魚類急性毒性試験

1．供試物質の概要

名　称	日　本　名	2,6-ジクロルトルエン		
	英　　名	2,6-Dichlorotoluene		
	一般名・商品名			
構造式		分子式・示性式	$C_{12}C_6H_3CH_3$	
		分　子　量	161.03	
		水への溶解度	不溶性	
		蒸　気　圧		
入手先	㈱和光純薬工業	製造年月日	年　月　日	
	TEL　（　）	ロット番号	WDL4949	
純　度	不純物：			
その他の物性等				

2．供試魚の概要

種名及び系統名	ヒメダカ（Oryzias latipes）		
由　来	市販・入手先名称：熊本県玉名郡長洲町梅田　村木養魚場 TEL 0968（78）0845		
飼育方法	馴致結果：　訓致中14日間における死亡率は２％以下であった。 餌の種類：　テトラミン　　量：0.3～0.4g／回　給餌頻度：１回／１日		
平均体長	2.0± 0.2cm（n＝22）	平均体重	0.069±0.022g（n＝22）

3．試験条件

試験温度	21±　　1℃	
希釈水	供給源	脱塩素水道水
	水　質	pH：6.9　　Ca／Mg比：3.8　　Na／K比：9.5 硬度：65　　アルカリ度：4.8 その他： 水質測定年月日：　平成４年12月17日

試験溶液	状　態	100ppmではビーカーの底に油状となって沈澱する。
	保管方法	ｼﾞﾒﾁﾙｽﾙﾎｷｼﾄﾞ(DMSO)を用いて36mg/ml液を作成し、冷蔵庫保管。
	調製方法	DMSOを用いて希釈（公比1.8）後、希釈水１Ｌに0.5ml添加した。
飼育方法	半止水式　２Ｌビーカーを用いた。ビーカーをパラフィルムで覆った。	
	半止水式の場合	換水方法：全量　　頻度：24時毎
	流水式の場合	供試物質供給システム： 流速：　　清掃の頻度：
光　源	蛍光灯・　照光周期：14時間明10時間暗で行った。	

4.試験結果

魚類急性毒性試験結果（予備試験）

供試物質名　：2,6-ジクロルトルエン
試験実施期間：平成４年　10月　19日　～　　平成４年　10月　23日　（　５日間）
試験実施機関：長崎県衛生公害研究所
濃度公比　　：10

区分	溶液量 L	物質濃度 mg/L	助剤濃度 mg/L	試験開始時			２４時間			４８時間			７２時間			９６時間		
				供試魚数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L
対照	1	0	0	5	6.9	9.6	5	7.0 6.9	7.3 9.2	5	7.4 7.1	7.3 9.2	5	7.2 7.0	7.5 9.3	5	7.0	6.7
助剤対照	1		1100	5	6.9	9.6	5	7.0 7.0	7.3 9.3	5	7.4 7.1	7.4 9.2	5	7.2 7.0	7.5 9.3	5	7.0	6.8
1	1	0.1	1100	5	7.0	9.6	5	7.0 6.9	6.9 9.3	5	7.1 7.0	7.6 9.2	5	7.1 7.1	7.4 9.3	5	7.2	6.5
2	1	1.0	1100	5	7.0	9.6	5	7.1 7.0	7.0 9.3	5	7.1 7.1	7.6 9.2	5	7.2 7.2	7.5 9.3	5	7.2	6.6
3	1	10	1100	5	7.0	9.6	1	7.2 7.0	7.4 9.3	0	7.2	8.9						
4	1	100	1100	5	7.0	9.6	0	7.2	8.7									
観察事項、pH変動の理由				100ppm：１時間後死亡。体表が溶ける。														

＊　供試物質濃度を測定した場合、その値を（　）の中に入れて物質濃度欄に記入すること。
＊＊　ｐＨ，ＤＯは上段に換水前、下段に換水後の測定値を記入すること。

試験開始後９６時間で大部分の魚が生存した最高濃度	1.0 mg/L
試験開始後４８時間で大部分の魚が死亡した最低濃度	10 mg/L

【本試験の設定濃度及び設定根拠】

公比	設定濃度区（mg/L）					設定根拠
	1	2	3	4	5	
1.8	1.7	3.1	5.6	10	18	予備試験の結果より

魚類急性毒性試験結果（本試験①）

供試物質名　：2,6-ジクロルトルエン
試験実施期間：平成４年　10月　26日　～　平成４年　10月　30日　（　5日間）
試験実施機関：長崎県衛生公害研究所
濃度公比　　：1.8

区分	溶液量 L	物質濃度 mg/L	助剤濃度 mg/L	試験開始時			２４時間			４８時間			７２時間			９６時間		
				供試魚数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L	生存数	ｐＨ	ＤＯ mg/L
対照	1	0	0	10	6.9	9.1	10	7.1 7.0	5.9 9.1	10	6.9 7.0	5.4 8.9	10	7.0 6.9	6.2 8.9	10	6.9	5.5
助剤対照	1	0	550	10	7.0	9.1	10	7.1 7.0	5.6 9.1	10	6.9 7.1	5.1 8.9	10	6.9 6.9	5.6 8.9	10	6.9	5.8
1	1	1.7	550	10	7.0	9.1	10	7.1 7.1	5.3 9.1	10	7.1 7.1	5.1 9.1	10	6.9 7.1	4.9 9.0	10	7.0	5.5
2	1	3.1	550	10	7.1	9.0	10	7.0 7.0	5.7 9.1	10	7.1 7.2	5.6 9.1	10	6.9 7.1	5.5 9.0	10	7.1	5.7
3	1	5.6	550	10	7.2	9.0	10	7.0 7.1	5.6 9.1	9	7.0 7.2	5.8 9.1	8	7.0 7.1	6.1 9.0	8	7.1	6.2
4	1	10	550	10	7.2	9.0	5	7.0 7.1	5.5 9.1	2	7.1 7.2	6.9 9.1	0	7.0	8.3			
5	1	18	550	10	7.2	9.0	0	7.2	8.0									
観察事項、ｐＨ変動の理由							10ppm:動きが少し鈍い 体表の変化はみられない			10ppm:２匹は浮いた状態 5.6ppm:反応が鈍く余り動きが悪い 3.1ppm:以下は元気			5.6ppm:表面に浮き動きがない			3.1ppm:動きが鈍い 1.7ppm:元気		

＊　供試物質濃度を測定した場合、その値を（　）の中に居れて物質濃度欄に記入すること。
＊＊　ｐＨ，Ｄｏは上段に換水前、下段に換水後の測定値を記入すること。

魚類急性毒性試験結果（本試験②）

供試物質名　：2,6-ジクロルトルエン

試験実施機関：長崎県衛生公害研究所

【魚類に対する影響】

区　分	物質濃度 mg/L	助剤濃度 mg/L	各観察時における　累積死亡率			
			２４時間	４８時間	７２時間	９６時間
対　照	0	0	0	0	0	0
助剤対照	0	550	0	0	0	0
1	1.7	550	0	0	0	0
2	3.1	550	0	0	0	0
3	5.6	550	0	１０	２０	２０
4	10	550	５０	８０	１００	１００
5	18	550	１００	１００	１００	１００
死亡率１００％の最低濃度　mg/L (m mol/L)			１８ (1.1×10^{-1})	１８ (1.1×10^{-1})	１０ (6.2×10^{-2})	１０ (6.2×10^{-2})
死亡率０％の最高濃度　mg/L (m mol/L)			５．６ (3.5×10^{-2})	３．１ (1.9×10^{-2})	３．１ (1.9×10^{-2})	３．１ (1.9×10^{-2})
LC_{50}　mg/L (m mol/L)			１０ (6.2×10^{-2})	７．９ (4.9×10^{-2})	６．４ (4.0×10^{-2})	６．４ (4.0×10^{-2})
９５％信頼限界			8.1<21.0	6.5<10	5.6<7.8	5.6<7.8
算出方法			プロビット法 ver.3	プロビット法 ver.3	プロビット法 ver.3	プロビット法 ver.3

その他の観察された影響及びそれらが認められた濃度	影響：　　mg/L　（　　m mol/L　）

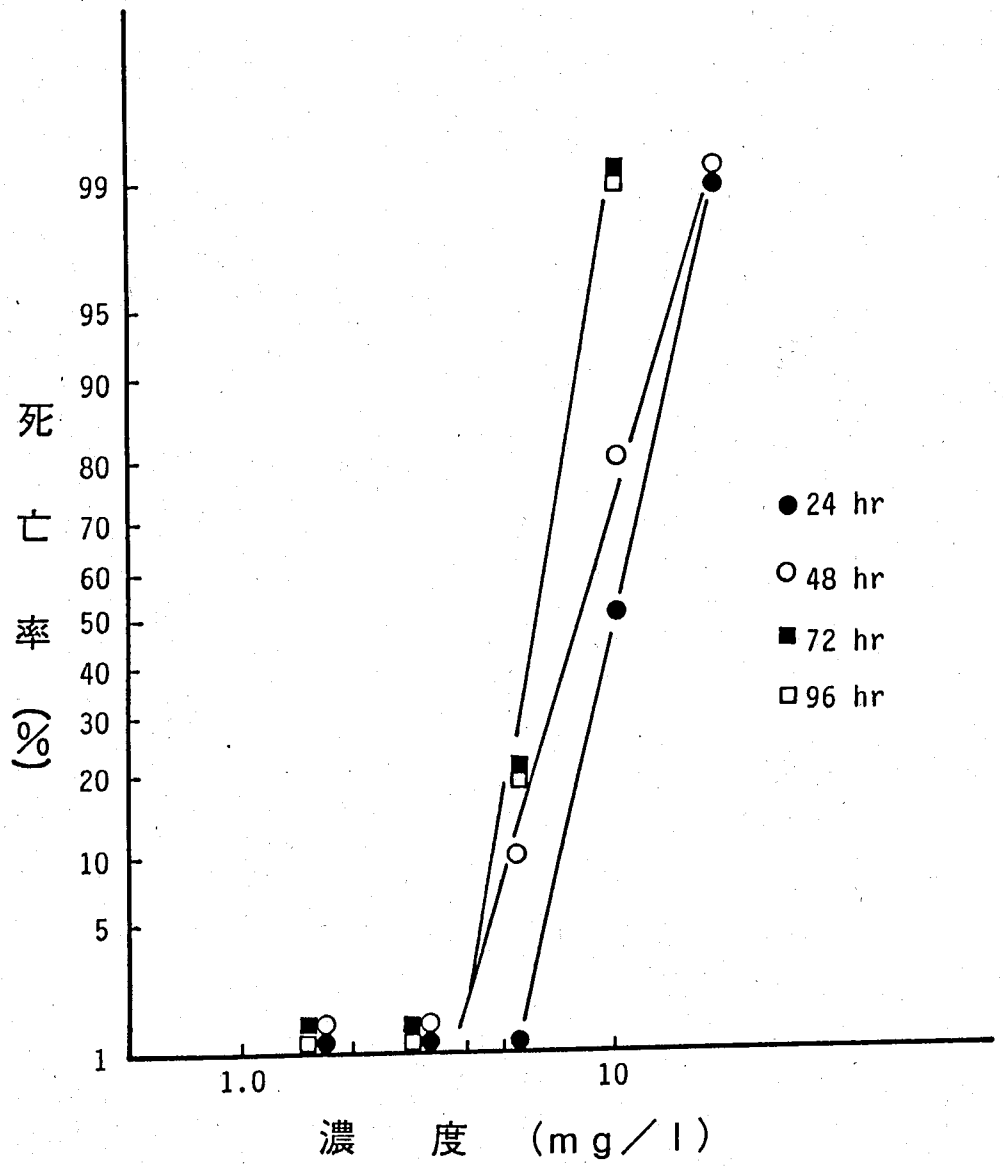
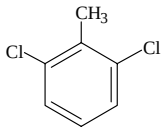


図　ヒメダカ急性毒性試験における２，６－ジクロルトルエン濃度と死亡率との関係

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	118-69-4
Chemical Name	2,6-Dichlorotoluene
Structural formula	

CONCLUSIONS AND RECOMMENDATIONS

Environment

The chemical is not readily biodegradable and has relatively high bioconcentration potential. Although toxicity of the chemical seems relatively high to Daphnia, PEC/PNEC ratio is less than 1 based on the local exposure scenario in the Sponsor country. It is currently considered of low potential risk and low priority for further work.

Human health

The chemical is moderately toxic in a repeated dose study (i.e. liver, kidney, thymus) and reproductive/developmental toxicity study (maternal toxicity). Occupational exposure is expected to be low as it is produced in closed system in Sponsor country. No consumer use is reported. Estimated daily intake through indirect exposure is also considered to be low. As the margin of safety is more than 200, it is currently considered of low potential risk and low priority for further work.

SHORT SUMMARY WHICH SUPPORTS THE REASONS FOR THE CONCLUSIONS AND RECOMMENDATIONS

2,6-Dichlorotoluene is stable liquid and the production volume is ca. 80 tonnes/year in 1996 in Japan. The chemical is used as intermediate for pesticide and pharmaceuticals. No consumer use is reported. The chemical is classified as “not readily biodegradable”. Bioconcentration factor is 246 – 828.

The potential environmental distribution of 2,6-dichlorotoluene obtained from a generic fugacity model (Mackey level III) showed the chemical would be distributed mainly to air and water. Predicted environmental concentration (PEC_{local}) of the chemical was estimated as 7.3×10^{-6} mg/l from Japanese local exposure scenario. In Japanese environmental survey, the chemical was not detected from surface water and sediments in 1982.

The main route of human exposure is inhalation with a limited numbers of workers potentially exposed during sampling operation. As there is no available data of the atmosphere concentration, the daily intake is calculated as 0.12 mg/kg/day as the worst case, based on the predicted high concentration and the possibility of exposure period. There is no available

information on consumer use. Indirect exposure via the environment, the daily intakes through drinking water and fish were estimated as 2.43 x 10^{-7} mg/kg/day and 9.07 x 10^{-6} mg/kg/day, respectively, based on PEC_{local} of 7.30 x 10^{-6} mg/l.

As the lowest acute and chronic toxicity data, 48 h EC50 (1.8 mg/l) value and 21 d NOEC (0.32 mg/l) of *Daphnia magna* were adopted, respectively. The assessment factors of 100 were used to both acute and chronic toxicity data to determine PNEC, because chronic toxicity data for fish was absent. Thus, PNEC of the chemical is 0.0032 mg/l. PEC/PNEC ratio is about 0.0023 and the bioconcentration factor of the chemical is moderate. Therefore, effects of the chemical on aquatic ecosystems are at low concern at present.

2,6-Dichlorotoluene had no genotoxic effects in bacteria and chromosomal aberration test *in vitro*. In a combined repeat dose and reproductive/developmental toxicity screening test, both male and female rats showed histopathological changes in liver, kidney and thymus, and maternal toxicity was observed. The no observed effect levels were obtained as 30 mg/kg/day for repeated dose toxicity and 100 mg/kg/day for reproductive toxicity.

For human health, the risk for workers is expected to be low because the margin of safety is 250. The risks for consumer and the general population through indirect exposure are also assumed to be low because the margin of safety through drinking water or fish is calculated to be 1.23 x 10^{8} or 3.31 x 10^{6}. Therefore, it is currently considered of low potential risk and low priority for further work.

IF FURTHER WORK IS RECOMMENDED, SUMMARISE ITS NATURE

2,6-dichlorotoluene is not readily biodegradable (OECD 301C: 0% after 28d) and stable in water. Direct photodegradation could be expected because 2,6-dichlorotoluene has absorption band in UV region.

2,6-dichlorotoluene is moderately bioaccumulative based on the test using carp (OECD 305C: BCF 380 – 570 at 0.02 mg/l).

The potential environmental distribution of 2,6-dichlorotoluene obtain from generic Mackay level III fugacity model is shown in Table 1. Parameters used for this model is shown as Annex to this report. The results show that, if 2,6-dichlorotoluene is released into air or soil, it is unlikely to be distributed into other compartment. If 2,6-dichlorotoluene is released into water, it is likely to be transported to air.

Table 1 Environmental distribution of 2,6-dichlorotoluene Using a generic level III fugacity model.

Compartment	Release 100% to air	Release 100% to water	Release 100% to soil
Air	89.8 %	24.4 %	0.2 %
Water	1.7 %	63.9 %	0.0 %
Soil	8.3 %	2.2 %	99.8 %
Sediment	0.3 %	9.4 %	0.0 %

As this chemical is used in closed systems as an intermediate and is not included in consumer products, its release to the environments may occur only from the production cites.

3.1.2 Predicted Environmental Concentration

As 2,6-dichlorotoluene is produced under the well controlled closed systems, amount of release to air phase is negligibly small. The waste of 2,6-dichlorotoluene from the production system is released to water phase after treated through its own waste-water treatment plant. Therefore, Predicted Environmental Concentration (PEC) will be calculated only for the water environment.

a. Local exposure

According to the report from a manufacturer in Japan, 72 kg/year (measured) of 2,6-dichlorotoluene was released with 3.4 x 1010 L/year of effluent into a bay in 1994. Local Predicted Environmental Concentration (PEClocal) is calculated to be 7.3 x 10-6 mg/L, employing the following calculation model and dilution factor of 290 (See Appendix 1).

Amount of release (7.2 x 107 mg/y)
Volume of effluent (3.4 x 1010 L/y) x Dilution Factor (290)

3.2 Effects on the Environments

3.2.1 Effects on aquatic organisms

Acute and chronic toxicity data of 2,6-Dichlorotoluene to aquatic organisms are summarized below (Table 2). Toxicity of this chemical seems relatively high to Daphnia. Predicted No Effect Concentration (PNEC) of this chemical was determined based on the toxicity data obtained by the

Environment Agency of Japan, because other data by different organizations were not available in the AQUIRE and IUCLID. As the lowest acute and chronic toxicity data, 48 h EC50 (immobility) value and 21 d NOEC (reproduction) of Daphnia magna were adopted, respectively (Table 2). The assessment factors of 100 were used to both acute and chronic toxicity data to determine PNEC, according to the OECD Provisional Guidance for Initial Assessment of Aquatic Effects (EXCH/MANUAL/96-4-5.DOC/May 1996), because chronic toxicity data for fish was absent.

From acute toxicity data (48 h EC50 of Daphnia): PNEC = 1.8/ 100 = 0.018 mg/l
From chronic toxicity data (21 d NOEC of Daphnia): PNEC = 0.32/ 100 = 0.0032 mg/l

Thus, PNEC of 2,6-Dichlorotoluene is 0.0032 mg/l.

Table 2 Acute and chronic toxicity data of 2,6-Dichlorotoluene to aquatic organisms at different trophic levels. The data were obtained by the Environmental Agency of Japan based on the OECD Test Guide Lines.

Species	Endpoint	Conc. (mg/l)	Remarks
Selenastrum capricornutum (algae)	Gro 72 h EC50	17.6	a, 1), A
	do. 72 h NOEC	10.0	c, 1), C
Daphnia magna (Water flea)	Imm 24 h EC50	1.8	a, 1), A
	Rep 21 d EC50	0.47	c, 1)
	Rep 21 d NOEC	0.32	c, 1), C
Oryzias latipes (fish, Medaka)	Mor 1 d LC50	10.0	a, 1)
	Mor 2 d LC50	7.9	a, 1)
	Mor 3 d LC50	6.4	a, 1)
	Mor 4 d LC50	6.4	a, 1), A

Notes: Gro; growth, Mor; mortality, Rep; reproduction,
No. 1, reference number, A), C); the lowest values among the acute or chronic toxicity data of algae, cladocera (water flea) and fishes to determine PNEC of 2,6-Dichlorotoluene.

References

1) Toxicity data of the tests were conducted by the Environment Agency of Japan based on OECD Test Guide Lines.

3.2.2 Terrestrial effects

No data available

3.2.3 Other effects

No data available

3.3 Initial Assessment for the Environment

Predicted No Effect Concentration (PNEC) of this chemical has been calculated as 0.0032 mg/l. PEC from Japanese local exposure scenario is 7.3×10^{-6} mg/l.

$$PEClocal / PNEC = 7.3 \times 10^{-6} / 0.0032 = 0.0023 < 1$$