In silico studies of dioxin-like toxicity of 75 individual chloronaphtalene congeners (PCNs)

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Introduction

Polychlorinated naphtalenes (PCNs) are relatively well known persistent, bioaccumulative and toxic pollutants, which have been affecting the environment since 20s of the last century. Those chemicals have been technically synthesized and used mainly in electrical equipment, wood preservation, as engine oil additives, refractive testing oils and pesticides. The most popular commercial mixtures of chloronaphtalenes were: Halowax (USA), Nibren Wax (Germany), Seekey Wax (Great Britain), Clonacire Wax (France), Cerifal Materials (Italy)[7-9]. Probably, even 150 000 tones of these chemicals had been produced in USA before it was officially prohibited in 1980. Data on production of PCNs in Europe and countries from other continents are not available[13].

Other significant antropogenic sources of chloronaphtalenes were thermal processes (waste incineration, municipal heating) and no-thermal technologies where chlorine was using (paper production, tap water chlorination)[12].

Some of chloronaphthalenes elicit toxic effects similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and these similarities might be expressed by means of toxic equivalency factors (TEFs). Determination of TEFs is based on the assumption, that all 'dioxin-like' compounds act through the AhR signal transduction pathway. After penetration of the cell, a molecule of TCDD analogue binds to the arylohydrocarbon receptor (AhR) localized in cytoplasm. After docking, the complex AhR-TCDD analogue is translocated to the nucleus, where it binds to dioxin response element (DRE) in DNA. DRE plays role of regulatory element for expression of many genes responsible for different toxic and non-toxic effects[11,18-20].

One of the describing effects is induction of P-450 depend izozyme CYP 1A1 (7-ethoxyresorufin-*O*-deethylase, EROD). It catalyzes reaction of deethylation of 7-ethoxyresorufin to resorufin. Resorufin are characterized by fluorescence abilities, which can be quantitatively measured by means of the fluorometer[2]. Based on this features, the EROD bioassay used rat hepatoma cells (H4IIE) was implemented by Nebert and Gelboin[18] and used to determination TEF values for new 'dioxin-like' compounds.

Another important in vitro assay indirectly measuring AhR binding affinity of potentially 'dioxin-like' compounds is test with luciferase implemented in 1993[1,21]. This bioassay uses recombinant cells consists of luciferase gene controlled by DRE. Luciferase catalyzes oxidation of luciferin to oxyluciferin. Oxidative form of luciferin produces light, which can be detected and quantitative measured by the luminometer[1,2,21].

Because of fact, that only for some congeners TCDD toxic equivalency factors (TEFs) were experimentally assigned[3,4,24], and the number of data on toxic effects exerted by particular CN congeners both *in vivo* and *in vitro* assays is rather limited, we tried to computationally predict 7-ethoxyresorufin-*O*-diethylase (EROD) and luciferase inducing potency for each of 75 PCNs. Based on these results, theoretical TEFs for all possible chloronaphtalene congeners were estimated.

Materials and Methods

The procedure of computational prediction of Ah-inducing potency consists of the three main steps: computational generation of the matrix of physico-chemical and quantum-chemical descriptors; compression of total structural information into few variables (factors) which were used as independent variables in QSAR modelling and prediction of values of EROD and luciferase induction for 75 chloronaphtalene congeners based on the constructed models.

The computed structural matrix was consisted of 75 rows (congeners) and 18 columns (descriptors). After initial control of data, the structural matrix was autoscaled in columns. The list of the structural descriptors applied in this study is given in table 1.

Table 1: Structural descriptors used in this study

Descriptor	Unit	Reference
Dipole moment	Debye	22
Symmetry	-	22
Electronic energy	eV	22
Total energy	eV	22
Standard heat of formation	kJ/mol	22
Energy of HOMO	eV	22
Energy of LUMO	eV	22
HOMO – LUMO gap	eV	22
Molecular weight	a.u.	22
Maximal positive charge at the carbon atom (Q+)	eV	22
Minimal negative charge on the chlorine atom (Q-)	eV	22
Solvent accessible surface area	$Å^2$	23
Van der Waals surface	$Å^2$	16
Solvent accessible surface volume	$Å^3$	23
Van der Waals volume	$Å^3$	16
Pattern of unsubstitution of vicinal carbon atoms with Cl	-	10
Wienner's index	-	25
Log of the octanol/water partition coefficent	-	17

To minimize of the number of variables and to eliminate redundancy between them, the Principal Component Analysis (PCA) with normalized VARIMAX rotation was implemented[15].

The linear modeling method – multiple regression of principal components (PCR) was used to define quantitative relationships between structural information and activity in EROD and luciferase bioassays[14,15]. Within the model, independent variables (PCs) were taken from principal component analysis, while the values of dependent variable were collected from published toxicological data[4,8,10]. Values of EROD-inducing potency and luciferase-inducing potency available for several CN congeners were transformed to linear function by logarithmic

transformation and conversion. After identification, efficiency of the model was evaluated by cross-validation technique[14,15].

Results and Discussion

Principal component analysis of the autoscaled data matrix compressed significant structural information into six principal components. Extracted components explain together 97.6 % of the total variance in structural data.

The first factor is a linear combination of descriptors mainly connected to chlorination degree of the naphtalene molecule, such as molecular weight, solvent accessible surface area, van der Waals surface, solvent accessible surface volume, van der Waals volume, Wienner's index, logarithm of the octanol/water partition coefficent, HOMO – LUMO gap (large positive loadings), and total energy, electronic energy, standard heat of formation, energy of LUMO, minimal Q- (large negative loadings). Second varivector (V) is determined by symmetry, third V by energy of HOMO, fourth V by index F, fifth by maximal Q+, while sixth by dipole moment.

In the next step these six extracted varivectors were used as independent variables in multiple regression model of EROD-inducing potency. Identified model of quantitative relationships between $[\log (EROD)]^{-1}$ and structural information represented by varivectors can be mathematically described by the equation:

$$\mathbf{1/log(EROD)} = \mathbf{-\ 0.189719}\ (\pm\ 0.013386) \mathbf{-\ 0.046294}\ (\pm\ 0.008755)\ \mathbf{V1} + \mathbf{0.023714}\ (\pm\ 0.011872)\ \mathbf{V2} + \mathbf{0.005144}\ (\pm\ 0.012877)\ \mathbf{V3} + \mathbf{0.001134}\ (\pm\ 0.010291)\ \mathbf{V4} - \mathbf{-\ 0.021506}\ (\pm\ 0.011826)\ \mathbf{V5} + \mathbf{0.004357}\ (\pm\ 0.014757)\ \mathbf{V6}$$

$$n = 17;\ R = 0.907;\ D = 82\ \%;\ F_{(6.10)} = 7.71;\ s = 0.038461;\ p < 0.01$$

Cross-validation procedure confirms predictive ability of the model and qualified it to prediction of unknown values of activity in the EROD test of 75 chloronaphtalene congeners.

Similar procedure was applied to prediction activity of chloronaphtalene congeners in luciferase test. The following equation describes the model:

$$\mathbf{1/log(luc)} = \mathbf{-\ 0.210631}\ (\pm\ 0.016661) \mathbf{-\ 0.060840}\ (\pm\ 0.012475)\ \mathbf{V1} + \mathbf{+\ 0.017996}\ (\pm\ 0.017741)\ \mathbf{V2} + \mathbf{0.004720}\ (\pm\ 0.017696)\ \mathbf{V3} + \mathbf{0.009733}\ (\pm\ 0.012430)\ \mathbf{V4}$$

$$n = 12;\ R = 0.906;\ D = 82\ \%;\ F_{(4.70)} = 8.02;\ s = 0.04421;\ p < 0.01$$

Results of predicted activity of chloronaphtalene congeners in the two bioassays are presented in table 2.

Table 2: Predicted values of 7-ethoxyresorufin-*O*-diethylase (EROD) and luciferase (luc) inducing potency for 75 congeners of chloronaphthalene

No.	Compound	EROD	luc
1	1-CN	8.98×10^{-10}	7.27×10^{-12}
2	2-CN	1.07×10^{-08}	9.55×10^{-10}
3	1,2-DiCN	2.13×10^{-06}	3.56×10^{-07}
4	1,3-DiCN	2.52×10^{-11}	5.86×10^{-09}
5	1,4-DiCN	5.02×10^{-08}	9.03×10^{-09}
6	1,5-DiCN	3.49×10^{-06}	2.66×10^{-07}
7	1,6-DiCN	1.25×10^{-07}	4.08×10^{-08}
8	1,7-DiCN	7.03×10^{-11}	7.37×10^{-09}
9	1,8-DiCN	1.85×10^{-07}	2.45×10^{-08}
10	2,3-DiCN	5.09×10^{-06}	2.74×10^{-06}
11	2,6-DiCN	2.06×10^{-06}	6.03×10^{-07}
12	2,7-DiCN	6.94×10^{-07}	5.13×10^{-07}
13	1,2,3-TrCN	6.06×10^{-06}	6.95×10^{-06}
14	1,2,4-TrCN	2.85×10^{-10}	6.80×10^{-08}
15	1,2,5-TrCN	1.38×10^{-06}	6.33×10^{-07}
16	1,2,6-TrCN	1.37×10^{-06}	9.13×10^{-07}
17	1,2,7-TrCN	1.09×10^{-07}	1.13×10^{-06}
18	1,2,8-TrCN	1.59×10^{-06}	1.22×10^{-06}
19	1,3,5-TrCN	6.50×10^{-11}	2.61×10^{-08}
20	1,3,6-TrCN	1.15×10^{-09}	1.03×10^{-07}
21	1,3,7-TrCN	1.94×10^{-09}	1.11×10^{-07}
22	1,3,8-TrCN	3.26×10^{-12}	1.13×10^{-08}
23	1,4,5-TrCN	2.02×10^{-08}	1.39×10^{-08}
24	1,4,6-TrCN	2.46×10^{-10}	1.94×10^{-08}
25	1,6,7-TrCN	4.01×10^{-08}	9.28×10^{-07}
26	2,3,6-TrCN	1.06×10^{-05}	6.60×10^{-06}
27	1,2,3,4-TeCN	4.10×10^{-05}	2.84×10^{-05}
28	1,2,3,5-TeCN	4.64×10^{-06}	1.94×10^{-05}
29	1,2,3,6-TeCN	5.97×10^{-05}	4.49×10^{-05}
30	1,2,3,7-TeCN	7.79×10^{-06}	2.60×10^{-05}
31	1,2,3,8-TeCN	3.85×10^{-05}	3.43×10^{-05}
32	1,2,4,5-TeCN	5.54×10^{-08}	1.54×10^{-06}
33	1,2,4,6-TeCN	2.02×10^{-07}	2.48×10^{-06}
34	1,2,4,7-TeCN	2.56×10^{-07}	3.38×10^{-06}
35	1,2,4,8-TeCN	8.79×10^{-08}	1.86×10^{-06}
36	1,2,5,6-TeCN	1.30×10^{-04}	5.88×10^{-05}
37	1,2,5,7-TeCN	2.39×10^{-07}	3.58×10^{-06}
38	1,2,5,8-TeCN	2.87×10^{-04}	6.72×10^{-05}
39	1,2,6,7-TeCN	1.19×10^{-06}	1.07×10^{-05}
40	1,2,6,8-TeCN	3.95×10^{-07}	4.66×10^{-06}
41	1,2,7,8-TeCN	5.14×10^{-05}	3.54×10^{-05}
42	1,3,5,7-TeCN	2.44×10^{-06}	3.96×10^{-05}
43	1,3,5,8-TeCN	5.14×10^{-09}	4.27×10^{-07}

44	1,3,6,7-TeCN	7.28×10^{-06}	2.00×10^{-05}
45	1,3,6,8-TeCN	4.63×10^{-07}	4.68×10^{-06}
46	1,4,5,8-TeCN	6.00×10^{-05}	2.10×10^{-05}
47	1,4,6,7-TeCN	5.34×10^{-06}	1.72×10^{-05}
48	2,3,6,7-TeCN	5.49×10^{-04}	3.29×10^{-04}
49	1,2,3,4,5-PeCN	3.19×10^{-05}	6.33×10^{-05}
50	1,2,3,4,6-PeCN	1.74×10^{-05}	6.07×10^{-05}
51	1,2,3,5,6-PeCN	1.89×10^{-05}	7.40×10^{-05}
52	1,2,3,5,7-PeCN	3.17×10^{-05}	2.19×10^{-04}
53	1,2,3,5,8-PeCN	1.59×10^{-05}	5.93×10^{-05}
54	1,2,3,6,7-PeCN	4.36×10^{-05}	1.28×10^{-04}
55	1,2,3,6,8-PeCN	2.84×10^{-05}	9.05×10^{-05}
56	1,2,3,7,8-PeCN	1.98×10^{-04}	1.80×10^{-04}
57	1,2,4,5,6-PeCN	5.43×10^{-06}	2.88×10^{-05}
58	1,2,4,5,7-PeCN	3.48×10^{-06}	7.52×10^{-05}
59	1,2,4,5,8-PeCN	3.90×10^{-07}	6.79×10^{-06}
60	1,2,4,6,7-PeCN	5.06×10^{-06}	1.00×10^{-04}
61	1,2,4,6,8-PeCN	2.27×10^{-06}	5.98×10^{-05}
62	1,2,4,7,8-PeCN	6.33×10^{-06}	3.24×10^{-05}
63	1,2,3,4,5,6-HxCN	1.09×10^{-04}	2.32×10^{-04}
64	1,2,3,4,5,7-HxCN	4.41×10^{-05}	3.80×10^{-04}
65	1,2,3,4,5,8-HxCN	1.57×10^{-04}	2.67×10^{-04}
66	1,2,3,4,6,7-HxCN	2.16×10^{-04}	8.30×10^{-04}
67	1,2,3,5,6,7-HxCN	4.88×10^{-04}	1.39×10^{-03}
68	1,2,3,5,6,8-HxCN	6.02×10^{-05}	4.51×10^{-04}
69	1,2,3,5,7,8-HxCN	5.72×10^{-05}	4.31×10^{-04}
70	1,2,3,6,7,8-HxCN	7.81×10^{-04}	8.09×10^{-04}
71	1,2,4,5,6,8-HxCN	4.78×10^{-05}	3.78×10^{-04}
72	1,2,4,5,7,8-HxCN	2.64×10^{-05}	2.88×10^{-04}
73	1,2,3,4,5,6,7-HpCN	1.17×10^{-04}	9.23×10^{-04}
74	1,2,3,4,5,6,8-HpCN	3.60×10^{-05}	5.25×10^{-04}
75	1,2,3,4,5,6,7,8-OCN	9.94×10^{-04}	3.24×10^{-03}

In most cases, both EROD and luciferase activity of individual PCNs are comparable. Generally, there is observable increase of the 'dioxin-like' activity in order of increasing chlorination degree. This factor seems to be a necessary condition of chloronaphtalene toxicity. PCNs consist of four chlorine atoms at least able to bonding with the dioxin receptor. Chloronaphtalene homologues differ from each other in activity. The most active, and probably also toxic compounds in the EROD and luciferase tests are CNs (in order of decreasing activity) nos. 75, 67 > 70, 73, 66, 48, 65, 63, 56 > 68, 69, 71, 74, 54, 72, 64. This data are comparable with earlier studies on chloronaphtalene toxicity. High activity of octachloronaphtalene (PCN#75) was reported by Campbell[5]. Also, relative high EROD and AHH inducing potency exhibit CNs nos. 67-69 and 73 in studies of Handberg et al. (1990). Addidtionally, as Campbell et al.[5,6] experimentally confirmed, PCN#73 has higher inducing potency than PCN#74 and PCN#63. It is interesting, that congeners no. 56 and 71, although they have been not previously reported as toxic, may also characterize by relatively high 'dioxin-like' activity. Theirs activity in EROD and luciferase

bioassays should be confirmed in the further investigation. Lower chlorinated PCNs, like mono-, di-, and trichloro homologues are not or rather weak inducers of 7-ethoxyresorufin-*O*-deethylase and luciferase, which is compatible with experiments[5,6].

As it previously suggested, differences of activity of higher chlorinated naphtalenes are influenced by the group of symmetry, value of energy of HOMO, number of vicinal carbon atoms unsubstituted with chlorine, and maximum positive charge together with dipole moment to a lesser extend.

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