, "De Haar Vissen" company, Wageningen, the Netherlands; acclimation for at least 12 days. Fish were healthy with less than 5% mortality in the 7 days preceding the test. Fish used in the range-finding test were 3.3±0.19 cm long and weighed 1.25±0.17 g, fish in the main test were 2.88±0.21 cm and weighed 0.68±0.14 g. Fish were fed daily with Trouvit until two days before testing.
Medium:
ISO medium, full composition in report, formulated with Milli-Ro water. Hardness and pH were measured before use in the test; temperature was measured daily; pH, nitrate, nitrite and ammonia were measured once a week.
Test solutions:
As dl-lactone was shown to be hydrolytically unstable, a semi-static test scheme was followed. Tests solutions were made daily by dissolving the exact amount of test substance by careful mixing. In the range-finder the lower concentrations were made up by dilution with test medium. Test media were not aerated during the test.
Procedure:
10-litre all-glass tanks filled to 6 l. 7 fish per concentration and control, introduced into the respective tank directly after preparation of the medium. Daily medium exchange for fresh test solutions. Photoperiod 16 h light, 8 h dark.
Range-finding test:
A range finder was performed with 4 concentrations of 100, 10, 1 and 0.1 mg/l and three fish per concentration.
Main test:
Based on no observed effects in all concentrations in the pretest, a limit test with 7 fish at 100 mg dl-lactone/l and 7 fish in the medium control was performed.
Measurement and recordings:
Fish were observed at approximately 2, 24, 48, 72 and 96 hours after first introduction into test media. Fish length and weight were measured in 10 specimens prior to the start of the test. Dissolved oxygen and pH were measured daily in all vessels, beginning at time = 0. Temperature was measured at

the beginning and at the end of the test in one control vessel.
Sampling and analytics:
Samples from the 100-mg/l and bank tanks were taken at t = 0 and t = 24 h from the approximate centre of the tank and analysed by HPLC wihtout storage.
HPLC conditions

Column	LiChrospher 100RP-18, 250*4 (i.d.) mm, d(rho)= 5 µm (Merck, Germany)
Mobile phase	20/80/0.1 (v/v/v) acetonitrile/Milli-Q water/formic acid
Flow	1 ml/min
Detection	SCIEX MSMS system API-300 mass spectrometer (Perkin Elmer, USA)
Interface	ion-spray, positive mode
Monit masses	MRM m/z 131.3 --> 113.0 (test substance) MRM m/z 127.2 --> 98.9 (internal standard)
Inject volume	100 µl
Int standard	4-hydroxy-6-methyl-2-pyrone (98%, Sigma-Aldrich, USA)

Result: No visible effects of dl-lactone exposure were noted, both in the range finder at all concentrations and in the main limit test.
dl-Lactone was completely soluble at all concentrations tested. The highest concentration in the range-finder was 100 mg/l nominal concentration and analysed at 93.2 mg/l after 24 hours. The concentration in the main test was analysed at 142 mg/l at t = 0, 145 mg/l at t = 24 h in the old water and 138 mg/l at t = 24 hours in the fresh medium.
Test substance: dl-Lactone from Roche Dalry, batch 805046, purity 100.0% according to certificate of analysis.
Conclusion: dl-Lactone was not acutely toxic to carp in a semi-static OECD test at 140 mg/l average concentration. The 96-hour NOEC and LC0 was 140 mg/l and the LC50 could not be determined as no single fish died. Moreover, dl-lactone was sufficiently (>80%) stable over the medium exchange period of 24 hours.
Reliability: (1) valid without restriction
OECD test under GLP with analytical confirmation.
Flag: Critical study for SIDS endpoint
24-AUG-2004 (8)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: > 100 - measured/nominal

Method: other: DIN 38412 part 15, static
GLP: no data
Test substance: other TS

Remark: No further data on method, year or GLP due to the characteristics of the source, a safety data sheet. The result is being cited as supportive evidence.
Result: In a safety data sheet for D-pantolactone (one of the two isomers in dl-lactone) from BASF Company in Germany, the acute toxicity of D-pantolactone to fish is stated as >100 mg/l (nominal concentration).
Test substance: Crystalline D-pantolactone, CAS 599-04-2, purity 99% (communication from BASF, 26-Aug-2004).

Reliability: (4) not assignable
The safety data sheet received from BASF is only a condensed source without any experimental details, as is typical for safety data sheets.
30-JUN-2005 (3)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: semistatic
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: ca. 130 - measured/nominal
EC50: > 130 - calculated
Limit Test: yes

Method: OECD Guide-line 202
Year: 1999
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The study was performed according to OECD Guideline 202 under GLP.
Daphnia:
Young daphnia with an age of <24 hours from the NOTOX breeding stock were used for the test. Each breeding batch was started with young animals of <3 d by placing approximately 250 of them into 10 l of medium in an all-glass culture vessel. After 7 days cultivation, hylf the medium was echanged twic weekly. Daphnids were fed daily with a suspension of fresh-water algae. Temeprature was kept in the range of 18-22 °C, constant within 1 °C. Cultures were used up to a maximum age of 4 weeks, then new batches were started.
Medium:
M7 medium as prescribed by Elendt-Schneider, full composition in report, formulated with Milli-Ro water.
Test solutions:
As dl-lactone was shown to be hydrolytically unstable, a semi-static test scheme was followed. Tests solutions were made daily by dissolving the exact amount of test substance by careful mixing. In the range-finder the lower concentrations were made up by dilution with test medium. Test media were not aerated during the test.
Procedure:
100-millilitre all-glass vessels filled up to the 80-ml mark were used. 10 daphnids were used per vessel, concentrations and blanks were tested in duplicate. Daphnids were introduced into the respective vessel directly after preparation of the medium. Daily medium exchange for fresh test solutions. Photoperiod 16 h light, 8 h dark.
Range-finding test:
A range finder was performed with 4 concentrations of 100, 10, 1 and 0.1 mg/l with 10 daphnia per concentration.
Main test:
Based on no observed effects in all concentrations in the pretest, a limit test with 2 x 10 daphnids at 100 mg dl-lactone/l and 2 X 10 daphnids in the medium control was performed.
Measurement and recordings:

Daphnids were observed at at the beginning, at 24 and 48hours after first introduction into test media. Dissolved oxygen and pH were measured daily in all vessels, beginning at time = 0. Temperature was measured at the beginning and at the end of the test in one control vessel.
Sampling and analytics:
Samples from the 100-mg/l and blank vesselwere taken at t = 0 and t = 24 h from the freshly prepared solutions and from the old solutions from the approximate centre of the vessel and analysed by HPLC without storage.
HPLC conditions

Column	LiChrospher 100RP-18, 250*4 (i.d.) mm, d(rho)= 5 µm (Merck, Germany)
Mobile phase	20/80/0.1 (v/v/v) acetonitrile/Milli-Q water/formic acid
Flow	1 ml/min
Detection	SCIEX MSMS system API-300 mass spectrometer (Perkin Elmer, USA)
Interface	ion-spray, positive mode
Monit masses	MRM m/z 131.3 --> 113.0 (test substance) MRM m/z 127.2 --> 98.9 (internal standard)
Inject volume	100 µl
Int standard	4-hydroxy-6-methyl-2-pyrone (98%, Sigma-Aldrich, USA)

Result: No visible effects in daphnids of dl-lactone exposure were noted, both in the range finder at all concentrations and in the main limit test.
dl-Lactone was completely soluble at all concentrations tested. The highest concentration in the range-finder was 100 mg/l nominal concentration and analysed at 93.2 mg/l after 24 hours (same medium as used for fish test). The concentration in the main test was analysed at 144 mg/l at t = 0, 123 mg/l at t = 24 h in the old water and 138 mg/l at t = 24 hours in the fresh medium.

Test substance: dl-Lactone from Roche Dalry, batch 805046, purity 100.0% according to certificate of analysis.

Conclusion: dl-Lactone was not acutely toxic to daphnids in a semi-static OECD test at 130 mg/l average concentration. The 48-hour NOEC was 130 mg/l and the EC50 could not be determined as no single daphnids became immobilised. Moreover, dl-lactone was sufficiently (>80%) stable over the medium exchange period of 24 hours.

Reliability: (1) valid without restriction
OECD test under GLP with analytical confirmation.

Flag: Critical study for SIDS endpoint

24-AUG-2004 (35)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 100 - measured/nominal

Method: other: DIN 38412 part 11, static
GLP: no data
Test substance: other TS

Remark: No further data on method, year or GLP due to the characteristics of the source, a safety data sheet. The result is being cited as supportive evidence.

Result: In a safety data sheet for D-pantolactone (one of the two isomers in dl-lactone) from BASF Company in Germany, the acute toxicity of D-pantolactone to daphnia is stated as >100 mg/l (nominal concentration).
Test substance: Crystalline D-pantolactone, CAS 599-04-2, purity 99% (communication from BASF, 26-Aug-2004).
Reliability: (4) not assignable
The safety data sheet received from BASF is only a condensed source without any experimental details, as is typical for safety data sheets.
30-JUN-2005 (3)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Selenastrum capricornutum (Algae)
Endpoint: other: biomass and growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 100 - measured/nominal
EbC50 : > 100 - calculated
ErC50 : > 100 - calculated
Limit Test: yes

Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1999
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The study was performed according to OECD Guideline 201 under GLP.
Algae:
Selenastrum capricornutum, strain CCAP 278/4, from NOTOX's own stock culture. This was originally started by inoculating growth medium with algal cells from a pure culture on agar. The suspensions were continuously aerated and exposed to light (4000-9000 lux) in a climate room at a temperature of 23±2 °C. Pre-culture: 4 days before the start of the test, cells from the algal stock culture were inoculated in culture medium at a cell density of 2*E+4 cells/ml. The pre-culture was maintained under the same conditions as used in the test. The cell density was meaured immediately before use.
Medium:
M-2 medium according to ISO standard "Algal growth inhibition test of November 1989, full composition in report, formulated with Milli-Ro water.
Test solutions:
Tests solutions were made up by dissolving the exact amount of test substance by careful mixing. In the range-finder the lower concentrations were made up by dilution with ISO M-2 medium. After preparation, volumes of 50 ml were added to each replicate vessel of the respective test concentrations. Subsequently, a volume of 0.118 ml of algal suspension was added, for which the cell density had previously been determined, so as to provide a densitiy of 10E+4 cells/ml at the start of the test.
Test procedure:
Test duration was 72 h, under continuous illumination using 30-W TLD "Gro-Lux" lamps (Sylvania) with a light intensity within the range of 3500-4000 lux. Test vessels were kept on a

continuous shaker in a climatised (22±0.5 °C) room. The pH was measured at the beginning and end of the test.
Range-finding test:
A range-finder was performed with 4 concentrations of 100, 10, 1 and 0.1 mg/l.
Main test:
Based on no observed effects in all concentrations in the pretest, a limit test with 6 replicates at 100 mg dl-lactone/l and 6 replicates of blank (medium only) control was performed. Additionally, 2 replicates of 100 mg dl-lactone/l without algae and 1 extra replicate of both concentrations (100 and 0 mg/l) necessary for sampling were made up.
Cell densities:
At the beginning of the test, cell density was counted using a microscope counting chamber. In parallel and thereafter, cell densities were determined by spectrophotometry at 720 nm (details in report).
Sampling and analytics:
Samples of 10 ml each were taken from the blank controls and the 100 mg/l solutions at 0, 24 and 72 hours after first introduction into test media. To follow the actual test substance concentration over time, a test vessel at 100 mg/l but without algae was also sampled at the start and end of the test period. Samples were analysed by HPLC immediately, without storage.
HPLC conditions

Column	LiChrospher 100RP-18, 250*4 (i.d.) mm, d(rho)= 5 µm (Merck, Germany)
Mobile phase	20/80/0.1 (v/v/v) acetonitrile/Milli-Q water/formic acid
Flow	1 ml/min
Detection	SCIEX MSMS system API-300 mass spectrometer (Perkin Elmer, USA)
Interface	ion-spray, positive mode
Monit masses	MRM m/z 131.3 --> 113.0 (test substance) MRM m/z 127.2 --> 98.9 (internal standard)
Inject volume	100 µl
Int standard	4-hydroxy-6-methyl-2-pyrone (98%, Sigma-Aldrich, USA)

Data handling:
Quantification of cell densities was based on a calibration curve of counted cell density versus extinction from six different cell densities. this correlation served to determine cell densities at the various time points in the test.
NOEC, areas under the growth curve, comparison of growth rates and calculation of EbC50 and ErC50 values were made as recommended in the OECD guideline 201.

Result: In the range-finding test, no significant effects were noted up to a concentration of 100 mg/l (full data in report). Therefore, a limit test scheme was adopted for the main test. In the limit test, the concentration remained above 80% of the measured initial concentration of 105 mg/l during the first 24 h. At 72 h, the concentration in the sample with the algae had decreased from 105 mg/l to 45 mg/l while in the nominal 100-mg/l solution without algae it had remained at 76 mg/l. The presence of an extra peak in the chromatograms indicated that the decrease in the algal solution was probably related to degradation, which had not happened in the algae-free solution. Based on the measured concentrations in the algal suspensions, the average measured exposure concentration was

78 mg/l.
At 100 mg/l nominal or starting concentration and an average exposure concentration of 78 mg/l over 72 h, dl-lactone had no significant effect on biomass and growth rate compared to controls. The NOEC was 100 mg/l nominal concentration respectively 78 mg/l average exposure concentration, neither an ErC50 nor an EbC50 could be determined due to lack of effects.

Test substance: dl-Lactone from Roche Dalry, batch 805046, purity 100.0% according to certificate of analysis.

Conclusion: dl-Lactone had no inhibitory effect on green algae in a standard test over 72 h at a nominal concentration of 100 mg/l and an average measured concentration of 78 mg/l.
In a comparison of dl-lactone concentrations in algal and algal-free vessels at 100 mg/l nominal concentration after 72 h, enhanced degradation was seen in the algal vessels, which suggests that the algae were actively degrading dl-lactone to an unidentified metabolite.

Reliability: (1) valid without restriction
OECD test under GLP with analytical confirmation.

Flag: Critical study for SIDS endpoint

16-NOV-2005 (9)

Species: Scenedesmus subspicatus (Algae)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC10: - measured/nominal
EC50: > 100 -

Method: other: DIN 38412 part 9, static
GLP: no data
Test substance: other TS

Remark: No further data on method, year or GLP due to the characteristics of the source, a safety data sheet. The result is being cited as supportive evidence.

Result: In a safety data sheet for D-pantolactone (one of the two isomers in dl-lactone) from BASF Company in Germany, the acute toxicity of D-pantolactone to green algae is stated as >100 mg/l (nominal concentration); it is not specified whether this concerns the biomass or growth rate endpoint or, probably, both.

Test substance: Crystalline D-pantolactone, CAS 599-04-2, purity 99% (communication from BASF, 26-Aug-2004).

Reliability: (4) not assignable
The safety data sheet received from BASF is only a condensed source without any experimental details, as is typical for safety data sheets.

30-JUN-2005 (3)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: other: ready biodegradation test toxicity/inhibition control
Species: activated sludge of a predominantly domestic sewage
Exposure period: 14 day(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: >= 22 - measured/nominal

Method: other: OECD Guideline 301B, toxicity control

Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Beside the standard toxcity control with 22 mg dl-lactone/l, 40 mg sodium acetate/l and 30 mg activated sludge/l and biodegradation measurement by carbon dioxide evolution, a single vessel was run with 100 mg dl-lactone/l and 1000 mg activated sludge/l in order to measure short-term (3-hour) adsorption by DOC. Following this vessel over longer time, a plateau of 79% DOC removal was reached from day 16, parallel to the standard CO2 test vessels, without any evidence for strong adsorption. This was taken as further evidence for easy biodegradability and for low toxicity towards activated sludge bacteria. Hence the formulation of NOEC >= 22 mg/l.
Test substance: dl-Lactone from DSM (until 2003 Roche) Dalry, sample no. 06085776, purity 99.6%, dated 24-Sep-2004.
Reliability: (1) valid without restriction
Test according to OECD guideline under GLP.
Flag: Critical study for SIDS endpoint
10-MAR-2005 (18)

Type: other: activated sludge bacteria
Species: activated sludge, domestic
Exposure period: 21 day(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 1000 - measured/nominal

Method: other: comparable to OECD Guide-line 302 B
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: A standard inherent degradation test was performed with pure dl-lactone from lab production in a Sapromat respirometer in a test setup corresponding to OECD 302C, except that the activated sludge was a 1:1 mixture (w/w) from a single small municipal sewage works and from the in-house pilot wastewater treatment plant receiving a continuous flow-proportional sample of industrial wastewater. Test concentrations were 10, 100 and 1000 mg dl-lactone/l. Degradation was followed by oxygen consumption and compared to the COD for dl-lactone and calculated as percent degradation.
Result: Degradation for both 10, 100 and 1000 mg dl-lactone/l reached 99% in 21 days. There was no inhibition of activated sludge bacteria in the presence of 10, 100 or 1000 mg dl-lactone/l.
Test substance: dl-Lactone from Roche, "pure", not otherwise characterised.
Conclusion: dl-Lactone was not inhibitory to non-adapted activated sludge bacteria in a respirometric inherent biodegradability test up to a starting concentration of 1000 mg/l.
Reliability: (2) valid with restrictions
Biodegradability and inhibition assessment from the in-house wastewater lab, not GLP but highly standardised test in a professional laboratory with many years of experience.
Flag: Critical study for SIDS endpoint
10-MAR-2005 (24)

Type: aquatic
Species: activated sludge
Exposure period: 30 minute(s)

Unit: mg/l **Analytical monitoring:** no data
EC50: > 100 - measured/nominal

Method: other: DIN/EN/ISO 8192-OECD 209-88/302/EWG, T. C aerobic
GLP: no data
Test substance: other TS

Remark: No further data on method, year or GLP due to the characteristics of the source, a safety data sheet. The result is being cited as supportive evidence.
Result: In a safety data sheet for D-pantolactone (one of the two isomers in dl-lactone) from BASF Company in Germany, the acute toxicity of D-pantolactone to aerobic activated sludge bacteria is stated as >100 mg/l (nominal concentration) over 30 minutes.
Test substance: Crystalline D-pantolactone, CAS 599-04-2, purity 99% (communication from BASF, 26-Aug-2004).
Reliability: (4) not assignable
The safety data sheet received from BASF is only a condensed source without any experimental details, as is typical for safety data sheets.
30-JUN-2005 (3)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: other: Roche inbred strain
Sex: no data
Vehicle: no data
Doses: no data
Value: = 9700 mg/kg bw

Method: other: Roche gavage oral toxicity test
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same rat strains.

Result:

Lethal dose	Time after gavage 24 h	10 d
LD10, mg/kg	6200	6200
LD50, mg/kg	9700±1600	9700±1600
LD90, mg/kg	15000	15000

Test substance: "dl-Lactone (pure)", Mag-No 4 3576 1, no information on actual percentage.
Conclusion: Pure dl-lactone has a low oral toxicity to rats with an LD50 of 9700 mg/kg bw; there is a comparatively wide range between LD10 and LD90 of 6200-15000 mg/kg bw. Moreover, the lethal doses being the same at 24 hours and 10 days, pure dl-lactone exerts its low toxicity rapidly, within the first 24 hours.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
Flag: Critical study for SIDS endpoint
25-AUG-2004 (12)

Type: LD50
Species: mouse
Strain: other: Roche inbred strain
Sex: no data
Vehicle: no data
Doses: no data

Value: = 4380 mg/kg bw

Method: other: Roche gavage oral toxicity test
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same mouse strains.

Result:

Lethal dose	Time after gavage	
	24 h	10 d
LD10, mg/kg	3400	2860
LD50, mg/kg	5300±840	4380±670
LD90, mg/kg	8300	6710

Test substance: "dl-Lactone (pure)", Mag-No 4 3576 1, no information on actual percentage.
Conclusion: Pure dl-lactone has a relatively low oral toxicity to mice with an LD50 of 4380 mg/kg bw; the range between LD10 and LD90 is 2860-6710 mg/kg bw. The lethal doses were approximately one-fifth higher at 24 hours than at 10 days, showing that while in mice pure dl-lactone exerts its low toxicity rapidly, for the greater part within the first 24 hours, there is a comparatively weak additional toxic effect after this initial period in mice.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
Flag: Critical study for SIDS endpoint
26-AUG-2004 (12)

Type: LD50
Species: rat
Strain: other: Roche inbred strain
Sex: no data
Vehicle: no data
Doses: highest dose 8000 mg/kg bw
Value: > 8000 mg/kg bw

Method: other: Roche gavage oral toxicity test
Year: 1976
GLP: no
Test substance: other TS

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same rat strains.

Result: Lethal dose | Time after gavage

	24 h	10 d
LD10, mg/kg	8000	8000
LD50, mg/kg	>8000	>8000
LD90, mg/kg	>8000	>8000

Test substance: "dl-Lactone (technical)", Mag-No 4 2175 8, no information on actual percentage.

Conclusion: Technical dl-lactone has a low oral toxicity to rats with an LD50 of greater than 8000 mg/kg bw. The LD10 was 8000 mg/kg bw; as 8000 mg/kg bw was the highest dose administered, no LD50 or higher-percentile lethal does can be derived from this test. Moreover, the lethal doses being the same at 24 hours and 10 days, it seems that technical dl-lactone exerts its low toxicity rapidly, within the first 24 hours, similar to pure dl-lactone.

Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.

25-AUG-2004 (12)

Type: LD50
Species: mouse
Strain: other: Roche inbred strain
Sex: no data
Vehicle: no data
Doses: no data
Value: = 4000 mg/kg bw

Method: other: Roche gavage oral toxicity test
Year: 1976
GLP: no
Test substance: other TS

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same mouse strains.

Result: Lethal dose | Time after gavage

	24 h	10 d
LD10, mg/kg	2540	2540
LD50, mg/kg	4000±650	4000±650
LD90, mg/kg	6300	6300

Test substance: "dl-Lactone (technical)", Mag-No 4 2175 8, no information on actual percentage.

Conclusion: Technical dl-lactone has a relatively low oral toxicity to mice with an LD50 of 4000 mg/kg bw; the range between LD10 and LD90 is 2540-6300 mg/kg bw. The lethal doses were the same at 24 hours and at 10 days, showing that technical dl-lactone exerts its toxicity rapidly, within the first 24 hours.

Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form,

the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.

26-AUG-2004 (12)

Type: other: LDlo in range-finder test to micronucleus mutagenicity assay
Species: mouse
Strain: other: NMRI BR
Sex: male/female
No. of Animals: 14
Vehicle: physiol. saline
Doses: 2000 and 1500 mg/kg bw
Value: = 2000 mg/kg bw

Method: other: OECD Guideline 474
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: A range-finding study for the dose in a micronucleus test in mice was performed with an administration by gavage of dl-lactone at doses of 2000 and 1500 mg/kg be in 10 ml of physiological saline; full details are given in chapter 5.
Remark: OECD study under GLP, but not according to an acute toxicity protocol.
Result: In a range-finder study with mice, out of 4 males and 4 females dosed with 2000 mg dl-lactone in physiological saline per kg bw, all treated animals showed abnormalities during an observation period of 3 days; 1 male died within 20 min, 2 more males and 1 female died within 1.5 hours. During the first 1.5 hours, all animals showed lethargy or convulsions, one male had tremors. At days 2 and 3 after gavage, all survivors showed no abnormalities. An additional 3 males and 3 females were dosed with 1500 mg dl-lactone in physiological saline per kg bw. All treated animals except one female showed lethargy within the first 20 min; after 1.5 hours, 2 one male and female each showed no signs while thothers were lethargic and had a rough coat. At days 2 and 3 after gavage, all survivors showed no abnormalities.
Test substance: dl-Lactone from Roche Dalry, batch no. BX226, purity 99.8% according to analytical certificate.
Conclusion: dl-Lactone had a lowest lethal dose of 2000 mg/kg bw in a range-finding study; 4/8 animals died within a short time (1.5 h) after administration. No animal from the 1500-mg/kg-bw group died within 3 days.
Reliability: (2) valid with restrictions
16-NOV-2005 (33)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

Type: other: NOEL cross-read from OECD skin irritation study
Species: rabbit
Strain: New Zealand white
Sex: male
No. of Animals: 3
Vehicle: other: moistened with water
Doses: 0.5 g/animal (body weights >= 1 kg) applied semi-occlusively for 4 hours
Value: >= 500 mg/kg bw

Method: other: OECD 404
Year: 2005
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: In an OECD skin irritation study, 0.5 g ld-Lactone was moistened with little milli-U water and applied during 4 hours under semi-occlusive covering to the clipped skin of rabbits weighing at least 1 kg. The test report notes that there was no sign of skin irritation in any animal nor any symptoms of systemic toxicity nor any mortality.
Conclusion: Based on a skin irritation study, dl-Lactone has no high dermal toxicity.
Reliability: (2) valid with restrictions
OECD study under GLP, but endpoint only cross-read.
11-MAR-2005 (55)

5.1.4 Acute Toxicity, other Routes

Type: LC50
Species: mouse
Strain: no data
Sex: no data
No. of Animals: 3
Vehicle: no data
Doses: 250, 750, 1000, 1500, 2000 mg/kg bw
Route of admin.: other: injection
Value: 750 - 1500 mg/kg bw

Method: as described in Klunk et al. (1982), Molec Pharmacol 22: 438-443
Year: 1982
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Two or three mice per treatment group were injected (not stated whether i.p., i.v. or i.m.) with dl-lactone in undescribed vehicle. Time to first clonic seizure (alternate contraction and relaxation) and/or to first tonic seizure (persistent contractions, usually resulting in death) was recorded and compared with that of other substituted butyrolactones in order to determine potency and mechanism respectively important structural determinants of physiological action. Full details of methods are given in a previous publication: Klunk WE et al. (1982), Molec Pharmacol 22: 438-443.
Result:

Mice per group	Dose		Time to first seizure of type	
	mg/kg bw	mmol/kg bw	clonic, s	tonic, s

2	250	1.92	-	(0/2)	-	(0/2)
3	750	5.77	340±131	(3/3)	-	(0/3)
3	1000	7.69	246±135	(3/3)	1275	(1/3)
3	1500	11.5	85±10	(3/3)	287±174	(3/3)
3	2000	15.4	101±28	(3/3)	39±126	(3/3)

Test substance: "DL-Pantolactone (alpha-hydroxy-beta,beta-dimethyl-gamma-butyrolactone) was obtained from Chemical Procurement Laboratories (College Point, NY)." No further information given.

Conclusion: dl-Lactone administered by injection leads to dose-dependent neurophysiological effects in mice. At 250 mg/kg bw no effects were observed (NOEL). All higher doses caused clonic convulsions with the first seizures appearing after a shorter delay the higher the dose; tonic convulsions started at a dose of 1000 mg/kg bw in one of three animals while both higher doses caused tonic seizures in all animals of the respective treatment groups with a highly dose-dependent reaction time. Judging from the remark in the paper (page 445) that "a tonic seizure [...] usually resulted in death", the LC0 of dl-lactone in mice by injection probably was 750 mg/kg bw and the LC50 between 750 and 1500 mg/kg bw.

Reliability: (4) not assignable
Peer-reviewed paper in scientific journal, clear presentation of results.

28-JUN-2005 (31)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: 0.06?, 600 mg/kg bw
Route of admin.: i.p.
Value: = 600 mg/kg bw

Method: no details available
Year: 1979
GLP: no data
Test substance: other TS

Remark: A huge difference of four magnitudes between the NOEL (0.06 mg/kg) and the LD50 (0.6 g/kg) is noted. As the original publication is in Russian, it cannot be said whether this wide gap is correctly cited or whether one of the units was translated erroneously, hence, this question cannot be resolved at present.

Result: The TNO (1995) Toxicity Profile on Pantolactone cites from a Russian publication (Moiseenok et al., 1979) as follows: "Intraperitoneal LD50 mouse (D-pantolactone): 0.6 g/kg bw (Moiseenok et al. 1979).
Intraperitoneal injection of D-pantolactone at 0.06 mg/kg bw did not affect the level of 'the acetylation coenzyme' in the liver or brain of mice. Earlier work [reference not given] is referred to where such treatment caused 'reduced behavioural reactions and body temperature', and potentiated the soporific action of other chemicals (Moiseenok et al. 1979)."

Test substance: D-Pantolactone, CAS 599-04-2, no further details given.

Conclusion: Based on a secondary source, D-lactone has a comparatively low acute intraperitoneal toxicity with an LD50 of 600 mg/kg bw.

Reliability: (4) not assignable
Short citation from a Russian publication, reliability cannot be judged.
22-SEP-2004 (36) (56)

Type: EC0
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: 250, 750, 1000, 1500, 2000 mg/kg bw
Route of admin.: other: injection
Value: = 250 mg/kg bw

Method: as described in Klunk et al. (1982), Molec Pharmacol 22: 438-443
Year: 1982
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Two or three mice per treatment group were injected (not stated whether i.p., i.v. or i.m.) with dl-lactone in undescribed vehicle. Time to first clonic seizure (alternate contraction and relaxation) and/or to first tonic seizure (persistent contractions, usually resulting in death) was recorded and compared with that of other substituted butyrolactones in order to determine potency and mechanism respectively important structural determinants of physiological action. Full details of methods are given in a previous publication: Klunk WE et al. (1982), Molec Pharmacol 22: 438-443.

Result:

Mice per group	Dose		Time to first seizure of type			
	mg/kg bw	mmol/kg bw	clonic, s		tonic, s	
2	250	1.92	-	(0/2)	-	(0/2)
3	750	5.77	340±131	(3/3)	-	(0/3)
3	1000	7.69	246±135	(3/3)	1275	(1/3)
3	1500	11.5	85±10	(3/3)	287±174	(3/3)
3	2000	15.4	101±28	(3/3)	39±126	(3/3)

Test substance: "DL-Pantolactone (alpha-hydroxy-beta,beta-dimethyl-gamma-butyrolactone) was obtained from Chemical Procurement Laboratories (College Point, NY)." No further information given.
Conclusion: dl-Lactone administered by injection leads to dose-dependent neurophysiological effects in mice. At 250 mg/kg bw no effects were observed (NOEL). All higher doses caused clonic convulsions with the first seizures appearing after a shorter delay the higher the dose; tonic convulsions started at a dose of 1000 mg/kg bw in one of three animals after more than 20 min while both higher doses caused tonic seizures in all animals of the respective treatment groups with a highly dose-dependent reaction time.
However, in comparison with five other substituted butyrolactones, dl-lactone was the weakest neurotoxicant, by a factor of five to the next weakest, as measured by the dose in millimoles/kg bodyweight (full data in paper).
Reliability: (4) not assignable
Peer-reviewed paper in scientific journal, clear presentation of results.

28-JUN-2005 (31)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: .5 g
Exposure: Semiocclusive
Exposure Time: 4 hour(s)
No. of Animals: 3
Vehicle: water
PDII: 0
Result: not irritating
EC classificat.: not irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 2005
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Test substance preparation
The powdery test substance was moistened with water (Milli-U), immediately before application, to ensure close contact with the animal's skin.

Test System
New Zealand White Albino Rabbit (SPF-Quality) from Charles River Deutschland, Kisslegg, Germany. The 3 males used within the study were at least 6 weeks old and body weights were at least 1.0 kg. Identification was by earmark.

Animal husbandry
Animals were housed in a controlled environment, in which optimal conditions were considered to be approximately 15 air changes per hour, a temperature of 21±3 °C (actual range: 19.4-21.1 °C), a relative humidity of 30-70% (actual range: 44-59%) and 12 hours artificial fluorescent light and 12 hours darkness per day. Accommodation was individually in labelled cages with perforated floors (Scanbur, Denmark, dimensions 56x44x37.5 cm).
Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.
Diet: Standard laboratory rabbit diet (Charles River Breeding and Maintenance Diet for Rabbits, Altromin, Lage, Germany) approximately 100 g per day. Certificates of analysis were examined and retained in the NOTOX archives. In addition, hay (BMI, Helmond, the Netherlands) was
provided at least three times a week. Free access to tap-water was given. Certificates of quarterly analysis were examined and retained in the NOTOX archives.
Results of analyses for ingredients and/or contaminants of diet and water were assessed and did not reveal any findings that were considered to have affected study integrity.

Treatment
All available data relevant to the potential dermal irritation/corrosivity of the substance indicated that no severe effects were to be expected. An in-vitro test was

considered, but a negative test result was anticipated that still would have to be confirmed in an in-vivo study. Since no severe harm for the animals was to be expected, this in-vivo skin irritation study was performed and was started by treatment of a single rabbit (sentinel). The two other animals were treated in a similar manner three weeks later, after considering the degree of skin irritation observed in the first animal.
Approximately 24 hours before treatment, the dorsal fur was clipped with electric clippers, exposing an area of approximately 150 square centimeters (10x15 cm^2). Whenever considered necessary the treated skin areas were re-clipped at least 3 hours before the observations, to facilitate scoring. A health inspection was performed prior to the commencement of treatment, to ensure that the animals were in a good state of health. Special attention was paid to the skin to be treated, which was intact and free from abnormalities.
Each animal was treated by dermal application of 0.5 grams of the test substance. The test substance was moistened with 0.1 ml of the vehicle and applied to the skin of one flank, using a Metalline (Lohmann GmbH, Neuwied, Germany) patch of 2x3 cm. The patch was mounted on Micropore (3M, St. Paul, Minnesota, USA) tape, which was wrapped around the abdomen and secured with Coban (3M, St. Paul, Minnesota, USA) elastic bandage. Four hours after the application, the dressing was removed and the skin cleaned of residual test substance using water.

Observations
Mortality/viability: Twice daily.
Signs of overt toxicity: At least once daily.
Body Weight: Day of treatment (prior to application) and at termination.
Irritation: The skin reactions were assessed at approximately 1, 24, 48 and 72 hours after the removal of the dressings and test substance. The irritation scores and a description of all other (local) effects were recorded. Adjacent areas of the untreated skin of each animal served as controls. The irritation was assessed according to the following numerical scoring system. At each observation, the highest scores given were recorded:
Erythema and eschar formation:
No erythema.....0
Very slight erythema (barely perceptible).....1
Well-defined erythema.....2
Moderate to severe erythema.....3
Severe erythema (beet redness).....4
(Where signs of necrosis or corrosion (injuries in depth) prevent erythema scoring, the maximum grade for erythema (= 4) is given.)
Oedema formation:
No oedema.....0
Very slight oedema (barely perceptible).....1
Slight oedema (edges of area well-defined by definite raising).....2
Moderate oedema (raised approximately 1 millimeter).....3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure)....4

Histopathology

No histopathology was performed.

Interpretation
The results were evaluated according to the OECD Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances (OECD, 1998) and the EC criteria for classification and labelling of dangerous substances and preparations (Council Directive 67/548/EEC and all adaptations to technical progress and amendments of this Directive published in the Official Journal of the European Communities).

Result: Irritation
No skin irritation was caused by 4 hours exposure to dl-Lactone.

Corrosion
There was no evidence of a corrosive effect on the skin.

Colouration/Remnants
No staining of the treated skin by the test substance was observed and no test substance remnants were seen.

Toxicity/Mortality
No symptoms of systemic toxicity were observed in the animals during the test period and no mortality occurred.

Test substance: dl-Lactone from DSM (until 2003 Roche) Dalry, sample no. 06085776, purity 99.6%, dated 24-Sep-2004.

Conclusion: Based on the test results and according to the OECD Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances (OECD, 1998), dl-Lactone does not have to be classified for skin irritation.

Reliability: (1) valid without restriction
OECD study under GLP.

15-MAR-2005 (55)

Species: human

GLP: no

Test substance: as prescribed by 1.1 - 1.4

Remark: May cause irritations after prolonged or intensive contact during occupational handling.

Test substance: As produced/used in synthesis of pantothenic acid/panthenol.

Reliability: (2) valid with restrictions
Occupational handling experience, considered reliable.

Flag: Critical study for SIDS endpoint

16-NOV-2005 (50)

5.2.2 Eye Irritation

Species: human

Test substance: as prescribed by 1.1 - 1.4

Remark: May cause irritations upon direct contact

Reliability: (2) valid with restrictions
Occupational handling experience, considered reliable.

16-NOV-2005 (50)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Concentration 1st: Induction 5 % intracutaneous
2nd: Induction 50 % occlusive epicutaneous
3rd: Challenge 50 % semiocclusive
No. of Animals: 15
Vehicle: other: water and FCA for the intracutaneous induction, water for epicutaneous induction and challenge
Result: not sensitizing
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
Year: 2005
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Test substance preparation
Vehicle: Water (Milli-U)
Rationale: The vehicle was selected based on trial formulations performed at NOTOX.
Preparation: The test substance formulations (w/w) were prepared within 4 hours prior to each treatment. Homogeneity was obtained to visually acceptable levels.

Test System
Species: Dunkin Hartley strain, albino guinea pig (SPF-quality); recognised by international guidelines as the recommended test system (eg, OECD, EC).
Source: Charles River Deutschland, Kisslegg, Germany.
Number of animals: Experimental group: 10 females;
Control group: 5 females (all females were nulliparous and non-pregnant).
Age: Young adult animals (approximately 4 weeks old) were selected.
Identification: Ear tattoo.
Reliability check: The results of a reliability test performed not more than 6 months previously are given in the Appendix to the test report. Similar procedures were used in the reliability test and in this study.

Animal husbandry
Conditions: Animals were housed in a controlled environment, in which optimal conditions were considered to be approximately 15 air changes per hour, a temperature of 21±3 °C (actual range: 19.3-21.8 °C), a relative humidity of 30-70% (actual range: 45-91%) and 12 hours artificial fluorescent light/12 hours darkness per day.
Accommodation: Group housing of maximally 5 animals per labelled cage (74 cm x 54 cm x 25 cm height) containing sterilised sawdust as bedding material (Woody-Clean type 3/4; Tecnilab-BMI BV, Someren, The Netherlands). Certificates of analysis were examined and retained in the NOTOX archives. The acclimatisation period was at least 5 days before the start of treatment under laboratory conditions.
Diet: Free access to standard guinea pig diet including

ascorbic acid (1000 mg/kg); (Charles River Breeding and Maintenance Diet for Guinea Pigs, Altromin, Lage, Germany). Certificates of analysis were examined and retained in the NOTOX archives. Hay (B.M.I., Helmond, The Netherlands) was provided at least twice a week.
Water: Free access to tap water. Certificates of quarterly analysis for tap-water were examined and retained in the NOTOX archives.
Results of analysis for ingredients and/or contaminants of diet, sawdust, and water were assessed and did not reveal any findings that were considered to have affected study integrity.

Preliminary irritation study
A preliminary irritation study was conducted in order to select test substance concentrations to be used in the main Study. The selection of concentrations was based on the following criteria: The concentrations are well-tolerated systemically by the animals. For the induction exposures: the highest possible concentration that produced mild to moderate irritation (grades 2-3). For challenge exposure: the maximum non-irritant concentration. Series of test substance concentrations were tested. Practical feasibility of administration determined the highest starting concentration for each route. The starting and subsequent concentrations were taken from the series: 100% (undiluted), 50%, 20%, 10%, 5%, 2%, 1% and if needed, further lower concentrations using the same steps. The test system and procedures were identical to those used during the main study, unless otherwise specified. The four animals selected were between 4 and 9 weeks old. No body weights were determined.
Intradermal injections: A series of four test substance concentrations was used, the highest concentration being the maximum concentration that could technically be injected. Each of two animals received two different concentrations in duplicate (0.1 ml/site) in the clipped scapular region. The injection sites were assessed for irritation 24 and 48 hours after treatment.
Epidermal application: A series of four test substance concentrations was used, the highest concentration being the maximum concentration that could technically be applied. Two different concentrations were applied (0.5 ml each) per animal to the clipped flank, using Metalline patches (2x3 cm) mounted on Medical tape, which were held in place with Micropore tape and subsequently Coban elastic bandage. The animals receiving intradermal injections were treated with the lowest concentrations and two further animals with the highest concentrations. After 24 hours, the dressing was removed and the skin cleaned of residual test substance using water.
Suppliers for materials: Lohmann GmbH, Neuwied, Germany (Metalline) and 3M, St. Paul, Minnesota, USA (Medical tape, Micropore and Coban).

Main study
Induction - Experimental animals
On day 1 the scapular region was clipped and three pairs of intradermal injections (0.1 ml/site) were made in this area as follows:
A) A 1:1 w/w mixture of Freunds' Complete Adjuvant (Difco, Detroit, USA) with water for injection (Fresenius AG, Bad

Homburg, Germany).
B) The test substance at a 5% concentration.
C) A 1:1 w/w mixture of the test substance, at twice the concentration used in (B) and Freunds' Complete Adjuvant.
Note: One of each pair was on each side of the midline and from cranial A) to caudal C).
On day 3 the dermal reactions caused by the intradermal injections were assessed for irritation.
On day 7 the scapular area between the injection sites was clipped and subsequently rubbed with 10% sodium-dodecyl-sulfate (SDS; Boom, Meppel, The Netherlands) in vaseline using a spatula. This concentration of SDS provokes a mild inflammatory reaction.
On day 8 the 10% SDS treated area between the injection sites was treated with 0.5 ml of a 50% test substance concentration using a Metalline patch (2x3 cm) mounted on Medical tape, which was held in place with Micropore tape and subsequently Coban elastic bandage. The dressing was removed after 48 hours exposure, the skin cleaned of residual test substance using water and the dermal reactions caused by the epidermal exposure were assessed for irritation.

Induction - Control animals
The control animals were treated as described for the experimental animals except that, instead of the test substance, vehicle alone was administered.

Challenge - All animals
On day 22 one flank of all animals was clipped and treated by epidermal application of a 50% test substance concentration and the vehicle (0.1 ml each), using Patch Test Plasters (Curatest, Lohmann, Almere, The Netherlands). The patches were held in place with Micropore tape and subsequently Coban elastic bandage. The dressing was removed after 24 hours exposure and the skin cleaned of residual test substance and vehicle using water. The treated sites were assessed for challenge reactions 24 and 48 hours after removal of the dressing.

Observations
Mortality/Viability: Twice daily
Overt toxicity: At least once daily.
Body weights: Prior to start and at termination of the study.
Skin reactions: Skin reactions were graded according to the following numerical scoring systems. Furthermore, a description of all other (local) effects was recorded.
Whenever necessary, the treated skin-areas were clipped at least 3 hours before the next skin reading to facilitate scoring.
Grading Irritation Reactions*:
Erythema and eschar formation:
No erythema.....0
Slight erythema (barely perceptible).....1
Well-defined erythema.....2
Moderate erythema.....3
Severe erythema (beet redness) to slight eschar formation (injuries in depth).....4
Oedema formation:
No oedema.....0

Slight oedema (barely perceptible).....1
Well-defined oedema (edges of area well-defined
by definite raising).....2
Moderate oedema (raised approximately
1 millimeter).....3
Severe oedema (raised more than 1 millimeter
and extending beyond the area of exposure).....4
(*. Intradermal reactions were assessed for erythema only or,
if necrosis is present, the diameter of necrosis.)

Grading Challenge Reactions:
No visible change.....0
Discrete or patchy erythema.....1
Moderate and confluent erythema.....2
Moderate erythema and swelling.....3
Intense erythema and swelling.....4

Interpretation
The results for the experimental animals at the challenge application(s) were compared with the results for the control animals. All skin reactions were considered signs of sensitisation provided that such reactions were less severe or were less persistent in the control group. A sensitisation rate (%) was calculated for each concentration as follows: the number of sensitised animals at one concentration as a proportion of the total number of animals of the experimental group. The results were evaluated according to the OECD Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances (OECD, 1998) and the EC criteria for classification and labelling of dangerous substances and preparations (Council Directive 67/548/EEC and all adaptations to technical progress and amendments of this Directive published in the Official Journal of the European Communities).

List of protocol deviations
Deviations from the maximum level for relative humidity occurred. Evaluation: Based on laboratory historical data these deviations were considered not to have affected the study integrity. The study integrity was not adversely affected by the deviations.

Result: Based on the pretest results, the test substance concentrations selected for the main study were a 5% concentration for the intradermal induction. No signs of irritation were observed up to the highest test substance concentration epidermally tested; therefore, the test site of all animals was treated with 10% SDS approximately 24 hours before the epidermal induction in the main study, to provoke a mild inflammatory reaction; then, a 10% test substance solution mixed 1:1 with Freund's Complete Adjuvant (FCA) was applied under occlusion for epidermal induction. Last, a 50% test substance concentration was selected for the epidermal challenge phase.
Main study, Induction
The skin effects caused by the intradermal injections exposure during the induction phase are summarised as follows: In the 5% test substance group, 8/10 animals showed grade 1 erythema, 1/10 showed dermal necrosis of 1 mm diameter and the remaining 1/10 showed no effects; all 5 vehicle-only controls showed no

effects. In the 10% test substance/FCA treatment croup, 5/10 animals showed grade 2 erythema, 1/10 showed grade 1 erythema and the remaining 4/10 showed necroses with diameters of 2, 3, 3 and 4 mm; in the 5 FCA-only controls, 3/5 showed grade 1 erythema and 2/5 showed grade 2 erythema.
The skin effects caused by the epidermal exposure during the induction phase are summarised as follows: 9/10 test animals showed no effects at all while the remaining 1/10 showed grade 1 erythema (this animal had shown grade 1 respectively grade 2 erythema on intradermal respectively epidermal induction), while all 5 control animal showed no effect.
Main Study, Challenge
No skin reactions were evident after the challenge exposure in both experimental and control animals. No mortality occurred and no symptoms of systemic toxicity were observed in the animals of the main study. Body weights and body weight gain of experimental animals remained in the same range as the controls over the study period.

Test substance: dl-Lactone from DSM (until 2003 Roche) Dalry, sample no. 06085776, purity 99.6%, dated 24-Sep-2004.

Conclusion: In a guinea pig maximisation test with intradermal and subsequent epicutaneous induction and epicutaneous challenge, there was no evidence that dl-Lactone had caused skin hypersensitivity in the guinea pig, since no responses were observed in the experimental animals in the challenge phase. This corresponds to a sensitisation rate of 0%, which in turn gives strong evidence for dl-Lactone not being a dermal sensitiser.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

18-JAN-2006 (54)

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF quality)
Route of administration: gavage
Exposure period: males: 28 days; females: 28-56 days, mean value 43 days
Frequency of treatment: once daily
Post exposure period: none
Doses: 0 (controls, vehicle only = milli-U water), 40, 200 and 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 200 mg/kg bw
LOAEL: = 1000 mg/kg bw

Method: other: OECD Guideline 422, "Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test", 22-Mar-1996
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Test substance formulation
Vehicle Water (Milli-Q) of 37°C adjusted to pH 4. Acetic acid was added to milli-Q water to obtain pH 4. Rationale for vehicle: Based on trial formulations performed at NOTOX and on information provided by the sponsor. During NOTOX Project

257568 (Determination of the hydrolysis of dl-lactone as a function of pH) it was determined that dllactone was hydrolytically stable at pH 4 and 50°C. Formulations in Milli-Q water are stable for 4 hours at room temperature and formulations in Milli-Q water adjusted to pH 4 are stable for 4 hours at room temperature and for 8 days at 37°C (determined during this project).

Animal husbandry
Conditions. A controlled environment was maintained in the room with optimal conditions of approximately 15 air changes per hour, a temperature of 17.1-24.2°C, a relative humidity of 33-76% and a 12 hour light/12 hour dark cycle. Temporary deviations from the maximum level for relative humidity (with a maximum of 6%) and light/dark cycle (with a maximum of 1 hour) occurred due to cleaning procedures or performance of functional observations in the room. Based on laboratory historical data these deviations are considered not to affect the study integrity.

Accommodation
Upon arrival, animals were housed in groups of 5 animals/sex/cage in suspended stainless steel cages. During the mating procedures, females were caged together with males on a one-to-one-basis in suspended stainless steel cages with wire mesh floors. Mated females and males were individually housed in labelled polycarbonate cages containing sawdust (SAWI bedding, Jelu Werk, Rosenberg, Germany) as bedding material. Certificates of analysis were examined and then retained in the NOTOX archives. Offspring was kept with the dam until termination. In order to reduce environmental influences as much as possible, cages were arranged in a latin square design over the cage rack during the study period. Each cage was identified with a colour-coded label according to dose group, showing the study number, animal identifications and other experimental details. From arrival until mating, males and females were housed in separate rooms. During the final stage of the pregnancy period (from approximately day 16 of gestation onwards) and during lactation, paper (Enviro-dri, BMI, Helmond, The Netherlands) was supplied to each dam for incorporation in the nest. The paper was analysed for contaminants. This was replaced when soiled.

Diet
Free access was allowed to standard pelleted laboratory animal diet (from Altromin (code VRF 1), Lage, Germany). Each batch was analysed for nutrients and contaminants were analysed on a regular basis. Results were examined and then retained in the NOTOX archives. Fresh diet was provided on a weekly basis, or at periodic intervals during pregnancy.

Water
Free access was allowed to tap water. Certificates of analysis (performed quarterly) were examined and then retained in the NOTOX archives. Analysis of bedding, diet, paper and water did not reveal any findings that were considered to have affected study integrity.

Test System

Rat: male and female Wistar rats Crl: (WI) BR (outbred, SPF-Quality). Untreated animals and virgin females were used at initiation of the studied test system.
Source: Charles River Deutschland, Sulzfeld, Germany.
Age at start F0-treatment: Approximately 10 weeks.
Number of F0-animals: 40 females and 40 males.
Acclimatisation F0: 5 days prior to start of treatment.
Health check F0: A health inspection was performed prior to commencement of treatment to ensure that the animals were in a good state of health.
Randomisation F0: 5 days before study start, by computer-generated random algorithm according to body weight, with all animals within ± 20% of the sex mean.
Identification F0: By tattoo on the tail.
Mating procedures F0: Females were paired on a one-to-one-basis with males from the same treatment group. Each morning following pairing, the trays under the cages were checked for ejected copulation plugs. The day on which a copulation plug was found was designated day 0 of gestation (=day 0 post-coitum). Once mating had occurred, the males and females were separated. - Mating had not been detected for females 53, 56, 57 (group 2), and 78 (group 4) after one week of pairing. On 14 October 2002, these females were paired with proven males of the same treatment group.
Parturition F0: The females were allowed to litter normally. Day 1 of lactation was defined as the day when a litter was found completed (i.e. membranes, placentas cleaned up, nest build up and/or feeding of pups started). Females that were littering were left undisturbed.
Lactation F0: Deficiencies in maternal care, such as inadequate construction or cleaning of the nest, pups left scattered and cold, physical abuse of pups or apparently inadequate lactation or feeding, were recorded.
Identification offspring: The offspring was individually identified by means of intracutaneous injection of Indian ink.

Allocation to treatment groups: 10 F0 males and 10 F0 females per treatment group

Group	Dose level, mg/kg bw/d	Individual numbers assigned F0 males	F0 females
1	0	01-10	41-50
2	40	11-20	51-60
3	200	21-30	61-70
4	1000	31-40	71-80

These dose levels were chosen based on the results of a dose range finding study (NOTOX Project 359325). Dose level Group 1: vehicle (milli-Q water) only.

Treatment F0 animals
Method: Oral gavage, using a rubber catheter attached to a plastic disposable syringe.
Frequency: Once daily, at approximately the same time each day.
Exposure period: The males were exposed for 2 weeks prior to mating, during mating and up to termination (28 days for all males). The females were exposed for 2 weeks prior to mating, during mating, during post-coitum and at least 4 days of lactation. The mean duration of treatment of females was 43 days, with a minimum of 28 days and a maximum of 56 days.
Dose volume: 5 ml/kg body weight. Actual dose volumes were

calculated according to the latest body weight.
Observations F0 animals
Mortality/Viability: Twice daily. Animals showing pain, distress or discomfort, which was considered not transient in nature or was likely to become more severe, were killed for humane reasons. The time of death was recorded as precisely as possible.
Clinical signs: Once daily detailed clinical observations were made in all animals. Once prior to start of treatment and once a week thereafter, this was also performed outside the home cage in a standard arena during the pre-mating period. The time of onset, degree and duration of clinical signs were recorded. Grading of the symptoms took place according to fixed scales. The definition of gradings within these scales was as follows: Fixed scale with maximum grade 1: grade 0 = absent, grade 1 = present. Fixed scale with max. grade 3 or 4 : grade 1 = slight, grade 2 = moderate, grade 3 = severe, grade 4 = very severe.
Cage debris of pregnant females was examined to detect potential abortions or premature births. Signs of difficult or prolonged parturition were recorded.
Functional Observations: The following tests were performed in 5 males and 5 females, randomly selected from each group: hearing ability; pupillary reflex; static righting reflex; motor activity test (recording period: 12 hours during overnight for individual animals, using a computerised monitoring system, Pearson Technical Services, Debenham, Stowmarket, England), during the motor activity test, males were caged individually and females were caged with their offspring. The assigned males were tested during week 4 of treatment and the assigned females were tested during lactation (all before blood sampling). In order to avoid hypothermia of pups, dams were removed from the pups for not more than 30-40 minutes.
Body weights: Males and females were weighed on the first day of exposure and weekly thereafter. Mated females were weighed on days 0, 7, 14 and 21 of gestation and during lactation on days 1 and 4.
Food consumption: Weekly, for males and females. During the mating period analysis of food consumption was suspended. Food consumption of mated females was measured on gestation days 0, 7, 14 and 21 and during lactation on days 1 and 4.
Water consumption: Subjective appraisal was maintained during the study, but no quantitative investigation introduced as no effect was suspected.
Reproduction processes: Male number paired with, mating date, confirmation of pregnancy and delivery day were recorded.

Observations offspring
Each litter was examined to determine the following if practically possible: - the numbers of live and dead pups at the First Litter Check (= check at day 1 of lactation) and daily thereafter (if possible, defects or cause of death were evaluated); - the individual weight of all live pups on days 1 and 4 of lactation; - sex of all pups (by assessment of the ano-genital distance); - the number of pups with physical or behavioural abnormalities, daily.

Clinical labooratory investigations F0 animals
Blood samples were collected from 5 males and 5 females

randomly selected from each group under isoflurane anaesthesia immediately prior to scheduled post mortem examination, between 07:30 and 09:30 am. The animals were fasted overnight (with a maximum of 20 hours) before blood sampling, but water was provided. Blood samples were drawn from the retro-orbital sinus of all rats/sex/group and collected into tubes prepared with EDTA for haematological parameters (0.25 ml), with citrate for clotting tests (1.0 ml) and Li-heparin-treated tubes for clinical biochemistry parameters (1.0 ml). The following parameters were determined.
Haematology: Erythrocytes count (RBC); Haemoglobin (HB); Haematocrit (HCT); Mean corpuscular volume (MCV); Mean corpuscular haemoglobin (MCH); Mean corpuscular haemoglobin concentration (MCHC); Platelet count; Red cell distribution width; Total leucocytes count (WBC); Differential leucocyte count; Clotting Potential; Prothrombin time (PT); Partial thromboplastin time (APTT).
Clinical Biochemistry: Alanine aminotransferase (ALAT); Alkaline phosphatase (ALP); Aspartate aminotransferase (ASAT); Bilirubin, total; Chloride; Cholesterol, total; Creatinine; Glucose; Phosphorus (inorganic); Protein, total; Protein, albumin; Urea; Calcium; Potassium; Sodium.

Pathology, F0 animals
Termination: All animals surviving to the end of the observation period and all moribund animals were anaesthetised using iso-flurane and subsequently exsanguinated. All animals were fasted overnight (with a maximum of 20 hours) prior to necropsy, but water was provided. Males were killed after the mating period when the minimum total dosing period of 28 days had been completed. Females with litter were killed at day 4 post partum or shortly thereafter. Females without litter were killed around the same time as the females with litter. In case a female was not pregnant, the uterus was stained using the Salewski technique in order to determine any very early post-implantation losses (=implantation site scars). Based on macroscopic findings (uterus enlarged and greenish contents), no Saleweski staining was performed on the uterus of female 63.
Macroscopic examination: After sacrifice or death all parental animals were subjected to macroscopic examination of the cranial, thoracic and abdominal tissues and organs, with special attention being paid to the reproductive organs. Descriptions of all macroscopic abnormalities were recorded. Samples of the following tissues and organs were collected and fixed in neutral phosphate buffered 4%
formaldehyde solution (except the epididymides and testes): From 5 surviving animals/sex/group and from all animals that died spontaneously or were killed in extremis:
Identification marks; not processed Ovaries; Adrenal glands; Pancreas; Aorta; Peyer's patches (jejunum, ileum) if detectable; Brain (cerebellum, mid-brain, cortex); Pituitary gland; Caecum; Preputial gland; Cervix; Prostate gland; Clitoral gland; Rectum; Colon; Salivary glands (mandibular, sublingual); Coagulation gland; Sciatic nerve; Duodenum; Seminal vesicles; Epididymides (fixed in Bouin’s); Skeletal muscle; Eyes with optic nerve and Harderian gland; Skin; Female mammary gland area; Spinal cord (cervical, midthoracic, lumbar); Femur including joint; Spleen; Heart; Sternum with bone marrow; Ileum; Stomach;

Jejunum; Testes (fixed in Bouin's); Kidneys; Thymus; Larynx; Thyroid including parathyroid; Lachrymal gland, exorbital; Tongue; Liver; Trachea; Lung (infused with formalin); Urinary bladder; Lymph nodes (mandibular, mesenteric); Uterus; Nasopharynx; Vagina; Oesophagus; All gross lesions.
From all adult animals: Cervix; Clitoral gland; Coagulation gland; Epididymides (fixed in Bouin's); Ovaries; Preputial gland; Prostate gland; Seminal vesicles; Testes (fixed in Bouin's); Uterus; Vagina; All gross lesions.
Organ weights: Terminal body weight was recorded for all parental animals. The following organ weights were recorded. From 5 surviving animals/sex/group: Adrenal glands; Brain; Epididymides (total weight for both); Heart; Kidneys; Liver; Spleen; Testes; Thymus. From all adult males: Epididymides (total weight for both); Testes.
Histotechnology: All organ and tissue samples, as defined under Histopathology (following), were processed, embedded and cut at a thickness of 2-4 µm and stained with haematoxylin and eosin. Of the selected 5 males/group of the control and high dose group, additional slides of the testes were prepared to examine staging of spermatogenesis. The testes was processed, sectioned at 3-4 µm, and stained with PAS/haematoxylin.
Histopathology
The following slides were examined by a pathologist: The preserved organs and tissues of the selected animals of groups 1 and 4. - The additional slides of the testes of the selected 5 males/group of groups 1 and 4 to examine staging of spermatogenesis. - The preserved organs and tissues of the animals of all dose groups which died spontaneously or were killed in extremis. - All gross lesions of all animals (all dose groups). - The preserved organs and tissues of all non-pregnant females and animals suspected of infertility. All abnormalities were described and included in the report.

Result:

Analysis of dose preparations
Accuracies were out of the 90-110% range on several days of analysis. It was considered not to be caused by inaccurate preparation of formulations but by analytical problems (i.e. sensitivity fluctuation in time of the LCMSMS system used). For formulations in Milli-U water (19 September 2002), accuracies at a target concentration of 8 mg/g ranged from 84 to 112%. Accuracies at a target concentration of 200 mg/g were between 88 and 91%. For formulations in Milli-U water adjusted to pH 4 (19 September 2002, 09 October 2002, 31 October 2002), accuracies at target concentrations of 8 mg/g ranged from 87 to 134%. Accuracies at a target concentration of 40 mg/g were between 88 and 98%. Accuracies at a target concentration of 200 mg/g were between 80 and 103%. The accuracies of group 4 formulations measured on 23 October 2002 were considered not reliable because concentrations were relatively low at t=0 (68-81% of target) but at target level after 8 days of storage.
Homogeneity: The relative standard deviation for the measurements (19 September 2002, 09 October 2002, 31 October 2002) ranged from 1.3 –12% indicating that formulations were homogeneous. The higher relative standard deviation at lower concentration was considered due to the analytical method used.
Stability: The measurements on 19 September 2002 showed that concentrations in formulations in Milli-U water

and in Milli-U water adjusted to pH 4 were stable for 4 hours at room temperature. Formulations in Milli-U water adjusted to pH 4 are stable for 8 days at 37°C (27 September 2002). The results of 17 October 2002 confirmed stability for 8 days at 37°C for Group 2. However for Group 4, stability was not confirmed. Therefore, it was decided to repeat stability testing for Group 4. Results of
these analyses (31 October 2002) showed an increase of 33% over 8 days at 37°C. This was due to the low values at t=0. The values at t=8 days were very close to the target (200 mg/g). Based on this it was concluded that the Group 4 formulations were stable over 8 days at 37°C and thus confirmed the results of 27 September 2002.
Conclusion: Taking the analytical problems into account, it could be concluded that formulations were prepared accurately and homogeneously. Furthermore, it could be concluded th at formulations in Milli-U water were stable for 4 hours at room temperature and that formulations in Milli-U water adjusted to pH 4 were stable for 4 hours at room temperature and for 8 days at 37°C.

Mortality
No unscheduled deaths occurred during the study period.

Clinical signs
Females of the highest dose group showed aggressive and restless behaviour during days 5 to 15 of treatment. Incidental findings that were noted included scabs, wound, hunched posture, piloerection, broken upper incisors, broken tail apex, and alopecia at several parts of the body. These findings are commonly noted in rats of this age and strain which are housed and treated under the conditions in this study. At the incidence observed, these were considered signs of no toxicological significance.

Functional observations
No changes were observed in hearing ability, pupillary reflex, static righting reflex and grip strength in the animals treated with dl-lactone, when compared to control animals. The variation in motor activity did not indicate a relation with treatment. Females of the highest dose group showed a decreased motor activity at the low sensors when compared to the control group. Since this change occurred in the absence of similar changes of the high sensors, they were considered to be of no toxicological relevance.

Body weight
Body weights and body weight gain were unaffected by treatment up to and including 1000 mg/kg bw/d. Males of the 200 mg/kg bw/d dose group showed statistically significant decreased body weights on day 1 of the mating period. In the absence of a clear dose response relationship, this finding was considered to be of no toxicological relevance.
On day 8 of the pre-mating period, males of the highest dose group showed a statistically significant increased body weight gain. As this finding was very slight and not considered to be adverse, it was considered to be of no toxicological significance.

Food consumption

Food consumption and relative food consumption were unaffected by treatment up to and including 1000 mg/kg bw/d. Statistically significant increased (relative) food consumption was observed on days 1-7 of the post-mating period in males at 40 mg/kg bw/d. This finding was not considered to be an adverse effect. No explanation for this increase can be given.

Clinical laboratory investigations
Haematology: Haematological parameters of treated rats were considered not to have been affected by treatment.
Clinical Biochemistry: The serum potassium level of males of the highest dose group was statistically significantly increased when compared to the control group. The statistically significantly increased serum sodium level of males of the highest dose group was considered to have arisen as a result of slightly low control values and thus considered to be of no toxicological significance. The values of glucose and inorganic phosphate achieving a level of statistical significance in treated males when compared to the control group, were considered to be of no toxicological significance as no clear dose-response relationship was observed.

Macroscopic examination
Macroscopic observations at necropsy did not reveal any alterations that were considered to have arisen as a result of treatment. Incidental findings included pelvic dilation of both kidneys, yellowish soft nodule at the tail of the left epididymis, dark red discolouration of the right clitoral gland, watery-clear cyst at the right ovary, haemorrhagic/clotted blood in the right uterus horn, enlarged spleen, many dark red foci on the right clitoral gland, uterus enlarged with greenish contents, hard and dark red discolouration of the papillary process of the liver, alopecia and gray-white discolouration of the medulla of the kidney. These findings are occasionally seen among rats used in these types of studies and in the absence of correlated microscopic findings they were considered changes of no toxicological significance.

Organ weights
No treatment-related changes were present. Males of the 200 mg/kg bw/d dose group showed statistically significantly decreased absolute and relative adrenals weight and females of the 40 mg/kg bw/d dose group showed statistically significantly increased relative adrenals weight. In the absence of a dose-response relationship, these findings were considered to be caused by chance and not related to treatment.

Microscopic examination
Microscopic findings: There were no treatment-related findings.
Staging of spermatogenesis: The assessment of the integrity of the spermatogenetic cycle did not provide any evidence of impaired spermatogenesis.

Reproduction
Reproduction parameters were unaffected by treatment up to 1000 mg/kg bw/d. Of the control group, two females were

non-pregnant. Of the 40 mg/kg bw/d dose group, one female did not mate and two females mated in the second mating period. Of the 200 mg/kg bw/d dose group, two females were non-pregnant. And of the 1000 mg/kg bw/d dose group, one female was non-pregnant. Mating performance, duration of gestation, fertility parameters and number of pups at birth were similar for the control and treated groups.

Breeding data
Breeding parameters were unaffected by treatment up to 1000 mg/kg bw/d. The number of dead and living pups at first litter check, postnatal loss between days 0-4 post-partum, living pups at day 4 post-partum and the viability index were similar for control and treated groups.

Test substance: dl-Lactone from Roche Dalry, batch BX226, purity 99.8% as per certificate of analysis.

Conclusion: dl-Lactone was administered by daily oral gavage to male and female Wistar rats at dose levels of 40, 200 or 1000 mg/kg bw/d. The males were exposed for 2 weeks prior to mating, during mating and up to termination (28 days for all males). The females were exposed for 2 weeks prior to mating, during mating, during post-coitum and at least 4 days of lactation. The mean duration of treatment of females was 43 days, with a minimum of 28 days and a maximum of 56 days.
Parental toxicity was assessed by observing mortality, clinical signs, body weights, food consumption, functional observations, clinical laboratory investigations, macroscopic examination, organ weights and microscopic examination. At 40 mg/kg bw/d, no parental toxicity was observed. At 200 mg/kg bw/d, no parental toxicity was observed. At 1000 mg/kg bw/d, parental toxicity consisted of clinical symptoms (aggressive and restless behaviour) in females during days 5 to 15 of treatment and of increased serum potassium level in males.
Reproductive toxicity was assessed by observing the mating performance, fertility indices and number of pups at birth. No reproductive toxicity was observed up to 1000 mg/kg bw/d.
Breeding toxicity was assessed by observing the number of postnatal and breeding loss during lactation. No breeding toxicity was observed up to 1000 mg/kg bw/d.
Developmental toxicity was assessed by observing clinical signs, body weights and macroscopic examination of the pups during their lactation period. No developmental toxicity was observed up to 1000 mg/kg bw/d.
In conclusion, gavage treatment of male and female Wistar rats with dl-Lactone at dose levels of 40, 200 or 1000 mg/kg bw/d for at least 28 days (during premating, mating, post-coitum and lactation) revealed slight parental toxicity in animals receiving 1000 mg/kg bw/d; this toxicity was transient during the study in the case of the females, while the males were killed after 28 days so that potential recovery could not be assessed. Reproductive, breeding and developmental parameters were unaffected up to 1000 mg/kg bw/d.
Based on the results in this combined repeated dose toxicity study with reproduction/developmental screening test, the definitive sub-chronic parental NOAEL was established as being 200 mg/kg bw/d while the LOAEL was 1000 mg/kg bw/d.

Reliability: (1) valid without restriction
OECD test under GLP.

Flag: Critical study for SIDS endpoint
15-MAR-2005 (5)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium, strains TA97, TA98, TA100, TA102, TA1535
Concentration: 0 (control), 50, 158.1, 500, 1581 and 5000 µg/plate
Cytotoxic Concentration: >5000 µg/plate
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1999
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Strains
Salmonella typhimurium strains TA1535, TA97, TA98, TA100 and TA102 were obtained from BN Ames. Nutrient broth cultures of each strain, supplemented with 9% DMSO, were stored in liquid nitrogen. Strain identities and characteristics were periodically checked by recommended procedures (full details in report).
For use in tests, cultures of the strains were grown overnight at 37°C in a shaking water bath in a nutrient broth liquid medium (described in full detail including sources of chemicals in the report). The growth of overnight cultures was controlled by measuring the optical density on a photometer at 650 nm. Each bacterial strain was diluted 10E-6 in 0.85% NaCl, 100 µl of the last dilution step was plated on a nutrient broth complete medium (details in report). Two replicate plates were incubated at 37°C, upside down, for 2 days. The number of colonies was registered and the number of cells plated on Vogel-Bronner minimal medium (full details in report) was calculated.
The sensitivity of the S. typhimurium strains was verified using the following positive controls: sodium azide with TA1535 and TA100, ICR191 with TA97, 2-nitrofluorene with TA98 and Mitomycin C with TA102. Moreover, 2-aminoanthracene was used with all strains with and without metabolic activation to examine the activity of the S9 mix; S9 from Molecular Toxicology, Boone NC, USA (all chemicals and S9 fully detailed in report).
A toxicity prescreen with plate incorporation and TA100 in duplicate was negative up to 5000 µg/plate and led to the selection of the tested doses as listed.
Standard Ames procedure
Test tubes containing 2 ml of 0.7% agar medium were autoclaved and kept in a prewarmed bath at 42-45°C; the following solutions were added in order.
0.2 ml of histidine/biotin mixture corresponding to 21 µg L-histidine and 24.4 µg biotin;
0.1 ml of test compound at different concentrations or of the solvent or 0.05 ml of the different reference substances;
0.1 ml of overnight cultures of the bacterial strain;
0.5 ml of S9 mix or, for tubes without metabolic activation, 0.5 ml sodium-phosphate-buffered saline at 0.2M, pH7.4.

The contents of the tubes were mixed and poured immediately onto Vogel-Bronner minimal agar plates. Three replicates for the test compound at each treatment and concentration and for the negative control or two replicates for the positive controls were incubated at 37°C, uspide down, for 2 days.
Liquid pre-incubation assay
The following solutions are added in order:
0.1 ml of test compound at different concentrations or of the solvent or 0.05 ml of the different reference substances;
0.5 ml of S9 mix or, for tubes without metabolic activation, 0.5 ml sodium-phosphate-buffered saline at 0.2M, pH7.4;
0.1 ml of overnight cultures of the bacterial strain.
The test tubes are incuibated and shaken for 30 min at 37°C.
2.2 ml soft agar supplemented with 21 µg L-histidine and 24.4 µg biotin was added afterwards and the content of the tubes were mixed and poured onto Vogel-Bronner minimal agar plates. Three replicates for the test compound at each treatment and concentration and for the negative control or two replicates for the positive controls were incubated at 37°C, uspide down, for 2 days.
Data reporting
COlonies are usually counted electronically using a Domino automatic image analysis system (Perceptive Instruments, Haverhill, England). Microscopic examination of the bacterial background lawn, resulting from the trace of histidine added, is an aid to determine the toxicity of the test compound. Toxicity was noted if apparent.

Result: The dose compund was soluble in water.
No toxic effects were seen up to the highest dose tested of 5000 µg/plate.
The test compound did not induce any dose-related increase in the number of revertant colonies per plate in any of the five tester strains (full details given in two tables, one each for standard Ames procedure and for liquid pre-incubation).
The mutant frequencies in the controls were in the range of the lab's historical control values and of data from literature. The positive controls induced significant increases in the mutant frequencies, verifying the sensitivity of the strains used.

Test substance: Ro 01-4479/000 (=dl-Lactone pure) from Roche Dalry, batch no. 805046, Analysis no. A9819037, assay 100% (anhydrous), expiry date 05/2002.

Conclusion: In a bacterial reverse mutation assay assay as described with five strains of Salmonella typhimurium, at doses of 50-5000 µg/plate, with and without metabolic activation by S9 mix, with standard Ames procedure and with liquid pre-incubation, dl-lactone did not show any indication of mutagenic activity.

Reliability: (1) valid without restriction
International standard test under GLP.

Flag: Critical study for SIDS endpoint

16-NOV-2005 (23)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: other: NMRI BR
Route of admin.: gavage
Exposure period: 24 and 48 hours

Doses: 1500 (two groups), 750 and 375 mg/kg bw
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals
NMRI BR (SPF) mice from Charles River, Sulzfeld, Germany were used. Animals were young adults (6-8 weeks old), females were nulliparous and non-pregnant.
The animals were housed in an air-conditioned room with approximately 15 air changes per hour, a temperature of 22±3 °C and a relative humidity between 30 and 70%; inspite of the relative humidity exceeding 70% for part of the test period, no abnormalities were noted in the animals and it was concluded that this deviation did not affect the integrity of the study. The animal room was illuminated for 12 hours per day with artificial fluorescent lighting and was dark for 12 hours.
The animals were housed in randomised groups of 5 each per sex per cage in labelled polycarbonate cages containing purified sawdust (Sawi, Jelu-Werk, Rosenberg, Germany) as bedding material. Paper bedding (BMI Helmond, The Netherlands) was provided for nest material. There was free access to standard pelleted diet (Altromin (code VRF 1), Lage, Germany) and to tap water. Certificates of analysis for all substrates, feed and water are retained in the NOTOX archives. For all animals there was an acclimatisation period of at least 5 days before start of treatment under laboratory conditions.
Treatment groups
Two dose groups, one of 4 males and 4 females at 2000 mg/kg bw and one of 3 males and 3 females at 1500 mg/kg bw, all administered by gavage in 10 ml of physiological saline, were used for the dose range-finding test. 5 males each per test group respectively were used as negative and positive controls as there were no obvious differences between sexes in the range-finding test. All animals were identifed by a unique number on the tail.
In the main test there were 6 groups of 5 male and 5 female mice each, labelled A through F. A was a negative control (vehicle only, 10 ml physiological saline/kg bw) group, B and C were high-dose treatment groups (1500 mg dl-lactone/kg bw in physiological saline, dose adjusted to a volume of 10 ml/kg bw; group B to be sampled at 24 hours post-dosing, group C at 48 hours post-dosing); D and E were middle- and low-dose treatment groups (D 750 mg and E 375 mg dl-lactone/kg bw in physiological saline, both groups D and E to be sampled at 24 hours post-dosing), while F was the positive control group (50 mg cyclophosphamide/kg bw,
dissolved in physiological saline, sampling at 48 hours; cyclophosphamide from Asta-Werke, Germany). Feed was withheld 3-4 hours prior to dosing. Administration was by oral gastric intubation.
Observations
The animals were observed at least once a day for signs of toxicity. Prior to dosing the animals were weighed.
Preparation of erythroblasts and erythrocytes

The test animals were killed by cervical dislocation 24 hours (groups A, B, D and E) respectively 48 hours (groups C and F) after dosing. In every instance, both femurs were removed and freed of blood and muscles. Then, both ends of the bone were shortened until a small opening to the marrow canal became visible. The prepared bones were flushed with foetal calf serum (FCS), the cell suspension was collected and centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the pellets re-suspended in FCS. A drop of the suspension was placed on the end of a previously cleaned and marked (NOTOX study number, animal number) microscopic slide, spread using a clean slide and air-dried, fixed with 100% methanol and automatically stained in a HEMA-tek Slide Stainer (Miles, Bayer Nederland, The Netherlands) and covered with a glass coverslip.
Before analysis, the unique marks of each slide were randomised by covering with an adhesive label bearing the NOTOX study number and a code. Slides were first screened at a magnification of x100 for suitable regions, then scored at x1000. The number of micronucleated polychromatic erythrocytes was counted in a total of 2000 polychromatic erythrocytes per slide. The ratio of polychromatic to normochromatic erythrocytes was determined in the first 1000 erythrocytes scanned. Micronuclei were only counted in polychromatic erythrocytes.
Statistics
After counting, the randomisation was unveiled and averages and standard deviations for the six groups were calculated. A test substance and/or dose would be considered positive if it induced a statistically significant (Wilcoxon Rank Sum test, two-sided test at $P < 0.05$) increase in the frequency of micronucleated polychromatic erythrocytes, at any dose or sampling time. Conversely, a test substance is considered negative if there is no such statistically signifcant difference at any dose or sampling time.
Acceptability criteria
A micronucleus test is considered acceptable if it meets the following criteria: 1) the positive control substance, cyclophosphamide, induces a significant increase in micronucleated polychromatic erythrocytes and the incidence of micronucleated polychromatic erythrocytes in the control animals is reasonably within the laboratory historical controls range (mean ± 3 SD).

Result: Dose range-finding study
4 males and 4 females were dosed with 2000 mg dl-lactone in physiological saline per kg bw. All treated animals showed abnormalities during an observation period of 3 days: 1 male died within 20 min, 2 more males and 1 female died within 1.5 hours. During the first 1.5 hours, all animals showed lethargy or convulsions, one male had tremors. At days 2 and 3 after gavage, all survivors showed no abnormalities.
3 males and 3 females were dosed with 1500 mg dl-lactone in physiological saline per kg bw. All treated animals except one female showed lethargy within the first 20 min; after 1.5 hours, 2 one male and female each showed no signs while thothers were lethargic and had a rough coat. At days 2 and 3 after gavage, all survivors showed no abnormalities.
Therefore, 1500, 750 and 375 mg/kg bw were chosen as the doses for the main test, with two high-dose groups, plus a vehicle negative and a cyclophosphamide positive control group. All

six groups consisted of 5 males and 5 females.
Micronucleus test
The mean bodyweights of all six groups, recorded just before dosing, were not statistically different (data available).
All animals treated with 375 and 750 mg/kg bw showed no abnormalities; this was also true for both the negative and positive controls. Among the 20 animals in the two 1500-mg/kg-bw-groups, 7/20 were lethargic at the beginning but all animals were normal after 19 hours.
Average numbers of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes and ratios of polychromatic to normochromatic erythrocytes:

Group	Dose, mg/kg bw	Sampling time, h	Number, mean±SD	Ratio, mean±SD
Males				
A, vehicle control	0	24	1.0±1.1	1.21±0.12
B, dl-lactone	1500	24	0.4±0.5	0.89±0.10
C, dl-lactone	1500	48	0.4±0.5	0.95±0.21
D, dl-lactone	750	24	1.2±1.3	1.08±0.15
E, dl-lactone	375	24	1.4±0.5	1.48±0.12
F, Cyclophosphamide	50	48	45.6±24.6**	0.32±0.10
Females				
A, vehicle control	0	24	0.2±0.4	1.31±0.17
B, dl-lactone	1500	24	0.8±1.1	1.17±0.09
C, dl-lactone	1500	48	1.0±1.4	1.17±0.11
D, dl-lactone	750	24	0.6±0.9	1.24±0.23
E, dl-lactone	375	24	0.8±0.8	1.13±0.10
F, Cyclophosphamide	50	48	20.4±2.6**	0.36±0.10

** Significantly different from negative (vehicle) control group, P <= 0.01.
All single data are available in the report.

Test substance: dl-Lactone from Roche Dalry, batch no. BX226, purity 99.8% according to analytical certificate.

Conclusion: dl-Lactone at an oral dose of 1500 mg/kg bw did not induce any increase in the incidence of micronucleated polychromatic erythrocytes in this in vivo mouse test. Therefore, lactone is regarded as negative regarding genotoxic effects in this model.
Further, the test groups treated with dl-lactonedid not show any decrease in the ratio of normochromatic to polychromatic erythrocytes, which reflects a lack of toxic effects of dl-lactone on erythropoiesis.
However, 4/8 animals in the high-dose range-finder at 2000 mg/kg bw died within 1.5 hours. No animal in either the lower (1500 mg/kg bw) dose range finding study (3 males, 3 females) nor in the treatment groups died.

Reliability: (1) valid without restriction
OECD study under GLP, reliability 1.

Flag: Critical study for SIDS endpoint

24-SEP-2004 (33)

5.7 Carcinogenicity

5.8.1 Toxicity to Fertility

Type: other: combined repeat dose and reproductive toxicity screening test

Species: rat
Sex: male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF quality)
Route of administration: gavage
Exposure Period: males: 2 weeks prior to mating, during mating and up to termination (28 days for all males)
females: 2 weeks prior to mating, during mating, during gestation and at least 4 days of lactation; mean treatment duration for females was 43 days
Frequency of treatment: once daily
Premating Exposure Period
male: 14 days
female: 14 days
No. of generation studies: 1
Doses: 0 (controls, vehicle only = milli-U water), 40, 200 and 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Parental: = 1000 mg/kg bw
NOAEL F1 Offspring: = 1000 mg/kg bw
Result: no reprotoxic effects noted up to the highest dose of 1000 mg/kg bw/d

Method: other: OECD Guideline 422, "Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test", 22-Mar-1996
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Test substance formulation
Vehicle Water (Milli-Q) of 37°C adjusted to pH 4. Acetic acid was added to milli-Q water to obtain pH 4. Rationale for vehicle: Based on trial formulations performed at NOTOX and on information provided by the sponsor. During NOTOX Project 257568 (Determination of the hydrolysis of dl-lactone as a function of pH) it was determined that dllactone was hydrolytically stable at pH 4 and 50°C. Formulations in Milli-Q water are stable for 4 hours at room temperature and formulations in Milli-Q water adjusted to pH 4 are stable for 4 hours at room temperature and for 8 days at 37°C (determined during this project).

Animal husbandry
Conditions. A controlled environment was maintained in the room with optimal conditions of approximately 15 air changes per hour, a temperature of 17.1-24.2°C, a relative humidity of 33-76% and a 12 hour light/12 hour dark cycle. Temporary deviations from the maximum level for relative humidity (with a maximum of 6%) and light/dark cycle (with a maximum of 1 hour) occurred due to cleaning procedures or performance of functional observations in the room. Based on laboratory historical data these deviations are considered not to affect the study integrity.

Accommodation
Upon arrival, animals were housed in groups of 5 animals/sex/cage in suspended stainless steel cages. During the mating procedures, females were caged together with males on a one-to-one-basis in suspended stainless steel cages with wire mesh floors. Mated females and males were individually

housed in labelled polycarbonate cages containing sawdust (SAWI bedding, Jelu Werk, Rosenberg, Germany) as bedding material. Certificates of analysis were examined and then retained in the NOTOX archives. Offspring was kept with the dam until termination. In order to reduce environmental influences as much as possible, cages were arranged in a latin square design over the cage rack during the study period. Each cage was identified with a colour-coded label according to dose group, showing the study number, animal identifications and other experimental details. From arrival until mating, males and females were housed in separate rooms. During the final stage of the pregnancy period (from approximately day 16 of gestation onwards) and during lactation, paper (Enviro-dri, BMI, Helmond, The Netherlands) was supplied to each dam for incorporation in the nest. The paper was analysed for contaminants. This was replaced when soiled.

Diet
Free access was allowed to standard pelleted laboratory animal diet (from Altromin (code VRF 1), Lage, Germany). Each batch was analysed for nutrients and contaminants were analysed on a regular basis. Results were examined and then retained in the NOTOX archives. Fresh diet was provided on a weekly basis, or at periodic intervals during pregnancy.

Water
Free access was allowed to tap water. Certificates of analysis (performed quarterly) were examined and then retained in the NOTOX archives. Analysis of bedding, diet, paper and water did not reveal any findings that were considered to have affected study integrity.

Test System
Rat: male and female Wistar rats Crl: (WI) BR (outbred, SPF-Quality). Untreated animals and virgin females were used at initiation of the studied test system.
Source: Charles River Deutschland, Sulzfeld, Germany.
Age at start F0-treatment: Approximately 10 weeks.
Number of F0-animals: 40 females and 40 males.
Acclimatisation F0: 5 days prior to start of treatment.
Health check F0: A health inspection was performed prior to commencement of treatment to ensure that the animals were in a good state of health.
Randomisation F0: 5 days before study start, by computer-generated random algorithm according to body weight, with all animals within ± 20% of the sex mean.
Identification F0: By tattoo on the tail.
Mating procedures F0: Females were paired on a one-to-one-basis with males from the same treatment group. Each morning following pairing, the trays under the cages were checked for ejected copulation plugs. The day on which a copulation plug was found was designated day 0 of gestation (=day 0 post-coitum). Once mating had occurred, the males and females were separated. - Mating had not been detected for females 53, 56, 57 (group 2), and 78 (group 4) after one week of pairing. On 14 October 2002, these females were paired with proven males of the same treatment group.
Parturition F0: The females were allowed to litter normally. Day 1 of lactation was defined as the day when a litter was

found completed (i.e. membranes, placentas cleaned up, nest build up and/or feeding of pups started). Females that were littering were left undisturbed.
Lactation F0: Deficiencies in maternal care, such as inadequate construction or cleaning of the nest, pups left scattered and cold, physical abuse of pups or apparently inadequate lactation or feeding, were recorded.
Identification offspring: The offspring was individually identified by means of intracutaneous injection of Indian ink.

Allocation to treatment groups: 10 F0 males and 10 F0 females per treatment group

Group	Dose level, mg/kg bw/d	Individual numbers assigned F0 males	F0 females
1	0	01-10	41-50
2	40	11-20	51-60
3	200	21-30	61-70
4	1000	31-40	71-80

These dose levels were chosen based on the results of a dose range finding study (NOTOX Project 359325). Dose level Group 1: vehicle (milli-Q water) only.

Treatment F0 animals
Method: Oral gavage, using a rubber catheter attached to a plastic disposable syringe.
Frequency: Once daily, at approximately the same time each day.
Exposure period: The males were exposed for 2 weeks prior to mating, during mating and up to termination (28 days for all males). The females were exposed for 2 weeks prior to mating, during mating, during post-coitum and at least 4 days of lactation. The mean duration of treatment of females was 43 days, with a minimum of 28 days and a maximum of 56 days.
Dose volume: 5 ml/kg body weight. Actual dose volumes were calculated according to the latest body weight.
Observations F0 animals
Mortality/Viability: Twice daily. Animals showing pain, distress or discomfort, which was considered not transient in nature or was likely to become more severe, were killed for humane reasons. The time of death was recorded as precisely as possible.
Clinical signs: Once daily detailed clinical observations were made in all animals. Once prior to start of treatment and once a week thereafter, this was also performed outside the home cage in a standard arena during the pre-mating period. The time of onset, degree and duration of clinical signs were recorded. Grading of the symptoms took place according to fixed scales. The definition of gradings within these scales was as follows: Fixed scale with maximum grade 1: grade 0 = absent, grade 1 = present. Fixed scale with max. grade 3 or 4 : grade 1 = slight, grade 2 = moderate, grade 3 = severe, grade 4 = very severe.
Cage debris of pregnant females was examined to detect potential abortions or premature births. Signs of difficult or prolonged parturition were recorded.
Functional Observations: The following tests were performed in 5 males and 5 females, randomly selected from each group: hearing ability; pupillary reflex; static righting reflex; motor activity test (recording period: 12 hours during overnight for individual animals, using a computerised

monitoring system, Pearson Technical Services, Debenham, Stowmarket, England), during the motor activity test, males were caged individually and females were caged with their offspring. The assigned males were tested during week 4 of treatment and the assigned females were tested during lactation (all before blood sampling). In order to avoid hypothermia of pups, dams were removed from the pups for not more than 30-40 minutes.
Body weights: Males and females were weighed on the first day of exposure and weekly thereafter. Mated females were weighed on days 0, 7, 14 and 21 of gestation and during lactation on days 1 and 4.
Food consumption: Weekly, for males and females. During the mating period analysis of food consumption was suspended. Food consumption of mated females was measured on gestation days 0, 7, 14 and 21 and during lactation on days 1 and 4.
Water consumption: Subjective appraisal was maintained during the study, but no quantitative investigation introduced as no effect was suspected.
Reproduction processes: Male number paired with, mating date, confirmation of pregnancy and delivery day were recorded.

Observations offspring
Each litter was examined to determine the following if practically possible: - the numbers of live and dead pups at the First Litter Check (= check at day 1 of lactation) and daily thereafter (if possible, defects or cause of death were evaluated); - the individual weight of all live pups on days 1 and 4 of lactation; - sex of all pups (by assessment of the ano-genital distance); - the number of pups with physical or behavioural abnormalities, daily.
Clinical labooratory investigations F0 animals
Blood samples were collected from 5 males and 5 females randomly selected from each group under isoflurane anaesthesia immediately prior to scheduled post mortem examination, between 07:30 and 09:30 am. The animals were fasted overnight (with a maximum of 20 hours) before blood sampling, but water was provided. Blood samples were drawn from the retro-orbital sinus of all rats/sex/group and collected into tubes prepared with EDTA for haematological parameters (0.25 ml), with citrate for clotting tests (1.0 ml) and Li-heparin-treated tubes for clinical biochemistry parameters (1.0 ml). The following parameters were determined.
Haematology: Erythrocytes count (RBC); Haemoglobin (HB); Haematocrit (HCT); Mean corpuscular volume (MCV); Mean corpuscular haemoglobin (MCH); Mean corpuscular haemoglobin concentration (MCHC); Platelet count; Red cell distribution width; Total leucocytes count (WBC); Differential leucocyte count; Clotting Potential; Prothrombin time (PT); Partial thromboplastin time (APTT).
Clinical Biochemistry: Alanine aminotransferase (ALAT); Alkaline phosphatase (ALP); Aspartate aminotransferase (ASAT); Bilirubin, total; Chloride; Cholesterol, total; Creatinine; Glucose; Phosphorus (inorganic); Protein, total; Protein, albumin; Urea; Calcium; Potassium; Sodium.

Pathology, F0 animals
Termination: All animals surviving to the end of the observation period and all moribund animals were anaesthetised using iso-flurane and subsequently exsanguinated. All animals

were fasted overnight (with a maximum of 20 hours) prior to necropsy, but water was provided. Males were killed after the mating period when the minimum total dosing period of 28 days had been completed. Females with litter were killed at day 4 post partum or shortly thereafter. Females without litter were killed around the same time as the females with litter. In case a female was not pregnant, the uterus was stained using the Salewski technique in order to determine any very early post-implantation losses (=implantation site scars). Based on macroscopic findings (uterus enlarged and greenish contents), no Saleweski staining was performed on the uterus of female 63.
Macroscopic examination: After sacrifice or death all parental animals were subjected to macroscopic examination of the cranial, thoracic and abdominal tissues and organs, with special attention being paid to the reproductive organs. Descriptions of all macroscopic abnormalities were recorded. Samples of the following tissues and organs were collected and fixed in neutral phosphate buffered 4% formaldehyde solution (except the epididymides and testes): From 5 surviving animals/sex/group and from all animals that died spontaneously or were killed in extremis: Identification marks; not processed Ovaries; Adrenal glands; Pancreas; Aorta; Peyer's patches (jejunum, ileum) if detectable; Brain (cerebellum, mid-brain, cortex); Pituitary gland; Caecum; Preputial gland; Cervix; Prostate gland; Clitoral gland; Rectum; Colon; Salivary glands (mandibular, sublingual); Coagulation gland; Sciatic nerve; Duodenum; Seminal vesicles; Epididymides (fixed in Bouin’s); Skeletal muscle; Eyes with optic nerve and Harderian gland; Skin; Female mammary gland area; Spinal cord (cervical, midthoracic, lumbar); Femur including joint; Spleen; Heart; Sternum with bone marrow; Ileum; Stomach; Jejunum; Testes (fixed in Bouin’s); Kidneys; Thymus; Larynx; Thyroid including parathyroid; Lachrymal gland, exorbital; Tongue; Liver; Trachea; Lung (infused with formalin); Urinary bladder; Lymph nodes (mandibular, mesenteric); Uterus; Nasopharynx; Vagina; Oesophagus; All gross lesions.
From all adult animals: Cervix; Clitoral gland; Coagulation gland; Epididymides (fixed in Bouin’s); Ovaries; Preputial gland; Prostate gland; Seminal vesicles; Testes (fixed in Bouin’s); Uterus; Vagina; All gross lesions.
Organ weights: Terminal body weight was recorded for all parental animals. The following organ weights were recorded. From 5 surviving animals/sex/group: Adrenal glands; Brain; Epididymides (total weight for both); Heart; Kidneys; Liver; Spleen; Testes; Thymus. From all adult males: Epididymides (total weight for both); Testes.
Histotechnology: All organ and tissue samples, as defined under Histopathology (following), were processed, embedded and cut at a thickness of 2-4 µm and stained with haematoxylin and eosin. Of the selected 5 males/group of the control and high dose group, additional slides of the testes were prepared to examine staging of spermatogenesis. The testes was processed, sectioned at 3-4 µm, and stained with PAS/haematoxylin.
Histopathology
The following slides were examined by a pathologist: The preserved organs and tissues of the selected animals of groups 1 and 4. - The additional slides of the testes of the selected

5 males/group of groups 1 and 4 to examine staging of spermatogenesis. - The preserved organs and tissues of the animals of all dose groups which died spontaneously or were killed in extremis. - All gross lesions of all animals (all dose groups). - The preserved organs and tissues of all non-pregnant females and animals suspected of infertility. All abnormalities were described and included in the report.

Result: Analysis of dose preparations
Accuracies were out of the 90-110% range on several days of analysis. It was considered not to be caused by inaccurate preparation of formulations but by analytical problems (i.e. sensitivity fluctuation in time of the LCMSMS system used). For formulations in Milli-U water (19 September 2002), accuracies at a target concentration of 8 mg/g ranged from 84 to 112%. Accuracies at a target concentration of 200 mg/g were between 88 and 91%. For formulations in Milli-U water adjusted to pH 4 (19 September 2002, 09 October 2002, 31 October 2002), accuracies at target concentrations of 8 mg/g ranged from 87 to 134%. Accuracies at a target concentration of 40 mg/g were between 88 and 98%. Accuracies at a target concentration of 200 mg/g were between 80 and 103%. The accuracies of group 4 formulations measured on 23 October 2002 were considered not reliable because concentrations were relatively low at t=0 (68-81% of target) but at target level after 8 days of storage.
Homogeneity: The relative standard deviation for the measurements (19 September 2002, 09 October 2002, 31 October 2002) ranged from 1.3 –12% indicating that formulations were homogeneous. The higher relative standard deviation at lower concentration was considered due to the analytical method used.
Stability: The measurements on 19 September 2002 showed that concentrations in formulations in Milli-U water and in Milli-U water adjusted to pH 4 were stable for 4 hours at room temperature. Formulations in Milli-U water adjusted to pH 4 are stable for 8 days at 37°C (27 September 2002). The results of 17 October 2002 confirmed stability for 8 days at 37°C for Group 2. However for Group 4, stability was not confirmed. Therefore, it was decided to repeat stability testing for Group 4. Results of these analyses (31 October 2002) showed an increase of 33% over 8 days at 37°C. This was due to the low values at t=0. The values at t=8 days were very close to the target (200 mg/g). Based on this it was concluded that the Group 4 formulations were stable over 8 days at 37°C and thus confirmed the results of 27 September 2002.
Conclusion: Taking the analytical problems into account, it could be concluded that formulations were prepared accurately and homogeneously. Furthermore, it could be concluded th at formulations in Milli-U water were stable for 4 hours at room temperature and that formulations in Milli-U water adjusted to pH 4 were stable for 4 hours at room temperature and for 8 days at 37°C.

Mortality
No unscheduled deaths occurred during the study period.

Clinical signs
Females of the highest dose group showed aggressive and restless behaviour during days 5 to 15 of treatment.

Incidental findings that were noted included scabs, wound, hunched posture, piloerection, broken upper incisors, broken tail apex, and alopecia at several parts of the body. These findings are commonly noted in rats of this age and strain which are housed and treated under the conditions in this study. At the incidence observed, these were considered signs of no toxicological significance.

Functional observations
No changes were observed in hearing ability, pupillary reflex, static righting reflex and grip strength in the animals treated with dl-lactone, when compared to control animals. The variation in motor activity did not indicate a relation with treatment. Females of the highest dose group showed a decreased motor activity at the low sensors when compared to the control group. Since this change occurred in the absence of similar changes of the high sensors, they were considered to be of no toxicological relevance.

Body weight
Body weights and body weight gain were unaffected by treatment up to and including 1000 mg/kg bw/d. Males of the 200 mg/kg bw/d dose group showed statistically significant decreased body weights on day 1 of the mating period. In the absence of a clear dose response relationship, this finding was considered to be of no toxicological relevance.
On day 8 of the pre-mating period, males of the highest dose group showed a statistically significant increased body weight gain. As this finding was very slight and not considered to be adverse, it was considered to be of no toxicological significance.

Food consumption
Food consumption and relative food consumption were unaffected by treatment up to and including 1000 mg/kg bw/d.
Statistically significant increased (relative) food consumption was observed on days 1-7 of the post-mating period in males at 40 mg/kg bw/d. This finding was not considered to be an adverse effect. No explanation for this increase can be given.

Clinical laboratory investigations
Haematology: Haematological parameters of treated rats were considered not to have been affected by treatment.
Clinical Biochemistry: The serum potassium level of males of the highest dose group was statistically significantly increased when compared to the control group. The statistically significantly increased serum sodium level of males of the highest dose group was considered to have arisen as a result of slightly low control values and thus considered to be of no toxicological significance. The values of glucose and inorganic phosphate achieving a level of statistical significance in treated males when compared to the control group, were considered to be of no toxicological significance as no clear dose-response relationship was observed.

Macroscopic examination
Macroscopic observations at necropsy did not reveal any alterations that were considered to have arisen as a result of treatment. Incidental findings included pelvic dilation of

both kidneys, yellowish soft nodule at the tail of the left epididymis, dark red discolouration of the right clitoral gland, watery-clear cyst at the right ovary, haemorrhagic/clotted blood in the right uterus horn, enlarged spleen, many dark red foci on the right clitoral gland, uterus enlarged with greenish contents, hard and dark red discolouration of the papillary process of the liver, alopecia and gray-white discolouration of the medulla of the kidney. These findings are occasionally seen among rats used in these types of studies and in the absence of correlated microscopic findings they were considered changes of no toxicological significance.

Organ weights
No treatment-related changes were present. Males of the 200 mg/kg bw/d dose group showed statistically significantly decreased absolute and relative adrenals weight and females of the 40 mg/kg bw/d dose group showed statistically significantly increased relative adrenals weight. In the absence of a dose-response relationship, these findings were considered to be caused by chance and not related to treatment.

Microscopic examination
Microscopic findings: There were no treatment-related findings.
Staging of spermatogenesis: The assessment of the integrity of the spermatogenetic cycle did not provide any evidence of impaired spermatogenesis.

Reproduction
Reproduction parameters were unaffected by treatment up to 1000 mg/kg bw/d. Of the control group, two females were non-pregnant. Of the 40 mg/kg bw/d dose group, one female did not mate and two females mated in the second mating period. Of the 200 mg/kg bw/d dose group, two females were non-pregnant. And of the 1000 mg/kg bw/d dose group, one female was non-pregnant. Mating performance, duration of gestation, fertility parameters and number of pups at birth were similar for the control and treated groups.

Breeding data
Breeding parameters were unaffected by treatment up to 1000 mg/kg bw/d. The number of dead and living pups at first litter check, postnatal loss between days 0-4 post-partum, living pups at day 4 post-partum and the viability index were similar for control and treated groups.

Pups
Development of pups was unaffected by treatment up to 1000 mg/kg bw/d. Mean body weights of pups were similar for control and treated groups. Incidental clinical symptoms consisted of (very) small pups, little or no milk and bruise on the head or snout. Macroscopic examination of the pups revealed tip of tail missing, tip of tail discoloured dark red, autolysis, small appearance and no milk. No relationship with treatment was established for these observations or they were considered to be within the normal biological variation for rats of this age and strain.

Test substance: dl-Lactone from Roche Dalry, batch BX226, purity 99.8% as per

certificate of analysis.

Conclusion: dl-Lactone was administered by daily oral gavage to male and female Wistar rats at dose levels of 40, 200 or 1000 mg/kg bw/d. The males were exposed for 2 weeks prior to mating, during mating and up to termination (28 days for all males). The females were exposed for 2 weeks prior to mating, during mating, during post-coitum and at least 4 days of lactation. The mean duration of treatment of females was 43 days, with a minimum of 28 days and a maximum of 56 days.
Parental toxicity was assessed by observing mortality, clinical signs, body weights, food consumption, functional observations, clinical laboratory investigations, macroscopic examination, organ weights and microscopic examination. At 40 mg/kg bw/d, no parental toxicity was observed. At 200 mg/kg bw/d, no parental toxicity was observed. At 1000 mg/kg bw/d, parental toxicity consisted of clinical symptoms (aggressive and restless behaviour) in females during days 5 to 15 of treatment and of increased serum potassium level in males.
Reproductive toxicity was assessed by observing the mating performance, fertility indices and number of pups at birth. No reproductive toxicity was observed up to 1000 mg/kg bw/d.
Breeding toxicity was assessed by observing the number of postnatal and breeding loss during lactation. No breeding toxicity was observed up to 1000 mg/kg bw/d.
Developmental toxicity was assessed by observing clinical signs, body weights and macroscopic examination of the pups during their lactation period. No developmental toxicity was observed up to 1000 mg/kg bw/d.
In conclusion, gavage treatment of male and female Wistar rats with dl-Lactone at dose levels of 40, 200 or 1000 mg/kg bw/d for at least 28 days (during premating, mating, post-coitum and lactation) revealed slight parental toxicity in animals receiving 1000 mg/kg bw/d; this toxicity was transient during the study in the case of the females, while the males were killed after 28 days so that potential recovery could not be assessed. Reproductive, breeding and developmental parameters were unaffected up to 1000 mg/kg bw/d.
Based on the results in this combined repeated dose toxicity study with reproduction/developmental screening test, the definitive suc-chronic parental NOAEL was established as being 200 mg/kg bw/d. The definitive reproductive, breeding and developmental NOAEL was established as being 1000 mg/kg bw/d.

Reliability: (1) valid without restriction
OECD test under GLP.

Flag: Critical study for SIDS endpoint

15-MAR-2005 (5)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF quality)
Route of administration: gavage
Exposure period: males: 2 weeks prior to mating, during mating and up to termination (28 days for all males)
females: 2 weeks prior to mating, during mating, during gestation and at least 4 days of lactation;

mean treatment duration for females was 43 days
Frequency of treatment: once daily
Doses: 0 (controls, vehicle only = milli-U water), 40, 200 and 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 1000 mg/kg bw
NOAEL Teratogenicity: = 1000 mg/kg bw
Result: no developmental toxic effects noted up to the highest dose of 1000 mg/kg bw/d

Method: other: OECD Guideline 422, "Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test", 22-Mar-1996
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Please see 5.8.1, Toxicity to Fertility (same study).
Result: For general results regarding test substance analysis, please see 5.8.1 (same study).

Mortality
No unscheduled deaths occurred during the study period.

Clinical signs
Females of the highest dose group showed aggressive and restless behaviour during days 5 to 15 of treatment. Incidental findings that were noted included scabs, wound, hunched posture, piloerection, broken upper incisors, broken tail apex, and alopecia at several parts of the body. These findings are commonly noted in rats of this age and strain which are housed and treated under the conditions in this study. At the incidence observed, these were considered signs of no toxicological significance.

Functional observations
No changes were observed in hearing ability, pupillary reflex, static righting reflex and grip strength in the animals treated with dl-lactone, when compared to control animals. The variation in motor activity did not indicate a relation with treatment. Females of the highest dose group showed a decreased motor activity at the low sensors when compared to the control group. Since this change occurred in the absence of similar changes of the high sensors, they were considered to be of no toxicological relevance.
Body weight
Body weights and body weight gain were unaffected by treatment up to and including 1000 mg/kg bw/d. Males of the 200 mg/kg bw/d dose group showed statistically significant decreased body weights on day 1 of the mating period. In the absence of a clear dose response relationship, this finding was considered to be of no toxicological relevance.
On day 8 of the pre-mating period, males of the highest dose group showed a statistically significant increased body weight gain. As this finding was very slight and not considered to be adverse, it was considered to be of no toxicological significance.

Food consumption
Food consumption and relative food consumption were unaffected

by treatment up to and including 1000 mg/kg bw/d. Statistically significant increased (relative) food consumption was observed on days 1-7 of the post-mating period in males at 40 mg/kg bw/d. This finding was not considered to be an adverse effect. No explanation for this increase can be given.

Clinical laboratory investigations
Haematology: Haematological parameters of treated rats were considered not to have been affected by treatment.
Clinical Biochemistry: The serum potassium level of males of the highest dose group was statistically significantly increased when compared to the control group. The statistically significantly increased serum sodium level of males of the highest dose group was considered to have arisen as a result of slightly low control values and thus considered to be of no toxicological significance. The values of glucose and inorganic phosphate achieving a level of statistical significance in treated males when compared to the control group, were considered to be of no toxicological significance as no clear dose-response relationship was observed.

Macroscopic examination
Macroscopic observations at necropsy did not reveal any alterations that were considered to have arisen as a result of treatment. Incidental findings included pelvic dilation of both kidneys, yellowish soft nodule at the tail of the left epididymis, dark red discolouration of the right clitoral gland, watery-clear cyst at the right ovary, haemorrhagic/clotted blood in the right uterus horn, enlarged spleen, many dark red foci on the right clitoral gland, uterus enlarged with greenish contents, hard and dark red discolouration of the papillary process of the liver, alopecia and gray-white discolouration of the medulla of the kidney. These findings are occasionally seen among rats used in these types of studies and in the absence of correlated microscopic findings they were considered changes of no toxicological significance.

Organ weights
No treatment-related changes were present. Males of the 200 mg/kg bw/d dose group showed statistically significantly decreased absolute and relative adrenals weight and females of the 40 mg/kg bw/d dose group showed statistically significantly increased relative adrenals weight. In the absence of a dose-response relationship, these findings were considered to be caused by chance and not related to treatment.

Microscopic examination
Microscopic findings: There were no treatment-related findings.
Staging of spermatogenesis: The assessment of the integrity of the spermatogenetic cycle did not provide any evidence of impaired spermatogenesis.

Reproduction
Reproduction parameters were unaffected by treatment up to 1000 mg/kg bw/d. Of the control group, two females were non-pregnant. Of the 40 mg/kg bw/d dose group, one female did

not mate and two females mated in the second mating period. Of the 200 mg/kg bw/d dose group, two females
were non-pregnant. And of the 1000 mg/kg bw/d dose group, one female was non-pregnant. Mating performance, duration of gestation, fertility parameters and number of pups at birth were similar for the control and treated groups.

Breeding data
Breeding parameters were unaffected by treatment up to 1000 mg/kg bw/d. The number of dead and living pups at first litter check, postnatal loss between days 0-4 post-partum,
living pups at day 4 post-partum and the viability index were similar for control and treated groups.

Pups
Development of pups was unaffected by treatment up to 1000 mg/kg bw/d. Mean body weights of pups were similar for control and treated groups. Incidental clinical symptoms consisted of (very) small pups, little or no milk and bruise on the head or snout. Macroscopic examination of the pups revealed tip of tail missing, tip of tail discoloured dark red, autolysis, small appearance and no milk. No relationship with treatment was established for these observations or they were considered to be within the normal biological variation for rats of this age and strain.

Test substance: dl-Lactone from Roche Dalry, batch BX226, purity 99.8% as per certificate of analysis.

Conclusion: dl-Lactone was administered by daily oral gavage to male and female Wistar rats at dose levels of 40, 200 or 1000 mg/kg bw/d. The males were exposed for 2 weeks prior to mating, during mating and up to termination (28 days for all males). The females were exposed for 2 weeks prior to mating, during mating, during post-coitum and at least 4 days of lactation. The mean duration of treatment of
females was 43 days, with a minimum of 28 days and a maximum of 56 days.
Parental toxicity was assessed by observing mortality, clinical signs, body weights, food consumption, functional observations, clinical laboratory investigations, macroscopic examination, organ weights and microscopic examination. At 40 mg/kg bw/d, no parental toxicity was observed. At 200 mg/kg bw/d, no parental toxicity was observed. At 1000 mg/kg bw/d, parental toxicity consisted of clinical symptoms (aggressive and restless behaviour) in females during days 5 to 15 of treatment and of increased serum potassium level in males.
Reproductive toxicity was assessed by observing the mating performance, fertility indices and number of pups at birth. No reproductive toxicity was observed up to 1000 mg/kg bw/d.
Breeding toxicity was assessed by observing the number of postnatal and breeding loss during lactation. No breeding toxicity was observed up to 1000 mg/kg bw/d.
Developmental toxicity was assessed by observing clinical signs, body weights and macroscopic examination of the pups during their lactation period. No developmental toxicity was observed up to 1000 mg/kg bw/d.
In conclusion, gavage treatment of male and female Wistar rats with dl-Lactone at dose levels of 40, 200 or 1000 mg/kg bw/d for at least 28 days (during premating, mating, post-coitum and lactation) revealed slight parental toxicity in animals receiving 1000 mg/kg bw/d; this toxicity was transient during

the study in the case of the females, while the males were killed after 28 days so that potential recovery could not be assessed. Reproductive,
breeding and developmental parameters were unaffected up to 1000 mg/kg bw/d.
Based on the results in this combined repeated dose toxicity study with reproduction/developmental screening test, the definitive suc-chronic parental NOAEL was established as being 200 mg/kg bw/d. The definitive reproductive, breeding and developmental NOAEL was
established as being 1000 mg/kg bw/d.

Reliability: (1) valid without restriction
OECD test under GLP.

Flag: Critical study for SIDS endpoint

15-MAR-2005 (5)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

5.11 Additional Remarks

Type: Biochemical or cellular interactions

Result: The BIBRA Toxicity Profile on Pantolactone states, "Numerous studies have shown that DL-, D- or undefined pantolactone can protect micro-organisms against the effects (often genotoxic) of certain chemicals or agents". Specifically, dl-lactone restituted the ability or inability to divide normally in Escherichia coli mutants exposed to ultraviolet radiation or thermal treatment.

Reliability: (2) valid with restrictions
Several independent reports in peer-reviewed literature, all with similar conclusions regarding antimutagenic respectively mutagenicity-reversing capabilities of (dl-)lactone, hence reliability estimated at 2.

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