

EVALUATION OF THE ESTROGENIC EFFECT OF POLYCHLORINATED BIPHENYLS WITH REGARD TO THEIR METABOLIC ACTIVATION BY APPLICATION OF IN SILICO TECHNOLOGIES

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Abstract

Hydroxylated polychlorinated biphenyls (OH-PCBs) major metabolites of PCBs, have been reported to present affinity to the estrogen receptor and induce receptor-mediated responses. The early identification of PCBs which may act as estrogen binders was investigated by making use of computational tools for chemical risk assessment. As an area of growing concern a strategy for development of such tools is one of the primary tasks of governmental and international organizations. In the current study the estrogenic potential of several PCBs is analyzed by using widely accepted and standardized computational program. The obtained results suggest that the system is equipped with scientific reliable modules for prediction of the estrogenic potential of diverse chemicals as well as robust machinery for adequate metabolism simulation.

Key words: estrogen receptor, QSAR, polychlorinated biphenyls (PCBs), metabolic activation, in silicon tools

1. INTRODUCTION

Endocrine disruption has become a major issue in environmental and human toxicology. The endocrine disruption hypothesis, as formulated in the early 1990s, proposes that natural and synthetic substances released into the environment by human activities are capable of disturbing endogenous hormone systems of exposed organisms, which in turn can lead to adverse health outcomes. The disruptive effects appear to be mediated principally by binding of the environmental compounds to cellular hormone receptors, or by interference with synthesis, plasma transport, catabolism and excretion of the endogenous hormones.

Polychlorinated biphenyls (PCBs) are widespread contaminants that were used as heat transfer fluids, hydraulic fluids, solvent extenders, plasticizers, flame-retardants, and dielectric fluids around the world until the 1970s. Despite the ban, they have been still detected in the environment due to their chemical stability and high persistency. In addition, PCBs are highly lipophilic, accumulate in the liver and adipose tissue. A large number of studies have reported that some PCB mixtures possess diverse deleterious effects including immunotoxicity, neurotoxicity, developmental toxicity, reproductive toxicity, and carcinogenicity (Safe et al. 1994). Hydroxylated PCBs (OH-PCBs), a major biological metabolite group of PCBs, are formed by the cytochrome P450 monooxygenase enzyme systems in humans and wildlife (Letcher et al., 2000). While many of these OH-PCBs are further conjugated with glucuronic acid, glutathione, or sulfate, which facilitates their excretion, some OH-PCBs persist in the body (Sadeghi-Aliabadi et al., 2007) which allow them to exert different toxic effects.

Hydroxylated PCBs have structural similarities to endogenous compounds, and *in vitro* and *in vivo* studies have shown that certain HO-PCBs may have adverse effects on estrogen hormone homeostasis (Arulmozhiraja et al., 2005). For example, some HO-PCBs showed estrogenic activities in different assay systems, the *Xenopus laevis* vitellogenin (VTG)-assay and yeast two-hybrid assay for estrogen receptor α (ER α) (Nomiya et al., 2010). Recently, Takeuchi et al. (2011) reported that several HO-PCBs were estrogenic on the basis of reporter gene assay.

In the last decade, much effort has been placed in developing alternatives to *in vitro* and *in vivo* testing (Mehling et al., 2012). Given that the number of HO-PCBs is large, there is increasing interest to use productive and cost-efficient *in silico* modeling approaches, such as quantitative structure–activity relationship (QSAR), to predict the estrogenic effect of HO-PCBs (Ekuase et al., 2011). These models combine the use of physical chemical or structural properties (descriptors) and computational methods to assign a molecule to a certain category or biological activity relevant for the prediction of a certain toxicological endpoint. Furthermore, it is clear that the role of metabolism of PCBs should be taken into account along with application of QSARs due to the fact that in general PCBs does not show estrogenic effect as parent structures.

The aim of the present study is to investigate the possibility of application of *in silico* technology for prediction the estrogenic effect of PCBs with regard to their metabolic activation.

2. MATERIALS AND METHODS

2.1. Estrogen binding data for PCBs

General representation of PCBs used in this study are shown in Fig.1. The estrogenic potency of the investigated HO-PCBs was obtained from published literature (Takeuchi et al., 2011). The estrogenic potency to the estrogen receptor (ER) was estimated by *in vitro* ER dependent reporter gene assays. The estrogenic activity in terms of 20% relative effective concentration (REC_{20} , M) was gained from the dose–response curve of the luminescence intensity and expressed in the corresponding $pREC_{20}$ ($-\log REC_{20}$).

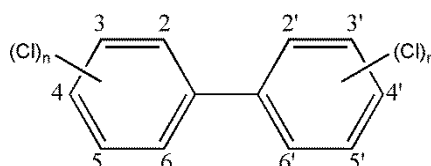


Fig.1. General structural representation of the substituted PCBs used in this study.

Experimental values for estrogen binding are listed in Table 1 (Takeuchi et al., 2011).

Table 1. Binding affinity of OH-PCBs to ER

No.	ChemicalName	$pREC_{20}$
1.	2-Chlorobiphenyl-4-ol	7.36
2.	5-Chlorobiphenyl-2-ol	5.31
3.	2',3'-Dichlorobiphenyl-2-ol	5.47
4.	2',5'-Dichlorobiphenyl-2-ol	5.38
5.	3',4'-Dichlorobiphenyl-2-ol	5.28
6.	2,2',5'-Dichlorobiphenyl-3-ol	6.40
7.	2',3,3'-Trichlorobiphenyl-2-ol	5.39
8.	2,2',4',6'-Tetrachlorobiphenyl-4-ol	8.89
9.	2',3,4,4'-Tetrachlorobiphenyl-2-ol	5.21

2.2. Metabolism of PCBs

In many cases metabolites are shown to be generally more reactive than their precursors (McLean et al., 1996). It is suggested that the toxicities of the PCB metabolites may contribute to the overall toxicity of the PCB parents. PCB congeners can exhibit significantly different bioactivities. Therefore, the toxic effects of PCBs are difficult to evaluate because of the contributions from their various metabolites.

Being highly stable PCBs can be metabolized through different pathways (Lehmle et al. 2010). PCBs have a large number of congeners and isomers, which makes their downstream metabolites more diverse. In general, halogenated aromatic compounds, undergo phase I and phase II metabolism. In the initial oxygenation step, the PCB parents oxidize into their corresponding hydroxylated and dihydroxylated metabolites via arene oxide intermediates (McLean et al., 1996). As shown by scientific researches, the aromatic hydroxylation of PCBs can occur via a non-arene oxide mechanism [Preston et al. 1983]. This phase I metabolism occurs in the presence of the cytochrome P-450 (CYP) enzyme. In the second step (phase II), the products are metabolized through glutathione conjugation or the mercapturic acid path.

2.3. *In silico* assessment of the estrogenic potential of xenobiotics

During the last three decades, substantial efforts have been made to develop various *in vivo* and *in vitro* tests allowing the detection of the potential endocrine effects of chemicals (Zacharewski, 1997). Despite the fact that those experiments are highly reliable they cannot be applied to evaluate the estrogenic potential of thousands of chemicals due to time and cost limitations. Computational (eco)toxicology in its various forms offers a practical approach to meet this challenge. The assumption that biological activity is implicit from chemical structure offer a process to formalize this knowledge and an attempt to form some direct relationships between chemical structures and biological effects. There are a number of computational packages for investigation of human health effects which includes modules for estrogen binding predictions [Cronin et al. 2003]. In general, these computational tools allow different biological effects to be predicted directly from chemical structure and have been used by regulatory agencies and industry alike because of their ease of use and rapid application. Among the available systems one should point out the growing use of non commercial platforms for biological endpoint predictions [Ambit, Toxtree].

2.4. OECD QSAR Toolbox

The Organisation for Economic Co-operation and Development (OECD) is running a project, funded by the European Union and in collaboration with regulatory bodies in Europe, Japan, and America to provide a tool suitable for use by the regulators. The system is designed, developed and currently maintained by the Laboratory of Mathematical Chemistry at “prof. Dr. Assen Zlatarov” University, Bourgas, Bulgaria [LMC]. The OECD QSAR Toolbox [OECD] is designed around the workflow a regulator/researcher would typically follow. The first step is to identify the chemical of interest, and the Toolbox allows standard options for chemicals input (SMILES, CAS, ECI code). When the chemical is entered it is possible to retrieve all available toxic data from the largest publicly available endpoint datasets incorporated in the system. If there is no information then the system provides option to investigate the chemical by using collection of profilers. Each profiler is related to a specific biological/toxic endpoint and it is organized as a combination of structural and/or molecular descriptors as rules.

The main advantage of the system is the opportunity to investigate a chemical with account to its metabolic fate. It is well known that the chemical in its parent form may not exert toxic effect however after metabolism a reactive metabolite can be produced which may damage biological macromolecules.

In the following two subsections details will be given for current versions of both *estrogen binding* profile and *in vitro metabolic simulator* incorporated in version 3.2 of the Toolbox.

2.4.1. Estrogen binding profile

The incorporated ER binding profiling scheme is based on structural and parametric rules extracted from literature sources and supported by experimental data. The ER-binding profiler classifies chemicals as non binders or binders depending on molecular weight (MW) and structural characteristics of the chemicals:

- Very strong binders: Chemicals with MW between 200 and 500 Da and two rings with a hydroxyl group connected to each of them.
- Strong binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 200 and 500 Da;
- Moderate binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 170 and 200 Da;
- Weak binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW less than 170 Da;

2.4.2. *In vitro* metabolism simulator

The current *in vitro* rat liver metabolic simulator represents electronically designed set of 509 structurally generalized, hierarchically arranged biotransformation reactions, which are characteristic for the metabolism for *in vitro* experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction. A training set of 647 xenobiotic chemicals of a wide structural diversity, with experimentally observed metabolic reactions and pathways has been built, using published data on their metabolism in rodent liver microsomes and S9 fraction. On the whole, the simulator contains 450 – 470 enzymatic phase I transformations, such as aliphatic C-oxidation, aromatic C-hydroxylation, oxidative N- and O-dealkylation, epoxidation, ester and amide hydrolysis, carbonyl group reduction, nitro and azo group reduction, N-hydroxylation, etc. Additionally, 15 – 20 enzymatic phase II

transformations, such as glucuronidation, sulfation, glutathione conjugation, N-acetylation, etc. are included with significantly lower priority than phase I ones.

The principal applicability of this simulator is associated with the reproduction as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit diverse array of *in vitro* biological effects.

3. RESULTS AND DISCUSSION

The applicability of the OECD QSAR Toolbox for predicting the estrogen binding potential for all PCBs listed in Table 1 is analyzed. A separate discussion for each chemical is presented in next subsections.

3.1. Monochlorobiphenyls

In this group the analysis is based on two chemicals - 2-Chlorobiphenyl and 5-Chlorobiphenyl. Summary for estrogen binding predictions and simulated metabolites is provided in Table 2.

Table 2. Estrogen binding predictions and simulated metabolites for 2-Chlorobiphenyl and 5-Chlorobiphenyl

PCBs	pREC ₂₀	Predicted ER binding effect ²	Simulated Metabolism ³
2-Chlorobiphenyl	N/A ¹	Negative	N/A
2-Chlorobiphenyl-4-ol	7.36	Positive	+
5-Chlorobiphenyl	N/A	Negative	N/A
5-Chlorobiphenyl-2-ol	5.31	Positive	+

¹N/A – Not available; ²Prediction according the ER binding profile in the OECD QSAR Toolbox; ³+/- simulated/not simulated metabolite

The estrogen binding prediction for both chemicals as parent structures is negative. The prediction is based on the fact that all requirements encoded in the profile are not met.

A total number of nine metabolites were simulated for 2-Chlorobiphenyl. Two of them are predicted as non ER binders and the rest seven are classified to be strong ER binders containing hydroxyl group. The metabolite 2-Chlorobiphenyl-4-ol is one of the simulated metabolites with positive prediction for estrogen binding.

As a result of metabolic simulation twenty six metabolites were generated for 5-Chlorobiphenyl. According to the profile three metabolites are predicted as non ER binders. The rest twenty three metabolites are predicted to be ER binders with strong activity. The observed and experimentally tested 5-Chlorobiphenyl-2-ol falls in this group. The obtained results for mono substituted PCBs suggests that the predictions provided by the system are reliable.

3.2. Dichlorobiphenyls

PCBs related to this group are 2',3'-Dichlorobiphenyl, 2',5'-Dichlorobiphenyl, and 3',4'-Dichlorobiphenyl. Summary for estrogen binding predictions and simulated metabolites is provided in Table 3.

Table 3. Estrogen binding predictions and simulated metabolites for Dichlorobiphenyls analyzed in this study

PCBs	pREC ₂₀	Predicted ER binding effect ²	Simulated Metabolism ³
2',3'-Dichlorobiphenyl	N/A ¹	Negative	N/A
2',3'-Dichlorobiphenyl-2-ol	5.47	Positive	+
2',5'-Dichlorobiphenyl	N/A	Negative	N/A
2',5'-Dichlorobiphenyl-2-ol	5.38	N/A	-
3',4'-Dichlorobiphenyl	N/A	Negative	N/A

3',4'-Dichlorobiphenyl-2-ol	5.28	Positive	+
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¹N/A – Not available; ²Prediction according the ER binding profile in the OECD QSAR Toolbox; ³+/- simulated/not simulated metabolite

Not surprisingly the parent chemicals 2',3'-Dichlorobiphenyl, 2',5'-Dichlorobiphenyl, and 3',4'-Dichlorobiphenyl in this group are predicted to be non ER binders due to the lack of any key structural feature responsible for estrogenic effect.

The application of the metabolic simulator results in generation of a total number of eight metabolites for 2',3'-Dichlorobiphenyl. The metabolite 2',3'-Dichlorobiphenyl-2-ol is simulated and the it is predicted to be strong ER binders by the profile. In this case the predicted ER binding activity can be explained by the structural similarity with the endogenous hormone estradiol. More specifically the similarity is toward specific distance between the OH groups in 3rd and 17th positions in the steroidal skeleton. It should be noted that the distance between hydroxyl group at 2nd position and the farthest hydrogen atom in 2',3'-Dichlorobiphenyl-2-ol is shorter however it is considered to be important for ER binding with lower binding activity as compared with estradiol.

As a result of metabolic simulation eight metabolites are generated for 2',5'-Dichlorobiphenyl. Negative ER binding prediction is assigned for four of them. The metabolite 2',5'-Dichlorobiphenyl-2-ol was not simulated however the rest four metabolites are predicted to be strong ER binders.

Regarding the results obtained for 3',4'-Dichlorobiphenyl a total number of fifteen metabolites are simulated. Two of them are predicted to be non ER binders. The simulated 3',4'-Dichlorobiphenyl-2-ol is one of the thirteen positive predicted metabolites. This result suggests that the system is well tuned in respect to the combination of the ER binding profile and the *in vitro* metabolic simulator applied for prediction for Dichlorobiphenyls.

3.3. Trichlorobiphenyls

In this group representative PCBs are 2,2',5'-Dichlorobiphenyl and 2',3,3'-Trichlorobiphenyl. Summary for estrogen binding predictions and simulated metabolites is provided in Table 4.

Table 4. Estrogen binding predictions and simulated metabolites for Trichlorobiphenyls analyzed in this study

PCBs	pREC ₂₀	Predicted ER binding effect ²	Simulated Metabolism ³
2,2',5'-Dichlorobiphenyl	N/A ¹	Negative	N/A
2,2',5'-Dichlorobiphenyl-3-ol	6.40	Positive	+
2',3,3'-Trichlorobiphenyl	N/A	Negative	N/A
2',3,3'-Trichlorobiphenyl-2-ol	5.39	N/A	-

¹N/A – Not available; ²Prediction according the ER binding profile in the OECD QSAR Toolbox; ³+/- simulated/not simulated metabolite

The investigated Trichlorobiphenyls as parent structures are predicted to be non ER binders. The lack structural features responsible for ER binding similarly with previously discussed PCBs is the reason for non binding effect.

The total number of the generated metabolites for 2,2',5'-Dichlorobiphenyl is twelve. Six of them do not correspond to none of the rules related to positive ER binding effect. The rest six metabolites are predicted to be strong ER binders. Among them 2,2',5'-Dichlorobiphenyl-3-ol was successfully generated and correctly predicted by the system.

Nine metabolites were generated for 2',3,3'-Trichlorobiphenyl. Three of them were predicted as non ER binders. The rest six metabolites were predicted to be strong ER binders. It should be noted that in this case the metabolite 2',3,3'-Trichlorobiphenyl-2-ol was not simulated. One explanation for missed metabolite could be the fact that hydroxylation reaction cannot be applied in 2nd position due to the presence of chlorine in 3rd position in the aromatic ring.

3.4. Tetrachlorobiphenyls

The group of Tetrachlorobiphenyls is represented in this study by 2,2',4',6'-Tetrachlorobiphenyl and 2',3,4,4'-Tetrachlorobiphenyl. Summary for estrogen binding predictions and simulated metabolites is provided in Table 5.

Table 5. Estrogen binding predictions and simulated metabolites for Tetrachlorobiphenyls analyzed in this study

PCBs	pREC ₂₀	Predicted ER binding effect ²	Simulated Metabolism ³
2,2',4',6'-Tetrachlorobiphenyl	N/A ¹	Negative	N/A
2,2',4',6'-Tetrachlorobiphenyl-4-ol	8.89	Positive	+
2',3,4,4'-Tetrachlorobiphenyl	N/A	Negative	N/A
2',3,4,4'-Tetrachlorobiphenyl-2-ol	5.21	Negative	+

N/A – Not available; ²Prediction according the ER binding profile in the OECD QSAR Toolbox; ³+/- simulated/not simulated metabolite

The prediction result for ER binding for both Tetrachlorobiphenyls as parent structures is negative. Nine metabolites were simulated for 2,2',4',6'-Tetrachlorobiphenyl. Four of them are predicted to be non ER binders and the rest five - strong ER binders. Among the last group 2,2',4',6'-Tetrachlorobiphenyl-4-ol was successfully simulated and correct predicted. It should be noted that the most potent ER binder among all investigated hydroxylated PCBs is 2,2',4',6'-Tetrachlorobiphenyl-4-ol. The highest potency can be explained with its structural similarity with the endogenous hormone estradiol. The hydroxyl group in 4th position (similar with OH group in 3rd position in estradiol) is not crowded by other substituents in ortho positions. On the other hand the influence of the distribution of electronic charges in the aromatic rings is assumed to be related to the highest ER binding potential.

The application of the metabolic simulator for 2',3,4,4'-Tetrachlorobiphenyl results in generation of a total number of ten metabolites. Half of them are predicted to be ER binders. The metabolite 2',3,4,4'-Tetrachlorobiphenyl-2-ol was successfully generated however the prediction result for ER binding is negative. According to the applied ER binding profile the prediction is negative because the effect of the hydroxyl group in 2nd position which is important for ER binding is prevented by the neighboring chlorine atoms.

4. CONCLUSIONS

The application of computational tools for chemical risk assessment allows identification of hazardous substances at very early stages of their assessment. The presented application of the platform OECD QSAR Toolbox for identification of hydroxylated polychlorinated biphenyls as estrogen binders encourage further using and refining of such kind of tools for their application in the field of computational (eco)toxicology. It should be pointed out that the virtual screening cannot be considered as tool to find all potentially estrogenic active chemicals from a screening database, nor is it suitable for replacing *in vitro* or *in vivo* testing. However, computational models are valuable tools for prioritizing potential estrogenic compounds for biological evaluation, thereby helping to find a majority of estrogenic compounds already at the beginning of a systematic *in vitro*/in vivo screening campaign.

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