FOREWORD

DISODIUM SUCCINATE

CAS N°: 150-90-3

INTRODUCTION
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:
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Director
Second International Organizations Division
Ministry of Foreign Affairs, Japan

4. Shared Partnership with:
5. Roles/Responsibilities of the Partners:
   • Name of industry sponsor /consortium
   • Process used

6. Sponsorship History
   • How was the chemical or category brought into the OECD HPV Chemicals Programme?
     The original draft documents were prepared by the Japanese government.

7. Review Process Prior to the SIAM:

8. Quality check process:


10. Date of last Update:

11. Comments:
**SIDS INITIAL ASSESSMENT PROFILE**

<table>
<thead>
<tr>
<th>CAS No.</th>
<th>150-90-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Name</td>
<td>Disodium succinate</td>
</tr>
<tr>
<td>Structural Formula</td>
<td>NaOOCCH₂CH₂COONa</td>
</tr>
</tbody>
</table>

**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 succinate 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 uvrA 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/1U) 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
The vapour pressure of $1.16 \times 10^{-5}$ 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.
1 IDENTITY

1.1 Identification of the Substance

CAS Number: 150-90-3
Chemical Name: Disodium succinate
Molecular Formula: C₄H₄Na₂O₄
Structural Formula: NaOOCCH₂CH₂COONa
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 HClO₄)

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

<table>
<thead>
<tr>
<th>Property</th>
<th>Protocol</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting Point</td>
<td>OECD TG 102</td>
<td>&gt;= 400 °C</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>Unknown</td>
<td>&gt;= 400 °C</td>
</tr>
<tr>
<td>Density</td>
<td>JIS K 7112-1980</td>
<td>1.886 g/cm³ at 25 °C</td>
</tr>
</tbody>
</table>
| Vapor Pressure                | OECD TG 104
  Calculated (MPBPWIN)        | < 0.00015 hPa at 100 °C
  1.16E-7 hPa                 |
| Partition Coefficient (Log P<sub>ow</sub>) | 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 succinate 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 $5.45 \times 10^{-12}$ atm m$^3$/mol. Vapor pressure is less than 0.015 Pa at 100 °C and is estimated to be $1.16 \times 10^{-5}$ 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 (CAS 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 LD₅₀ 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

<table>
<thead>
<tr>
<th>Route</th>
<th>Animals</th>
<th>Type</th>
<th>Values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Rat</td>
<td>LD₅₀</td>
<td>&gt; 2,000 mg/kg bw (disodium succinate hexahydrate)</td>
<td>MHLW, Japan: 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 1,200 mg/kg bw (disodium succinate)</td>
<td></td>
</tr>
</tbody>
</table>

Studies in Humans

There is no available information on humans.

Conclusion

The oral LD₅₀ 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

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Test system</th>
<th>Highest concentration</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ames test (reverse mutation)</td>
<td>S. typhimurium (TA98, TA100, TA1535, TA1537)</td>
<td>5,000 µg/plate (disodium succinate hexahydrate)</td>
<td>Negative (+ &amp; - MA*)</td>
<td>MHLW, Japan: 2002</td>
</tr>
<tr>
<td>Ames test (reverse mutation)</td>
<td>S. typhimurium (TA97, TA94, TA98, TA100, TA1535, TA1537)</td>
<td>5,000 µg/plate (disodium succinate)</td>
<td>Negative (+ MA)</td>
<td>Ishidate et al.: 1984</td>
</tr>
<tr>
<td>Ames test (reverse mutation)</td>
<td>S. typhimurium (TA97, TA102)</td>
<td>10,000 µg/plate (disodium succinate)</td>
<td>Negative (+ &amp; - MA)</td>
<td>Fujita et al.: 1994</td>
</tr>
<tr>
<td><strong>Non-bacterial in vitro test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosomal aberration test</td>
<td>CHL cells</td>
<td>5,000 µg/mL (disodium succinate hexahydrate)</td>
<td>Negative (+ &amp; - MA)</td>
<td>MHLW, Japan: 2002</td>
</tr>
<tr>
<td>Chromosomal aberration test</td>
<td>CHL cells</td>
<td>15,000 µg/mL (disodium succinate)</td>
<td>Equivocal (polyploidy) (- MA)</td>
<td>Ishidate et al.: 1984</td>
</tr>
</tbody>
</table>

*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 uvrA 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 µg of 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 \textit{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\textsubscript{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\textsubscript{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\textsubscript{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 \textit{Salmonella typhimurium} TA98, TA100, TA1535, and TA1537, and \textit{Escherichia coli} WP2 uvrA 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

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Test duration</th>
<th>Result (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aquatic plants, e.g. algae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green algae (Selenastrum capricornutum)</td>
<td>72 h</td>
<td>Growth rate method</td>
<td>MOE, Japan(2001)</td>
</tr>
<tr>
<td></td>
<td>Open system</td>
<td>ErC$_{50}$ &gt; 998</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NOErC = 998</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biomass method</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EbC$_{50}$ &gt; 998</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NOEbC = 998</td>
<td></td>
</tr>
<tr>
<td><em>Invertebrates</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnids (Daphnia magna)</td>
<td>48 h</td>
<td>Immobilization</td>
<td>MOE, Japan(2001)</td>
</tr>
<tr>
<td></td>
<td>Static</td>
<td>EC$_{0}$ = 997</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC$_{50}$ &gt; 997</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21 d</td>
<td>Mortality</td>
<td>MOE, Japan(2001)</td>
</tr>
<tr>
<td></td>
<td>Semi-static</td>
<td>LC$_{0}$ &gt; 95.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reproduction</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>EC$_{50}$ &gt; 95.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NOEC = 95.2</td>
<td></td>
</tr>
<tr>
<td><em>Fish</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medaka (Oryzias latipes)</td>
<td>96 h</td>
<td>LC$_{0}$ = 47.0</td>
<td>MOE, Japan(2001)</td>
</tr>
<tr>
<td></td>
<td>Flow-through</td>
<td>LC$_{50}$ &gt; 95.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC$_{100}$ &gt; 95.4</td>
<td></td>
</tr>
</tbody>
</table>

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, \textit{Daphnia magna}, static).

A test with fish (OECD TG 203, \textit{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, \textit{Selenastrum capricornutum}, open system) were reported. In daphnids, the effect of disodium succinate on reproduction of \textit{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 \(1.16 \times 10^{-5}\) 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, \textit{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, \textit{Daphnia magna}, static). For fish (OECD TG 203, \textit{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, \textit{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, \textit{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


Dye, WS. et al. (1944) Growth, 8, 1-11.


Hansh, C. et al. (1995) SRC PHYS PROP DATA BASE.


NITS (1975) National Technical Information Service PB254541.

### IUCLID 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

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

Butanediolic 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
28.07.2003

29.07.2003
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
28.07.2003

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
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.14.1 Water Pollution</td>
<td></td>
</tr>
<tr>
<td>1.14.2 Major Accident Hazards</td>
<td></td>
</tr>
<tr>
<td>1.14.3 Air Pollution</td>
<td></td>
</tr>
<tr>
<td>1.15 Additional Remarks</td>
<td></td>
</tr>
<tr>
<td>1.16 Last Literature Search</td>
<td></td>
</tr>
<tr>
<td>1.17 Reviews</td>
<td></td>
</tr>
<tr>
<td>1.18 Listings E.G. Chemical Inventories</td>
<td></td>
</tr>
</tbody>
</table>
2.1 MELTING POINT

Value : >= 400 ° C
Sublimation : 
Method : OECD Guide-line 102 "Melting Point/Melting Range"
Year : 2000
GLP : no
Test substance : Disodium succinate.
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 BOILING POINT

Value : >= 400 ° C at 1013 hPa
Decomposition : 
Method : OECD Guide-line 103 "Boiling Point/boiling Range"
Year : 2000
GLP : no
Test substance : Disodium succinate.
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

2.3 DENSITY

Type : density
Value : = 1.886 at 25° C
Method : other
Year : 1994
GLP : no
Test substance : Disodium succinate.
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

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE
### 2. PHYSICO-CHEMICAL DATA

**Value:** \( \leq 0.00015 \text{ hPa at 100° C} \)

**Decomposition:** other (calculated)

**Method:** OECD Guide-line 104 "Vapor Pressure Curve"

**Year:** 2000

**GLP:** no

**Test substance:** Disodium succinate.

**Source:** Chemicals Evaluation and Research Institute (CERI), Japan.

**Reliability:** (2) valid with restrictions

**Flag:** Critical study for SIDS endpoint

**Date:** 28.07.2003

---

#### 2.5 PARTITION COEFFICIENT

**Log pow:** < \(-0.59\) at ° C

**Method:** other (measured)

**Year:** 1995

**GLP:**

**Test substance:**

**Reliability:** (2) valid with restrictions

**Flag:** Critical study for SIDS endpoint

**Date:** 28.07.2003

---

**Log pow:** = \(0.39\) at ° C

**Method:** other (calculated)

**Year:** 2003

**GLP:**

**Test substance:**

**Remark:** Calculated by Kowwin v. 1.9

**Reliability:** (2) valid with restrictions

**Date:** 29.07.2003

---

**Log pow:** = \(-0.59\) at ° C

**Method:**

**Year:** 1995

**GLP:**

**Test substance:**


**Test substance:** Other (Succinic acid)

**Reliability:** (3) invalid

**Date:** 28.07.2003

---

#### 2.6.1 WATER SOLUBILITY
### 2. PHYSICO-CHEMICAL DATA

<table>
<thead>
<tr>
<th>Value</th>
<th>&gt;= 100 g/l at 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative</td>
<td>of very high solubility</td>
</tr>
<tr>
<td>( P_{ka} )</td>
<td>at 25 °C</td>
</tr>
<tr>
<td>( \text{pH} )</td>
<td>= 8.6 at 100 g/l and 25 °C</td>
</tr>
<tr>
<td>Method</td>
<td>OECD Guide-line 105 &quot;Water Solubility&quot;</td>
</tr>
<tr>
<td>Year</td>
<td>2000</td>
</tr>
<tr>
<td>GLP</td>
<td>no</td>
</tr>
<tr>
<td>Test substance</td>
<td>Disodium succinate.</td>
</tr>
</tbody>
</table>
| Remark                 | The \( P_{ka} \) of succinic acid is 4.207 and 5.636 (25 degree C).
|                        | The \( \text{pH} \) of succinic acid is 4.00 and 5.24 (25 degree C).
|                        | Ref: Kagakubinran, Maruzen Co., Ltd. |
| Source                 | Chemicals Evaluation and Research Institute (CERI), Japan. |
| Test substance         | Aldrich Chemical Co., Inc. |
| Purity                 | 100.1 % (titration by HClO4). |
| Reliability            | (2) valid with restrictions |
| Flag                   | Critical study for SIDS endpoint |

<table>
<thead>
<tr>
<th>Value</th>
<th>ca. 200 g/l at 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative</td>
<td></td>
</tr>
<tr>
<td>( P_{ka} )</td>
<td>at 25 °C</td>
</tr>
<tr>
<td>( \text{pH} )</td>
<td>at and °C</td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1996</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
<tr>
<td>Reliability</td>
<td>(3) invalid</td>
</tr>
</tbody>
</table>

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. ENVIRONMENTAL FATE AND PATHWAYS

3.1.1 PHOTODEGRADATION

<table>
<thead>
<tr>
<th>Type</th>
<th>air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light source</td>
<td></td>
</tr>
<tr>
<td>Light spect.</td>
<td>nm</td>
</tr>
<tr>
<td>Rel. intensity</td>
<td>based on Intensity of Sunlight</td>
</tr>
<tr>
<td>Indirect photolysis</td>
<td></td>
</tr>
<tr>
<td>Sensitizer</td>
<td>OH</td>
</tr>
<tr>
<td>Conc. of sens.</td>
<td>1500000 molecule/cm3</td>
</tr>
<tr>
<td>Rate constant</td>
<td>0.0000000000007123 cm3/(molecule*sec)</td>
</tr>
<tr>
<td>Degradation</td>
<td>= 50 % after 15 day</td>
</tr>
<tr>
<td>Deg. Product</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>other (calculated)</td>
</tr>
<tr>
<td>Year</td>
<td>2003</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td>AOPWIN v. 1.90</td>
</tr>
<tr>
<td>Remark</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td>Reliability</td>
<td></td>
</tr>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
<tr>
<td>28.07.2003</td>
<td></td>
</tr>
</tbody>
</table>

3.1.2 STABILITY IN WATER

<table>
<thead>
<tr>
<th>Type</th>
<th>abiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1/2 pH4</td>
<td>&lt; 10 at 50 degree C</td>
</tr>
<tr>
<td>t1/2 pH7</td>
<td>&lt; 10 at 50 degree C</td>
</tr>
<tr>
<td>t1/2 pH9</td>
<td>&lt; 10 at 50 degree C</td>
</tr>
<tr>
<td>Degradation</td>
<td>&lt; 10 % after 5 day at pH and degree C</td>
</tr>
<tr>
<td>Deg. Product</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>OECD Guide-line 111 &quot;Hydrolysis as a Function of pH&quot;</td>
</tr>
<tr>
<td>Year</td>
<td></td>
</tr>
<tr>
<td>GLP</td>
<td>no</td>
</tr>
<tr>
<td>Test substance</td>
<td>Chemicals Evaluation and Research Institute (CERI), Japan.</td>
</tr>
<tr>
<td>Source</td>
<td>Disodium succinate.</td>
</tr>
<tr>
<td>Test substance</td>
<td>Aldrich Chemical Co., Inc.</td>
</tr>
<tr>
<td></td>
<td>Purity: 100.1 % (titration by HClO4).</td>
</tr>
<tr>
<td>Conclusion</td>
<td>This chemical is stable at pH 4, 7 and 9 at 50 degree C for 5 days.</td>
</tr>
<tr>
<td>Reliability</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
<tr>
<td>29.07.2003</td>
<td></td>
</tr>
</tbody>
</table>

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

28.07.2003
3.3.2 DISTRIBUTION

<table>
<thead>
<tr>
<th>Media</th>
<th>air - biota - sediment(s) - soil - water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Calculation according Mackay, Level III</td>
</tr>
<tr>
<td>Year</td>
<td>2003</td>
</tr>
<tr>
<td>Remark</td>
<td>Parameters used are:</td>
</tr>
<tr>
<td></td>
<td>Molecular weight: 162.05</td>
</tr>
<tr>
<td></td>
<td>Melting point: 400 degree C</td>
</tr>
<tr>
<td></td>
<td>Water solubility: 100 g/m³</td>
</tr>
<tr>
<td></td>
<td>log Kow: -0.59</td>
</tr>
<tr>
<td>Result</td>
<td>Release 100% to:</td>
</tr>
<tr>
<td></td>
<td>Air: 50.4% in water, 49.3% in soil, 0.2% in sediment;</td>
</tr>
<tr>
<td></td>
<td>Water: 99.6% in water, 0.4% in sediment;</td>
</tr>
<tr>
<td></td>
<td>Soil: 44.9% in water, 55.9% in soil, 0.2% in sediment;</td>
</tr>
<tr>
<td></td>
<td>Sediment: 57.6% in water, 42.2% in soil, 0.2% in soil.</td>
</tr>
<tr>
<td>Reliability</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
<tr>
<td>28.07.2003</td>
<td></td>
</tr>
</tbody>
</table>

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

<table>
<thead>
<tr>
<th>Type</th>
<th>aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>activated sludge, non-adapted</td>
</tr>
<tr>
<td>Concentration</td>
<td>100mg/l related to COD (Chemical Oxygen Demand) related to</td>
</tr>
<tr>
<td>Contact time</td>
<td>14 day</td>
</tr>
<tr>
<td>Degradation</td>
<td>= 64 - 78 % after 14 day</td>
</tr>
<tr>
<td>Result</td>
<td>readily biodegradable</td>
</tr>
<tr>
<td>Control substance</td>
<td>Aniline</td>
</tr>
<tr>
<td>Kinetic</td>
<td>%</td>
</tr>
<tr>
<td>Deg. Product</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>OECD Guide-line 301 C &quot;Ready Biodegradability: Modified MITI Test (I)&quot;</td>
</tr>
<tr>
<td>Year</td>
<td>1994</td>
</tr>
<tr>
<td>GLP</td>
<td>yes</td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
<tr>
<td>Remark</td>
<td>The concentration of aniline was 100 mg/L. The concentration of activated sludge was 30 mg/L.</td>
</tr>
<tr>
<td>Result</td>
<td>The biodegradation rates of this chemical were as follows:</td>
</tr>
<tr>
<td></td>
<td>74, 78 and 64 % by BOD after 14 days</td>
</tr>
<tr>
<td></td>
<td>100, 100 and 100 % by HPLC analysis after 14 days.</td>
</tr>
<tr>
<td></td>
<td>100, 100 and 100 % by TOC analysis after 14 days.</td>
</tr>
<tr>
<td>Source</td>
<td>Chemicals Evaluation and Research Institute (CERI), Japan.</td>
</tr>
<tr>
<td>Test substance</td>
<td>Aldrich Chemical Co., Inc.</td>
</tr>
<tr>
<td></td>
<td>Purity: 98.9 % (titration by HClO4).</td>
</tr>
<tr>
<td></td>
<td>Impurity: water 0.2 %</td>
</tr>
<tr>
<td>Conclusion</td>
<td>Readily biodegradable.</td>
</tr>
<tr>
<td>Reliability</td>
<td>(1) valid without restriction</td>
</tr>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
<tr>
<td>28.07.2003</td>
<td></td>
</tr>
</tbody>
</table>

3.6 BOD5, COD OR BOD5/COD RATIO
### 3.7 BIOACCUMULATION

<table>
<thead>
<tr>
<th>BCF</th>
<th>: 3.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elimination</td>
<td>:</td>
</tr>
<tr>
<td>Method</td>
<td>: other</td>
</tr>
<tr>
<td>Year</td>
<td>: 2003</td>
</tr>
<tr>
<td>GLP</td>
<td>:</td>
</tr>
<tr>
<td>Test substance</td>
<td>:</td>
</tr>
<tr>
<td>Remark</td>
<td>: An estimated BCF value of 3.16 was calculated by BCFWIN v. 2.14. A log Kow value of -0.59 was used.</td>
</tr>
<tr>
<td>Reliability</td>
<td>: (2) valid with restrictions</td>
</tr>
<tr>
<td>Date</td>
<td>: 28.07.2003</td>
</tr>
</tbody>
</table>

### 3.8 ADDITIONAL REMARKS
4.1 ACUTE/PROLONGED TOXICITY TO FISH

<table>
<thead>
<tr>
<th>Type</th>
<th>flow through</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Oryzias latipes (Fish, fresh water)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>96 hour(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>Yes</td>
</tr>
<tr>
<td>LC50</td>
<td>&gt; 95.4 mg/L</td>
</tr>
<tr>
<td>LC100</td>
<td>&gt; 95.4 mg/L</td>
</tr>
<tr>
<td>LC0</td>
<td>= 47.0 mg/L</td>
</tr>
<tr>
<td>Method</td>
<td>OECD Guide-line 203 &quot;Fish, Acute Toxicity Test&quot;</td>
</tr>
<tr>
<td>Year</td>
<td>2001</td>
</tr>
<tr>
<td>GLP</td>
<td>Yes</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: Disodium succinate hexahydrate (CAS No.: 6106-21-4, Nacalai Tesque, Inc. (Japan), Lot. No.: MOT9476, Purity = 100.2%</td>
</tr>
</tbody>
</table>

Method:

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.
OECD SIDS DISODIUM SUCCINATE

4. ECOTOXICITY ID 150-90-3
DATE: 27.01.2003

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%.

<table>
<thead>
<tr>
<th>Nominal Conc., mg/L</th>
<th>Measured Conc., mg/L</th>
<th>Percent of Nominal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Hour</td>
<td>96 Hours</td>
</tr>
<tr>
<td>Control</td>
<td>&lt;0.600</td>
<td>&lt;0.600</td>
</tr>
<tr>
<td>6.25</td>
<td>6.14</td>
<td>5.13</td>
</tr>
<tr>
<td>12.5</td>
<td>12.2</td>
<td>10.9</td>
</tr>
<tr>
<td>25.0</td>
<td>24.3</td>
<td>22.8</td>
</tr>
<tr>
<td>50.0</td>
<td>46.6</td>
<td>47.3</td>
</tr>
<tr>
<td>100</td>
<td>94.8</td>
<td>96.1</td>
</tr>
</tbody>
</table>

*: 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 100 mg/L at 96th hr.

<table>
<thead>
<tr>
<th>Measured Conc. (mg/L)</th>
<th>Cumulative Number of Dead (Percent Mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24hr</td>
</tr>
<tr>
<td>Control</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5.64</td>
<td>0 (0)</td>
</tr>
<tr>
<td>11.6</td>
<td>0 (0)</td>
</tr>
<tr>
<td>23.6</td>
<td>0 (0)</td>
</tr>
<tr>
<td>47.0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>95.4</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Measured Conc. (mg/L)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24hr</td>
</tr>
<tr>
<td>Control</td>
<td>n</td>
</tr>
<tr>
<td>5.64</td>
<td>n</td>
</tr>
<tr>
<td>11.6</td>
<td>n</td>
</tr>
<tr>
<td>23.6</td>
<td>n</td>
</tr>
<tr>
<td>47.0</td>
<td>n</td>
</tr>
<tr>
<td>95.4</td>
<td>n</td>
</tr>
</tbody>
</table>

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)
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: 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: 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+/-1°C
  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 butanediolic 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%.

<table>
<thead>
<tr>
<th>Nominal Conc. mg/L</th>
<th>Measured Conc., mg/L</th>
<th>Mean* mg/L</th>
<th>Percent of Nominal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.</td>
<td>0 Hour</td>
<td>48 Hour</td>
<td>Fresh</td>
</tr>
<tr>
<td>Fresh</td>
<td>Old</td>
<td>Fresh</td>
<td>Old</td>
</tr>
<tr>
<td>Control</td>
<td>&lt;15.0</td>
<td>&lt;15.0</td>
<td>---</td>
</tr>
<tr>
<td>592</td>
<td>591</td>
<td>567</td>
<td>579</td>
</tr>
<tr>
<td>769</td>
<td>766</td>
<td>748</td>
<td>757</td>
</tr>
<tr>
<td>1,000</td>
<td>999</td>
<td>995</td>
<td>997</td>
</tr>
</tbody>
</table>

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 - 20.6°C

- 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.

<table>
<thead>
<tr>
<th>Measured Conc. mg/L</th>
<th>Cumulative Number of Dead or Immobilized Daphnids (Percent Mortality or Immobility)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.</td>
<td>24 Hour</td>
</tr>
<tr>
<td>Control</td>
<td>0 ( 0  )</td>
</tr>
<tr>
<td>579</td>
<td>0 ( 0  )</td>
</tr>
<tr>
<td>757</td>
<td>0 ( 0  )</td>
</tr>
<tr>
<td>997</td>
<td>0 ( 0  )</td>
</tr>
</tbody>
</table>

- Calculation of toxic values: Mean measured concentration

**Source**: Ministry of Environment, Japan (2001)

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

**Flag**: Critical study for SIDS endpoint

**DATE**: 27.01.2003
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+/-2°C
     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%.

<table>
<thead>
<tr>
<th>Nominal conc. mg/L</th>
<th>Measured Conc., mg/L 0 Hour</th>
<th>72 Hour</th>
<th>Mean* mg/L</th>
<th>Percent of nominal conc. 0 Hour</th>
<th>72 Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control &lt;15.0</td>
<td>&lt;15.0</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>250</td>
<td>249</td>
<td>246</td>
<td>247</td>
<td>99.7</td>
<td>98.3</td>
</tr>
<tr>
<td>500</td>
<td>504</td>
<td>498</td>
<td>501</td>
<td>101</td>
<td>99.6</td>
</tr>
<tr>
<td>1,000</td>
<td>1,000</td>
<td>997</td>
<td>998</td>
<td>100</td>
<td>99.7</td>
</tr>
</tbody>
</table>

*: 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

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

<table>
<thead>
<tr>
<th>Mean Measured Conc. mg/L</th>
<th>Growth rates and percent inhibition (Average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. Rate Im(24-48hr) Inhibition(%) Rate Im(0-72hr) Inhibition(%)</td>
<td></td>
</tr>
<tr>
<td>mg/L</td>
<td>u(24-48hr) Im(24-48hr) u(0-72hr) Im(0-72hr)</td>
</tr>
</tbody>
</table>
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

Species : Navicula sp. (Algae)
Endpoint : Growth rate
Exposure period : 7 day
Unit : mg/l
Analytical monitoring : Other
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

Species : Other algae: Chaetoceros gracilis
Endpoint : Growth rate
Exposure period : 7 day
Unit : mg/l
Analytical monitoring :
Method : Other
Year : 1993
- 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: 15°C
  h) Light Condition: 5,000 lux, continuously

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

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

Species:
Other algae: Pavlova lutheri

Endpoint:
Growth rate

Exposure period:
7 day

Unit:
mg/l

Analytical monitoring:
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: 15°C
  h) Light Condition: 5,000 lux, continuously

Result:
At the highest concentration, i.e., 300 mg/L, growth rate inhibition was...
<table>
<thead>
<tr>
<th>Source</th>
<th>Ministry of Environment, Japan (2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reliability</td>
<td>(4) not assignable</td>
</tr>
<tr>
<td>Remark: Details on the test condition and the results are not available.</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>21.01.2003</td>
</tr>
</tbody>
</table>

**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: 15°C  
  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.  

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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+/-10C
  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.
OECD SIDS DISODIUM SUCCINATE

4. ECOTOXICITY

DATE: 27.01.2003

Identification: ID 150-90-3

- 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 21 days 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%.

<table>
<thead>
<tr>
<th>Nominal Conc. (mg/L)</th>
<th>Measured Conc., mg/L</th>
<th>Date</th>
<th>0 Fresh</th>
<th>1 Fresh</th>
<th>8 Old</th>
<th>9 Old</th>
<th>16 Old</th>
<th>17 Old</th>
<th>TWM* mg/L</th>
<th>% of Nominal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control &lt;3.00</td>
<td>&lt;3.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>25.1</td>
<td>17.0</td>
<td>25.3</td>
<td>11.3</td>
<td>25.4</td>
<td>14.0</td>
<td></td>
<td>19.1</td>
<td>76.5</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>49.7</td>
<td>41.3</td>
<td>49.9</td>
<td>37.7</td>
<td>51.2</td>
<td>41.4</td>
<td></td>
<td>82.8</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>99.3</td>
<td>90.9</td>
<td>100</td>
<td>91.2</td>
<td>102</td>
<td>88.6</td>
<td></td>
<td>95.2</td>
<td>95.2</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Nominal Conc. (mg/L)</th>
<th>Measured Concentration as a Percentage of Nominal</th>
<th>Date</th>
<th>0 Fresh</th>
<th>1 Fresh</th>
<th>8 Old</th>
<th>9 Old</th>
<th>16 Old</th>
<th>17 Old</th>
<th>% of Nominal</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.0</td>
<td>101</td>
<td>68.2</td>
<td>101</td>
<td>45.3</td>
<td>25.4</td>
<td>76.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>99.5</td>
<td>82.6</td>
<td>99.7</td>
<td>75.5</td>
<td>102</td>
<td>82.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>99.3</td>
<td>90.9</td>
<td>100</td>
<td>91.2</td>
<td>102</td>
<td>88.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 - 20.6°C  
- 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.

<table>
<thead>
<tr>
<th>Measured Conc. (mg/L)</th>
<th>Cumulative Number of Dead Parental Daphnids (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>19.1</td>
<td>0 0 0 0 0 0 0 0 0 1</td>
</tr>
<tr>
<td>45.0</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>95.2</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measured Conc. (mg/L)</th>
<th>Cumulative Number of Dead Parental Daphnids (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 0 0 0 0 0 0 0 0 0 1</td>
</tr>
<tr>
<td>19.1</td>
<td>1 1 1 1 1 1 2 2 2 2 2</td>
</tr>
<tr>
<td>45.0</td>
<td>1 1 1 1 1 2 2 2 2 2 2</td>
</tr>
<tr>
<td>95.2</td>
<td>0 0 0 0 0 1 1 1 2 2 2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Measured Conc. (mg/L)</th>
<th>Mean Cumulative Numbers of Juveniles Produced per Adult (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 --- 13.6 13.6 13.6 42.2 42.2 42.2 56.1</td>
</tr>
<tr>
<td>19.1</td>
<td>0 --- 22.6 22.6 23.1 49.6 51.4 51.4 64.5</td>
</tr>
<tr>
<td>45.0</td>
<td>0 --- 23.5 23.5 45.1 59.4 59.4 59.4 80.1</td>
</tr>
<tr>
<td>95.2</td>
<td>0 --- 23.5 23.5 29.0 56.9 56.9 61.4 75.0</td>
</tr>
</tbody>
</table>
### Measured Mean Cumulative Numbers of
### Conc. Juveniles Produced per Adult (days)
### mg/L 15 16 17 18 19 20 21

<table>
<thead>
<tr>
<th>Conc. mg/L</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.6</td>
<td>68.6</td>
<td>91.9</td>
<td>93.8</td>
<td>93.8</td>
<td>93.8</td>
<td>112</td>
</tr>
<tr>
<td>19.1</td>
<td>73.5</td>
<td>73.5</td>
<td>95.8</td>
<td>98.6</td>
<td>98.6</td>
<td>108</td>
<td>121</td>
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<tr>
<td>45.0</td>
<td>81.3</td>
<td>81.3</td>
<td>102</td>
<td>103</td>
<td>104</td>
<td>118</td>
<td>121</td>
</tr>
<tr>
<td>95.2</td>
<td>77.6</td>
<td>82.0</td>
<td>106</td>
<td>108</td>
<td>108</td>
<td>129</td>
<td>141</td>
</tr>
</tbody>
</table>

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

### Measured Conc.1), mg/L

<table>
<thead>
<tr>
<th>Vessel No.</th>
<th>Control</th>
<th>19.1</th>
<th>45.0</th>
<th>95.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>146</td>
<td>85</td>
<td>144</td>
<td>114</td>
</tr>
<tr>
<td>2</td>
<td>98</td>
<td>109</td>
<td>148</td>
<td>139</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>---</td>
<td>---</td>
<td>184</td>
</tr>
<tr>
<td>4</td>
<td>128</td>
<td>135</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>119</td>
<td>145</td>
<td>145</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>74</td>
<td>150</td>
<td>96</td>
<td>154</td>
</tr>
<tr>
<td>7</td>
<td>134</td>
<td>99</td>
<td>97</td>
<td>118</td>
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<tr>
<td>8</td>
<td>---</td>
<td>161</td>
<td>76</td>
<td>147</td>
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<td>9</td>
<td>139</td>
<td>84</td>
<td>142</td>
<td>152</td>
</tr>
<tr>
<td>10</td>
<td>82</td>
<td>---</td>
<td>119</td>
<td>119</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Type</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>rat</td>
</tr>
<tr>
<td>Strain</td>
<td>Crj: CD(SD)</td>
</tr>
<tr>
<td>Sex</td>
<td>male/female</td>
</tr>
<tr>
<td>Number of animals</td>
<td>5</td>
</tr>
<tr>
<td>Vehicle</td>
<td>other: distilled water</td>
</tr>
<tr>
<td>Value</td>
<td>&gt; 1200 mg/kg bw</td>
</tr>
<tr>
<td>Method</td>
<td>OECD Guide-line 401 &quot;Acute Oral Toxicity&quot;</td>
</tr>
<tr>
<td>Year</td>
<td>2002</td>
</tr>
<tr>
<td>GLP</td>
<td>yes</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: See Remark</td>
</tr>
</tbody>
</table>
| 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          |

### 5.1.2 ACUTE INHALATION TOXICITY

<table>
<thead>
<tr>
<th>Type</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>mouse</td>
</tr>
<tr>
<td>Strain</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Route of admin.</td>
<td>i.v.</td>
</tr>
<tr>
<td>Exposure time</td>
<td></td>
</tr>
<tr>
<td>Value</td>
<td>= 4500 mg/kg bw</td>
</tr>
<tr>
<td>Remark</td>
<td>Test substance: Sodium succinate</td>
</tr>
<tr>
<td>Source</td>
<td>Merk Index: 1989</td>
</tr>
<tr>
<td>Reliability</td>
<td>(4) not assignable</td>
</tr>
</tbody>
</table>

### 5.1.3 ACUTE DERMAL TOXICITY

### 5.1.4 ACUTE TOXICITY, OTHER ROUTES
5. TOXICITY

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species : rat
Sex : male/female
Strain : Crl: 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 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

### 5.5 GENETIC TOXICITY ‘IN VITRO’

<table>
<thead>
<tr>
<th>Type</th>
<th>Bacterial reverse mutation assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>System of testing</td>
<td>Salmonella typhimurium TA100, TA1535, TA98, TA1537, Escherichia coli WP2uvrA</td>
</tr>
<tr>
<td>Concentration</td>
<td>0, 156, 313, 625, 1250, 2500, 5000 µg/plate</td>
</tr>
<tr>
<td>Cycotoxic conc.</td>
<td>See Result</td>
</tr>
<tr>
<td>Metabolic activation</td>
<td>with and without</td>
</tr>
<tr>
<td>Result</td>
<td>negative</td>
</tr>
<tr>
<td>Method</td>
<td>other: Guidelines for Screening Mutagenicity Testing of Chemicals (Chemical Substances Control Law of Japan) and OECD Test Guideline 471 “Bacterial Reverse Mutation Test”</td>
</tr>
<tr>
<td>Year</td>
<td>2002</td>
</tr>
<tr>
<td>GLP</td>
<td>yes</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: Source: disodium succinate hexahydrate (CAS No.6106-21-4), Nippon Shokubai Co., Ltd., Purity 99.9%, Lot No. 9P01B</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>No data</td>
</tr>
<tr>
<td>Result</td>
<td>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.</td>
</tr>
</tbody>
</table>
| 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:
05.12.2002
Critical study for SIDS endpoint

Type:
Ames test
System of testing:
Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98
Concentration:
Max dose was 5000 µg/plate (six different concentrations)
Cytotoxic 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

Type:
Ames test
System of testing:
Salmonella typhimurium TA97, TA102
Concentration:
0, 100, 500, 1000, 5000, 10000 µg/plate
Cytotoxic 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).

| 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 |

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).

| 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).
5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENITY

5.8 TOXICITY TO REPRODUCTION

<table>
<thead>
<tr>
<th>Type</th>
<th>One generation study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>rat</td>
</tr>
<tr>
<td>Sex</td>
<td>male/female</td>
</tr>
<tr>
<td>Strain</td>
<td>Crj; CD(SD)</td>
</tr>
<tr>
<td>Route of admin.</td>
<td>gavage</td>
</tr>
<tr>
<td>Exposure period</td>
<td>Males; for 52 days</td>
</tr>
<tr>
<td></td>
<td>Females; from 14 days before mating to day 4 of lactation</td>
</tr>
<tr>
<td>Frequency of treatment</td>
<td>Once daily</td>
</tr>
<tr>
<td>Premating exposure period</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 days</td>
</tr>
<tr>
<td>Female</td>
<td>14 days</td>
</tr>
<tr>
<td>Duration of test</td>
<td>Males; for 53 days</td>
</tr>
<tr>
<td></td>
<td>Females; from 43 to 47 days</td>
</tr>
<tr>
<td>Doses</td>
<td>0(vehicle), 100, 300, 1000 mg/kg bw/day</td>
</tr>
<tr>
<td>Control group</td>
<td>yes, concurrent vehicle</td>
</tr>
<tr>
<td>NOAEL Parental</td>
<td>&gt;= 600 mg/kg bw</td>
</tr>
<tr>
<td>NOAEL F1 Offspring</td>
<td>&gt;= 600 mg/kg bw</td>
</tr>
<tr>
<td>Method</td>
<td>OECD combined repeated dose and reproductive/developmental toxicity screening test</td>
</tr>
<tr>
<td>Year</td>
<td>2002</td>
</tr>
<tr>
<td>GLP</td>
<td>yes</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: see Remark</td>
</tr>
<tr>
<td>Remark</td>
<td>1) Test substance: disodium succinate hexahydrate (CAS No. 6106-21-4), Nippon Shokubai Co., Ltd., Purity 99.9%, Lot No. 9P01B</td>
</tr>
<tr>
<td></td>
<td>2) Route: oral(gavage)</td>
</tr>
<tr>
<td></td>
<td>3) No. of animals/group: males 12, females</td>
</tr>
<tr>
<td>Result</td>
<td>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.</td>
</tr>
</tbody>
</table>

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

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

**Remark:** See the section 5.8 "Toxicity to Reproduction"

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 disodium 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

5.11 EXPERIENCE WITH HUMAN EXPOSURE
6. REFERENCES


(6) Merck Index (1996); approx.


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


