We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,600
Open access books available

177,000
International authors and editors

195M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Chapter

The Role of Metabolism in the Estrogenic Activity of Endocrine-Disrupting Chemicals

Darja Gramec Skledar and Lucija Peterlin Mašič

Abstract

Exposure to several natural and synthetic chemicals can disrupt the endocrine system and thus present a threat to human health. In vivo, such chemicals can be metabolized, which can change the endocrine activity of the parent chemical. Metabolism is usually considered to be a detoxification process, as it generally appears to reduce the estrogenic activity of a chemical and accelerate its elimination from the body. This is seen for bisphenol A (BPA), a known agonist of the estrogen receptor, whereby BPA glucuronide has no effects on this receptor. In contrast, numerous metabolites that show significantly greater estrogenic activities from their parent chemicals have been described in the literature. An example is the ipso metabolite of BPA, 4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, which shows >100-fold estrogenic activity compared to BPA. Consideration of metabolic pathways in in vitro models is therefore of great importance for reliable analysis and correct in vitro to in vivo correlations. The inclusion of metabolic aspects in these assays will reduce false-positive data for chemicals that are detoxified in vivo and false-negative data for proestrogens. Different approaches for this incorporation of metabolic systems for determination of estrogenic activities are already in use and are described in the present chapter.

Keywords: endocrine-disrupting chemicals, metabolic bioactivation, bisphenols, estrogenic activity, in vitro assays

1. Introduction

According to the World Health Organization definition from 2002, an endocrine-disrupting chemical (EDC) is an exogenous substance, or a mixture of substances, that alters the function(s) of the endocrine system and consequently that causes adverse health effects in the intact organism or its progeny or in (sub) populations [1]. The occurrence of EDCs in the environment is widespread, and exposure to EDCs is connected with many modern illnesses, such as cancers and metabolic syndrome [2, 3]. Effects of some EDCs on nuclear receptors have been reported even at very low doses, and thus EDCs have promoted increased concern among scientists and regulators [4–6]. As a result, over the last two decades, numerous in vitro and in vivo assays have been developed for the identification of EDCs [7, 8]. In vitro assays represent an important step, especially in the early stages of testing, as they can provide valuable information about the mechanisms of
endocrine disruption (e.g., binding to the estrogen receptor [ER]). The main drawback of commonly used in vitro systems for estrogenic activity is that they do not consider pharmacokinetic parameters and especially the metabolism of a tested chemical [9].

Metabolism is the enzymatic process by which lipophilic compounds are transformed into hydrophilic metabolites, which can then be rapidly excreted with the urine. The liver is the main site of drug metabolism, although metabolic reactions also occur in extrahepatic tissues, like the gut and the airways [10]. Phase I metabolic reactions mainly consist of oxidation, reduction, and hydrolysis. During phase I metabolism, new polar functional groups, like hydroxyl or carboxyl groups, are introduced into the parent molecules, which results in either excretion of the modified chemical with the urine or in further metabolism by phase II metabolic reactions. During phase II metabolism (i.e., conjugation reactions), chemicals are conjugated with endogenous hydrophilic molecules (e.g., glucuronic acid, sulfate, glycine, and acetyl group), which strongly increases their hydrophilicity and facilitates their excretion. Metabolism can also influence the biological activity of a chemical. In most cases, metabolism works as a detoxification system, as it can convert biologically active chemicals into less active or even inactive metabolites [11]. In contrast, some chemicals that have no initial biological activity can be metabolically activated to a biologically active chemical (i.e., prodrugs) [12]. The principle of prodrugs is often used in the pharmaceutical industry, with the aim being to improve the pharmacokinetics of drugs [13]. For example, enalapril is a medication that is used for the treatment of arterial hypertension, and in the body, it is metabolized by esterases to the pharmacologically active compound enalaprilat [14]. Additionally, some chemicals can be metabolized to reactive metabolites, as seen for the thiophene moiety [12], and can have detrimental effects.

Thus, metabolism can strongly influence the endocrine activities of many chemicals. Rybacka and coworkers developed an in silico model for the prediction of endocrine-disrupting potential of industrial chemicals and their metabolites [15]. More than 6000 industrial chemicals were evaluated using this model, and 9% of them were predicted to be positive for interactions with the ER. However, when metabolism was incorporated into this model, this doubled the number of chemicals that were defined as positive for interactions with the ER [15]. Metabolism can, for example, significantly affect antiestrogenic and antiandrogenic activities of the new
brominated flame retardants 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and bis(2-ethylhexyl) tetrabromophthalate (TBPH). While these parent chemicals show no activity toward ERs and androgen receptors, their metabolites 2,3,4,5-tetrabromobenzoic acid (TBBA) and 2-ethylhexyltetrabromobenzoate ester (TBMEPH) show antiestrogenic and antiandrogenic activities with IC$_{50}$ values in the low micromolar (Figure 1) [16].

However, as in vitro assays do not generally consider metabolic transformations of chemicals, it can be difficult to detect chemicals without estrogenic activities that are converted to metabolites with estrogenic activities (i.e., proestrogens). Moreover, in vitro estrogenic activities can be overestimated for compounds that are metabolized to inactive metabolites in vivo [17]. This can result in poor in vitro to in vivo predictions, as observed with benzyl-butyl phthalate, which has estrogenic activity in the T47D-KBluc in vitro assay, but not in the in vivo uterotrophic assay. This can be explained by the rapid hydrolysis of benzyl-butyl phthalate to its monoester in vivo, which has no estrogenic activity [9]. In contrast, methoxychlor metabolites have higher estrogenic activity than methoxychlor itself, which is why the in vivo estrogenic activity for methoxychlor is higher than that predicted from in vitro assays (Figure 2) [9].

![Figure 2. Metabolism of proestrogens to active metabolites with estrogenic activity. In all of these examples, hydroxylation at the para position of the aromatic ring is responsible for the estrogenic activity.](image_url)
2. Metabolism of 17β-estradiol

17β-Estradiol (E2) metabolism has been extensively studied, especially in connection with risk of premenopausal breast cancer [18, 19]. E2 can undergo aromatic hydroxylation that is catalyzed by cytochromes P450, for the C2 and C4 positions of the steroid rings, to form catechol structures, or for the C16 position of the steroid rings, to form 16-hydroxyestrone structures [20]. Estrogen metabolites with a catechol structure are further metabolized by catechol-O-methyltransferase to their methylated metabolites, as shown in Figure 3. The 16-hydroxyestrone structures are further metabolized to 17-epiestriols and estriols (Figure 3). As determined in the urine of premenopausal women, in vivo the 16-hydroxylation pathway of estradiol metabolism is the most important, followed by the 2-hydroxylation pathway, while the 4-hydroxylation pathway represents only minor metabolic conversion [20].

Different environmental factors and polymorphic variations in the genes that encode metabolic enzymes have also been reported to affect estradiol metabolism [24]. Smoking and high physical activity, for example, both favor the 2-hydroxylation metabolic pathway, with the 16-hydroxylation pathway decreased [25, 26]. Moreover, Gu and coworkers reported on connections between smoking and increased metabolism of estradiol, increased activity of the 4-hydroxylation metabolic pathway, and decreased methylation of estrogen metabolites [25].

The catechol estrogens 2-hydroxyestradiol and 4-hydroxyestradiol can bind to the ER of MCF-7 cells, and their relative binding activities compared to E2 were reported to be 23 and 26%, respectively [27]. These work as agonists of ER-dependent gene expression in the MCF-7 cell line [28].

The estrogenic activities of E2 and its metabolites were evaluated in luciferase reporter gene assays using T47D breast cancer cells (i.e., the ER-CALUX assay) [21]. The estrogenic activities of the E2 metabolites were compared with that of E2, which can be represented as the “E2 equivalency factor” (EEF) (Figure 3). E2 was the most potent compound, while its metabolites had reduced estrogenic

Figure 3. Endogenous metabolism of E2 together with the E2 equivalency factors (EEF) for the various metabolites, as determined with the ER-CALUX assay [20–23]. COMT, catechol-O-methyltransferase.
activities. Here, the 4-hydroxy metabolites, and especially 4-hydroxyestradiol, had higher estrogenic activities than the 2-hydroxy metabolites [21]. Both of these forms of hydroxylated estradiols (i.e., 4-hydroxyestradiol, 2-hydroxyestradiol) increased uterine weight of neonatal rats, which confirmed their estrogenic activities \textit{in vivo} [29]. 4-Hydroxyestradiol had greater \textit{in vivo} estrogenic activity than 2-hydroxyestradiol (203, 107% increases in uterine weight, respectively) [29].

3. Impact of metabolism on estrogenic activity

As mentioned above, metabolites with altered estrogenic activity can be formed during metabolism. In some cases, estrogenic activities can be predicted from the structures of the metabolites. Some structural characteristics of the molecule are known to be important for binding to the ER, such as the aromatic ring with an OH group at the \textit{para} position [30]. Hydroxylation of the \textit{para} position of the aromatic ring, which is a common phase I metabolic reaction, often enhances estrogenicity. In contrast, glucuronides are devoid of estrogenic activity.

3.1 Metabolic activation of proestrogens

A number of chemicals that have been reported to be without estrogenic activities \textit{in vitro} can affect the estrogen system \textit{in vivo}. Methoxychlor is a broad-spectrum pesticide, and as such it is one of the most studied proestrogens. While methoxychlor has no estrogenic activity, its mono-demethylated and bis-demethylated metabolites have significant estrogenic activities [31]. Mollergues et al. determined the estrogenic activity of methoxychlor using the ER-CALUX assay with and without the β-naphthoflavone-/phenobarbital-induced S9 fraction [32]. In this assay, methoxychlor showed weak estrogenic activity (EC\textsubscript{50} 4.6 μM). This estrogenic activity was significantly increased when the activated S9 fraction was added to the assay (EC\textsubscript{50} 0.15 μM), which suggested the formation of active metabolites of methoxychlor. The pure methoxychlor metabolite 4,4\textsubscript{0}-(2,2,2-trichloroethane-1,1-diyl) diphenol was also tested, and its estrogenic activity was comparable with that of methoxychlor treated with the S9 fraction (EC\textsubscript{50} 0.05 μM), which suggested that 4,4\textsubscript{0}-(2,2,2-trichloroethane-1,1-diyl) diphenol is the main metabolite formed with the S9 fraction [32]. As the methoxychlor metabolites mono-OH methoxychlor and bis-OH-methoxychlor are formed both \textit{in vitro} and \textit{in vivo}, addition of the metabolic enzymes here (i.e., the S9 fraction) improved the \textit{in vivo} prediction [33]. Additionally, Sumida and coworkers added a source of metabolic enzymes (i.e., human and rat S9 and liver microsomes) to MCF-7 and Hela reporter gene assays to determine the influence of metabolism on the estrogenic activity of trans-stilbene and methoxychlor [33]. While these parent compounds were without estrogenic activities, the addition of the metabolic enzymes resulted in significant increases in estrogenicity [33].

Chalcone is an α,β-unsaturated ketone, and it is a structural fragment in many natural chemicals. Chalcone itself is without estrogenic activity. However, estrogenic activities were shown for its hydroxylated metabolites using a luciferase reporter assay with the MCF-7 cell line (Figure 2) [34]. Similarly, CYP-mediated hydroxylation on the aromatic ring of 4-phenyl-3-buten-2-ol results in estrogenic activity of its 4-hydroxyl metabolite (Figure 2) [34]. Benzophenone-3 is a widely used UV filter in sunscreens. While the estrogenic activity of benzophenone-3 determined with a reporter assay using CHO cells was weak, its main metabolite 2,4-dihydroxybenzophenone showed >20-fold ER\textsubscript{α} agonist activity (EC\textsubscript{50} 2.2, 0.099 μM, respectively) and 100-fold ER\textsubscript{β} agonist activity (EC\textsubscript{50} 3.3, 0.033 μM,
respectively) (Figure 2) [35]. This enhanced estrogenicity is, however, not surprising, as a 4-hydroxyl group on the phenol ring is essential for strong estrogenic activity.

Permethrin and bifenthrin are pyrethroid pesticides [36]. In in vitro reporter gene assays using the BG-1 cell line (i.e., CALUX bioassays), neither of them showed ER agonist activity, while bifenthrin acted as an ER antagonist. However, both of these pesticides induced expression of chloriogenin (i.e., an E2-responsive protein) in in vivo assays in the juvenile fish *Menidia beryllina*, which indicated estrogenic activities for both of the parent pesticides or their metabolites in vivo [36]. These differences between the in vitro and in vivo data for permethrin might be due to the formation of metabolites with higher estrogenic activities [37, 38]. Similarly, a higher estrogenic activity of the parent bifenthrin that was shown in an in vivo study was also seen for the bifenthrin metabolites [39].

3.2 Metabolic inactivation

Inconsistencies in the estrogenic activities obtained in vitro and in vivo were also seen for different phthalates [40]. Here, the tested phthalates (i.e., *n*-butyl benzyl phthalate, dicyclohexyl phthalate, 2-ethylhexyl phthalate, di-*n*-butyl phthalate) showed estrogenic activities in an in vitro MCF-7 proliferation assay (i.e., the E-screen assay), but they did not induce expression of calbindin-D<sub>9K</sub> mRNA in neonatal rat uterus [40]. The explanation here might lie in the rapid in vivo metabolism of phthalates to monoester metabolites, which have no estrogenic activity [41]. *n*-Butyl benzyl phthalate is rapidly metabolized to mono-*n*-butyl phthalate, mono-benzyl phthalate, hippuric acid, phthalic acid, and benzoic acid [42]. In two in vitro assays (i.e., E-screen assay, progesterone receptor assay on MCF-7 cell line), *n*-butyl benzyl phthalate showed estrogenic activities, while its metabolites were without activity (Figure 4) [42].

Loss of estrogenic activity has also been described for various glucuronides. For example, the soy isoflavones genistein and daidzein are known as estrogens [43]. In contrast, their glucuronides are not estrogenic [44]. Despite this, glucuronides have shown very low estrogenic activities in in vitro reporter gene assays using U2OS cells (~0.002–0.0005-fold that of their aglycones), which can be explained by their intracellular deconjugation to their corresponding aglycones [44]. Beekmann and
coworkers used a cell-free microarray assay for real-time co-regulator-nuclear receptor interactions, which enabled the detection of estrogenicity as an agonistic response to the ER ligand-binding domain (LBD) [45]. They reported 0.125-fold to 0.00022-fold potencies for the glucuronides in comparison with their corresponding aglycones for the modulation of ERα-LBD and ERβ-LBD-co-regulator interactions [45].

Similarly, in vitro estrogenic activities were shown for \( p \)-nonylphenol and \( p \)-octylphenol with yeast cells, with \( EC_{50} \) values in the high nanomolar range (\( EC_{50} \) 110, 700 nM, respectively) [46]. Their glucuronides were without agonistic and antagonistic estrogenic and androgenic activities [46]. Due to the rapid glucuronidation of \( p \)-nonylphenol and \( p \)-octylphenol in vivo, these in vitro estrogenicities probably do not reflect any hazards in vivo [46].

4. Bisphenols

The bisphenols are chemicals that contain two phenol rings that are connected through a bridging atom, which can be a carbon or a sulfur, as for BPA and bisphenol S (BPS; bis(4-hydroxyphenyl)sulfone), respectively [47]. BPA is the best known bisphenol as it is used as a monomer in the production of polycarbonate plastic and epoxy resins, and it is also known to be an endocrine disruptor [16, 48–54]. Both the metabolism and endocrine effects of BPA have been studied in detail, although there remains a lack of information on some of its analogs.

For the bisphenols, their metabolism has a protecting role. Their conjugation with glucuronic acid and sulfate is the main in vivo transformation for all of the bisphenols. In contrast to BPA as an agonist for the ERs, BPA glucuronide is without estrogenic activity [55]. Similar situations have been defined for the other bisphenol glucuronides, such as BPS glucuronide [56] and BPAF glucuronide [11]. A lack of estrogenic activity has also been shown for BPA sulfate [57]. In contrast to these conjugates, which are the main metabolites of the bisphenols, numerous oxidative metabolites of BPA have been detected. However, these were mainly reported for in vitro studies, whereas in vivo only hydroxylated BPA has been detected in feces in mice [58] and rats [59]. The estrogenic activity of the main BPA oxidative metabolite is lower than that of BPA [60]. Among the oxidative metabolites, two have shown significantly greater estrogenic activities than BPA: its ipso metabolite 4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene (MBP) and hydroxycumyl alcohol. The MBP estrogenic activity has been determined in several in vitro and in vivo assays, in which it has shown up to 500-fold estrogenic activity over BPA (Figure 5, Table 1) [16, 62–65]. Additionally, hydroxycumyl alcohol has shown ~100-fold estrogenic activity over BPA [66]. However, there are questions about the in vivo relevance of these data. Indeed, although MBP has very high estrogenic activity, it has not been detected in vivo, as BPA glucuronidation is a much faster metabolic reaction in vivo.

Figure 5.
BPA metabolic activation through formation of the potent estrogenic metabolite MBP [61].

DOI: http://dx.doi.org/10.5772/intechopen.81918
Hashimoto and coworkers evaluated the estrogenic activity of 13 BPA-related compounds using a yeast two-hybrid system without and with the addition of an S9 fraction [67]. Most of these chemicals showed enhanced estrogenic activities when the S9 fraction was added. This might be explained by the formation of oxidative metabolites with enhanced estrogenic activities [61]. A similar situation was reported for bisphenol B (BPB; 2,2-bis(4-hydroxyphenyl)butane), which is a more potent ER agonist than BPA [60]. Metabolism can also affect this BPB estrogenic activity. Here, after incubation of BPB with an S9 fraction, there was enhanced estrogenicity, which was probably due to the formation of BPB dimers [62, 68]. Nevertheless, in vivo, glucuronidation is the predominant metabolic pathway for the bisphenols, while their oxidative metabolites are in most cases detected only in vitro.

5. Testing strategies

In vitro systems for evaluation of estrogenic activity are usually limited to target chemicals, without taking into account that many chemicals are extensively metabolized, which can have significant effects on their estrogenic activities. For some of the cell lines used for endocrine testing, metabolic activities have been confirmed. However, for reliable data, it is important that the metabolic capacity of any in vitro system is in good agreement with the in vivo conditions, although that can be difficult to ascertain. Bursztyka and coworkers [69] evaluated the metabolic capacities toward the known endocrine disruptors genistein and BPA for various cell lines that are commonly used in endocrine testing (i.e., the HepG2, MCF7, and HC11 cell lines) [69]. Phase I metabolic activities were not seen for these tested cell lines. The HepG2 and MCF7 cell lines showed glucuronidation and sulfation activities toward genistein and BPA, while the HC11 cell line was without metabolic activity. However, phase II metabolism in these cell lines was not representative of in vivo conditions. So, while sulfate conjugates of genistein and BPA were the main metabolites detected in these cell lines, it is known that genistein and BPA are mainly metabolized to glucuronide conjugates in vivo [69].

Testing strategies that include metabolic aspects have not been well defined. While different approaches can be used, they generally have many drawbacks. Jacobsen et al. [70] used two approaches: a compound-by-compound approach and an effects-based approach (Figure 6) [70].

### Table 1.

| Assay system                      | EC50 (μM) | Relative potency | Reference |
|----------------------------------|-----------|-----------------|-----------|
| Yeast reporter assay             | 0.014     | 3.6             | [16]      |
| MCF7-luc                         | 0.0011    | 0.52            | [63]      |
| ERE-luc in NIH3T3 cells          | 0.00068   | 1.0             | [63]      |
| Yeast two-hybrid assay           | 0.0083    | 14              | [63]      |
| Yeast estrogen screen assay      | 0.71      | 160             | [63]      |
| Uterotrophic assay               | NR        | NR              | [65]      |
| Vitellogenin induction           | NR        | NR              | [64]      |

Estrogenic agonistic activity of MBP compared to BPA, according to various in vitro and in vivo assays [61].
In the compound-by-compound approach, the relevant metabolites are identified, isolated or synthesized, and then evaluated for their endocrine activities, together with the parent chemical. Moreover, mixtures of a parent chemical and its metabolites can be tested, to determine the effects of such mixtures (e.g., synergism, additive, and antagonistic). This approach is commonly used [16, 56, 63, 71]. A drawback to this approach is that there is a need to conduct metabolic studies, as in many cases the metabolites are not known. Additionally, in vitro studies of metabolism can result in a “pallet” of metabolites, where not all will be relevant in vivo [61].

In the effects approach, the endocrine activity of the parent chemical is compared to the endocrine activity of the parent chemical treated with a metabolizing system, which is usually an S9 fraction [70]. Incorporation of such metabolic systems into in vitro assays is a common practice when testing for genotoxicity, like for the Ames test, where the S9 fraction is added as a source of metabolic enzymes, although such metabolic systems are still rarely used for the evaluation of endocrine activities of different chemicals [72].

In a recent study, a rat S9 fraction was incorporated in a CALUX reporter gene assay (i.e., the U2-OS cell line), and 27 chemicals were evaluated [73]. Selective inclusion of cofactors enables the evaluation of the different phases of metabolism on the estrogenic activities (i.e., phase I, nicotinamide adenine dinucleotide phosphate [reduced]; phases I and II, nicotinamide adenine dinucleotide phosphate [reduced], UDP-glucuronic acid, phosphoadenosylphosphosulfate, glutathione). These data showed that the endocrine activities for 23 of the 27 chemicals tested
were affected by metabolism. Phase II metabolism resulted in decreased estrogenic activities, which is expected and is in agreement with \textit{in vivo} data. For example, BPA and genistein showed lower estrogenic activities after phase II metabolism. In contrast, after phase I metabolism, 5 of the chemicals showed lower estrogenic activities, and 11 of the chemicals showed higher estrogenic activities [73].

However, also the effects approach has many weaknesses, as, for example, the metabolites formed \textit{in vitro} are not necessarily formed \textit{in vivo}. These data should therefore be interpreted with caution. The optimal path might therefore be the inclusion of both strategies (i.e., chemical by chemical, effects approach) [70]. Moreover, Jacobsen and coworkers proposed a five-step scheme for the evaluation of the endocrine effects of chemicals and their metabolites [70]:

1. The endocrine activity of the parent chemical should be determined. Then, based on all of the available information, an \textit{in vitro} metabolizing system for the production of the metabolites should be selected (e.g., S9 fractions, microsomes, as the most common systems).

2. The compatibility of the metabolizing system and the test system for determination of the endocrine activities should then be evaluated.

3. The mixture of metabolites formed with the metabolizing system should then be evaluated for endocrine activity.

4. The influence of the metabolite mixture on the endocrine activity of the parent chemical should then be evaluated.

5. The endocrine activities of the pure metabolites and their mixtures should then be evaluated.

\section*{6. Conclusions}

Metabolism is considered as a detoxification system that lowers biological activities and facilitates excretion of foreign chemicals. This appears to be true for many estrogenic chemicals, including E2, which is metabolized to numerous metabolites that have significantly lower estrogenic activities. However, some chemicals that are without estrogenic activity can be metabolized to potent estrogens. Some metabolic pathways in particular, such as hydroxylation on the para position of the aromatic ring, are dangerous in terms of the metabolic activation of proestrogens. Metabolic aspects should therefore be considered when testing chemicals for estrogenic activities.

\section*{Acknowledgements}

This study was supported by the Slovenian Research Agency (Grant Nos. Programme P1-0208 and Programme P1-0245).

\section*{Conflict of interest}

The authors declare that they have no conflicts of interest.
Author details

Darja Gramec Skledar and Lucija Peterlin Mašič*
Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

*Address all correspondence to: lucija.peterlin@ffla.uni-lj.si

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G. Global Assessment of the State-of-the-Science of Endocrine Disruptors. Geneva: World Health Organisation; 2002. pp. 1-180

[2] Rochester JR. Bisphenol A and human health: A review of the literature. Reproductive Toxicology. 2013;42:132-155

[3] Kabir ER, Rahman MS, Rahman I. A review on endocrine disruptors and their possible impacts on human health. Environmental Toxicology and Pharmacology. 2015;40(1):241-258

[4] Acconcia F, Pallottini V, Marino M. Molecular mechanisms of action of BPA. Dose-Response. 2015;13(4):1559325815610582

[5] Usman A, Ahmad M. From BPA to its analogues: Is it a safe journey? Chemosphere. 2016;158:131-142

[6] Welshons WV, Nagel SC, vom Saal FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology. 2006;147(6):56-69

[7] Svobodová K, Čajthaml T. New in vitro reporter gene bioassays for screening of hormonal active compounds in the environment. Applied Microbiology and Biotechnology. 2010;88(4):839-847

[8] Gelbke HP, Kayser M, Poole A. OECD test strategies and methods for endocrine disruptors. Toxicology. 2004;205(1–2):17-25

[9] Conley JM, Hannas BR, Furr JR, Wilson VS, Gray LE. A demonstration of the uncertainty in predicting the estrogenic activity of individual chemicals and mixtures from an in vitro estrogen receptor transcriptional activation assay (T47D-KBluc) to the in vivo uterotrophic assay using oral exposure. Toxicollogical Sciences. 2016;153(2):382-395

[10] Gramec Skledar D, Troberg J, Lavdas J, Peterlin Mašič L, Finel M. Differences in the glucuronidation of bisphenols F and S between two homologous human UGT enzymes, 1A9 and 1A10. Xenobiotica. 2015;45(6):511-519

[11] Li M, Yang YJ, Yang Y, Yin J, Zhang J, Feng YX, et al. Biotransformation of Bisphenol AF to its major glucuronide metabolite reduces estrogenic activity. PLoS One. 2013;8(12)

[12] Gramec D, Peterlin Mašič L, Sollner Dolenc M. Bioactivation potential of thiophene-containing drugs. Chemical Research in Toxicology. 2014;27(8):1344-1358

[13] Zawilska JB, Wojcieszak J, Olejniczak AB. Prodrugs: A challenge for the drug development. Pharmacological Reports. 2013;65(1):1-14

[14] MacFadyen RJ, Meredith PA, Elliott HL. Enalapril clinical pharmacokinetics and pharmacokinetic-pharmacodynamic relationships. An overview. Clinical Pharmacokinetics. 1993;25(4):274-282

[15] Rybacka A, Rudén C, Tetko IV, Andersson PL. Identifying potential endocrine disruptors among industrial chemicals and their metabolites—Development and evaluation of in silico tools. Chemosphere. 2015;139:372-378

[16] Fic A, Zegura B, Gramec D, Mašič LP. Estrogenic and androgenic activities of TBBA and TBMEPH, metabolites of novel brominated flame retardants, and selected bisphenols, using the XenoScreen XL YES/YAS assay. Chemosphere. 2014;112:362-369
[17] Soto AM, Maffini MV, Schaeberle CM, Sonnenschein C. Strengths and weaknesses of in vitro assays for estrogenic and androgenic activity. Best Practice & Research. Clinical Endocrinology & Metabolism. 2006;20(1):15-33

[18] Brinton LA, Trabert B, Anderson GL, Falk RT, Felix AS, Fuhrman BJ, et al. Serum estrogens and estrogen metabolites and endometrial cancer risk among postmenopausal women. Cancer Epidemiology, Biomarkers & Prevention. 2016;25(7):1081-1089

[19] Ziegler RG, Fuhrman BJ, Moore SC, Matthews CE. Epidemiologic studies of estrogen metabolism and breast cancer. Steroids. 2015;99:67-75

[20] Eliassen AH, Ziegler RG, Rosner B, Veenstra TD, Roman JM, Xu X, et al. Reproducibility of fifteen urinary estrogens and estrogen metabolites over a 2- to 3-year period in premenopausal women. Cancer Epidemiology, Biomarkers & Prevention. 2009;18(11):2860-2868

[21] Hoogenboom LAP, de Haan L, Hooijerink D, Bor G, Murk AJ, Brouwer A. Estrogenic activity of estradiol and its metabolites in the ER-CALUX assay with human T47D breast cells. APMIS. 2001;109(2):101-107

[22] Ziegler RG, Rossi SC, Fears TR, Bradlow HL, Adlercreutz H, Sepkovic D, et al. Quantifying estrogen metabolism: An evaluation of the reproducibility and validity of enzyme immunoassays for 2-hydroxyestradiol and 16alpha-hydroxyestrone in urine. Environmental Health Perspectives. 1997;105(3):607-614

[23] Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Letters. 2005;227(2):115-124

[24] Samavat H, Kurzer MS. Estrogen metabolism and breast cancer. Cancer Letters. 2015;356:231-243

[25] Gu F, Caporaso NE, Schairer C, Fortner RT, Xu X, Hankinson SE, et al. Urinary concentrations of estrogens and estrogen metabolites and smoking in caucasian women. Cancer Epidemiology, Biomarkers & Prevention. 2013;22(1):58-68

[26] Matthews CE, Fortner RT, Xu X, Hankinson SE, Eliassen AH, Ziegler RG. Association between physical activity and urinary estrogens and estrogen metabolites in premenopausal women. The Journal of Clinical Endocrinology and Metabolism. 2012;97(10):3724-3733

[27] Schütze N, Vollmer G, Wünsche W, Grote A, Feit B, Knuppen R. Binding of 2-hydroxyestradiol and 4-hydroxyestradiol to the estrogen receptor of MCF-7 cells in cytosolic extracts and in nuclei of intact cells. Experimental and Clinical Endocrinology. 1994;102(5):399-408

[28] Schütze N, Vollmer G, Knuppen R. Catecholestrogens are agonists of estrogen receptor dependent gene expression in MCF-7 cells. The Journal of Steroid Biochemistry and Molecular Biology. 1994;48(5-6):453-461

[29] Newbold RR, Liehr JG. Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. Cancer Research. 2000;60(2):235-237

[30] Devillers J, Marchand-Geneste N, Carpy A, Porcher JM. SAR and QSAR modeling of endocrine disruptors. SAR and QSAR in Environmental Research. 2006;17(4):393-412

[31] Kupfer D, Bulger WH. Metabolic activation of pesticides with proestrogenic activity. Federation Proceedings. 1987;46(5):1864-1869
Estrogen

[32] Mollergues J, van Vught-Lussenburg B, Kirchnawy C, Bandi RA, van der Lee RB, Marin-Kuan M, et al. Incorporation of a metabolizing system in biodetection assays for endocrine active substances. ALTEX. 2016;34(3):389-398

[33] Sumida K, Ooe N, Nagahori H, Saito K, Isobe N, Kaneko H, et al. An in vitro reporter gene assay method incorporating metabolic activation with human and rat S9 or liver microsomes. Biochemical and Biophysical Research Communications. 2001;280(1):85-91

[34] Kohno Y, Kitamura S, Sanoh S, Sugihara K, Fujimoto N, Ohta S. Metabolism of the alpha,beta-unsaturated ketones, chalcone and trans-4-phenyl-3-buten-2-one, by rat liver microsomes and estrogenic activity of the metabolites. Drug Metabolism and Disposition. 2005;33(8):1115-1123

[35] Watanabe Y, Kojima H, Takeuchi S, Uramaru N, Sanoh S, Sugihara K, et al. Metabolism of UV-filter benzophenone-3 by rat and human liver microsomes and its effect on endocrine-disrupting activity. Toxicology and Applied Pharmacology. 2015;282(2):119-128

[36] Brander SM, He G, Smalling KL, Denison MS, Cherr GN. The in vivo estrogenic and in vitro anti-estrogenic activity of permethrin and bifenthrin. Environmental Toxicology and Chemistry. 2012;31(12):2848-2855

[37] McCarthy AR, Thomson BM, Shaw IC, Abell AD. Estrogenicity of pyrethroid insecticide metabolites. Journal of Environmental Monitoring. 2006;8(1):197-202

[38] Nillos MG, Chajkowski S, Rimoldi JM, Gan J, Lavado R, Schlenk D. Stereoselective biotransformation of permethrin to estrogenic metabolites in fish. Chemical Research in Toxicology. 2010;23(10):1568-1575

[39] DeGroot BC, Brander SM. The role of P450 metabolism in the estrogenic activity of bifenthrin in fish. Aquatic Toxicology. 2014;156:17-20

[40] Hong EJ, Ji YK, Choi KC, Manabe N, Jeung EB. Conflict of estrogenic activity by various phthalates between in vitro and in vivo models related to the expression of Calbindin-D9k. The Journal of Reproduction and Development. 2005;51(2):253-263

[41] Okubo T, Suzuki T, Yokoyama Y, Kano K, Kano I. Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. Biological & Pharmaceutical Bulletin. 2003;26(8):1219-1224

[42] Picard K, Lhuguenot JC, Lavier-Canivenc MC, Chagnon MC. Estrogenic activity and metabolism of n-butyl benzyl phthalate in vitro: Identification of the active molecule(s). Toxicology and Applied Pharmacology. 2001;172(2):108-118

[43] Vitale DC, Piazza C, Melilli B, Drago F, Salomone S. Isoflavones: Estrogenic activity, biological effect and bioavailability. European Journal of Drug Metabolism and Pharmacokinetics. 2013;38(1):15-25

[44] Islam MA, Bekele R, Van den Berg JH, Kuswanti Y, Thapa O, Soltani S, et al. Deconjugation of soy isoflavone glucuronides needed for estrogenic activity. Toxicology In Vitro. 2015;29(4):706-715

[45] Beekmann K, de Haan LH, Actis-Goretta L, Houtman R, van Bladeren PJ, Rietjens IM. The effect of glucuronidation on isoflavone induced estrogen receptor (ER)α and ERβ mediated coregulator interactions. The Journal of Steroid Biochemistry and Molecular Biology. 2015;154:245-253
Moffat GJ, Burns A, Van Miller J, Joiner R, Ashby J. Glucuronidation of nonylphenol and octylphenol eliminates their ability to activate transcription via the estrogen receptor. Regulatory Toxicology and Pharmacology. 2001; 34(2):182-187

Schmidt J, Masic LP. Organic synthetic environmental endocrine disruptors: Structural classes and metabolic fate. Acta Chimica Slovenica. 2012;59(4):722-738

Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D. Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology. 1993;132(6):2279-2286

Perez P, Pulgar R, Olea-Serrano F, Villalobos M, Rivas A, Metzler M, et al. The estrogenicity of bisphenol A-related diphenylalkanes with various substituents at the central carbon and the hydroxy groups. Environmental Health Perspectives. 1998;106(3):167-174

Yamasaki K, Sawaki M, Takatsuki M. Immature rat uterotrophic assay of bisphenol A. Environmental Health Perspectives. 2000;108(12):1147-1150

Markey CM, Michaelson CL, Veson EC, Sonnenschein C, Soto AM. The mouse uterotrophic assay: A reevaluation of its validity in assessing the estrogenicity of bisphenol A. Environmental Health Perspectives. 2001;109(1):55-60

Chen MY, Ike M, Fujita M. Acute toxicity, mutagenicity, and estrogenicity of bisphenol A and other bisphenols. Environmental Toxicology. 2002;17(1):80-86

Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, et al. In vivo effects of bisphenol A in laboratory rodent studies. Reproductive Toxicology. 2007; 24(2):199-224

Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, et al. In vitro molecular mechanisms of bisphenol A action. Reproductive Toxicology. 2007;24(2):178-198

Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SC, Fennell TR. Metabolism and disposition of bisphenol A in female rats. Toxicology and Applied Pharmacology. 2000;168(3):225-234

Skledar DG, Schmidt J, Fic A, Klopvic I, Trontelj I, Dolenc MS, et al. Influence of metabolism on endocrine activities of bisphenol S. Chemosphere. 2016;157:152-159

Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y, Ozawa S. Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. Toxicology In Vitro. 2002;16(5):549-556

Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, et al. Biotransformations of bisphenol A in a mammalian model: Answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environmental Health Perspectives. 2003;111(3):309-319

Knaak JB, Sullivan LJ. Metabolism of bisphenol A in the rat. Toxicology and Applied Pharmacology. 1966;8(2):175-184

Kitamura S, Suzuki T, Sanoh S, Kohta R, Jinno N, Sugihara K, et al. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. Toxicological Sciences. 2005;84(2):249-259
Estrogen

[61] Gramec Skledar D, Peterlin Mašič L. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environmental Toxicology and Pharmacology. 2016;47:182-199

[62] Yoshihara S, Makishima M, Suzuki N, Ohta S. Metabolic activation of bisphenol A by rat liver S9 fraction. Toxicological Sciences. 2001;62(2):221-227

[63] Yoshihara S, Mizutare T, Makishima M, Suzuki N, Fujimoto N, Igarashi K, et al. Potent estrogenic metabolites of bisphenol A and bisphenol B formed by rat liver S9 fraction: Their structures and estrogenic potency. Toxicological Sciences. 2004;78(1):50-59

[64] Ishibashi H, Watanabe N, Matsumura N, Hirano M, Nagao Y, Shiratsuchi H, et al. Toxicity to early life stages and an estrogenic effect of a bisphenol A metabolite, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene on the medaka (Oryzias latipes). Life Sciences. 2005;77(21):2643-2655

[65] Okuda K, Takiguchi M, Yoshihara S. In vivo estrogenic potential of 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, an active metabolite of bisphenol A, in uterus of ovariectomized rat. Toxicology Letters. 2010;197(1):7-11

[66] Nakamura S, Tezuka Y, Ushiyama A, Kawashima C, Kitagawara Y, Takahashi K, et al. Ips0 substitution of bisphenol A catalyzed by microsomal cytochrome P450 and enhancement of estrogenic activity. Toxicology Letters. 2011;203(1):92-95

[67] Hashimoto Y, Moriguchi Y, Oshima H, Kawaguchi M, Miyazaki K, Nakamura M. Measurement of estrogenic activity of chemicals for the development of new dental polymers. Toxicology In Vitro. 2001;15(4–5):421-425

[68] Okuda K, Fukuuchi T, Takiguchi M, Yoshihara S. Novel pathway of metabolic activation of bisphenol A-related compounds for estrogenic activity. Drug Metabolism and Disposition. 2011;39(9):1696-1703

[69] Bursztyka J, Perdu E, Pettersson K, Pongratz I, Fernandez-Cabrera M, Olea N, et al. Biotransformation of genistein and bisphenol A in cell lines used for screening endocrine disruptors. Toxicology In Vitro. 2008;22(6):1595-1604

[70] Jacobsen NW, Brooks BW, Halling-Sørensen B. Suggesting a testing strategy for possible endocrine effects of drug metabolites. Regulatory Toxicology and Pharmacology. 2012;62(3):441-448

[71] Kang JS, Choi JS, Kim WK, Lee YJ, Park JW. Estrogenic potency of bisphenol S, polyethersulfone and their metabolites generated by the rat liver S9 fractions on a MVLN cell using a luciferase reporter gene assay. Reproductive Biology and Endocrinology. 2014;12:102

[72] Jacobs MN, Janssens W, Bernauer U, Brandon E, Coecke S, Combes R, et al. The use of metabolising systems for in vitro testing of endocrine disruptors. Current Drug Metabolism. 2008;9(8):796-826

[73] van Vugt-Lussenburg BMA, van der Lee RB, Man HY, Middelhof I, Brouwer A, Besselink H, et al. Incorporation of metabolic enzymes to improve predictivity of reporter gene assay results for estrogenic and anti-androgenic activity. Reproductive Toxicology. 2018;75:40-48