要　旨

試験委託者

環境庁

表　題

2,6-ジ-*tert*-ブチル-*p*-クレゾールの藻類（*Selenastrum capricornutum*）に対する生長阻害試験

試験番号

９Ｂ４４７Ｇ

試験方法

本試験は，OECD 化学品テストガイドライン No. 201「藻類生長阻害試験」（1984年）に準拠して実施した。

1) 被験物質:　2,6-ジ-*tert*-ブチル-*p*-クレゾール

2) 暴露方式：　止水式（密閉），振とう培養（100rpm）

3) 供試生物:　*Selenastrum capricornutum* (ATCC22662)

4) 暴露期間：　72時間

5) 試験濃度（設定値）：

対照区，助剤対照区，1.00，2.15，4.64，10.0 mg/L

（分散可能最高濃度）

（公比：2.2，助剤濃度一定：100 mg/L，ジメチルホルムアミド および HCO-40使用）

6) 試験液量：　100 mL（ＯＥＣＤ培地）／容器

7) 連数：　３容器／濃度区

8) 初期細胞濃度：1×10^4 cells/mL

9) 試験温度：　23±2 ℃

10) 照明：　4000 lux （±20％の変動内，フラスコ液面付近）で連続照明

11) 分析法:　ＨＰＬＣ法

結　果

1) 試験液中の被験物質濃度

被験物質の測定濃度が開始時において設定値の±20％を超えたものがなかったため，下記の生長阻害濃度の算出には設定値を採用した。

2) 生長曲線下面積の比較による阻害濃度

50％生長阻害濃度 EbC50(0-72)：　>10.0　mg/L（95％信頼区間：算出不可）

最大無作用濃度 NOECb(0-72)：　1.00　mg/L

3) 生長速度の比較による阻害濃度

50％生長阻害濃度 ErC50(24-48)：　>10.0　mg/L（95％信頼区間：算出不可）

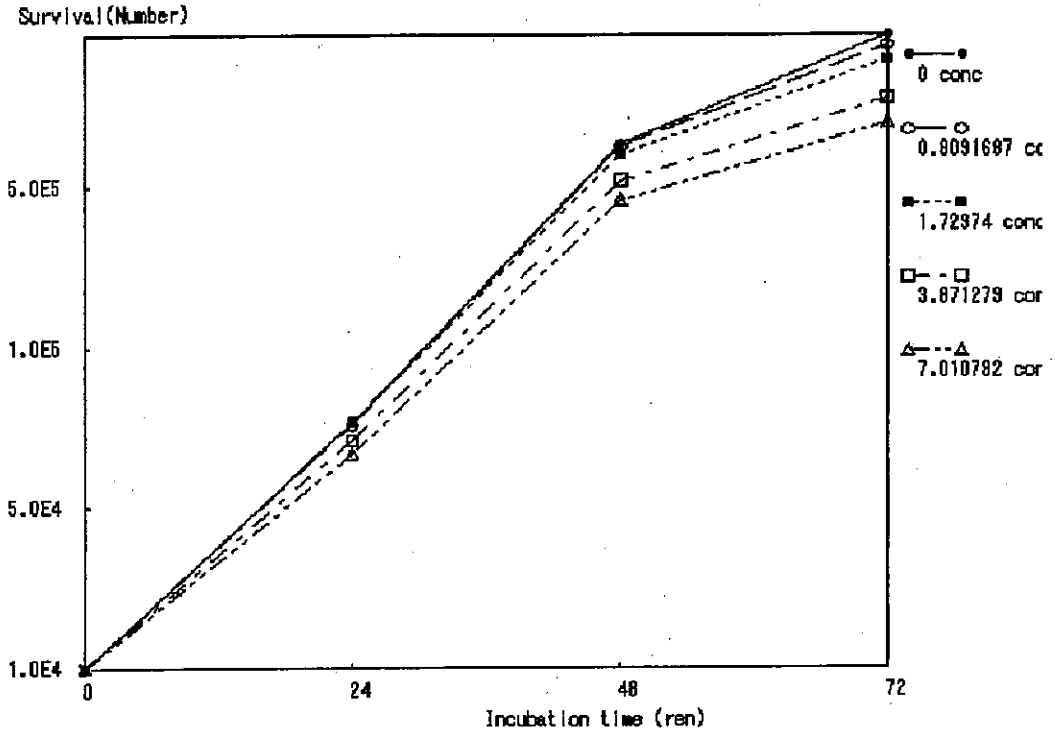
最大無作用濃度 NOECr(24-48)：　>10.0　mg/L

50％生長阻害濃度 ErC50(24-72)：　>10.0　mg/L（95％信頼区間：算出不可）

最大無作用濃度 NOECr(24-72)：　1.00　mg/L

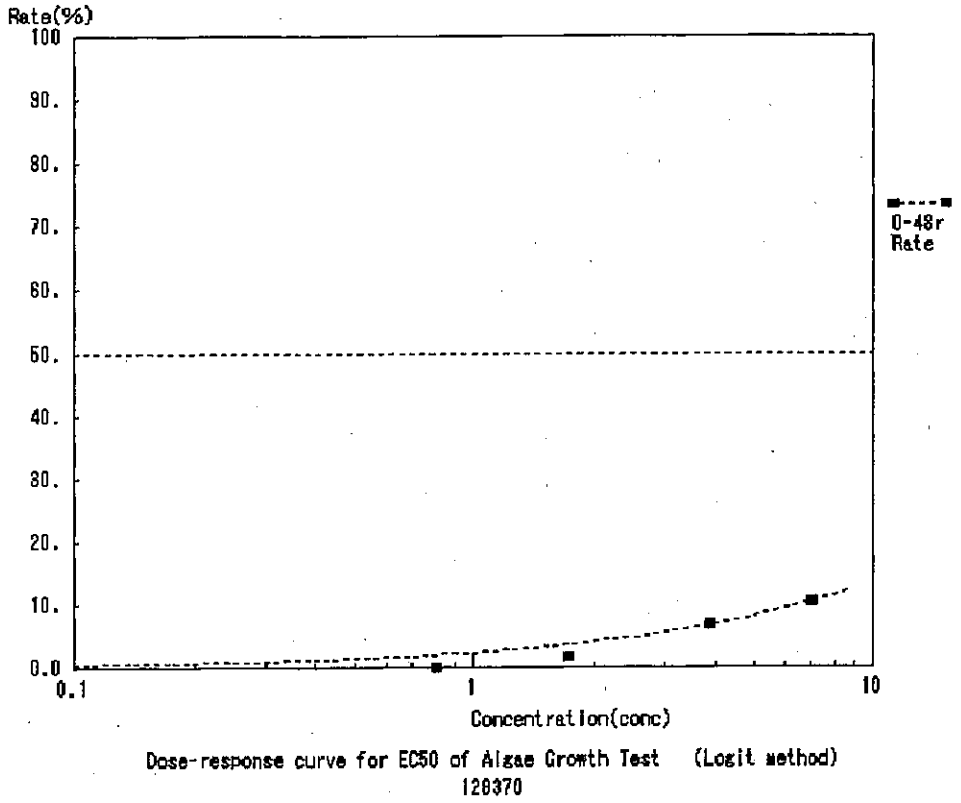
2,6-ジ-tert-ブチル-p-クレゾール(CAS.128-37-0)

①生長曲線



Time course pattern of Algae Growth Test
128370

②阻害率曲線



Dose-response curve for EC50 of Algae Growth Test (Logit method)
120370

③毒性値

48hErC50(実測値に基づく)＞7.0mg/L
48hNOECr(実測値に基づく)＝1.7mg/L

要　旨

試験委託者

環境庁

表　題

2,6-ジ-*tert*-ブチル-*p*-クレゾールのオオミジンコ（*Daphnia magna*）に対する急性遊泳阻害試験

試験番号

９Ｂ４６９Ｇ

試験方法

本試験は，OECD 化学品テストガイドライン No.202「ミジンコ類，急性遊泳阻害試験および繁殖試験」(1984年)に準拠して実施した。

1) 被験物質：　2,6-ジ-*tert*-ブチル-*p*-クレゾール
2) 暴露方式：　止水式，水面をテフロンシートで被覆
3) 供試生物：　オオミジンコ（*Daphnia magna*）
4) 暴露期間：　48時間
5) 試験濃度（設定値）：
　対照区，助剤対照区，0.200，0.360，0.630，1.10，2.00 mg/L
　公比：1.8
　助剤濃度一定：40.0 mg/L（HCO-40 およびジメチルホルムアミド使用）
6) 試験液量：　100 mL／容器
7) 連数：　４容器／濃度区
8) 供試生物数：　20頭／濃度区（5頭／容器）
9) 試験温度：　20±1℃
10) 照明：　16時間明／8時間暗
11) 分析法：　ＨＰＬＣ法

結　果

1) 試験液中の被験物質濃度

被験物質の測定濃度がすべて設定値の±20%以内であったため，各影響濃度の算出には設定値を採用した。

2) 24 時間暴露後の結果

半数遊泳阻害濃度（EiC50）：　>2.00　mg/L　（95%信頼限界：　算出不可 ）

最大無作用濃度（NOECi）：　0.630 mg/L

100%阻害最低濃度：　>2.00　mg/L

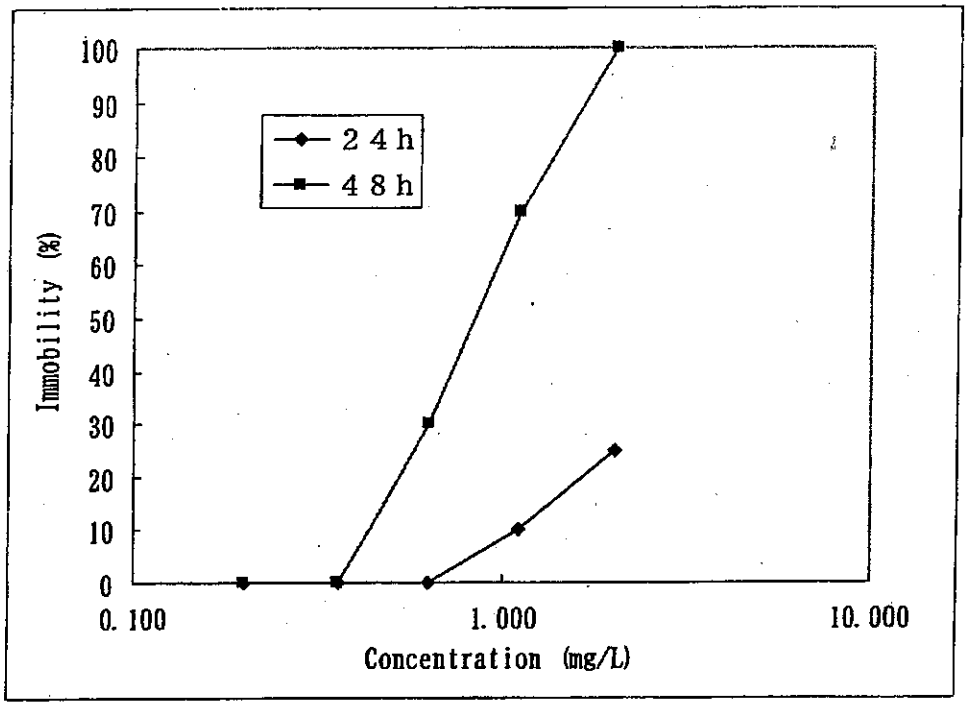
3) 48 時間暴露後の結果

半数遊泳阻害濃度（EiC50）：　0.835 mg/L　（95%信頼限界：0.709～0.985 mg/L）

最大無作用濃度（NOECi）：　0.360 mg/L

100%阻害最低濃度：　2.00　mg/L

Figure 1 Concentration-Response (Immobility) Curve



要　旨

試験委託者

環境庁

表　題

2,6-ジ-*tert*-ブチル-*p*-クレゾールのオオミジンコ（*Daphnia magna*）に対する繁殖阻害試験

試験番号

９Ｂ４９１Ｇ

試験方法

本試験は，OECD 化学品テストガイドラインNo. 211「オオミジンコ繁殖試験」（1998年）に準拠して実施した。

1) 被験物質：　2,6-ジ-*tert*-ブチル-*p*-クレゾール
2) 暴露方式：　半止水式（24時間毎に試験液の全量を交換）
　　水面をテフロンシートで被覆
3) 供試生物：　オオミジンコ（*Daphnia magna*）
4) 暴露期間：　21日間
5) 試験濃度(設定値)：
　　対照区，助剤対照区，0.008，0.025，0.080，0.250，0.800 mg/L
　　公比：3.2
　　助剤濃度一定：100 mg/L（HCO-60 および ｼﾞﾒﾁﾙﾎﾙﾑｱﾐﾄﾞ使用）
6) 試験液量：　80 mL／容器
7) 連数：　10容器／濃度区
8) 供試生物数：10頭／濃度区（1頭／容器）
9) 試験温度：　20±1℃
10) 照明：　16時間明／8時間暗
11) 分析法：　ＨＰＬＣ法

結　果

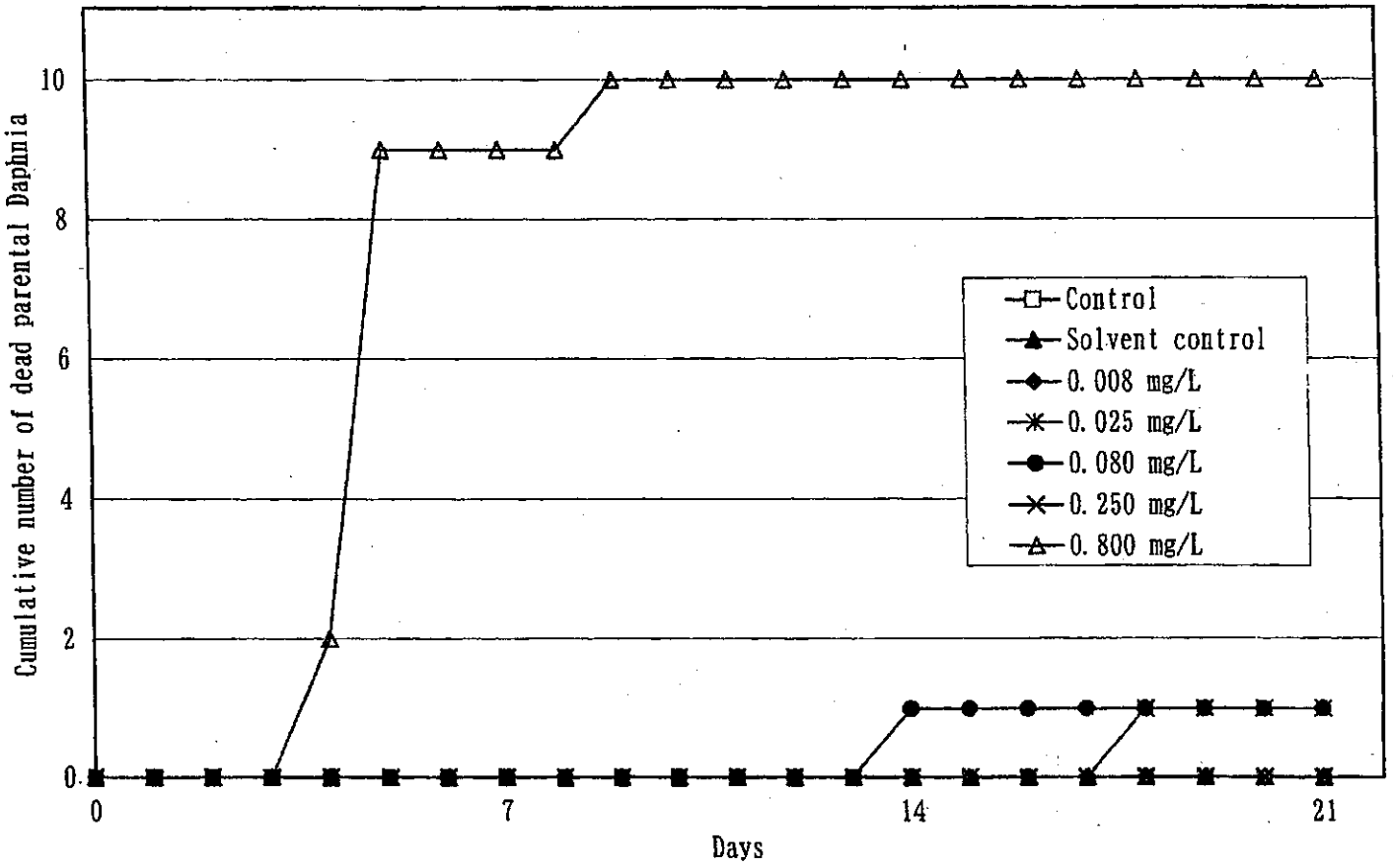
1) 試験液中の被験物質濃度

被験物質の測定濃度が設定値の±20％を超えたものがあったため，各影響濃度の算出には測定値（時間加重平均値）を採用した。

2) 21 日間暴露の各影響濃度結果を以下に示す。

親ミジンコの半数致死濃度（LC50）：	0.390 mg/L
	(95％信頼限界 ： 0.218 ～0.698 mg/L)
50％ 繁殖阻害濃度（EC50）：	0.096 mg/L
	(95％信頼限界 ： 0.086～0.116 mg/L)
最大無作用濃度(NOEC) ：	0.069 mg/L
最小作用濃度(LOEC) ：	0.218 mg/L

Figure 1 Cumulative Numbers of Dead Parental *Daphnia*



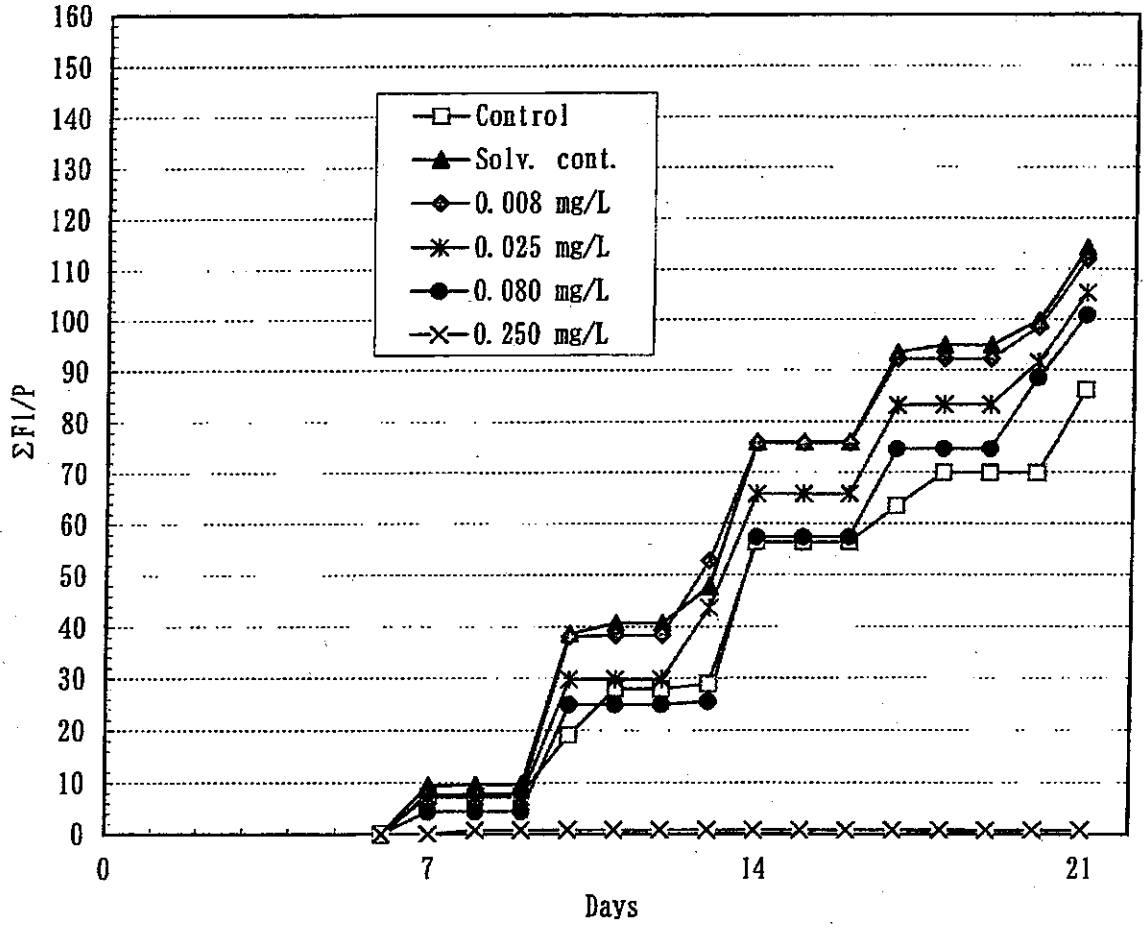
Values in legend are given in the nominal concentration.

Table 4 Mean Cumulative Numbers of Juveniles Produced per Adult Alive for 21 Days (ΣF1/P)

Nominal Conc.	Days															
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	0.0	7.3	7.9	7.9	19.1	28.0	28.0	28.9	56.4	56.4	56.4	63.5	69.9	69.9	69.9	86.2
Solv. cont.	0.0	9.5	9.7	9.7	38.6	40.7	40.7	47.9	76.0	76.0	76.0	93.5	94.9	94.9	99.8	114.3
0.008 mg/L	0.0	7.8	7.8	7.8	38.1	38.4	38.4	52.9	75.8	75.8	75.8	92.2	92.2	92.2	98.5	112.0
0.025 mg/L	0.0	7.2	7.2	7.2	29.8	29.8	29.8	43.7	65.8	65.8	65.8	83.2	83.3	83.3	91.6	105.3
0.080 mg/L	0.0	4.4	4.4	4.4	25.0	25.0	25.0	25.6	57.4	57.4	57.4	74.7	74.7	74.7	88.6	100.9
0.250 mg/L	0.0	0.0	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
0.800 mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-: All parental *Daphnia* were dead during a 21-days testing period.

Figure 2 Time Course of ΣF1/P for Each Concentration Level



Values in legend are given in the nominal concentration.

要　旨

試験委託者

環境庁

表　題

2,6-ジ-*tert*-ブチル-*p*-クレゾールのヒメダカ（*Oryzias latipes*）に対する急性毒性試験

試験番号

９Ｂ５１３Ｇ

試験方法

本試験は，OECD 化学品テストガイドライン No.203「魚類毒性試験」（1992年）に準拠して実施した。

1) 被験物質：　2,6-ジ-*tert*-ブチル-*p*-クレゾール

2) 暴露方式：　半止水式（24時間毎に試験液の全量を交換），水面をテフロンシートで被覆

3) 供試生物：　ヒメダカ（*Oryzias latipes*）

4) 暴露期間：　96時間

5) 試験濃度（設定値）：対照区，助剤対照区，0.500，0.900，1.60，2.80，5.00mg/L
公比；1.8，最大助剤濃度；100 mg/L（ﾒﾁﾙｾﾛｿﾙﾌﾞ，HCO-40使用）

6) 試験液量：　5.0L／容器

7) 連数：　１容器／濃度区

8) 供試生物数：　10尾／濃度区

9) 試験温度：　24±1℃

10) 照明：　室内光，16時間明／8時間暗

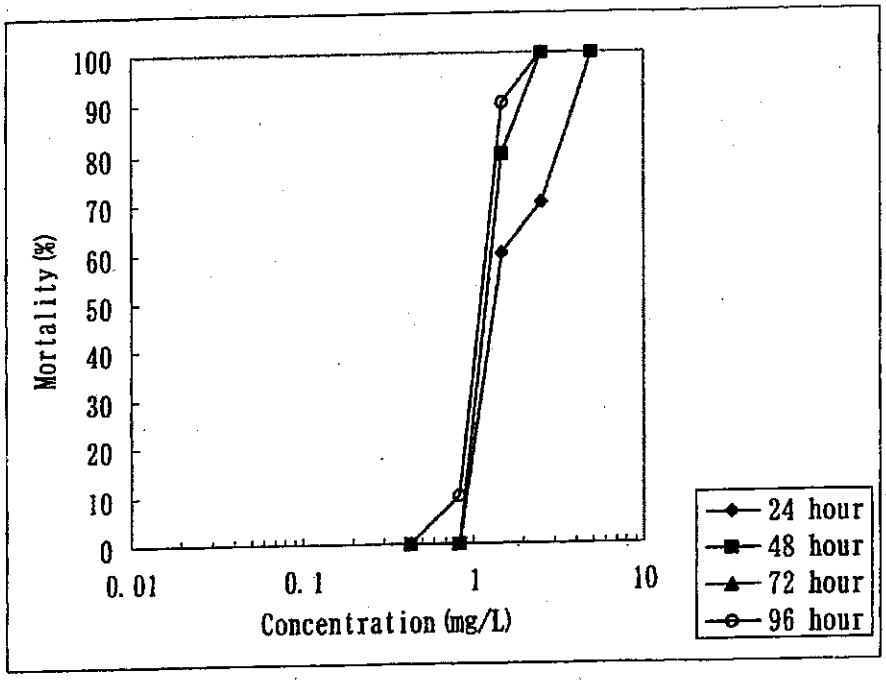
11) 分析法：　ＨＰＬＣ法

結　果

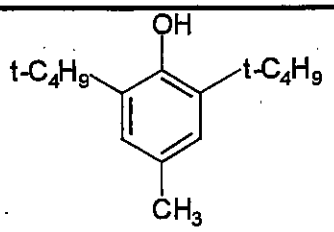
1) 試験液中の被験物質濃度：試験区において設定濃度に対して±20%を越える分析結果があったため，以下の値は測定濃度の幾何平均値を基に示した。

2) 96 時間の半数致死濃度（LC50）：1.10 mg/L（95%信頼区間：0.895mg/L～1.36mg/L）

Figure 1 Concentration-Response (Mortality) Curve



SIDS INITIAL ASSESSMENT PROFILE

CAS No.	128-37-0
Chemical Name	2,6-di-tert.-butyl-p-cresol (BHT) Butylated Hydroxytoluene
Structural Formula	

RECOMMENDATIONS

The chemical is a candidate for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

BHT is of low acute toxicity. BHT caused acute toxic effects in mammalians but there were no specific clinical symptoms. In rats, the oral LD_{50} was > 2930 mg/kg bw, the LD_{50} after dermal exposure was > 2000 mg/kg bw. It was slightly irritating to the skin and eyes of rabbits.

On chronic oral exposure of rats, liver and thyroid are the main targets. Doses above 25 mg/kg bw/day BHT resulted in thyroid hyperactivity, enlargement of the liver, induction of several liver enzymes. 25 mg/kg bw/day BHT can be considered as NOAEL for chronic exposure. The haemorrhagic effects of high repeated doses of BHT seen in certain strains of mice and rats, but not in other species, may be related to its ability to interact with prothrombin and vitamin K.

BHT showed no potential to cause point mutations in several bacterial and mammalian *in vitro* test systems.

Overall, the available studies demonstrate that BHT has no clastogenic activity *in vitro* or *in vivo*. Most *in vitro* chromosome aberration assays were negative as were sister chromatid exchange assays and DNA damage and repair assays. *In vivo*, micronucleus assays with mice, cytogenetic assays with rats and mice, dominant lethal assays with rats and mice, and the heritable translocation assay with mice were also negative.

BHT is not a genotoxic carcinogen. Carcinogenic effects observed in one long-term study with rats probably were caused by the specific study conditions. However, it cannot be completely ruled out that the hepatotoxic effects caused by high and chronic doses of BHT may result in persistent cell proliferation, which is known as a possible mechanism of non-genotoxic carcinogens. In addition, depending on the application regime, BHT may exert either anticarcinogenic or tumour-promoting activity at relatively high doses. For the possible carcinogenic and tumour-promoting effect of BHT, a threshold level of 100 mg/kg bw/day can be assumed. At this dose, no increase in the incidence of liver carcinoma, but a slight increase in liver adenoma were observed after chronic exposure starting *in utero* as a worst case scenario.
The only effects on reproduction were lower numbers of litters of ten or more pups at birth at doses of 100 mg/kg bw/day and above. The NOAEL was 25 mg/kg bw/day.

From studies with mice and rats there is no evidence of teratogenic effects of BHT. During pregnancy BHT had maternal effects on mice above oral doses of 240 mg/kg bw/day. The NOEL for developmental toxicity was 800 mg/kg bw day.
Despite of being in wide dispersive use as ingredient of various products for many years only very few cases of

allergic reaction in humans after dermal exposure or oral intake have been described. For the use of BHT as antioxidant in foodstuff an acceptable daily intake (ADI) of 0 - 0.3 mg/kg bw/day has been established.

Environment

BHT has a melting point of ca. 70 °C, a water solubility in the range of 0.6-1.1 mg/l (20-25 °C), a density of 1.03 g/cm³, and a vapor pressure of 1.1 Pa (20 °C). The measured log Kow is determined to be 5.1.

According to a Mackay Level I model calculation, the main target compartment for BHT is air (79-87 %), followed by soil (6.1-10.2 %) and sediment (5.7-9.5 %). Due to the instability of BHT in aqueous solution the estimations reflect a tendency for BHT distribution among environmental compartments. BHT is relatively unstable under environmental conditions. Extent and products of decomposition are dependent on several factors like irradiation, pH, temperature, moisture, presence of soil and soil microorganisms, and oxygen content. In air BHT is indirectly photodegradable by hydroxyl radicals with $t_{1/2}$ = 7.0 hours. In aqueous solution BHT is decomposed in natural sunlight with irradiation (ca. 75 %) and without (ca. 40 %), forming different, partly unidentified metabolites. BHT is also not stable in soil. Within one day of incubation 63-82 % of BHT were decomposed in non-sterilized and 25-35 % in sterilized soils. A mineralization up to 30 % was observed under non-sterilized conditions. Depending on the exposure pathways, the compartments air, hydrosphere and soil can be environmental target compartments for this substance and its metabolites. BHT is not readily biodegradable in water according to a modified MITI-I test (4.5 % degradation after 28 days). A wide range of bioconcentration factors (BCF) was found in different experiments. Bioconcentration factors (BCF) in the range of 230-2500 have been determined for fish after 56 days. The BCF values determined after a 28 days exposure period in a model ecosystem with soil were 2-17 for fish, 30 for snails and 38 for algae. It can be assumed that BHT has a moderate to high bioaccumulation potential in aquatic species.

For the toxicity of BHT on aquatic species reliable experimental results from tests with fish, daphnia, and algae are available. Only those effect values are considered for the assessment that did not exceed the low water solubility of BHT (0.6 - 1.1 mg/l) and were based on measured concentrations. The lowest reliable acute toxicity values are:

fish (*Brachydanio rerio*): 96h LC0 ≥ 0.57 mg/l;

invertebrates (*Daphnia magna*): 48h EC0 ≥ 0.17 mg/l;

algae (*Scenedesmus subspicatus*): 72h E_rC_8 = 0.4 mg/l. This value can be used as a NOEC.

In a 21 days reproduction test with *Daphnia magna* a NOEC = 0.07 mg/l was determined. Using an assessment factor of 50,a PNECaqua = 0.0014 mg/l is derived from this long term NOEC.

Exposure

In 2000, the world production capacity of BHT amounts to about 62,000 t/a by more than 20 producers. BHT is a registered antioxydant, licenced for food products, animal feed, cosmetics, and packaging material. It is also used in petroleum products, synthetic rubbers, plastics, elastomers, oils, waxes, soaps, paints, and inks.

Releases into the environment may occur during production of BHT as well as during its use in different applications as stabilizer and during the use of the products that contain the substance. A significant release into the environment is expected from migration of BHT onto the surface of products containing the substance.

NATURE OF FURTHER WORK RECOMMENDED

Environment: The substance is a candidate for further work. Releases into the environment during use of BHT and from products containing the substance have to be assumed but are not quantifiable. In the environment, BHT is rapidly decomposed forming several, partly unidentified, metabolites. BHT is not readily biodegradable, a moderate to high bioaccumulation potential has to be assumed. The NOEC from the long-term toxicity to daphnids was 0.07 mg/l, resulting in a PNEC of 0.0014 mg/l. Therefore, the performance of an environmental risk assessment is recommended. Especially the questions concerning exposure, bioaccumulation as well as toxicity of the metabolites should be clarified.

Human Health: No recommendation for further work, because all SIDS endpoints are adequately covered and because exposure is controlled in occupational settings.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Testing of the ecotoxicological behavior of BHT exhibits difficulties due to its extremely low water solubility of about 0.6 to 1.1 mg/l at room temperature and its tendency to decompose to various degradation products depending on the actual conditions (see chapt. 2.1.2). Based on the instability, low recovery rates of applied BHT from the test solutions are not surprising. Due the aforementioned instability of BHT the data obtained in testing cover not only the toxicity of BHT but also the toxicity of the oxidation/degradation products of BHT.

For the acute toxicity of BHT in aquatic species reliable experimental results from short-term tests with fish, daphnia, algae and microorganisms are available. Only those effect values were considered for the assessment that did not exceed the low water solubility of the compound (0.6 - 1.1 mg/l) and were based on measured concentrations.

A test on the acute toxicity of BHT to fish was conducted according to the European protocol EEC C.1 (equivalent to OECD guideline 203). Semistatic exposure (renewal of test solution every 24 hours) of *Brachydanio rerio* in a limit test with water-saturated concentration of the test substance had no adverse effect within the test period of 96 hours. The BHT concentration measured in the test solution after 24 hours of exposure was 0.57 mg/l (Bayer AG 1994).

In a test according to the European protocol EEC C.2 (equivalent to OECD guideline 202, part 1) the acute toxicity of BHT in invertebrates was determined. *Daphnia magna* were exposed in a limit test with water-saturated concentration of the test substance. No toxic effects were observed during incubation and the 48h EC_0 based on the measured BHT concentration (geometric mean of TS concentrations measured at the start and after 48 h of exposure) was reported to be $\geq$ 0.17 mg/l (Bayer AG 1994).

A further acute test with *Daphnia pulex* is also available for BHT (Passino, Smith, 1987). This test was performed according to ASTM method. Acetone was used as solvent with a concentration of 0.5 ml/l. A 48h EC_{50} of 1.44 mg/l related to nominal concentration was found. However the concentration of acetone in this study was higher than proposed by OECD, US-EPA and EU (0.1 ml/l). As there are other valid tests performed without solvent available that are regarded to be more relevant, the test is not used for the effect assessment of BHT.

In a long-term test *Daphnia magna* were exposed to three BHT concentrations (0.1 mg/l; 0.316 mg/l and 1.0 mg/l; nominal) for a test duration of 21 days. The test was performed according to OECD guideline 202 (part 2) under semistatic conditions. The NOEC (endpoint: reproduction) based on measured test substance concentrations (geometric mean of TS concentrations measured at the start and after 48 h and 72 h of exposure at water exchange) was reported to be 0.07 mg/l (Bayer AG 1994).

The acute toxicity of BHT to algae was determined in a test according to the European protocol EEC C.3 (equivalent to OECD guideline 201). The saturated BHT-water concentration was tested in a limit test with the algae *Scenedesmus subspicatus*. Only a slightly lower growth rate was observed after 72 h of incubation (8 % inhibition). Based on measured concentrations (geometric mean of TS concentrations measured at the start and after 72 h of exposure) an E_rC_8 of 0.4 mg/l is derived (Bayer AG 1994). This value can be used as a NOEC.

In a test with activated sludge according to Directive 88/302/EEC, Part C (respiration inhibition test) a 3h EC_0 = 1000 mg/l was determined (Bayer AG 2000).

For the protozoan species *Tetrahymena pyriformis* a 24h EC_{50} of 1.7 mg/l was found in a cell multiplication inhibition test (Yoshioka et al.,1985).

The lowest available long-term NOEC of 0.07 mg/l, found in a 21 days reproduction test with *Daphnia magna* is used for the derivation of the $PNEC_{aqua}$. The application of an assessment factor of 50 is justified, as results from two long-term tests (daphnia and algae) are available, resulting in a $PNEC_{aqua}$ of 0.0014 mg/l.

4.2 Terrestrial Effects

No reliable data available.

4.3 Other Environmental Effects

In a feeding study with "White Leghorn" chicken (*Gallus domesticus*) the influence of BHT on aflatoxin toxicity has been studied. 0.1 % BHT in the diet (8x the standard BHT concentration of 0.013 % in feed) had no significant inhibiting effect on weight gain, whereas 0.4% (30 x the standard BHT concentration in feed) resulted in a temporary depression (36 %) of weight gain from day 1 until 3 weeks; from week 3 to week 6 the weight gain was normal (98 % of control) and at the end of BHT treatment (6 weeks) the total weight gain amounted to 78 % of control. Both BHT dosages improved body weight gain and feed efficiency of chicken treated with 3000 ppb aflatoxin in feed (Larsen et al., 1985).

要　　約

試 験 委 託 者 ： 環境省

表　　題 ： プロピレンテトラマーの藻類（*Selenastrum capricornutum*）に対する生長阻害試験

試 験 番 号 ： A020372－1

試 験 方 法 ：

1) 適用ガイドライン： OECD 化学品テストガイドライン No. 201「藻類生長阻害試験」（1984年）
2) 暴 露 方 式 ： 止水式（密閉系），連続振とう培養（100rpm）
3) 供 試 生 物 ： *Selenastrum capricornutum*（株名：ATCC22662）
（現在 *Pseudokirchneriella subcapitata*と学名が変更されている。）
4) 暴 露 期 間 ： 72時間
5) 試 験 濃 度 （設定値）： 対照区，助剤対照区，0.0400 mg/L（試験液調製可能最高濃度での限度試験）
助剤濃度一定：100 μL/L（ジメチルホルムアミド使用）
6) 試 験 液 量 ： 100 mL（OECD培地）／容器
7) 連　　数 ： ３容器／試験区
8) 初期細胞濃度 ： 前培養した藻類　1×10^4 cells/mL
9) 試 験 温 度 ： 23±2 ℃
10) 照　　明 ： 4000 lux （±20％の変動内，フラスコ液面付近）で連続照明
11) 分 析 法 ： ガスクロマトグラフィー質量分析（GC／MS）

試 験 結 果 ：

1) 試験液および試験培養液中の被験物質濃度

被験物質濃度分析の結果，測定値の設定値に対する割合は，暴露開始時の試験液において134 %，暴露終了時の試験培養液において検出限界以下であった。水中からの50％揮散速度は約６時間であることから，濃度減少の主な原因は揮散と考えられた。阻害濃度の算出には開始時の測定値を用いた。

2)　生長曲線下面積の比較による阻害濃度

50％生長阻害濃度 EbC50 (0-72h) : >0.0534 mg/L (95％信頼区間：算出不可)

最大無作用濃度 NOECb (0-72h) :　>0.0534 mg/L

3)　生長速度の比較による阻害濃度

50％生長阻害濃度 ErC50 (24-48h) : >0.0534 mg/L (95％信頼区間：算出不可)

最大無作用濃度 NOECr (24-48h) :　>0.0534 mg/L

50％生長阻害濃度 ErC50 (24-72h) : >0.0534 mg/L (95％信頼区間：算出不可)

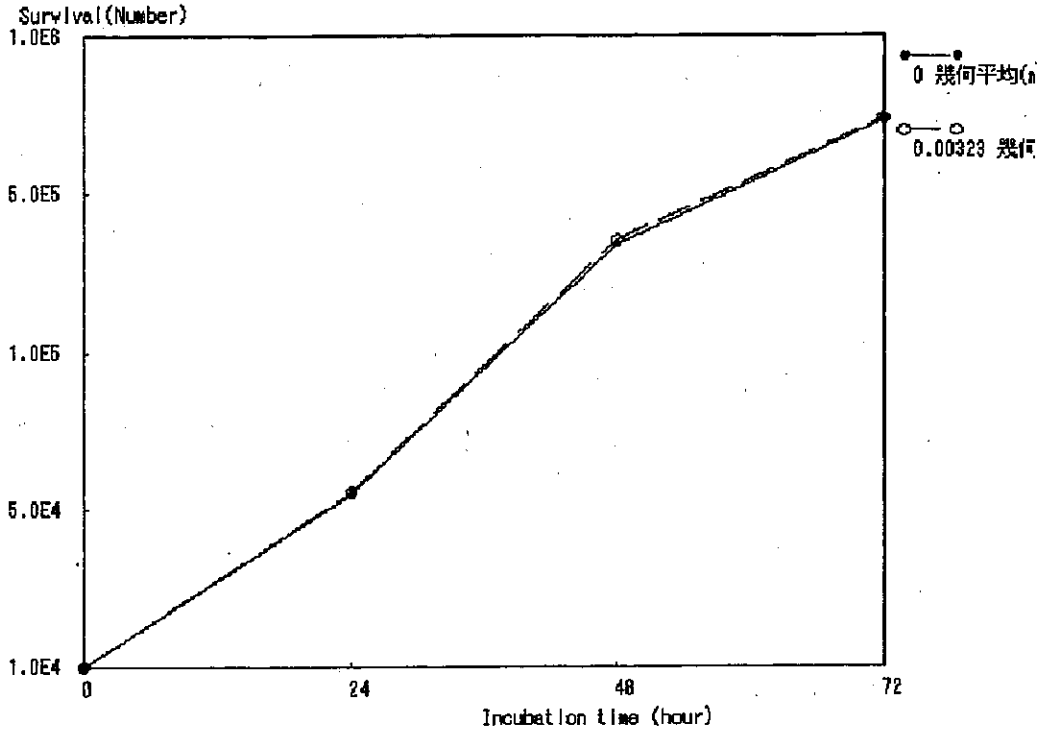
最大無作用濃度 NOECr (24-72h) :　>0.0534 mg/L

4)　藻類の形態観察

暴露終了時の顕微鏡下での細胞形態観察の結果，0.0400 mg/Lの濃度区では細胞形態の変化（収縮，膨張，破裂等）や細胞凝集は認められず，また，対照区および助剤対照区との相違もなかった。

プロピレンテトラマー(CAS.6842-15-5)

①生長曲線



②毒性値

48hErC50(実測値に基づく)＞0.0032mg/L
48hNOECr(実測値に基づく)＝0.0032mg/L

要　約

試験委託者：　環境省

表　　題：　プロピレンテトラマーのオオミジンコ（*Daphnia magna*）に対する急性遊泳阻害試験

試験番号：　A020372－2

試験方法：

1) 適用ガイドライン：　OECD 化学品テストガイドライン No. 202「ミジンコ類，急性遊泳阻害試験および繁殖試験」（1984年）

2) 暴露方式：　半止水式（24時間後に試験液の全量を交換）
水面をテフロンシートで被覆

3) 供試生物：　オオミジンコ（*Daphnia magna*）

4) 暴露期間：　48時間

5) 試験濃度（設定値）：　対照区，助剤対照区，
0.0040，0.0072，0.0128，0.0224，0.0400 mg/L
公比：1.8
ただし0.0400 mg/Lは試験液調製可能最高濃度
助剤濃度一定：100μL/L（ジメチルホルムアミド使用）

6) 試験液量：　100 mL／容器

7) 連数：　４容器／試験区

8) 供試生物数：　20頭／試験区（5頭／容器）

9) 試験温度：　20±1℃

10) 照明：　室内光，16時間明（800 lux以下）／8時間暗

11) 分析法：　ガスクロマトグラフィー質量分析（GC／MS）

試　験　結　果　：

1) 試験液中の被験物質濃度

試験液の分析の結果，測定値の設定値に対する割合は，暴露開始時において 66～91%，換水前において 30～40%であった。水中からの 50%揮散速度は約 6 時間であることから，減少の主な原因は，揮散と考えられた。

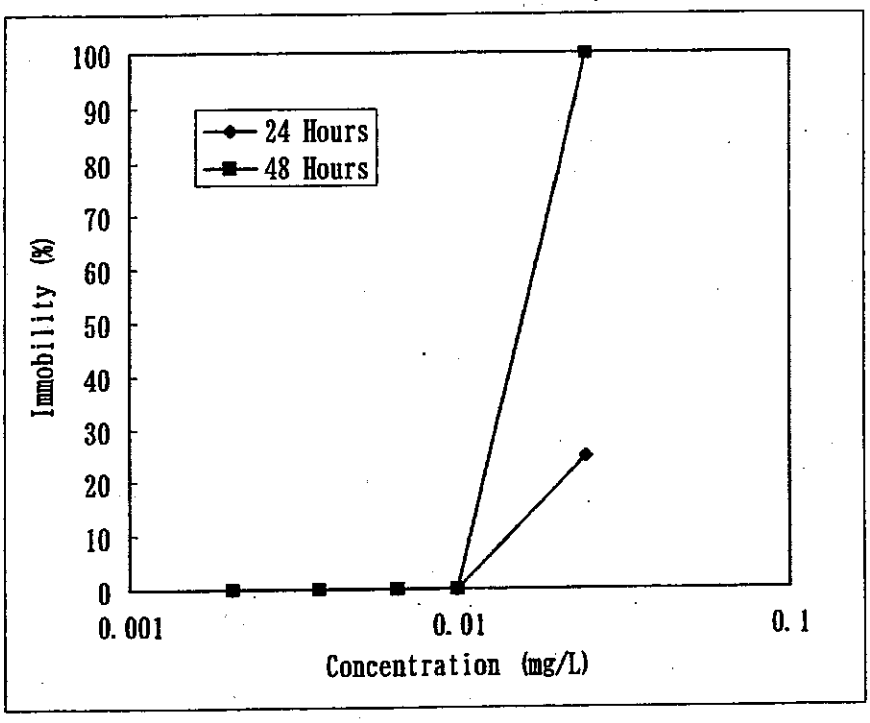
2) 24時間暴露後の結果

	(mg/L)	95%信頼区間 (mg/L)
半数遊泳阻害濃度 (EiC50)	> 0.0240	算出不可
0%阻害最高濃度	0.0099	—
100%阻害最低濃度	> 0.0240	—

3) 48時間暴露後の結果

	(mg/L)	95%信頼区間 (mg/L)
半数遊泳阻害濃度 (EiC50)	0.0154	0.0099 ～ 0.0240
0%阻害最高濃度	0.0099	—
100%阻害最低濃度	0.0240	—

Figure 1 Concentration-Immobility Curve



要　約

試験委託者：　環境省

表　　題：　プロピレンテトラマーのオオミジンコ（*Daphnia magna*）に対する繁殖阻害試験

試験番号：　A020372－3

試験方法：

1) 適用ガイドライン：　OECD 化学品テストガイドライン No. 211「オオミジンコ繁殖試験」（1998年）

2) 暴露方式：　半止水式（毎日試験液の全量を交換）
水面をテフロンシートで被覆

3) 供試生物：　オオミジンコ（*Daphnia magna*）

4) 暴露期間：　21日間

5) 試験濃度：　対照区，助剤対照区，
（設定値）　0.0020，0.0042，0.0090，0.0190，0.0400 mg/L　（公比：2.1）
ただし，0.0400 mg/Lは試験液調製可能最高濃度
助剤濃度一定：100μL/L（ｼﾞﾒﾁﾙﾎﾙﾑｱﾐﾄﾞ使用）

6) 試験液量：　80 mL／容器

7) 連　　数：　10容器／試験区

8) 供試生物数：　10頭／試験区（1頭／容器）

9) 試験温度：　20±1℃

10) 照　　明：　室内光，16時間明（800 lux以下）／8時間暗

11) 分析法：　ガスクロマトグラフィー質量分析（GC／MS）

試　験　結　果　：

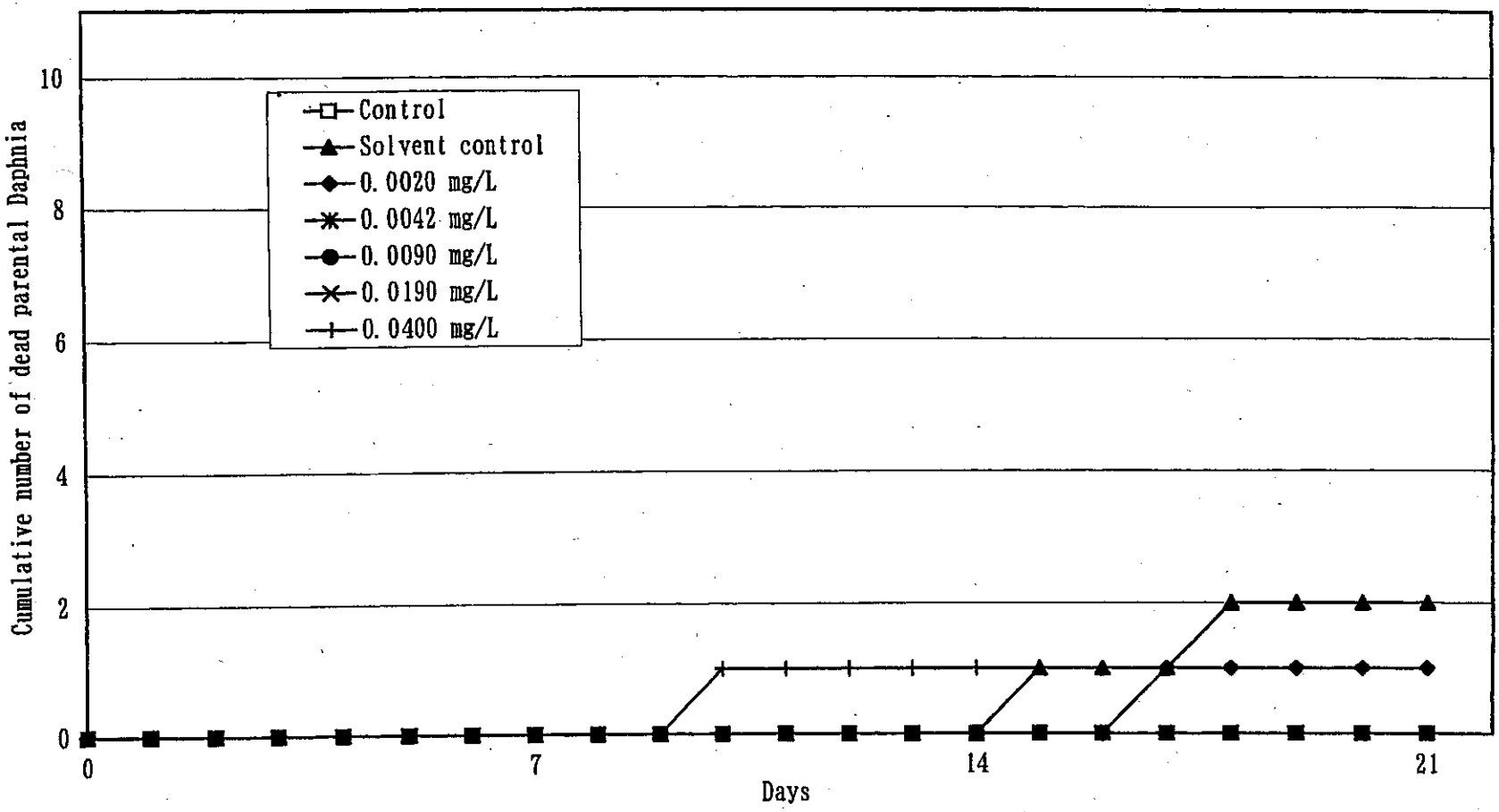
1) 試験液中の被験物質濃度

試験液の分析の結果，測定値の設定値に対する割合は，調製時において 81～104%，換水前において 11～29%であった。水中からの 50%揮散速度は約 6 時間であることから，減少の主な原因は，揮散と考えられた。

2) 21日間暴露後の結果

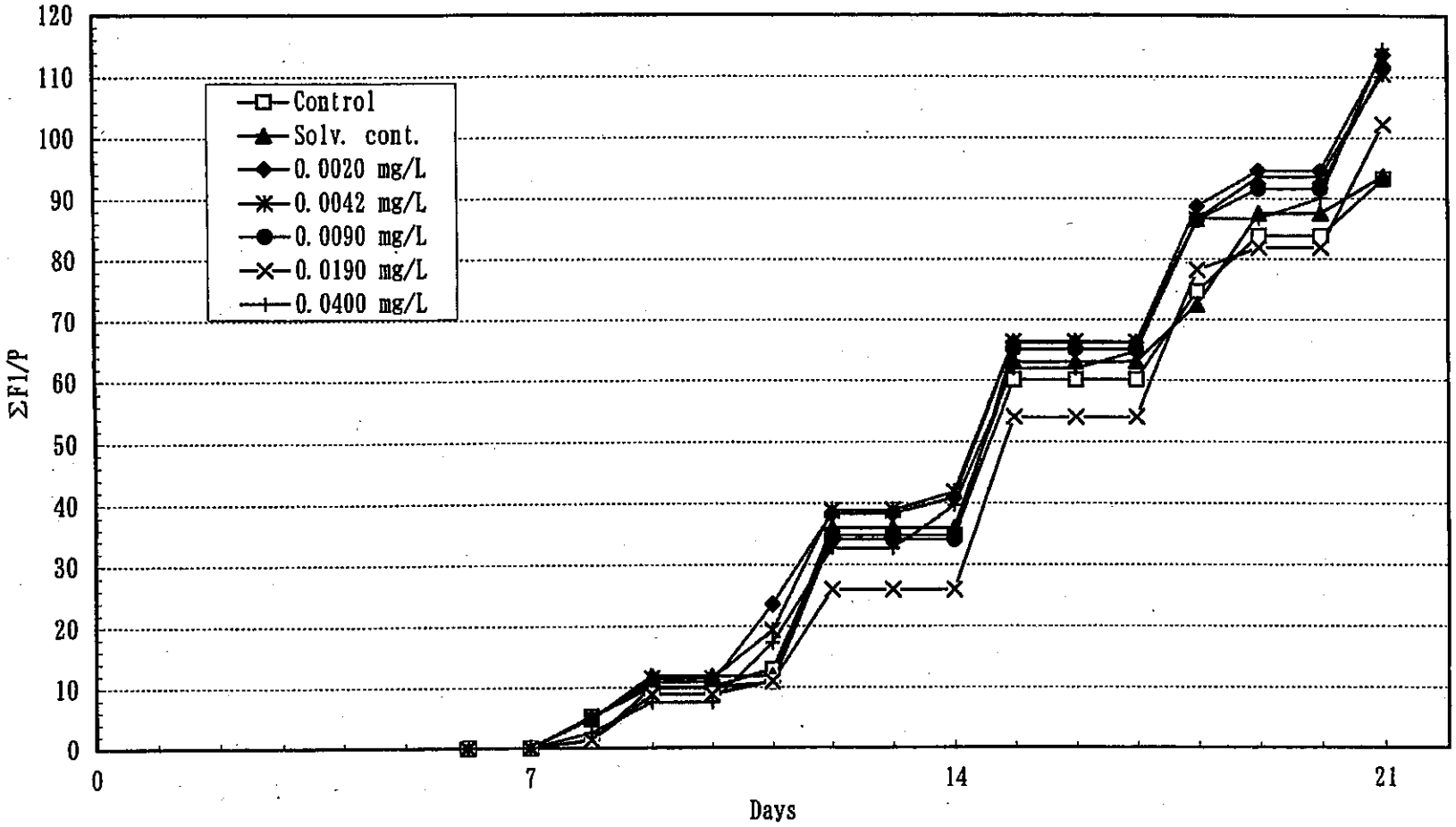
	(mg/L)	95%信頼区間 (mg/L)
親ミジンコの半数致死濃度 (LC50)	> 0.0196	算出不可
50%繁殖阻害濃度 (EC50)	> 0.0196	算出不可
最大無作用濃度 (NOEC)	> 0.0196	—
最小作用濃度 (LOEC)	> 0.0196	—

Figure 1 Cumulative Number of Dead Parental *Daphnia*



Values in legend are given in the nominal concentration.

Figure 2 Time Course of ΣF1/P for Each Concentration Level



Values in legend are given in the nominal concentration.

要　　約

試 験 委 託 者 ： 環境省

表　　題 ： プロピレンテトラマーのヒメダカ（*Oryzias latipes*）に対する急性毒性試験

試 験 番 号 ： Ａ０２０３７２－４

試 験 方 法 ：

1) 適用ガイドライン： OECD 化学品テストガイドライン No. 203「魚類急性毒性試験」（1992年）
2) 暴 露 方 式 ： 半止水式（24時間毎に試験液の全量を交換）
水面をテフロンシートで被覆
3) 供 試 生 物 ： ヒメダカ（*Oryzias latipes*）
4) 暴 露 期 間 ： 96時間
5) 試 験 濃 度 （設定値）： 対照区，助剤対照区，0.0400 mg/L（試験液調製可能最高濃度の限度試験）
助剤濃度：100μL/L（ｼﾞﾒﾁﾙﾎﾙﾑｱﾐﾄﾞ使用）
6) 試 験 液 量 ： 5.0 L／容器
7) 連　　数 ： １容器／試験区
8) 供試生物数 ： 10尾／試験区
9) 試 験 温 度 ： 24±1 ℃
10) 照　　明 ： 室内光，16時間明（1000 lux以下）／8時間暗
11) 分 析 法 ： ガスクロマトグラフィー質量分析（ＧＣ／ＭＳ）

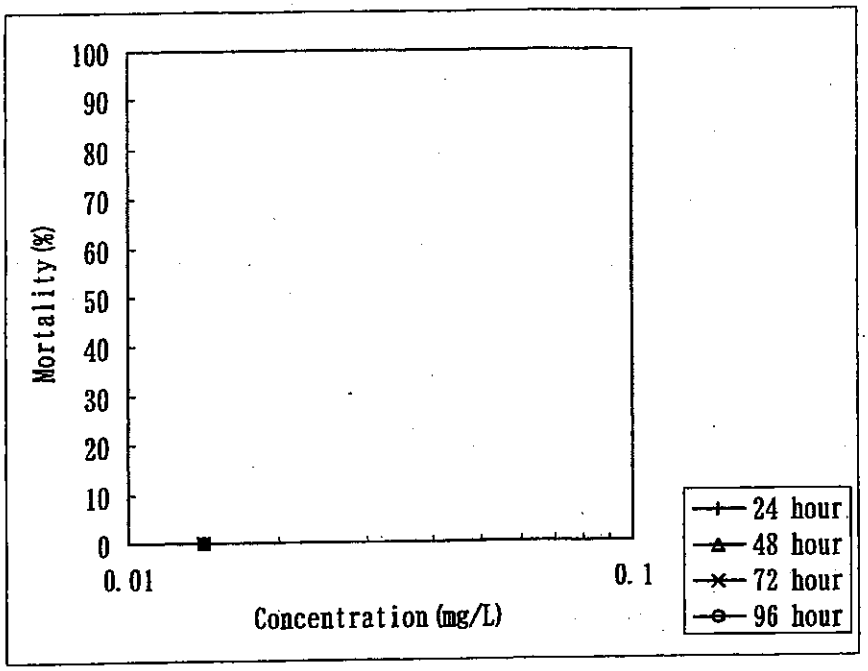
試 験 結 果 ：

1) 試験液中の被験物質濃度

試験液の分析の結果，測定値の設定値に対する割合は，暴露開始時において75％，24時間後において17％であった。水中からの50％揮散速度は約６時間であることから，減少の主な原因は，揮散と考えられた。

2) 96時間暴露後の半数致死濃度(LC50)： >0.0143 mg/L（95％信頼区間：算出不可）

Figure 1 Concentration-Mortality Curve



要　旨

試験委託者

環境省

表　題

2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)の藻類(*Selenastrum capricornutum*)に対する生長阻害試験

試験番号

NMMP／E00／1090

試験方法

本試験は、OECD化学品テストガイドラインNo.201「藻類生長阻害試験」(1984年)に準拠して実施した。

1) 被験物質　:2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)
2) 培養方式　:振とう培養 (100rpm)
3) 供試生物種　:*Selenastrum capricornutum* (ATCC-22662)
4) 温度　:23±2 ℃
5) 暴露期間　:72 時間
6) 試験液量　:100 mL(OECD培地)
7) 照明　:4000 ～ 5000 lux(連続照明)
8) 初期細胞濃度　:1×10^4 cells/mL
9) 試験濃度(設定)　:対照区、0.10mg/L、0.23mg/L、0.53mg/L、1.23mg/L、2.83mg/L 6.52mg/L および 15.0mg/L (公比:2.3)
10)試験液中の被験物質の分析　:HPLC法(暴露開始時、終了時)

結　果

1) 生長曲線下の面積の比較による生長阻害濃度

EbC50(0-72) ＝ 2.16mg/L(95%信頼区間:1.94mg/L～2.40mg/L)

無影響濃度(NOEC(面積法 0-72))＝ 0.23mg/L

2) 生長速度の比較による生長阻害濃度

ErC50(24-48)＝ 6.43mg/L(95%信頼区間:5.94mg/L～6.96mg/L)

無影響濃度(NOEC(速度法 24-48))＝ 1.23mg/L

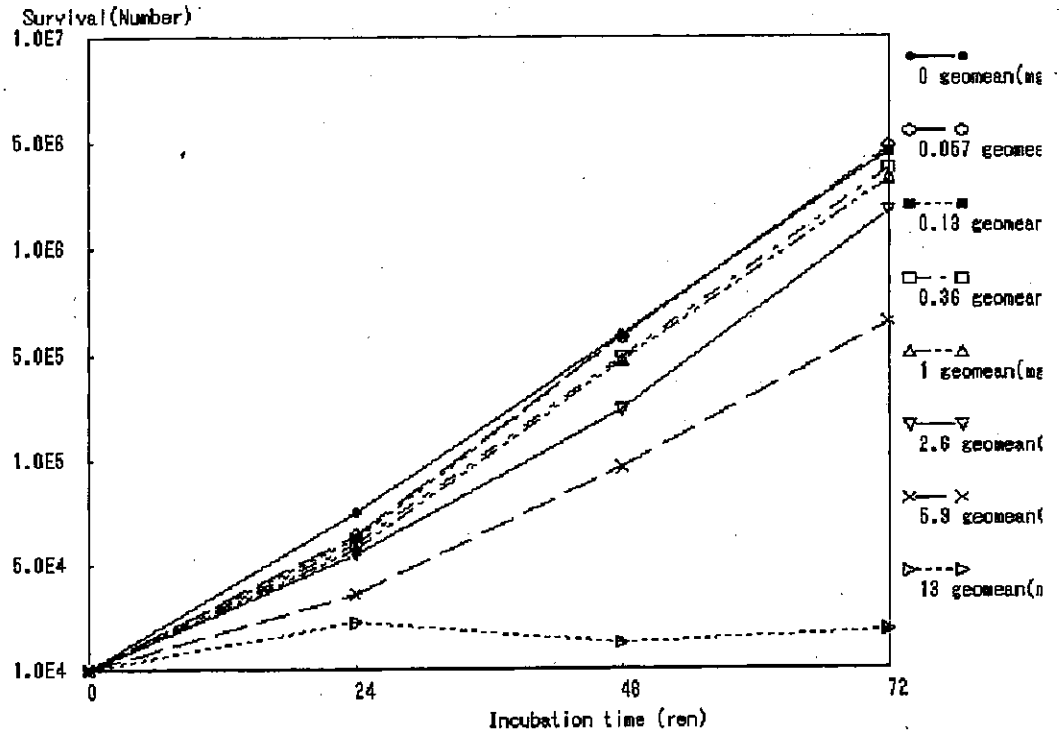
ErC50(24-72)＝ 7.31mg/L(95%信頼区間:6.84mg/L～7.84mg/L)

無影響濃度(NOEC(速度法 24-72))＝ 2.83mg/L

(上記濃度は、全て設定濃度に基づく値)

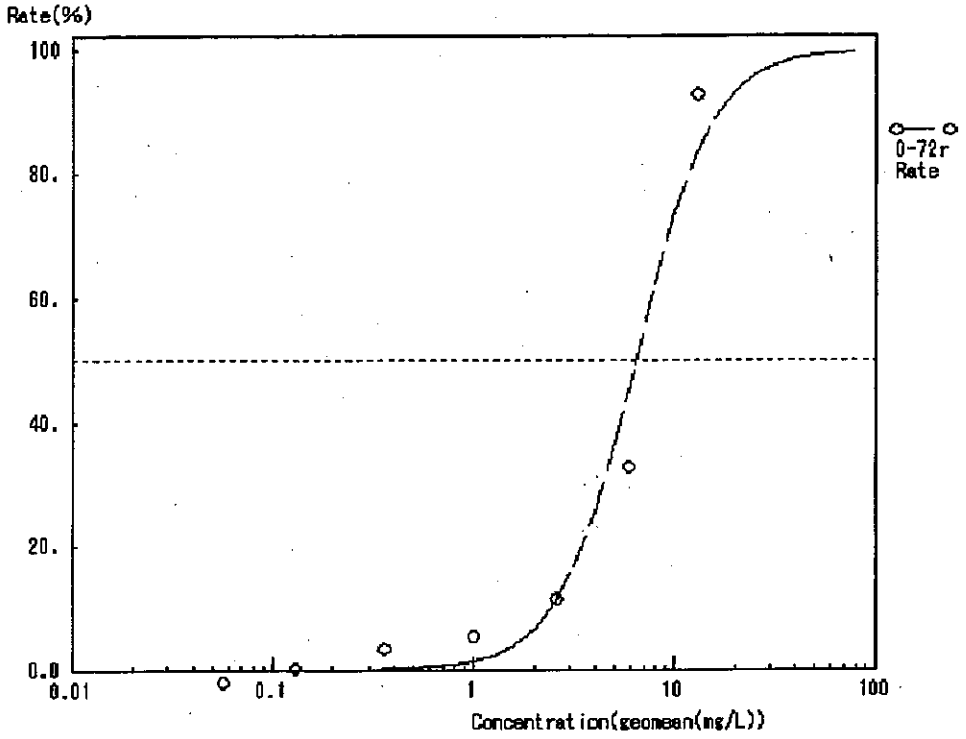
2,2'-ジメチル-4,4'-メチレンビス<シクロヘキシルアミン> (CAS.6864-37-5)

①生長曲線



Time course pattern of Algae Growth Test
6864375

②阻害率曲線



③毒性値

72hErC50(実測値に基づく)＝6.1mg/L
72hNOECr(実測値に基づく)＝0.36mg/L

CAS:6864-37-5

要　旨

試験委託者

環境省

表　題

2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)のオオミジンコ（*Daphnia magna*)に対する急性遊泳阻害試験

試験番号

NMMP／E00／2090

試験方法

本試験は、OECD 化学品テストガイドライン No.202「ミジンコ類、急性遊泳阻害試験および繁殖試験」(1984年)に準拠して実施した。

1) 被験物質　:2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)
2) 暴露方法　:止水式
3) 供試生物　:オオミジンコ（*Daphnia magna*)
4) 暴露期間　:48 時間
5) 連数　　:1濃度区に付き4連
6) 生物数　　:20 頭／1濃度区(1連に付き5頭で1濃度区 20 頭)
7) 試験濃度　:対照区、2.12mg/L、3.81mg/L、6.86mg/L、12.4mg/L、22.2mg/L および40.0mg/L (公比 1.8)(設定濃度)
8) 試験液量　:100 mL
9) 照明　　:室内光、16 時間明／8 時間暗
10)試験水温　:20±1℃

結　果

1) 24 時間暴露後の結果

24 時間半数遊泳阻害濃度(EiC50)＝5.11mg/L(95%信頼区間: 3.81mg/L～6.86mg/L)

2) 48 時間暴露後の結果

48 時間半数遊泳阻害濃度(EiC50)＝4.57mg/L(95%信頼区間: 3.81mg/L～6.86mg/L)

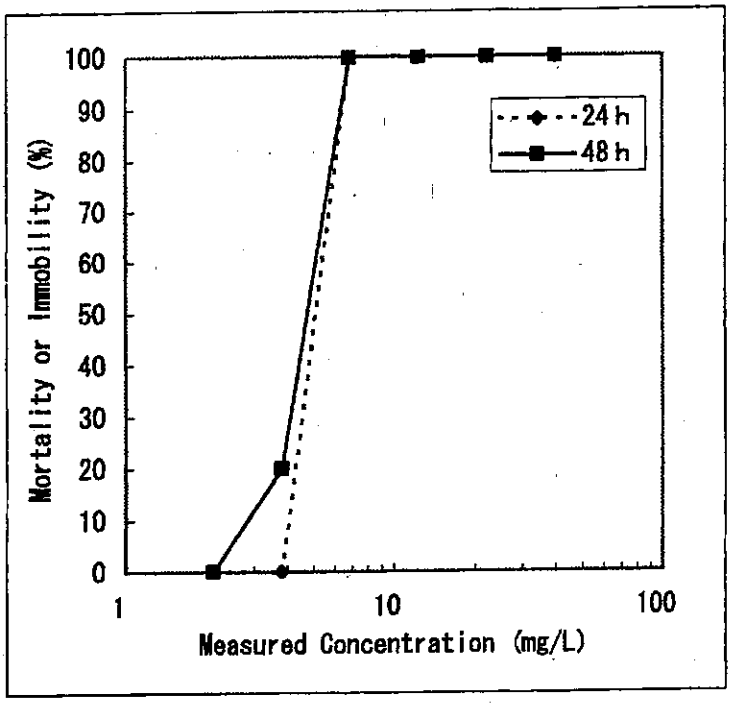
最大無作用濃度(NOECi)＝2.12mg/L

100%阻害最低濃度＝6.86mg/L

(上記濃度は、全て設定濃度に基づく値)

Figure 1 Concentration-Response Curve of 2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine)

Mortality or Immobility in *Daphnia magna*



要　旨

試験委託者

環境省

表　題

2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)のオオミジンコ（*Daphnia magna*)に対する繁殖阻害試験

試験番号

NMMP／E00／3090

試験方法

本試験は、OECD 化学品テストガイドライン No.211「オオミジンコ繁殖試験」(1998年)に準拠して実施した。

1) 被験物質　:2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)
2) 暴露方法　:半止水式(週3回、試験液の全量を交換)
3) 供試生物　:オオミジンコ（*Daphnia magna*)
4) 暴露期間　:21 日間
5) 試験濃度　:対照区、0.21mg/L、0.38mg/L、0.69mg/L、1.23mg/L、2.22mg/L および 4.00mg/L(公比 1.8、設定濃度)
(追加濃度区) 対照区、7.20mg/L、13.0mg/L、23.3mg/L(公比1.8、設定濃度)
6) 試験液量　:1容器(連)につき 80 mL
7) 連数　:10 容器(連)／濃度区
8) 供試生物数　:10 頭／濃度区(1連につき 1 頭)
9) 試験水温　:20±1℃
10)照明　:室内光、16 時間明／8 時間暗
11)被験物質の分析　:高速液体クロマトグラフ法

結　果

1) 試験液中の被験物質濃度

実測濃度が設定濃度の±20%以内であったので結果の算出には設定濃度を用いた。

2) 21 日間の親ミジンコの半数 致死濃度(LC50)

= 5.48mg/L (95%信頼区間 : 2.55mg/L～33.0mg/L)

3) 21 日間の 50% 繁殖阻害濃度(ErC50)

= >7.20mg/L

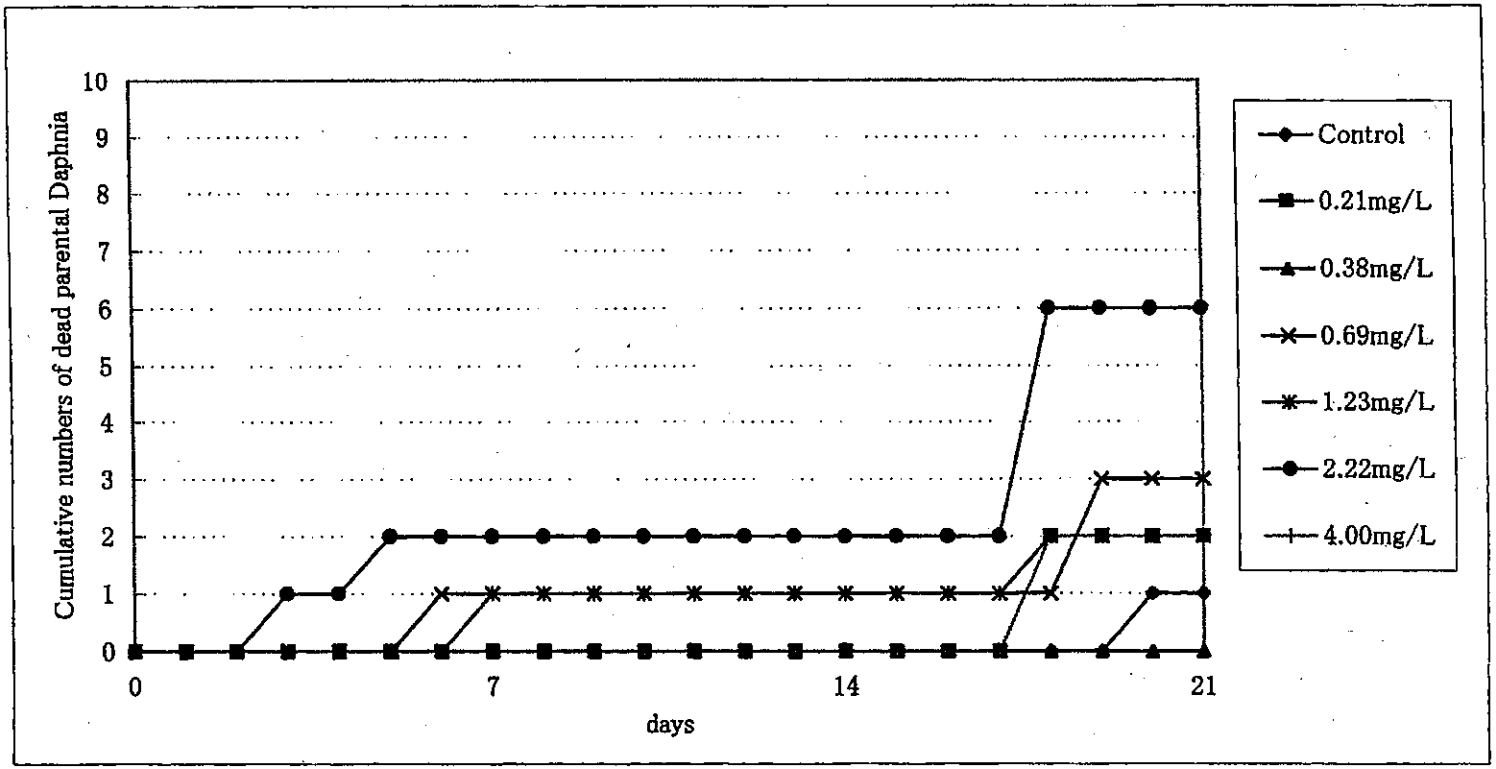
4) 21 日間の最大無作用濃度(NOECr) = 4.00mg/L

5) 21 日間の最小作用濃度(LOECr) = 7.20mg/L

(上記濃度は、設定濃度に基づく値である)

Figure 1 Cumulative Numbers of Dead Parental *Daphnia*

Original Test



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CAS:6864-37-5

Figure 1 Continued

Supplemental Test

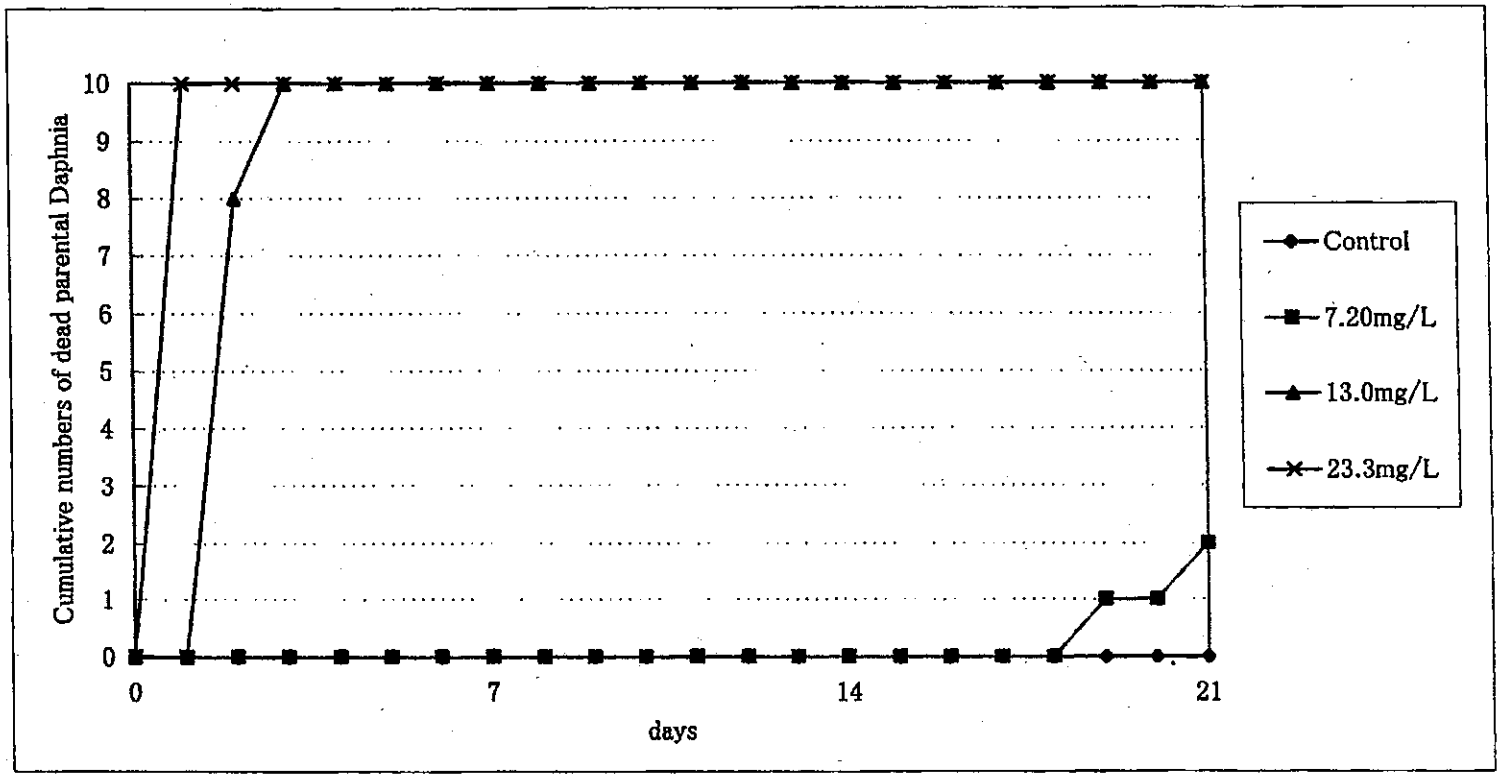


Figure 2 Mean Cumulative Numbers of Juveniles Produced per Adult (ΣF1/P) during 21 days

Original Test

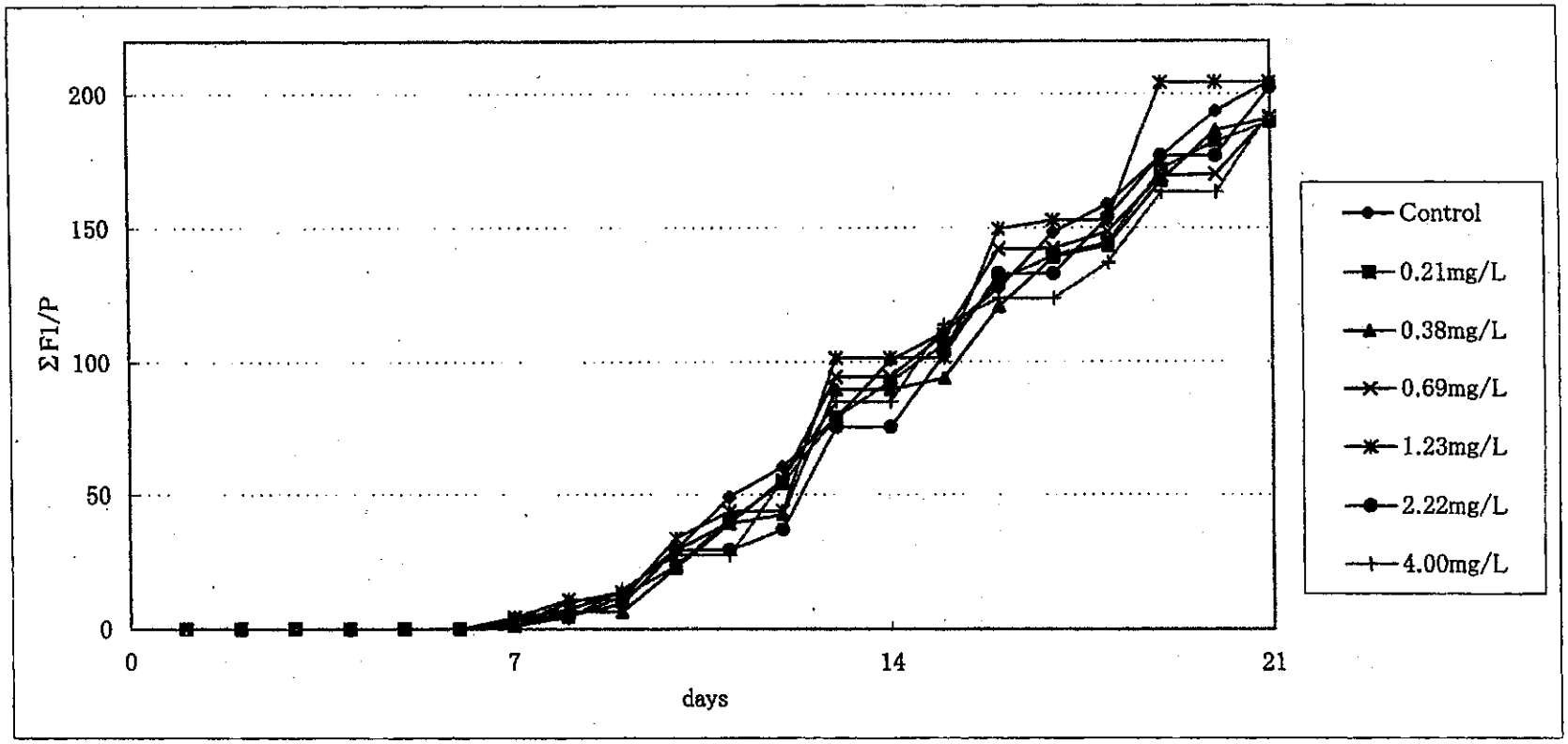
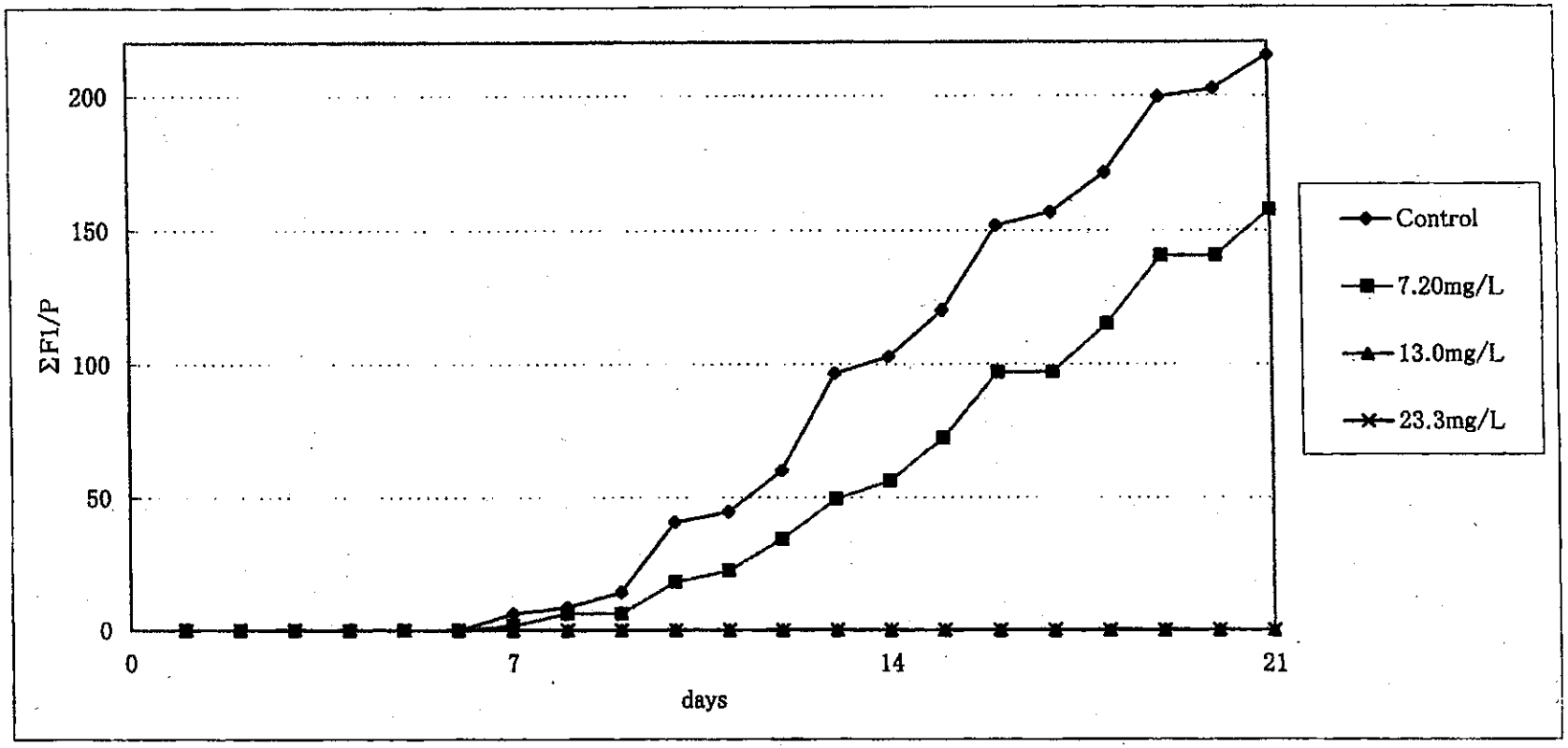


Figure 2 Continued

Supplemental Test



要　旨

試験委託者

環境省

表　題

2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)のヒメダカ(*Oryzias latipes*)に対する急性毒性試験

試験番号

NMMP／E00／4090

試験方法

本試験は、OECD 化学品テストガイドライン No.203「魚類毒性試験」(1992年)に準拠して実施した。

被験物質	:2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)
方式	:半止水式(24時間換水)
供試生物	:ヒメダカ(*Oryzias latipes*)
試験濃度	:対照区、1.6mg/L、2.9mg/L、5.1mg/L、9.3mg/L、16.7mg/L、30.0mg/L およびpH 調整(中和)した100mg/L (設定濃度)
曝露期間	:96 時間
試験液量	:3.0L
生物数	:10 尾／濃度区
照明	:室内光、16 時間明／8 時間暗
エアレーション	:なし
温度	:24±1℃

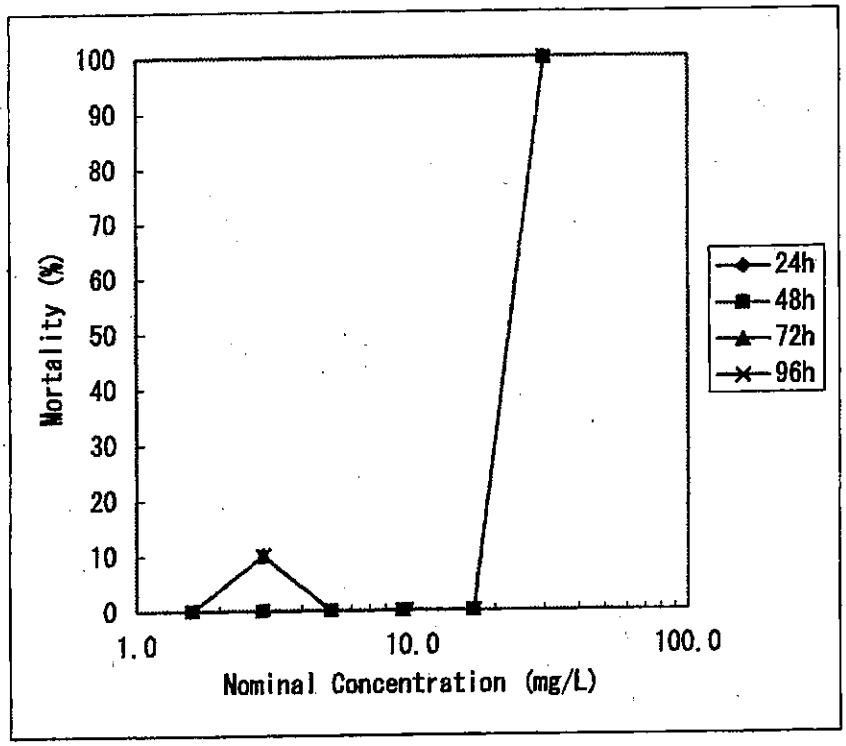
結　果

試験の結果、2,2'-ジメチル-4,4'-メチレンビス(シクロヘキシルアミン)の設定濃度に基づく96時間の半数致死濃度(LC50)は22.4mg/Lであり、その95%信頼区間は16.7～30.0mg/Lであった。

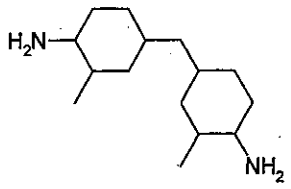
pH調整(中和)した試験液では96時間の半数致死濃度(LC50)は>100.0mg/Lであった。

Figure 1. Concentration-Response Curve of 2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine)

Mortality in Medaka



SIDS INITIAL ASSESSMENT PROFILE

CAS No.	6864-37-5
Chemical Name	2,2 -dimethyl-4,4 methylenebis(cyclohexylamine)
Structural Formula	

RECOMMENDATIONS

The chemical is currently of low priority for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

In humans (epoxy resins production workers) scleroderma-like skin changes have been described revealing 2,2 dimethyl-4,4 methylenebis(cyclohexylamine) as most probable causative agent. In DMD production workers unspecific skin changes, but no scleroderma-like symptoms were seen. DMD is harmful via the oral route and toxic via the dermal and inhalation route:

LD_{50} rat (oral): > 320 < 460 mg/kg bw, symptoms: unspecific;
LC_{50} rat (inhalation, liquid aerosol): 420 mg/m^3/4h, symptoms: irritation of the airways;
LD_{50} rabbit (dermal): > 200 < 400 mg/kg bw, symptoms: cyanosis, necrotic changes at the test site.

The substance is highly corrosive to skin (full thickness necrosis after 3 minutes of exposure) and may cause severe damage to eyes. In the guinea pig maximization test the substance showed no sensitizing effect. In a well conducted rat 90-day inhalation study (OECD TG 413) body weight development was impaired, local irritative effects observed for the skin and upper airways (nasal mucosa) and target organ toxicity indicative of a mild anemic effect as well as effects on the liver, testes and kidneys were seen at 48 mg/m^3. No histopathological correlate was found with respect to increased absolute lung weights. At 12 mg/m^3 the only effect seen was an increase in GPT levels in males. The NOAEC was 2 mg/m^3.

In a subchronic oral toxicity study with rats (OECD TG 408), the animals were exposed to 0, 2.5, 12 and 60 mg/kg bw/day by gavage over 3 months. Liver, white and red blood cells, kidneys, adrenal glands and heart were the target organs for toxic effect showing also histopathological alterations. At the high dose level (60 mg/kg bw/day) body weight development/food consumption were clearly impaired and the general state of health was poor. The absolute testes weight was decreased and an atrophy of the seminiferous tubuli and a reduced content of the seminal vesicle were noted. These changes were interpreted as consequence of the marked impairment on body weight. While the toxic effects at the mid dose of 12 mg/kg bw/day were generally less pronounced, a NOAEL was achieved at 2.5 mg/kg bw/day.

The substance showed no genotoxic effects in the Ames test (OECD TG 471), cytogenetic assay with CHO cells (OECD TG 473) and HGPRT assay (OECD TG 476) when tested up to the cyto-/bacteriotoxic range.

In rat 90-day oral and inhalation studies the substance showed no direct adverse effects to the male and female reproductive organs (testes, ovaries and uterus examined). The observed effects on testes being a secondary non-specific consequence of the severe systemic toxicity (e.g. decrease in body weight) seen at the same dose level. A fertility study is not required under SIDS due to the existence of good 90 day repeated dose toxicity studies with

histopathological evaluation of the sex organs.

In a developmental toxicity study (OECD TG 414) the test substance (0, 5, 15 or 45 mg/kg bw/day) was administered from day 6 to 19 post-coitum orally by gavage to rats. The NOAEL for maternal toxicity was 5 mg/kg bw/day. Slight fetotoxicity (retardation of ossification of skull bones) without teratogenicity was observed at 45 mg/kg bw/day, together with severely reduced body weight of the dams. The NOAEL for developmental toxicity was 15 mg/kg bw/day.

Environment

2,2 -dimethyl-4,4 methylenebis(cyclohexylamine) has a water solubility of 3.6 g/l, a vapour pressure of 0.08 Pa and a measured log Kow of 2.51. However, due to the Lewis base character of the substance the experimental determination of the log Kow is inaccurate.

From the physico-chemical properties the hydrosphere is identified as target compartment for the substance. According to OECD criteria the substance is not biodegradable even with adapted inoculum (OECD TG 302B <1 % after 28 days) and can only be poorly eliminated in sewage water treatment plants. Due to the chemical structure of 2,2 -dimethyl-4,4 methylenebis(cyclohexylamine) hydrolysis is not likely to occur under environmental conditions. In the atmosphere the substance is quickly degraded by photochemical attack (half life =3.1 hours). The log K_{OC} was calculated to 3.26. It has to be considered however, that as a basic compound cyclohexylamine can additionally be bound to the soil by ion exchange. The following aquatic effects concentrations are available:

Leuciscus idus: LC_{50} (96 h) > 22 < 46 mg/l,
Daphnia magna: EC_{50} (48h) = 15.2 mg/l,
Scenedesmus subspicatus: ErC_{50} (72 h) > 5 mg/l; EbC_{50} (72 h) = 2.1 mg/l

With these data the substance is considered as toxic to aquatic organisms. With an assessment factor of 1000 a PNECaqua of 2.1 µg/l can be derived. Results from prolonged or chronic studies are not available. No data are available on terrestrial organisms.

Exposure

The global production volume of 2,2'-dimethyl-4,4'methylenebis(cyclohexylamine) (DMD) in 2000 amounts to 1000 – 5000 t. The total volume was produced in Germany by one company. The substance is mainly used as a hardener in epoxy resins and polyamides. No relevant releases to the environment could be identified. The exposure of workers at the manufacturing and processing site is controlled.

NATURE OF FURTHER WORK RECOMMENDED

No further work is recommended unless information regarding significant exposure becomes available.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

The following aquatic effects concentrations of DMD were found in acute toxicity studies on fish, daphnia, algae and bacteria (BASF AG 1987, 1988c, 1988d, 1989):

Leuciscus idus:	LC_{50} (96 h) > 22 < 46 mg/l
Daphnia magna:	EC_{50} (24 h) = 25.5 mg/l
	EC_{50} (48 h) = 15.2 mg/l
Scenedesmus subspicatus:	ErC_{50} (72 h) > 5 mg/l
	EbC_{50} (72 h) = 2.1 mg/l
	ErC_{10} (72 h) = 1.25 mg/l
	EbC_{10} (72 h) = 0.44 mg/l
Pseudomonas pudita:	EC_{50} (17 h) = 96 mg/l

Based on these data DMD is considered as toxic to aquatic organisms (lowest EC/LC_{50} > 1 < 10 mg/l). Results from prolonged or chronic studies are not available (BASF AG 1987, 1988c, 1988d, 1989).

The lowest effect value found was the 72h-EbC_{50} for *Scenedesmus subspicatus* of 2.1 mg/l. Although growth rate can regarded as more reliable parameter in algae growth inhibition tests, the ErC_{50} is not used as basic value for the PNECaqua derivation as no exact value was found for this endpoint and the difference between the two values is only a factor of 2.

With an assessment factor of 1000 a PNECaqua of 2.1 µg/l can be derived. This assessment factor is proposed as only short-term tests are available.

4.2 Terrestrial Effects

No relevant releases to the environment could be identified. Therefore, studies on terrestrial organisms are considered not to be necessary.

4.3 Other Environmental Effects

None.

4.4 Initial Assessment for the Environment

The worldwide production volume of DMD was 1000 - 5000 t in 2000. The total volume was produced in Germany by one company. The substance is used as hardener in epoxy resins and polyamides. No relevant releases into the environment could be identified.

The substance has a low bioaccumulation potential in aquatic organisms. According to OECD criteria the substance is not biodegradable even with adapted inoculum (OECD 302 B: < 1 % after 28 days) and can only be poorly eliminated in sewage treatment plants. Due to the chemical structure hydrolysis is not likely to occur under environmental conditions. The half-life for photochemical oxidative degradation in the atmosphere was calculated to 3.1 h.