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RESULTS OF THE HARMFUL EFFECTS OF SELECTED WATER POLLUTANTS (ANILINES, PHENOLS, ALIPHATIC COMPOUNDS) TO *DAPHNIA MAGNA*

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Abstract—Using the acute *Daphnia* test (according to DIN 38412, Part II), the EC 50, EC 0 and EC 100 after 24 and 48 h test time were calculated or determined for 70 substances—phenols, anilines and aliphatic compounds. The results of the tests are given in three tables according to substance group. Evaluation showed that the toxicity of the substances (particularly in the case of anilines) may be higher or even substantially greater when the test period is extended from 24 to 48 h.

Key words—*Daphnia magna*, acute *Daphnia* test, 24 and 48 h EC 50, EC 0 and EC 100, phenols, anilines, aliphatic compounds

INTRODUCTION

This work is part of the research project commissioned by the Federal Environmental Agency (Umweltbundesamt) "*Damaging effects of environmental chemicals in the Daphnia reproduction test as a basis for evaluation of the environment hazard in aquatic systems*".

Seventy substances were examined using the *Daphnia* test in order to calculate and/or determine the data on the EC 50, EC 0 and EC 100 for test periods of 24 and 48 h. The substances to be tested were selected from three classes of substances (phenols, anilines and aliphatic compounds).

Determining the effect of potential pollutants on the ability of *Daphnia magna* Straus to swim was carried out in accordance with the DIN 38412, Part II (*Daphnia* short-time test) (German Institute of Standardization, 1982).

MATERIALS AND METHODS

Test conditions

The parameters which could influence the test results were observed as stipulated in the procedure: 6–24 h old daphnids—which came from our own culture (Bringmann and Kühn, 1982)—were used as test animals as they react most sensitively to toxic influences at that stage in their development.

The dilution water used had a specific composition: acid capacity $K_{S4.3}$ of 0.8 mmol l^{-1} total hardness of 2.4 mmol l^{-1}, a calcium to magnesium ratio of 4:1, a sodium to potassium ratio of 10:1 and thus a resulting initial pH value of 8.0 ± 0.2.

The test solutions were kept at 20°C in an incubator. The test periods lasted 24 and 48 h. No feed was given during the test period.

Test procedure

From the solutions with the pollutants, dilution series were prepared using "dilution water". The concentration steps of the test solution were selected so as to give 3–4 EC values in a range between EC 0 and EC 100, of which at least one value was below and one above EC 50. The test vessels were two 50 ml beakers and for volatile or strongly smelling substances, two 50 ml bottles with ground-glass stoppers each with 20 ml useful capacity.

Two parallel preparations were made for each concentration step. Loading amounted to one test animal per 2 ml test medium as ten 6–24 h old daphnids were placed in each test and control vessel, i.e. 20 animals per concentration step.

After a test period of 24 h and again after 48 h, the number of animals in the control and test solutions that could still swim were counted. After 48 h it was also possible to measure the pH value in order to determine whether the hydrogen ion concentrations were in an acceptable range for the daphnids or whether the result had to be considered as having been influenced by a change in pH during the course of the test. Furthermore, the oxygen content was determined at the end of the test period in order to ensure that it had not fallen below a minimum oxygen concentration of 2 mg l^{-1}.

Evaluation of the test results

The test was considered as valid when fewer than 10% of the animals in the control solutions were unable to swim, when the pH value was not below 7.0 and the O_2 value was not below 4.0 mg l^{-1}.

In the case of defined concentrations of a substance, a specific percentage of daphnids was incapable of swimming at the end of the test period.

The EC 0 and EC 100 values were taken from the results obtained for the test solution, the 24 and 48 h EC 50s calculated arithmetically from the concentration/effect ratio.

RESULTS

The results of the effect of the substances tested on the ability of *Daphnia magna* to swim are listed by chemical substance group in Tables 1–3.

The following data are given for each substance tested:

EC 0, EC 50 and EC 100 determined or calculated after 24 h test period;

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Table 1. List of the toxicological test results with *Daphnia magna*

Phenols	Test period (h)	EC 50	P 95% in mg l⁻¹	EC 0	EC 100	EC 50 48 h:24 h	Ratio between dilution steps
Phenol	24	21	16–29	9	>100	1:2.1	1:1.4
	48	10	8.8–12	3.5	16		
p-Chlorophenol	24	3.4	2.8–4.0	1.5	11	1:1.4	1:1.4
	48	2.5	2.3–2.7	1.5	4		
2,3-Dichlorophenol	24	4.1	3.3–4.9	2	7.7	1:1.3	1:1.4
	48	3.1	2.8–3.4	2	5.5		
2,4-Dichlorophenol	24	2.5	2.1–2.9	1.0	5.1	1:1.8	1:1.4
	48	1.4	1.2–1.7	0.7	3.6		
2,6-Dichlorophenol	24	6.0	3.4–10	1.9	60	1:1.8	1:√10
	48	3.4	—	1.9	6		
2,4,5-Trichlorophenol	24	1.59	1.35–1.64	0.93	2.6	1:1.7	1:1.4
	48	0.9	0.72–1.2	0.34	1.8		
2,4,6-Trichlorophenol	24	3.7	3.1–4.6	1.0	11	1:1.7	1:1.4
	48	2.2	1.8–2.6	1.0	5.5		
2,3,4,5-Tetrachlorophenol	24	Not available					
	48						
Pentachlorophenol	24	0.67	0.54–0.83	0.19	2.0	1:1.2	1:1.4
	48	0.55	0.45–0.67	0.19	2.0		
o-Bromophenol	24	1.6	1.0–2.6	0.27	18	1:1.8	1:2
	48	0.9	0.7–1.1	0.3	2.2		
m-Aminophenol	24	2.2	1.0–4.8	0.057	1800	1:2	1:√10
	48	1.1	0.6–2.2	0.057	180		
p-Aminophenol	24	0.31	0.27–0.36	0.14	0.73	1:1.3	1:1.4
	48	0.24	0.2–0.29	0.097	0.52		
3-(Dimethylamino)phenol	24	Insoluble					
	48						
o-Nitrophenol	24	27	20–37	7	200	1:1.6	1:1.4
	48	17	11–25	2.5	143		
p-Nitrophenol	24	11	9.4–1.3	5.6	21	1:2.3	1:1.4
	48	4.7	3.1–7.1	1.0	15		
2,4,6-Trinitrophenol	24	123	110–137	78	214	1:1.4	1:1.4
	48	90	80–102	56	153		
p-Cresol	24	14	13–15	6.3	50	1:1.8	1:2
	48	7.7	—	3.1	12.5		
2,4,6-Trimethylphenol	24	5.7	4.1–7.9	1.6	50	1:1.7	1:2
	48	~3.3	—	1.6	6.3		
3,5-Dimethoxyphenol	24	6.9	4.7–10	0.8	200	1:2.6	1:2
	48	2.7	2.4–3.1	0.4	12.5		
p-Hydroxyphenol	24	0.32	0.27–0.38	0.13	0.71	1:1.1	1:1.4
	48	0.29	0.25–0.33	0.13	0.71		
p-Hydroxybenzoic acid	24	181	167–198	104	286	1:1.0	1:1.4
	48	173	—	19	204		
p-Ethylphenol	24	10	8–13	3.8	29	1:1.8	1:1.4
	48	5.7	—	3.8	7.4		
4-Tert-butylphenol	24	4.2	3.8–4.7	2.6	7.1	1:1.1	1:1.4
	48	3.9	3.4–4.5	2.6	7.1		
o-Phenylphenol	24	2.1	1.8–2.4	0.75	4.0	1:1.4	1:1.4
	48	1.5	1.0–2.4	0.38	2.9		
4-Acetamidophenol	24	13	6–32	3.2	3162	1:1.4	1:√10
	48	9.2	6.1–14	1.0	32		
4-Chloro-3,5-dimethylphenol	24	4.7	4.2–5.3	2.9	8	1:1	1:1.4
	48	4.5	3.8–5.3	2.0	5.7		
2-Amino-4-methylphenol	24	3.6	2.7–4.9	2.5	71	1:1.2	1:1.4
	48	3.0	2.2–4.1	1.8	9.5		
4-Acetamidophenol	24	13	6–32	3.2	3162	1:1.4	1:√10
	48	9.2	6.1–14	1.0	32		
2-Methyl-4,6-dinitrophenol	24	2.8	2.5–3.0	1.3	5.0	1:1	1:2
	48	2.7	—	1.3	5.0		
3-Hydroxyphenylurea	24	218	136–348	19	2400	1:2.3	1:2
	48	93	46–186	2.3	2400		
3-(Trifluoromethyl)phenol	24	11	10–12	7.3	20	1:1	1:1.4
	48	11	10–12	7.3	20		
4-Hydroxybenzonitrile	24	17	14–20	6.8	71	1:1.1	1:1.4
	48	15	13–17	6.8	51		
3,5-Di-tert-butylphenol	24	3.4	3.0–4.0	1.3	7	1:2	1:1.4
	48	1.7	1.1–2.6	0.34	3.6		
2,4,6-Triiodophenol	24	Insoluble					
	48						

Table 2. List of toxicological test results with *Daphnia magna*

Anilines	Test period (h)	EC 50	*P* 95% in mg l^{-1}	EC 0	EC 100	EC 50 48 h:24 h	Ratio between dilution steps
Aniline*	24	0.9	0.5–1.6	0.38	380	1:3	1:√10
	48	0.3	0.08–1.0	0.01	1.2		
o-Chloroaniline	24	4.2	2.4–7.5	1.2	36	1:2.3	1:1.4
	48	1.8	1.5–2.2	0.3	4.7		
m-Chloroaniline	24	1.8	1.1–3.1	0.05	50	1:5.1	1:2
	48	0.35	0.27–0.46	0.024	0.78		
p-Chloroaniline	24	13	5–32	0.1	100	1:42	1:2
	48	0.31	0.12–0.78	0.0008	50		
2,4-Dichloroaniline	24	4.3	3.3–5.5	1.3	10	1:1.6	1:2
	48	2.7	2.1–3.5	0.63	10		
2,6-Dichloroaniline	24	7.1	5.6–8.9	2.5	20	1:5.1	1:2
	48	1.4	0.45–4.6	1.3	20		
o-Bromoaniline	24	5.6	3.7–8.3	2	30	1:1.9	1:1.4
	48	3	2.6–3.6	1	11		
2,4-Dinitroaniline	24	12	10–13	7.3	20	1:1.2	1:1.4
	48	9.6	8.1–11	3.7	20		
p-Methylaniline	24	†		1.6	500		1:√10
	48	†		0.0002	50		
2,4-Dimethylaniline	24	18	9–39	3.1	200	1:1.8	1:2
	48	9.9	5.6–17	3.1	200		
o-Ethylaniline	24	19	14–25	5	40	1:1.4	1:2
	48	14	12–17	5	40		
p-Ethylaniline	24	19	8–45	0.2	>100	1:9.5	1:2
	48	2	0.6–7	0.2	100		
Benzidine	24	1.5	0.5–4.1	0.5	>33	1:2.5	1:2
	48	0.6	0.2–1.5	0.5	8.3		
3-Trifluoromethylaniline	24	6.6	2.6–17	1.1	35	1:2.4	1:2
	48	2.7	2.0–3.7	0.3	35		
5-Chloro-2,4-dimethoxyaniline	24	7.22	3.8–13.8	0.63	>20	1:4.5	1:√10
	48	1.62	0.59–4.44	0.02	6.3		
4-Chloro-2-nitroaniline	24	≈3.7	—	2.6	5.1	1:1.2	1:1.4
	48	3.2	2.9–3.5	2.6	5.1		
3-Chloro-4-methylaniline	24	0.82	0.59–1.14	0.23	30	1:1.3	1:2
	48	0.62	0.47–0.81	0.12	30		
m-Aminobenzoylhydrazine	24	27	15–48	3.1	800	1:11.7	1:2
	48	2.3	1.0–5.1	0.1	25		
p-Methoxyaniline	24	52	25–109	1.0	>500	1:27.4	1:2
	48	1.9	1.3–2.8	0.24	15.6		
4-Aminoacetophenone	24	†	—	2.3–3.8	380	—	1:√10
	48	5.0	2.2–12	1.2	380		

*Test vessels: bottles closed with ground-glass stoppers.
†Cannot be calculated from the specific values.

EC 0, EC 50 and EC 100 determined or calculated after 48 h test period;
Confidence interval, *P*, 95% for the EC 50 values after 24 and 48 h test periods;
Concentration steps in the dilution series;
Calculated ratio of the substance concentrations for the 48 h EC 50 (=1) to the 24 h EC 50.

Out of the 70 substances tested, the 24 h EC 50 values for pentachlorophenol, *p*-aminophenol, *p*-hydroxyphenol, aniline and 3-chloro-4-methylaniline and the 48 h EC 50 values for 2,4,5-trichlorophenol and bromophenol as for *m*- and *p*-chloroaniline and benzidine, were in the highly toxic concentration range below 1 mg l^{-1} (Rudolph and Boje, 1986).

Furthermore, the ratio between the substance concentration of the 48 h EC 50 (=1) of each substance tested and the substance concentration of the 24 h EC 50 of the corresponding substance was calculated. These ratios indicate the reduction of the concentration having a harmful effect on the daphnids if the test period is increased by 24 to 48 h.

As can be seen from Table 4, this ratio for the group of phenols and aliphatic compounds is in almost all cases below 1:2, whereas for the anilines it can be as much as 1:40, i.e. only after a test period of 48 h does the value calculated for the EC 50 reach the concentration range which is regarded as highly toxic. One extreme example should be mentioned here: for *p*-chloroaniline 13 mg l^{-1} were calculated for the 24 h EC 50 and 0.3 mg l^{-1} were calculated for the 48 h EC 50, i.e. a 40 times lower substance concentration.

CONCLUSION

The acute *Daphnia* test is one test procedure for the ecotoxicological evaluation of substances at level one of the Chemicals Act.

The results of these investigations show that an extension of the test period by 24 h is always to be recommended. Both the 24 h EC 50 and the 48 h EC 50 should be calculated and the respective EC 0 and EC 100 values should be determined.

Table 3. List of the toxicological rest results with *Daphnia magna*

Aliphatic compounds	Test period (h)	EC 50	*P* 95% in mg l^{-1}	EC 0	EC 100	EC 50 48 h:24 h	Ratio between dilution steps
Methanol	24	>10,000	—	>10,000	—	—	1:2
	48	>10,000	—	>10,000	—		
Ethanol	24	>10,000	—	>10,000	—	—	1:2
	48	>10,000	—	>10,000	—		
n-Propanol	24	4262	3917–4636	3135	6500	1:1.2	1:1.2
	48	3644	3339–3977	2177	5417		
n-Butanol	24	2237	2117–2363	1677	2700	1:1.1	1:1.1
	48	1983	1897–2072	1260	2455		
sec-Butanol	24	4465	4047–4926	2603	7143	1:1.1	1:1.4
	48	4227	1859–7143	1859	7143		
Isobutanol	24	1463	1257–1703	781	2143	1:1	1:1.4
	48	1439	1070–1933	558	2143		
Tert-butanol	24	5504	4607–6577	2915	8000	1:1	1:1.4
	48	5504	4607–6577	2915	8000		
1,2-Propandiol	24	>10,000	—	—	>10,000	—	1:1.4
	48	>10,000	—	—	>10,000		
1,3-Propandiol	24	8285	7357–9331	3644	>10,000	1:1.1	1:1.4
	48	7417	6430–8555	2603	>10,000		
1-Chlorobutane*	24	3020	—	2041	4000	1:1	1:1.4
	48	3020	—	2041	4000		
1-Chlorohexadecane	24	Insoluble					
	48						
2-Chloroethanol	24	1254	1029–1527	558	3000	1:5.5	1:1.4
	48	227	187–275	145	558		
Chloroacetic acid	24	99	94–104	81	107	1:1.3	1:1.4
	48	77	71–85	55	107		
Chloroaceticethylester	24	3.3	2.58–3.9	1.9	7.4	1:2.1	1:1.4
	48	1.6	1.1–2.4	1.0	7.4		
2,3-Dibromopropanol	24	703	598–826	383	>750	1:2.1	1:1.4
	48	338	289–395	140	536		
1,2-Dichloroethane*	24	383	340–431	186	714	1:1.2	1:1.4
	48	324	285–368	186	714		
Methylene chloride*	24	1959	1871–2050	1447	2500	1:1.2	1:1.2
	48	1682	1532–1847	1005	2500		
1,1,1-Trichloroethane	24	Not amenable to testing because of volatility					
	48						
2,2,2-Trichloroethanol	24	223	—	182	357	1:1.1	1:1.4
	48	198	189–208	130	255		
Methylamine 40%-Lsg.‡	24	180	155–209	95	260	1:1.1	1:1.4
	48	163	147–180	95	260		
Methylamine 40%-Lsg.†	24	850	690–1047	226	1214	1:1.2	1:1.4
	48	702	484–1018	161	867		
Pentanol	24	714	607–841	510	>1000	1:1	1:1.4
	48	714	607–841	364	>1000		

*Test vessels: bottle closed with ground-glass stoppers.
†Neutralized.
‡Not neutralized.

Table 4. Calculated pollutant concentrations for the 24 and 48 h EC 50. Test organism: *Daphnia magna*

	24 h-EC 50 ($mg\,l^{-1}$)	48 h-EC 50 ($mg\,l^{-1}$)	Ratio of substance concentrations of the EC 50 48 h:24 h
Anilines			
Aniline	0.9	0.3	1:3.0
o-Chloroaniline	4.2	1.8	1:2.3
m-Chloroaniline	1.8	0.35	1:5.1
p-Chloroaniline	13	0.31	1:42
2,4-Dichloroaniline	4.3	2.7	1:$\leqslant$2
2,6-Dichloroaniline	7.1	1.4	1:5.1
o-Bromoaniline	5.6	3	1:$\leqslant$2
p-Ethylaniline	19	2	1:9.5
Benzidine	1.5	0.6	1:2.5
m-Trifluoromethylaniline	6.6	2.7	1:2.44
5-Chloro-2,4-dimethoxyaniline	7.2	1.6	1:4.5
4-Chloro-2-nitroaniline	3.7	3.2	1:$\leqslant$2
3-Chloro-4-methylaniline	0.82	0.62	1:$\leqslant$2
m-Aminobenzoylhydrazine	27	2.3	1:11.7
p-Methoxyaniline	52	1.9	1:27.4
Aliphatic compounds			
Chloroaceticethylester	3.3	1.6	1:2.1
Phenols			
p-Chlorophenol	3.4	2.5	1:$\leqslant$2
2,3-Dichlorophenol	4.1	3.1	1:$\leqslant$2
2,4-Dichlorophenol	2.5	1.4	1:$\leqslant$2
2,6-Dichlorophenol	6.0	3.4	1:$\leqslant$2
2,4,5-Trichlorophenol	1.5	0.9	1:$\leqslant$2
2,4,6-Trichlorophenol	3.7	2.2	1:$\leqslant$2
Pentachlorophenol	0.67	0.55	1:$\leqslant$2
o-Bromophenol	1.6	0.9	1:$\leqslant$2
m-Aminophenol	2.2	1.1	1:$\leqslant$2
p-Aminophenol	0.31	0.24	1:$\leqslant$2
2,4,6-Trimethylphenol	5.7	3.3	1:$\leqslant$2
3,5-Dimethoxyphenol	6.9	2.7	1:2.6
p-Hydroxyphenol	0.32	0.29	1:$\leqslant$2
o-Phenylphenol	2.1	1.5	1:$\leqslant$2
4-Tert-butylphenol	4.2	3.9	1:$\leqslant$2
2-Amino-4-methylphenol	3.6	3.0	1:$\leqslant$2
2-Methyl-4,6-dinitrophenol	2.8	2.7	1:$\leqslant$2
3,5-Di-tert-butylphenol	3.4	1.7	1:$\leqslant$2

Of the 18 anilines tested, 5 substance concentrations for the 24 h EC 50 and 15 substance concentrations for the 48 h EC 50 were in the highly toxic range; one substance from the 20 aliphatic compounds which were tested; and of the 31 phenols tested, 13 substance concentrations for the 24 h EC 50 and 18 substance concentrations for the 48 h EC 50 were $<4\,mg\,l^{-1}$.

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