要　旨

試験委託者　　環境省

表　　題　　3，4－ジクロロニトロベンゼンの藻類（*Pseudokirchneriella subcapitata*）に対する生長阻害試験

試験番号　　Ｎｏ．２００４－生６８

試験法ガイドライン

本試験は、厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」（薬食発第 1121002 号、平成 15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日）に準拠して実施した。

1）被験物質	：	3，4－ジクロロニトロベンゼン
2）暴露方式	：	止水式、振盪培養（100rpm）
3）供試生物	：	*Pseudokirchneriella subcapitata*（ATCC 22662）
4）暴露期間	：	72時間
5）試験濃度（設定値）	：	対照区，0.22，0.46，1.0，2.2，4.6，10 mg/L 公比；　2.2
6）試験溶液量	：	100 mL（OECD 培地）／容器
7）連数	：	3 容器／試験濃度区、6 容器／対照区
8）初期細胞濃度	：	0.5×10^{4} cells/mL
9）試験温度	：	23±2 ℃
10）照明	：	60 ～ 120 μE/m²/s（フラスコ液面付近）で連続照明
11）ｐＨ	：	試験溶液のｐＨ調整は行わない
12）分析法	：	HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質濃度は暴露開始時および暴露終了時の測定値を用いて幾何平均値(揮散が主因と思われる濃度減少が認められたため)を求め、各影響濃度を算出した。

2)生長速度の比較による阻害濃度

50 %生長阻害濃度 E_rC_{50}(0-72)　：2.50 mg/L(95%信頼限界：2.29 ～ 2.73 mg/L),Logit

最大無影響濃度 NOEC(Rate 0-72)　：0.72 mg/L

3)生長曲線下面積の比較による阻害濃度

50 %生長阻害濃度 E_bC_{50}(0-72)　：1.07 mg/L(95%信頼限界：1.00 ～ 1.15 mg/L),Logit

最大無影響濃度 NOEC(Area 0-72)　：0.34 mg/L

Figure 1. Algal Growth Curve of *Pseudokirchneriella subcapitata*
(Mean cell counts *vs* time during the 72-hour exposure)

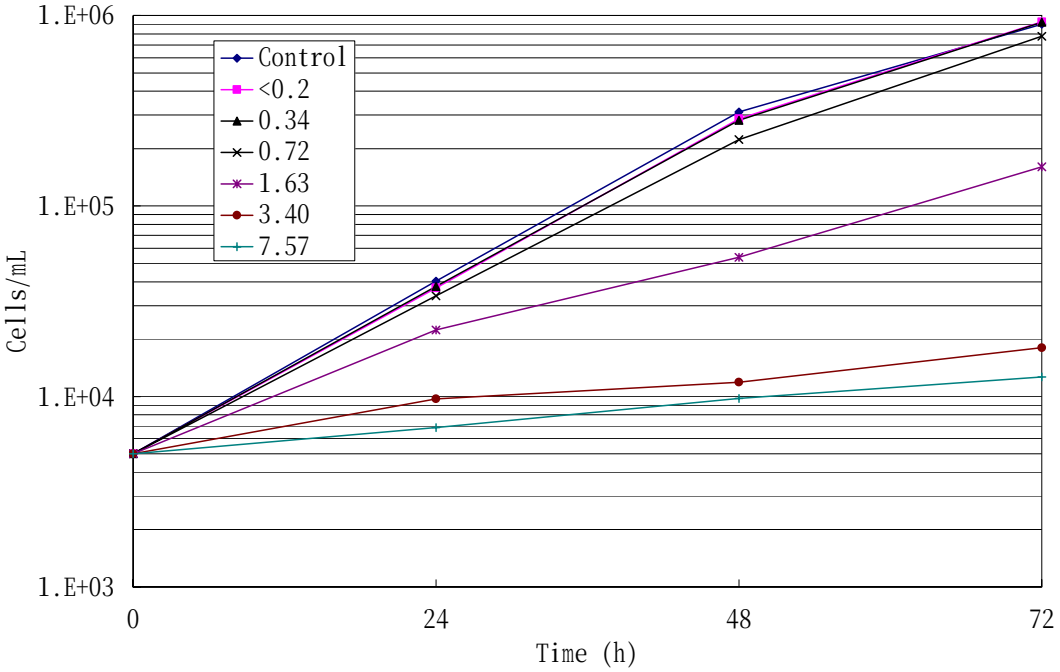


Figure 2. Concentration-Inhibition Curve Based on I_μ values Calculated from the Growth Rates

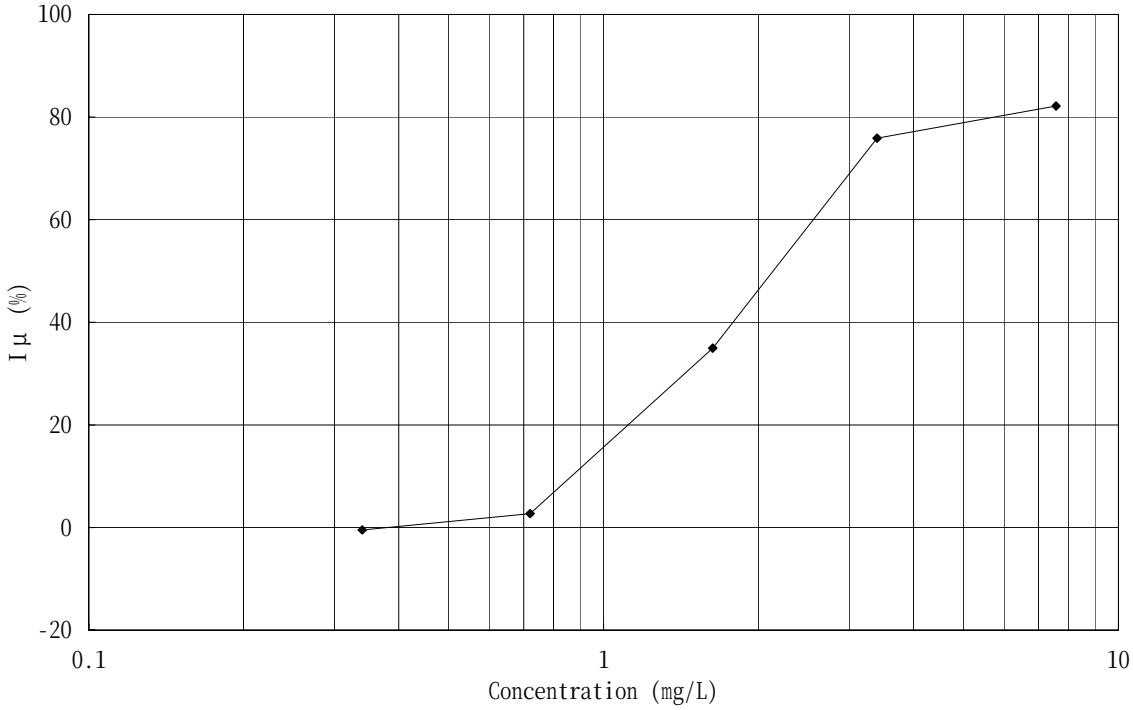
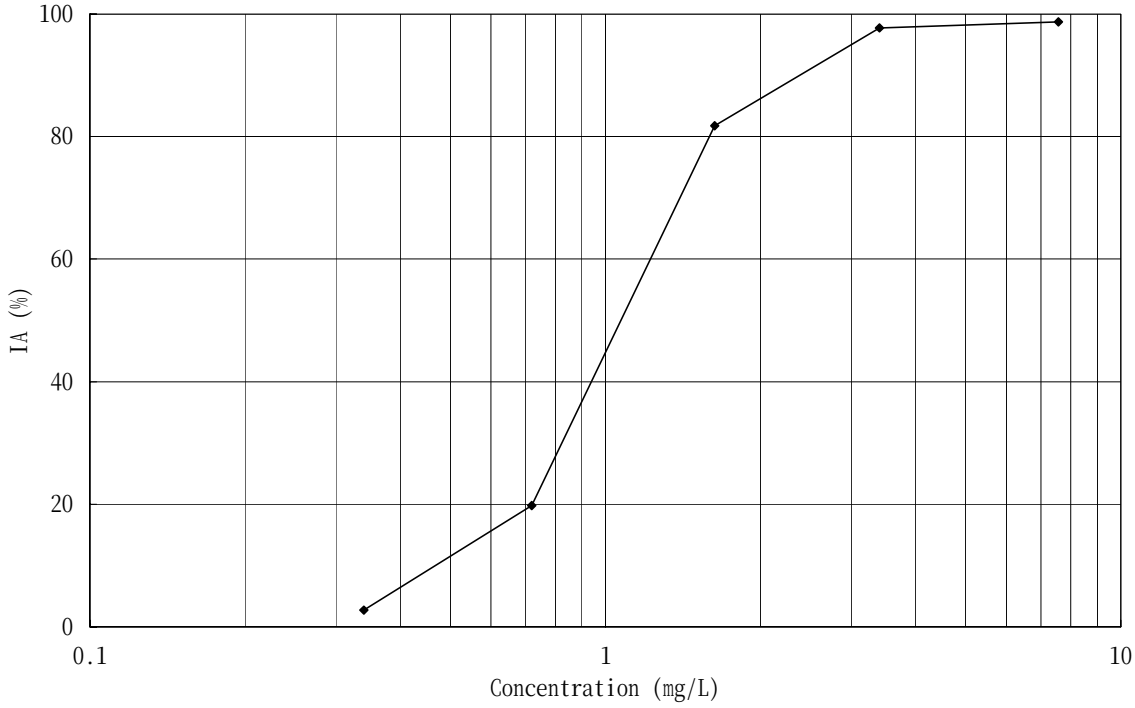


Figure 3. Concentration-Inhibition Curve Based on I_A Values Calculated from the Area under the Growth Curves



要　旨

試験委託者　環境省

表　題　3，4－ジクロロニトロベンゼンのオオミジンコ（*Daphnia magna*）に対する急性遊泳阻害試験

試験番号　Ｎｏ．２００４－生６９

試験法ガイドライン

本試験は、厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」（薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日）に準拠して実施した。

1）被験物質	：	3，4－ジクロロニトロベンゼン
2）暴露方式	：	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）試験濃度（設定値）	：	対照区，1.0，1.8，3.2，5.6，10 mg/L
		公比；　1.8
6）試験溶液量	：	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／8 時間暗
11）給餌	：	無給餌
12）ｐＨ	：	試験溶液のｐＨ調整は行わない
13）分析法	：	HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質の濃度は暴露開始時および暴露終了時の測定値を用いて算術平均値(分析の測定誤差と考えられたため)を求め、各影響濃度を算出した。

2)24 時間暴露後の結果

50 %遊泳阻害濃度（EC_{50}）：6.21 mg/L(95%信頼限界：4.56 ～ 8.45 mg/L),Binomial

0 %阻害最高濃度　　：4.56 mg/L

100 %阻害最低濃度　　：8.45 mg/L

3)48 時間暴露後の結果

50 %遊泳阻害濃度（EC_{50}）：5.23 mg/L(95%信頼限界：4.63 ～ 6.14 mg/L),Probit

0 %阻害最高濃度　　：2.86 mg/L

100 %阻害最低濃度　　：8.45 mg/L

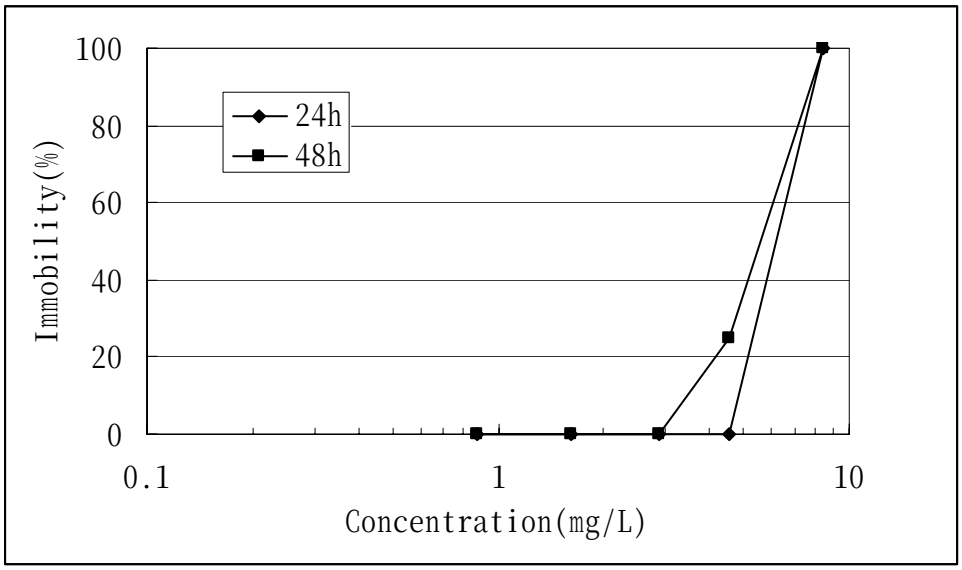
Table 8. Total Hardness(as $CaCO_3$)

(Static Condition)

Nominal Concentration (mg/L)	Mean[a] Measurd Concentration (mg/L)	Total Hardness(as $CaCO_3$),mg/L	
		0 Hour new	48 Hours old
Control	—	255	260
1.0	0.87	258	261
1.8	1.62	255	263
3.2	2.86	258	264
5.6	4.56	259	264
10	8.45	259	260

new: Freshly prepared test solutions
old: Test solutions after 48 hour exposure
a: Arithmetic mean
—: Not calculated

Figure 1. Concentration-Response (Immobility) Curve



要　旨

試験委託者　　環境省

表　題　　３,４－ジクロロニトロベンゼンのヒメダカ(*Oryzias latipes*)に対する急性毒性試験

試験番号　　Ｎｏ．２００４－生７０

試験法ガイドライン

本試験は厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」(薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日)に準拠して実施した。

1)被験物質	：	３,４－ジクロロニトロベンゼン
2)暴露方式	：	半止水式（48時間目に試験溶液の全量を交換）
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, 1.0, 1.8, 3.2, 5.6, 10 ㎎/L
		公比；　1.8
6)試験溶液量	：	3 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／8 時間暗
11)給餌	：	無給餌
12)通気	：	なし
13)ｐH	：	試験溶液のｐH調整は行わない
14)分析法	：	HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質の濃度は、一部に分析誤差も考えられるものの、揮散による影響が変動の主因と判断し、各測定値の時間加重平均値(暴露開始時と 48 時間換水前および 48 時間換水後と暴露終了時の対数平均を算出し、それらの算術平均値)を採用した。

2)50 %死亡濃度

24 時間 50 %死亡濃度(LC_{50}): >8.48 mg/L

48 時間 50 %死亡濃度(LC_{50}): >8.48 mg/L

72 時間 50 %死亡濃度(LC_{50}): 7.59 mg/L(95%信頼限界：4.70 ～ 9.52 mg/L),Probit

96 時間 50 %死亡濃度(LC_{50}): 4.65 mg/L(95%信頼限界：3.66 ～ 6.05 mg/L),Probit

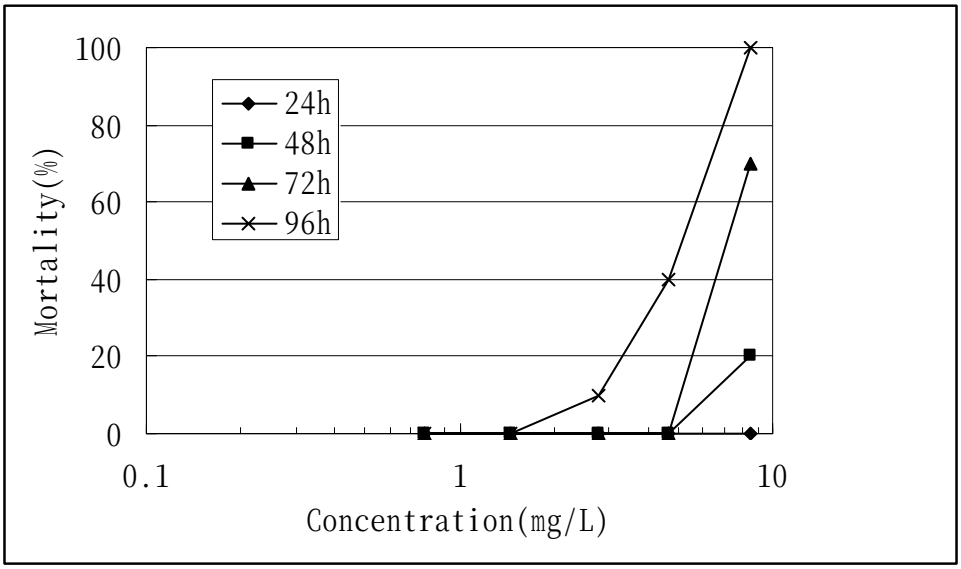
Table 8. pH Values

(Semi-Static Condition)

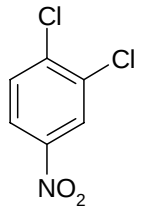
Nominal Concentration (mg/L)	Mean[a] Measured Concentration (mg/L)	pH					
		0 Hour	24 Hours	48 Hours		72 Hours	96 Hours
		new		old	new		old
Control	—	7.6	7.0	6.9	7.6	7.0	7.0
1.0	0.77	7.6	7.0	6.9	7.5	7.0	7.1
1.8	1.46	7.6	7.1	6.9	7.5	7.0	7.0
3.2	2.80	7.6	7.1	7.0	7.5	7.0	7.1
5.6	4.63	7.6	7.1	6.9	7.6	6.9	7.0
10	8.48	7.7	7.0	6.8	7.6	7.0	7.0

new: Freshly prepared test solutions
old: Test solutions after 48 hour exposure
a: Time-weighted mean
—: Not calculated

Figure 1. Concentration—Response (Mortality) Curve



SIDS INITIAL ASSESSMENT PROFILE

CAS No.	99-54-7
Chemical Name	1,2-Dichloro-4-nitrobenzene
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

1,2-Dichloro-4-nitrobenzene is absorbed from the gastro-intestinal tract and although there are some species differences in experimental animals from the available data it can be concluded that 1,2-dichloro-4-nitrobenzene is excreted mainly via urine in the form of the mercapturic acid derivate N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine. Data on humans were not identified in the available literature.

There are no valid acute inhalation studies available. Based on the results of the acute dermal toxicity study with rats the LD50 is > 2000 mg/kg bw. From studies with rabbits no LD50 could be derived, the lowest Lethal Dose Level (LDLo) was 950 mg/kg bw. The acute oral toxicity in rats ranges from 625 to 950 mg/kg bw. 1,2-Dichloro-4-nitrobenzene causes the formation of methaemoglobin. Predominant signs of intoxication were lethargy, increasing weakness, collapse and coma.

1,2-Dichloro-4-nitrobenzene gave no skin irritation effects when tested for 4 hours under semiocclusive conditions according to OECD TG 404 and showed slightly irritating effects, which disappeared within 72 hours under occlusive conditions according to the method of Federal Register 38 No. 187. 1,2-Dichloro-4-nitrobenzene is slightly irritating to the eyes when tested according to OECD TG 405. 1,2-Dichloro-4-nitrobenzene was not found to induce dermal sensitization when tested according to OECD TG 406. In addition, 1,2-dichloro-4-nitrobenzene was not found to induce dermal sensitization in humans in a limited study.

The main targets identified in animal studies after repeated oral administration as well as after inhalation exposure are the haematological system and in addition the kidneys after oral application and the liver after inhalation. From a 28-day oral study performed according to OECD TG 407 a NOAEL of 4 mg/kg bw/day was derived. The NOAEL following subchronic inhalation exposure study of limited validity (limited documentation) was 0.4 mg/m³ (4 hours per day).

Changes in haematological parameters (e.g. methaemoglobinaemia, Heinz bodies) are the main target in the only available report on exposure of workers. As these findings relate to mixed exposures they cannot be clearly attributed to 1,2-dichloro-4-nitrobenzene, but would be plausible, because they were also observed in animal experiments. In the recent open literature reports of human poisoning could not be identified.

1,2-Dichloro-4-nitrobenzene exhibits mutagenic activity in *Salmonella typhimurium* but not in the HPRT test in Chinese Hamster Ovary (CHO) cells. 1,2-Dichloro-4-nitrobenzene induced chromosomal aberrations in V79 cells with metabolic activation only at the highest concentration, which was cytotoxic. In insects (*Drosophila melanogaster*) 1,2-dichloro-4-nitrobenzene revealed no mutagenic activity in the SLRL-test after application over 3 days with slight increased toxicity, but revealed mutagenic activity following a single i.p. injection of a clearly toxic dose. 1,2-Dichloro-4-nitrobenzene showed no clastogenic activity *in vivo* in a chromosomal aberrations test with rats. Overall in non-toxic doses, there was no evidence for genotoxicity *in vivo* under the conditions tested.

Studies dealing specifically with toxicity to reproduction were not identified. The subacute study with 1,2-dichloro-4-nitrobenzene yielded no damage of the reproductive organs in rats despite clear systemic toxicity up to the maximum tolerated dose of 100 mg/kg bw.

1,2-Dichloro-4-nitrobenzene commercial grade (85% 1,2-dichloro-4-nitrobenzene and 15% 1,2-dichloro-3-nitrobenzene) caused effects on development at maternally toxic doses probably due to methaemoglobinaemia in dams and foetuses. A significant dose-response trend for variations (dilated ureters) was seen in the foetuses of the >= 30 mg/kg bw/day-groups and significant reduced body weight gain of dams at dose levels of 30 mg/kg bw/day on gd 6-10 with an even stronger effect at 100 mg/kg bw/day. Thus, 10 mg/kg bw/day was determined as the NOAEL for maternal and developmental toxicity.

Environment

1,2-Dichloro-4-nitrobenzene is a yellow substance with a melting point of 43 °C, a boiling point of 255 °C, a flash point of 155 °C, and an ignition temperature of 420 °C. With a density of 1.56 g/cm³ at 15 °C and 1.487 g/cm³ at 50 °C 1.2-dichloro-4-nitrobenzene is heavier than water. The substance is slightly soluble in water with 121 mg/l at 20 °C. The vapour pressure was determined to be 2 Pa at 25 °C. A log Kow of 3.04 at 25 °C was experimentally determined.

With regard to its chemical structure 1,2-dichloro-4-nitrobenzene is not expected to hydrolyse under environmental conditions. According to the Mackay level I fugacity model, the main target compartments for 1,2-dichloro-4-nitrobenzene are air (48 %) and water (44 %). The measured Henry's law constant of 0.82 $Pa*m^3*mol^{-1}$ indicates that the compound has a low to moderate potential for volatilization from surface waters.

In the atmosphere slow photodegradation takes place by reaction with photochemically produced OH radicals. The atmospheric half-life is calculated to be 321 days with an atmospheric concentration of 0.5 x 10^6 hydroxyl radicals/cm^3 as a 24 h average. 1,2-Dichloro-4-nitrobenzene will undergo direct photolysis in air due to absorbance of environmental UV light, however, the respective half-life is not known. In water, no photolysis will occur to a significant extent.

1,2-Dichloro-4-nitrobenzene is not readily biodegradable (Manometric respirometry test: biodegradation < 10 % after 21 days based on BOD; OECD TG 301 C biodegradation 0 % within 28 days, presumably due to inhibition of inoculum). 1,2-Dichloro-4-nitrobenzene is biodegradable by adapted microorganisms under aerobic conditions and by non-adapted inocula under anaerobic conditions (primary degradation). Sewage from adapted wastewater treatment plants has significant potential to primary degrade 1,2-dichloro-4-nitrobenzene (Test method "Simulation of an industrial waste water treatment plant": after 3 days 100 %).

Bioconcentration factors determined for fish were in the range of 26 – 65. A measured Koc (Koc = 417) for sediment suggests the substance to have a medium geoaccumulation potential.

Concerning the acute toxicity of 1.2-dichloro-4-nitrobenzene towards aquatic species reliable experimental results of tests with fish, *Daphnia*, and algae are available. The acute toxicity determined for fish (*Leuciscus idus*) was of 3.1 mg/l (48 h LC_{50}) [DIN 38412 L15] and *Daphnia* (*Daphnia magna*) of 3 mg/l (24 h-EC_{50}) [DIN 38412 L11]. In the growth inhibition test with algae (*Scenedesmus obliquus)* the value 5.8 mg/l was achieved after 48 h (48 h-ErC_{50}) [OECD TG 201]. For the algae *Chlorella fusca* a value of 0.32 mg/l was found after 24 h (24 h-ErC50).
In a chronic (21 d) study with *Daphnia magna* a NOEC of 0.025 mg/l was determined for the most sensitive endpoint reproduction rate. An E_rC_{10} > 0.1 mg/l was reported for the algae *Scenedesmus subspicatus* after 48 hours
For terrestrial organisms the lowest measured 6d-EC_{50} for was 27 mg/l for the plant *Phaseolus aureus*.
Applying an assessment factor of 50 to the lowest available chronic value of 25 µg/l (21d reproduction in *D. magna*), a $PNEC_{aqua}$ of 0.5 µg/l is obtained.

Exposure

About 36,800 tonnes of 1,2-dichloro-4-nitrobenzene were produced worldwide (excluding Eastern Europe) in 2001. 1,2-Dichloro-4-nitrobenzene is a basic chemical for the synthesis of intermediates which are further processed to herbicides, bactericides, and dyestuffs. A direct use of 1,2-dichloro-4-nitrobenzene is not known in the Sponsor country. 1,2-Dichloro-4-nitrobenzene is not contained in products registered in the Danish, Finnish, Norwegian, Swedish and Swiss Product Registers.

In the Sponsor country, 1,2-dichloro-4-nitrobenzene is manufactured and processed in closed systems. From this site the effluent concentrations was below the detection limit of 2 µg/l.

In Gemany in 1999, the 90-percentile of the 1,2-dichloro-4-nitrobenzene concentrations in the River Rhine was < 0.5 µg/l and in the River Danube < 0.02 µg/l. For the River Elbe the maximum was < 0.02 µg/l.
A non-quantifiable contamination of the terrestrial compartment by 1,2-dichloro-4-nitrobenzene might result from the application of herbicides manufactured from 3,4-dichloroaniline. This assumption is based on the observation that during the biodegradation of such herbicides 3,4-dichloroaniline is formed that in trace amounts may be oxidized biotically or abiotically to 1,2-dichloro-4-nitrobenzene. However, a significant exposure of the terrestrial compartment by this source is not expected.

Exposure is well controlled in occupational settings of the main producer in the Sponsor country and the exposure of workers is well below the workplace guidance value (ARW) of 1 mg/m^3 for 1,2-dichloro-4-nitrobenzene recommended by the German Association of the Chemical Industry (VCI).
The levels of 3,4-dichloro-aniline-adducts in blood and of 3,4-dichloro-aniline in urine of manufacturing and processing plants workers were never higher than 5 % of the tolerance values (no health effect for worker in case that value is not exceeded).

Based on the very low emissions of 1,2-dichloro-4-nitrobenzene into air and water by the manufacturing and processing plants in the Sponsor country, on the very low environmental concentrations, and on the low bioaccumulation potential, a significant indirect exposure of the general public via the environment or via the food chain is not expected.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

The chemical possesses properties indicating a hazard for human health (principally haematological toxicity, and developmental toxicity, probably linked to methemoglobinemia) and the environment. Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low, exposure is controlled in occupational settings, and exposure of consumers is not known to occur. Therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

1,2-Dichloro-4-nitrobenzene exhibits mutagenic activity in *Salmonella typhimurium* but not in the HPRT test in *Chinese Hamster* Ovary (CHO) cells. 1,2-Dichloro-4-nitrobenzene induced chromosomal aberrations in V79 cells with metabolic activation only at the highest concentration, which was cytotoxic. In insects (*Drosophila melanogaster*) 1,2-dichloro-4-nitrobenzene revealed no mutagenic activity in the SLRL-test after application over 3 days with slight increased toxicity, but revealed mutagenic activity following a single i.p. injections of a clearly toxic dose. 1,2-Dichloro-4-nitrobenzene showed no clastogenic activity in vivo in a chromosomal aberrations test with rats. Overall, in non-toxic doses, there was no evidence for genotoxicity in vivo under the conditions tested. Studies dealing specifically with toxicity to reproduction were not identified. The subacute study with 1,2-dichloro-4-nitrobenzene yielded no damage of the reproductive organs in rats despite clear systemic toxicity up to the maximum tolerated dose of 100 mg/kg bw/day.

1,2-Dichloro-4-nitrobenzene commercial grade (85 % 1,2-dichloro-4-nitrobenzene and 15 % 1,2-dichloro-3-nitrobenzene) caused effects on development at maternally toxic doses probably due to methaemoglobinaemia in dams and foetuses. A significant dose-response trend for variations (dilated ureters) was seen in the foetuses of the >= 30 mg/kg bw/day-groups and significantly reduced body weight gain of dams at dose levels of 30 mg/kg bw/day on gd 6 - 10 with an even stronger effect at 100 mg/kg bw/day. Thus, 10 mg/kg bw/day was determined as NOAEL for maternal and developmental toxicity.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

In this chapter in general only the lowest valid test concentrations of acute and chronic testing are presented.

Acute Toxicity Test Results

Acute toxicity to fish (*Leuciscus idus*) has been tested in accordance to the German standard method for water, wastewater and sludges DIN 38412 Part 15. A 48 h LC_{50} of 3.1 mg/l was measured (Knie et al., 1983). With *Leuciscus idus melanotus* in a static system Hoechst AG (1980) observed an acute toxicity (96 h LC_{50}) of 5.2 mg/l. Acute toxicity to fish (*Oryzias latipes*) has also been tested in a static system according to Japanese Industrial Standard (JIS) K 0102-1986-71. The result (48 h LC_{50}) was 7 mg/l (MITI, 1992).

With *Daphnia* acute tests were performed according to standard procedures. In a study according to the German standard method for water, wastewater and sludges DIN 38412 Part 11 the toxicity to *Daphnia magna* was tested during 24 h resulting in an EC_{50} of 3 mg/l (Knie et al., 1983). Using a method analog to OECD Guideline 202 Zhao et al. (1997) reported an EC_{50} (48 h) of 8.2 mg/l.

In a one generation, non-guideline study with the green alga *Chlorella fusca* (= *Scenedesmus vacuolatus*) a 24 h E_rC_{50} = 0.32 mg/l was obtained in a growth inhibition test (Schmitt et al., 2000). With *Scenedesmus obliquus* the 48h-E_rC_{50} of 5.8 mg/l was found (Liu and Lang, 1995).

All effect values are related to nominal concentrations. As 1,2-dichloro-4-nitrobenzene is low to moderately volatile (Henry's law constant 0.82 Pa*m³/mol; Thomas, 1990) it cannot be excluded that a decrease in test substance concentration has occurred during the studies that have been performed in open systems. In a stability experiment performed with 1,2-dichloro-3-nitrobenzene in an open system a continuous decrease in substance concentration was observed. After 1, 2, 4 and 8 days the concentration of 1,2-dichloro-3-nitrobenzene has decreased by 6 %, 17 %, 22 % and 36 %. The authors attributed this decline in concentration to evaporation (Canton et al., 1985). Although this stability experiment cannot be directly transferred to the 1,2-dichloro-4-nitrobenzene, it can

give an indication of the degree of test substance loss during the above mentioned ecotoxicity studies. Although neither an exact validated vapor pressure is available for 1,2-dichloro-3-nitrobenzene nor a measured Henry's law constant, it can be estimated that the volatility of 1,2-dichloro-3-nitrobenzene is in the same order than the volatility of 1,2-dichloro-4-nitrobenzene. Therefore, it can be concluded from the above mentioned stability experiment that within a period of 4 days the decrease in 1,2-dichloro-4-nitrobenzene concentration will be </= 22 % and therefore the nominal concentrations are acceptable. This is also confirmed by volatilisation studies performed for other substances with Henry's law constants in a similar range (e.g. 3-methylbut-2-en-1-ol: Henry´s law constant 0.73 - 1.4 Pa*m^3/mole, 93 % recovery rate after 4 days).

Chronic Toxicity Test Results

In a reproduction (21 d) study with *Daphnia magna* performed in a semistatic system in closed vessels Kuehn et al. (1988) found a NOEC of 0.025 mg/l for the most sensitive endpoint reproduction rate. The stability of the test substance concentration was confirmed by analytical monitoring. In the same publication also an E_rC_{10} > 0.1 mg/l was determined for the algae *Scenedesmus subspicatus* after 48 hours

Thus the lowest chronic value is the NOEC of 25 µg/l *for Daphnia magna*. is. Since there are chronic studies from 2 trophic levels, an assessment factor of 50 (following the EU Technical Guidance Document) is applied and a **$PNEC_{aqua}$ of 0.5 µg/l** is obtained.

Toxicity to Microorganisms

Regarding the toxicity to microorganisms, a O_2-consumption test in accordance to Robra with *Pseudomonas putida* during 30 minutes was performed and an EC_{10} of 44 mg/l was determined (Knie et al., 1983).

For the protozoan species *Tetrahymena pyriformis* a 40 h EC_{50} of 13 mg/l was found in a population growth inhibition test (Schultz 1999).

In an 88 h assay with the fungus *Rhizoctonia solani* Eckert (1962) observed an EC_{50} of 21 mg/l. Hafsah, Tahara, and Mizutani (1984) reported that the fungus *Mucor javanicus* showed 55 % growth inhibition at 50 mg/l of 1,2-dichloro-4-nitrobenzene.

4.2 Terrestrial Effects

Acute Toxicity Test Results

No test result with plants according to OECD-Guideline 208 (Terrestrial plant growth test) is known. In humid sand, with the endpoint growth of seedlings the 6d-EC_{50} of 1,2-dichloro-4-nitrobenzene was 27 mg/l for *Phaseolus aureus* and 56 mg/l for *Cucumis sativus* (Eckert, 1962).

For the endpoint growth of seedlings (biomass), the EC_{50} of *Lactuca sativa* was measured for various chloro(nitro)benzenes and other compounds including e.g. the isomer 1,2-dichloro-3-nitrobenzene, but not 1,2-dichloro-4-nitrobenzene. For 1,2-dichloro-3-nitrobenzene the EC_{50} was > 0.32 and < 1 mg/l after 16 to 21 days (Hulzebos et al. 1993). An equation for the calculation of the EC was derived (log EC_{50} = -0.46 log K_{ow} + 2.38 [µmol/l], Hulzebos et al., 1993), which was used to calculate the EC_{50} of 1,2-dichloro-4-nitrobenzene (log K_{ow} = 3.04) to be about 1.8 mg/l.

4.3 Other Environmental Effects

No data available.

Table 13 Acute and chronic toxicities of 1,2-dichloro-4-nitrobenzene

Trophic level	*Species*	Test	Result	Source	IUCLID
Fish	*Leuciscus idus*	48 h LC_{50}	3.1 mg/l	Knie et al. (1983)*	4.1
Fish	*Leuciscus idus melanotus*	96 h LC_{50}	5.2 mg/l	Hoechst AG (1980)	4.1
Fish	*Oryzias latipes*	48 h LC_{50}	7 mg/l	MITI (1992)	4.1
Daphnia	*Daphnia magna*	24 h EC_{50}	3 mg/l	Knie et al. (1983)*	4.2
Daphnia	*Daphnia carinata*	48 h EC_{50}	8.2 mg/l	Zhao et al. (1997)	4.2
Algae	*Chlorella fusca* (= *Scenedesmus vacuolatus*)	24 h E_rC_{50}	0.32 mg/l	Schmitt et al. (2000)	4.3
Algae	*Scenedesmus obliquus*	48h E_rC_{50}	5.8 mg/l	Liu and Lang (1995)*	4.3
Algae	*Scenedesmus subspicatus*	48h E_rC_{10}	>0.1 mg/l	Kuehn et al. (1988)*	4.3
Bacteria	*Pseudomonas putida*	30 min EC_{10}	44 mg/l	Knie et al. (1983)	4.4
Protozoa	*Tetrahymena pyriformis*	40 h EC_{50}	13 mg/l	Schultz (1999)	4.4
Fungus	*Rhizoctonia solani*	88 h EC_{50}	21 mg/l	Eckert (1962)	4.4
Fungus	*Mucor javanicus*	6 d E_rC_{55}	50 mg/l	Hafsah, Tahara, and Mizutani (1984)	4.4
Daphnia	*Daphnia magna*	21 d NOEC	0.025 mg/l	Kuehn et al. (1988)*	4.5.2
Terrestrial plant	*Phaseolus aureus*	6d EC_{50}	27 mg/l	Eckert (1962)	4.6.2

*Studies used for assessment

5 RECOMMENDATIONS

Environment:

The chemical possesses properties indicating a hazard for the environment. Based on data presented by the sponsor country, exposure to the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the sponsor.

Human Health:

The chemical possesses properties indicating a hazard for human health (principally haematological toxicity, and developmental toxicity, probably linked to methemoglobinemia). Based on data presented by the sponsor country, exposure is controlled in occupational settings, and exposure of

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	other: not specified
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	2.9
LC50	:	3.1
LC100	:	3.3
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN-Standard 38412 L15 (Fish short-time test)
Year	:	1983
GLP	:	no data
Test substance	:	other TS: no purity given

Method	:	Method of the German Standards Institution, Berlin, Germany
Reliability	:	(2) valid with restrictions Test procedure according to national standard method
Flag	:	Critical study for SIDS endpoint

23.10.2003 (66)

Type	:	static
Species	:	Leuciscus idus melanotus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	= 4.5
LC50	:	= 5.2
LC100	:	= 5.6
EC0	:	= 4
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1980
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Test condition	:	- Fish were obtained from commercial source and kept 20 d before start of experiment in dechlorinated water in test facility - Body weight 1.3 -2.6 g (mean 1.9) - Body length 4.9 -6.2 cm - Fed with Tetra Min (Tetra-Werke, Melle, Germany) - For experiments deionized tap water amended with 192 mg/l NaHCO3, 120 mg/l CaSO4 x 2 H2O, 120 mg/l MgSO4, and 8 mg/l KCl. The total hardiness was 9.5 °d and the carbonate hardiness 6.4 °d - pH (including fish) 7.0 - 8.4 - Aquaria 40 x 25 x 30 cm3 containing 20 l test medium at 20 +/- 1 °C, aeration 100 ml/min, oxygen > 7 mg/l - 12 h light (700 lux), 12 h dark - 65 h before start of test fish were put into aquaria - Test substance suspneded with Ultra turrax and aliquot grought into aquarium to give final concentration (including suspended test substance)
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods
Flag	:	Critical study for SIDS endpoint

23.10.2003 (67)

Type	:	static
Species	:	Oryzias latipes (Fish, fresh water)

Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	= 7.01
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: Japanese Industrial Standard (JIS K 0102-1986-71) "Testing methods for industrial waste water"
Year	:	1992
GLP	:	no data
Test substance	:	other TS: no purity given
Test condition	:	- Orange-red killifish (Oryzias latipes) was obtained from Nakashima fish farm, Daimyojin Nagasu-cho Tamana-gun Kumamot 869-01 Japan - After external desinfection, the fish were reared in a flow through system for 3 - 5 weeks - Fish were reared in an acclimatization tank for 28 d at 25 +/- 2 °C - Water was groundwater from the Kurume Research Laboratories - Water temperature, pH, dissolved oxygen were continuously measured - Total hardness, COD, chloride, and other parameters were measured every 6 months - Incubation of each 10 fish in round glass vessels containing 4 l of liquid each - Incubation temperature 25 +/- 2 °C - 48 h LC50 was estimated by Doudoroff method or Probit method
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods
Flag	:	Critical study for SIDS endpoint

23.10.2003 (15)

Type	:	static
Species	:	Leuciscus idus melanotus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	= 6.3
LC50	:	= 8
LC100	:	= 10
EC0	:	= 4
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1980
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	- Fish were obtained from commercial source and kept 20 d before start of experiment in dechlorinated water in test facility - Body weight 1.3 -2.6 g (mean 1.9) - Body length 4.9 -6.2 cm - Fed with Tetra Min (Tetra-Werke, Melle, Germany) - For experiments deionized tap water amended with 192 mg/l NaHCO3, 120 mg/l CaSO4 x 2 H2O, 120 mg/l MgSO4, and 8 mg/l KCl. The total hardiness was 9.5 °d and the carbonate hardiness 6.4 °d - pH (including fish) 7.0 - 8.4 - Aquaria 40 x 25 x 30 cm3 containing 20 l test medium at 20 +/- 1 °C, aeration 100 ml/min, oxygen > 7 mg/l - 12 h light (700 lux), 12 h dark - 65 h before start of test fish were put into aquaria - Test substance suspneded with Ultra turrax and aliquot grought into aquarium to give final concentration (including suspended test substance)
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods

23.10.2003 (68)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	= 2
LC100	:	= 50
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1989
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
Test according to national standards

23.10.2003 (69) (8)

Type	:	
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	6.4
Method	:	
Year	:	1994
GLP	:	
Test substance	:	

Result : -log LC50 = 4.48 (mol/l), which equals 6.4 mg/l
Reliability : (4) not assignable
Original reference in Chinese. Most of the data have later been published by Lang et al. (1996) and Zhao et al. (1997)

16.04.2003 (70) (71)

Type	:	semistatic
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	6.4
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: see Test condition
Year	:	1996
GLP	:	no data
Test substance	:	other TS: no purity given

Remark : Since various data of Lang et al. (1996) match (3 digits) these published by Yuan et al. in 1994 and 1995 [Yuan X, Lang P, Long F, Lu G (1994) The Relationship Between Toxicities of Nitroaromatic Hydrocarbons to Photobacterium phosphoreum and Other Aquatic Organisms. Jilin Daxue Ziran Kexue Xuebao (4): 97 - 100; Yuan X, He Y, Lang P (1995) QSAR Study and the Toxicity of Nitroaromatic Compounds to Bacteria in the Songhua River. Huanjing Kexue 16 (5): 18 - 21], it is assumed that Lang et al. (1996) use data of the work of Yuan et al. (1994, 1995). However, the work of Yuan et al. (1994, 1995) is not cited by Lang et al. (1996)
Result : -log EC50 = 4.48 (mol/l), which equals 6.4 mg/l.
Calculation with the energy values of the lowest unoccupied molecular orbital yielded -log LC50 (mol/l) = 4.16, which equals LC50 = 13.3 mg/l
Test condition : The test was performed under the following conditions:
- 1 year old carps, average weight and length 23.8 +/- 6.4 g / 11.6 +/- 2.3

cm
- Sterilized and reared 2 weeks in 5 % (w/v) salt water
- Test water was dechlorinated tap water with 21.45 mg/l chlorine
- Temperature 15 - 18 °C
- pH 7.0 - 7.5
- Oxygen-content 6.35 mg/l at 12.3 °C
- Direct sunlight was avoided
- 5 concentrations were established
- Test aquaria contained 20 l test water and 10 fish
- Test water was replaced twice a day and 10 l, each time
- Acetone used as solvent (0.05 - 0.1 % v/v)

Reliability : (3) invalid
It is not clear how much chlorine was in the "dechlorinated" tap water (see Test conditions, the level reported is toxic to fish).
Although Lang et al. (1996) do not cite previous publications, there is strong evidence, that the data reported by Lang et al. (1996) have been published previously.

04.07.2003 (72)

Type : other: semistatic (water renewal after 12 hours)
Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : 6.4
Method : other: comparable to OECD-Guideline 203 (Fish: Acute Toxicity Test, 1992)
Year : 1994
GLP : no data
Test substance : other TS: no purity given

Remark : Data which are described to be measured by Zhao et al. (1997) have been published by Yuan et al. in 1994 and 1995. Neither the work of Yuan et al. (1994, 1995) nor the work of Lang et al. (1996) is cited by Zhao et al. (1997)
Result : -log LC50 = 4.48 (mol/l), which equals 6.4 mg/l
Test condition : - 60 fish used in each test (fish length 5 cm / fish weight 5 g)
- 10 fish in 16 l of test water
- Temperature 20 +/- 1 °C
- Stock solution was prepared in acetone

Reliability : (3) invalid
The study of Zhao et al. 1997 contains all (except one) carp data of a publication of Lang et al. (1996). However, these authors give a completly different description of their experiments acompared to one used by Zhao et al. (1997) e.g. source, size and age of carps. Since such a similarity in results (3 digits, 18 compounds) is extremely unlikely, it is thought that Zhao et al. (1997) have used published data.

16.04.2003 (31)

Type : other: not specified
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : .5
LC0 : 7
LC100 : 10
Limit test : no
Analytical monitoring : no
Method : other: UBA-Proposal "Letale Wirkung bei Brachidanio rerio" (1982.06.01)
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Test substance	:	The stock solution was prepared by dissolving 1 g test substance in 1 g ethanol before it was diluted to the final concentration (1 g/l) with 2 g/l emulgator W. For this solution, the calculated COD was 958 mg/l, the COD measured 270 mg/l. The pH of this solution was 4.7.
Reliability	:	(3) invalid Test procedure according to national standards. Problems with emulsifying 1,2-dichloro-4-nitrobenzene

16.04.2003 (57)

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	.1
LC0	:	9
LC100	:	10
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: DIN 38412, Part 15 (Fish, short-time test)
Year	:	1984
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Test condition	:	- Brachydanio rerio 30 +/- 5 mm - Temperature: 23 +/-2°C - Aquarium 8.4 l containing 3.5 l tap water - Tap water was filtered through activated carbon and ion exchange resin (to remove copper). It had a Ca/Mg ratio of 4/1 and a German Hardiness of 15 °dH. pH of the filtered tap water was 7.0 +/- 0.2 - 10 animals in each aquarium, aerated - Stock solution was prepared by dissolving 1 g test substance in 1 g acetone before it was diluted to the final concentration (1 g/l) with 1 g/l emulgator W. For this solution, the calculated COD was 958 mg/l, the COD measured 253 mg/l. The pH of this solution was 6.5.
Reliability	:	(3) invalid Test procedure according to national standards. Problems with emulsifying 1,2-dichloro-4-nitrobenzene

16.04.2003 (58)

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	= 10
LC100	:	= 17.8
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1986
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Test condition	:	- Brachydanio rerio 30 +/- 5 mm - Temperature: 23 +/-2°C - Aquarium containing 5 l water and 10 fish - No stock solution prepared but 1,2-dichloro-4-nitrobenzene given directly into the water
Reliability	:	(4) not assignable Only raw data available

14.04.2003 (73)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	7.5
LC50	:	10
LC100	:	13.3
Limit test	:	
Analytical monitoring	:	no
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1986
GLP	:	no
Test substance	:	other TS: no purity given
Test condition	:	- Temperature: 23 +/-2°C - Aquarium containing 10 l water and 10 fish - No stock solution prepared but 1,2-dichloro-4-nitrobenzene given directly into the water
Reliability	:	(4) not assignable Only raw data available

27.06.2003 (74)

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	4
LC50	:	> 4
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: see below
Year	:	1975
GLP	:	no
Test substance	:	no data
Method	:	Standard Methods for the Examination of Water and Wastewater 13th Edition (1971), American Public Health Assn., NY 10019
Reliability	:	(4) not assignable Insufficient data

15.04.2003 (59)

Type	:	
Species	:	other: fish
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	4
Method	:	other: tested with standard EEC or OECD methods or comparable
Year	:	2001
GLP	:	no data
Test substance	:	other TS: no purity given
Remark	:	Ecotoxicological data were taken from literature. Major criteria for the selection of toxicity data were reliability and comparability of test methods. Very old data were generelly discarded.
Reliability	:	(4) not assignable Secondary literature

10.04.2003 (75)

Type	:	static
Species	:	Lepomis macrochirus (Fish, fresh water)

Exposure period : 3 hour(s)
Unit : mg/l
EC0 : 5
Method :
Year : 1957
GLP :
Test substance :

Method : Endpoint: Behaviour (Observation of stress effect)
Remark : Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability : (4) not assignable
Original literature not available
29.06.2003 (76)

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 1 hour(s)
Unit : mg/l
EC0 : 5
Method :
Year : 1957
GLP :
Test substance :

Method : Endpoint: Behaviour (Observation of stress effect)
Remark : Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Result : The same result was obtained after 3 h of incubation
Reliability : (4) not assignable
Original literature not available
29.06.2003 (76)

Type : static
Species : Petromyzon marinus
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : 5
Method :
Year : 1957
GLP :
Test substance :

Method : Endpoint: Behaviour (Observation of stress effect)
Remark : Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability : (4) not assignable
Original literature not available
29.06.2003 (76)

Type : static
Species : Oncorhynchus tschawytscha (Fish, fresh water, marine)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : 10
Method :
Year : 1969
GLP :
Test substance :

Method : Endpoint: Behaviour

Remark	:	Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability	:	(4) not assignable Original literature not available

29.06.2003 (77)

Type	:	static
Species	:	Ptychocheilus oregonensis (Fish, fresh water)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	10
Method	:	
Year	:	1969
GLP	:	
Test substance	:	

Method	:	Endpoint: Behaviour
Remark	:	Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability	:	(4) not assignable Original literature not available

29.06.2003 (77)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	other: not specified
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	= 2
EC50	:	= 3
EC100	:	= 11
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 11 (Daphnia short-time test)
Year	:	1983
GLP	:	no data
Test substance	:	no data

Method	:	Method of the German Standards Institution Berlin, Germany
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods. Basic data given
Flag	:	Critical study for SIDS endpoint

10.04.2003 (66)

Type	:	static
Species	:	other: Daphnia carinata
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	8.2
Analytical monitoring	:	no data
Method	:	other: comparable to OECD 202 part 1 (Daphnia: Acute toxicity, 1984)
Year	:	1996
GLP	:	no data
Test substance	:	other TS: no purity given

Result	:	- measured log 1/IC50 = 4.37, corresponds to measured IC50 = 8.2 mg/l - calculated log 1/IC50 = 4.16, corresponds to calculated IC50 = 13 mg/l
Test condition	:	The test was performed under the following conditions:

- Temperature 22 +/- 1 °C, with a photoperiod of 14 hours light / 10 hours dark
- Cultured parthenogenetically, fed with a diet of green algae
- Each test used 60 Daphnia carinata (6 - 24 hours old), 10 of them in each 25 ml
- Daphnias were not fed during tests
- Stock solution prepared in acetone
- The results were considered valid if dissolved oxygen measured at the end of the test was at least equal to 60 % saturation, and if percentage of immobilization observed for the controls was zero

Reliability : (2) valid with restrictions
Comparable to guideline study, only basic data given
Flag : Critical study for SIDS endpoint
10.04.2003 (31)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
LC50 : ca. 6
Limit Test : no
Analytical monitoring : no data
Method : other: Static test in open system
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Test condition : - The same test procedure was conducted in two parallel test systems. Every test vessel was filled with 10 ml test solution and per concentration 10 Daphnias were used.
- A control test was also run in addition to the treatment series.
- The concentrations tested are presented in form of the dilution factor and they range from 1:1 up to 1:100 of the test solution which contained about 117 mg/l

Reliability : (2) valid with restrictions
Basic data given
10.04.2003 (50)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : > .1
Method : other: DIN-Standard 38 412 L11 (Daphnia, Short-time toxicity test)
Year : 1988
GLP : no data
Test substance : other TS: no purity given

Method : Method of the German Standards Institution, Berlin, Germany
Remark : Although the water solubility of 1,2-dichloro-4-nitrobenzene is about 120 mg/l, and results > 0.1 mg/l are reported, the authors report that due to their method of solvatation the highest concentration checked was 0.1 mg/l (in Ethanol)
It was clarified by one author, that due to an Electronic Data Processing error the authors report some experiments with 28 and 56 mg/l, however, the correct data were 0.025 and 0.05 mg/l, respectively
Result : Reported concentration range in data compilation: 0.0008 - 0.10 mg/l.
Test condition : The test was performed under the following conditions:
- Test organism: Daphnia magna Strauss, strain = IRCHA
- The test consists of 4 parallel test beakers per concentration level and at least 4 for the control

- Test system: Each beaker was filled with 24 h-old Daphnia (1 organism/50 ml), the total number per concentration level was 20 organisms
- Test temperature 25 +/- 1 °C
- Dilution water: Source = Synthetic fresh water, hardness = 2.5 mmol/l Ca + Mg, Na/ K ratio = 10:1, pH = 8.0 +/- 0.2
- pH-values and oxygen-concentration were measured during the test in two test beakers per concentration level. The detected variation of these parameters had no negative influence on the organisms

Reliability : (2) valid with restrictions
Test procedure according national standard method. Reported in sufficient detail. Some minor contradictions in the report have been clarified by one of the authors

23.10.2003 (44)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : 11.3
Analytical monitoring : no data
Method : other: comparable to OECD 202 part 1 (Daphnia: Acute toxicity)
Year : 1994
GLP : no data
Test substance : other TS: i.e. > 95 % purity

Result : - measured log 1/IC50 = 4.23 (both publications), corresponds to measured IC50 = 11 mg/l
- calculated log 1/IC50 = 4.41 [Zhao Y-H, He Y-B, Wang L-S (1995) Predicting Toxicities of Substituted Aromatic Hydrocarbons to Fish by Toxicities to Daphnia magna or Photobacterium phosphoreum. Toxicol Environ Chem 51: 191 -195], corresponds to calculated IC50 = 7.5 mg/l
- calculated log 1/IC50 = 4.63 [Zhao Y-H, Wang L-S (1995) Quantitative Structure-Activity Relationships of Hydrophobic Organic Chemicals. Toxicol Environ Chem 50: 167 - 172], corresponds to calculated IC50 = 4.5 mg/l

Test condition : The test was performed under the following conditions:
- Temperature 22 +/- 1 °C, with a photoperiod of 14 hours light / 10 hours dark
- Cultured parthenogenetically, fed with a diet of green algae
- Each test used 60 organisms (6 - 24 hours old), 10 of them in each 25 ml
- Daphnia magna were not fed during tests
- The results were considered valid if dissolved oxygen measured at the end of the test was at least equal to 60 % saturation, and if percentage of immobilization observed for the controls was zero

Reliability : (2) valid with restrictions
Comparable to guideline study, only basic data given. Experimental result is exactly the same in both studies, thus it is assumed that one experimental result was published twice

23.10.2003 (29) (30)

Type : other: not indicated
Species : other: Daphnia
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 3
Analytical monitoring : no data
Method : other: tested with standard EEC or OECD methods or comparable procedures
Year : 2001
GLP : no data
Test substance : other TS: no purity given

Remark	:	Ecotoxicological data were taken from literature. Major criteria for the selection of toxicity data were reliability and comparability of test methods.
Reliability	:	(4) not assignable Secondary literature, origin of data not reported

27.06.2003 (75)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	other algae: Scenedesmus obliquus
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= 5.8
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 201 (Algae, Growth inhibition test, 1981)
Year	:	1995
GLP	:	no data
Test substance	:	other TS: no purity given
Result	:	Result reported: - log EC50 (mol/l) = 4.52 which equals 5.8 mg/l
Test condition	:	The test was performed under the following conditions: - Temperature 20 °C +/- 1 °C - pH 7.2 +/- 0.2 - Continuous light provided by white Neon lamps (3,600 lux), - Stock solution prepared in aceton (1 ml/l) - Initial cell concentration was approx. 10,000 cells/ml
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

23.04.2003 (78)

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC10	:	> .1
EC50	:	> .1
Method	:	other: DIN 38 412, Part 9 (Cell multiplication inhibition test)
Year	:	1988
GLP	:	no data
Test substance	:	other TS: no purity given
Method	:	Method of the German Standards Institution, Berlin, Germany
Result	:	Effect levels determined the endpoint biomass and the results were the following: EC10 = > 0.10 mg/l EC50 = > 0.10 mg/l
Test condition	:	- The concentration range tested was 0.0008 - 0.10 mg/l - The cell material was used after 72 h of preculture to inoculate the dilution preparation after the cell concentration had been fixed at 1.0E5/ml - Test preparations: - Wide-neck bottles of 250 ml with ground-glass stoppers were used as the test vessels. - The test and control preparations were incubated under constant lighting and shaken daily. - Before beginning the test pH was adjusted to 8 Although some experiments were reported to be done in the range of 10 -

100 mg/l, it was clarifed by one of the authors that the concentration range tested was 0.0008 - 0.10 mg/l

Reliability : (2) valid with restrictions
Test procedure in accordance to national standard methods

Flag : Critical study for SIDS endpoint

07.08.2003 (44)

Species : Chlorella fusca (Algae)
Endpoint : growth rate
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : = .32
Limit test :
Analytical monitoring : no data
Method : other: cf. Test conditions
Year : 2000
GLP : no data
Test substance : other TS: no purity given

Remark : New accepted scientific name for Chlorella fusca is Scenedesmus vacuolatus

Test condition : Test measures inhibition of one generation reproduction cycle within 24 h according to Altenburger et al. 1990 [Altenburger R, Boedecker W, Faust M, Grimme LH (1990) Evaluation of the Isobologram Method for the Assessment of Mixtures of Chemiclas. Combination of Effect Studies with Pesticides in Algal Biotests. Ecotox Environ Safety 20: 98 - 114].
The test was performed under the following conditions:
- Temperature 28 °C +/- 0.5 °C, pH 6.7
- Incubation in gastight vessels
- Initial cell concentration was approx. 1E5 cells/ml
- one day = 1 generation under the experimental conditions, cell number increases by a factor of 12 during incubation
- 14 h light, 10 h dark cycle

Reliability : (2) valid with restrictions
Basic data given

07.08.2003 (23)

Species : other algae
Endpoint : other
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 1.055
Limit test :
Analytical monitoring : no data
Method : other: tested with standard EEC or OECD methods or comparable procedures
Year : 2001
GLP : no data
Test substance : other TS: no purity given

Remark : Ecotoxicological data were taken from literature. Major criteria for the selection of toxicity data were reliability and comparability of test methods. Very old data were generelly discarded.

Result : Result reported as Log 1/C [mmol/l] = 2.26 which equals 1.055 mg/l

Reliability : (4) not assignable
Secondary literature

15.04.2003 (75)

Species : other algae: Selenastrum obliquus
Endpoint :
Exposure period : 96 hour(s)

Unit	:	mg/l
EC50	:	6 - 15
Method	:	
Year	:	1995
GLP	:	
Test substance	:	
Remark	:	From log kow (log kow = 3.29) cited from Zhao et al. (1993) [Zhao Y, Wang L, Gao H, Zhang Z (1993) Quantitative Structure - Activity Relationships - Relationship between Toxicity of Organic Chemicals to Fish and to Photobacterium phosphoreum. Chemosphere 26 (11): 1971 - 1979] Chinese letters of 3,4-Dichloronitrobenzene (1,2-dichloro-4-nitrobenzene) were identified
Result	:	Reported results measured and calculated: log EC50 = 4.5 - 4.12 (mol/l) which equals 6 - 15 mg/l
Test condition	:	- Culturing ot the algae: 24 +/- 1 °C; 12 h light, 12 h dark; 4000 lux +/- 10 %, pH 7.5 +/- 0.2 - 10000 cells/ml - Spectrometric determination at 650 nm
Reliability	:	(4) not assignable Original reference in Chinese

15.04.2003 (79)

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	1
Limit test	:	
Analytical monitoring	:	no data
Method	:	
Year	:	2000
GLP	:	no data
Test substance	:	
Remark	:	From log kow Chinese letters of 3,4-Dichloronitrobenzene (1,2-dichloro-4-nitrobenzene) were identified in the publication of Zhang et al. (1995) [Zhang Y, Yu H, Han S, Zhao Y, Wang L (1995) The Toxicity of Substituted Aromatic Compounds to Algae and Quantitative Structure-Activity Relationship Studies. Huanjing Huaxue 14 (2): 140 - 144]
Result	:	measured log (1/EC50) = 2.283 mmol/l, which equals 1 mg/l; calculated log (1/EC50) = 2.042 mmol/l, which equals 1,7 mg/l
Test condition	:	- Spectrometric determination at 686 nm
Reliability	:	(4) not assignable Original reference in Chinese

15.04.2003 (35)

Species	:	other algae: Scenedesmus obliquus
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= 5.8
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 201 (Algae, Growth inhibition test, 1981)
Year	:	1995
GLP	:	no data
Test substance	:	other TS: no purity given
Test condition	:	The test was performed under the folowing conditions: Temperature 20 °C +/- 1 °C, continuous light provided by white Neon

lamps (4.000 lux),
- Initial cell concentration was approx. 1 E4 cells/ml
- Growth was monitored by electron microscope (400 times)

Reliability : (4) not assignable
Secondary literature. Although about 60 % of the "measured" EC50 data are also reported in the paper of Liu and Lang (1995) [Liu J, Lang P (1995) Toxicities of Nitroaromatic Compounds to Scenedesmus obliquus and Toxic Symptoms. Huanjing Kexue 16: 7 - 10], there is no reference that these data have been published elsewhere.

23.10.2003 (80)

Species : other algae: Scenedesmus obliquus
Endpoint : growth rate
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 5.8
Limit test :
Analytical monitoring : no data
Method : other: OECD Guideline 201 (Algae, Growth inhibition test, 1984)
Year : 1995
GLP : no data
Test substance : other TS: no purity given

Test condition : The test was performed under the following conditions:
Temperature 24 °C +/- 1 °C, in a schedule of 12 hours light /12 hours dark
- Stock solution prepared in aceton
- Initial cell concentration approx. 1 E4 cells/ml
- The cell density was measured after 0, 24, 48, 72 and 96 hours
- The optical density was determined at 650 nm

Reliability : (4) not assignable
Secondary literature. The authors use the data published by Liu and Lang (1995) [Liu J, Lang P (1995) Toxicities of Nitroaromatic Compounds to Scenedesmus obliquus and Toxic Symptoms. Huanjing Kexue 16: 7 - 10], although there is no reference the Liu and Lang (1995).

23.10.2003 (31)

Species : Haematococcus pluvialis (Algae)
Endpoint : other: O2 production of algae
Exposure period : 4 hour(s)
Unit : mg/l
EC50 : = 2
Limit test :
Analytical monitoring : no data
Method : other: Manometric determination, cf. Test conditions
Year : 1983
GLP : no data
Test substance : no data

Remark : Test criteria: inhibitory effect on oxygen production
Test condition : Oxygen production measured (manometric determination) according to Tuempling (1972) in Warburg vessels [Tuempling, vW (1972) Ein manometrisches Verfahren zur Bestimmung der autotrophen Bioaktivität. Fortschritte Wasserchemie 14: 205 - 213]
- Cell densitiy: 80.000 cells/ml
- Incubation volume 5 ml
- Volume of a Warburg vessel ca. 40 ml
- Since the algae nutrient solution contains high buffer capacity, no neutralization assumed to be needed

Reliability : (4) not assignable
Documentation insufficient for assessment

23.10.2003 (66)

Type	:	soil
Species	:	other fungi: Trichoderma viride
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	ca. 250
EC5	:	ca. 70
EC95	:	ca. 1000
Analytical monitoring	:	no data
Method	:	other: Growth inhibition test
Year	:	1968
GLP	:	no data
Test substance	:	other TS: "chemically pure"
Remark	:	Measurement of toxicity applied via the vapor phase: vapor pressure not taken into account for 1,2-dichloro-4-nitrobenzene. Concentration in results part denotes concentration in soil used for exposure
Test condition	:	Fungitoxicity of vapor released from a nonsterile compost soil amended with 1,2-dichloronitrobenzene - Dilution series by adding to soil with 1 g/kg 1,2-dichloronitrobenzene unamended soil in the ratio of 1 : 1 - At each stage of the dilution, triplicate 100 g samples were transfered to 16 oz wide mouth jars containing 10 ml of water. Screw caps were applied immediately and replaced 3 -4 h later with inverted culture plates with inoculum discs on potato-dextrose agar. - Radial growth was measured after incubation at 24 °C
Reliability	:	(3) invalid Unsuitable test system

23.10.2003 (83)

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(s)
Unit	:	mg/l
NOEC	:	.025
Analytical monitoring	:	yes
Method	:	other: Provisional procedure proposed by the Federal Environmental Agency for extended toxicology with Daphnia magna (01.01.1984)
Year	:	1988
GLP	:	no data
Test substance	:	other TS: no purity given
Method	:	Determination of NOEC for reproduction rate, mortality and the time of the first appearance of offspring. Analytical monitoring of test substance concentration by GC
Remark	:	The substance was tested far below the water solubility limit (circa 120 mg/l) with a maximum test concentration of 0.1 mg/l. It is not stated why higher concentrations have not been used.
Result	:	Tested concentration range: 0.0032 - 0.10 mg/l. The author assumes the water solubility limit with 0.1 mg/l Results of analytical monitoring Nom conc. instant analysis analysis after 2 d

		0.1 0.1 -0.12 0.09 - 0.1 0.05 0.05 - 0.06 0.04 - 0.05 0.025 0.020 - 0.030 0.020 - 0.026 0.012 0.010 - 0.02 0.010 - 0.012 Analysis after 2 d: In general, highest analytical values were obtained in controls. The lower values were measured in used Daphnia medium
Test condition	:	The test was performed under the following conditions: - Semistatic test - Test organism: Daphnia magna Strauss, strain IRCHA - 100 µg/l of testsubstance was dissolved in Ethanol - The test consists of 4 parallel test vessels (gastight) per concentration level and at least 4 for the control - Test system: Each vessel was filled with 24 h-old Dapnia (1 organism/50 ml), the total number per concentration level was 20 organisms. - The parent animals in the control and test vessels were pipetted 3 times a week into freshly prepared test and control media. - Test temperature 25 +/- 1 °C - Dilution water: Source = Synthetic fresh water, Hardness = 2.5 mmol/l Ca + Mg, Na/K ratio = 10:1, pH = 8.0 +/- 0.1 - pH-values and oxygen concentration were measured during the test in two test-vessels per concentration level. The detected variation of these parameters had no negative influence on the organisms
Reliability	:	(2) valid with restrictions Test procedure according national standard method. Reported in sufficient detail. Some minor contradictions in the report have been clarified by one of the authors
Flag	:	Critical study for SIDS endpoint

23.10.2003 (44)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	Phaseolus aureus (Dicotyledon)
Endpoint	:	growth
Exposure period	:	6 day(s)
Unit	:	mg/l
EC50	:	26.9
Method	:	other: germination and growth of seedlings in sand
Year	:	1962
GLP	:	no data
Test substance	:	no data
Result	:	Reduction in fresh weight after 6 days (compared to controls) was used to measure inhibition. Although the author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmol/l. Result refers to concentration of aqueous solution.
Test condition	:	The test was performed under the following conditions: - Pregermination of the seeds for 24 hours - Test chemical suspended in 0.1 strength Hoagland's solution - 15 seeds were cultured in each tall form beaker (150 ml) containing 220 g (dry weight) of sand - 36 ml of test chemical was added - Incubation in the dark in a Mangelsdorf seed-germinator, temperature 25 °C, relative humidity approaching 100 %, incubation time 6 days
Reliability	:	(2) valid with restrictions Study with acceptable restrictions, up to date method by the time the study

要　旨

試験委託者　　環境省

表　　題　　２，６－ジ－ｓｅｃ－ブチルフェノールの藻類（*Pseudokirchneriella subcapitata*）に対する生長阻害試験

試験番号　　Ｎｏ．２００４－生７４

試験法ガイドライン

本試験は、厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」（薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日）に準拠して実施した。

1) 被験物質	:	２，６－ジ－ｓｅｃ－ブチルフェノール
2) 暴露方式	:	止水式、振盪培養（100rpm）
3) 供試生物	:	*Pseudokirchneriella subcapitata*（ATCC 22662）
4) 暴露期間	:	72時間
5) 試験濃度(設定値)	:	対照区，0.046，0.10，0.22，0.46，1.0，2.2 mg/L 公比；　2.2
6) 試験溶液量	:	100 mL（OECD 培地）／容器
7) 連数	:	3 容器／試験濃度区、6 容器／対照区
8) 初期細胞濃度	:	0.5×10^4 cells/mL
9) 試験温度	:	23±2 ℃
10) 照明	:	60 ～ 120 μE/m²/s （フラスコ液面付近）で連続照明
11) ｐＨ	:	試験溶液のｐＨ調整は行わない
12) 分析法	:	HPLC 法

結　果

1)試験溶液中の被験物質濃度

暴露終了時の被験物質濃度は暴露開始時に比較して 30 %程度の濃度低下が認められたが、藻体への吸着もしくは藻体分離操作時のプラスチック容器への吸着による減少と考えられたことから、暴露開始時の測定値を用いて、各影響濃度を算出した。

2)生長速度の比較による阻害濃度

50 %生長阻害濃度 E_rC_{50}(0-72)　：1.13 mg/L(95%信頼限界：0.975 ～ 1.35 mg/L),Probit

最大無影響濃度 NOEC(Rate 0-72)　：0.083 mg/L

3)生長曲線下面積の比較による阻害濃度

50 %生長阻害濃度 E_bC_{50}(0-72)　：0.278 mg/L(95%信頼限界：0.251 ～ 0.307 mg/L),Probit

最大無影響濃度 NOEC(Area 0-72)　：0.083 mg/L

Figure 1. Algal Growth Curve of *Pseudokirchneriella subcapitata*
(Mean cell counts *vs* time during the 72-hour exposure)

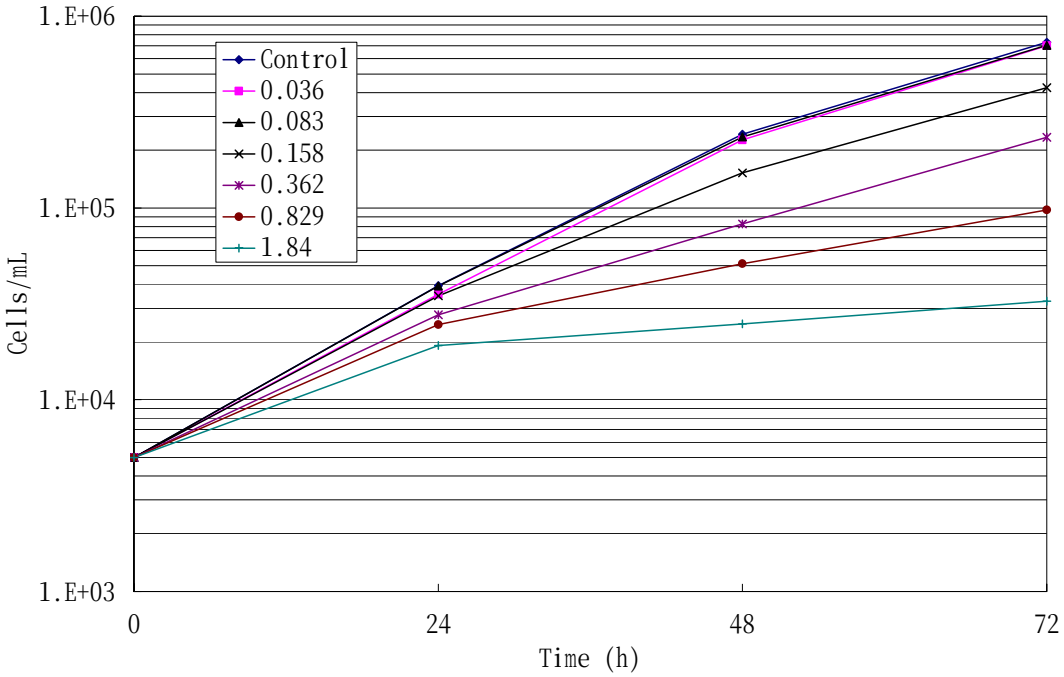


Figure 2. Concentration-Inhibition Curve Based on I_μ values Calculated from the Growth Rates

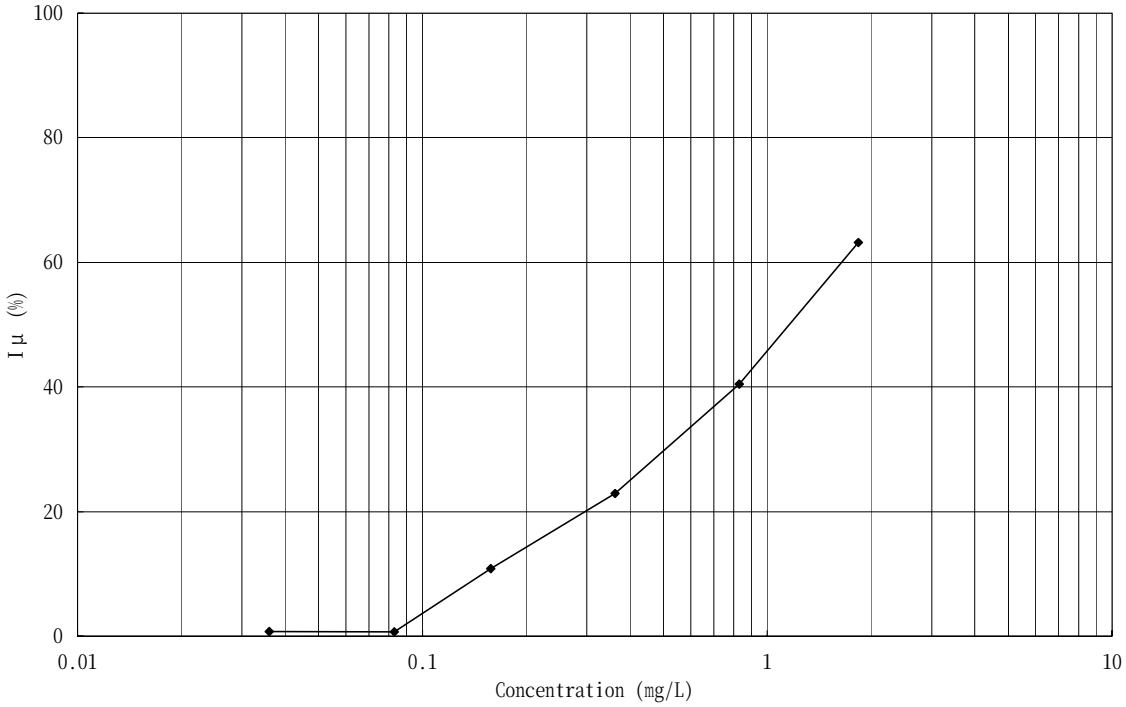
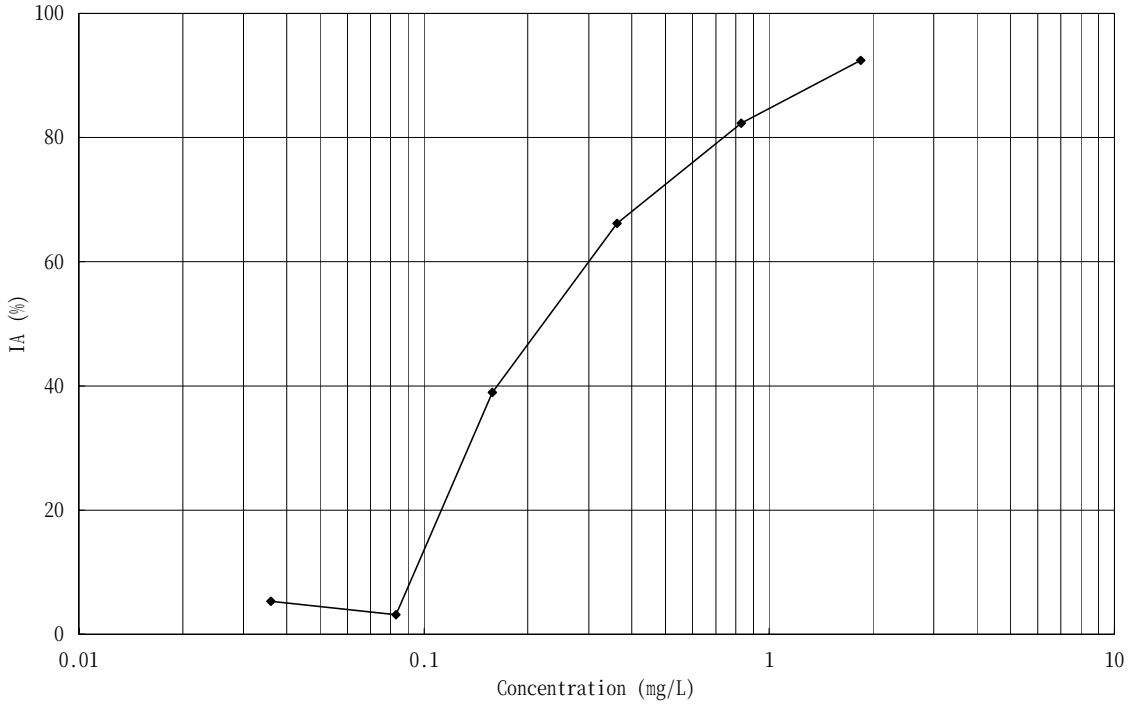


Figure 3. Concentration-Inhibition Curve Based on I_A Values Calculated from the Area under the Growth Curves



要　旨

試験委託者　　環境省

表　題　　２，６－ジー ｓｅｃ－ブチルフェノ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　　Ｎｏ．２００４－生７５

試験法ガイドライン

本試験は、厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」（薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日）に準拠して実施した。

1）被験物質　：　２，６－ジー ｓｅｃ－ブチルフェノール
2）暴露方式　：　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）試験濃度（設定値）：　対照区，0.32，0.56，1.0，1.8，3.2 mg/L
　　公比；　1.8
6）試験溶液量　：　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／8 時間暗
11）給餌　：　無給餌
12）ｐＨ　：　試験溶液のｐＨ調整は行わない
13）分析法　：　HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質の濃度は暴露開始時および暴露終了時の測定値を用いて幾何平均値(揮散が主因と思われる濃度減少が認められたため)を求め、各影響濃度を算出した。

2)24 時間暴露後の結果

50 %遊泳阻害濃度（EC_{50}）：1.56 mg/L(95%信頼限界：1.29 ～ 1.90 mg/L),Logit

0 %阻害最高濃度：0.301 mg/L

100 %阻害最低濃度：2.88 mg/L

3)48 時間暴露後の結果

50 %遊泳阻害濃度（EC_{50}）：0.955 mg/L(95%信頼限界：0.817 ～ 1.12 mg/L),Logit

0 %阻害最高濃度：0.301 mg/L

100 %阻害最低濃度：1.71 mg/L

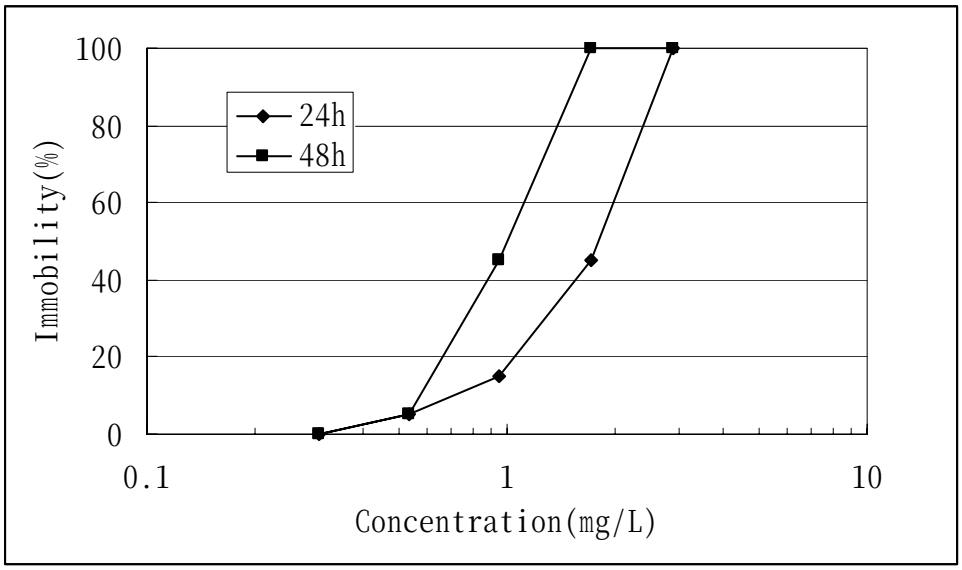
Table 8. Total Hardness(as $CaCO_3$)

(Static Condition)

Nominal Concentration (mg/L)	Mean[a] Measurd Concentration (mg/L)	Total Hardness(as $CaCO_3$),mg/L	
		0 Hour new	48 Hours old
Control	—	254	254
0.32	0.301	256	255
0.56	0.534	256	256
1.0	0.954	257	257
1.8	1.71	256	256
3.2	2.88	257	256

new: Freshly prepared test solutions
old: Test solutions after 48 hour exposure
a: Geometric mean
—: Not calculated

Figure 1. Concentration-Response (Immobility) Curve



要　旨

試験委託者　　環境省

表　題　　2,6－ジーｓｅｃ－ブチルフェノールのヒメダカ(*Oryzias latipes*)に対する急性毒性試験

試験番号　　Ｎｏ．２００４－生７６

試験法ガイドライン

本試験は厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」（薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日)に準拠して実施した。

1)被験物質	：	2,6－ジーｓｅｃ－ブチルフェノール
2)暴露方式	：	半止水式（24時間毎に試験溶液の全量を交換）
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，0.010，0.022，0.046，0.10，0.22，0.32，0.46，1.0 mg/L 公比；　2.2(ただし、0.22 ～ 0.46 mg/Lは公比 1.3の変則公比)
6)試験溶液量	：	3 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／8 時間暗
11)給餌	：	無給餌
12)通気	：	なし
13)ｐＨ	：	試験溶液のｐＨ調整は行わない
14)分析法	：	HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質の濃度は、一部に分析誤差も考えられるものの、揮散による影響が変動の主因と判断し、各測定値の時間加重平均値(暴露開始時と 24 時間換水前および 48 時間換水後と 72 時間換水前の対数平均を算出し、それらの算術平均値)を採用した。

2)50 %死亡濃度

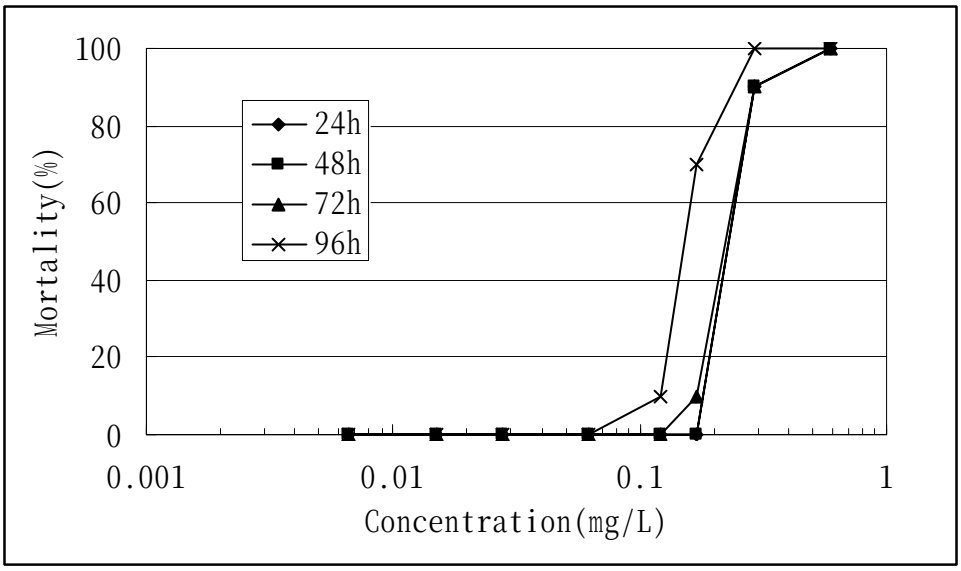
24 時間 50 %死亡濃度(LC_{50}): 0.241 mg/L(95%信頼限界：0.149 ～ 0.287 mg/L),Logit

48 時間 50 %死亡濃度(LC_{50}): 0.241 mg/L(95%信頼限界：0.149 ～ 0.287 mg/L),Logit

72 時間 50 %死亡濃度(LC_{50}): 0.223 mg/L(95%信頼限界：0.181 ～ 0.282 mg/L),Logit

96 時間 50 %死亡濃度(LC_{50}): 0.154 mg/L(95%信頼限界：0.129 ～ 0.190 mg/L),Logit

Figure 1. Concentration—Response (Mortality) Curve



要　旨

試験委託者　　環境省

表　　題　　２，６－ジー ｔ ｅ ｒ ｔ－ブチル－４－エチルフェノールの藻類（*Pseudokirchneriella subcapitata*）に対する生長阻害試験

試験番号　　Ｎｏ．２００４－生７７

試験法ガイドライン

本試験は、厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」（薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日）に準拠して実施した。

1) 被験物質	：	２，６－ジー ｔ ｅ ｒ ｔ－ブチル－４－エチルフェノール
2) 暴露方式	：	止水式、振盪培養（100rpm）
3) 供試生物	：	*Pseudokirchneriella subcapitata*（ATCC 22662）
4) 暴露期間	：	72時間
5) 試験濃度(設定値)	：	対照区，0.57 mg/L(限度試験)
6) 試験溶液量	：	100 mL（OECD 培地）／容器
7) 連数	：	6 容器／試験濃度区、6 容器／対照区
8) 初期細胞濃度	：	0.5×10^4 cells/mL
9) 試験温度	：	23±2 ℃
10) 照明	：	60 ～ 120 μE/m²/s （フラスコ液面付近）で連続照明
11) ｐＨ	：	試験溶液のｐＨ調整は行わない
12) 分析法	：	HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質濃度は揮散が主因と考えられる濃度減少のため、暴露開始時および暴露終了時の測定値を用いて幾何平均値を求め、各影響濃度を算出した。

2)生長速度の比較による阻害濃度

50 %生長阻害濃度 E_rC_{50}(0-72)　：　>0.516 mg/L

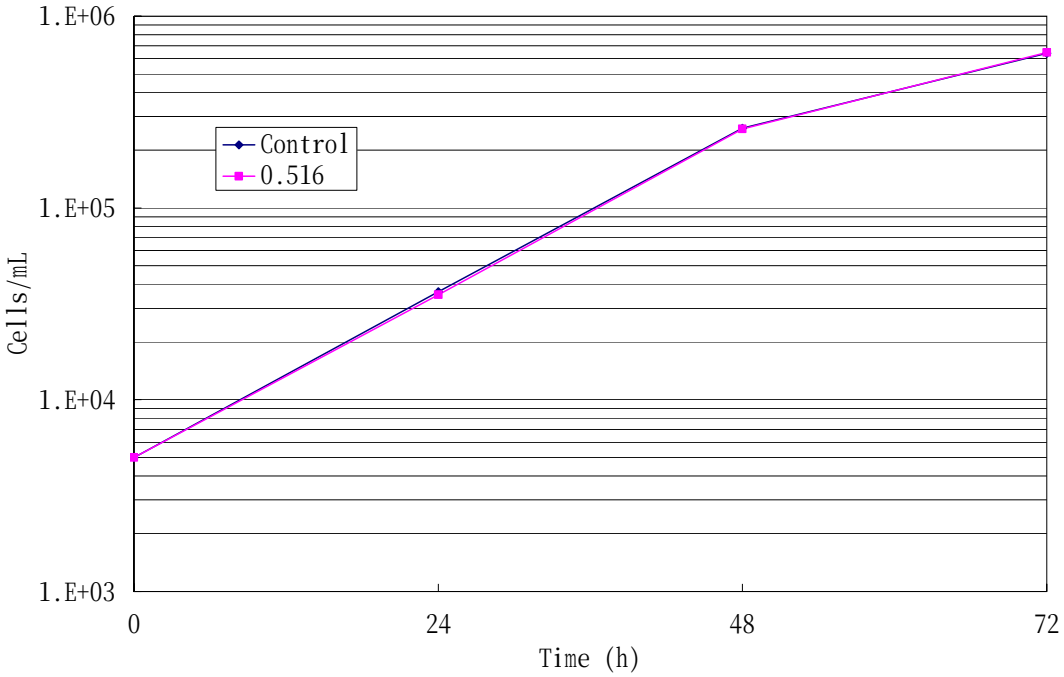
最大無影響濃度 NOEC(Rate 0-72)　：　0.516 mg/L

3)生長曲線下面積の比較による阻害濃度

50 %生長阻害濃度 E_bC_{50}(0-72)　：　>0.516 mg/L

最大無影響濃度 NOEC(Area 0-72)　：　0.516 mg/L

Figure 1. Algal Growth Curve of *Pseudokirchneriella subcapitata*
(Mean cell counts *vs* time during the 72-hour exposure)



要　旨

試験委託者　　環境省

表　題　　２，６－ジー ｔｅｒｔ－ブチル－４－エチルフェノ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　　Ｎｏ．２００４－生７８

試験法ガイドライン

本試験は、厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」（薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日）に準拠して実施した。

1）被験物質　：　２，６－ジー ｔｅｒｔ－ブチル－４－エチルフェノール
2）暴露方式　：　半止水式（24時間目に試験溶液の全量を交換）
3）供試生物　：　オオミジンコ（*Daphnia magna*）
4）暴露期間　：　48 時間
5）試験濃度（設定値）：　対照区，0.10，0.19，0.32，0.58 mg/L
　　公比；　1.8
6）試験溶液量　：　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／8 時間暗
11）給餌　：　無給餌
12）ｐＨ　：　試験溶液のｐＨ調整は行わない
13）分析法　：　HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質の濃度は、揮散による影響が変動の主因と判断し、各測定値の時間加重平均値(暴露開始時と 24 時間換水前および 24 時間換水後と暴露終了時の対数平均を算出し、それらの算術平均値)を採用した。

2)24 時間暴露後の結果

50 %遊泳阻害濃度（EC_{50}）：>0.469 mg/L

0 %阻害最高濃度　　：0.469 mg/L

100 %阻害最低濃度　　：>0.469 mg/L

3)48 時間暴露後の結果

50 %遊泳阻害濃度（EC_{50}）：>0.469 mg/L

0 %阻害最高濃度　　：0.270 mg/L

100 %阻害最低濃度　　：>0.469 mg/L

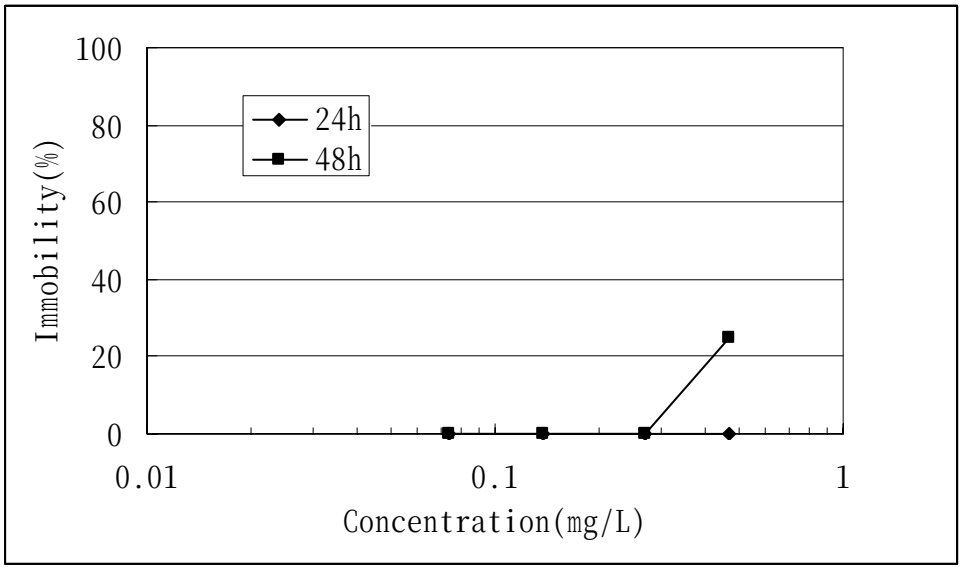
Table 8. Total Hardness(as $CaCO_3$)

(Semi-Static Condition)

Nominal Concentration (mg/L)	Mean[a] Measured Concentration (mg/L)	Total Hardness(as $CaCO_3$),mg/L			
		0 Hour	24 Hours		48 Hours
		new	old	new	old
Control	—	245	248	246	244
0.10	0.074	244	246	247	246
0.19	0.137	246	247	246	247
0.32	0.270	245	245	248	246
0.58	0.469	246	246	245	246

new: Freshly prepared test solutions
old: Test solutions after 24 hour exposure
a: Time- weighted mean
—: Not calculated

Figure 1. Concentration-Response (Immobility) Curve



要　旨

試験委託者　　環境省

表　題　　2,6－ジーｔｅｒｔ－ブチル－4－エチルフェノールのヒメダカ(*Oryzias latipes*)に対する急性毒性試験

試験番号　　Ｎｏ．２００４－生７９

試験法ガイドライン

本試験は厚生労働省医薬食品局長、経済産業省製造産業局長、環境省総合環境政策局長連名通知「新規化学物質等に係る試験の方法について」(薬食発第 1121002 号、平成15・11・13 製局第 2 号、環保企発第 031121002 号、平成 15 年 11 月 21 日)に準拠して実施した。

1)被験物質	:	2,6－ジーｔｅｒｔ－ブチル－4－エチルフェノール
2)暴露方式	:	半止水式 (24時間毎に試験溶液の全量を交換)
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, 0.12, 0.21, 0.37, 0.66 mg/L 公比； 1.8
6)試験溶液量	:	5 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／8 時間暗
11)給餌	:	無給餌
12)通気	:	なし
13)ｐH	:	試験溶液のｐH調整は行わない
14)分析法	:	HPLC 法

結　果

1)試験溶液中の被験物質濃度

被験物質の濃度は、揮散による影響が変動の主因と判断し、各測定値の時間加重平均値(暴露開始時と 24 時間換水前および 72 時間換水後と暴露終了時の対数平均を算出し、それらの算術平均値)を採用した。

2)50 %死亡濃度

24 時間 50 %死亡濃度(LC_{50}): >0.588 mg/L

48 時間 50 %死亡濃度(LC_{50}): >0.588 mg/L

72 時間 50 %死亡濃度(LC_{50}): >0.588 mg/L

96 時間 50 %死亡濃度(LC_{50}): 0.588 mg/L

Figure 1. Concentration—Response (Mortality) Curve