Revue Roumaine de Chimie, **2009**, *54*(8), 659–669

HYDRACTINIA ECHINATA TEST SYSTEM. I. TOXICITY DETERMINATION OF SOME BENZENIC, BIPHENYLIC AND NAPHTHALENIC PHENOLS. COMPARATIVE SAR-QSAR STUDY

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Received August 20, 2007

Structure-activity/toxicity relationships (SAR) of some phenolic derivatives (xenoestrogens) are presented in this work. The experimental data were determinated using the *Hydractinia echinata* organisms, by comparison to other aquatic organisms, *e.g. Tetrahymena pyriformis*, *Daphnia magna*, *Pimephales promelas*, and *Vibrio fischeri*. The results are explained on the basis of the lipophilic/hydrophilic balance, the push-pull electronic mechanism, and steric influences. The toxicities of some not-tested derivatives were calculated using the ESIP (Elementar Specific Influence Parameters) algorithm (model KÖLN). A series of QSAR equations have been developed, which correlate the effectivity ($Mlog1/MRC_{50}$) and the xenoestrogens' affinity (ER $Mlog1/IC_{50}$) for the estrogenic receptor with descriptors such as: the logarithm of the 1-octanol/water partition coefficient (log*P*), the molecular volume (V_m), the molecular area (MSA), the molecular polarizability (a_E), the lowest unoccupied molecular orbital energy (E_{lumo}) and the highest occupied molecular orbital (E_{homo}). Some combinations of descriptors of binary type product, log$P$*E_{lumo}, have also been used.

INTRODUCTION

The basic concept in the analysis of the chemical structure – biological activity relation may be formulated in several ways: molecules with identical structure determine identical biologic activities,[1] derivatives with similar structures must have similar toxic action mechanisms,[2] or, compounds with similar biological activity have in the main, common features.[3]

The presence of phenols is general and permanent since they appear both in nature and as synthesis products. They are used in almost all human activity fields: domestic industry,[4-6] pharmaceutics,[7-10] agriculture, paper industry, synthetic resins,[2, 11] etc. Their impact on the human and the animal environment is difficult to estimate, depending by several factors such as: the degree and the direction of exposal, metabolism, bioconcentration, manner of life and social development level, etc.[5]

At the cell's level, the phenols are characterized by a great variety of toxicity mechanisms[1, 12] and have a full range of activities which are still unexplained.[13] The phenolic hydroxyl group may bond to an enzymatic receptor, with a basic configuration: Tyr108, Arg 503, Arg 504,[14] its bonding capacity being influenced both by the steric nature of an *orto*- substituent and by its own capacity to form hydrogen bonds. The toxicity increases with the size of the *para* position's alkyl radical.[15-16]

Among the well known phenol's biological activities, the estrogenic one is also to be considered.[6] The interference of the external chemical derivatives – xenoestrogens – in the biological processes set-up by the estrogenic hormones, affects the development or the reproduction functions of the amphibians, shellfishes, fishes, and mammalian organisms. The main mechanism consists in the bonding of the xenoestrogens to the estrogenic receptor (ER) in competition with the endogen hormone.[5, 15, 17]

As part of the endocrine troubles, the estrogenic effect is associated to the presence of the phenolic structure, where the ability of the hydroxy group to form hydrogen bonds as well and the existence of a hydrophobic region play a very important role.[1, 3]

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ER belongs to the steroid/thyroids nuclear receptor's super family.[18] Having a three-dimensional configuration, ER accepts molecules (ligands) of different sizes and with different bond formation possibilities, from estrogenic hormones to various natural and synthetic derivatives.[3, 16, 19-21] In one word, ER is extensive, hydrophobic and volume-tolerant.[13]

Since the ligand's nature causes a certain answer, the study of its affinity to ER as well as of its structural characteristics, is important both for the direct identification of some new ligands in the development of therapeutic agents which bear their effect via ER,[18] and of the xenobiotics with latent-possible structures for the establishment of such bonds.[20]

The cells to which human estrogenic receptors have been transferred indicate that the alkylphenols prove modest estrogenic effects.[17] However, their lipophilic nature as well as their great half-value life, determine their accumulation in the fat tissues, their concentration and their bioavailability increase and thus their lower affinity to the receptor is set off.[20, 22]

Among the test systems presented in this work, only *P. promelas* presents an endocrine system and an ER accordingly.[23] However, Fülöp[24] shows that in the unicellular organism's case of *Tetrahymena pyriformis,* the appearance of ER is possible, with all its specific attributes, by the transformation of an already existing primary feeding receptor. This means that at the living organism cell level, some modifications take place, depending by the nature of the external influences.

The aim of this work was to determine the toxicities of some phenols, derivatives of benzene, biphenyl and naphthalene, using the *H. echinata* organism. The obtained results were compared to those presented by some 4 other aquatic organisms, as well as to the human estrogen receptor ER. Comparative researches proved that, by instance, some phenols have the same way of action (polar narcosis) in bacteria, protozoa and fishes.[25]

The explanation of the relations between the structure and the reactivity of these phenols is grounded on the influence of the complex formed from the lipophilic/hydrophilic, electronic and steric equilibriums, as well as by the establishment of some quantitative relations using the algorithm proposed by the ESIP (Elementar Influence of Specific Parameters) model.[26] By this way, the toxicity assessment of some not-tested derivatives becomes possible.

RESULTS

The ESIP algorithm,[26] was applied for the calculation of the toxicities for *H. echinata, T. pyriformis, P. promelas, V. fischeri,* and *D. magn* organisms for a series of 88 phenols. The toxic influence of a test substance can be subdivided into that of ESIP, and the sum of the ESIP parameters (multiplied by their frequency in the substance) results in the $ClogMRC_{50}$ value (C means calculated). The ESIP parameters involved in the calculations are shown in Table 1.

Table 1

Elementar influence of specific parameters, ESIP, used in the toxicity calculation for *H. echinata (H.e.), T. pyriformis (T.p.), P. promelas (P.p.) , V. fischeri (V. f.), D. magna (D. m.),* organisms

No	Structural element	ESIP				
		H.e	*T. p.*	*P. p.*	*V. f.*	*D. m.*
1	Saturated C	0.50	0.45	0.50	0.54	0,71/0,72
2	Aromatic C in benzenes	0.24	0.42	0,49/0,47	0.51	0.49
3	Aromatic C in biphenyls	0.34	0.34	0.41	0.39	0.31
4	Aromatic C in naphthalenes	0.32		0.40		0.41
5	Aromatic C in systems with 3 condensed rings	0.36				0.35
6	Aromatic C in systems with 4 condensed rings	0.24				0.32
7	OH group in monoalcools	-0.93	-0.26	-0.35		
8	OH group in alcandioles	-0.48				
9	OH group in polyalcanoles	-0.38				
10	OH group in phenylcarbynoles	0.45				
11	NH_2 group in alkyl-monoamines	2.95	2.34	2.67		
12	NH_2 group in alkyl-diamines	1.52				
13	OH phenolic group in benzenes	1.38	0.04	0.37	0.63	0.38
14	OH phenolic group in biphenyls	0.27	0.19	-0.40	0.34	1.08
16	OH phenolic group in naphthalenes	0.57	0.79	0.56	0.14	
17	OH phenolic group in *ortho*-dihydroxybenzenes	1.83	0.46	0.62	0.25	
18	OH phenolic group in *meta*-dihydroxybenzenes	0.48	-0.02	0.10	-0.02	0.18
19	OH phenolic group in *para*-dihydroxybenzenes	2.33	0.53	1.78	1.69	
20	OH phenolic group in naphthalenedioles	1.05			-0.22	
21	OH phenolic group in trihydroxybenzenes	1.24	0.37			

The values of the experimental toxicities, Mlog1/MRC_{50} and of the calculated ones, Clog1/MRC_{50}, for the series of 88 phenols using the *H. echinata* test system (as well as other aquatic organisms) are presented in Table 2.

Table 2

The values of the experimental toxicities, Mlog1/MRC_{50} [mol/L], by comparison to the calculated ones, Clog1/MRC_{50} according to the ESIP model's algorithm for *H.ydractinia e.* and other organisms, and the bond affinity, Mlog1/IC_{50} [mol/L] for the estrogenic receptor, ER

No	Derivative	Cs	Car	*Hydractinia e.*		*Tetrahymena p.*		*Pimephales p.* Clog1/MRC_{50}		*Vibrio f.*		*Daphnia m.*		ER log1/IC_{50}
				M	C	M	C	M	C	M	C	M	C	M
	Benzene		6	1.65	1.44			3.40	2.96			3.39	2.94	
1	Phenol		6	2.89*	2.82	2.79	2.58	3.41	3.21	3.42	3.68	3.32	3.32	1.02
2	2-Methylph		6	3.21*	2.82	2.72	2.58	3.77	3.21	3.75	3.68	3.64	3.32	
3	3-Methylph		6	3.00*	2.82	2.93	2.58	3.29	3.21	3.99	3.68	3.54	3.32	
4	4-Methylph		6	3.14*	2.82	2.83	2.58	3.58	3.21	4.72	3.68	3.34	3.32	
5	2,3-DiMeph	1	6		3.32	3.12	3.03		3.71	4.58	4.21		4.04	
6	2,4-DiMeph	1	6		3.32	3.14	3.03	3.86	3.71	4.44	4.21		4.04	
7	2,5-DiMeph	1	6		3.32	3.08	3.03		3.71	4.14	4.21		4.04	
8	2,6-DiMeph	1	6		3.32		3.03	3.75	3.71		4.21		4.04	
9	3,4-DiMeph	1	6		3.32	3.12	3.03	3.90	3.71	5.44	4.21		4.04	
10	3,5-DiMeph	1	6		3.32	3.11	3.03		3.71	3.99	4.21		4.04	
11	2-Ethylph	1	6		3.32	3.16	3.03		3.71		4.21		4.04	
12	3-Ethylph	1	6		3.32	3.29	3.03		3.71		4.21		4.04	
13	4-Ethylph	1	6		3.32	3.21	3.03	4.07	3.71		4.21	4.09	4.04	2.77
14	2,4,6-TriMeph	2	6	3.60*	3.82	3.42	3.48	4.02	4.21	4.08	4.75	4.49	4.75	
15	2,3,5-TriMeph	2	6		3.82	3.36	3.48		4.21	4.19	4.75		4.75	
16	2,3,6-TriMeph	2	6		3.82	3.42	3.48	4.22	4.21	4.32	4.75		4.75	
17	3,4,5-TriMeph	2	6		3.82	3.93	3.48		4.21		4.75		4.75	
18	4-n-Propylph	2	6	3.80	3.82	3.64	3.48	4.09	4.21		4.75		4.75	
19	2-Isopropylph	2	6	3.23*	3.82	3.80	3.48		4.21		4.75		4.75	
20	3-Isopropylph	2	6	3.60*	3.82	3.61	3.48		4.21		4.75		4.75	
21	4-Isopropylph	2	6	3.27*	3.82	3.47	3.48	4.47	4.21		4.75		4.75	
22	4-n-Butylph	3	6	4.39*	4.32		3.93		4.71		5.28		5.47	
23	2-sec-Butylph	3	6		4.32		3.93				5.28		5.47	2.86
24	4-sec-Butylph	3	6		4.32	3.98	3.93		4.71		5.28		5.47	3.24
25	2-tert-Butylph	3	6		4.32	4.25	3.93		4.71		5.28		5.47	2.62
26	3-tert-Butylph	3	6		4.32	3.73	3.93		4.71		5.28		5.47	2.64
27	4-tert-Buph	3	6		4.32	3.91	3.93	4.46	4.71	5.85	5.28	4.55	5.47	2.99
28	2-tert-Bu-4-Meph	4	6		4.81	4.30	4.37	4.89	5.21		5.82		6.18	
29	4-n-Pentylphl	4	6	4.81*	4.81		4.37		5.21		5.82	5.10	6.18	
30	4-tert-Pentylph	4	6		4.81	4.23	4.37	4.80	5.21		5.82		6.18	
31	4-cyclo-Pentylph	4	6		4.81	4.29	4.37		5.21		5.82		6.18	
32	2,6-Diisopropylph	5	6	3.73	5.31		4.82		5.21		6.36		6.90	
33	6-tert-Bu-2,4-diMeph	5	6		5.31	4.25	4.82		5.21		6.36		6.90	
34	4-n-Heptylph	6	6	6.06*	5.81		5.27		6.20		6.90		7.62	
35	4-n-Octylph	7	6	5.98*	6.31		5.27		6.70		7.44		8.34	
36	4-tert-Octylph	7	6		6.31	5.10	5.27	5.95	6.70		7.44		8.34	4.85
37	2,6-ditert-Buph	7	6	4.61*	6.81		6.17		7.20		7.98		9.06	
38	3,5-ditert-Buph	7	6		6.81	4.64	6.17		7.20		7.98		9.06	
39	4-n-Nonylph (tec)	8	6	4.95*	6.81	5.47	6.17	6.20	7.20		7.98		9.06	5.02
40	*p*262NP	8	6	4.47	6.81									
41	*p*363NP	8	6	6.31*	6.81									
42	*p*353NP	8	6	7.65	6.81									
43	2,6-ditert-Bu-4-Meph	8	6		6.81	4.79	6.17	5.78	7.20		7.98		9.06	3.31
44	2,4,6-tritert-Buph	11	6		8.31	3.36	7.52	6.63	8.69		9.60		11.22	
45	4,4'-Bisph A	2	12	4.74	6.62	4.97	6.05	4.70	7.43		8.43		8.07	3.96
46	2-Allylph		8		3.29	3.33	3.43	3.95	4.16		4.69	4.13	4.30	
47	Diethylstilbestrol	3	14	6.67	7.60		7.35		8.87		9.98		9.77	7.22
48	Dicumarol		14			4.70	6.01	4.82	7.38		8.37		7.62	
49	Anisole					2.52				3.76				
	Biphenyl		12	4.05	3.84							3.70	4.87	
50	2-Phenylph		12	4.01*	4.32	4.09	4.25	4.50	4.50	4.92	5.12	4.80	4.80	3.00
51	3-Phenylph		12		4.32	4.35	4.25		4.50		5.12		4.80	3.21

Table 2 (continued)

52	4-Phenylph		12	4.31*	4.32	4.38	4.25	4.44	4.50		5.12		4.80	3.37
53	4-Benzylph		12		4.32	4.19	5.14		6.12	5.85	6.72		6.26	
54	2,6-Diphenylph		18		5.76	5.11	6.79		7.34		7.90		7.74	
55	2,2'-Biph		12		4.26	3.88	4.25		4.50		4.85		4.80	3.97
	Naphthalene		10	3.18	3.20							3.89	4.05	
56	1-Naphthol		10	3.86*	3.78	3.76	3.77	4.49	4.56	4.60	5.21			
57	2-Naphthol		10	3.69*	3.78	3.79	3.77	4.62	4.56	5.82	5.21			
58	1-MeOHnaphth					3.85								
59	2-MeOHnaphth					3.97								
60	1,2-DiHybz		6	5.11	5.11	3.75	3.47	4.08	4.08	3.54	3.54			
61	1,2-DiHy-3-Mebz		6		5.11	3.28	3.47		4.08		3.54			
62	1,2-DiHy-4-Mebz		6		5.11	3.37	3.47		4.08	5.36	3.54			
63	2-MeOHph			2.83*		2.49								
64	1,3-DiHybz		6	2.24*	2.40	2.35	2.46	3.04	3.04	3.00	3.00	3.30	3.30	
65	1,3-DiHy-5-Mebz		6	2.56*	2.40	2.61	2.46		3.04		3.00		3.30	
66	1,3-DiHy-5-pentylbz	4	6		4.20	4.31	4.30		5.03		5.14		6.16	
67	1,3-DiHy-4-hexylbz	5	6		4.69	4.80	4.69		5.53		5.68		6.88	
68	3-MeOHph							3.22		3.35				
69	2,6-DiMeOHtoluene							3.88						
70	1,4-DiHybz		6	6.10*	6.10	3.47	3.59	6.40	6.40	6.42	6.42			
71	MeHydr		6		6.10	4.86	3.59		6.40	5.49	6.42			
72	2,3-DiMeHydr	1	6		6.60	4.41	4.04		6.90		6.96			
73	TriMeHydr	2	6		7.10	4.34	4.49		7.40		7.49			
74	TetraMeHydr	3	6		7.60	4.28	4.94		7.89		8.03			
75	Tert-BuHydr	3	6	5.03*	7.60		4.94		7.78		8.03			
76	2,6-di-tert-BuHydr	7	6		9.59		6.73		9.89	4.72	10.17			
77	PhenylHydr		12		8.71	5.01	5.11		8.46		8.01			
78	MeOHHydr					5.20								
79	4-MeOHph					2.86		3.05		4.53				
80	p-DiMeOHbz							3.07						
81	Naphthdiol-1,5		10	5.27*	5.31					4.64	4.64			
82	Naphthdiol-2,7		10	5.35*	5.31						4.64			
83	1,2,3-TriHybz		6	5.15	5.15	3.85	3.65							
84	1,2,4-TriHybz		6		5.15	3.44	3.65							
85	1,3,5-TriHybz					1.74								
86	2,6-DiMeOHph					2.40								
87	3,5-DiMeOHph					2.99								
88	3-EtOH-4-MeOHph					2.70								

Cs – number of saturated Carbon atoms involved in ESIP's algorithm simulation; Car – number of aromatic Carbon atoms involved in ESIP's algorithm simulation; M/C values indicate the logarithm of the inversed concentration [mol/L] of a substance which reduces the metamorphosis induction by 50 % (termed Mlog1/MRC_{50}M); M- measured or mediated; C – calculated; Mlog1/IC50 – measured affinity of a compound to ER as the inversed logarithm of the concentration [mol/L]
*p*262NP - 4(2',6'-dimethyl-2'-heptyl phenol)*; p*363NP- 4(3',6'-dimethyl-3'-heptyl phenol)*; p*353NP- 4(3',5'-dimethyl-3'-heptyl phenol); bz – benzene; ph – phenol; naphth – naphthalene; Hydr- hydroquinone; Me- methyl; MeOH- Methoxy; EtOH- Ethoxy; Bu- Butyl; Hy- Hydroxy
* represents an average measured value, characterizing the reproductibilicity of the experimental measurements:
Phenol (3,15/2,66/2,85); 2-Meph (3,18/3,24); 3-Meph (3,00/3,00); 4-Meph (3,14/3,14); 2,4,6-Trimeph (3,19/4,00); 2-Isopropylph (2,93/3,52); 3-Isopropylph (3,34/3,85); 4-Isopropylph (3,19/3,35); 4n-Buph (4,27/4,21/4,45/4,57); 4n-Pentylph (4,68/4,74/5,01/4,98); 4n-Heptylph (5,66/6,19/6,12/6,18/6,14); 4n-Octylph (5,83/6,21/5,96/5,92/5,98); 2,6-ditert-Buph (3,98/5,08/4,76); *p*363NP (6,25/6,37); 2-Phenylph (3,98/3,93/4,12); 4-Phenylph (4,02/4,59); 1-Naphthol (3,71/4,01); 2-Naphthol (3,89/3,48); 2MeOHph (2,89/2,77); 1,3-Dihydroxybz (1,78/2,70); 1,3-Dihydroxy-5-mebz (2,38/2,74); 1,4-Dihydroxybz (6,14/6,06); tert-Buhydr (5,05/5,00); Naphthalindiol-1,5 (5,20/5,33); Naphthalindiol-2,7 (5,52/5,17)

The values of the individual descriptors used in correlations are presented in Table 3.

The QSAR linear regression equations (1-10) (Table 4) and (1-17) (Table 5) are of type:

$$Y = b_1X + b_0$$

where Y=Mlog1/MRC_{50} or Mlog1/IC_{50}, X=Clog1/MRC_{50}, Mlog1/IC_{50}, an individual descriptor or the binary product of two descriptors, b_1 represents the slope of the straight line and b_0 represents the intersection to the origin. The exactness of each regression is estimated by the correlation coefficient r^2. The number of the derivatives involved in the correlation (n_{cor}) is also indicated, by comparison to the total number (n_{tot}) resulted from the sum of n_{cor} and of the exceptions.

Table 3

Individual descriptor parameters used in QSAR equations (literature data)[34-39]

Nr	Derivative	logP	E_{homo} [eV]	E_{lumo} [eV]	aE [Å^3]	V_m [Å^3]	MSA [Å^2]
1	Phenol	1.50	-9.11	0.40	8.97	91.44	121.00
2	2- Methylphenol	1.98	-8.96	0.40	10.53	107.35	141.17
3	3- Methylphenol	1.98	-8.84	0.39	10.44	107.54	142.51
4	4- Methylphenol	1.97	-8.88	0.43	10.52	105.90	143.08
5	2,3-Dimethylphenol	2.77*		0.38			
6	2,4-Dimethylphenol	2.35	-8.85	0.40	12.27	124.29	162.21
7	2,5-Dimethylphenol	2.77*		0.38			
8	2,6-Dimethylphenol	2.36*	-8.89	0.39	11.97	122.98	161.54
9	3,4-Dimethylphenol	2.23	-8.80	0.43	12.18	124.56	163.05
10	3,5-Dimethylphenol	2.77*	-8.97	0.39	11.82	122.99	163.16
11	2-Ethylphenol	2.47		0.40			
12	3-Ethylphenol	2.50		0.40			
13	4-Ethylphenol	2.50	-8.91	0.43	11.90	123.69	165.60
14	3,4,5-Trimethylphenol	2.87*	-8.90	0.42	13.67	140.08	182.32
15	2,4,6-Trimethylphenol	2.73		0.38			
16	2,3,5-Trimethylphenol	2.92*	-8.90	0.36	13.53	140.17	178.44
17	2,3,6-Trimethylphenol	2.67		0.41			
18	4-n-Propylphenol	3.20	-8.91	0.43	13.39	141.99	185.68
19	2-Isopropylphenol	2.88	-8.99	0.41			
20	3-Isopropylphenol	3.05*	-9.02	0.42			
21	4-Isopropylphenol	2.90*	-8.92	0.44			
22	4-n-Butylphenol	3.64*	-8.90	0.44	14.69	157.89	
23	2-sec-Butylphenol		-8.98	0.42	14.38	155.54	202.06
24	4-sec-Butylphenol	3.58*	-8.91	0.45			
25	2-tert-Butylphenol	3.31		0.42			
26	3-tert-Butylphenol	3.45*		0.43			
27	4-tert-Butylphenol	3.31	-8.99	0.47	14.53	156.64	200.28
28	2-tert-Butyl-4-methylphenol	4.10*	-8.70	0.50			
29	4-n-Pentylphenol	4.06*	-8.90	0.44	16.13	175.63	229.46
30	4-tert-Pentylphenol	3.83					
31	4-cyclo-Pentylphenol	3.69*					
33	6-tert-Butyl-2,4-Dimethylphenol	4.75*					
34	4-n-Heptylphenol	5.15*					
36	4-tert-Octylphenol	5.76					
37	2,6-ditert-Butylphenol	4.17					
38	3,5-ditert-Butylphenol	5.13*					
41	p-363-Nonilphenol	6.40*	-8.92	0.43	21.44	241.09	
44	2,4,6-tritert-Butylphenol	7.42*					
45	4,4'-Bisphenol A	3.43*	-8.97	0.27			
46	2-Allylphenol	2.55*	-9.02	0.36	12.83	135.85	175.52
47	Diethylstilbestrol	7.03*	-8.65	-0.03			
50	2-Phenylphenol	3.09	-8.74	0.05	16.31	160.92	197.27
51	3-Phenylph	3.23		-0.15			
52	4-Phenylphenol	3.20	-8.68	-0.10			
53	4-Benzylphenol	3.69*					
54	2,6-Diphenylph	5.25*					
56	1-Naphthol	2.84	-8.46	-0.25	15.30	134.56	169.22
57	2-Naphthol	2.65*					
58	1-Methoxynaphthalene	3.24*					
59	2-Methoxynaphthalene	3.24*					
60	1,2-Dihydroxybenzene	0.88*	-8.88	0.30			
61	1,2-Dihydroxy-3-methylbenzene	1.38*					
62	1,2-Dihydroxy-4- methylbenzene	1.37*					
63	2-Methoxyphenol	1.59*					
64	1,3-Dihydroxybenzene	0.80	-8.98	0.28			
65	1,3-Dihydroxy-5-methylbenzene	1.31*	-8.95	0.26			
66	1,3-Dihydroxy-5-pentylbenzene	3.42					
67	1,3-Dihydroxy-4-hexylbenzene	3.45					
68	3-Methoxyphenol	1.58					
70	1,4-Dihydroxybenzene	0.59	-8.72	0.23			

Table 3 (continued)

71	Methyl- hydroquinone	0.98*		
72	2,3-Dimethyl-hydroquinone	1.24*		
73	Trimethyl-hydroquinone	1.69*		
77	Phenylhydroquinone	2.43*		
78	Methoxyhydroquinone	0.47*		
79	4-Methoxyphenol	1.34*	-9.11	0.31
83	1,2,3- Trihydroxybenzene	0.21*	-9.16	0.03
84	1,2,4- Trihydroxybenzene	0.21*		0.16
85	1,3,5- Trihydroxybenzene	0.16*		0.25
86	2,6- Dimethoxyphenol	1.10*		
87	3,5- Dimethoxyphenol	1.60*		
88	3-Ethoxy-4-methoxyphenol	1.69*		

* calculated values

Table 4

Observed toxicities (M) vs. calculated ones (C) according to the ESIP's model algorithm for *H. echinata* and other organisms, and vs. the bond affinities of the ER estrogenic receptor of the derivatives of Table 2

QSAR equation	Organism	b_1	b_0	r^2	n_{cor}	n_{tot}	Exceptions
			Mlog1/MRC_{50} vs Clog1/MRC_{50}				
1	*H. echinata*	0.946	0.193	0.944	29	35	32,37,39,40,45,73
2	*T. piryformis*	0.624	1.353	0.854	56	58	44 - 47
3	*P. promelas*	0.645	1.476	0.934	27	29	45, 47
4	*V. fischeri*	0.772	0.980	0.752	21	24	9, 61, 75
5	*D. magna*	0.588	1.583	0.899	10	10	
			Mlog1/MRC_{50} vs Mlog1/IC_{50} *				
6	*H. echinata*	1.655	-3.674	0.985	6	6	
7	*T. piryformis*	1.171	-1.645	0.734	14	14	
8	*P. promelas*	1.113	-2.017	0.764	9	9	
9	*V. fischeri*	0.859	-1.726	0.853	3	3	
10	*D. magna*	1.373	-3.309	0.873	4	4	

* In the correlations with the bond affinities for ER, besides the M values, C values are also used for the presented organisms

Table 5

QSAR equations of the phenols measured toxicities vs. the individual descriptors and combinations for *H. echinata*, and ER affinities vs. individual descriptors respectively

QSAR equation	Mlog1/MRC_{50} vs	b_1	b_o	r^2	n_{cor}	n_{tot}	Exceptions
1	logP	0.741	1.639	0.940	22	22	
2	Nr. Csat	0.562	2.438	0.934	14	18	32, 37, 47, 74
3	aE(a.u.)	0.262	0.304	0.900	11	11	
4	V_m(Ä^3)	0.023	0.619	0.970	11	11	
5	MSA(Ä^2)	0.017	0.703	0.932	9	9	
6	Ehomo*logP	-0.084	1.568	0.936	18	22	59,63,69,82
7	Elumo*logP	1.490	1.824	0.837	11	24	45,47,49-51,55, 59, 63, 64, 69, 82-84
8	Ehomo*aE	-0.019	1.329	0.916	8	9	49
9	Elumo*aE	0.388	1.515	0.940	8	8	
10	Ehomo*Vm	-2.603	0.630	0.970	11	11	
11	Elumo*Vm	0.051	0.844	0.981	9	11	49, 55
12	Ehomo*MSA	-1.949	0.696	0.909	9	9	
13	Elumo*MSA	0.095	1.427	0.948	7	9	49, 55
	Mlog1/MRC_{50} vs					ER	
14	logP	0.996	-0.260	0.929	11	11	
15	aE(a.u.)	0.263	-0.950	0.781	7	7	
16	V_m(Ä^3)	-6.799	3.939	0.840	6	7	1
17	MSA(Ä^2)	0.023	-1.507	0.872	5	5	

DISCUSSION

The presence of a hydroxylic group on the benzenic ring leads to a higher molecular reactivity in the case of *H. echinata* organisms. However, this behavior is not noticed in the case of *P. promelas* and *D. magna* systems, where it seems that the initial lipophilic effect is not essentially modified.

As a matter of fact, the measured toxicity values of phenol and of the methylphenol derivatives are – with one exception- identical. This phenomenon is also noticed in the case of chlorine, amino, nitro, carboxyl, sulfonic, methoxy, etc, monosubstituted derivatives (unpublished results). In this case, a "standard toxicity" should be considered. In the case of some combinations of these substituents, the term "limited izotoxicity" can be introduced. Even if this later has already been noticed, there are no literature mentions on this subject.[29-31]

In these conditions, the first saturated carbon atom will participate to the quantification, starting by the dimethyl- and the ethylphenols, the second one from the trimethylphenol, etc. This calculation type is applied, in the same manner to all the derivatives containing saturated chains. The position alkyl-isomers will have identical calculated toxicities.

For the *H. echinata, T. pyriformis* and *P. promelas* test systems, the molecule's hydrophobic/ lipophilic character will increase depending by the number of the carbon atoms of the lateral chain.[3, 5, 16, 31] The phenol's relative activity is dependent by the extent of the *para* position's substituent,[15, 16] as well as by its branchement.

Till n-nonil-phenol, the hydrophilic/ hydrophobic equilibrium appears antagonic (per total) to the metamorphosis: as the lipophilic character of the molecule increases, the molecule diffuses more easily through the lipidic membranal barrier and interacts to the endogen/cellular receptor active center. The toxicity values increase constantly.

The technical nonil-phenol, a variable mixture of isomers, seems to be the border of the number of C_{sat} atoms, from which the lipophilic antagonic influence decreases.

But, the tests performed with the two isomers *p*363NP 4[3',6'-dimethyl-3'-heptil-phenol] (6,31, value used in the correlations) and *p*353NP 4[3',5'-dimethyl-3' heptil-phenol] (7,65), show that the toxicity increases by 1.4 ÷ 1.7 log u. as compared to n-nonil-phenol as the branchement of the *para*'s hydrocarbonated chain is more intense[32] as well as geometrically closer to the atomic nucleus. By comparison, the *p*262NP [4(2',6'-dimethyl-2'-heptil phenol] isomer's (4,47) diffusion, is "hindered" by the cellular membrane and a greater percentage of molecules remains in this area.[22]

From the existent data (*H. echinata, T, pyriformis, P. promelas*) it follows that the hydrophilic/ hydrophobic equilibrium turns critical from the 9th saturated carbon atom of the lateral chain, the values being quite close as compared to the 4-tert-octyl-phenol's molecule, which has a maximum of estrogenicity.[21] Thus, one could consider that a linear or a branched chain having 8-9 carbon atoms in the *para*- position represents the hydrocarbonated interface which affects the molecular lipophilic character for these organisms. The critical equilibrium may also appear quickly (*V. fischeri, D. magna*).

On the other hand, in the case of the 2,6-di-Isopropil– and 2,6-di-tert-Buthyphenol (*H. echinata*), 2,6-ditert-Butyl-4-methyl- and 2,4,6-tritert-Butylphenol (*T. pyriformis, P. promelas*) derivatives, one could notice that the molecular reactivity by the OH phenolic group is particularly affected by *orto, orto'* sterical hindrances.

In the case of Allylphenol (*T. pyriformis, P. promelas, D. magna*), the two nonsaturated carbon atoms of the lateral chain have been assimilated to the $C_{6\text{-}ar}$ benzenic ones, so the total number of the aromatic carbon atoms considered in the quantification is 8. The lipophilic character of the molecule increases as compared to the phenolic's one and thus the toxicity is greater.

Although the toxicity of anisole or the phenyl-methyl-ether (*T. pyriformis, V. fischeri*) is quite close to the phenol's one, its quantification will be different because the etheric oxygen behaves only as a hydrogen bond acceptor through its unshared pairs of electrons.[3]

2-Phenylphenol is in all cases more toxic than phenol, but the *orto*- sterical influence does not act differently (*H. echinata, T. pyriformis*) as compared to the *para*- position. In the case of 2,6-di-Phenylphenol, the toxicity is affected as well by the *orto*, *orto'* sterical hindrances as by a reduced membranar diffusion, the quantification involving 12 $C_{12\text{-}ar}$ biphenilic atoms and 6 $C_{6\text{-}ar}$ benzidinic ones. 4-Benzylphenol (*T. pyriformis, V. fischeri*) contains a methylenic bridge C_{sp^3}, which wasn't been taken into account during quantification.

2,2'-Biphenol, tested only with *T. pyriformis* and exhibiting a close toxicity to the phenyl-phenols was considered for the moment as a monohydroxylic compound. The reciprocal steric hindrance between the neighboring 2,2' hydroxy groups affects the extended conjugation, but the complex of equilibrium, specific to the phenyl-phenols is not essentially modified.

Excepting the *V. fischeri* test system, the naphthol's position isomers exhibit quite similar toxicities. In some cases their toxicity is quite close to those of the biphenilic derivatives. This matter of fact leads to the conclusion that the derivatives having two separate or two condensed aromatic rings might be treated on the whole. But, the differentiation is obviously illustrated by the ESIP values, for all the test systems where these data are present.

Similarly to anisole, the studied methoxynaphthalenes (*T. pyriformis*) present a similar toxicity to their corresponding naphthols.

The dihydroxy-benzenes present some toxicity values which are obviously connected to the *orto, meta, para* isomery. A new hydroxyl group in the 2 or 4 positions, could increase the OH initial group's reactivity in the formation of hydrogen bonds with an estrogenic receptor.[3, 13] Moreover, this additional group has the capacity to form its own hydrogen bonds.[33]

Excepting *T. pyriformis*, 1,4-dihydroxybenzene presents a greater toxicity, by about 3 log u. as compared to the phenol's one. The existence of two identical hydroxyl groups, a wholly symmetrical and flat molecule, as well as the absence of sterical hindrances, are considered to be the premises of a p-pi extended conjugation according to a *push-pull electronic mechanism*: an OH group is electron donating and becomes electropositive, and the second one, an electron accepting group becomes electronegative. This phenomenon, which is probably alternant and permanent even in the absence of a reaction partner, induces a strong hydrogen bond donor character. The molecule is flat, strongly polar, has a maximal electronic delocalization, and consequently, a *total symmetry.*

The tert-butyl (*H. echinata*) and methyl- (*V. fischeri*) substituents lower the toxicity by 1 log u., due to the fact that the neighboring OH group is sterically shifted from the molecular plane and its ability to participate to the *push-pull* extended conjugation is partially reduced. Two neighboring tert-butyl groups (*V. fischeri*) induce an additional decrease of the toxicity, and this by almost 2 log u.

In the case of *T. piriformis*, a single alkyl or phenyl substituent induce a greater toxicity by increasing the lipophilicity of the derivative. Subsequent alkylic substitutions increase the lipophilic character, but they affect the reactivity of the OH groups through sterical influences, and consequently, the toxicity decreases continually. The methyl group is a strong electron donating group, and induces a greater toxicity, probably through the molecular polarity increase.

However, the toxicity decreases dramatically by the transformation of a phenolic OH in an alcoxy group (*P. promelas*, *V. fischeri*), and of both OH groups (*P. promelas*) respectively. The molecule proves itself an energetic ligand when it is able to keep intact its two OH groups.

1,2-dihydroxybenzene (catechole) is characterized by a *push-pull electronic mechanism* slightly lowered as compared to the 1,4-derivative's, due to the reciprocal steric influences and to the inner cyclization between the two hydroxy groups. As consequence, excepting *T. pyriformis* test system, the toxicity is reduced by one unit order.

The presence of a methyl group in positions 3- or 4- will not modify essentially the toxicity in the case of *T. pyriformis*, but in the case of the *V. fischeri* test system, the toxicity increases by about two units order when the methyl group is located in the 4- position.

In exchange, 2-methoxyphenol (Guajacol) exhibits a lower toxicity, precisely by 2.30 log u. in the case of *H. echinata* and by 1.25 log u. for *T. pyriformis* test system. This proves in what extent the reciprocal electronic influence between the two free OH groups[10, 20] is lowered by the transformation of OH in methoxy and the increase of the steric hindrance. These two examples support the idea that the xenoestrogen is more effective by the increase of the hydrogen bond donor effect.

1,3-dihydroxybenzene (resorcinol) is the less toxic among the dihydroxy isomers, and even less toxic than phenol. The OH groups are not activated by the *push-pull electronic mechanism*, the hydrophilic /hydrophobic balance of the molecule is strongly shifted to the hydrophilic character, and therefore the molecule will remain preferentially in the extracellular aqueous phase. The lipophilic/ hydrophobic influence and hence the increase of toxicity is noticed depending by the bulkiness of the alkyl substituent (*H. echinata, T. pyriformis*). This phenomenon is noticed in the case of 3-methoxyphenol (*P. promelas*, *V. fischeri*) as well as in the case of 2,6-dimethoxytoluene (*P. promelas*), derivative which does not posses a free hydroxy group.

Due to the *push-pull electronic mechanism*, 1,5-naphthalenediol and even the 2,7- derivative (*H. echinata*), exhibit greater toxicities as compared to those of the mononaphthols. However, the experimental data are insufficient for eventual correlations and results extents.

In the case of 1,2,3-trihydroxybenzene the substituents exhibit reciprocal steric hindrances and the inner cyclizations are more intense. Therefore, its hydrophilicity is affected, but the toxicity at the 1,2-dihydroxybenzene's level (*H. echinata, T. pyriformis*) suppose the existence of a stronger polarity and hence the existence of a *push-pull electronic mechanism*. This latter might alternating manifest, by instance between the hydroxyl group 2 (exhibiting a donor effect) and one of the two marginal OH groups (1 or 3) as electron acceptors. In these conditions, the *conjugation pseudo-meta* could by imagined, presuming an electronic displacement (from 2 to 1) in a first stage, the electronic deficit of the 2' position can be quickly set off by the electrons displacement from the 3 position. The molecule's polarity is due to the fact that the OH group located in position 3 has a positive charge, the center of the negative charges being situated at the 1 and 2 positions group's level.

In the case of 1,2,4- derivative (*T. pyriformis*) one should expect a greater toxicity due to the increased conjugation possibilities. However, it seems that, due to geometrical reasons, the possibilities of inner cyclizations are reduced, hence the hydrophilicity of the derivative is increased and so, the toxicity measured value is slightly lower than in the case of the 1,2,3-substituted derivative. Accordingly, the 1,3,5-derivative exhibit the lower toxicity of the series, the molecule being strongly hydrophilic.

The transformation of the OH groups into alcoxy induces lower toxicities, and this is more obvious in the case of the 2,6- derivative, where sterical hindrance occurs, as compared to the 3,5- derivative.

At the cell's level, the biological activity of the alkyl- and of the hydroxyphenols in the *H. echinata*, *T. pyriformis, V. fischeri* and *D. magna* test systems could be explained by their interaction with an estrogenic type receptor. To support this affirmation, one should consider that:

P. promelas (fish) presents a properly outlined endocrine system, where the ER existence is connected to the organism's hormonal activity. The toxicity's evolution in the others test systems is entirely similar to the *P. promelas*' s, and this is only possible through the existence of a receptor which, if not identical, at least similar for all the considered organisms. This receptor may appear as a consequence of the transformation in the molecule of an existing one, by instance the feeding receptor, as mentioned in the case of *T. pyriformis*.[24]

In the present work, the studied phenols fulfill all the prescribed conditions for the corresponding ER ligand: an OH-phenolic group, through which hydrogen bonds may be formed, is attached to a rigid and flat hydrocarbonate cycle. To this latter, a critical biophoric structure, which affects the molecule's general lipophilic character, is also attached: a saturated or aromatic hydrocarbonated moiety, an additional phenolic or etheric OH group.

The present receptor exhibits the specific features of the estrogenic one: is extensive, hydrophobic and volume-tolerant.

The equations 1-5 (Table 4) correlate the measured values M to the calculated ones, C, for *H. echinata* and the other organisms. These equations show that the ESIP model can be applied in different test systems. The correlation coefficients r^2 exhibit values ranging between 0.752 in the case of *V. fischeri* and 0.944 for *H. echinata*, when slopes of about 1 are obtained. The exceptions are usually in the case of the derivatives which present important steric hindrances at the phenolic hydroxyl's level.

According to equations 6-10, the correlations between the measured effectiveness, $Mlog1/MRC_{50}$, and the affinity for the estrogenic receptor, $Mlog1/IC_{50}$, of the studied organisms, indicate a similar evolution, even if the number of experimental measurements is relatively small. By this way, the idea that in the xenoestrogens presence, organisms with no hormonal system can build up an estrogenic type receptor (if needed) is confirmed.[24]

For *H. echinata* and the estrogenic receptor, the correlations between the measured values, M, and the individual parameters logP, aE, V_m and MSA are represented by the equations 1, 3-5 and 14-17 respectively (Table 5). In the case of ER, the correlation coefficients are smaller, and this is probably due to the concurrent reaction among the xenoestrogen and the pre-existent ligand.

Equations 6-13 (Table 5) indicate a good dependence between the effectiveness and the binary products of the above descriptors with E_{lumo} and E_{homo}. The phenomenon may be explained by taking into account the possibilities of the OH-phenolics' group to equally form with a receptor, an electrophilic or nucleophilic bond[15] during the bioreactive interaction with an endogenic macromolecule. The phenolics' hydrogen substitution by methyl groups will significantly decrease the chemical affinity for the active center, and the toxicity will decrease consequently.

Equation 2 (Table 5) indicate a high correlation (r^2=0.934) between the effectiveness and the

number of C_{sat}, excepting the derivatives with strong *ortho/ortho'* steric hindrances or the DES non-congeneric derivative.

One could notice that a certain number of disagreements also appear. Some of them are due even to the measured values. Otherwise, this phenomenon is often encountered and somehow expected in the case of *in vivo* experimental measurements. The similar evolution of the effectiveness in different organisms allows the reciprocal use of the experimental data and the prediction/estimation of the toxicity values (according to the ESIP's model algorithm) in the case of some substances which haven't been tested yet.

EXPERIMENTAL

Excepting the nonil-phenolic isomers,[27] the tested derivatives have been purchased from Merck Co, Fluka, Riedel de Haën, AcrÕs Organic Co, Loba Chemie Fischamend Co, Monicolor Co, Reactivul Bucureşti and were used such as, from solutions of known concentrations, in synthetic sea water or methanol.

The biologic data on *H. echinata* and the experimental procedures were presented in a previous work.[26] The tested derivatives are presented in Table 2. For the considered organisms, the measured effectivities, $Mlog1/MRC_{50}$ and the phenols affinities for the estrogenic receptor ER[21], $Mlog1/IC_{50}$ (mol/L), have similar evolutions, but the absolute values are different. In the case of *H. echinata*, the reproducibility of the measurements is shown in the fifth column of Table 2, by the presence of an asterisk (*). The measured value, M, is the average of these measurements. The calculated values, C, result by the sum of the least square differences between M and C, in the case of the derivatives for which this difference does not exceed 0.50 log u. Table 2 also indicates the number of aliphatic, C_{sat}, and benzene aromatic, $C_{6\text{-}ar}$, biphenilic, $C_{12\text{-}ar}$ and naphthalenic, $C_{10\text{-}ar}$, carbon atoms involved in the extension of the ESIP model's algorithm to the new hydroxy-phenolic sub-structures.

The 8-hydroxy-phenolic sub-structures are shown in Table 1, and are differentiated by the number of the hydroxyl group as well as by the hydrocarbonated radical's.

All the calculated values have a dynamic character: they change with the number of experimental determinations and accordingly to the involvement of some new experimental data.

The semiempirical calculations were carried out using the Hyperchem 5.0. package. Linear regressional analysis was performed by Statistica 7.1. software (Statsoft inc., USA).

The molecular descriptors used in the QSAR correlations were: the molecular hydrophobicity, the molecular steric character, the stereoelectronic parameter, and the energy of the highest occupied molecular orbital.

The molecular hydrophobicity or the penetration of the toxicant to the substrate's active center,[28] is described by the octanol/ water repartition coefficient, logP, with measured (m) and calculated (c*) values, ranging in 0.16 ÷ 7.42 log u., and by the molecular polarisability a_E (8.97 ÷ 21.44 $Å^3$). In the correlations, no distinctions are made among the m and c* values.

The molecular steric character is represented by the molecular volume V_m (91.44 ÷241.09 $Å^3$), as well as by the molecular surface MSA (121.00 ÷ 229.46 $Å^2$).

The energy of the lowest unoccupied molecular orbital, E_{lumo} (–0.03 ÷ 0.50 eV), represents the electrophilicity or the molecule's tendency to form a hydrogen bond with the substance's active centre with an electron of its E_{homo} orbital. E_{lumo} quantifies the molecule's electron affinity and reflects the phenol's tendency to the direct attack of the high density electron centers of endogenic macromolecules, as well as their ability to undergo a metabolic activation by a monoelectronic reduction.

The energy of the highest occupied molecular orbital, E_{homo} (-9.11 ÷ -8.46 eV), represents the nucleophilicity or the molecule's tendency to donate electrons and to form a hydrogen bond with the substrate's active centre. This parameter quantifies the ionization potential, and represents by this way a measure of the compound's susceptibility to oxidation.

Combined descriptors such as the binary product, $logP*E_{lumo}$, represent some interactions between two individual parameters. The values of the individual descriptors used in correlations are shown in Table 3.

CONCLUSIONS

The present work emphasizes the *in vivo* prescreening possibilities of the *H. echinata* (hydrozoa) organism test system in toxicity determinations. The calculated values are quantified by the ESIP's algorithm (Köln model) for the hydrocarbonated and hydroxylic molecular sub-structures, and present a dynamic character: they alter depending on the determinations number or by new data.

The toxicity of the monohydroxylic, *para*-alkylic phenols increases till the critical hydrophilic/ hydrophobic balance, corresponding to a number of nine C_{sat} carbon atoms, especially if the branchement is geometrically closer of the benzenic nucleus. The toxicity is strongly affected by the *orto, orto'* steric hindrances at the OH's group level.

The hydroxybiphenyls and the naphthols are more effectives than the phenols due to the fact that the hydrocarbonated structures are more lipophilic.

The dihydroxyphenols' and naphthalenediols' toxicity depends by the reciprocal position of the two phenolic groups between which the molecular polar character increases by a *push-pull electronic mechanism*, by the hydrocarbonated substituent's volume, and by their greater hydrophilicity, as compared to the phenol's one.

The *push-pull electronic mechanism* is also exercised in the case of the trihydroxylic phenols, which are the most hydrophilic derivatives of the considered series.

The QSAR equations confirm the proposed mechanisms. The derivatives which are not included in the quantifications are precisely those which exhibit extreme steric or electronic influences.

Even if the ER's existence is not known such as in the case of *H. echinata*, the parallelism of the determined effectiveness by this test system to those test systems in which RE were used by transfer or in which the organism possessed such receptors (*P. promelas*), allows us to presume/to accept the appearance of such a receptor as in the case of *T. pyriformis*. However, comparatively, the *H. echinata* test system may offer the same important results but in more favorable conditions (materials and time) than the *P. promelas* test system, a fish situated on an upper position on the evolution scale.

The reproducibility of the *H. echinata* test system may be estimated by repeating the experiments at large periods of time, using organisms of different cultures and generations.

The biological differences among *H. echinata* and mammalians are too important to attempt average similarities or extrapolations. However, the results are important due to their contribution in the explanation of some reaction mechanisms and in the possible decrease of the number of experimental determinations, which is a major concern of the experimental test in this field of scientific research. Thus, the meaning and the special importance of the "3 R's" concept: reduction, refinement and replacement,[34] concerning the experiments involving living organisms, is confirmed. By this way, this concept is awarded by theoretical background and practical dimensions. One could also presume that the described test system may be used by instance in the monitoring of the medicinal drug concentrations and implicitly in studies of the evolutions of some human diseases.

Aknowledgements: A part of this work was supported through a CNCSIS grant type A, 71GR/2006, and 66GR/09.05.2007, CNCSIS Code 1443 by the Roumanian Ministry of Education and Research of Romania.

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