

3. 藻類生長阻害試験

試験方法および結果の要約

供試生物	分類 : 単細胞緑藻類 学名 : <i>Pseudokirchneriella subcapitata</i> 入手先 : American Type Culture Collection (ATCC22662株) 感受性 : 重クロム酸カリウムの72時間50%生長阻害濃度 (EbC50) 平均値 ± 標準偏差 = 0.428 ± 0.0683 mg/L, n=18
試験方法	「新規化学物質等に係る試験の方法について<藻類生長阻害試験, ミジンコ急性遊泳阻害試験及び魚類急性毒性試験>」(平成15年11月21日 薬食発第 1121002号, 平成15・11・13製局第2号, 環企発第031121002号, 最終改正:平成17年4月1日)
試験条件	培養方式: 止水式(密閉系), 振とう培養(100rpm) 試験容器: 500mL容ガラス製共栓付き三角フラスコ ヘッドスペース容量: 当社測定値 490 mL 連数: 6容器/対照区および助剤対照区, 3容器/濃度区 培地: 化審法テストガイドライン推奨培地 試験液量: 100mL/容器 暴露期間: 72時間 生物数: 5×10 ³ cells/mL (初期細胞濃度) 照明: 65 μE/m ² /s (装置中央フラスコ液面付近) で連続照明 装置内変動: ±8%以内 温度: 23±2 °C 測定値: 22.8~23.5°C pH: 試験液のpH調整なし 測定値: 8.3~10.2 (72時間後)
助剤の種類と濃度	種類: テトラヒドロフラン 濃度: 65 μL/L
試験濃度	対照区, 助剤対照区, 5.00, 7.10, 10.0, 14.1, 20.0* mg/L (公比: 1.4) * 試験液調製可能最高濃度
結果および考察	mg/L (95%信頼区間)
速度法	半数生長阻害濃度 ErC50(0-72h): >4.36 (算出不可) 最大無影響濃度 NOECr(0-72h): 1.15
面積法	半数生長阻害濃度 EbC50(0-72h): >4.36 (算出不可) 最大無影響濃度 NOECb(0-72h): 1.15
収量法	半数生長阻害濃度 ECy50(0-72h): >4.36 (算出不可) 最大無影響濃度 NOECy(0-72h): 1.15
統計的手法	半数生長阻害濃度: 直線回帰分析 有意差検定: Williamsの多重比較検定
特記事項	被験物質の培地に対する溶解度 20 mg/L (当社測定値) 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 67~82 %, 暴露終了時の試験培養液において 9~11 %であった。暴露開始時における濃度減少は高濃度区側より低濃度区側が大きく, その原因として試験操作時の水中からの揮散が考えられた。この濃度減少は本被験物質の性質上避けられないと判断した。また, 暴露期間中の濃度減少は水中からの揮散が考えられた。阻害濃度の算出には測定値の平均値(時間加重平均)を用いた。

Table 1 Measured Concentration of the Test Substance in Test Cultures

Nominal Concentration (mg/L)	Measured Concentration (mg/L) (Percent of Nominal)				Mean ^a Measured Concentration (Percent of Nominal) (mg/L)
	0 Hour	24 Hour	48 Hours	72 Hours	
Control	<0.0001	<0.0001	<0.0001	<0.0001	---
Solvent Control	<0.0001	<0.0001	<0.0001	<0.0001	---
5.00	3.37 (67)	0.364 (7)	0.554 (11)	0.450 (9)	0.767 (15)
7.10	4.74 (67)	0.576 (8)	0.873 (12)	0.636 (9)	1.15 (16)
10.0	6.79 (68)	0.865 (9)	1.15 (11)	0.864 (9)	1.62 (16)
14.1	10.4 (74)	1.30 (9)	1.65 (12)	1.28 (9)	2.43 (17)
20.0	16.4 (82)	2.68 (13)	3.11 (16)	2.15 (11)	4.36 (22)

a : time weighted mean

Table 2 Cell Densities of *Pseudokirchneriella subcapitata* during the 72-Hour Exposure

Nominal Concentration [Mean ^a Measured Conc.] (mg/L)	Vessel No.	Cell Densities (cells/mL)			
		0 Hour*	24 Hours	48 Hours	72 Hours
Control	1	5000	29200	228600	753800
	2	5000	28600	216600	700800
	3	5000	27600	202600	624800
	4	5000	24200	188600	693800
	5	5000	32500	234600	713800
	6	5000	28800	194600	593800
	Average	5000	28500	210900	680100
	SD	0	2700	18700	59500
Solvent Control	1	5000	28500	199600	517800
	2	5000	27900	207600	683800
	3	5000	29300	212600	620800
	4	5000	31600	209600	677800
	5	5000	32100	229600	515800
	6	5000	25600	192600	695800
	Average	5000	29200	208600	618600
	SD	0	2400	12600	83000
5.00 [0.767]	1	5000	25700	191600	660800
	2	5000	27100	181600	619800
	3	5000	26600	180600	615800
	Average	5000	26500	184600	632100
7.10 [1.15]	1	5000	22900	174600	638800
	2	5000	24900	179600	604800
	3	5000	30000	203600	601800
	Average	5000	25900	185900	615100
10.0 [1.62]	1	5000	27500	202600	418800
	2	5000	25800	182600	477800
	3	5000	25700	192600	428800
	Average	5000	26300	192600	441800
14.1 [2.43]	1	5000	22000	141600	333800
	2	5000	23400	148600	357800
	3	5000	22200	163600	378800
	Average	5000	22500	151300	356800
20.0 [4.36]	1	5000	12500	106600	326800
	2	5000	21700	112600	332800
	3	5000	22000	109600	300800
	Average	5000	18700	109600	320100
SD	0	5400	3000	17000	

a : time weighted mean

SD : Standard deviation

* : Nominal initial densities

Table 3 Growth Rate of Control

Vessel No.	Growth Rate			Average	SD	CV(%)
	μ (0-24h)	μ (24-48h)	μ (48-72h)			
1	0.0735	0.0857	0.0497	0.0682	0.0177	26.0
2	0.0727	0.0844	0.0489			
3	0.0712	0.0831	0.0469			
4	0.0657	0.0856	0.0543			
5	0.0780	0.0824	0.0464			
6	0.0730	0.0796	0.0465			
Average	0.0724	0.0835	0.0488			
SD	0.0040	0.0023	0.0030			
CV(%)	5.5	2.8	6.1			

SD: Standard deviation

CV: Coefficient of variation

Table 4 Growth Inhibition (%) of *Pseudokirchneriella subcapitata*

Nominal Concentration		Growth Rate		Area under the growth curves		Yield	
[Mean ^a Measured Conc.]		Rate	Inhibition(%) ^{*1}	Area	Inhibition(%) ^{*1}	Yield	Inhibition(%) ^{*1}
(mg/L)	Vessel No.	μ (0-72h)	I_{μ} (0-72h)	A (0-72h)	I_A (0-72h)	Y (0-72h)	I_Y (0-72h)
Control	1	0.0697		14933000		748800	
	2	0.0686		13994000		695800	
	3	0.0671		12722000		619800	
	4	0.0685		13133000		688800	
	5	0.0689		14676000		708800	
	6	0.0663		12187000		588800	
	Average SD	0.0682 0.0012	-	13608000 1102000	-	675100 59500	-
Solvent Control	1	0.0644		11388000		512800	
	2	0.0683		13558000		678800	
	3	0.0670		12955000		615800	
	4	0.0682		13622000		672800	
	5	0.0644		12170000		510800	
	6	0.0686		13286000		690800	
	Average SD	0.0666 0.0020	-	12830000 883000	-	613600 83000	-
5.00 [0.767]	1	0.0678		12845000		655800	
	2	0.0669		12146000		614800	
	3	0.0669		12062000		610800	
	Average SD	0.0672 0.0005	-0.9	12351000 430000	3.7	627100 24900	-2.2
7.10 [1.15]	1	0.0674		12106000		633800	
	2	0.0666		11866000		599800	
	3	0.0665		12528000		596800	
	Average SD	0.0668 0.0005	-0.3	12167000 335000	5.2	610100 20600	0.6
10.0 [1.62]	1	0.0615		10248000		413800	
	2	0.0633		10435000		472800	
	3	0.0618		10085000		423800	
	Average SD	0.0622 0.0010	6.6**	10256000 175000	20.1**	436800 31600	28.8**
14.1 [2.43]	1	0.0583		7632000		328800	
	2	0.0593		8122000		352800	
	3	0.0601		8705000		373800	
	Average SD	0.0592 0.0009	11.1**	8153000 537000	36.5**	351800 22500	42.7**
20.0* [4.36]	1	0.0581		6480000		321800	
	2	0.0583		6917000		327800	
	3	0.0569		6468000		295800	
	Average SD	0.0578 0.0008	13.2**	6622000 256000	48.4**	315100 17000	48.6**

a time weighted mean

*1 Values are the growth inhibition (%) relative to the solvent control.

SD Standard deviation

★ The maximum attainable concentration under the present test conditions and preparation methods.

* Indicates a significant difference ($\alpha=0.05$) from the solvent control. (There was no sign in this test.)** Indicates a significant difference ($\alpha=0.01$) from the solvent control.

Table 5 Calculated EC50 and NOEC

Based on I_r (0-72h) value (Growth rates)

ErC50 (0-72h) (mg/L)	95-Percent Confidence Limits (mg/L)	NOECr (0-72h) (mg/L)
>4.36	--	1.15

Based on I_A (0-72h) value (Areas under growth curve)

EbC50 (0-72h) (mg/L)	95-Percent Confidence Limits (mg/L)	NOECb (0-72h) (mg/L)
>4.36	--	1.15

Based on I_y (0-72h) value (Yield)

EyC50 (0-72h) (mg/L)	95-Percent Confidence Limits (mg/L)	NOECy (0-72h) (mg/L)
>4.36	--	1.15

The EC50 values and associated 95% confidence limits could not be determined by least squares linear regression analysis because the growth inhibition (%) at the maximum concentration level was less than 50%.

-- not calculated

The NOEC values were determined by an analysis of variance (ANOVA), Williams test, subsequent to Bartlett test for homogeneity of variances. Statistical analyses were performed using Yukms Statlight #4 software (Yukms Corp., Tokyo) and all tests of significance were at $\alpha=0.05$, except Bartlett test, which was at $\alpha=0.01$.

Table 6 Temperature, Light Intensity and Revolutions in the Incubation Chamber

Exposure Period (Hours)	Temperature (°C)	Light Intensity ($\mu E/m^2/s$)	Revolutions (rpm)
0	23.4	65-66	100
24	23.5	64-65	100
48	23.3	64-65	100
72	22.8	65-66	100

Table 7 pH Values of Test Cultures

Nominal Concentration [Mean ^a Measured Conc.] (mg/L)	pH		
	0 Hour	72 Hours (Vessel No.)	
Control	8.2	10.2	(1)
Solvent Control	8.1	9.9	(1)
5.00 [0.767]	8.1	10.0	(1)
7.10 [1.15]	8.0	9.8	(1)
10.0 [1.62]	8.0	8.5	(1)
14.1 [2.43]	8.0	8.3	(1)
20.0 [4.36]	8.0	8.4	(1)

a : time weighted mean

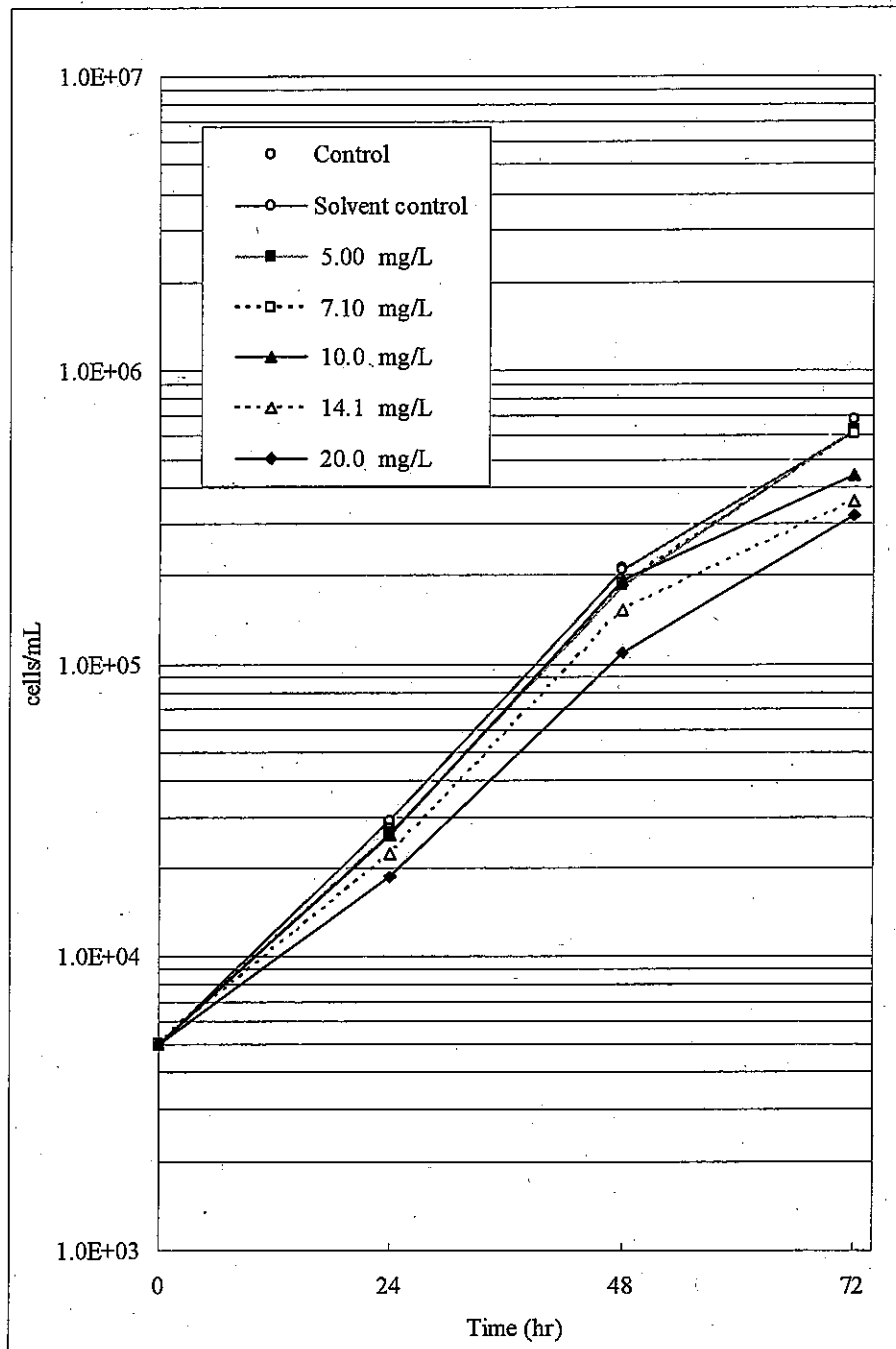
Table 8 Appearance of Test Solutions after Preparation

Nominal Concentration [Mean ^a Measured Conc.] (mg/L)	Suspended	Floating	Precipitation	Oily Substances	Color
	solids	solids			
Control	none	none	none	none	c-
Solvent Control	none	none	none	none	c-
5.00 [0.767]	none	none	none	none	c-
7.10 [1.15]	none	none	none	none	c-
10.0 [1.62]	none	none	none	none	c-
14.1 [2.43]	none	none	none	none	c-
20.0 [4.36]	none	none	none	none	c-

a : time weighted mean

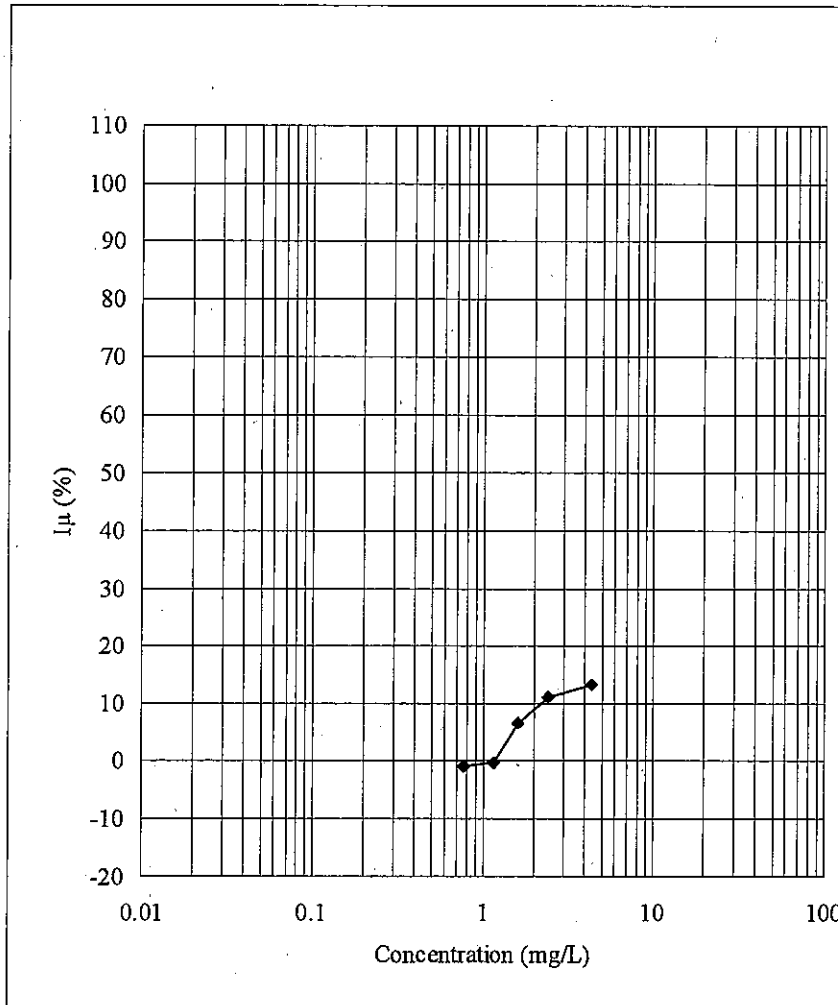
c- : colorless

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



Values in legend are given in the nominal concentration.

Figure 2 Concentration-Inhibition Curve Based on I_{μ} values Calculated from the Growth Rates



4. ミジンコ急性遊泳阻害試験

試験方法および結果の要約

供試生物	和名 : オオミジンコ 学名 : <i>Daphnia magna</i> 入手先 : 国立環境研究所 感受性 : 重クロム酸カリウムの48時間半数遊泳阻害濃度 平均値±標準偏差=0.75±0.14mg/L, n=16															
試験方法	「新規化学物質等に係る試験の方法について<藻類生長阻害試験, ミジンコ急性遊泳阻害試験及び魚類急性毒性試験>」(平成15年11月21日薬食発第1121002号, 平成15・11・13製局第2号, 環保企発第031121002号, 最終改正: 平成17年4月1日)															
試験条件	暴露方式: 半止水式 (24時間後に試験液の全量を交換) 試験容器: 共栓付き100 mL容ガラス三角フラスコ (被験物質の著しい揮散が考えられるため, 試験容器を試験液で満たし(130 mL), 共栓で蓋をして空隙をほぼなくした) 連数 : 4容器/試験区 飼育水 : Elendt M4 溶液 (希釈水) 試験液量: 130mL/容器 暴露期間: 48時間 生物数 : 20頭/試験区 (5頭/容器) 照明 : 室内光, 16時間明(800 lux以下)/8時間暗 給餌 : 無し <table border="1" data-bbox="560 1198 1370 1352"> <tr> <td>温度 : 20±1 °C</td> <td>測定値 : 19.9 °C</td> </tr> <tr> <td>D O : 飽和の60%以上</td> <td>測定値 : 8.3~ 8.8 mg/L</td> </tr> <tr> <td>p H : 6.0~9.0</td> <td>測定値 : 8.0~ 8.4</td> </tr> <tr> <td>硬度 : 250 mg/L以下 CaCO₃換算 (希釈水)</td> <td></td> </tr> </table>	温度 : 20±1 °C	測定値 : 19.9 °C	D O : 飽和の60%以上	測定値 : 8.3~ 8.8 mg/L	p H : 6.0~9.0	測定値 : 8.0~ 8.4	硬度 : 250 mg/L以下 CaCO ₃ 換算 (希釈水)								
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硬度 : 250 mg/L以下 CaCO ₃ 換算 (希釈水)																
助剤の種類と濃度	使用せず															
試験濃度	対照区, 0.500, 1.00, 2.00, 4.00, 8.00 mg/L (公比: 2.0)															
結果および考察	<table border="1"> <thead> <tr> <th></th> <th colspan="2">mg/L (95%信頼区間)</th> </tr> </thead> <tbody> <tr> <td>24時間</td> <td>半数遊泳阻害濃度 EC50 :</td> <td>2.57 (1.75~3.30)</td> </tr> <tr> <td></td> <td>0%阻害最高濃度 :</td> <td>1.75</td> </tr> <tr> <td>48時間</td> <td>半数遊泳阻害濃度 EC50 :</td> <td>2.31 (2.02~2.67)</td> </tr> <tr> <td></td> <td>0%阻害最高濃度 :</td> <td>0.945</td> </tr> </tbody> </table>		mg/L (95%信頼区間)		24時間	半数遊泳阻害濃度 EC50 :	2.57 (1.75~3.30)		0%阻害最高濃度 :	1.75	48時間	半数遊泳阻害濃度 EC50 :	2.31 (2.02~2.67)		0%阻害最高濃度 :	0.945
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	0%阻害最高濃度 :	0.945														
統計的手法	24時間 EC50 : Binomial法, 48時間 EC50 : Probit法															
特記事項	被験物質の希釈水に対する溶解度 18 mg/L (当社測定値) 各試験液濃度の設定値に対する割合は, 試験液調製時において82~99%, その24時間後において80~93%であった。															

Table 1 Measured Concentration of the Test Substance in Test Water
(Semi-Static Condition)

Nominal Concentration (mg/L)	Measured Concentration (mg/L) (Percent of Nominal, %)				Mean ^a Measured Concentration (mg/L) (Percent of Nominal, %)
	0 Hour New	24 Hours Old	24 Hours New	48 Hours Old	
Control	< 0.002*	< 0.002*	< 0.002*	< 0.002*	--
0.500	0.496 (99)	0.466 (93)	0.491 (98)	0.465 (93)	0.479 (96)
1.00	0.981 (98)	0.933 (93)	0.937 (94)	0.931 (93)	0.945 (95)
2.00	1.83 (92)	1.70 (85)	1.76 (88)	1.71 (86)	1.75 (88)
4.00	3.47 (87)	3.23 (81)	3.33 (83)	3.19 (80)	3.30 (83)
8.00	6.76 (85)	6.46 (81)	6.54 (82)	6.53 (82)	6.57 (82)

a: time weighted mean

New: freshly prepared test solutions

Old: test solutions on 24 hours after preparation of new solutions

*: detection limit under the test conditions

Table 2 The Number of Immobilized *Daphnia magna* (Percent Immobility)

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Cumulative Number of Immobilized <i>Daphnia</i> (Percent Immobility)	
		24 Hours	48 Hours
Control	--	0 (0)	0 (0)
0.500	0.479	0 (0)	0 (0)
1.00	0.945	0 (0)	0 (0)
2.00	1.75	0 (0)	2 (10)
4.00	3.30	18 (90)	19 (95)
8.00	6.57	20 (100)	20 (100)

a: time weighted mean

Table 3 Calculated EC50 Values

Exposure Period (Hours)	EC50 (mg/L)	95-Percent Confidence Limits (mg/L)	Statistical Method
24	2.57	1.75 - 3.30	Binomial ^{*1}
48	2.31	2.02 - 2.67	Probit ^{*1}

*1: Using the concentrations of 0.479 - 6.57 mg/L

Table 4 Highest Concentration in 0% Immobility and Lowest Concentration in 100% Immobility

Exposure Period (Hours)	Highest Concentration in 0% Immobility (mg/L)	Lowest Concentration in 100% Immobility (mg/L)
24	1.75	6.57
48	0.945	6.57

Table 5 Appearance of Test Solutions

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Appearance of Test Solutions			
		0 Hour New	24 Hours Old	24 Hours New	48 Hours Old
Control	--	C-	C-	C-	C-
0.500	0.479	C-	C-	C-	C-
1.00	0.945	C-	C-	C-	C-
2.00	1.75	C-	C-	C-	C-
4.00	3.30	C-	C-	C-	C-
8.00	6.57	C-	C-	C-	C-

a: time weighted mean

New: freshly prepared test solutions

Old: test solutions on 24 hours after preparation of new solutions

Color:

C-; colorless

Table 6 Temperature of Test Solutions

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Temperature (°C)			
		0 Hour New	24 Hours Old	24 Hours New	48 Hours Old
Control	--	19.9	19.9	19.9	19.9
0.500	0.479	19.9	19.9	19.9	19.9
1.00	0.945	19.9	19.9	19.9	19.9
2.00	1.75	19.9	19.9	19.9	19.9
4.00	3.30	19.9	19.9	19.9	19.9
8.00	6.57	19.9	19.9	19.9	19.9

a: time weighted mean

New: freshly prepared test solutions

Old: test solutions on 24 hours after preparation of new solutions

Table 7 Dissolved Oxygen Concentrations in Test Solutions

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Dissolved Oxygen Concentration (mg/L)			
		0 Hour New	24 Hours Old	24 Hours New	48 Hours Old
Control	--	8.8	8.5	8.6	8.4
0.500	0.479	8.8	8.5	8.6	8.6
1.00	0.945	8.8	8.5	8.5	8.6
2.00	1.75	8.7	8.5	8.5	8.6
4.00	3.30	8.6	8.4	8.4	8.6
8.00	6.57	8.4	8.3	8.3	8.4

a: time weighted mean

New: freshly prepared test solutions

Old: test solutions on 24 hours after preparation of new solutions

Table 8 pH Values of Test Solutions

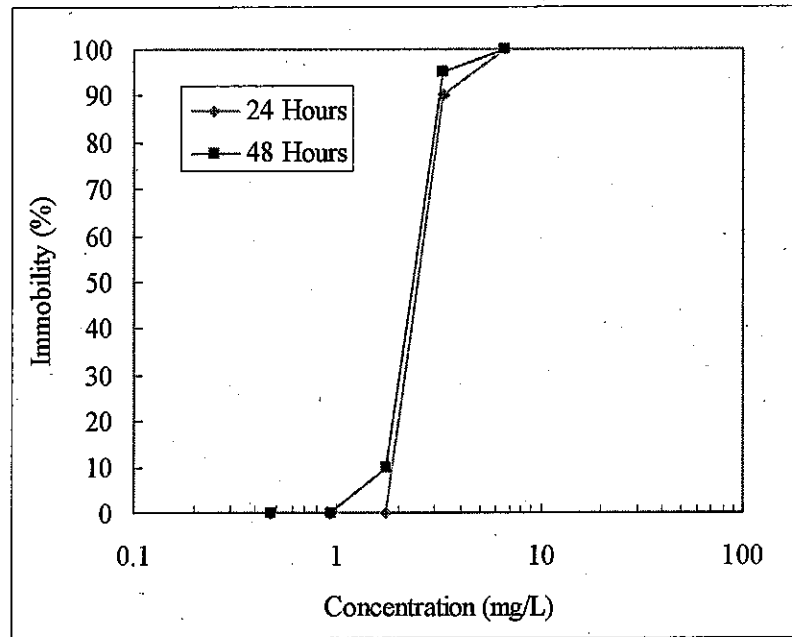
Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	pH			
		0 Hour New	24 Hours Old	24 Hours New	48 Hours Old
Control	--	8.4	8.3	8.3	8.3
0.500	0.479	8.3	8.2	8.2	8.2
1.00	0.945	8.3	8.2	8.1	8.2
2.00	1.75	8.3	8.2	8.0	8.2
4.00	3.30	8.3	8.2	8.1	8.2
8.00	6.57	8.3	8.3	8.1	8.2

a: time weighted mean

New: freshly prepared test solutions

Old: test solutions on 24 hours after preparation of new solutions

Figure 1 Concentration-Immobilization Curve



5. 魚類急性毒性試験

試験方法および結果の要約

供試生物	和名 : ヒメダカ 学名 : <i>Oryzias latipes</i> 入手先 : 綱島フィッシング (神奈川県横浜市港北区綱島西五丁目18番1号) 感受性 : 硫酸銅 (II) の96時間半数致死濃度 LC50 : 0.77 mg/L (95%信頼区間 : 0.58~1.0 mg/L)								
試験方法	「新規化学物質等に係る試験の方法について<藻類生長阻害試験, ミジンコ急性遊泳阻害試験及び魚類急性毒性試験>」(平成15年11月21日薬食発第1121002号, 平成15・11・13製局第2号, 環境企発第031121002号, 最終改正:平成17年4月1日)								
試験条件	<p>暴露方式 : 半止水式 (24時間毎換水)</p> <p>試験容器 : 5.0 L容ガラス製水槽 (テフロンシートで水面被覆)</p> <p>連数 : 1 容器/試験区</p> <p>飼育水 : 脱塩素水道水 (希釈水)</p> <p>試験液量 : 5.0 L/容器</p> <p>暴露期間 : 96時間</p> <p>生物数 : 10尾/試験区</p> <p>照明 : 室内光, 16時間明 (1000 lux以下)/8時間暗</p> <p>給餌 : 無し</p> <table border="1"> <tr> <td>温度 : 24±1 °C</td> <td>測定値 : 23.6~23.9 °C</td> </tr> <tr> <td>D O : 飽和の60%以上</td> <td>測定値 : 6.6~ 8.4 mg/L</td> </tr> <tr> <td>p H : 6.5~8.5</td> <td>測定値 : 7.1~ 7.6</td> </tr> <tr> <td>硬度 : 30~100 mg/L CaCO₃換算</td> <td>測定値 : 49 mg/L (希釈水)</td> </tr> </table>	温度 : 24±1 °C	測定値 : 23.6~23.9 °C	D O : 飽和の60%以上	測定値 : 6.6~ 8.4 mg/L	p H : 6.5~8.5	測定値 : 7.1~ 7.6	硬度 : 30~100 mg/L CaCO ₃ 換算	測定値 : 49 mg/L (希釈水)
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p H : 6.5~8.5	測定値 : 7.1~ 7.6								
硬度 : 30~100 mg/L CaCO ₃ 換算	測定値 : 49 mg/L (希釈水)								
助剤の種類と濃度	使用せず								
試験濃度	対照区, 1.00, 1.80, 3.20, 5.60, 10.0 mg/L (公比 : 1.8)								
結果および考察	<table border="1"> <tr> <td></td> <td>mg/L (95%信頼区間)</td> </tr> <tr> <td>96時間</td> <td>半数致死濃度 (LC50) : 6.82 (4.76~9.78)</td> </tr> <tr> <td></td> <td>0%死亡最高濃度 : 4.76</td> </tr> <tr> <td></td> <td>100%死亡最低濃度 : 9.78</td> </tr> </table>		mg/L (95%信頼区間)	96時間	半数致死濃度 (LC50) : 6.82 (4.76~9.78)		0%死亡最高濃度 : 4.76		100%死亡最低濃度 : 9.78
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	0%死亡最高濃度 : 4.76								
	100%死亡最低濃度 : 9.78								
統計的手法	LC50 : Binomial法								
特記事項	被験物質の希釈水に対する溶解度 18 mg/L (当社測定値) 各試験液濃度の設定値に対する割合は, 試験液調製時において88~106%, 24時間後において75~101%であった。濃度減少の主な原因はヒメダカへの移行 (取り込みや, 体表への吸着) の可能性が考えられる。								

Table 1 Measured Concentration of the Test Substance in Test Water

Nominal Concentration (mg/L)	Measured Concentration, mg/L (Percent of Nominal)				(Semi-Static Condition) Mean ^a Measured Concentration (mg/L)
	0 Hour (new)	24 Hours (old)	24 Hours (new)	48 Hours (old)	
Control	<0.00006	<0.00006	<0.00006	<0.00006	----
1.00	0.925 (93)	0.750 (75)	0.879 (88)	0.822 (82)	0.842 (84)
1.80	1.91 (106)	1.47 (82)	1.77 (98)	1.66 (92)	1.70 (94)
3.20	2.91 (91)	2.55 (80)	2.89 (90)	2.69 (84)	2.76 (86)
5.60	5.06 (90)	4.24 (76)	5.07 (91)	4.69 (84)	4.76 (85)
10.0	10.3 (103)	8.67 (87)	10.1 (101)	10.1 (101)	9.78 (98)

a : time weighted mean

new : freshly prepared test solutions

old : test solutions on 24 hours after preparation of new solutions

The test water for analysis was sampled at two renewal sets of four during 96-hour exposure.

Table 2 Mortality of the Medaka (*Oryzias latipes*) Exposed to the Test Substance

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Cumulative Mortality (Percent Mortality)			
		24 Hours	48 Hours	72 Hours	96 Hours
Control	---	0 (0)	0 (0)	0 (0)	0 (0)
1.00	0.842	0 (0)	0 (0)	0 (0)	0 (0)
1.80	1.70	0 (0)	0 (0)	0 (0)	0 (0)
3.20	2.76	0 (0)	0 (0)	0 (0)	0 (0)
5.60	4.76	0 (0)	0 (0)	0 (0)	0 (0)
10.0	9.78	8 (80)	10 (100)	10 (100)	10 (100)

a: time weighted mean

Table 3 Calculated LC50 Values

Exposure Period (Hours)	LC50 (mg/L)	95-Percent Confidence Limits (mg/L)	Statistical Method
24	7.80*	--	Binomial
48	6.82*	4.76 - 9.78	Binomial
72	6.82*	4.76 - 9.78	Binomial
96	6.82*	4.76 - 9.78	Binomial

--: Could not be determined.

*: Using the concentrations of 0.842 - 9.78 mg/L

Table 4 Highest Concentration in 0% Mortality and the Lowest Concentration in 100% Mortality

Exposure Period (Hours)	Highest Concentration in 0% Mortality (mg/L)	Lowest Concentration in 100% Mortality (mg/L)
24	4.76	>9.78
48	4.76	9.78
72	4.76	9.78
96	4.76	9.78

Table 5 Observed Toxicological Symptoms

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Symptoms (Symptom-number of fish)			
		24 Hours	48 Hours	72 Hours	96 Hours
Control	---	N	N	N	N
1.00	0.842	N	N	N	N
1.80	1.70	N	N	N	N
3.20	2.76	N	N	N	N
5.60	4.76	ASR-6	ASR-10(SUR-1)	ASR-9(SUR-1) AP-1	ASR-8(SUR-1) ASL-1,AP-1
10.0	9.78	AP-2	--	--	--

a : time weighted mean

N : No toxicological symptom was observed.

ASR: abnormal swimming (reduced activity)

AP: paralyzation

SUR: surfacing

ASL: abnormal swimming (loss of equilibrium)

---: No observation was made because all fish were dead at this observation time.

Table 6 Appearance of Test Solutions

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Appearance of Test Solutions							
		0 Hour	24 Hours		48 Hours		72 Hours		96 Hours
		new	old	new	old	new	old	new	old
Control	----	C-	C-	C-	C-	C-	C-	C-	C-
1.00	0.842	C-	C-	C-	C-	C-	C-	C-	C-
1.80	1.70	C-	C-	C-	C-	C-	C-	C-	C-
3.20	2.76	C-	C-	C-	C-	C-	C-	C-	C-
5.60	4.76	C-	C-	C-	C-	C-	C-	C-	C-
10.0	9.78	C-	C-	C-	--	--	--	--	--

a : time weighted mean

new : freshly prepared test solutions

old : test solutions on 24 hours after preparation of new solutions

--: No observation was made because all fish were dead at this observation time.

Color

C- : Colorless

Table 7 Temperature of Test Solutions

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Temperature (°C)								
		0 Hour		24 Hours		48 Hours		72 Hours		96 Hours
		new	old	new	old	new	old	new	old	
Control	----	23.6	23.6	23.7	23.8	23.6	23.9	23.7	23.8	
1.00	0.842	23.7	23.7	23.6	23.7	23.8	23.9	23.8	23.8	
1.80	1.70	23.7	23.7	23.6	23.7	23.8	23.9	23.8	23.9	
3.20	2.76	23.7	23.7	23.6	23.7	23.8	23.9	23.9	23.9	
5.60	4.76	23.7	23.7	23.6	23.7	23.8	23.9	23.9	23.9	
10.0	9.78	23.7	23.7	23.7	--	--	--	--	--	

minimum:23.6
maximum:23.9

a : time weighted mean

new : freshly prepared test solutions

old : test solutions on 24 hours after preparation of new solutions

-- : No measurement was made because all fish were dead at this observation time.

Table 8 Dissolved Oxygen Concentrations in Test Solutions

Nominal Concentration (mg/L)	Mean ^a Measured Concentration (mg/L)	Dissolved Oxygen Concentration (mg/L)								
		0 Hour		24 Hours		48 Hours		72 Hours		96 Hours
		new	old	new	old	new	old	new	old	
Control	---	8.3	6.7	8.3	6.8	8.3	7.0	8.3	7.0	
1.00	0.842	8.4	6.6	8.3	6.8	8.3	7.0	8.4	7.0	
1.80	1.70	8.4	6.6	8.3	6.7	8.3	6.8	8.3	6.8	
3.20	2.76	8.2	6.8	8.3	6.8	8.3	7.0	8.3	7.0	
5.60	4.76	8.1	6.8	8.1	6.7	8.2	6.8	8.1	6.8	
10.0	9.78	8.2	7.0	7.9	--	--	--	--	--	

minimum:6.6
maximum:8.4

a : time weighted mean

new : freshly prepared test solutions

old : test solutions on 24 hours after preparation of new solutions

-- : No measurement was made because all fish were dead at this observation time.

Table 9 pH Values of Test Solutions

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	new	old	new	old	
Control	----	7.5	7.1	7.5	7.2	7.6	7.3	7.6	7.2	
1.00	0.842	7.6	7.1	7.6	7.1	7.5	7.2	7.6	7.2	
1.80	1.70	7.6	7.1	7.5	7.1	7.5	7.2	7.6	7.2	
3.20	2.76	7.6	7.1	7.5	7.1	7.5	7.2	7.6	7.2	
5.60	4.76	7.6	7.2	7.6	7.1	7.5	7.2	7.5	7.2	
10.0	9.78	7.6	7.2	7.6	--	--	--	--	--	

minimum:7.1
maximum:7.6

a : time weighted mean

new : freshly prepared test solutions

old : test solutions on 24 hours after preparation of new solutions

-- : No measurement was made because all fish were dead at this observation time.

Figure 1 Concentration-Mortality Curve

