FOREWORD INTRODUCTION

DISODIUM SUCCINATE

CAS N°: 150-90-3

SIDS Initial Assessment Report

For

SIAM 16

Paris, France, 27-30 May 2003

1. Chemical Name:	Disodium succinate
2. CAS Number:	150-90-3
3. Sponsor Country:	Japan
	Contact Point: Mr. Yasuhisa Kawamura Director Second International Organizations Division Ministry of Foreign Affairs, Japan
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5. Roles/Responsibilities of the Partners:	
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SIDS INITIAL ASSESSMENT PROFILE

CAS No.	150-90-3
Chemical Name	Disodium succinate
Structural Formula	$NaOOCCH_2CH_2COONa$

SUMMARY CONCLUSIONS OF THE SIAR

Analogue Rationale

Disodium succinate is stable as a hexahydrate and has been produced as disodium succinate hexahydrate (CAS No.: 6106-21-4) in Japan. Many toxicity studies were conducted using disodium succinate hexahydrate as the test substance, because there should be no difference between disodium suucinate and disodium succinate hexahydrate in terms of environmental behavior, aquatic toxicity, and mammalian toxicity.

Human Health

There is no available information on toxicokinetics and metabolism.

An oral acute toxicity study [OECD TG 401] of disodium succinate hexahydrate showed that this chemical did not cause any changes even at 2,000 mg/kg. The oral LD50 value was considered to be greater than 2,000 mg (equivalent to 1,200 mg of disodium succinate)/kg bw in male and female rats.

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [OECD TG 422], Crj: CD (SD) IGS rats were given disodium succinate hexahydrate by gavage at 0, 100, 300, or 1,000 mg/kg bw/day. Males were dosed for 52 days from day 14 before mating and females were dosed from day 14 before mating to day 4 of lactation throughout the mating and pregnancy period. Blood urea nitrogen levels were increased in females at 1,000 mg/kg bw/day. Higher levels of urinary protein were found in one and two of the five males at 300 and 1,000 mg/kg bw/day, respectively, whereas no animals with these high levels were found in the control and 100 mg/kg bw/day groups. These findings suggest adverse effects of this compound on the kidney. Therefore, the NOAEL of disodium succinate hexahydrate for repeated dose toxicity was considered to be 100 mg (equivalent to 60 mg of disodium succinate)/kg bw/day for male rats and 300 mg (equivalent to 180 mg of disodium succinate)/kg bw/day for female rats.

In a reverse gene mutation assay [OECD TG 471], disodium succinate hexahydrate was not mutagenic in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvr*A with and without an exogenous metabolic activation. In a chromosomal aberration test [OECD TG 473], disodium succinate hexahydrate did not induce structural chromosomal aberrations or polyploidy with and without an exogenous metabolic activation in cultured Chinese hamster lung (CHL/IU) cells.

There is no data available on the carcinogenicity.

The above-mentioned combined study [OECD TG 422] showed that the reproduction/developmental parameters, i.e., mating, pregnancy, delivery, lactation, and viability and body weight of pups, were not affected by disodium succinate hexahydrate at up to 1,000 mg/kg bw/day. The NOAEL of disodium succinate hexahydrate for reproduction/developmental toxicity was considered to be 1,000 mg (highest dose tested, equivalent to 600 mg of disodium succinate)/kg bw/day in rats.

There is no available information on eye and skin irritation and sensitization.

Environment

Disodium succinate is a white powder with a melting point of more than 400 degree C, a water solubility of more than 100 g/L. This chemical is stable at pH 4, 7 and 9 at 50 degree C for 5 days, and readily biodegradable. A

vapour pressure of $1.16x10^{-5}$ Pa is calculated. A Log Pow of < -0.59 is estimated and the bioaccumulation potential of disodium succinate is expected to be low.

The toxicity of disodium succinate on aquatic organisms has been studied in three freshwater species belonging to three trophic levels. The toxicity tests were conducted using disodium succinate hexahydrate instead of the test substance because disodium succinate hexahydrate is not different to the test substance in aqueous solution and disodium succinate is stable as hexahydrate.

In an algal growth inhibition test (OECD TG 201, *Selenastrum capricornutum*, open system), the 72 h ErC50 and the 72 h EbC50 were >998 mg/L. For daphnids, a 48 h EC0 of 997 mg/L and a 48 h EC50 > 997 mg/L were reported (OECD TG 202, *Daphnia magna*, static). For fish (OECD TG 203, *Oryzias latipes*, flow-through) a 96 h LC0 of 47.0 mg/L, 96 h LC50 >95.4 mg/L and 96 h LC100 > 95.4 mg/L were determined.

Regarding chronic toxicity to algae, a 72 h NOErC of 998 mg/L and a NOEbC 998 mg/L (OECD TG 201, *Selenastrum capricornutum*, open system) were reported. For daphnids, the 21 d EC50 was more than 95.2 mg and a 21 d NOEC of 95.2 mg/L on reproduction and a 21 d LC50 >95.2 mg/L for parent daphnids were reported (OECD TG 211, *Daphnia magna*, semi-static).

There is no information available on toxicity to terrestrial or other organisms.

Exposure

Disodium succinate anhydrate and hexahydrate is used as a seasoning agent and raw material for plating reagent. This chemical is permitted for use as a food additive and no limit value for food additives exists in Japan. This chemical is naturally contained in shellfish. In Japan disodium succinate is being produced in its hexahydrate form and the annual production volume in Japan is ca. 3,000 tonnes.

The main target environmental compartment of this chemical is water and once it is released into the aquatic phase, partitioning to other compartments is unlikely to occur.

Occupational exposure to this chemical through inhalation and dermal routes is possible. Consumer exposure to this chemical through ingestion is possible.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

The chemical is currently of low priority for further work based on its low hazard potential.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 150-90-3
Chemical Name: Disodium succinate
Molecular Formula: $C_4H_4Na_2O_4$
Structural Formula: $NaOOCCH_2CH_2COONa$
Synonyms: Butanedioic acid disodium salt
Di-sodium succinate
Disodium succinate
Sodium succinate
Succinic acid, disodium salt
Succinic acid disodium salt
Succinic acid sodium salt
Soduxin

Substance type: organic

Physical status: powder

1.2 Purity/Impurities/Additives

Purity: 100% (titration by $HClO_4$)

1.3 Physico-Chemical properties

Disodium succinate is a white powder and it is very soluble in water (>100 g/L at 25 °C). Other physical-chemical properties are shown in Table 1.

Table 1 Summary of physico-chemical properties

Property	Protocol	Results
Melting Point	OECD TG 102	>= 400 °C
Boiling Point	Unknown	>= 400 °C
Density	JIS K 7112-1980	1.886 g/cm^3 at 25 °C
Vapor Pressure	OECD TG 104 Calculated (MPBPWIN)	< 0.00015 hPa at 100 °C 1.16E-7 hPa
Partition Coefficient (Log P_{ow})	Estimated	< -0.59
Water Solubility	OECD TG 105	> 100 g/L at 25°C

1.4 Analogue rationale

Disodium succinate is stable as a hexahydrate and has been produced as disodium succinate hexahydrate (CAS No.: 6106-21-4) in Japan. Many toxicity studies were conducted using disodium succinate hexahydrate as the test substance, because there should be no difference between

disodium suucinate and disodium succinate hexahydrate in terms of environmental behavior, aquatic toxicity, and mammalian toxicity.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

This chemical is produced as hexahydrate (CAS No. 6106-21-4) in Japan. The production of CAS No. 6106-21-4 is 3,000 tons/year in Japan.

In Japan, this chemical is used as a seasoning agent or as a raw material for plating reagent. It is added as a seasoning agent to sake (Japanese wine), soy sauce, boiled fish paste, ham, cracker and wasabi preserved in sake lees. In Europe, this chemical is not approved as a food additive but is registered as a flavouring agent.

2.2 Environmental Exposure and Fate

Disodium succinate dissociates in water releasing two sodium ions with two pKa values of 4.2 and 5.6 (CERI, 2000).

A study on hydrolysis was conducted and no abiotic degradation was reported (pH 4, 7 and 9 at 50 °C over 5 days).

Disodium succinate is readily biodegradable (64 - 78% of BOD, 100% of HPLC and 100% of TOC were observed after 14 days in a test according to OECD TG301C). The estimated $LogP_{ow}$ is less than -0.59. The BCF is estimated to be low according to the low $LogP_{ow}$ value.

This chemical degrades by photochemically induced OH radicals in the atmosphere with a half-life of 360 hours. No estimation for the reaction with ozone is possible. The Henry's Law constant is estimated to be 5.45×10^{-12} atm m^3/mol. Vapor pressure is less than 0.015 Pa at 100 °C and is estimated to be 1.16×10^{-5} Pa at 25 °C. The water solubility is more than 100 g/L at 25 °C.

The main target environmental compartment of this chemical is water and, based on fugacity model (Mackay level III), once this chemical is released into the aquatic phase partitioning to other compartment is unlikely to occur.

2.3 Occupational Exposure

In Japan, this chemical is synthesized by hydrogenation of maleic anhydride. Production processes, hydrogenation with hydrogen, neutralization with sodium hydroxide, purification and crystallization, and drying are performed in closed batch systems with remote control and the possibility of worker exposure to this chemical in these processes is very low.

During the packing process of this chemical under local exhaust ventilation, worker exposure through inhalation of dust is possible, since this chemical is non-volatile. The EHE_{inh} for a worker during the packing operation (the duration is 6.5 hours/day) is estimated to be 0.06 mg/kg/day using the EASE model, assuming that this work is performed through direct handling under local exhaust ventilation, and that succinic acid easily aggregates. The EHE_{der} for the worker during the same packing operation is 7.8 mg/kg/day, assuming that dermal contact to both hands is incidental. As the workers wear gloves, goggles, and dust masks during the packing operation, the actual exposure is probably less than these values.

No occupational exposure standard value for this chemical was located.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

Many toxicity studies were conducted using disodium succinate hexahydrate (CAC No. 6106-21-4), because there should be no difference between disodium succinate and disodium succinate hexahydrate in mammalian toxicity.

Disodium succinate (CAS No. 150-90-3) and disodium succinate hexahydrate (CAS No. 6106-21-4) were assessed. The NOAEL of disodium succinate hexahydrate was converted into the NOAEL of disodium succinate based on the molecular weights of each chemical.

3.1.1 Toxicokinetics, Metabolism and Distribution

There is no data available.

3.1.2 Acute Toxicity

Studies in Animals

An acute toxicity study in rats was identified as a key study because it was well conducted according to an OECD acute oral toxicity test guideline [TG 401] [MHLW, Japan: 2002] under GLP (Table 2).

In this study, Crj:CD (SD) rats (five animals/sex/group) were administered disodium succinate hexahydrate by gavage at a single dose of 0 (vehicle: distilled water) or 2,000 mg/kg bw. No deaths or abnormal findings were found in any groups. There was no difference in body weight gain between groups. The oral LD_{50} value was considered to be greater than 2,000 mg/kg bw in male and female rats (2,000 mg of disodium succinate hexahydrate is equivalent to 1,200 mg of disodium succinate).

Table 2: Acute toxicity of disodium succinate in rodents

Route	Animals	Type	Values	References
Oral	Rat	LD_{50}	> 2,000 mg/kg bw (disodium succinate hexahydrate) > 1,200 mg/kg bw (disodium succinate)	MHLW, Japan: 2002

Studies in Humans

There is no available information on humans.

Conclusion

The oral LD_{50} value was considered to be greater than 2,000 mg/kg bw in male and female rats (2,000 mg of disodium succinate hexahydrate is equivalent to 1,200 mg of disodium succinate).

3.1.3 Repeated Dose Toxicity

Studies in Animals

One study is available for repeated dose toxicity. This study was conducted according to an OECD combined repeated dose toxicity study with the reproduction/developmental toxicity screening test guideline [TG 422] [MHLW, Japan, 2002] under GLP. This study was identified as a key study because it was well conducted. The details of this study are as follows.

Crj: CD (SD) IGS rats (12 animals/sex/dose) were administered disodium succinate hexahydrate by gavage at doses of 0 (vehicle: distilled water), 100, 300, or 1,000 mg/kg bw/day. Males were dosed for 52 days from day 14 before mating and females were dosed from day 14 before mating to day 4 of lactation throughout the mating and pregnancy period. Hematological, blood biochemical, and histopathological examinations were performed in both sexes, and urinalysis was conducted in males.

No deaths were found in any groups. Slight loose stool was observed in one and four males at 100 and 1,000 mg/kg bw/day, respectively, and one female at 1,000 mg/kg bw/day. Salivation was found in one male and one female at 1,000 mg/kg bw/day. There were no effects of this compound on the body weight, food consumption, hematology, or blood coagulation. The blood sodium levels were higher in males at 300 and 1,000 mg/kg bw/day. The blood urea nitrogen levels were increased in females at 1,000 mg/kg bw/day. Higher levels (300 mg/dL and higher) of urinary proteins were found in one and two of the five males at 300 and 1,000 mg/kg bw/day, respectively, whereas no animals with these high levels were found in the control and 100 mg/kg bw/day groups. These findings suggest adverse effects of this compound on the kidney. Increased weight of the adrenal gland was observed in males at 1,000 mg/kg bw/day. No compound-related effects on the histopathological findings were observed. Based on the higher levels of urinary protein in males and blood urea nitrogen in females, the NOAEL of disodium succinate hexahydrate for repeated dose toxicity was considered to be 100 mg/kg bw/day for male rats and 300 mg/kg bw/day for female rats (100 and 300 mg of disodium succinate hexahydrate are equivalent to 60 and 180 mg of disodium succinate , respectively).

Studies in Humans

There is no available information on humans.

Conclusion

In an oral repeated dose toxicity study in rats, higher levels of urinary protein in males and blood urea nitrogen in females were observed at 300 and 1,000 mg/kg bw/day, respectively. The NOAEL of disodium succinate hexahydrate for repeated dose toxicity was considered to be 100 mg/kg bw/day for male rats and 300 mg/kg bw/day for female rats (100 and 300 mg of disodium succinate hexahydrate are equivalent to 60 and 180 mg of disodium succinate , respectively).

3.1.4 Mutagenicity

Table 3: Summary of genotoxicity assays

Type of test	Test system	Highest concentration	Result	Reference
Bacterial test				
Ames test *(reverse mutation)*	*S. typhimurium* (TA98, TA100, TA1535, TA1537) *E. coli* (WP2 *uvr* A)	5,000 µg/plate (disodium succinate hexahydrate)	Negative (+ & - MA*)	MHLW, Japan: 2002
Ames test *(reverse mutation)*	*S. typhimurium* (TA97, TA94, TA98, TA100, TA1535, TA1537)	5,000 µg/plate (disodium succinate)	Negative (+ MA)	Ishidate et al.: 1984
Ames test *(reverse mutation)*	*S. typhimurium* (TA97, TA102)	10,000 µg/plate (disodium succinate)	Negative (+ & - MA)	Fujita et al.: 1994
Non-bacterial in vitro test				
Chromosomal aberration test	CHL cells	5,000 µg/mL (disodium succinate hexahydrate)	Negative (+ & - MA)	MHLW, Japan: 2002
Chromosomal aberration test	CHL cells	15,000 µg/mL (disodium succinate)	Equivocal (polyploidy) (- MA)	Ishidate et al.: 1984

*MA: Metabolic activation

In vitro bacterial tests

Three studies were reported (Table 3). No positive results were obtained. The study by MHLW, Japan (2002) was identified as a key study because this study was well conducted according to a current protocol [OECD TG 471; Japanese Guideline for Screening Mutagenicity Testing Chemicals (Chemical Substances Control Law of Japan)] under GLP. Disodium succinate hexahydrate was not mutagenic with and without S9 mix in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvr*A at up to 5,000 µg/plate (5,000 µg of disodium succinate hexahydrate is equivalent to 3,000 µg of disodium succinate). These results were supported by the results of Ishidate et al. (1984) and Fujita et al (1994).

Non-bacterial in vitro test

Two non-bacterial *in vitro* tests were reported (Table 4). Although Ishidate et al. (1984) reported an equivocal result (5% of polyploidy) at the very high concentration of 15,000 µg/mL, no detailed information on the test procedures was available. MHLW, Japan (2002) conducted a chromosomal aberration test using cultured Chinese hamster lung (CHL/IU) cells according to OECD TG 473 under GLP. This study was identified as a key study because all experimental conditions and reporting were sufficient. Disodium succinate hexahydrate did not induce structural chromosomal aberrations and polyploidy with and without S9 mix at up to 5,000 µg/mL (5,000 µgof disodium succinate hexahydrate is equivalent to 3,000 µg of disodium succinate). No cytotoxicity was observed at up to 5,000 µg/mL after 6h short-term or 24-48h continuous treatment.

In vivo Studies

There is no available information.

Conclusion

Disodium succinate was not genotoxic with and without an exogenous metabolic activation in bacterial tests as well as a chromosomal aberration test *in vitro*.

3.1.5 Carcinogenicity

There are no data available.

3.1.6 Reproduction/developmental toxicity

Studies in Animals

One study is available for reproductive and developmental toxicity. This study was conducted according to an OECD combined repeated dose toxicity study with the reproduction/developmental toxicity screening test guideline [TG 422] [MHLW, Japan: 2002] under GLP. This study was identified as a key study because it was well conducted. Details of the study are as follows.

Crj: CD (SD) IGS rats (12 animals/sex/dose) were administered disodium succinate hexahydrate by gavage at doses of 0 (vehicle: distilled water), 100, 300, or 1,000 mg/kg bw/day. Males were dosed for 52 days from day 14 before mating and females were dosed from day 14 before mating to day 4 of lactation throughout the mating and pregnancy period. No compound-related effects on the estrous cycle, copulation index, fertility index, gestation length, gestation index, number of corpora lutea, or number of implantation sites were found in dams. No compound-related effects on the number, sex ratio, or viability were observed in pups on days 0 and 4 of lactation. Anophthalmia and polydactyly were observed in one pup at 300 mg/kg bw/day. These anomalies are considered to be spontaneous, because the incidences of these anomalies were extremely low and these are of types seen in historical control data. There were no compound-related changes in body weights of pups. No abnormal findings considered to be attributable to administration of this compound were observed in dead pups during lactation and pups at scheduled sacrifice. Based on these findings, the NOAEL of disodium succinate hexahydrate for reproduction/developmental toxicity was considered to be 1,000 mg/kg bw/day in rats (highest dose tested) (1,000 mg of disodium succinate hexahydrate is equivalent to 600 mg of disodium succinate).

Studies in Humans

There is no available information on humans.

Conclusion

In an OECD combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, there was no evidence of reproduction/developmental toxicity in rats. The NOAEL for reproduction/developmental toxicity was considered to be 1,000 mg/kg bw/day in rats (1,000 mg of disodium succinate hexahydrate is equivalent to 600 mg of disodium succinate).

3.1.7 Other human health related information

There is no available information.

3.1.8 Information on structurally related chemicals

Succinic acid (CAS No.: 110-15-6)

Succinic acid is a natural constituent of plants and animals. This chemical is involved in the citric acid cycle [NTIS, 1975]. The Oral LD_{50} value in rats was reported to be 2,260 mg/kg bw [KODAK, Company Reports]. This chemical was not mutagenic in the Ames test and a chromosomal aberration test [Ishidate et al., 1984]. Subcutaneous injections of this compound at 31 mg/kg bw/day for three weeks did not change the typical diestrous vaginal smears in two months old ovariectomized rats [Dye et al., 1944]. Application of this compound at 750 µg produced severe damage in the rabbit eyes [AJOPAA, 1946].

Monosodium succinate (CAS No.: 2922-54-5)

The oral LD_{50} value was greater than 8,000 mg/kg bw in rats [Maekawa et al: 1990]. In a 13-week oral toxicity study in rats, the only suppression of body weight gain was found at greater than 2.5% in the drinking water [Maekawa et al: 1990]. In a 2-year toxicity/carcinogenicity study in rats, this chemical had neither toxic nor carcinogenic activity when it was given continuously at levels of 1 or 2% in drinking water [Maekawa et al: 1990].

3.2 Initial Assessment for Human Health

There is no available information on toxicokinetics and metabolism.

The oral acute toxicity study [OECD TG 401] of disodium succinate hexahydrate showed that this chemical did not cause any changes even at 2,000 mg/kg. The oral LD_{50} value was considered to be greater than 2,000 mg/kg bw in male and female rats (2,000 mg of disodium succinate hexahydrate is equivalent to 1,200 mg of disodium succinate). There is no available information on the eye and skin irritation and sensitization.

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [OECD TG 422], Crj: CD (SD) IGS rats were given disodium succinate hexahydrate by gavage at 0, 100, 300, or 1,000 mg/kg bw/day. Males were dosed for 52 days from day 14 before mating and females were dosed from day 14 before mating to day 4 of lactation throughout the mating and pregnancy period. Slight loose stool was observed in four of the 12 males at 1,000 mg/kg bw/day. No compound-related changes in the body weight, food consumption, hematology, blood coagulation, or histopathological findings were found. The blood urea nitrogen levels were increased in females at 1,000 mg/kg bw/day. Higher levels (300mg/dL and higher) of urinary protein were found in one and two of the five males at 300 and 1,000 mg/kg bw/day, respectively, whereas no animals with these high levels were found in the control and 100 mg/kg bw/day groups. These findings suggest the adverse effects of this compound on the kidney. Therefore, the NOAEL of disodium succinate hexahydrate for repeated dose toxicity was considered to be 100 mg/kg bw/day for male rats and 300 mg/kg bw/day for female rats (100 and 300 mg of disodium succinate hexahydrate are equivalent to 60 and 180 mg of disodium succinate, respectively).

In a reverse gene mutation assay [OECD TG 471], disodium succinate hexahydrate was not mutagenic in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvr*A with and without an exogenous metabolic activation. In a chromosomal aberration test [OECD TG 473], disodium succinate hexahydrate did not induce structural chromosomal aberrations or polyploidy with and without an exogenous metabolic activation in cultured Chinese hamster lung (CHL/IU) cells.

There is no data available on the carcinogenicity.

The above-mentioned combined study [OECD TG 422] showed the reproduction/developmental parameters, i.e., mating, pregnancy, delivery, lactation, and viability and body weight of pups, were not affected by administration of disodium succinate hexahydrate at up to 1,000 mg/kg bw/day. The NOAEL of disodium succinate hexahydrate for reproduction/developmental toxicity was considered to be 1,000 mg/kg bw/day in rats (1,000 mg of disodium succinate hexahydrate is equivalent to 600 mg of disodium succinate).

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

The toxicity of disodium succinate on aquatic organisms has been studied in three freshwater species belonging to three trophic levels as shown in Table 4. These tests were conducted using disodium succinate hexahydrate (CAS No.: 6106-21-4) instead of the test substance, because disodium succinate is stable as hexahydrate, available commercially, and there should be no difference regarding environmental behavior and aquatic toxicity.

Therefore concentrations are represented as disodium succinate (anhydrate).

Table 4: Summary of effects of disodium succinate on aquatic organisms

Organisms	**Test duration**	**Result (mg/L)**	**Reference**
Aquatic plants, e.g. algae			
Green algae (*Selenastrum capricornutum*)	72 h Open system	Growth rate method ErC_{50} > 998 NOErC = 998 Biomass method EbC_{50} > 998 NOEbC = 998	MOE, Japan(2001)
Invertebrates			
Daphnids (*Daphnia magna*)	48 h Static	Immobilization EC_0 = 997 EC_{50} > 997	MOE, Japan(2001)
	21 d Semi-static	Mortality LC_0 > 95.2 Reproduction EC_{50} > 95.2 NOEC = 95.2	MOE, Japan(2001)
Fish			
Medaka (*Oryzias latipes*)	96 h Flow-through	LC_0 = 47.0 LC_{50} > 95.4 LC_{100} > 95.4	MOE, Japan(2001)

In an algal growth inhibition test (OECD TG 201, open system), the acute toxicity results (72 h ErC_{50} and 72 h EbC_{50}) to *Selenastrum capricornutum* were were determined to be >998 mg/L by both the biomass method and the growth rate method. In addition, toxicity to four marine algal species, (*Navicula*) sp, *Chaetoceros gracilis*, *Pavlova lutheri* and *Tetraselmis tetrathele*, was reported by OHGAI et al. (1993). Growth inhibition (on growth rate for seven days) was observed at the concentration of 300 mg/L of disodium succinate, however the pH was extremely decreased (pH = 3.8) in the test solutions at 300 mg/L. The details regarding this test were not available, and its reliability could not be determined.

Regarding acute toxicity to daphnids, a 48 h EC_0 of 997 mg/L and a 48 h EC_{50} > 997 mg/L were reported (OECD TG 202, *Daphnia magna*, static).

A test with fish (OECD TG 203, *Oryzias latipes*, flow-through) resulted in a 96 h LC_0 of 47.0 mg/L, 96 h LC_{50} > 95.4 mg/L and 96 h LC_{100} > 95.4 mg/L. In this test, only one individual out of twenty died at the highest concentration of 95.4 mg/L.

All acute toxicity tests (except that using marine species) were conducted in compliance with GLP and the results were estimated based on the mean measured concentrations.

Chronic Toxicity Test Results

Regarding chronic toxicity to algae, a 72 h NOErC of 998 mg/L and a NOEbC of 998 mg/L (OECD TG 201, *Selenastrum capricornutum*, open system) were reported. In daphnids, the effect of disodium succinate on reproduction of *Daphnia magna* (OECD TG 211, semi-static) was investigated. The 21 d EC_{50} was more than 95.2 mg/L and a 21 d NOEC of 95.2 mg/L were reported (MOE Japan, 2001). For mortality of parent daphnids, the 21 d LC_{50} was more than 95.2 mg/L. In these toxicity tests, no adverse effects of the chemical on reproduction of daphnids and/or growth of algae were observed at the highest concentrations.

4.2 Terrestrial Effects

There is no available information.

4.3 Initial Assessment for the Environment

Disodium succinate is a white powder with a melting point of more than 400 degree C, a water solubility of more than 100 g/L. This chemical is stable at pH 4, 7 and 9 at 50 degree C for 5 days, and readily biodegradable. A vapour pressure of $1.16x10^{-5}$ Pa is calculated. A Log Pow of < -0.59 is estimated and the bioaccumulation potential of disodium succinate is expected to be low.

The toxicity of disodium succinate on aquatic organisms has been studied in three freshwater species belonging to three trophic levels. The toxicity tests were conducted using disodium succinate hexahydrate instead of the test substance because disodium succinate hexahydrate is not different to the test substance in aqueous solution and disodium succinate is stable as hexahydrate.

In an algal growth inhibition test (OECD TG 201, *Selenastrum capricornutum*, open system), the 72 h ErC50 and the 72 h EbC50 were >998 mg/L. For daphnids, a 48 h EC0 of 997 mg/L and a 48 h EC50 > 997 mg/L were reported (OECD TG 202, *Daphnia magna*, static). For fish (OECD TG 203, *Oryzias latipes*, flow-through) a 96 h LC0 of 47.0 mg/L, 96 h LC50 >95.4 mg/L and 96 h LC100 > 95.4 mg/L were determined.

Regarding chronic toxicity to algae, a 72 h NOErC of 998 mg/L and a NOEbC 998 mg/L (OECD TG 201, *Selenastrum capricornutum*, open system) were reported. For daphnids, the 21 d EC50 was more than 95.2 mg and a 21 d NOEC of 95.2 mg/L on reproduction and a 21 d LC50 > 95.2 mg/L for parent daphnids were reported (OECD TG 211, *Daphnia magna*, semi-static).

There is no information available on toxicity to terrestrial or other organisms.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work based on a low hazard potential.

6 REFERENCES

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MOE (Ministry of the Environment) Japan (2001) Unpublished data.

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Ohgai, M. et al. (1993) Nippon Suisan Gakkaisi, 59, 647-652.

I U C L I D

Data Set

Existing Chemical	:	ID: 150-90-3
CAS No.	:	150-90-3
EINECS Name	:	Disodium succinate
EINECS No.	:	205-778-7
Molecular Formula	:	C4H4O4.Na2

Producer Related Part

Company	:	National Institute of Health Sciences
Creation date	:	29.01.2003

Substance Related Part

Company	:	National Institute of Health Sciences
Creation date	:	29.01.2003
Memo	:	
Printing date	:	27.01.2003
Revision date	:	
Date of last Update	:	24.01.2003
Number of Pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7
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 OECD AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

Butanedioic acid disodium salt
Reliability **:** (1) valid without restriction
28.07.2003

Butanedioic acid, disodium salt
Reliability **:** (1) valid without restriction
28.07.2003

Di-sodium succinate
Reliability **:** (1) valid without restriction
28.07.2003

Sodium succinate
Reliability **:** (1) valid without restriction
28.07.2003

Soduxin
Reliability **:** (1) valid without restriction
28.07.2003

Succinic acid disodium salt
Reliability **:** (1) valid without restriction
28.07.2003

Succinic acid sodium salt
Reliability **:** (1) valid without restriction
28.07.2003

Succinic acid, disodium salt
Reliability **:** (1) valid without restriction
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1.3 IMPURITIES

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

Type	**:**	use
Category	**:**	Food/foodstuff additives
Remark	**:**	This chemical is used as a seasoning and raw material for plating reagent. It is added as a seasoning to sake (Japanese wine), soy sauce, boiled fish paste, ham, cracker and wasabi preserved in sake lees.
Reliability	**:**	(1) valid without restriction
Flag	**:**	Critical study for SIDS endpoint

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1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value	:	>= 400 ° C
Sublimation	:	
Method	:	OECD Guide-line 102 "Melting Point/Melting Range"
Year	:	2000
GLP	:	no
Test substance	:	
Source	:	Chemicals Evaluation and Research Institute (CERI), Japan
Test substance	:	Disodium succinate. Aldrich Chemical Co., Inc. Purity: 100.1 % (titration by HClO4)
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

29.07.2003 (2)

2.2 BOILING POINT

Value	:	>= 400 ° C at 1013 hPa
Decomposition	:	
Method	:	OECD Guide-line 103 "Boiling Point/boiling Range"
Year	:	2000
GLP	:	no
Test substance	:	
Source	:	Chemicals Evaluation and Research Institute (CERI), Japan
Test substance	:	Disodium succinate. Aldrich Chemical Co., Inc. Purity: 100.1 % (titration by HClO4)
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

29.07.2003 (6)

2.3 DENSITY

Type	:	density
Value	:	= 1.886 at 25° C
Method	:	other
Year	:	1994
GLP	:	no
Test substance	:	
Source	:	Chemicals Evaluation and Research Institute (CERI), Japan
Test substance	:	Disodium succinate. Aldrich Chemical Co., Inc. Purity: 98.9 % (Titration by HClO4) Impurity: water 0.2 %
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

29.07.2003 (3)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = .000000116 hPa at 25° C
Decomposition :
Method other (calculated)
Year : 2003
GLP : no
Test substance :
Conclusion A vapour pressure of 1.16 E-5 Pa was calculated by MPBPWIN v. 1.40.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
28.07.2003

Value : <= .00015 hPa at 100° C
Decomposition :
Method OECD Guide-line 104 "Vapor Pressure Curve"
Year : 2000
GLP : no
Test substance :
Source : Chemicals Evaluation and Research Institute (CERI), Japan.
Test substance : Disodium succinate.
Aldrich Chemical Co., Inc.
Purity: 100.1 % (titration by HClO4).
Reliability : (2) valid with restrictions
29.07.2003 (4)

2.5 PARTITION COEFFICIENT

Log pow : < -.59 at ° C
Method other (measured)
Year : 1995
GLP :
Test substance :
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
28.07.2003

Log pow : = .39 at ° C
Method other (calculated)
Year : 2003
GLP :
Test substance :
Remark : Calculated by Kowwin v. 1.9
Reliability : (2) valid with restrictions
29.07.2003 (7)

Log pow : = -.59 at ° C
Method
Year : 1995
GLP :
Test substance :
Remark : Ref. Hansch, C, et al. (1995).
Test substance : Other (Succinic acid)
Reliability : (3) invalid
28.07.2003

2.6.1 WATER SOLUBILITY

Value	:	>= 100 g/l at 25 ° C
Qualitative	:	of very high solubility
Pka	:	at 25 ° C
PH	:	= 8.6 at 100 g/l and 25 ° C
Method	:	OECD Guide-line 105 "Water Solubility"
Year	:	2000
GLP	:	no
Test substance	:	
Remark	:	The pKa of succinic acid is 4.207 and 5.636 (25 degree C). Ref: Critical Stability Constants, vol 3 by Arthur E. Martell and Robert M. Smith (1989). The pHa of succinic acid is 4.00 and 5.24 (25 degree C). Ref: Kagakubinran, Maruzen Co., Ltd. Succinic acid will exists primarily in the ionised form under environmental pHs (SRC).
Source	:	Chemicals Evaluation and Research Institute (CERI), Japan.
Test substance	:	Disodium succinate. Aldrich Chemical Co., Inc. Purity: 100.1 % (titration by HClO4).
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

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Value	:	ca. 200 g/l at 25 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	at and ° C
Method	:	
Year	:	1996
GLP	:	
Test substance	:	
Reliability	:	(3) invalid

29.07.2003 (6)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	1500000 molecule/cm3
Rate constant	:	.0000000000007123 cm3/(molecule*sec)
Degradation	:	= 50 % after 15 day
Deg. Product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	
Test substance	:	
Remark	:	AOPWIN v. 1.90
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

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3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	< 10 at 50 degree C
t1/2 pH7	:	< 10 at 50 degree C
t1/2 pH9	:	< 10 at 50 degree C
Degradation	:	< 10 % after 5 day at pH and degree C
Deg. Product	:	
Method	:	OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year	:	
GLP	:	no
Test substance	:	
Source	:	Chemicals Evaluation and Research Institute (CERI), Japan.
Test substance	:	Disodium succinate. Aldrich Chemical Co., Inc. Purity: 100.1 % (titration by HClO4).
Conclusion	:	This chemical is stable at pH 4, 7 and 9 at 50 degree C for 5 days.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

29.07.2003 (5)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

28.07.2003

3.3.2 DISTRIBUTION

Media	:	air - biota - sediment(s) - soil - water
Method	:	Calculation according Mackay, Level III
Year	:	2003
Remark	:	Parameters used are: Molecular weight: 162.05 Melting point: 400 degree C Water solubility: 100 g/m3 log Kow: -0.59
Result	:	Release 100% to; Air: 50.4% in water, 49.3% in soil, 0.2% in sediment; Water: 99.6% in water, 0.4% in sediment; Soil: 44.9% in water, 54.9% in soil, 0.2% in sediment; Sediment: 57.6% in water, 42.2% in soil, 0.2% in soil.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

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3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, non-adapted
Concentration	:	100mg/l related to COD (Chemical Oxygen Demand) related to
Contact time	:	14 day
Degradation	:	= 64 - 78 % after 14 day
Result	:	readily biodegradable
Control substance	:	Aniline
Kinetic	:	**%** **%**
Deg. Product	:	
Method	:	OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year	:	1994
GLP	:	yes
Test substance	:	
Remark	:	The concentration of aniline was 100 mg/L. The concentration of activated sludge was 30 mg/L.
Result	:	The biodegradation rates of this chemical were as follows: 74, 78 and 64 % by BOD after 14 days 100, 100 and 100 % by HPLC analysis after 14 days. 100, 100 and 100 % by TOC analysis after 14 days.
Source	:	Chemicals Evaluation and Research Institute (CERI), Japan.
Test substance	:	Aldrich Chemical Co., Inc. Purity: 98.9 % (titration by HClO4). Impurity: water 0.2 %
Conclusion	:	Readily biodegradable.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

28.07.2003 (1)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

BCF	**:**	= 3.16
Elimination	**:**	
Method	**:**	other
Year	**:**	2003
GLP	**:**	
Test substance	**:**	
Remark	**:**	An estimated BCF value of 3.16 was calculated by BCFWIN v. 2.14. A log Kow value of -0.59 was used.
Reliability	**:**	(2) valid with restrictions

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3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
LC50	:	> 95.4 mg/L
LC100	:	> 95.4 mg/L
LC0	:	= 47.0 mg/L
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	2001
GLP	:	Yes
Test substance	:	other TS: Disodium succinate hexahydrate (CAS No.: 6106-21-4, Nacalai Tesque, Inc. (Japan), Lot. No.: MOT9476, Purity = 100.2%
Method	:	-Test Organisms: a) Supplier: Test organisms were obtained from Nakajima Yougyo-jo (Private Fish Farm, Japan), before one month and a half of a test. b) Size (length and weight): 2.3 cm (2.2 - 2.4 cm) in length; 0.16 g (0.13 - 0.23 g) in weight c) Age: Not described d) Any pretreatment: Test organisms were acclimated for 28 days before testing. During acclimation, test fishes were fed with TETRAMINE equivalent to 3% of weight per day. These test organisms were not fed for 24 hours before the test started. The mortality of the test organisms for 7 days before testing was less than 5%. LC50(96 hr) for a reference substance (copper sulfate pentahydrate) was 0.707 mg/L. -Test substance: The acute toxicity to fish was calculated based on butanedioic acid, disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate. a) Empirical Formula: Na2C4H16O10 b) Molecular Weight: 270.14 g/mol c) Purity: =100.2 % -Test Conditions: a) Dilution Water Source: Dilution water was prepared from tap water (Kurume city, Japan). The tap water was dechlorinated and treated by activated carbon. After that Residual Chlorine was removed from the water. Before using the dilution water, aeration was fully carried out. b) Dilution Water Chemistry: pH: = 7.4 Total hardness (as CaCO3): = 61.0 mg/L c) Exposure Vessel Type: 1.8 L test solution in a 3 L glass beaker d) Nominal Concentrations: control, 6.25, 12.5, 25.0, 50.0 and 100 mg/L e) Vehicle/Solvent and Concentrations: Any solvent was not used. f) Stock Solutions Preparations and Stability: Test chemical was refrigerated. The stability of the chemical was confirmed by IR spectrum. The IR spectrum at the end of the test was same at the start of test. g) Number of Replicates: 1 h) Fish per Replicates: 10 i) Flow-Through Rate of Test Water: The flow through rate of test medium was 30mL/min.

j) Water Temperature: 24+/-1ºC
k) Light Condition: 16:8 hours, light-darkness cycle
l) Feeding: None
m) Aeration : Test solution was not aerated during the test period.

-Analytical Procedure: The tested concentrations were measured at the start and the end of the test period using HPLC.

-Statistical Method:
a) Data Analysis: During test period the test organisms were lived more than 50% in all concentrations, therefore the LC50 is more than the highest concentration.
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Arithmetic mean

Remark : The acute toxicity to fish was calculated based on measured concentration of butanedioic acid, disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate.

Result : - Measured Concentrations: The test concentrations were measured at 0 h and 96 h. For all of them, the deviations from the nominal were less than +/- 20%.

Nominal Conc.	Measured Conc., mg/L			Percent of Nominal	
mg/L	0 Hour	96 Hours	Mean*	0 Hour	96Hours
Control	<0.600	<0.600	---	---	---
6.25	6.14	5.13	5.64	98.2	82.1
12.5	12.2	10.9	11.6	97.6	87.4
25.0	24.3	22.8	23.6	97.3	91.3
50.0	46.6	47.3	47.0	93.2	94.6
100	94.8	96.1	95.4	94.8	96.1

*: Mean measured concentration (Arithmetic Mean)

- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for old and renewal solution with control and each concentration at the start of test and every 24 hours.
pH: 7.6 - 7.8
DO: 7.5 - 8.3 mg/L
Water Temperature: 24.1 - 24.9ºC

-Effect Data(mortality):
LC50 (96hr) > 95.4 mg/L (mc)
LC0 (96hr) = 47.0 mg/L (mc)
LC100 (96hr) > 95.4 mg/L (mc)
mc: based on measured concentration

- Cumulative Mortality: None of test organisms were killed during exposure period at control,6.25, 12.5, 25.0, 50.0 mg/L. The lowest concentration from which the test organisms were killed was 100mg/L at 96th hr.

Measured Conc. mg/L	Cumulative Number of Dead (Percent Mortality) 24hr	48hr	72hr	96hr
Control	0 (0)	0 (0)	0 (0)	0 (0)
5.64	0 (0)	0 (0)	0 (0)	0 (0)
11.6	0 (0)	0 (0)	0 (0)	0 (0)
23.6	0 (0)	0 (0)	0 (0)	0 (0)
47.0	0 (0)	0 (0)	0 (0)	0 (0)
95.4	0 (0)	0 (0)	0 (0)	1 (10)

-Other Effect: Toxicological symptom was not observed at any concentration.

Measured Conc. mg/L	Symptoms 24hr	48hr	72hr	96hr
Control	n	n	n	n
5.64	n	n	n	n
11.6	n	n	n	n
23.6	n	n	n	n
47.0	n	n	n	n
95.4	n	n	n	n

n: No abnormalities are detected

- Calculation of toxicity values: The calculation of toxicity values was the nominal concentration. The reason is that all of the deviations from the nominal concentration were less than +/-20%.

Source : Ministry of Environment, Japan (2001)
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
15.01.2003 (10)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
: mg/l
Analytical monitoring : Yes
EC0 : = 997
EC50 : > 997
Method : OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilization Test"
Year : 2001
GLP : Yes

Test substance : other TS: Disodium succinate hexahydrate (CAS No.: 6106-21-4, Nakarai Kagaku, Inc. (Japan), Lot. No.: MOT9476, Purity = 100.2%

Method : - Test Organisms:
a) Age: < 24 hours old
b) Supplier/Source: Test organisms were obtained from the University of Sheffield (UK) and had been reproduced in the testing laboratory for 10 years.
c) Any pretreatment: Parental daphnids were acclimated for 29 days on test condition before testing. During acclimation, test daphnids were fed with Chlorella vulgaris, 0.1 - 0.2 mg carbon/day/individual. The mortality of the daphnids was less than 5% for 2 weeks before testing. Any resting-egg and male daphnia was not observed. EC50(48hr, immobility) for reference substance (potassium dichromate) was 0.171mg/L.

-Test substance: The acute toxicity to daphnids was calculated based on butanedioic acid, disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate.
Disodium succinate hexahydrate
a) Empirical Formula: $Na_2C_4H_{16}O_{10}$
b) Molecular Weight: 270.14 g/mol
c) Purity: =100.2 %

-Test Conditions:
a) Dilution Water Source: Dilution water was prepared from tap water (Kurume city, Japan). The tap water was dechlorinated and treated by activated carbon. After that Residual Chlorine was removed from the water.
b) Dilution Water Chemistry:
pH: = 7.4
Total hardness (as $CaCO_3$): = 61.0 mg/L
c) Exposure Vessel Type: 100 mL test solution in a 100 mL glass beaker
d) Nominal Concentrations: control, 592, 769 and 1,000 mg/L
e) Vehicle/Solvent and Concentrations: Any solvent was not used.
f) Stock Solutions Preparations and Stability: Test chemical was refrigerated. The stability of the chemical was confirmed by IR spectrum. The IR spectrum at the end of the test was same at the start of test.
g) Number of Replicates: 4
h) Individuals per Replicates: 5
i) Water Temperature: 20+/-1ºC
j) Light Condition: 16:8 hours, light-darkness cycle
k) Feeding: None
l) Aeration : not described

- Analytical Procedure: Test concentrations were measured at the start and the end of the test using HPLC.

- Statistical Method:
a) Data Analysis: During test period the immobility of test organisms was not observed in any concentration, therefore the EC 50 is more than the highest concentration.
b) Method of Calculating Mean Measured Concentrations: time-weighted mean

Remark : The acute toxicity to fish was calculated based on butanedioic acid,

disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate.

Result : - Measured Concentrations: The test concentrations were measured at the start and the end of the test. For all of them, the deviations from the nominal were less than +/-20%.

Nominal Conc. mg/L	Measured Conc., mg/L 0 Hour Fresh	48 Hour Old	Mean* mg/L	Percent of Nominal 0 Hour Fresh	24 Hour Old
Control	<15.0	<15.0	---	---	---
592	591	567	579	99.8	95.7
769	766	748	757	99.6	97.2
1,000	999	995	997	99.9	99.5

Fresh: freshly prepared test solution. Old: test solution after 48 hours exposure
*: Mean measured concentration (time-weighted mean)

- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for control and each concentration at the start and the end of the test.
pH: 7.7 - 7.9
DO: 7.0 - 8.8 mg/L
Water Temperature: 20.5 - 20.6ºC

-Effect Data:
EC0 (48hr) = 997 mg/L (mc)
EC50 (48hr) > 997 mg/L (mc)
mc: based on the mean measured concentrations

-Mortality or Immobility: No test organism was Immobilized at any concentration.

Measured Conc. mg/L	Cumulative Number of Dead or Immobilized Daphnids (Percent Mortality or Immobility) 24 Hour	48 Hour
Control	0 (0)	0 (0)
579	0 (0)	0 (0)
757	0 (0)	0 (0)
997	0 (0)	0 (0)

- Calculation of toxic values: Mean measured concentration

Source : Ministry of Environment, Japan (2001)
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
15.01.2003 (10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	Growth rate
Exposure period	:	72 hours
Unit	:	mg/L
Analytical monitoring	:	Yes
NOEC	:	= 998 mg/L (both biomass method and rate method)
EC50	:	> 998 mg/L (both biomass method and rate method)
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	2001
GLP	:	Yes
Test substance	:	other TS: Disodium succinate hexahydrate (CAS No.: 6106-21-4, Nakarai Kagaku, Inc. (Japan), Lot. No.: MOT9476, Purity = 100.2%
Method	:	- Test Organisms: a) Supplier/Source: Obtained from American Type Culture Collection and reproduced in aseptic culture. b) Method of Cultivation: Sterile c) Stain Number: ATCC22662 d) Pre-culture (duration, medium, etc.): Test alga was pre-incubated for 3 days under the same method of test in OECD medium. EbC50 (0-72 hr) for a reference substance (potassium dichromate) was 0.427 mg/L. -Test substance: The acute toxicity to algae was calculated based on butanedioic acid, disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate. Disodium succinate hexahydrate a) Empirical Formula: Na2C4H16O10 b) Molecular Weight: 270.14 g/mol c) Purity: =100.2 % - Test Conditions: a) Medium: OECD medium b) Exposure Vessel Type: 100 mL Medium in a 500mL Erlenmeyer Flask with silicon cap (open system) c) Nominal Concentrations: control, 250, 500 and 1000 mg/L d) Vehicle/Solvent and Concentrations: Any solvent was not used. e) Stock Solutions Preparations and Stability: Test chemical was refrigerated. The stability of the chemical was confirmed by IR spectrum. The IR spectrum at the end of the test was same at the start of test. f) Number of Replicates: 3 g) Initial Cell Number: 10,000 cells/mL h) Water Temperature: 23+/-2ºC i) Light Condition: 4,000 - 5,000 lux, continuously j) Shaking: 100 rpm - Analytical Procedure: Test concentrations were measured at the start and the 72nd hour using HPLC. - Statistical Method: a) Data Analysis: The calculated inhibition rate at the highest concentration based on growth rate inhibition and biomass were less than 50%, therefore the EC50 was more than the highest concentration. The NOEC values were determined by analysis of variance (ANOVA).

b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): time-weighted mean

Remark : The toxicity to alga was calculated based on butanedioic acid, disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate.

Result : - Measured Concentrations: The tested concentrations were measured at the start and the 72nd hour. For all of them, the deviations from the nominal concentration were less than +/-20%.

Nominal conc. mg/L	Measured Conc., mg/L 0 Hour	72 Hour	Mean* mg/L	Percent of nominal 0 Hour	72 Hour
Control	<15.0	<15.0	---	---	---
250	249	246	247	99.7	98.3
500	504	498	501	101	99.6
1,000	1,000	997	998	100	99.7

*: Mean measured concentration (time-weighted mean)

- Water chemistry (pH) and temperature in test: pH and water temperature were measured for control and each concentration at the start and the end of test period.
pH: 7.7 - 7.9 (at the start of the test)
10.3 - 10.5 (at the end of the test)
water temperature: 23.0 - 23.6ºC

-Effect Data: biomass Area Method
EbC50(0-72hr) > 998 mg/L (mc)
NOEbC (0-72hr) >= 998 mg/L (mc)
Rate Method
ErC50(24-48hr) > 998 mg/L (mc)
NOErC (24-48hr) = 998 mg/L (mc)
ErC50(0-72hr) > 998 mg/L (mc)
NOErC (0-72hr) = 998 mg/L (mc)
mc: nominal concentration

- Percent Growth Inhibition of Selenastrum capricornutum

Measured Conc. mg/L	Area under the growth curves (Average) Area A (0-72hr)	Inhibition (%)* IA (0-72hr)
Control	25,400,000	---
247	27,900,000	-10.2
501	28,600,000	-12.8
998	28,300,000	-11.7

Mean Measured Conc.	Growth rates and percent inhibition (Average) Rate u(24-48hr)	Inhibition(%) Im(24-48hr)	Rate u(0-72hr)	Inhibition(%) Im(0-72hr)

Control	0.0771	---	0.0665	---
247	0.0778	-0.875	0.0642	3.37
501	0.0779	-0.954	0.0650	2.21
998	0.0771	-0.138	0.0660	0.745

Growth Curves: During the test period algae grew almost linearly(log scale) in each concentration.

Source : Ministry of Environment, Japan (2001)
Reliability : (1) Valid without restriction
Flag : Critical study for SIDS endpoint
21.01.2003 (8)

Species : Navicula sp. (Algae)
Endpoint : Growth rate
Exposure period : 7 day
Unit : mg/l
Analytical monitoring :
Method : Other
Year : 1993
GLP :
Test substance : Other TS
Method : - Test Organisms: Navicula ramosissima
a) Supplier/Source: The alga used for the test was cultured at least for 5 years in the laboratory.
b) Stock Culture: PESSi

- Test Conditions:
a) Medium: PESSi
b) Media Type: Sea water
c) Exposure Vessel Type: 10 mL Medium in a 80mL test tube with glass screw cap
d) Nominal Concentrations: control, 3, 10, 30, 100, 300 and 1,000 mg/L
e) Number of Replicates: 3
f) Initial Cell Number: 10,000 cells/mL
g) Water Temperature: 15ºC
h) Light Condition: 5,000 lux, continuously

Result : As for alga, growth was promoted by 30 and 100 mg/L. At the highest concentration, i.e., 300mg/L, growth rate inhibition was observed.

- pH in test: In control and exposure except 300mg/L, the pH was 7.4 - 8.1. In high concentration, i.e., 300mg/L, the pH was 3.8.

Source : Ministry of Environment, Japan (2001)
Reliability : (4) not assignable
Remark: Details on the test condition and the results are not available.
15.01.2003 : (9)

Species : Other algae: Chaetoceros gracilis
Endpoint : Growth rate
Exposure period : 7 day
Unit : mg/l
Analytical monitoring :
Method : Other
Year : 1993

GLP	:	
Test substance	:	Other TS
Method	:	- Test Organisms: a) Supplier/Source: The alga used for the test was cultured at least for 5 years in the laboratory. b) Stock Culture: PESSi - Test Conditions: a) Medium: PESSi b) Media Type: Sea water c) Exposure Vessel Type: 10 mL Medium in a 80mL test tube with glass screw cap d) Nominal Concentrations: control, 3, 10, 30, 100, 300 and 1,000 mg/L e) Number of Replicates: 3 f) Initial Cell Number: 10,000 cells/mL g) Water Temperature: 15ºC h) Light Condition: 5,000 lux, continuously
Result	:	As for alga, growth was promoted by 30 mg/L. At the highest concentration, i.e., 300mg/L, growth rate inhibition was observed. - pH in test: In control and exposure except 300mg/L, the pH was 7.4 - 8.1. In high concentration, i.e., 300mg/L, the pH was 3.8.
Source	:	Ministry of Environment, Japan (2001)
Reliability		(4) not assignable - Remark: Details on the test condition and the results are not available.
21.01.2003		(9)
Species	:	Other algae: Pavlova lutheri
Endpoint	:	Growth rate
Exposure period	:	7 day
Unit	:	mg/l
Analytical monitoring	:	
Method	:	Other
Year	:	1993
GLP	:	
Test substance	:	Other TS
Method	:	- Test Organisms: a) Supplier/Source: The alga used for the test was cultured at least for 5 years in the laboratory. b) Stock Culture: PES - Test Conditions: a) Medium: PES b) Media Type: Sea water c) Exposure Vessel Type: 10 mL Medium in a 80mL test tube with glass screw cap d) Nominal Concentrations: control, 3, 10, 30, 100, 300 and 1,000 mg/L e) Number of Replicates: 3 f) Initial Cell Number: 10,000 cells/mL g) Water Temperature: 15ºC h) Light Condition: 5,000 lux, continuously
Result	:	At the highest concentration, i.e., 300mg/L, growth rate inhibition was

observed.

- pH in test: In control and exposure except 300mg/L, the pH was 7.4 - 8.1. In high concentration, i.e., 300mg/L, the pH was 3.8.

Source : Ministry of Environment, Japan (2001)
Reliability : (4) not assignable
Remark: Details on the test condition and the results are not available.
21.01.2003 (9)

Species : Other algae: Tetraselmis tetrathele
Endpoint : Growth rate
Exposure period : 7 day
Unit : mg/l
Analytical monitoring :
Method : Other
Year : 1993
GLP :
Test substance : Other TS
Method : - Test Organisms:
a) Supplier/Source: The alga used for the test was cultured at least for 5 years in the laboratory.
b) Stock Culture: PES

- Test Conditions:
a) Medium: PES
b) Media Type: Sea water
c) Exposure Vessel Type: 10 mL Medium in a 80mL test tube with glass screw cap
d) Nominal Concentrations: control, 3, 10, 30, 100, 300 and 1,000 mg/L
e) Number of Replicates: 3
f) Initial Cell Number: 10,000 cells/mL
g) Water Temperature: 15ºC
h) Light Condition: 5,000 lux, continuously

Result : As for alga, growth was promoted by 100 mg/L. At the highest concentration, i.e., 300mg/L, growth rate inhibition was observed.

- pH in test: In control and exposure except 300mg/L, the pH was 7.4 - 8.1. In high concentration, i.e., 300mg/L, the pH was 3.8.

Source : Ministry of Environment, Japan (2001)
Reliability : (4) Not assignable
Remark: Details on the test condition and the results are not available.
21.01.2003 (9)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)
Endpoint : Reproduction rate

Exposure period	:	21 day
Unit	:	mg/l
Analytical monitoring	:	Yes
NOEC	:	= 95.2
LOEC	:	not available
EC50	:	> 95.2
Method	:	other: OECD guide-line 211
Year	:	2001
GLP	:	yes
Test substance	:	other TS: Disodium succinate hexahydrate (CAS No.: 6106-21-4, Nakarai Kagaku, Inc. (Japan), Lot. No.: MOT9476, Purity = 100.2%
Method	:	-Test Organisms: a) Age: < 24 hours old b) Supplier/Source: Test organisms were obtained from the University of Sheffield (UK) and had been reproduced in the testing laboratory for 10 years. c) Any pretreatment: Parental daphnids were acclimated for 38 days on test conditions before testing, any groups showing high mortality were not used for testing. The mortality of the daphnids was less than 5% for 2 weeks before testing. EC50(48 hr, immobility) for a reference substance (potassium dichromate) was 0.171mg/L. -Test substance: The chronic toxicity to daphnids was calculated based on butanedioic acid, disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate. Disodium succinate hexahydrate a) Empirical Formula: Na2C4H16O10 b) Molecular Weight: 270.14 g/mol c) Purity: =100.2 % - Test Conditions: a) Dilution Water Source: Dilution water was prepared from tap water (Kurume city, Japan). The tap water was dechlorinated and treated by activated carbon. After that Residual Chlorine was removed from the water. b) Dilution Water Chemistry: pH: = 7.4 Total hardness (as CaCO3): = 61.0 mg/L c) Exposure Vessel Type: 80 mL test solution in a glass beaker d) Nominal Concentrations: control, solvent control, 25.0, 50.0 and 100 mg/L e) Vehicle/Solvent and Concentrations: Any solvent was not used. f) Stock Solutions Preparations and Stability: Test chemical was refrigerated. The stability of the chemical was confirmed by IR spectrum. The IR spectrum at the end of the test was same at the start of test. g) Number of Replicates: 10 h) Individuals per Replicates: 10 i) Renewal Rate of Test Water: once per day j) Water Temperature: 20+/-1oC k) Light Condition: 16:8 hours, light-darkness l) Feeding: 0.1 - 0.2 mg carbon/day/individual (Chlorella vulgaris: Green Algae) m) Aeration: not described - Analytical Procedure: The test concentrations were measured for both renewal and old test solution at the start of test and 1st, 8th, 9th, 16th and 17th day using HPLC.

- Statistical Method:
a) Data Analysis:
LC50 and EC50: During test period the test organisms were not killed more than 50% in any concentration. The effects on reproduction were less than 50%. From these reason LC50 and EC50 is more than highest concentration.
NOEC and LOEC: The cumulative number of juveniles produced per adult in control and test vessels after 21days was tested by Bartlett's test and one-way analysis of variance. The cumulative number of dead parental daphnids after 21 days was tested by Kruskal-Wallis test. NOEC and LOEC were determined by these results and juvenile and parental daphnids condition of activity.
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Time-weighted Mean

Remark : NOEC was determined based on the cumulative number of alive juveniles produced per adult alive.
The chronic toxicity to daphnids was calculated based on the mean measured concentrations as butanedioic acid, disodium salt (anhydride), but this chemical is stable as hexahydrate, i.e., disodium succinate hexahydrate. Then, the test was carried out with disodium succinate hexahydrate.

Result : - Effect: reproduction- Measured Concentrations: The test concentrations were measured for both renewal and old test solution at the start of test and 1st, 8th, 9th, 16th and 17th day. Some of them, the deviation from the nominal concentration were not less than +/-20%.

Nominal Conc.	Measured Conc., mg/L							
mg/L Date	0 Fresh	1 Old	8 Fresh	9 Old	16 Fresh	17 Old	TWM* mg/L	% of Nominal
Control	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	---	---
25.0	25.1	17.0	25.3	11.3	25.4	14.0	19.1	76.5
50.0	49.7	41.3	49.9	37.7	51.2	41.4	82.8	90.0
100	99.3	90.9	100	91.2	102	88.6	95.2	95.2

Fresh: Start of renewal period
Old: End of renewal period*: Time-weighted mean of measured concentration during 21 days

- Measured Concentration as a Percentage of Nominal

Nominal Conc.	Measured Concentration as a Percentage of Nominal					
mg/L Date	0 Fresh	1 Old	8 Fresh	9 Old	16 Fresh	17 Old
25.0	101	68.2	101	45.3	25.4	76.5
50.0	99.5	82.6	99.7	75.5	102	82.8
100	99.3	90.9	100	91.2	102	88.6

Fresh: Start of renewal period
Old: End of renewal period

- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for control and each concentration at the start of test and before and after renewal of the test solutions.
pH: 7.6 - 7.8
DO: 7.7 - 8.9 mg/L
Water Temperature: 20.1 - 20.6ºC

- Total hardness: 37.0 - 45.4 mg/L

-Effect Data:
LC50 (21day) >95.2 (mc)
EC50 (21day) >95.2 (mc)
NOEC (21day) = 95.2 (mc)
LOEC (21day) not available
mc: based on the mean measured concentrations

- Cumulative Number of Died Parental Daphnids: No test organism was killed at control solvent control, 0.13, 0.24 and 0.38 mg/L. The lowest concentration that test organisms were dead was at 25.0 mg/L after 10days.

Measured Conc. (mg/L)	Cumulative Number of Dead Parental Daphnids (days) 1	2	3	4	5	6	7	8	9	10
Control	0	0	0	0	0	0	0	0	0	0
19.1	0	0	0	0	0	0	0	0	0	1
45.0	0	0	0	0	0	0	0	0	0	0
95.2	0	0	0	0	0	0	0	0	0	0

Measured Conc. (mg/L)	Cumulative Number of Dead Parental Daphnids (days) 11	12	13	14	15	16	17	18	19	20	21
Control	0	0	0	0	0	0	0	0	0	0	1
19.1	1	1	1	1	1	1	1	2	2	2	2
45.0	1	1	1	1	1	1	2	2	2	2	2
95.2	0	0	0	0	0	0	1	1	1	2	2

-Effect Data(reproduction):Juveniles were first produced on the 8th day at every concentration.

Measured Conc. mg/L	Mean Cumulative Numbers of Juveniles Produced per Adult (days) 0 --- 7	8	9	10	11	12	13	14
Control	0 --- 0	13.6	13.6	13.6	42.2	42.2	42.2	56.1
19.1	0 --- 0	22.6	22.6	23.1	49.6	51.4	51.4	64.5
45.0	0 --- 0	23.5	23.5	45.1	59.4	59.4	59.4	80.1
95.2	0 --- 0	23.5	23.5	29.0	56.9	56.9	61.4	75.0

Measured Conc. mg/L	Mean Cumulative Numbers of Juveniles Produced per Adult (days) 15	16	17	18	19	20	21
Control	68.6	68.6	91.9	93.8	93.8	93.8	112
19.1	73.5	73.5	95.8	98.6	98.6	108	121
45.0	81.3	81.3	102	103	104	118	121
95.2	77.6	82.0	106	108	108	129	141

-Cumulative numbers of juveniles produced per adult alive for 21days

	Measured Conc.1), mg/L			
Vessel No.	Control	19.1	45.0	95.2
1	146	85	144	114
2	98	109	148	139
3	85	---	---	184
4	128	135	---	---
5	119	145	145	---
6	74	150	96	154
7	134	99	97	118
8	---	161	76	147
9	139	84	142	152
10	82	---	119	119
Mean	112	121	121	141
S. D.	27.3	30.5	28.0	23.7

1): Time-weighted mean measured concentration.

- Calculation of toxicity values: The calculation of toxicity values was the mean measured concentrations.

Source : Ministry of Environment, Japan (2001)
Reliability : (1) Valid without restriction
Flag : Critical study for SIDS endpoint
15.01.2003 (10)

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Crj: CD(SD)
Sex	:	male/female
Number of animals	:	5
Vehicle	:	other: distilled water
Value	:	> 1200 mg/kg bw
Method	:	OECD Guide-line 401 "Acute Oral Toxicity"
Year	:	2002
GLP	:	yes
Test substance	:	other TS: See Remark
Remark	:	1) Test substance: disodium succinate hexahydrate (CAS No. 6106-21-4), Nippon Shokubai Co.,Ltd., Purity 99.9%, Lot No. 9P01B 2) Route: oral(gavage) 3) Dosage: 0(vehicle), 2000mg/kg bw 4) No. of animals/group: male 5, female 5
Result	:	There were no deaths and abnormal findings in either sex during the observation period. Body weights of these groups increased as same as the control group. Furthermore, the necropsy revealed that there were no abnormalities at the termination of the 14-day observation period. Estimated LD50 value of disodium succinate hexahydrate is greater than 2000 mg/kg. It becomes greater than 1200 mg/kg bw as disodium succinate.
Conclusion	:	The oral LD50 value in rats is greater than 2000 mg/kg as sodium succinate hexahydrate (1200 mg/kg bw as disodium succinate for both sexes.
Reliability	:	(1) valid without restriction Well conducted study, carried out by Biosafety Research Center, Food, Drugs and Pesticides (An-Pyo Center)(Japan).
Flag	:	Critical study for SIDS endpoint

25.12.2002 (13)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Route of admin.	:	i.v.
Exposure time	:	
Value	:	= 4500 mg/kg bw
Remark	:	Test substance: Dodium succinate
Source	:	Merk Index: 1989
Reliability	:	(4) not assignable

06.11.2002 (15)

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	Males; for 52 days Females; from 14 days before mating to day 4 of lactation
Frequency of treatment	:	Once daily
Post obs. period	:	1 day
Doses	:	0(vehicle), 100, 300, 1000 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL	:	>= 600 mg/kg bw
Method	:	OECD combined study TG422
Year	:	2002
GLP	:	Yes
Test substance	:	other TS: See Remark
Remark	:	1)Test substance: disodium succinate hexahydrate (CAS No. 6106-21-4), Nippon Shokubai Co., Ltd., Purity 99.9%, Lot No. 9P01B 2)Route: oral (gavage) 3)No. of animals/group: males 12, females 12
Result	:	The NOAEL of disodium succinate hexahydrate was greater than or equal to 1000 mg/kg bw/day for males and females. It becomes greater than or equal to 600 mg/kg bw/day as disodium succinate. 1) Mortality and clinical signs No death occurred in males and females in any group throughout the treatment period. As the changes in clinical signs, loosening of stool was observed in 1 and 4 males in the 100 and 1000 mg/kg groups and 1 female in the 1000 mg/kg group, respectively. This finding was a mild one and not accompanied by dirty hair in any group. One male in the 100mg/kg group and 1 female in the 1000 mg/kg group showed this finding in 1 day only, but 3 males except one male in the 1000 mg/kg group showed this finding in 3 to 4 days. In addition, for males, there were alopecia in 2 and 3 animals in the 300 and 1000 mg/kg groups, eschar in 1 and 2 animals in the 300 and 1000 mg/kg groups, ocular discharge in 1 animal each in the 300 and 1000 mg/kg groups, nasal discharge in 2 animals each in the 300 and 1000 mg/kg groups and salivation in 1 animal in the 1000 mg/kg group, respectively. For females, there was alopecia in 1, 2, 3 and 1 animals in the control, 100, 300 and 1000 mg/kg groups, eschar in 1 animal each in the 100 and 1000 mg/kg groups and salivation in 1 animal in the 1000 mg/kg group, respectively. 2) Body weight There was no significant difference between the treatment groups and the

control group in both males and females throughout the treatment period.

3) Food consumption
There was no significant difference between the treatment groups and the control group in both males and females throughout the treatment period.

4) Hematology
In the hematology and the blood coagulation test, there was no significant difference between the treatment groups and the control group in all test items in both males and females.

5) Clinical chemistry
In males, sodium showed high values in the 300 and 1000 mg/kg groups compared with the control group. In addition, chloride showed high values in the 300 mg/kg group, which was a slight and insignificant change. Also, total bile acid showed low values in the 1000 mg/kg group. The values in the control group, however, scattered large, and those of most animals in the 1000 mg/kg group were within the scatter in the control group.
In females, creatinine showed high values in the 300 mg/kg group, which was a change not related to the dose. In the 1000 mg/kg group, urea nitrogen showed high values.

6) Urinalysis (conducted only for males)
There was moderate occult blood in 1 of 5 animals in the 300 mg/kg group and severe one in 1 of 5 animals in the 1000 mg/kg group. The protein of 300 mg/dL or more were observed in 1 of 5 animals in the 300 mg/kg group and 2 of 5 animals in the 1000 mg/kg group. In addition, the yellow-brownish urine was observed in 2 of 5 animals in the 1000 mg/kg group, which were the changes within the normal values. Also, the abnormally high volumes of 24-hour urine were observed in 1 animal each in the 100 and 300 mg/kg groups, but there was no intergroup difference in the results of urine volume and urinary osmotic pressure in the animals excepting these 2 animals.

7) Organ weight
In males, absolute adrenal weight showed significantly high values in the 1000 mg/kg group compared with the control group.
In females, there was no significant difference in any organ determined between the treatment groups and the control group.

8) Findings at necropsy
In males, red patches in the liver were observed in 1 animal in the 300 mg/kg group, white patches/region in the liver, deverticulum in the small intestine and hypertrophy of the testis in 1 animal each in the 1000 mg/kg group and nodes of the epididymis in 2 animals in the 1000 mg/kg group.
In females, adhesion of the spleen was observed in 1 animal in the control group, black patches in the glandular stomach in 2 and 1 animals in the 300 and 1000 mg/kg groups, white patches in the liver in 1 animal in the 300 mg/kg group and yellow patches, hypophysial cyst and alopecia in 1 animal each in the 1000 mg/kg group, respectively.

9) Histopathology
There was no finding indicating a significant increase in the incidence in both males and females in the treatment groups compared with the control group.
In males, atrophy of the seminiferous tubule was observed in 1 animal in the 300 mg/kg group and dilation of the seminiferous tubule in 1 animal in the 1000 mg/kg group. Dilation of the seminiferous tubule was hemilaterally observed, and no abnormality was observed in the cells constituting the seminiferous epithelium. Spermatic granuloma in the epididymis was observed in 2 animals in the 1000 mg/kg group but not observed in other

treatment groups. Necrosis of the liver was observed in 1 animal in the 1000 mg/kg group. No gastric finding was observed in all groups including the control group. Lymphocytic infiltration in the prostate was observed in 4 and 1 animals in the control group and the 1000 mg/kg group, respectively. Prostatitis was observed in 1 animal in the 1000 mg/kg group. In the animal showing prostatitis, there were the findings of pyelitis and accompanied proliferation of transitional epithelium in the kidney and the findings of lymphocytic infiltration and proliferation of transitional epithelium in the urinary bladder. Other findings were those also observed in the control group or solitary occurrence.
In females, necrosis of the mucosa in the glandular stomach were observed in 2 and 1 animals in the 300 and 1000 mg/kg groups, focal necrosis of the liver in 1 animal in the 1000 mg/kg group and necrosis of the liver in 1 animal in the 300 mg/kg group. In addition, proliferation of lymphatic tissues in the small intestine was observed in 1 and 4 animals in the control group and the 1000 mg/kg group, respectively. Other histopathological findings observed were those also observed in the control group or in a few animals only.
For the testis in the control group and the 1000 mg/kg group, the number of seminiferous epithelial cells in the seminiferous tubule in the stage VIII was determined. As a result, the numbers of spermatogonia (type A), spermiocytes in the preleptotene stage, spermiocytes in the pachytene stage, round spermatids and Sertoli's cells showed no differences compared with the control group.

Conclusion : Based on the higher levels of urinary protein at 300 mg/kg bw/day, the NOAEL for repeated dose toxicity was considered to be 100 mg/kg bw/day as disodium succinate hexahydrate (60 mg/kg bw/day as disodium sccinate).

Reliability : (1) valid without restriction
Well conducted study, carried out by Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center)(Japan).

Flag : Critical study for SIDS endpoint
25.12.2002 (13)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Bacterial reverse mutation assay
System of testing : Salmonella typhimurium TA100, TA1535, TA98, TA1537, Escherichia coli WP2uvrA
Concentration : 0, 156, 313, 625, 1250, 2500, 5000 µg/plate
Cycotoxic conc. : See Result
Metabolic activation : with and without
Result : negative
Method : other: Guidelines for Screening Mutagenicity Tesing of Chemicals (Chemical Substances Control Law of Japan) and OECD Test Guideline 471 "Bacterial Reverse Mutation Test"
Year : 2002
GLP : yes
Test substance : other TS: Source: disodium succinate hexahydrate (CAS No.6106-21-4), Nippon Shokubai Co., Ltd., Purity 99.9%, Lot No. 9P01B
Statistical methods : No data
Result : The test substance was not mutagenic in Salmonella typhimurium TA100, TA1535, TA98, TA1537 and Escherichia coli WP2uvrA, with or without an exogenous metabolic activation system.
No toxicity was observed up to 5000 µg/plate, with or without metabolic activation.
Test condition : Procedures: Pre-incubation method
Solvent: Saline
Positive controls: -S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2uvrA), Sodium azide (TA1535) and 2-Methoxy-6-chloro-9-[3-(2-

chloroethyl)-aminopropylamino]acridine•2HCl (TA1537)
+S9 mix; Benzo[a]pyrene (TA100 and TA98) and 2-Aminoanthracene (TA1535, TA1537, WP2uvrA)
S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone
Plates/test: 3
Number of replicates: 2

Reliability : (1) valid without restriction
Well conducted study, carried out by Bozo Research Inc. (Japan).

Criteria for positive response : The number of colonies found was twice the number in the control.

Flag : Critical study for SIDS endpoint

05.12.2002 (13)

Type : Ames test
System of testing : Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98
Concentration : Max dose was 5000 µg/plate (six different concentrations)
Cycotoxic conc. : See Result
Metabolic activation : with
Result : negative
Method :
Year :
GLP : no
Test substance : other TS: Source: Japan Food Additives Association., Purity 98.6%
Statistical methods : No data
Result : The test substance was not mutagenic in Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, TA1538 with an exogenous metabolic activation system.
No toxicity was observed up to 5000 µg/plate with metabolic activation.
Test condition : Procedures: Pre-incubation method
Solvent: Phosphate buffer
S9: Rat liver, induced with Kanechlor KC-400
Plates/test: 2

Criteria for positive response : The number of colonies found was twice the number in the control.

Reliability : (2) valid with restrictions
Well conducted study, carried out by National Institute of Hygienic Science (Japan).

05.12.2002 (12)

Type : Ames test
System of testing : Salmonella typhimurium TA97, TA102
Concentration : 0, 100, 500, 1000, 5000, 10000 µg/plate
Cycotoxic conc. : See Result
Metabolic activation : with and without
Result : negative
Method :
Year :
GLP : no
Test substance : other TS: Source: disodium succinate, Wako Pure Industries, Ltd., Lot No. WDJ8980
Statistical methods : Kruskal-Wallis test and Moore's regression analysis
Result : The test substance was not mutagenic in Salmonella typhimurium TA97 and TA97, with or without an exogenous metabolic activation system.
No toxicity was observed up to 10000 µg/plate, with or without metabolic activation.
Test condition : Procedures: Pre-incubation method
Solvent: Distilled water
Positive controls: -S9 mix; 9-Aminoacridine (TA97), Mitomycin C (TA102) +S9 mix; 2-Aminoanthracene (Both strains)
S9: Rat liver, induced with aroclor 1254
Plates/test: 3

Criteria for positive response	:	The number of colonies found was twice the number in the control.
Reliability	:	(2) valid with restrictions Well conducted study, carried out by The Tokyo Metropolitan Research Laboratory of Public Health (Japan).

05.12.2002 (11)

Type	:	Chromosomal aberration test
System of testing	:	Type of cell used: Chinese hamster lung (CHL/IU) cell
Concentration	:	-S9 mix (24 and 48 hr continuous treatment); 0, 313, 625, 1250, 2500, 5000 µg/mL -S9 mix (6 hr short-term treatment); 0, 313, 625, 1250, 2500, 5000 µg/mL +S9 mix (6 hr short-term treatment); 0, 313, 625, 1250, 2500, 5000 µg/mL
Cycotoxic conc.	:	No cytotoxicity was observed up to a concentration of 5000 µg/mL, at short-term or continuous treatment.
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Guidelines for Screening Mutagenicity Testing of Chemicals (Chemical Substances Control Law of Japan) and OECD Test Guideline 473 "In vitro Mammalian Chromosomal Aberration Test"
Year	:	2002
GLP	:	yes
Test substance	:	other TS: Source: disodium succinate hexahydrate, Nippon Shokubai Co., Ltd., Purity 99.9%, Lot No. 9P01B
Statistical methods	:	No data
Result	:	The test substance did not induce structural chromosomal aberrations and/or polyploidy in CHL cells, with or without an exogenous metabolic activation system.
Test condition	:	Solvent: Saline Positive controls: -S9 mix, Mitomycin C +S9 mix, Cyclophosphamide S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone Plates/test: 2
Reliability	:	(1) valid without restriction Well conducted study, carried out by Bozo Research Inc. (Japan).
Flag	:	Critical study for SIDS endpoint

05.12.2002 (13)

Type	:	Chromosomal aberration test
System of testing	:	Type of cell used: Chinese hamster lung (CHL) cell
Concentration	:	-S9 mix (24 and 48 hr continuous treatment); Max dose was 15000 µg/mL (3 different doses)
Cycotoxic conc.	:	See Result
Metabolic activation	:	without
Result	:	ambiguous
Method	:	
Year	:	
GLP	:	no
Test substance	:	other TS: Source: Japan Food Additives Association., Purity 98.6%
Result	:	The test substance did not induce structural chromosomal aberrations in CHL cells. However, an equivocal result was obtained about polyploidy. The maximum dose was selected by estimated 50% cell-growth inhibition.
Test condition	:	Solvent: Saline
Reliability	:	(2) valid with restrictions Well conducted study, carried out by National Institute of Hygienic Science (Japan).

05.12.2002 (12)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENITY

5.8 TOXICITY TO REPRODUCTION

Type	:	One generation study
Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	Males; for 52 days Females; from 14 days before mating to day 4 of lactation
Frequency of treatment	:	Once daily
Premating exposure period		
Male	:	14 days
Female	:	14 days
Duration of test	:	Males; for 53 days Females; from 43 to 47 days
Doses	:	0(vehicle), 100, 300, 1000 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL Parental	:	>= 600 mg/kg bw
NOAEL F1 Offspring	:	>= 600 mg/kg bw
Method	:	OECD combined repeated dose and reproductive/developmental toxicity screening test
Year	:	2002
GLP	:	yes
Test substance	:	other TS: see Remark
Remark	:	1)Test substance: disodium succinate hexahydrate (CAS No. 6106-21-4), Nippon Shokubai Co., Ltd., Purity 99.9%, Lot No. 9P01B 2)Route: oral(gavage) 3)No. of animals/group: males 12, females
Result	:	The NOAEL of disodium succinate hexahydrate was greater than or equal to 1000 mg/kg bw/day for males and females. It becomes greater than or equal to 600 mg/kg bw/day as sodium succinate. 1) Copulation and fertility Copulation and conception were all established, and both the copulation index and the fertility index were 100% in all groups. In the observation of estrous cycle, there was no intergroup difference in the mean estrous cycle. The abnormal estrous cycle was observed in 1 animal each in the 100 and 1000 mg/kg groups. There was no intergroup difference in the incidence of abnormal estrous cycle. 2) Parturition and lactation The gestation period was significantly shortened in the 100 and 1000 mg/kg groups compared with the control group. There was no abnormality in the conditions of parturition, and the numbers of corpora lutea, implantation sites, delivered offspring and live delivered offspring showed almost the same values. There were no intergroup differences in the delivery index, implantation index, parturition index, live birth index, sex ratio and viability index of neonates on day 4 of lactation. 3) Morphology, body weight and necroptic findings of neonates

In the external examination in neonates, anophthalmia and polydactyly were observed in 1 animal each in the 300 mg/kg group.
Body weight during lactation period was significantly low on days 0 and 4 of lactation in males and day 4 in females in the 100 mg/kg group and on day 4 in males in the 300 mg/kg group, which was the change not associated with the dose.
In the necropsy of dead offspring during the lactation period, pyelectasia was observed in 1 animal in the 100 mg/kg group.
In the necropsy on day 4 of lactation, red patches on the plantar were observed in 15 males and 13 females in the 100 mg/kg group, and the number of occurrences significantly increased in both males and females compared with the control group. However, this finding occurred in litters in 2 broods in both males and females. In addition, dilation of the ureter was observed in 3, 4 and 3 males and 5, 1 and 2 females in the 100, 300 and 1000 mg/kg groups, respectively, and the number of occurrences significantly increased in the male 100 mg/kg group. However, dilation of the ureter in the female 100 mg/kg group was observed in litters in 4 of 5 animals. Other findings included thymic remnant in the neck in 3, 1, 2 and 3 male animals in the control, 100, 300 and 1000 mg/kg groups and in 1, 2 and 2 female animals in the control, 100 and 300 mg/kg groups, nodes in the liver in 1 animal each in the male and female 1000 mg/kg groups, white patches in the liver in 1 animal in the male 100 mg/kg group, pyelectasia in 3 animals each in the male 100, 300 and 1000 mg/kg groups and in 2 and 1 animal in the female 300 and 1000 mg/kg groups, anophthalmia in 1 animal in the female 300 mg/kg group, cysts in the hindlimbs in 1 animal in the female 100 mg/kg group and polydactyly in 1 animal in the female 300 mg/kg group.

Conclusion : There is no evidence that this chemical has reproductive/developmental toxicity in rats. The NOAEL for reproduction/developmental toxicity was considered to be 1000 mg/kg bw/day as disodium succinate hexahydrate (600 mg/kg bw/day as disodium succinate.

Reliability : (1) valid without restriction
Well conducted study, carried out by Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center)(Japan).

Flag : Critical study for SIDS endpoint

25.12.2002 (13)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Remark : See the section 5.8 "Toxicity to Reproduction"

25.11.2002

5.10 OTHER RELEVANT INFORMATION

Type : other: Promotion effect

Remark : F344 male rats were treated drinking water with a carcinogen (0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine) for the first 4 weeks, and then they were given powdered basal diet containing 5% succinic acid, mono- and di-sodium salts for 32 weeks. The urinary pH and sodium ion concentration were significantly increased, in mono- and di-sodium salts groups compared to the values of succinic acid group. And the incidence and number of urinary bladder tumors were significantly increased. The urinary bladder tumor growth might be related with pH and sodium ion concentration.

Test substance : other TS: Source; Wako Pure Chemical Industries

Reliability : (3) invalid

05.12.2002 (14)

5.11 EXPERIENCE WITH HUMAN EXPOSURE

(1) Chemicals Evaluation and Research Institute (CERI), Japan. (2000). Unpublished data. Test report number 21110.

(2) Chemicals Evaluation and Research Institute (CERI), Japan. (2000a). Unpublished data. Test report number K-1110.

(3) Chemicals Evaluation and Research Institute (CERI), Japan. (2000b). Unpublished data. Test report number K-1110.

(4) Chemicals Evaluation and Research Institute (CERI), Japan. (2000c). Unpublished data. Test report number K-1110.

(5) Chemicals Evaluation and Research Institute (CERI), Japan. (2000d). Unpublished data. Test report number K-1110.

(6) Merck Index (1996); approx.

(7) Meylan, WM. and Howard, PH. (1995).

(8) MEYLAN, WM. and HOWARD, PH. (1995)

(9) Ohgai, M., et al. (1993) Nippon Suisan Gakkaisi, 59 (4), 647-652.

(10) MOE, Japan (2001) Ministry of the Environment, unpublished data.

(11) Fujita, H., et al. (1994) Ann. Rep. Tokyo Metr. Res. Lab. P.H., 45, 191-199.

(12) Ishidate, M., et al. (1984) Fd Chem. Toxic., 22, 623-636.

(13) MHLW, Japan (2002) Ministry of Health, Labor and Welfare, Toxicity Testing Reports of Environmental Chemicals 9, 383-412.

(14) Otoshi, T. et al. (1993) Carcinogenesis, 14, 2277-2281.

(15) Budavari, S. (ed) (2001) The MERK INDEX-An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ, Merk and Co., Inc., No. 8754.