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METABOLIC ESTROGENIC ACTIVITY OF SOME ENDOCRINE DISRUPTOR CHEMICALS

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Summary. There is growing interest in the possible health threat posed by endocrine-disrupting chemicals (EDCs), which are substances in the environment, food, and consumer products that interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control or reproduction. In order to understand health risks of estrogenic endocrine disruptors, as much information as possible regarding their biological activity is needed, i.e., their extent of exposure and mechanisms of action via pathways involved in hormone action and homeostasis, including the biological activities of possible metabolites. It is concluded that an underestimation of the overall risk of exposure to estrogens may well result when assessing the contribution of the parent compounds individually only, and experimental or computational studies should be included to elucidate whether compounds can be bioactivated into more estrogenic metabolites. The aim of this work was to predict the metabolic estrogenic activity of some endocrine disruptor chemicals.

Key words: endocrine disruptor chemicals, metabolic estrogenic activity, microbial metabolism

ЕСТРОГЕННА АКТИВНОСТ НА МЕТАБОЛИТИТЕ НА НЯКОИ СЪЕДИНЕНИЯ, ДЕЙСТВАЩИ ВЪРХУ ЕНДОКРИННАТА СИСТЕМА

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Резюме. Наблюдава се нарастващ интерес относно евентуална заплаха за здравето, предизвикана от вещества, водещи до ендокринни смущения. Тези вещества се срещат в околната среда, храните и продуктите от бита, които се намесват в хормония биосинтез, метаболизма или предизвикват действия, които водят до отклонение от нормалния хомеостатичен контрол или възпроизвеждане. За да се определи здравословният риск от действието на съединенията с естрогенна активност, е необходимо търсенето на повече информация по отношение на тяхната биологична активност, т.е. степента на експозиция и механизмите им на действие, чрез пътища, включени в хормонното действие и хомеостаза, включително и биологичната активност на възможните метаболити. Заключението е, че при изследване действието на веществата с естрогенна активност е от значение оценяването не само на изходните съединения, но и експерименталното и теоретичното предсказване на възможната биоактивация на техните метаболити, които могат да проявят по-силно естрогенно действие. Целта на настоящата работа беше да се предскаже естрогенната активност на метаболитите за някои съединения с ендокринно действие.

Ключови думи: съединения с естрогенна активност, естрогенна активност на метаболити, микробиален метаболизъм

Introduction

There has been growing social concern that industrially and naturally occurring compounds in the environment may have adverse effects on the endocrine systems of humans and wildlife. These compounds are called endocrine disrupters or endocrine disrupting chemicals (EDCs). Many EDCs affect sex hormone systems, including estrogen, and it is already known that various compounds such as insecticides, plasticizers, and

surfactants have estrogenic activity. Revealing the metabolism and identifying the metabolites of toxic compounds such as EDCs are important research subjects, because many compounds are affecting biological activities through metabolism [23].

In addition to the developmental and reproductive effects, there is also a growing concern that metabolic disorders may be linked with EDCs. Global obesity rates have risen dramatically over the past three decades in adults, children, and adolescents, especially in developed countries. Obesity is frequently associated with metabolic disorders (including type 2 diabetes, metabolic syndrome, cardiovascular and pulmonary complications, and liver disease) as well as other health issues such as psychological /social problems, reproductive defects, and some forms of cancer. The increased incidence of metabolic diseases also correlates with substantial changes in the chemical environment resulting from new industrial and agricultural procedures initiated over the past 40 years. This change in the environment has led to the hypothesis that some of the numerous environmental pollutants are EDCs, interfering with various aspects of metabolism and adding another risk factor for obesity [1, 13].

Hormones function mainly through interactions with their cognate receptors, which can be classified into two large groups: (a) membrane bound receptors, which respond primarily to peptide hormones such as insulin, and (b) nuclear receptors (NRs), which are activated by interaction with small lipophilic hormones such as sex steroid hormones. EDCs may possess multiple mechanisms of action; however, because many EDCs are small lipophilic compounds, one privileged route is through their direct interaction with a given NR, which presumably perturbs or modulates downstream gene expression. The ability of EDCs to interact with these NRs is supported by, and explains, the wide range of metabolic perturbations reported in both experimental and epidemiological studies. It also reinforces the concept of associating endocrine and metabolic disruption [2].

Quantitative structure – activity relationship (QSAR) models have proven their utility, from both the pharmaceutical and toxicological perspectives, for identification of chemicals that might interact with NRs. While their primary function in the pharmaceutical enterprise is lead discovery and optimization, QSAR models have played an essential role in toxicology as a priority setting tool for risk assessment. For example, public health concern about EDCs resulted in Federal legislation mandating the environmental protection agency (EPA) to regulate potential EDCs in drinking water and food additives [25]. Under this requirement, up to 87,000 existing chemicals will be experimentally evaluated for their potential to disrupt activities in the estrogen, androgen, and thyroid hormone systems [16]. In an attempt to reduce the time and expense in this prodigious task of screening and testing such a large number of chemicals, QSAR models are being developed to prioritize chemicals as to their endocrine disrupting potential for further experimental evaluation [6].

QSAR models offer numerous additional benefits beyond prediction [14], such as: (1) leveraging existing structure–activity data; (2) providing insights into mechanisms of action (e.g. agonist versus antagonist) or identifying alternative mechanisms (e.g. metabolism); (3) identifying key structural features associated with high/low activity; (4) suggesting new design strategies and synthetic targets; (5) narrowing the dose range for a planned assay; (6) assisting in generation of new hypotheses to guide further research; (7) revealing chemicals that deviate from the QSAR model and, therefore, from the presumed biological model.

The aim of this work was to predict the metabolic estrogenic activity of some endocrine disruptor chemicals.

Material and methods

1. OECD (Q)SAR Toolbox

The OECD (Q)SAR Application Toolbox is software tool used in regulatory toxicology to fill gaps in (eco)toxicity data. They include different SAR and QSAR models for estimating (eco)toxicological endpoints. (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on hazards of chemicals, while reducing time, monetary cost and animal testing currently needed [15].

2. Observed microbial metabolism

Degradation pathways used by microorganism to obtain carbon and energy from 200 chemicals are stored in a special file format that allows easy computer access to catabolic information. The collection includes the catabolism of C1-compounds, aliphatic hydrocarbons, alicyclic rings, fu-

rans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, sulfonates, nitrates, nitro-derivatives, nitriles, and compounds containing more than one functional group. Most of pathways are related to aerobic conditions. Different sources including monographs, scientific articles and public web sites such as the UM-BBD [7] were used to compile the database.

3. Microbial metabolism simulator

The original CATABOL simulator of microbial metabolism is implemented in the system [4, 5, 9]. Single pathway catabolism is simulated using the abiotic and enzyme-mediated reactions via the hierarchically ordered principal molecular transformations extracted from documented metabolic pathway database. The hierarchy of the transformations is used to control the propagation of the catabolic maps of the chemicals. The simulation starts with the search for match between the parent molecule and the source fragment associated with the transformation having the highest hierarchy. If the mach is not found, search is performed with the next transformation, etc. When the match is identified, the transformation products are generated. The procedure is repeated for the newly formed products. Predictability (probability that the metabolite is observed, given that the metabolite is predicted) evaluated on the bases of documented catabolism for 200 chemicals stored in the database of "Observed microbial catabolism" is 83%.

4. Ligand-binding pocket and interaction mechanisms

The region of the receptor-binding site not occupied by the receptor and thus available to the ligands and their analogues is called Receptor-excluded volume (RExV) [22]. An attempt to determine the volume of this map was done by superimposing molecular skeletons of known high affinity ligands [12]. Based on the models for the ligand-binding pocket, one could anticipate that the binding affinity is conditioned by electronic and hydrophobic interactions. Besides the traditional two sites interacting with the nucleophiles on A and D rings, a third receptor site situated at one of the binding pockets (11β) could interact with the ligand. These three sites are denoted as A, B and C and as anticipated overall A > B ≈ C, i.e. the A site provides the strongest interaction energy, whereas B and C sites have approximately same strength [10]. Four electronic interaction mechanisms could be anticipated based on these three sites: A–B, A–C, A–B–C (AD) and A only [21].

Results and Discussion

The process of the endocrine disruption is based on binding of endocrine disruptors to the ligand binding domain in the estrogen receptor.

Estrogen receptor (ER) binding is a molecular initiating event much like protein binding [19] that may led to a series of adverse outcomes, which are typically linked to reproductive and development hazards. It is an endpoint where several comprehensive databases exist, which has led to the development of several approaches for using (Q)SARs to predict ER-binding and possible subsequent endocrine disruption [3]. Popular among these are the "four phase" assessment that includes Comparative Molecular Field Analysis (CoMFA) [24] and the Common Reactivity Pattern Approach (COREPA) [18]. The present work showed that the QSAR approach in the OECD (Q)SAR Toolbox has been used for identifying interaction mechanisms for some endocrine disruptors in Table 1.

Since the ER-binding is a receptor mediated event, particular organic functional groups, size and shape are critical to binding potency. A schematic representation of an ER-binding pocket with its three sites of interaction (A, B, C) is shown in Fig. 1.

Table 1. Observed values and predicted ER binding for selected estrogenic compounds

№	CAS number	Name of compounds	Observed values RBA, %	Predicted ER binding
1	108-95-2	Phenol	0	Weak binder, OH
2	80-05-7	Bisphenol A	0.008	Very strong binder, OH
3	136-83-4	2-Nonylphenol	–	Strong binder, OH
4	88-04-0	3,5-dimethyl-4-chlorophenol	–	Weak binder, OH
5	120-32-1	2-Benzyl-4-chlorophenol	–	Strong binder, OH
6	103-90-2	Paracetamol	–	Weak binder, OH

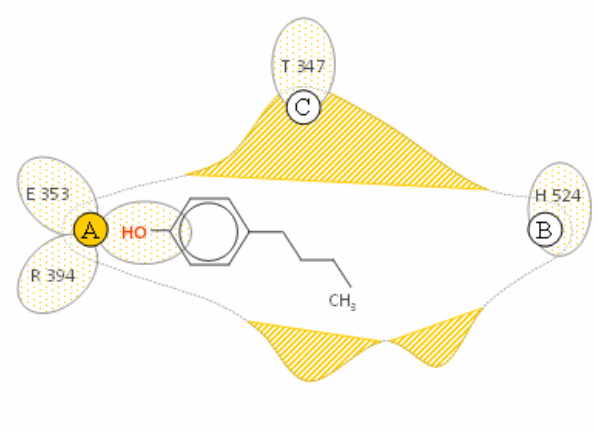


Fig. 1. *Mechanisms of ER-binding*

Chemicals with a single 5-or 6-member carbon ring structure with an unhindered hydroxyl-group (-OH) (a hydroxyl group in the para- or meta-position on the ring and without substituents ortho to the hydroxyl group) [20] are ER-binders. Binding potency is related to the size and shape of non-hydroxylated-ring aspect of the molecule, which can be grossly measured by molecular weight (MW).

Biotransformations are chemical reactions that are catalysed by microorganisms in terms of growing or resting cells or that are catalysed by isolated enzymes.

Biotransformation involves two stages: phase I, mainly involving enzymatic activity from the cytochrome P450 (CYP) family; and phase II, catalysed by conjugation enzymes like glutathione S-transferase (GST), UDP-glucuronosyltransferase, and N-acetyltransferase (NAT). Phase I enzymes promote the activation of drugs and pro-carcinogens for the genotoxic electrophilic intermediaries. Meanwhile, phase II enzymes generally act as inactivating enzymes, that is, they catalyse the binding of intermediary metabolites to cofactors, transforming them into more hydrophilic products, thus facilitating their elimination [17]. Therefore, the coordinated expression and regulation of xenobiotic metabolizing enzymes (XMEs) in both phase I and phase II and their metabolic equilibrium in the cells of target organs can be important factors in determining susceptibility to cancer as related to exposure to carcinogens [11].

In our previous experimental work, we measured the percentage removal of substrate and reaction rate of endocrine disruptors through oxidation by immobilized on a polypropylene membrane enzyme laccase from Trametes versicolor under standard conditions [8].

The listed values show that our immobilized laccase is able to oxidize a wide range of phenolic substrates. Taking as reference the phenol removal, i.e. the removal percentage of 40% after 30 min of enzyme treatment under standard conditions, it is possible to conclude that some endocrine disruptors (nonylphenol and chlorophene) are oxidized by laccase at a similar rate, other derivatives (paracetamol and chloroxyphenol) at a smaller rate, and others (bisphenol A) at higher rate.

Observed and predicted microbial metabolism of some selected estrogenic compounds were predicted on the OECD (Q)SAR Toolbox (Table 2).

Phenol and its metabolites are shown as weak binder to the estrogenic receptor. A part of its metabolites have non-cyclic structures, i.e. estrogenic activity is not observed.

Bisphenol A is predicted to have a very strong binder to the estrogenic receptor but its metabolites demonstrated different behaviour – metabolites with non-cyclic structures, weak binder, very strong binder and strong binder.

2-Nonylphenol has a strong binder to the estrogenic receptor but its metabolites have non-cyclic structures, weak binder, moderate binder and strong binder.

3,5-dimethyl-4-chlorophenol is predicted to be weak binder as phenol but metabolites have moderate binder, non-cyclic structure or structure without OH or NH_2 groups.

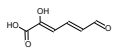
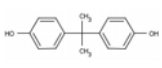
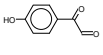
Metabolites of 2-benzyl-4-chlorophenol are predicted to be with non-cyclic structures, without OH or NH_2 groups, moderate binder and strong binder and the parent structure has a strong binder.

Paracetamol has a weak binder to the estrogenic receptor as phenol and 3,5-dimethyl-4-chlorophenol. Behaviour of its metabolites to the estrogenic receptor is similar to that of phenol.

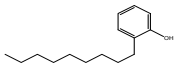
Conclusion

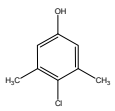
Metabolic perturbations are only one small aspect of the EDC-related problems to be solved, but what we know now may be only the tip of the iceberg. In the present context of endemic metabolic disorders, with severe economic, social, and professional consequences, every action first to understand and then to control risk factors is beneficial on all counts.

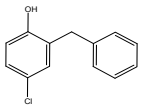
Table 2. *Observed and predicted microbial metabolism and estrogenic activity of metabolites for some selected estrogenic compounds*

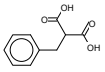
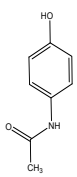
№	CAS number	Name of compounds	Observed microbial metabolism	Predicted microbial metabolism
1	108-95-2	Phenol 	9 metabolites: 1)  weak binder, OH 2)  non-cyclic structure 3)  non-cyclic structure 4)  non-cyclic structure 5)  non-cyclic structure 6)  non-cyclic structure 7)  non-cyclic structure 8)  non-cyclic structure 9)  non-cyclic structure	10 metabolites: 1)  weak binder, OH 2)  non-cyclic structure 3)  non-cyclic structure 4)  non-cyclic structure 5)  non-cyclic structure 6)  non-cyclic structure 7)  non-cyclic structure 8)  non-cyclic structure 9) non-cyclic structure 10)  non-cyclic structure
2	80-05-7	Bisphenol A 	17 metabolites: 1)  Strong binder, OH 2)  Very strong binder, OH	26 metabolites: 1)  Weak binder, OH 2)  Weak binder, OH 3)  Weak binder, OH

			3)  Very strong binder, OH 4)  Weak binder, OH 5)  Weak binder, OH 6)  Weak binder, OH 7)  Weak binder, OH 8)  non-cyclic structure 9) non-cyclic structure 10)  Weak binder, OH 11)  non-cyclic structure 12)  Weak binder, OH 13)  Weak binder, OH 14)  Very strong binder, OH 15)  Very strong binder, OH 16)  Very strong binder, OH	4)  Weak binder, OH 5)  Non-cyclic structure 6)  Weak binder, OH 7)  Weak binder, OH 8)  Non-cyclic structure 9)  Non-cyclic structure 10)  Non-cyclic structure 11)  Non-cyclic structure 12)  Non-cyclic structure 13)  Non-cyclic structure 14)  Non-cyclic structure 15) Non-cyclic structure 16) Non-cyclic structure 17)  Weak binder, OH 18)  Weak binder, OH 19)  Non-cyclic structure 20)  Non-cyclic structure

			17)  Weak binder, OH	21)  Non-cyclic structure 22)  Non-cyclic structure 23)  Non-cyclic structure 24)  Non-cyclic structure 25)  Non-cyclic structure 26)  Non-cyclic structure
3	136-83-4	2-Nonylphenol 	0	26 metabolites: 1)  Strong binder, OH 2)  Strong binder, OH 3)  Strong binder, OH 4)  Strong binder, OH 5)  Strong binder, OH 6)  Strong binder, OH 7)  Strong binder, OH 8)  Strong binder, OH 9)  Strong binder, OH 10)  Strong binder, OH 11)  Moderate binder, OH

				12)  Moderate binder, OH 13)  Strong binder, OH 14)  Strong binder, OH 15)  Weak binder, OH 16)  Moderate binder, OH 17)  Non-cyclic structure 18)  Non-cyclic structure 19)  Non-cyclic structure 20)  Non-cyclic structure 21)  Non-cyclic structure 22)  Non-cyclic structure 23)  Non-cyclic structure 24)  Non-cyclic structure 25)  Non-cyclic structure 26)  Non-cyclic structure
4	88-04-0	3,5-dimethyl-4-chlorophenol 	0	11 metabolites: 1)  Moderate binder, OH 2)  Non-cyclic structure 3)  Without OH or NH_2 group

				4)  Non-cyclic structure 5)  Non-cyclic structure 6)  Non-cyclic structure 7)  Non-cyclic structure 8)  Non-cyclic structure 9)  Non-cyclic structure 10)  Non-cyclic structure 11)  Non-cyclic structure
5	120-32-1	2-Benzyl-4-chloro-phenol 	0	22 metabolites: 1)  Strong binder, OH 2)  Without OH or NH_2 group 3)  Without OH or NH_2 group 4)  Without OH or NH_2 group 5)  Without OH or NH_2 group 6)  Without OH or NH_2 group 7)  Without OH or NH_2 group

				 8) Without OH or NH_2 group  9) Without OH or NH_2 group  10) Without OH or NH_2 group  11) Moderate binder, OH  12) Non-cyclic structure  13) Non-cyclic structure  14) Non-cyclic structure  15) Non-cyclic structure  16) Non-cyclic structure  17) Non-cyclic structure  18) Non-cyclic structure  19) Non-cyclic structure  20) Non-cyclic structure  21) Non-cyclic structure  22) Non-cyclic structure
6	103-90-2	Paracetamol 	0	8 metabolites:  1) Weak binder, NH2;weak binder, OH  2) weak binder, OH

				3)  weak binder, OH 4)  Non-cyclic structure 5)  Non-cyclic structure 6)  Non-cyclic structure 7)  Non-cyclic structure 8)  Non-cyclic structure

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