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Acute Toxicity and QSAR of Chlorophenols on *Daphnia magna*

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Chlorophenols which are released into natural waters from various industrial processes and from agricultural uses have been recognized as a group of chemical substances potentially hazardous to the aquatic environment (Jones 1981). Therefore it is important to estimate their toxic impact on biota.
Thus, the scope of our research was to obtain acute toxicity data for seventeen chlorophenols towards Daphnia magna and to explore the possibilities of deriving QSAR's (quantitative structure-activity relationship) from the above values.

MATERIALS AND METHODS

Monochlorophenols (MCP), dichlorophenols (DCP), trichlorophenols (TCP), tetrachlorophenols (TTP), and pentachlorophenol (PCP) (all above 95 % purity) were bought from different commercial sources. The above compounds were diluted with reconstituted hard water. The chemical composition of hard reconstituted water for toxicity tests (pH = 7.8 - 8.2 ; Hardness = 200 mg/L expressed as $CaCo_3$) was $NaHCO_3$ (200 mg), $CaCl_2.2H_2O$ (297 mg), K_2SO_4 (26 mg), and deionized water up to 1,000 mL. All chemicals used were analytical grade. They were dissolved in deionized water (10 μs/cm). Prior to use, the appropriate volume was aerated for one day.
MCP was dissolved in reconstituted water. Acetone was used as dispersent-solvent for DCP, TCP, TTP and PCP. The volume of acetone never exceeded 0.1 mL/L of reconstituted water (ISO 1980).
Methodology adopted for static tests with Daphnia magna was that published by the French Standards Association (NF 1974) with slight modifications regarding the conditions in which they were raised. Daphnid offspring, at least from the third generation, were obtained from acyclical parthenogenesis in 10-L tanks, with aerated hard water (7.5 ± 0.4 mg/L as Ca ; 5.2 ± 0.3 mg/L as Mg ; pH = 7). The breeding room was kept at a regulated temperature of 22 ± 1°C under 16 : 8 L/D and the daphnids were fed with Chlorella vulgaris (1.0 X 10^6 cells/daphnid/hour).
Preliminary tests were performed in order to determine the range of concentrations at which definitive tests should be carried out. To this end, single series of geometrically spaced concentrations of test solutions were used (0.1, 0.35, 1, 3.5, 10, 35,

and 100 mg/L). The definitive tests aimed at determining the percentages of Daphnia which were immobilized by different concentrations of toxic solutions after 24 h exposure in order to determine the 24 h-IC50. Thus, the range of concentrations were chosen in such a way that 3 or 4 percentages of immobilization between 10 % and 90 % should be obtained. For each concentration, there were 4 test tubes. Ranges of test tubes were filled with increasing quantities of the solutions to be tested and dilution water was added up to 8 mL. Five daphnids (< 72 hours old) were then placed into each test tube and more reconstituted water was added to make the volume up to 10 mL. Control tubes (reconstituted water and dispersent-solvent) were included in the tests. The test vessels, covered with plastic stoppers, were put in the dark, at 20 ± 1°C without aeration. During the observation of test populations which were carried out after 24 h exposure the daphnids were not fed. Those that were unable to swin within 15 sec after stimulation by gentle agitation of water were considered as immobilized, even if they could still move their antennae. Dissolved oxygen, pH, and temperature were measured by the end of the toxicity tests. Percentages of immobilization (between 10 and 90 % on the basis of total number of Daphnia per concentration) were recorded and plotted as a function of concentration on log-probit paper. The points obtained were fitted together into a straight line from which the IC50 was directly read as the abscissa of the point corresponding to 50 % immobilization.

The results were considered valid if dissolved oxygen measured at the end of test was at least equal to 25 % of saturation (2.27 mg/L of O_2 at 20°C), if percentage of mortality observed for the controls was zero (ISO 1980), and if IC50 of the reference compound (potassium dichromate) ranged between 0.9 and 1.5 mg/L after 24 h exposure.

Each chemical tested was assayed in duplicate for a minimum of 3 replicates on different days. The 24 h-IC50 and 95 % confidence intervals of chlorophenols tested in this study are given in mg/L. The acute data are also expressed as logarithms of their inverse millimolar concentration C (mmol/L) for toxicity/structure evaluations (Table 1).

RESULTS AND DISCUSSION

Our results suggest that the number and the position of chlorine substituents in the phenol affect the chlorophenols' toxicity towards Daphnia magna. Thus if, on the whole, acute toxicity of the above compounds increases with the degree of chlorination, para-isomers appear as more toxic than ortho-isomers. Similar observations have been reported by Liu et al. (1982) in another bioassay using Bacillus sp. (TL 81) as test species.

Therefore, it appeared interesting to estimate the correlation between chemical structure of chlorophenols and their toxicity towards daphnids.

For this study the following six parameters were taken into account (Table 2) : the logarithm of the octanol-water partition coefficient (log P) obtained from Ribo & Kaiser (1983), the dis-

Table 1. 24 h-IC50 (and 95 % confidence intervals) of 17 chlorophenols to *Daphnia magna* expressed in mg L^{-1} and as logarithms of the inverse millimolar concentrations C (mmol L^{-1})

Compound Name	24 h-IC50 (ppm)	95 % confidence intervals (ppm)	log 1/C
2-MCP	17.95	(16.60 - 19.30)	0.855
3-MCP	15.78	(13.80 - 17.75)	0.911
4-MCP	8.07	(5.79 - 10.34)	1.202
2,3-DCP	5.19	(4.09 - 6.30)	1.497
2,4-DCP	2.68	(2.48 - 2.89)	1.784
2,6-DCP	9.38	(8.69 - 10.08)	1.240
3,4-DCP	2.77	(2.55 - 2.98)	1.770
3,5-DCP	2.09	(1.85 - 2.33)	1.892
2,3,4-TCP	2.24	(2.00 - 2.48)	1.945
2,3,5-TCP	2.28	(2.06 - 2.50)	1.938
2,3,6-TCP	7.38	(6.25 - 8.52)	1.427
2,4,5-TCP	2.08	(1.88 - 2.29)	1.977
2,4,6-TCP	5.47	(4.93 - 6.01)	1.557
3,4,5-TCP	0.88	(0.82 - 0.93)	2.351
2,3,4,5-TTP	1.76	(1.54 - 1.98)	2.120
2,3,5,6-TTP	2.27	(1.87 - 2.66)	2.009
PCP	0.76	(0.62 - 0.89)	2.545

Table 2. Numerical values used to evaluate QSAR's

Compound Name	Log P	pK	DpH *	F (10^{-2})	ΣD (10^{-1})	$^1\chi$
2-MCP	2.17	8.495	1.405	0.09	2.315	3.805
3-MCP	2.50	8.854	1.046	0.33	2.333	3.788
4-MCP	2.35	9.180	0.720	0.44	2.333	3.788
2,3-DCP	3.19	6.443	3.457	0.57	2.461	4.215
2,4-DCP	3.08	7.677	2.223	0.45	2.499	4.198
2,6-DCP	2.86	6.796	3.104	0.68	2.480	4.215
3,4-DCP	3.37	7.387	2.513	0.68	2.479	4.198
3,5-DCP	3.52	6.920	2.980	0.68	2.517	4.181
2,3,4-TCP	4.07	7.657	2.243	0.84	2.607	4.626
2,3,5-TCP	4.21	7.366	2.534	0.62	2.645	4.609
2,3,6-TCP	3.88	7.131	2.769	0.58	2.624	4.626
2,4,5-TCP	3.72	7.432	2.468	0.68	2.645	4.609
2,4,6-TCP	3.69	7.420	2.480	0.70	2.664	4.609
3,4,5-TCP	4.39	7.744	2.156	1.01	2.625	4.609
2,3,4,5-TTP	4.95	6.958	2.942	1.17	2.753	5.036
2,3,5,6-TTP	4.90	5.481	4.419	1.15	2.772	5.036
PCP	5.01	4.921	4.979	1.91	2.881	5.464

* DpH = pK phenol (9.9) - pK compound

Table 3. Relationships between acute toxicity values on Daphnia magna and molecular parameters of the 17 tested compounds

Equation N°	Parameters	log 1/C =	r*	s**	F***
(1)	log P	$0.468 \log P + 0.005$	0.879	0.233	51.11
(2)	pK	$- 0.279\ pK + 3.740$	-0.628	0.381	9.74
(3)	DpH	$0.328\ DpH + 0.849$	0.738	0.330	17.94
(4)	F	$0.959\ F + 0.998$	0.825	0.276	31.98
(5)	ΣD	$2.476\ \Sigma D - 4.648$	0.839	0.266	35.72
(6)	1χ	$0.835\ 1\chi - 2.005$	0.822	0.278	31.35
(7)	log P, log P^2	$1.047 \log P - 0.08 \log P^2 - 0.99$	0.889	0.232	26.37
(8)	F, F^2	$1.960\ F - 0.502\ F^2 + 0.610$	0.869	0.251	21.52
(9)	ΣD, ΣD^2	$12.798\ \Sigma D - 2.001\ \Sigma D^2 - 17.872$	0.848	0.268	17.92
(10)	log P, DpH	$0.366 \log P + 0.134\ DpH + 0.025$	0.910	0.210	33.56
(11)	log P, N	$0.509 \log P - 0.241\ N + 0.167$	0.908	0.212	32.82
(12)	log P, N_1	$0.478 \log P - 0.043\ N_1 + 0.035$	0.880	0.240	24.11
(13)	log P, DpH, N_1	$0.326 \log P + 0.159\ DpH + 0.091\ N_1 - 0.035$	0.913	0.214	21.73
(14)	log P, DpH, N_2	$0.369 \log P + 0.126\ DpH + 0.033\ N_2 - 0.019$	0.910	0.218	20.90
(15)	log P, DpH, N	$0.4 \log P + 0.15\ DpH - 0.27\ N + 0.21$	0.944	0.173	35.49

* Correlation coefficient
** Standard error
*** F-test

sociation constant (pK) obtained from Doedens (1967), the dissociation constant (DpH) increment relative to phenol, the melting point (F) obtained from Weast (1984), the perimeter of the efficient section of the molecule (ΣD) established according to Benoit-Guyot et al. (1984), and the molecular connectivity index of the first order $^1\chi$ calculated according to Hall & Kier (1984). The level of their intercorrelations appears in Table 4.

Table 4 . Matrix of the values of the correlation coefficients (n = 17)

	log P	pK	DpH	F	ΣD	$^1\chi$
log P	1	- 0.717	0.635	0.851	0.957	0.954
pK		1	- 1	- 0.780	- 0.772	- 0.789
DpH			1	0.750	0.646	0.789
F				1	0.866	0.896
ΣD					1	0.990
$^1\chi$						1

Thus, a high level of intercorrelation exists between $^1\chi$ - log P, $^1\chi$ - ΣD, and ΣD - log P.

Our main equations for QSAR's established on the basis of the six above parameters for the 17 chlorophenols studied appear in Table 3. Among equations with only one parameter, log P gives the highest correlation coefficient (r = 0.879). When each parameter is associated with its square value, the best results are obtained with log P (r = 0.889) and F (r = 0.869). But, when two variables are taken into account, combined use of log P and DpH in the same equation improves the correlation coefficient (r = 0.910). It has been shown that the presence of chloro substituents in para- and/or ortho-position, on the phenol nucleus, affects chlorophenols' toxicity. So, we have included in our equations, indicators for the presence of chlorine atoms in these positions. Thus, N_1 and N_2 are respectivly indicators for chlorine atoms in para- and ortho-positions, and N is an indicator for the number of chlorine atoms in position 2 and 6. Our results show that the occurrence of chlorine atoms in positions 2 and 6 increases the link between the structure of chlorophenols and their toxicity to Daphnia magna (Eq. 15 : r = 0.944). This ortho-effect has been explained in terms of hydrogen bonding and shielding of the OH group by the chloro substituents (Ribo & Kaiser 1983).

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REFERENCES

Benoit-Guyot JL, Andre C, Taillandier G, Rochat J, Boucherle A (1984) Toxicity and QSAR of chlorophenols on Lebistes reticulatus. Ecotoxicol Environ Saf 8(3):227-235

Doedens JD (1967) Chlorophenols. In Kirk-Othmer Encyclopedia of Chemical technology. 2nd ed, Vol 5, Parolla EA, Schetty GO, Dankberg FL, Kerstein JJ and Strauss LL eds:325-338

Hall LH, Kier LB (1984) Molecular connectivity of phenols and their toxicity to fish. Bull Environ Contam Toxicol 32(3):354-362

ISO (1980)Détermination de l'inhibition de la mobilité de Daphnia magna Straus (Cladocera, Crustacea). Norme Internationale ISO/6341

Jones PA (1981) Chlorophenols and their impurities in the canadian environment. Report EPS 3-EC-81-2, Environment Canada. Environmental Protection Service, p 434

Liu D, Thomson K, Kaiser KLE (1982) Quantitative structure-toxicity relationship of halogenated phenols on bacteria. Bull Environ Contam Toxicol 29:130-136

NF (Norme Française) (1974) Détermination de la mobilité de Daphnia magna Straus (Crustacé, Cladocère). Norme Française T 90-301

Ribo JM, Kaiser KLE (1983) Effects of selected chemicals to photoluminescent bacteria and their correlations with acute and sublethal effects on other organisms. Chemosphere 12(11/12): 1421-1442

Weast RC (1984) CRC Handbook of chemistry and physics. 64 th ed CRC Press, Boca Raton, Florida

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