ELECTRONIC PARAMETERS RESPONSIBLE FOR THE BIOLOGICAL ACTIVITY OF POLYCHLORINATED NAPHTHALENES

Jesus Olivero-Verbel[1], Leonardo Pacheco-Londoño[1], and Kurunthachalam Kannan[2]

[1]Environmental and Computational Chemistry Group. University of Cartagena. A.A. 6541. Cartagena, Colombia. E-mail: jesusolivero@yahoo.com www.reactivos.com
[2]Food Safety and Toxicology Center. Michigan State University. East Lansing, MI. 48824. USA.

Introduction

Polychlorinated naphthalenes (PCNs) are persistent compounds derived industrially from the chlorination of naphthalene and also formed during the combustion of municipal waste[1]. Due to their planarity and molecular similarity to dioxins, these molecules can induce cellular biochemical changes by activating aryl hydrocarbon receptor (AhR). In some locations, PCNs can contribute a greater proportion of the dioxin-like activity than those by polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-dioxins (PCDDs) or dibenzofurans PCDFs[2]. Molecular properties can be used to understand gas chromatographic retention or toxic potentials of congeners in a homologous series. In our earlier study, we have described quantitative structure retention relationships of PCNs[3]. The objective of this investigation was to evaluate the different molecular parameters responsible for the bioactivity of PCNs.

Methods

Structure-activity relationships for the bioactivity of PCNs were developed by following a three step-process: 1. Database selection of biological activity for different PCNs. 2. Molecular descriptor generation and 3. Discriminant analysis to classify biological data based on molecular descriptors. Biological activity information was taken from Villeneuve *et al.*,[4] and consisted of the potencies relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for 20 individual PCNs using the H4IIE-luc in vitro assay. This cell line has been transfected with an Ah receptor (AhR)-controlled luciferase reporter gene construct that responds to AhR agonists, and consequently measure the AhR-dependent activity, or dioxin-like activity. Molecular descriptor generation is a process to obtain several parameters based on the molecular electronic or topological architecture. Calculated molecular descriptors for 20 PCNs were derived from quantum chemical calculations using the Gaussian 94 Software (Gaussian, Inc., Pittsburgh PA, 1998). PCN geometries were fully optimized and single point data were calculated using the *Ab initio* molecular orbital method at the HF/3-21G level. The following electronic descriptors were obtained: Total energy (E_T), zero point energy (E_{zero}), rotational entropy (S_{ROT}), energy of the highest occupied molecular orbital (E_{HOMO}), energy of the lowest unoccupied molecular orbital (E_{LUMO}), the difference between E_{HOMO} and E_{LUMO} (GAP), dipolar moment (DM), quadrupole moments (QM) for different planes and atomic charges (Q), such as the most negative (MNC) and most positive charge (MPC). Using both the biological data and the calculated molecular descriptors, a discriminant analysis was performed in order to classify the PCNs as active (relative potency to TCDD $\geq 1.5 \times 10^{-4}$) or inactive (relative potency to TCDD $< 1.5 \times 10^{-4}$) groups. Best model was selected based on statistical significance and the percentage of cases correctly classified.

Results and Discussion

Discriminant analysis for the classification of 20 PCNs as active or inactive AhR agonist is shown in Table 1. A classification function was obtained based on two descriptors: The most positive charge

in the molecule (MPC) and the sum of the atomic charges for the atoms in the beta position (ΣQß). The information encoded by each of these descriptors is independent from each other (correlation between both descriptors was 0.267). The Wilks's lambda value showed that the eigenvalue for the discriminant function was significant. The canonical correlation value indicates that the discrimination function has a moderate capability to determine the group differences. According to the standardized coefficients, the most important descriptor to discriminate the two levels of activity was ΣQß.

Table 1. Discriminant analysis for activity of PCNs

Discriminant Function	Eigenvalue	Relative Percentage		Canonical Correlation
1	0.889	100.00	0.686	
Functions Derived	Wilks Lambda	Chi-Square	DF	P-Value
1	0.529	10.812	2	0.005

Classification function coefficients for activity of PCNs		
Discrimination	0	1
ΣQß	11.974	2.145
MPC	17.496	199.798
Constant	-29.688	-33.561

Standardized Coefficients of the discriminant function for Activity of PCN	
(ΣQb).	0.947
MPC	-0.662

The results of the classification of PCNs as inactive (0) or active (1) from the discriminant analysis is shown in Table 2.

Table 2. Classification table of PCNs based on molecular structure

Actual Activity	Group Size	Predicted Activity	
		0	1
0	14	13 (92.86 %)	1 (7.14 %)
1	6	0 (0.00 %)	6 (100.00 %)

Molecule	Actual Group	Predicted Group	Descriptor value	
			ΣQß	MPC
2-CN (2-chloronaphtlanene)	0	0	0,769	0,275
1,4-CN	0	0	1,083	0,287
1,5-CN	0	0	1,085	0,287
2,3-di-CN	0	0	0,616	0,283
1,2,7-tri-CN	0	0	0,661	0,314
1,2,3,4-tetra-CN	0	0	0,763	0,290
1,2,4,6-tetra-CN	0	0	0,723	0,317
1,2,5,6-tetra-CN	0	0	0,736	0,300

1,2,6,8-tetra-CN	0	0	0,724	0,315
2,3,6,7-tetra-CN	0	*1	0,292	0,292
1,2,3,5,8-penta-CN	0	0	1,048	0,331
1,2,3,6,7-penta-CN	1	1	0,370	0,323
1,2,3,4,6,7-hexa-CN	1	1	0,443	0,326
1,2,3,5,6,7-hexa-CN	1	1	0,445	0,328
1,2,3,5,6,8-hexa-CN	1	1	0,651	0,332
1,2,3,6,7,8-hexa-CN	1	1	0,440	0,299
1,2,4,5,6,8-hexa-CN	0	0	0,850	0,326
1,2,3,4,5,6,7-hepta-CN	1	1	0,512	0,335
1,2,3,4,5,6,8-hepta-CN	0	0	0,706	0,326
1,2,3,4,5,6,7,8-octa-CN	0	0	0,575	0,144

*. Incorrectly classified.

In Table 2, only one PCN (5 %) was incorrectly classified by the molecular descriptors. The relationship between ΣQß and MPC is shown in Figure 1. It is clear that the lack of activity is mainly determined by ΣQß values greater than 0.6, and MPC values greater than 0.27. As presented in Figure 1, the classification of 2,3,6,7-tetra-CN as an active compound by the discriminant function was the result of having descriptor values typical for this group.

The structure and the atomic charges for the a- and b-chlorines in the 1,2,3,6,7-penta-CN are shown in Figure 2. It is clear that the charges in b-chlorines are dependent on the presence or absence of adjacent a-chlorines. This fact *per se* establishes that PCN activity is based on the relative positioning of the different chlorine atoms in the whole molecule, and not in the presence of particular arrangements at one side of the naphthalene.

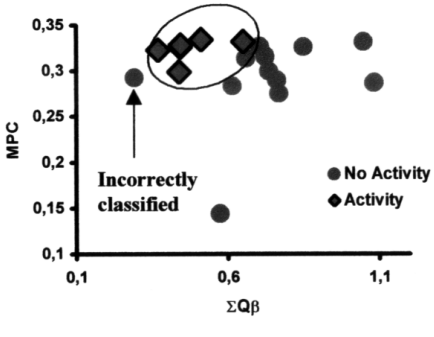

Figure 1. Plot of MPC vs. SQß.

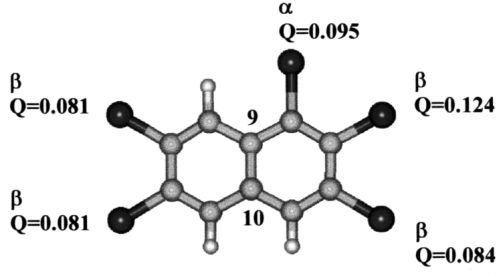

Figure 2. Molecular structure of 1,2,3,6,7-penta-CN and atomic charges for the chlorine atoms

The presence of ΣQß in the discriminant function might be related to the electronic architecture of PCNs required for activity. This electronic arrangement could also reflect the mass distribution along the molecule required to conformationally interact with the AhR. In the case of dioxins, this interaction which depends on specific charge distribution patterns, has been quantitatively described by the molecular quadrupole moments[5]. In order to obtain structure bioactivity relationships for active PCNs, a multiple linear regression was performed using the relative potency to TCDD (REP) and the molecular descriptors as independent and dependent variables, respectively. The best model is presented below.

$$\text{Log (REP)} = 13.3 \pm 6.4 + 35.8 \pm 10.0(\Sigma Q_{9\text{-}10}) - 0.3 \pm 0.2(S_{ROT})$$

R=0.902, SE=0.253, N=6, F=6.55, P=0.08

A plot of observed vs. calculated values for the prediction model is shown in Figure 3. The sum of the atomic charges for carbons 9 and 10 ($\Sigma Q_{9\text{-}10}$) and the molecular rotational entropy (S_{ROT}) were the molecular descriptors that better describe the biological activity of PCNs. The correlation between these two descriptors was -0.45, indicating the absence of multicollinearity.

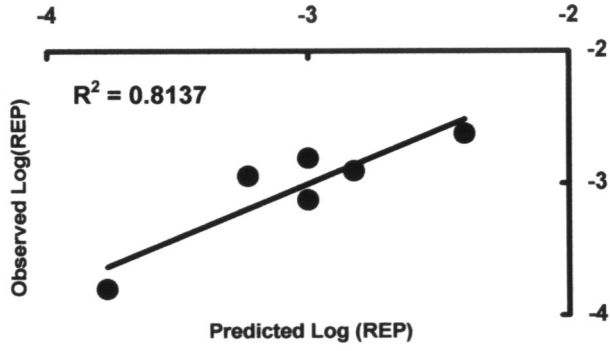

Figure 3. Plot of observed vs. predicted bioactivity (REP) derived from the best QSAR multiregression model for 6 PCN congeners.

The descriptor $\Sigma Q_{9\text{-}10}$ reflects the electronic nature around both aromatic rings, whereas S_{ROT} is a thermodynamic parameter related to molecular symmetry. These results are in agreement with the discriminant analysis, and support the idea that biological activity of PCNs is regulated by the presence of particular arrangements of chlorine atoms that create specific electronic environments around both rings.

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