Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: A review of the data and identification of knowledge gaps

Donald R. Mattison\textsuperscript{1,2}, Nataliya Karyakina\textsuperscript{1}, Michael Goodman\textsuperscript{2}, and Judy S. LaKind\textsuperscript{4,5,6}

\textsuperscript{1}\textit{Risk Sciences International}, Ottawa, ON, Canada, \textsuperscript{2}\textit{McLaughlin Centre for Population Health Risk Assessment}, University of Ottawa, Ottawa, ON, Canada, \textsuperscript{3}\textit{Department of Epidemiology, Emory University School of Public Health, Atlanta, Georgia, USA}, \textsuperscript{4}\textit{LaKind Associates, LLC, Catonsville, Maryland, USA}, \textsuperscript{5}\textit{Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, USA}, and \textsuperscript{6}\textit{Department of Pediatrics, Milton S. Hershey Medical Center, Penn State College of Medicine, Hershey, Pennsylvania, USA}

Abstract

Chemicals with estrogenic activity are derived from many different natural and synthetic processes and products, including endogenous production (e.g., estradiol, conjugated estrogens), drugs (e.g., ethinyl estradiol, conjugated estrogens), plants used as foods (phytoestrogens such as genistein, daidzein, S-equol), and man-made chemicals (xenoestrogens such as bisphenol A). Human exposure to low doses of endogenous estrogens, estrogenic drugs, phytoestrogens, and xenoestrogens has the potential to improve health or disrupt normal endocrine activity, as well as impact the diverse systems with which estrogens interact, including the cardiovascular system, and lipid and carbohydrate metabolism. Mechanisms of action and diversity of adverse and non-adverse effects following human exposure to low doses of estrogen active chemicals (EACs, defined as chemicals which interact with an estrogen receptor [ER]) are poorly understood. This review summarizes our current understanding of the pharmacological action with a focus on pharmacokinetics (PK) and toxicokinetics (TK) of several representative EACs in both physiological and pathological processes. The goal of this review is to assess the current state-of-the-science on: (i) the potential for EACs to interfere with endocrine activity, (ii) factors which contribute to endocrine-related clinical outcomes, and (iii) existing knowledge gaps. While classical PK approaches (compartmental or non-compartmental) can be used to characterize absorption, distribution, metabolism, and elimination of EACs, many of the detailed pharmacological characteristics necessary to understand benefit-risk balance have not yet been clarified. Pharmacological complexities mirror the complexity of determining whether and under what conditions exposure to estrogens in drugs, foods or to xenoestrogenic chemicals are beneficial or harmful to human health.

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Keywords

bisphenol A, conjugated estrogens, daidzein, daidzin, estradiol, ethinyl estradiol, equol, genistein, genistin, pharmacokinetics, pharmacodynamics, physiologically based pharmacokinetics

History

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Phytoestrogens: Genistein, daidzein

Absorption

Distribution

Metabolism

Elimination

Mechanism of action

Phytoestrogens: S-equol

Absorption

Distribution

Metabolism

Excretion

Mechanism of action

Summary

Knowledge gaps

PBPK model for genistein

Genistein PBPK knowledge gaps

Xenoestrogens: Bisphenol A

Absorption

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PBPK model for BPA

PBPK knowledge gaps

Summary

Conclusions

Declaration of interest

References

Introduction

Chemicals with estrogenic activity (estrogen active chemicals, EACs) are derived from thousands of natural and synthetic processes and sources including endogenous production, pharmaceuticals, phytoestrogens consumed as food and anthropogenic chemicals found in the environment, in consumer products and in foods (Shanle and Xu 2011). Thus, human exposure to estrogenic chemicals is ubiquitous. Chemicals characterized as “estrogenic” may alter the metabolism or synthesis of endogenous estrogens (EFSA 2013, Heldring et al. 2007, JRC 2013, Rhomberg and Goodman 2012, Vandenberg et al. 2012) or bind to estrogen receptors (ERs) located at various locations in and on cells (Heldring et al. 2007, Huang et al. 2010, Marino et al. 2012, O’Malley et al. 2012, Shanle and Xu 2011, Teeguarden et al. 2013a, Teeguarden and Hanson-Drury 2013). Estrogenic compounds have also been a critical component of the discussion on endocrine disruption, in which chemicals interfere with the endocrine system potentially causing numerous adverse health outcomes, including obesity, type 2 diabetes mellitus (DM), and cardiovascular disease (CVD) (Moyer and U.S. Preventive Services Task Force 2013, Nelson et al. 2012, Sarrel et al. 2013, Turgeon et al. 2004, 2006).

With recent advances in research on various types of estrogenic compounds, it has become clear that basic characteristics of EACs—including their pharmacokinetics (what the body does to the drug [PK]), pharmacodynamics (what the drug does to the body [PD]), and relation to health outcomes—are complex and poorly understood (Moyer and U.S. Preventive Services Task Force 2013, Nelson et al. 2012, Phillips et al. 2008, Sarrel et al. 2013, Turgeon et al. 2004, 2006). Without a firm understanding of disposition (absorption (A), distribution (D), metabolism (M), and elimination (E), together ADME, and PD), as well as the influence of such important factors as sex, age, hormonal status, and the features of exposure such as timing, dose, and duration, it will be difficult to interpret the health-based information that is appearing in the epidemiological and experimental clinical literature.

The goal of this review is to assess the current state-of-the-science on: (i) the potential for EACs to interfere with endocrine activity, (ii) factors which contribute to endocrine-related clinical outcomes, and (iii) existing knowledge gaps. While classical PK approaches (compartmental or non-compartmental) can be used to characterize absorption, distribution, metabolism, and elimination of EACs, many of the detailed pharmacological characteristics necessary to understand benefit-risk balance have not yet been clarified. This review is not meant to be exhaustive; rather, we have chosen to highlight concepts which summarize our current knowledge concerning PK properties of endogenous and exogenous EACs as well as the areas of variability and uncertainty. Detailed physiologically based pharmacokinetic (PBPK) models for E2 (Plowchalk and Teeguarden 2002), Genistein (Ga) (Schlosser et al. 2006, Zager et al. 2007), and bisphenol A (BPA) (Fisher et al. 2011, Teeguarden et al. 2005, 2013, Yang et al. 2013) have become available over the past decade. We discuss these models to highlight the limitations in our biological and pharmacological understanding of estrogens with the aim of performing more advanced predictions of variability in EACs disposition and factors contributing to intra- and inter-individual variability in human response. Additionally, these models provide an understanding of the differences in PK and PD for exposures which produce circulating concentrations of the EAC in the normal ranges as well as above and below typical concentrations. It is important to acknowledge that in all instances the quality of the models and their predictions depends critically on analytical chemistry, the ability to measure and characterize accurately the concentrations of the parent EACs and their metabolites, many of which are not active as estrogens (Birnbaum et al. 2012, Calafat et al. 2013, Christensen et al. 2013).

Literature search and review

A review of the published human studies examining the PK of the selected EACs was undertaken in a systematic way to identify existing evidence on similarities (and differences) in their pharmacologic parameters due to endogenous exposure (e.g., background levels of E2) and following oral exposure through diet or pharmaceuticals. The search strategy was designed to identify published studies examining relevant PK endpoints (absorption, distribution, metabolism, and elimination) with a focus on particular population groups who may be at increased risk of adverse health effects due to EAC exposure (premenopausal (PreM), postmenopausal (PostM) women and elderly). It is recognized that a major concern with EACs is their potential effect on fetuses, neonates, and children. However, these age ranges were excluded from our review due to the distinct anatomical, physiological, metabolic characteristics across life stages that contribute to unique differences in their susceptibility to chemical exposures compared to adults. In addition, the preponderance of data for adults, especially for pharmaceuticals, would introduce serious data gaps.
The search strategy utilized Ovid MEDLINE and Ovid EMBASE which were searched for publications dating from January 1947 to August 2013; a subsequent updated search for literature published up to April 2014 was conducted to ensure that the database remained current during the revision of the manuscript. Articles were identified through the use of key words and relevant terms for EACs, including: estrogen, estradiol (E2), conjugated estrogens (CEs), ethinyl estradiol (EE), daidzein (Da), Ga, phytoestrogens, equol, BPA, PK, absorption, distribution, metabolism, elimination, excretion, bioavailability, toxicokinetics (TK), PBPK, pharmacokinetic modeling, women, human. Search terms were grouped according to the Boolean operators OR and AND to develop the search profile. The titles and abstracts of all articles identified in the primary search were examined by two reviewers (DRM and NK) to determine the potential eligibility of each study for inclusion in the review. Following the primary screening process, the full articles were obtained. In addition to searching the main databases, the reference lists of retrieved articles were also manually searched for additional relevant studies. In order to determine which publications would be included in this review, the following inclusion and exclusion criteria applied.

Inclusion: Publications appearing in peer-reviewed journals with a particular focus on reviews published on the relevant EAC were included in this analysis. Original human studies on the selected EACs (E2, EE, CE, Da, Ga, equol, and BPA) based on the relevant oral exposure patterns (therapeutics, dietary, environmental exposure) with an assessment of PK parameters were included.

Exclusion: Studies were excluded if they were on animals or fetuses, neonates, and children. Many of the PK studies on animals have been carried out with high exposure levels that are not necessarily relevant to humans, who are generally exposed to low levels of EACs. In addition, due to interspecies differences in the PK and TK, limitations of extrapolation from nonhuman data, inherent challenges such as methodological set-up or dose/concentrations used, the relevance of animal data for humans remains an important component of the exclusion criteria. However, in some cases, studies on monkeys (as in the case of BPA) were included in order to more fully understand PK parameters relevant to human health effects.

Important limitations of the existing human studies include considerable variation in experimental conditions, including model selection, dose/concentrations applied, forms and types of the administered compounds, exposures to mixtures of compounds, timing of exposure, lack of data on dietary matrix, sampling strategy, control of co-variables, and presence of contaminants. Levels of exposure are one of the important factors which influence the internal exposure levels. Despite the fact that most of the human studies were designed to mimic typical human exposures, some of the studies evaluated higher than typical exposures. However, it was important to consider the possible differences in PK of the EACs in the normal ranges as well as above and below typical exposure concentrations. For example, in the National Health and Nutrition Examination Survey (NHANES) 2009–2010, the mean urine concentration for Da in the total population of age 6 and older was 69.3 μg/L, however, the range from the 50th percentile to the 95th percentile was 57.2–1850 μg/L (CDC 2013). Decreased fractional absorption of isoflavones with an increase in ingested dose due to the rate limiting saturation process was reported in human studies (Setchell et al. 2003b) introducing an overall uncertainty in the estimation of the optimal dose required to produce putative beneficial or adverse health effects to humans. In addition, many of the xenoestrogen studies were designed to begin at levels higher than typical human exposures but have, using tracers, evolved to levels which mimic typical human exposures.

The same two reviewers independently assessed the strengths and limitations of the included studies based on the class of EAC being considered with a focus on endpoints pertaining to the present research questions and also considering the opinion provided in the respective reviews.

Using our search algorithm, 29671 studies were identified and of those, 7336 were retained from the primary screening process. Most studies were excluded during primary screening because they were either irrelevant or represented a duplicate record; 285 were included in the present review.

### Background information on estrogens selected for this review

In this paper, we explore the current state-of-the-science of the PK of four types of estrogenic compounds that are derived from disparate sources and result in widely varying levels of human exposure. These include (Table 1):

- **Endogenous estrogen:** E2, the primary endogenous estrogen produced predominantly by the ovary in Prem women.
- **Pharmaceutical:** EE, a derivative of E2 used in contraceptives and hormone replacement therapy (HRT) and CE used in HRT.
- **Phytoestrogens:** Ga and Da (and its metabolite S-equol), phytoestrogens found in many soy and legume-based food sources.
- **Xenoestrogen:** BPA, a synthetic chemical used in the production of polycarbonate bottles, coatings in cans, thermal paper, and other items.

Estrogens influence and control many processes by interacting with ER in various cellular locations, including the nucleus, cytoplasm, and cell membranes (Figure 1) (Heldring et al. 2007, Marino et al. 2012). In the nucleus, the ER are ligand-regulated transcription factors in which E2-ER binding produces modification of the conformation of the receptor allowing association with specific DNA sites and the recruitment of cell-specific transcriptional coregulatory proteins, either coactivators (for gene induction) or corepressors (for gene silencing) (Huang et al. 2010, O’Malley et al. 2012).

Cellular signaling of estrogens is mediated, in part, through two ERs—ERα and ERβ—which are products of separate genes located on different chromosomes. A unique characteristic of the ERs is that they have a ligand cavity which is substantially larger than E2. This large receptor ligand binding cavity allows many different molecules to bind to and activate the ER, and consequently alter gene expression via either gene induction or silencing. When ERα and ERβ are coexpressed in cells, ERβ can antagonize or inhibit ERα-dependent transcription (Heldring et al. 2007). ERs also exist outside of the nucleus...
Table 1. Physico-chemical characteristics of the selected EACs. (from PubChem at: http://pubchem.ncbi.nlm.nih.gov/).

<table>
<thead>
<tr>
<th>Group of chemicals</th>
<th>Endogens estrogens</th>
<th>Pharmaceutical estrogens</th>
<th>Phytoestrogens</th>
<th>Isoflavones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>Estratriene-3,17</td>
<td>19-Nor-17α-pregna-3,17</td>
<td>Genistein</td>
<td>Da: 5,7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one</td>
</tr>
<tr>
<td>(IUPAC)</td>
<td>Estratriene-3,17-</td>
<td>beta-diol</td>
<td>Genistin</td>
<td>Da: 7-hydroxy-3-(4-hydroxyphenyl) chromen-4-one</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>17β-Estradiol</td>
<td>Ethinyl estradiol</td>
<td>Daidzin</td>
<td>(3S)-3-(4-hydroxyphenyl)-3,4-dihydro-2H-chromen-7-ol</td>
</tr>
<tr>
<td></td>
<td>1,3,5(10) -</td>
<td>Conjugged estrogens</td>
<td>S(-) Equol</td>
<td>(3R)-3-(4-hydroxyphenyl)-3,4-dihydro-2H-chromen-7-ol</td>
</tr>
<tr>
<td></td>
<td>Estratriene-3,17-</td>
<td></td>
<td>S(-) Equol</td>
<td>4-[2-(4-hydroxyphenyl)propan-2-yl]phenol</td>
</tr>
<tr>
<td></td>
<td>1,3,5(10)-trien-20-</td>
<td></td>
<td>R(+) Equol</td>
<td>(Not detected in humans)</td>
</tr>
<tr>
<td></td>
<td>yne-3,17-diol</td>
<td></td>
<td></td>
<td>(Produced by intestinal bacteria in humans)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₁₈H₂₄O₂</td>
<td>C₂₀H₂₄O₂</td>
<td>C₁₅H₁₀O₄</td>
<td>C₁₅H₁⁴O₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Da</td>
<td>C₁₅H₁⁴O₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S(-) Equol</td>
<td>Ga: 7β-hydroxy-3-(4-hydroxyphenyl)-7-[(2S,3R,4S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxyan-2-yl]oxychromen-4-one</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Da: 254</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Da: 242</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dg: 416</td>
<td>228</td>
</tr>
<tr>
<td>Chemical structure</td>
<td>Estrone sulfate (E1S)</td>
<td>Equilin sulfate (EqS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic estrogen</td>
<td>Estrone sulfate</td>
<td>Equilin sulfate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>molecule</td>
<td>Estrone sulfate</td>
<td>Equilin sulfate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular mass (g)</td>
<td>272</td>
<td>296</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility in water (mg/L)</td>
<td>Practically insoluble in water 3.90 mg/L at 27°C</td>
<td>Practically insoluble in water 11.3 mg/L at 27°C</td>
<td>Soluble in water at 25°C</td>
<td>2.84 (Ga, calculated)</td>
</tr>
<tr>
<td>Octanol/Water</td>
<td>4.01</td>
<td>3.67</td>
<td>2.84 (Ga, calculated)</td>
<td>Low solubility in water 120 mg/L at 25°C</td>
</tr>
<tr>
<td>Partition Coefficient (Po/w)</td>
<td>Soluble in ethanol (1 part in 28), chloroform (1 part in 435), diethyl ether (1 part in 150), acetone and dioxane (Budavari 1996, Reynolds 1998)</td>
<td>Soluble in acetone (1 part in 5), ethanol (1 part in 6), chloroform (1 part in 20), dioxane (1 part in 4), diethyl ether (1 part in 4), and vegetable oils (Budavari 1996, Reynolds 1998)</td>
<td>Ga: soluble in water</td>
<td>Da: low soluble in water</td>
</tr>
<tr>
<td>Solubility in organic solvents</td>
<td>Ga: soluble in water</td>
<td>Ga: low soluble in water</td>
<td>Da: 270</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>EqS: 370</td>
<td>EqS: 370</td>
<td>Da: 254</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>EqS: 372</td>
<td>EqS: 370</td>
<td>Dg: 416</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>EqS: 370</td>
<td>EqS: 370</td>
<td>Dg: 416</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>EqS: 370</td>
<td>EqS: 370</td>
<td>Dg: 416</td>
<td>228</td>
</tr>
</tbody>
</table>
Endogenously, the major source of E2 is the ovary; the production of E2 in the follicle and its distribution through the circulation allows the ovary to influence tissues and organs including the hypothalamus, pituitary, and uterus. Studies with E2 used as a therapeutic agent provide information on the role of endogenous hormones, given externally as drugs, to modify the health of women of reproductive age (Stanczyk et al. 2013a) or PostM women (Moyer and U.S. Preventive Services Task Force 2013, Nelson et al. 2012, Turgeon et al. 2004, 2006). As treatment occurs via multiple routes (oral, dermal, vaginal, uterine, subcutaneous), it is also possible to characterize the various health benefits and risks of different routes of exposure; this has clear implications for our understanding of risks and benefits associated with exposure to other estrogens.

**Ethinyl estradiol (Table 1)**

EE, a synthetic derivative of E2, has been used therapeutically for fertility regulation since the 1930’s, with both beneficial and adverse effects observed in women of reproductive age (Brunton et al. 2010, Melmed et al. 2012, Stanczyk et al. 2013a). The discovery in the late 1930’s that the addition of the ethynyl group to E2 produced an orally-active estrogen with increased bioavailability provided the basis for development of a broad range of orally active estrogens and set the stage for development of oral contraceptives (OCs). EE is used in most OCs and also in some HRT preparations. However, because of its stability, bioavailability, and potency, very small doses are typically used in OC and HRT (μg quantities of EE compared to mg quantities of crystalline E2 needed in oral preparations). Adapting dose, formulation, and routes of administration to maximize benefit-risk has required substantial research and understanding of PK and PD (Darwish et al. 2014, Stanczyk et al. 2013b, Zimmerman et al. 2013). Studies with EE can provide insight to the dose-response relationships which may be observed with diverse estrogenic compounds, including environmental estrogens (Stanczyk et al. 2013a). PBPK models for EE (which to our knowledge have not been developed) would be beneficial in extending our understanding of PK and PD of estrogens.

**Conjugated estrogens (Table 1)**

CEs are a complex mixture of the classical estrogens and a group of unique ring B unsaturated CEs which are produced by pregnant mares and harvested from their urine (Bhavnani 1998, 2000, 2002). CEs have been used via oral or topical routes in HRT for decades and are associated with both beneficial and adverse effects in early menopausal and late post-menopausal women (Moyer and U.S. Preventive Services Task Force 2013, Turgeon et al. 2004, 2006).

This mixture of estrogens, alone or with a progestational agent (typically medroxyprogesterone acetate), was evaluated in the Women’s Health Initiative (WHI) research program and was found to produce a range of unexpected clinical study results. The WHI made clear that the endocrine status of an individual exposed to an estrogen has a strong influence on the types of responses observed (Moyer and U.S. Preventive Services Task Force 2013, Nelson et al. 2012, Sarrel et al. 2013, Turgeon et al. 2004, 2006).

Use of the CE in therapeutics is supported by many different PK studies, typically focusing on the major chemical constituents

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**Endogenous and therapeutic estrogens**

**17β-Estradiol (Table 1)**

E2 is an endogenous estrogenic hormone produced in several organs and tissues including the gonads and adrenal and adipose tissue (Melmed et al. 2012). E2 is also used in therapeutic products such as contraceptives and HRT (Archer 2013, Bhavnani 1998, Stanczyk et al. 2013a). Consequently, there has been substantial interest in understanding the PK and PD of E2 to characterize its role in physiology and endocrinology, as well as to support its use in therapeutic products (Archer 2013, Brunton et al. 2010, Melmed et al. 2012, Stanczyk et al. 2013a, Sutter et al. 2014, Deshpande et al. 2014). PBPK models for E2 have been developed (Bouzom et al. 2012, Chow and Pang 2013, Huang 2012, Jones et al. 2012, Lipscomb et al. 2012) and form the basis for evaluating, from a biologically-motivated perspective, the disposition of a diverse group of estrogen-active compounds (Plowchalk and Teeguarden 2002).

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**Figure 1.** Molecular pathways involved in the regulatory actions of ERs. In addition to the classical (direct) ligand-dependent DNA binding and induction or silencing gene transcription, there are ER outside of the nucleus in the cytoplasm and/or on the cell membrane which can bind EACs and produce rapid physiological responses in the cell which are independent of gene transcription (e.g., activation of phosphatases, kinases, or ion channels). There are also ligand-independent pathways, for example growth factor signaling may phosphorylate and activate ER in the absence of a ligand through other signaling pathways. ERE Estrogen response element(s), ER Estrogen receptor(s), TF Transcription factor(s), SM Second messenger(s), GF Growth factor, P Phosphorilation, NO Nitric oxide. From: Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M, Gustafsson J-A.  (2007). Estrogen receptors: how do they signal and what are their targets. Physiological Reviews 87:905–931. Reproduced with permission from American Physiological Society (APS).
O-desmethylangolensin (ODMA) (Day et al. 2000).

lactase-phlorizin hydrolase metabolizes Da to S-equol and to tional supplements or extracts (Nielsen and Williamson 2007, foods derived from legumes and co-occurs with genistin. It Da, also an isoflavone phytoestrogen, is found in plants and expostures and metabolism, however, further work is needed. After ingestion, both daidzin and genistin are hydrolyzed form of endogenous and therapeutic estrogens, as well as xenoestrogens (Miksicek 1995, Ross and Kasum 2002) (Table 1). Health effects of isoflavones are thought to result from their biological activity as antioxidants, free-radical scavengers, estrogens, or antiproliferative agents (Ross and Kasum 2002). Polyphenols are generally found in plants as glycosides (attached to sugars) with two specific representatives of this group genistin and daidzin discussed here. While the glycoside structure of genistin and daidzin was initially thought to limit their absorption and bioavailability, subsequent studies have demonstrated that this structural feature may actually increase their bioavailability depending on the dietary source (Ross and Kasum 2002). After ingestion, both daidzin and genistin are hydrolyzed forming the principal bioactive aglycones, Da, and Ga. Both Da and Ga can be absorbed or metabolized further by intestinal microflora to other compounds (Lampe et al. 1998). Women may metabolize isoflavones more efficiently than men (Chetty et al. 2012, Lu and Anderson 1998, Setchell et al. 1984, Soldin and Mattison 2009, Soldin et al. 2011) and this has also been observed for other substrates (Chetty et al. 2012, Soldin and Mattison 2009, Soldin et al. 2011).

Genistein
Ga, an isoflavone phytoestrogen found in diverse plants including soy beans and foods derived from soy, is considered to have beneficial health effects and is sold as a nutritional supplement (Setchell et al. 2001). However, the results of population health studies are conflicting (Newbold et al. 2001, Nielsen and Williamson 2007, Ross and Kasum 2002). The presence of Ga in foods and putative health effects of those foods has stimulated study of the PK and PD of Ga (Holst and Williamson 2004, 2008, Manach et al. 2005, Scalbert and Williamson 2000, Williamson and Manach 2005) as well as studies to characterize PBPK of Ga (Schlosser et al. 2006, Zager et al. 2007). Given the complexity of exposures and metabolism, however, further work is needed.

Daidzein
Da, also an isoflavone phytoestrogen, is found in plants and foods derived from legumes and co-occurs with genistin. It is contained in commercially available phytoestrogen nutritional supplements or extracts (Nielsen and Williamson 2007, Setchell et al. 2001). The intestinal brush border enzyme lactase-phlorizin hydrolyase metabolizes Da to S-equol and to O-desmethylangolensin (ODMA) (Day et al. 2000).

S-equol
Only 30–40% of individuals have the ability to metabolize Da to S-equol. S-equol is a more potent estrogen than Da and ODMA (Jackson et al. 2011, Lampe et al. 1998, Setchell et al. 2001) and has also been studied for characterization of PK and PD (Gardner et al. 2009, Minatoya et al. 2013, Schwen et al. 2012a, b, Setchel and Clerici 2010a, Setchell et al. 2009a, b, Shi et al. 2009, 2014, Tseng et al. 2013).

Xenoestrogen bisphenol A
BPA is a synthetic high-production volume chemical used in the manufacture of polycarbonate plastics and epoxy resins (Table 1) (LaKind and Naiman 2008). Because of its extensive use in food-related storage items, human exposure to BPA is ubiquitous (Krishnan et al. 2010). BPA is thought to have estrogenic and other endocrine activity and consequently has been extensively studied to characterize exposure (EFSA 2013, JRC 2013, Hengstler et al. 2011, Krishnan et al. 2010, LaKind and Naiman 2008, 2011, LaKind et al. 2012a, b, Teeguarden et al. 2013a, b) as well as PK (Doerge et al. 2010a, b, 2011a, b, 2012, Fisher et al. 2011, Mazur et al. 2012), and health effects including diabetes (LaKind et al. 2012a, LaKind et al. 2014, Lang et al. 2008), obesity (Hugo et al. 2008), CVDs (LaKind et al. 2014, Lang et al. 2008, Melzer et al. 2010), hepatotoxicity (Lang et al. 2008), gonadal toxicity in men (Li et al. 2010, Meeker et al. 2010), as well as PBPK modeling (Fisher et al. 2011, Kawamoto et al. 2007, Shin et al. 2004, 2010, Teeguarden et al. 2005, 2013, Yang et al. 2013).

Pharmacokinetics of estrogenic compounds
This section summarizes our current understanding of the disposition of the selected EACs. While information related to other routes of exposure will be briefly discussed, ingestion is the primary focus of this review because it is the predominant route of exposure to therapeutic agents, phytoestrogens, and BPA (Morgan et al. 2011).

Endogenous and therapeutic estrogens: 17ß-Estradiol
The endogenous estrogens in humans include 17ß-E2, estriol (E3), and their hydroxylated metabolites and conjugates (Kuhl 2005) (Figure 2). In PreM women, the predominant estrogen in circulation is E2. Both E2 and E1 are synthetized in the human ovary, and E3 is formed through conjugates (Kuhl 2005) (Figure 2). In PreM women, the pre dominant estrogen in circulation is E2. Both E2 and E1 are synthetized in the human ovary, and E3 is formed through 16α-hydroxylation of E1 and E2 (Speroff et al. 1999). In PostM women, E1, which is formed in the adipose tissue from adrenal androstenedione, replaces E2 as the primary estrogen (Korenman et al. 1978, Speroff et al. 1999). E3 is short-acting and is the least potent endogenous estrogen in the serum of healthy pre-menopausal women, but is produced in high quantity by the placenta and, unlike E1, is not converted to E2 (Kuhl 2005).

Throughout the ovarian cycle, circulating E2 acts at multiple sites, including: hypothalamus, pituitary, uterus, breast, kidneys, liver, adipose tissue, and central nervous, immune, cardiovascular, and gastrointestinal systems (Melm et al. 2012), suggesting the multiple sites at which EACs could act. In the pituitary, E2 and gonadotropin releasing hormone (GnRH) influence the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH), initially providing nega-
tive feedback in the follicular phase of the cycle (Figure 3). As the follicle grows and the concentration of E2 in the blood increases above \( \sim 200 \text{ pg/ml} \), the pituitary response switches from negative to positive feedback providing a stimulus to increase secretion of FSH and LH which, in turn, drive increased E2 production and ovulation. This biphasic response of the pituitary to increasing concentrations of E2 emphasizes the point that response to estrogenic chemicals is dependent on the status of the tissue at the time the estrogenic exposure occurs (i.e., responses are specific to the hormonal history of the tissue).

The bioavailability of E2 as an oral therapeutic product is quite low; when used orally, it is rapidly metabolized in the intestine and liver with only about 5% of the administered dose available as circulating E2. Reducing the particle size (micronization) of orally administered E2 has been shown to improve absorption—micronized E2 is absorbed more efficiently than the crystalline form (with the respective hepatic concentrations about four-fold higher compared to the peripheral blood) (Kuhl 2005). As a result, the adverse effects of orally administered E2 on hepatic metabolism, changes in lipid and lipoprotein levels and blood coagulation profiles are much larger than observed with other routes of administration (topical, subcutaneous, vaginal, intrauterine), which avoids exposing the liver to high concentrations which result from GI absorption and passage through the liver.

Absorption and distribution

After oral administration and absorption, E2 circulates in the plasma as “free” E2 and reversibly bound to sex hormone-binding globulin (SHBG) and albumin (Stanczyk et al. 2013a). In PreM women, E2 in the blood is bound to serum albumin (\( \sim 60\% \)) with low affinity and to SHBG (\( \sim 35\% \)) with high affinity, with approximately 1% – 5% in the “free” form (Anderson 1974, Siiteri et al. 1982, Westphal 1986). Because E2 binds to serum albumin with low affinity, it can be dissociated to free E2, providing a pool of albumin-bound hormone able to exert an estrogenic effect (Stanczyk et al. 2013a). Binding to the SHBG and albumin restrict the free fraction of E2 in plasma which is considered to be the biologically and pharmacologically active fraction and appears to play a significant role in the transport and distribution of the hormone as well as reduction in the rate of hepatic degradation of E2. A buffering role of protein binding in stabilizing the free E2 concentration has been suggested, as protein binding reduces the metabolic clearance rate of steroids, which in turn reduces the production rate required to achieve a given free E2 concentration (Anderson 1974). It was found that oral E2 treatment increases serum SHBG level, resulting in a reduced free E2 fraction (Stanczyk et al. 2013a). Both the “free” and the albumin-bound fractions of E2 are available for further metabolism and tissue uptake (Mendel 1992, Pardridge and Mietus 1979). Due to the dual effects of obesity—increasing estrogen production and depressing SHBG concentrations—in both pre- and postmenopausal healthy women, but especially after menopause, obesity can lead to a marked increase in the amount of non-bound (free fraction) E2 and associated health consequences (Lukanova et al. 2004, McTiernan et al. 2006, Pasquali et al. 1997, Sitteri et al. 1987).
Figure 3. Hypothalamic-pituitary-ovarian axis. Gonadotropin-releasing hormone is produced by neurons in the hypothalamus and released into the hypothalamus and pituitary portal system where it influences pituitary release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH are released into the circulation where they influence follicle growth, ovulation and production of estradiol and progesterone. The secretion is regulated in a complex feedback loop which effectively regulates the serum concentrations of hormones within a physiological concentration range.


Metabolism

More than 95% of orally administered E2 is converted to numerous oxidative and conjugated metabolites mainly through the 2-hydroxylation and 16α-hydroxylation metabolic pathways, as well as the sulfation and glucuronidation pathways in the liver (Stanczyk et al. 2013a; Figure 4). After oral administration, about 15% of the administered E2 is metabolized to E1 through the action of highly active hydroxysteroid dehydrogenase (17β-HSD type 2) which is expressed in the gastrointestinal tract as well as other organs and tissues (Stanczyk et al. 2013a, Yen et al. 1975). Following oral administration of 1 mg micronized E2 to a PostM woman, serum E2 concentrations were 30–50 pg/mL, whereas the corresponding range of E1 concentrations was 150–300 pg/mL (Stanczyk 2001). About 25% of the administered E2 is metabolized to estrone sulfate (E1S) which itself has no biologic activity (Longcope et al. 1985, Stanczyk et al. 2013a).

Approximately 99% of the E1S is bound with a relatively high affinity to albumin, resulting in a relatively long half-life for the circulating conjugates. The reported half-lives for E2 and E1 are 20–30 min, while the reported half-life of E1S is 10–12 h (Kuhl 2005). There is a dynamic inter-conversion between E2, E1, and E1S from which E2 is continuously delivered by transformation of E1S to E1 and then conversion of E1 to E2 via the action of 17β-HSD type 1 dehydrogenase (Kuhl 2005; Figure 4). Both E2 and E1 can also undergo 16α-hydroxylation to form E3, which cannot be retransformed to E2 (Figure 2), but rather undergoes extensive conjugation and then is rapidly excreted in urine as E3 glucuronide (E3G) (Kuhl 2005).

Elimination

The route of administration, sex, and age impact the disposition of E2 (Greenblatt et al. 1980, Longcope et al. 1968, 1985, Stanczyk et al. 2013a). Calculated on the basis of a 2-hour infusion of E2, the metabolic clearance rate (MCR) of E2 was significantly lower than the MCR of E1 (p < 0.05). Clearance was also lower in women than in men, whereas no sex difference was observed in the MCR of E1 (Chetty et al. 2012, Hembree et al. 1969, Soldin et al. 2011, Soldin and Mattison 2009). The MCRs of E2 in pre- and post-M women are similar, but E2 is metabolized more rapidly in the early versus late stage of menopause (Hembree et al. 1969). Differences in estrogen disposition among PreM, early and
late PostM women may account, in part, for the differences in physiological responses observed in the WHI between early and late PostM women (Turgeon et al. 2004, 2006).

In general, endogenous estrogens (E1, E2, and E3) and their glucuronide and sulfate conjugates are excreted in bile (−6%) and urine (−54%) (Stanczyk et al. 2013a). The enterohepatic recirculation of E2 and E1 (Figure 5) delays their elimination from the body, with the range of terminal elimination half-lives about 13 to 20 h (Stanczyk et al. 2013a). The main urinary estrogens include E1: 13.5–29.7%, E2: 5.2–10.3%, E3: 2.0–5.9%, 16α-hydroxyestrone (16α-OH-E1): 1.0–2.9%; 2-hydroxyestrone (2-OH-E1): 2.6–10.0%, 2-hydroxyestradiol (2-OH-E2): 0.58–1.44%, 2-methoxyestrone (2-M-E1): 0.36–2.42%, 2-methoxyestradiol (2-M-E2): 0.05–0.13% (Longcope et al. 1985).

Mechanism of action

E2 regulates numerous endocrine functions through interaction with two ER isoforms (Figure 1), ERα and ERβ, which are members of a superfamily of nuclear receptors. The type of ER and concentration of these receptor subtypes vary with tissue type, leading to isoform, and tissue specific estrogen response (Brandenberger et al. 1997, Huang et al. 2010, Kuiper et al. 1997, O’Malley et al. 2012, Plowchalk and Teeguarden 2002). The biological effects of endogenous estrogens are also mediated by rapid non-genomic mechanisms involving cell membrane receptors which are coupled with G-proteins and can activate intracellular signal cascades (Kuhl 2005; Figure 1). ERs have also been observed in the cytoplasm and on the cell membrane, and it is likely that they have different tissue distributions; however, this area needs further development and is a source of uncertainty (Heldring et al. 2007, Marino et al. 2012, Shanle and Xu 2011). Many other factors are also involved in the control of estrogen-induced biological responses. For example, findings on clinical efficacy of intranasal, transdermal, and oral administration of E2 show that the total exposure to the intracellular E2 (area under the concentration–time curve, AUC) is an important predictor of physiological response (reviewed by Kuhl 2005). In this context, it has been suggested, but disputed, that the short-term presence of high concentrations and the long-term presence of low concentrations of E2 may cause a similar expression of estrogen-dependent gene products during a time interval from 12 to 48 h. The proliferation rate of ER-sensitive human breast cancer (MCF7) cells was similar after 1 h treatment with 7 nM E2 or 24 h treatment with 0.29 nM E2 (Cavaillès et al. 2002).

Summary

The PK of orally administered E2 has been extensively studied (Järvinen et al. 1999, 2000, Schubert et al. 1994, Sierra-Ramírez et al. 2011, Stadberg et al. 1999, Stanczyk et al. 2013a, Yen et al. 1975). Following oral treatment, E2 is rapidly absorbed from the gastrointestinal tract. However, the bioavailability of E2 is quite low (~5%) because of substantial presystemic metabolism by the intestine and liver. After absorption, ~38% of circulating E2 is bound to SHBG and ~60% to albumin, leaving less than 2% free (or unbound) in the circulation. While it is generally thought that only the free form of a drug is available for extraction or uptake by tissues for effect or metabolism, PBPK simulations suggest that protein-bound E2 may be taken up by the liver and other tissues (Plowchalk and Teeguarden 2002). Initial metabolism of E2 occurs in the gastrointestinal tract followed by the liver where E2 is metabolized to estrogenically inactive glucuronide and sulfate metabolites that are excreted in the bile, urine, and/or feces. The conjugates dissolved in the bile are hydrolyzed in the colon by bacterial enzymes and re-absorbed, and this enterohepatic recirculation contributes to the increased serum concentrations of E2 and E1, delays ultimate excretion and lengthens systemic exposure and associated clinical outcomes (Figure 5). It is of interest that E1S has no estrogenic activity (Stanczyk et al. 2013a), similar to the glucuronide of BPA. However, at the same time, E1S is thought to act as a reservoir (precursor) for the formation of active estrogens (Kuhl 2005), mainly via the action of steroid sulfatase (Stanway et al. 2007). The route of administration plays an important role, and oral treatment has a considerably stronger impact on hepatic function than parenteral therapies which circumvent first-pass liver metabolism.

Knowledge gaps

The disposition, systemic exposure, and tissue-specific effects of E2 depend on the sex, age, and endocrine status of the individual (Kuhl 2005, Stanczyk et al. 2013a), the formulation (Järvinen et al. 1999) and dose administered (Järvinen et al. 1999, 2000, Stadberg et al. 1999, Yen et al. 1975), the route and duration of administration (Järvinen et al. 1999, Longcope et al. 2002).
et al. 1985, Morton et al. 2009, Sierra-Ramirez et al. 2011), and food consumed which may alter presystemic or systemic disposition (Schubert et al. 1994). The levels of free E2 and E1 in target tissues or in target cells within these tissues could be very different from blood levels. Thus, measurement of free or total estrogen concentrations in plasma may be an inadequate parameter for relating exposure of target tissues to estrogens with the risk of adverse health effects. Actual tissue concentrations and endocrine history may be needed to define the impact of specific EAC exposures. Identification of the genetic and environmental factors influencing E2 metabolism and profile of estrogens and their metabolites in the blood or urine are important because these factors could profoundly modify the biological and health effects of estrogens. These critical questions concerning exposure and health effects emphasize the need for detailed biologically based models, such as the PBPK models which have been developed, with some success, for E2, Ga, and BPA.

**PBPK model for estradiol**

Recognizing that biological responses to E2 are dependent on the concentration, characteristics, duration, and timing of the exposure, the E2 PBPK model was developed as a foundational model to explore these factors using data from male and female (ovariectomized [Ovx] and intact [NI]) rats and male and female (PreM and PostM) humans (Plowchalk and Teeguarden 2002). The model includes protein binding (in the human to SHBG and albumin and in the rat to α-fetoprotein and albumin), allows uptake of both free and protein-bound E2 into tissues, and includes first-pass effects and metabolism as well as tissue ER binding and response to E2 (uterine wet weight, with pituitary and gonads as additional target tissues for dosimetry (Plowchalk and Teeguarden 2002). This allows integration of the pituitary-ovarian-uterine axis for assessment of the dynamic interaction among these three organs which characterizes EAC impact on reproductive function (Melmed et al. 2012).

In developing the model and exploring its performance, Plowchalk and Teeguarden (2002) noted several factors which pointed to biological knowledge gaps. For example, hepatic uptake was greater than expected based on the free fraction of E2. While it is generally believed that only the free fraction of drugs, hormones, or chemicals in blood are available for uptake by an organ, and toxicological, pharmacological, or endocrinological action (Plowchalk and Teeguarden 2002), earlier data from other investigators have suggested that protein-bound E2 may be available for uptake (Plowchalk and Teeguarden 2002). In fact, in the E2 PBPK model hepatic uptake was substantially greater than would be expected based on the unbound concentration of E2.

Plasma uterine and liver E2 concentrations agreed with published data from steady-state infusion studies in both male and female rats (Plowchalk and Teeguarden 2002). However, uterine E2 concentrations in the Nl rat, assuming the uterus as a diffusion-limited organ, resulted in substantial under-prediction of uterine concentrations from the measured or observed concentrations. As constructed, the E2 PBPK model only considered a single type of ER and did not include the possibility of, or account for, changing ER concentrations in target tissues. However, the uterus contains nuclear, cytoplasmic, and cellular membrane ER; including both ERα and ERβ (Melmed et al. 2012) and under conditions of changing E2 the uterine estrogen receptor concentrations can change (Shanle and Xu 2011). In addition, endogenous estrogen production in the Ni rat will modify uterine estrogen receptor concentrations (Melmed et al. 2012). Consequently, estrogen receptor concentrations in the Ovx rat differ substantially from estrogen receptor concentrations in the Nl rat.

Accurate model prediction of E2 concentrations in the uterus and pituitary required knowledge of ER concentrations in those tissues. Adjustment of parameters between Ovx and NI animals was necessary for accurate predictions of plasma and tissue E2 concentrations. This highlights the necessity for obtaining better data on the disposition of E2 in Nl rats.

**PBPK model for E2—knowledge gaps**

Human oral administration models (using micronized E2) did not fit the data as well as the data derived from i.v. routes of administration. It has been demonstrated that bioavailability and first pass effects of micronized E2 are different from those observed with standard oral forms of E2 (Archer 2013, Stanczyk et al. 2013a). This indicates the need to better understand intestinal and hepatic function as they impact on oral exposures to estrogens (interestingly, the developers of the PBPK models for all EACs discussed in this review have identified intestinal and hepatic function as an area requiring further study and functional characterization to improve the performance of the models). Specifically, sensitivity analysis suggests that extra-hepatic clearance and hepatic blood flow, ER content and ER binding affinity should be studied more carefully, and included in the PBPK models in greater, physiologically accurate detail.

**Endogenous and therapeutic estrogens: Ethinyl estradiol**

EE is a semi-synthetic derivative of E2 (Table 1). The introduction of an ethinyl group at C17α into the E2 molecule produces a potent, orally-active estrogen that is much more resistant to metabolism or inactivation than E2 (Kuhl 2005).

**Absorption**

EE is rapidly absorbed, with peak plasma EE levels occurring within 1 to 2 h after ingestion of 30 μg EE (Boyd et al. 2003, Stanczyk et al. 2013a). A secondary peak can often be observed 10 to 14 h after oral administration of EE as a consequence of enterohepatic recirculation (Stanczyk et al. 2013a). The mean oral bioavailability of EE is approximately 45%, with large inter- and intra-individual variability in the range of 20%–65% (Orme et al. 1989), which might be attributed to inter- and intra-individual differences in the level and activity of cytochrome P450 (CYP 3A4) and extent of 2-hydroxylation of EE (Goldzieher and Stanczyk 2008). A two-fold increase in EE blood concentrations was observed in women following 1 year of treatment cycles (COC containing EE/drospirenone [DROSIP]) with mean accumulation ratio of 2.1 (Blode et al. 2000). Inter-individual day-to-day variability has been reported; for example, in a study of three subjects who ingested the same formulation containing EE and norethindrone (NE)
on the same day during three consecutive menstrual cycles, the AUCs of EE in one participant varied by almost a factor of four (Brody et al. 1989, Goldzieher and Stanczyk 2008). Further, studies of populations of different ethnicities have shown that plasma EE concentrations differ substantially from one group to another (Goldzieher and Stanczyk 2008, de Visser et al. 2003). Back et al. (1987) measured the plasma concentrations of levonorgestrol (LNG) and EE in a randomized crossover study in healthy female volunteers (n = 5) given a combination oral contraceptive tablet (250 μg LNG and 50 μg EE) by the oral route and per vagina and also receiving the same dose intravenously. The bioavailability of EE after oral dosing was 62% and after vaginal administration was 74%; however, T_{max} (time to achieve peak concentration) was longer after vaginal administration, which suggests that while bioavailability was essentially similar, the absorption rate was slower by vaginal route (van den Heuvel et al. 2005).

Distribution

EE does not bind to SHBG (Stanczyk et al. 2013a). After oral ingestion and absorption, EE circulates in the blood mostly as conjugates and oxidative metabolites of EE.

Metabolism

In contrast to E2, 2-hydroxylation of EE is the most important pathway of EE metabolism, as the 16α-hydroxylation pathway is blocked due to steric hindrance from the ethinyl group at C17 (Kuhl 2005, Stanczyk et al. 2013a). EE is rapidly conjugated partly to EE glucuronides (EE-3-glucuronide [EE3G] and EE-17-glucuronide [EE17G]), which are biologically inactive, and to EE sulfates (EES) and EE-17-sulfate (EE17S). EES can be partially deconjugated to EE during enterohepatic recirculation (Figure 5) leading to an approximate 12–20% contribution to the circulating unconjugated EE (Goldzieher and Stanczyk 2008, Stanczyk et al. 2013a, Back et al. 1980). This process is thought to increase the therapeutic effectiveness of EE to some extent. Antibiotic therapy may cause a reduction of the effectiveness of OCs due to decrease in the circulating concentrations of estrogens and progestagens by eliminating the bacteria which are primarily responsible for the hydrolysis stage of enterohepatic recirculation (Figure 5) and causing hepatic microsomal enzyme CYP induction; and by interference with absorption from the gastrointestinal tract and increased excretion of the oral contraceptive (Adlercreutz et al. 1984, DeRossi and Hersh 2002, Hämäläinen et al. 1987). However, evidence on the association between antibiotic use and OC effectiveness is controversial (Toh et al. 2011).

Elimination

The terminal elimination phase of EE is characterized by a half-life in the range of approximately 5–30 h (Goldzieher and Stanczyk 2008, Stanczyk et al. 2013a). About 62% of the total EE and its metabolites are eliminated in feces and about 38% in urine (Stanczyk et al. 2013a). The main urinary metabolites are glucuronides (about 80%) and sulfates (about 8% to 10%); about 6 and 9% of the unconjugated EE is excreted in urine and feces, respectively (Stanczyk et al. 2013a). In bile, the predominant EE forms are EEG and EES (Back et al. 1979, Cargill et al. 1969, Stanczyk et al. 2013a). In contrast to oral administration of E2, considerably more of the total EE is eliminated in feces compared with urine which suggests that E2 and EE differ in routes of elimination following oral administration (Stanczyk et al. 2013a).

Mechanism of action

EE exerts its biological effects via interactions with various ERs by the same mechanism as E2 (Stanczyk et al. 2013a). EE is much more active than E2 because of the ability of the ethinyl group at C17α to prevent the oxidation of the 17β-hydroxy group and irreversibly inhibit CYP enzymes, which are involved in the metabolism of EE (Kuhl 2005). Because of the rapid first-pass metabolism and low bioavailability compared with EE, higher doses of oral E2 are required to achieve the same biological effects as EE (Stanczyk et al. 2013a).

Summary

The PK of orally administered EE has been extensively studied mainly in PreM women. While E2 is readily absorbed following oral administration, it is also quickly inactivated in the intestine and liver. Substitution of the estrane steroid at C17 with an ethinyl group renders EE much more resistant to metabolism and elimination than the parent molecule E2. Following oral treatment, EE is rapidly absorbed in the small intestine and reaches a serum peak concentration about 2 h later, with a second peak several hours after the initial EE peak as a result of enterohepatic recirculation (Figure 5). Variations exist in the overall absorption process, and can be further modified by drugs (i.e., antibiotics) that affect enterohepatic recirculation, or liver enzyme activities (Blode et al. 2012c). EE bioavailability is about 45% (ranging from 20–65%), greater than that for E2 (5%). When administered orally, the EE effects on hepatic function are substantial due to high levels of EE in the portal blood, with increased risk of intravascular coagulation. At the same time, large differences in oral bioavailability might affect the effective dose estimation. The ability of EE to accumulate within a treatment cycle (about two-fold) was reported in some studies (Blode et al. 2012b, Endrikat et al. 2002), however, no EE accumulation during the treatment cycle was observed by DiLiberti et al. (2011) which might be related to the particular study design. Metabolism of EE is similar to E2 with CYP-mediated 2-hydroxylation; however, metabolism of 16α-hydroxylation is blocked as a result of steric hindrance (Kuhl 2005). EE can inhibit its own hydroxylation at C2 through the decreased activity of P450 enzymes (Blode et al. 2012c, Kuhl 2005). The diminished metabolism results in a marked hepatic effect of EE as compared to E2 (facilitated by the adverse effects on blood hemostatic parameters or lipids and lipoproteins levels) (Aten and Eisenfeld 1982, Sitruk-Ware et al. 2007a, 2007b). Hepatic function is most affected following the oral EE administration, while other routes of administration minimize those effects (Fait et al. 2006). About 40% of E2 is bound to SHBG; in contrast, essentially none of the EE is bound to SHBG. Conjugation of EE and its oxidative metabolites occurs with glucuronide and sulfate, and both conjugated forms are estrogencically inactive. More EE is eliminated in the feces than E2. EE binds both ERα and ERβ.
and is a more potent estrogen than E2. The similarity in ER binding between EE and E2 results in similar effects on the hypothalamus and pituitary (suppression of the mid-cycle ovulatory surge of LH and FSH). The PKs of EE are comparable between pre- and postmenopausal women (Blode et al. 2008); however, the significant variability and differences in EE PK of participants from various geographical regions and ethnic groups have been described (Goldzieher 1989, Goldzieher and Stanczyk 2008). Blode et al. (2008) suggested that differences in EE metabolism might be one of the main reasons for large intra- and inter-individual variability which is also reflected in the large range of absolute bioavailability (25–65%) reported in the literature (Brody et al. 1989). However, no differences in PK parameters of EE were observed across ethnic groups (Blode et al. 2012b) which might be due to the different design of the studies and lack of standardized timing of samples.

Knowledge gaps

A major problem when addressing the issue of how EE PK data compare to other estrogenic chemicals (in other words, in using EE as a foundational chemical with which to compare properties of other estrogens) is the limited comparative data from EE-only studies. Available clinical studies have been conducted using EE combined with a series of different progestagens added (to protect the uterus from endometrial hyperplasia in HRT or suppress ovulation in OC). Since progestagens are known to exhibit different PK properties and interactions with estrogens, it is difficult to predict the type of interaction and possible PD effects within different combinations of doses of EE and doses of progestagens in the preparations used. In addition, the different estrogens (and/or progestins) are not recognized in the same way in all cells and do not have similar effects. It was reported that “the risk of VTE was lowest with drugs containing the progestogens levonorgestrel, norgestimate, and norethisterone, higher with the etonogestrel and norelgestromin, and possibly highest with pills containing gestodene, desogestrel, and drospirenone” (EMA 2013). Edelman et al. (2013) reported that obesity affected the oral PK parameters of EE due to alterations in drug clearance; however, the degree or magnitude of obesity did not directly correlate with the observed PK changes, and severity of changes in the PK parameters correlate with end-organ functional activity. Carefully conducted PK studies in women of differing body weights could help to ensure efficacy in future contraceptive development. More research is needed to better define the particular phenotypes that might be at greatest risk for adverse effects including reduced clinical efficacy.

Unfortunately, while there has been substantial work on EE PK and PD (Stanczyk et al. 2013a), at present there is no published PBPK model available for EE. Development of a PBPK model of EE to the growing suite of models for EACs would be of substantial benefit in understanding intestinal and hepatic metabolism and differing effects of administration by oral and other routes of exposure. Additionally, such models could also improve our broader understanding of adverse effects based on hepatic and cardiovascular exposure to this potent estrogen.

Endogenous and therapeutic estrogens: Conjugated estrogens

Several estrogen preparations are available for use following menopause, and among these, CE have been the most frequently used since the early 1940s (Stern 1982). CE, according to the Unites States Pharmacopeia (Wyeth 2007), are a mixture of conjugated equine estrogens obtained from natural sources and occurring as the sodium salts of the watersoluble estrogen sulfates derived from pregnant mare’s urine (Figure 6). In addition to the classical estrogens which are also produced in humans (e.g., the ring B saturated estrogens (as % of CE): E2–0.56%, E1–49.1%, and 17α-E2–3.7%), there are a group of unique ring B unsaturated estrogens such as equilin and equilenin (equilin [Eq]-22.8%, 17β-dihydroequilin [17β-Eq]-1.5%, 17α-dihydroequilenin [17α-Eq]-13.5%, equilenin [Eqn]-2.8%, 17β-dihydroequilin [17β-Eqn]-0.7%, 17α-dihydroequilenin [17α-Eqn] – 1.6%, and Delta8-estrone [Δ8-E1]-3.9%), some of which are interconvertible (Figure 6; Kuhl 2005). The human estrogens cannot be converted to the ring B unsaturated estrogens (Figure 6) (Kuhl 2005).

Absorption

CE sulfates are water-soluble and undergo hydrolysis in the gastrointestinal tract with the formation of unconjugated estrogens. Relatively slow absorption of conjugated and unconjugated estrogens with maximum levels of Eq (560 pg/mL) and E1 (1400 pg/mL) after 3 to 5 h was reported in healthy PostM women following oral ingestion of CE (10 mg); trace concentrations of both estrogens were detected in the blood 24 h after ingestion (Bhavnani et al. 1981). Following intravenous administration of CE at the same dose, maximum concentrations of Eq (4 ng/mL) and E1 (11.2 mg/mL) were reached 20 min after administration (Bhavnani et al. 1981) which

![Figure 6. Structure of equine estrogens. From: Perrella J, Berco M, Cecutti A, Gerulath A, Bhavnani BR. (2003). Potential role of the interaction between equine estrogens, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in the prevention of coronary heart and neurodegenerative diseases in PostM women. Lipids Health Dis 2:4](http://www.lipidworld.com/content/2/1/4). © 2003 Perrella et al. 2003; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article’s original URL.)
indicates that both hydrolysis and absorption depend on the route of administration. After absorption from the gastrointestinal tract, these estrogens are sulfated, most likely during the first pass through the liver, and circulate in the sulfated form (Bhavnani et al. 1998). EqS and E1S are the main circulating forms of Eq and E1, respectively. Oral ingestion of E1S leads to rapid appearance of unconjugated E1 and E2 in the blood; EqS can be also absorbed from the gastrointestinal tract without prior hydrolysis (Bhavnani 1998).

Distribution

CE in both unconjugated and conjugated forms circulate in the blood either bound to serum albumin or SHBG, with dynamic interactions between different estrogens and blood proteins (Rosenthal et al. 1972). The sulfate esters of Eq, E1, and E2 do not bind to SHBG (Pan et al. 1985, Rosenthal et al. 1972), but the sulfated forms of Eq and E1 interact with serum albumin with high affinity (Pan et al. 1985). Up to 74% of the total EqS and 85%-90% of E1S have been observed to bind to serum albumin (Pan et al. 1985, Rosenthal et al. 1972). The unconjugated Eq, E1, 17ß-dihydroequilin, and E2 bind to serum albumin with low affinity but interact with SHBG with high affinity (Pan et al. 1985, Wu et al. 1976). As a result, only a small percent of the total equine estrogens in blood are present in the unbound (“free”) form (Bhavnani et al. 1998). Due to differences in bioavailability and inter- conversion rates of the individual components in the CE formulations, the ratios between the serum concentrations of the various estrogens differ from those in the formulation. While the proportion of E1 in the total dose of CE corresponds to the proportion in the plasma circulation (about 50%), the percentage of circulating Eq is higher than that in the formulation itself (Kuhl 2005). HRT with CE increases SHBG levels and the proportion of the bound estrogens which impacts the biological activity of each of the various estrogens present in CE in estrogen target tissues (Bhavnani et al. 1998).

Metabolism

Both types of equine estrogens (the ring B unsaturated estrogens and ring B saturated estrogens (Figure 6) undergo extensive metabolism and metabolic interconversion in the liver with enterohepatic recirculation via sulfate and glucuronide conjugation, biliary secretion of conjugates into the intestine, and hydrolysis in the intestine followed by reabsorption (Bhavnani 1998). EqS is the major component of CE and is metabolized in PostM women to the potent estrogens 17ß-EqS (Bhavnani and Cecutti 1993, Bhavnani et al. 1983, Dorfman and Dorfman 1954; Figure 6). The estrogenic effects of EqS are facilitated by its metabolism to 17ß-EqS (Bhavnani 1998). HRT with CE increases SHBG levels and the proportion of the bound estrogens which impacts the biological activity of each of the various estrogens present in CE in estrogen target tissues (Bhavnani et al. 1998).

Elimination

Troy et al. (1994) reported slow renal elimination of conjugated and unconjugated estrogens (T_{half-life} 12–16 h) and a long mean residency time (16–29 h) in healthy PostM women. Following intravenous administration of [13C] EqS and [13C]-17ß-dihydroequilin to PostM women, over 40 and 46% of the total dose, respectively, was excreted in the urine in 3 days with more than 73 and 63% of the administered dose excreted as the glucuronides and about 17 and 16% of total dose as sulfates; only 1.3 and 1.7% were found in the unconjugated fraction, respectively (Bhavnani et al. 2002). No statistically significant sex differences in the elimination rate and MCR of Eq and E1S were reported (Bhavnani et al. 1998).

Mechanism of action

Results of bioassays suggest that all estrogens in CE in their unconjugated form are biologically active and can interact with human ERα and ERβ in a stereo-specific and structure-dependent manner with, in general, higher potency for ERβ. Available data indicate that the increased in vivo estrogenic activity of CE might be associated with the formation of 17ß-reduced metabolites of ring B unsaturated estrogens (Bhavnani et al. 2002). In contrast to E2, ring B unsaturated estrogens exerted their biological effects mainly through the interaction with the ERβ and not the ERα (Bhavnani and Stanczyk 2013). However, no association between the strength of binding affinity and functional activity of CEs has been observed (Bhavnani et al. 2008). Bhavnani et al. (2008) suggest that the biological activity of different equine estrogens (17ß-EqS and EqS) depends on the existing ratios of ERα and ERβ, baseline levels of E2 at the estrogen target cells and the structure of the estrogen ligand in the particular individual (Bhavnani et al. 2008). In addition, different components of CE formulation have different potencies on different receptor isoforms (e.g., ERα or ERβ, as well as the other ERs) which may partly explain why particular cells and tissues have different sensitivities to endocrine effects. All CE are antioxidants with a number of ring B unsaturated estrogens having higher antioxidant activity compared to the E1 and E2 (Bhavnani and Stanczyk 2013; Figure 6). The ability of CE to prevent osteoporosis and cardioprotective effects in some women might be facilitated by its ability to prevent the formation of oxidized LDL and HDL (Bhavnani and Stanczyk 2013).

Summary

Following oral exposure, CE undergoes rapid absorption and first-pass metabolism. CE can be absorbed directly as CE sulfates in the gastrointestinal tract and also after hydrolysis in the gastrointestinal tract as the unconjugated estrogens. In the liver, these estrogens are sulfated rapidly and circulate in the blood. The unconjugated CE are cleared from the circulation more quickly compared to their sulfate forms (Bhavnani et al. 1998, 2000) which might be partially explained by the fact that the sulfate forms of these estrogens can bind to albumin with a higher affinity than their unconjugated form (Bhavnani 1998).

Knowledge gaps

The PKs of the CE are complex due to the large number of biologically-active components with varying degrees of binding affinities for transport proteins, bioavailability, different rates of metabolism and elimination, component-specific combinations and associated estrogenic activity. Because of this complexity, the exact mechanism of CE action for biological and clinical effects (e.g., relief of the vasomotor symptoms in
PostM women, alterations of risks for osteoporosis and CVD) has not been fully elucidated. The assumption that biological activity of all estrogens is always related to binding affinity does not hold with respect to the CE where the structure rather than the binding affinity is thought to have a major influence on estrogenic activity (Bhavnani et al. 2008).

Historically, CE has been used extensively for HRT and prevention of osteoporosis and CVD in Peri- and PostM women. However, no overall long-term benefit of HRT on coronary heart disease (CHD) was reported in the randomized therapy in Heart and Estrogen/progestin Replacement Study (HERS) (Grady et al. 2002, Herrington et al. 2000, Hulley et al. 1998). It has been suggested that the addition of medroxyprogesterone acetate to the CE used in HERS may have negated any cardiovascular benefit of estrogen (Grady et al. 2002). CE are a complex mixture containing at least 10 biologically active estrogen compounds (Bhavnani et al. 1998). The mechanisms by which estrogenic compounds interact remain poorly understood and further clarification is needed regarding whether reported effects are related to the type and amount or biological activities of the individual estrogen components of CE or to combinations with progesterin. Clearly, given the concerns about phytoestrogens and xenoestrogens, elucidating these mechanisms for pharmaceutical estrogens represents an opportunity to better understand the benefits and risks of complex estrogen mixtures and diverse types of estrogens, as an example the complex estrogens found in the environment and foods containing isoflavones.

At present, there is no published PBPK model for CE. This is especially unfortunate given the questions concerning the biological and health consequences of exposures to complex estrogen mixtures. However, such a model, comprising ~10 estrogenically active components, would certainly be a challenge, both from the pharmacokinetic and pharmacodynamic perspectives (Tan et al. 2011). Such models for the CE could substantially advance our understanding of both the PK and PD of complex estrogen mixtures irrespective of their source: therapeutic, food, or environmental.

Phytoestrogens: Genistein, daidzein

Structurally, soya isoflavones, mainly Da and Ga and the estrogenic metabolite of Da, S-equol, are remarkably similar to estrogens (Table 1) (Setchell 1998); this feature allows them to bind to ERs exerting either estrogenic or antiestrogenic activity (Setchell et al. 2005). Human exposure to these plant estrogens is common due to widespread use of different soy-based products. Additionally, they are found in supplements with various proposed health benefits.

Absorption

Dg and Gg (Figure 7) are present in most soy products as a complex mixture of glucoside conjugates that are not bioavailable (Setchell et al. 2002b). Following ingestion, daidzin and genistin glucosides are hydrolyzed by intestinal beta-glycosidases to the aglycones Ga and Da (active estrogenic forms) which are further metabolized by intestinal microflora (Decroos et al. 2005, Lu and Anderson 1998; Figure 8). PK data on soy isoflavones administered in different forms show that the bioavailability of Ga and Da does not differ significantly; however, the rates of absorption for the glucoside and agluco-side forms of isoflavones are different (Setchell et al. 2003a). Setchell et al. (2001) reported that $T_{\text{max}}$ values for Ga and Da were 5.2 and 6.6 h, respectively, whereas the corresponding values for genistin and daidzin were delayed to 9.3 and 9.0 h, respectively, possibly, due to hydrolysis by intestinal brush border beta-glycosidases (Day et al. 1998). A nonlinear relation between bioavailability and ingested dose was observed suggesting rate-limited and saturable uptake (Setchell et al. 2003b), and, subsequently, a more appropriate strategy for optimization of the dose used in clinical and dietary studies is needed, to provide appropriate internal concentrations of the isoflavones.

Distribution

Most of the absorbed Ga and Da are conjugated in plasma (Nielsen and Williamson 2007). Unconjugated Ga represents only about 1.1–1.5% of the total plasma pool of Ga (Setchell et al. 2001), much like that observed for other endogenous and therapeutic estrogens. The low level of unconjugated isoflavones reflects the rapid hepatic glucuronidation of these compounds (McCarty 2006). About 50% of the unconjugated Ga in serum is bound to albumin (McCarty 2006, Nagel et al. 1998).

Metabolism

After ingestion, isoflavone glucosides undergo hydrolysis to their aglycones in the apical membrane of the lumen of the small intestine, and by bacterial glucosidases (Setchell et al. 1984, Vitale et al. 2013, Xu et al. 1995; Figure 8). Da is metabolized to form ODMA (non-estrogenic metabolite) and S-equol (estrogenic metabolite), whereas Ga is metabolized to the non-estrogenic p-ethyl phenol. Additional metabolites which have been identified in human plasma or urine include dihydrodaidzein, dihydrogenistein, dihydroequol, and 6-hydroxy-O-desmethylangolensin (Manach et al. 2005).
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Elimination

Elimination of Ga and Da is similar with half-lives of 6–8 h (Manach et al. 2005). In general, urinary excretion of isoflavones is almost complete within 48 h after ingestion (Lu and Anderson 1998, Phipps et al. 2002, Xu et al. 2000). Significantly longer excretion times have been observed in patients with renal disease (Fanti et al. 1999). This is concordant with the understanding of the clearance of many drugs for which renal clearance is the major route of elimination. The significantly slower clearance rate reported for Ga compared to Da might explain the higher (1.5–2.0 times) plasma Ga concentrations compared to Da. For fecal excretion, large (10–20-fold) inter-individual differences have been reported (Xu et al. 1995). A bi-phasic peak in plasma and urine Ga and Da concentrations has been observed in a number of subjects, suggesting enterohepatic recirculation (illustrated in Figure 8).

Mechanism of action

There are many mechanisms by which isoflavones may affect estrogen action. Most obviously, isoflavones may bind ERs, with much greater affinity to ERβ compared with ERα (Kuiper et al. 1998). In addition to direct ER binding, isoflavones may influence sex steroid action by their inhibitory effects on many enzymes involved in steroid metabolism (Adlercreutz et al. 1993, Kao et al. 1998). Isoflavones have been reported to down-regulate aromatase mRNA in human granulosa-luteal cells which may affect in situ steroidogenesis (Rice et al. 2006, Vitale et al. 2013). Phytoestrogens can also induce rapid non-genomic actions that include effects on plasma membrane and on cell signaling pathways (Kim et al. 1998 and Nilsson et al. 2011, cited by Vitale et al. 2013). Ga, the most extensively studied soy isoflavone, exerts conformational binding to the estrogen receptor that identifies it as a natural selective estrogen receptor modulator (Brzozowski et al. 1997, Pike et al. 1999).

For the phytoestrogens under consideration here, the binding to ER differs and as a result biological responses differ, as well (McCarty 2006). At low concentrations, unconjugated free Ga has agonist binding activity for both ERα and ERβ but with about an order of magnitude lower affinity for the “classical” ERα. As a result, physiological effects mediated through the activation of ERβ might explain Ga’s clinical activities. For example, hepatocytes do not express ERβ which explains why soy isoflavones, in contrast to oral E2, do not affect serum lipid profiles or induce the prothrombotic effects associated with increased risk for thromboembolic disorders. Vascular endothelium expresses both ERα and ERβ which can explain the positive effects of soy isoflavones on endothelial function in PostM women. The osteoblasts express ERβ which might mediate the beneficial impact of soy isoflavones on bone metabolism. The suggestion (Setchell and Clerici 2010b) that soy-derived products might decrease prostate cancer risk is based on the observation that ERβ appears to play an anti-proliferative role in healthy prostate. Ongoing clinical studies with administration of Ga in early prostate cancer may clarify this hypothesis.

Phytoestrogens: S-equol

Equol is a major metabolite of daidzin and Da with the conversion from the glycoside greater than from aglycoside (Figure 7; Axelson et al. 1982, Setchell et al. 2001). Equol could be responsible for the clinical effectiveness of soy-based products.
(the so-called “equol-hypothesis”) (Setchell et al. 2002a, Setchell and Clerici 2010b). Equol exists in two enantiomeric forms, R(+)−equol and S(−)-equol but it has been reported that in humans and animals only the S(−)-equol (referred to here as “equol”) can be produced by the intestinal microflora (Setchell et al. 2005). Little is known about the PK of the diastereoisomers, despite existing interest in the potential of both forms for the prevention and treatment of estrogen- and androgen-dependent conditions such as prostate cancer (Setchell et al. 2009a). Clinical studies with soy isoflavone supplementation show inconsistent results which may reflect, at least in part, variations in the plasma levels of free equol achieved by the different supplementation regimens. These levels might also depend on the ability of humans to convert Da to equol which varies from person to person (“equol–producers” or “equol–non-producers”).

Absorption
Equol is readily absorbed from the gastrointestinal tract and conjugated to glucuronic acid in the liver (Axelson et al. 1982). The decreased fractional absorption of isoflavones with an increase dose observed in human studies is due to saturation of the absorption processes (Setchell et al. 2003b).

Distribution
Approximately 50% of the absorbed equol appears in the free form in comparison with 18.7% Da (Nagel et al. 1998) or E2 (4.6%) and may explain the enhanced potency of equol (Setchell et al. 2002a). Equol binds to SHBG and competitively inhibits E2 and testosterone binding in a dose-dependent manner (Martin et al. 1996).

Metabolism
Gut microflora play a critical role in the biotransformation of Da to equol as evident from studies demonstrating that germ-free animals as well as infants below the age of 4 months who have a poorly developed gut microflora do not produce equol when fed a soy diet (Magee 2011, Setchell et al. 1997). Equol serum levels are higher after ingestion of daidzin Dg compared to the corresponding levels following ingestion of aglycone Da, possibly because Dg has longer transit time in the gastrointestinal tract (Zubik and Meydani 2003). Equol does not undergo further biotransformation (Setchell et al. 2002b).

The ability to produce equol differs among adult populations; on average, 33% of North American women versus 60% of Asian adults (Liu et al. 2010, Magee 2011, Tanaka et al. 2009) are able to metabolize daidzin and Da to equol, possibly explaining discrepancies in health effects of soy-containing food. A greater number of equol producers have been observed among PostM women compared to PreM women, suggesting that age might be a determinant for the metabolism of isoflavones (Faughnan et al. 2004, Nielsen and Williamson 2007). Inter-individual variations ability to produce equol has been attributed to a number of factors, such as availability of specific intestinal equol-producing bacteria, gut transit time, host genetics, and dietary composition (presence of daidzin and Da as a substrate) and modifications (e.g., fiber, meat, fat, and alcohol intake) (Lampe 2009, Setchell et al. 1984, 2002a, b).

Excretion
In healthy humans, equol is excreted almost exclusively as the highly water soluble monoglucuronide conjugate (99%); no sex- or hormone-related differences have been reported but studies with larger numbers of participants are needed (Axelson et al. 1982). Reported maximum urinary excretion occurs between 24 and 72 h after soy product ingestion (Axelson et al. 1982). Fecal excretion of equol is a relatively minor route of elimination and has been shown to be higher on 5–6 days compared with 4 days after intake, suggesting that much of the fecal equol represents biliary excretion (Watanabe et al. 1998).

Mechanism of action
Equol is a selective ERβ agonist with weaker activity for ERα (Setchell et al. 2005) and more estrogenic than Da (Kelly et al. 1995). Equol binds to ERβ with approximately 20% as much affinity as E2 (Larkin et al. 2008, Muthyala et al. 2004, Setchell et al. 2005) and is reported to be antagonistic to E2 by competing for cytoplasmic ERs (Tang and Adams 1980). In addition to its estrogenic properties, equol potently antagonizes dihydrotestosterone (DHT) in vivo (Lund et al. 2004), the suggested mechanism by which equol enhances prostate health (Douglas et al. 2013). Further, equol in vitro inhibits binding of E2 and testosterone to SHBG in a dose-dependent manner (Magee 2011, Martin et al. 1996).

Summary
Isoflavones contain an aromatic ring with hydroxyl group (Table 1, Figure 7) similar to E2 and also bind to both ERα and ERβ with a significantly higher affinity for the ERβ than for the ERα. However, the affinity of isoflavones to the ERs is lower than that of E2 (Kuiper et al. 1998). Ga has the highest estrogen receptor binding affinity to ERβ among isoflavones which can be associated with the presence of an aromatic ring with three hydroxyl groups. In soy proteins and most soy-based products, genistin and daidzin are conjugated to sugars; consequently for absorption and metabolism, they require further hydrolysis in the gastrointestinal tract through the action of the brush border membrane and the bacterial β-glucosidases (Day et al. 1998). Hydrolysis also leads to formation of glycitein which is not further converted. Glucosides are metabolized by intestinal microflora forming the active compounds Ga and Da. Both Da and Ga are readily absorbed reaching Cmax (peak serum or plasma concentration) between 2 and 8 h after ingestion and are then slowly eliminated (Setchell et al. 2002a). The higher clearance rate and volume of distribution contribute to lower serum concentration of Da compared to Ga (Setchell et al. 2003b). No correlation between concentrations of Da and Ga in serum and urine has been observed, suggesting that the urinary isoflavone concentration provides only an approximate indication of the dietary isoflavone intake and that bioavailability cannot be assessed from urine excretion only (Setchell et al. 2003b). Considerable inter-individual variation in plasma concentrations and excretion profiles for Da and Ga and their metabolites has been observed (Setchell et al. 2003b). Some individuals produced little or no ODMA and S-equol, and inter-individual variation in ability to metab-
olize Da to S-equol could influence the potential health protective effects of soybean isoflavones, soy-based pharmaceuticals or nutritional supplements. Only 20%–30% of the Western population that consumes soy foods is able to produce S-equol (Setchell and Cole 2006). The PK of the isoflavones from food sources is similar among healthy women; saturable rate-limiting uptake with higher dietary intake and a nonlinear relationship between bioavailability and dose ingested were reported. Setchell et al. (2002b) suggested that consuming modest amounts of isoflavones will better contribute to the steady state plasma isoflavone concentrations and relevant health effects due to rate-limiting uptake of aglycones. However, bioavailability of the ingested soy products depends on the effect of certain food matrices on the dynamics of absorption (Coldham et al. 2000, Setchell et al. 2003a). A high-fiber diet has been implicated in increased elimination of estrogens, possibly by decreasing gut transit time (Lewis et al. 1997).

Knowledge gaps

Both beneficial and adverse health effects of isoflavones are dependent on the plasma concentration and duration of systemic exposure at the target organ (Polkowski and Mazurek 2000). Available data concerning the PK parameters of Ga, Da, or equol, including bioavailability, are sometimes contradictory depending on experimental conditions (model selection, dose/concentration, forms and types of the administered compound, time of exposure, dietary matrix, sampling time, etc.; Setchell et al. 2003a, b). The inability to distinguish between subjects who are “equol-producers” and who are “non-equol producers” in previous clinical studies might explain the inconsistency in reported data on the PKs, PDs, adverse effects and health benefits of soy products (Setchell et al. 2002a). Intra-individual variability is another important knowledge gap; several factors might contribute to this including variation in the isoflavone content in food, bioavailability of isoflavones from the food matrix, lactase persistence status of the test population, variations in the gastrointestinal microbiome and intestinal brush border flora, and variations in the gastric transit times. It is generally considered that glycosylation of the (iso) flavonoids facilitates a delay of the intestinal absorption until metabolism in the large intestine by colonic microflora releases aglycones (Kühnau 1976). The mammalian β-glucosidase lactase phlorizin hydrolase (LPH) is present on the luminal side of the brush border in the small intestine and can deglycosylate isoflavonoid glycosides within the gut lumen before absorption (Semenza 1987). This enzyme is genetically regulated with levels normally declining during adolescence and over 75% of the world’s population has the non-persistent LPH phenotype (Flatz 1987). LPH persistence genotype/pheno type of the test population can also affect inter-individual absorption of isoflavones from the food matrix. More studies are needed to characterize the profile of conjugated and unconjugated isoflavones present in human plasma following different forms of isoflavones ingested. Investigation into genetic determinants influencing the bioavailability of isoflavones is another important area of further research (Setchell et al. 2002a). Questions related to whether data from administration of pure compounds can directly be extrapolated to isoflavones within the food matrix and whether the food matrix can alter the PK and bioavailability of isoflavones remain to be answered. Further, it is unclear what would be the optimal doses required to deliver particular health effects to humans. In addition, the safety issues involved in consuming pharmacologic doses are presently unknown. Very few studies have assessed the long-term impact of various isoflavone dietary regimens on exposure to free Ga or equol. It should be mentioned that short-term pharmacokinetic studies do not take into account possible adaptive changes in enzyme expression that could influence plasma levels (McCarty 2006). Therefore, long-term studies in diverse populations, including equol versus non-equol producers, are needed to elucidate the effectiveness of soy protein diets in the treatment or prevention of hormone-dependent conditions, as well as the beneficial or adverse effects and therapeutic value of these compounds (Setchell et al. 2003b).

PBPK model for genistein

There is widespread human exposure to Ga via diet. Thus, it is important that a PBPK model that improves our ability to characterize specific target tissue responses to this phytoestrogen with detailed tissue dosimetry has been developed (Schlosser et al. 2006, Zager et al. 2007). The PBPK model includes enterohepatic recirculation, plasma protein binding of Ga and its conjugates, multi-compartment transport through bile and gut, and simple diffusion into tissues. The model was developed from data from male and female Wistar rats and male and female Sprague–Dawley rats (Schlosser et al. 2006). The PBPK model contains two parallel sub-models: one for the parent Ga and the other for all conjugates of Ga. The model also includes explicit treatment of gut uptake and elimination as well as renal elimination, which is necessary given the gastrointestinal microbiome and intestinal brush border metabolism of Ga (Nielsen and Williamson 2007). Because of first pass metabolism, the majority of Ga in the plasma is conjugated, and consequently tissue uptake is predominantly from conjugates. Sex differences in tissue partitioning and other parameters were also included in the PBPK model.

Genistein PBPK knowledge gaps

As with the E2 PBPK model described above, limitations and errors in the Ga model predictions define areas for future research to improve our understanding of disposition of—and PD responses to—Ga. For example, while HPLC measurement of Ga and Ga metabolite plasma concentrations were available, they did not sum to the total 14C activity observed; instead, the total 14C activity was utilized.

Further, the complexity of the model requires data for many biological parameters, some of which are not available. In addition, some of the Ga metabolites may be active as estrogens while others are inactive; the model does not allow for that distinction. As currently implemented, the conjugates compartment contains all metabolites. This substantially limits the ability of the model to account for active metabolites and conjugates with different metabolic, transport, elimination, and biological behavior.

The model uses partition coefficients developed for Da. While Da and Ga are similar in structure and should have similar partition coefficients, this assumption represents an area of
biological uncertainty. Further uncertainties include a dearth of data on the partition coefficients of the diverse metabolites as well as the influence of the role of transport processes or ERs on tissue uptake and elimination of the phytoestrogens and their metabolites.

Schlesser et al. (2006) noted substantial difficulty parameterizing the model to adequately describe absorption from the gut following oral treatment. Plasma protein binding of parent and conjugates was poorly characterized, so alternative data were utilized, including plasma protein binding of Da to human plasma proteins (Csárdás et al. 2002). Finally, sex differences were observed for model parameters; however, the biological basis for those differences remains obscure. A more fully developed human PBPK model could be helpful for clarifying the main physiological effects of low nanomolar concentrations of soy isolavones and the impact of optimal intakes on health outcomes.

**Xenoestrogens: Bisphenol A**

BPA is a high production volume chemical used to produce polycarbonate plastic and which has broad human exposure. BPA’s chemical structure (Table 1) allows it to bind to the ERs, and BPA is considered a ubiquitous xenoestrogen although its ER affinity is orders of magnitude lower than that of E2 and other endogenous estrogens (Krishnan et al. 1993, Kuiper et al. 1998).

The ADME of BPA has been characterized in humans and nonhuman primates by oral routes of exposure (Doerge et al. 2010a, Patterson et al. 2013, Taylor et al. 2011, Tominaga et al. 2006, Völkel et al. 2002, 2005).

**Absorption**

BPA absorption from the gastrointestinal tract after ingestion has been studied in rodents, nonhuman primates and humans (Willhite et al. 2008). The very low absolute bioavailability of aglycone BPA in primates and humans (0.1–0.2% of total BPA dose) reflects extensive presystemic metabolism in the gastrointestinal tract and liver (Teeguarden et al. 2011). The bioavailability of BPA depends on the route of exposure and age (Pottenger et al. 2000, Völkel et al. 2011). Recently, inhalation and absorption through the skin have been proposed as potentially important routes of exposure (Liao and Kannan 2011) as inhaled and absorbed unconjugated BPA might circulate in the bloodstream longer compared to ingested BPA, which is subject to first-pass metabolism and elimination (Birnbaum et al. 2012).

**Distribution**

More than 90% of BPA in plasma is bound (Teeguarden et al. 2005). Less than 1% of the BPA circulates in an unbound and unconjugated biologically active “free” form, which is consistent with a low bioavailability following oral exposure (Völkel et al. 2002, 2005, 2008).

**Metabolism**

Following oral administration, BPA undergoes rapid and almost complete (98%) metabolism, primarily in the gastrointestinal tract (Inoue et al. 2003) and in the liver (Inoue et al. 2001) with formation of bisphenol A monoglucuronide (BPAG) and bisphenol A sulphate (BPAS). Uridine diphosphate (UDP) and glucuronosyltransferase (UGT) isoforms and sulfotransferase have been shown to metabolize BPA in the gastrointestinal tract and liver following oral administration of BPA to adult rats (Taylor et al. 2011), monkeys (Doerge et al. 2010b, 2011b), mice (Taylor et al. 2011), and humans (Völkel et al. 2002). UGT isoforms have been observed in fetal liver at levels 10–30% of those observed in adults, increasing to adult levels after 2–3 months of age (Hines 2008; Figure 9).

BPAS is another relatively minor metabolite of BPA in rats, monkeys (Doerge et al. 2010a) and humans (Ye et al. 2005, 2006) and is only formed with higher doses of BPA, likely due to saturation of the glucuronidation pathway (Völkel et al. 2002). Ginsberg and Rice (2009) suggested that human liver, kidney, and placenta can deconjugate BPAG by β-glucuronidase with formation of the free BPA; however, given the exceedingly low circulating concentrations of BPA, these tissue studies do not appear to be consistent with human observations. Lacroix et al. (2011) did not observe “back-conversion” of BPAG to the free BPA in vivo. Significant interspecies differences in metabolism of BPA in rodents, humans, and nonhuman primates have been reported. In rats, BPA undergoes enterohepatic recirculation, prolonging systemic exposure to higher blood concentrations of BPA relative to nonhuman primates and humans (Teeguarden et al. 2005, Völkel et al. 2002). However, enterohepatic recirculation in rodents may have only a modest impact on unconjugated serum BPA (Pottenger et al. 2000).

**Elimination**

In humans, BPAG is rapidly excreted in urine within 24 h with 84–97% eliminated in 5–7 h following oral administration which suggests that enterohepatic recirculation in humans does not occur (Völkel et al. 2002, 2005). No differences in the blood concentrations of BPAG were observed in males and females, suggesting that there is no significant sex difference in BPA metabolism (Völkel et al. 2002). However, a significant interspecies difference between excretion of BPA in rodents, humans, and nonhuman primates has been reported (WHO 2011, Willhite et al. 2008). The major elimination pathway of BPAG in rodents is in the bile (Inoue et al. 2001) and subsequently in the feces as BPA (Pottenger et al. 2000), whereas in humans BPAG is eliminated almost completely in urine (Figure 9; Völkel et al. 2002).

**Mechanism of action**

A number of molecular and cell-based studies suggest that BPA is a weak estrogen based on the 1000–10 000-fold lower relative binding affinity of BPA for the classical nuclear receptors ERα and ERβ compared to E2 (Gutendorf and Westendorf 2001, Hewitt and Korach 2011, Kuiper et al. 1998). BPA binds classical and nonclassical membrane ERs as well as the G-protein–coupled receptor 30 (GPR30) and acts through non-genomic pathways (Rubin 2011). However, the estrogenic mechanism via classical nuclear and nonclassical membrane-bound receptors remains the most accepted hypothesis. The relative binding affinities of BPA for ERα and ERβ, relative to E2 (with E2 = 1), have been reported as 0.00023 and 0.0026,
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respectively (Gutendorf and Westendorf 2001). Similarly, the relative binding affinities for both ERα and ERβ for other compounds of interest are: Ga-0.0001/0.032; E1-0.007/0.065; E3-0.07/0.26; and EE-1.16/1.44, respectively.

Like many other estrogens, the initial presystemic metabolism of BPA changes the structure and ER binding affinity of BPA conjugates. The primary metabolite BPAG circulating in the blood does not bind to ER (Krishnan et al. 2010, Völkel et al. 2002, 2005) in either in vitro or in vivo test systems (Matthews et al. 2001). BPAS does not bind ER and is also not an estrogenic compound (Willhite et al. 2008). Understanding this initial presystemic metabolism is crucial to our characterization of risks from BPA exposure.

Summary

After oral administration at low doses, BPA is extensively absorbed from the gastrointestinal tract. Presystemic metabolism in the gut and liver transforms BPA to BPAG, which has no significant estrogenic activity. In rodents, after oral exposure, BPAG is excreted in bile with subsequent enterohepatic recirculation and reabsorption and fecal excretion.

Knowledge gaps

Although BPA TK has been studied in both primates and humans, a number of inconsistencies exist in the reported findings due to limitations in the experimental study designs which make many of findings of limited value for low-level human exposure risk assessment. The main limitations include: analytical methodology (e.g., use of total radioactivity) or lack of measurements of both aglycone BPA (i.e., unconjugated and active) and conjugated (i.e., inactive glucuronides and sulfates) forms of BPA, and the evaluation of internal dosimetry in the “low dose” intervals (Teeguarden and Hanson-Drury 2013). Inadequate reporting of the sample preparation and analytical methods in some studies limited an opportunity to ascertain if the methods used were fully validated, or if there was appropriate use of solvent standards, blanks, and matrix controls to assure data quality. Evidence exists supporting a possibility for contamination of blood by aglycone during sample collection and analysis (Markham et al. 2010, Ye et al. 2013). An adequate assessment of the potential impact of either postexposure sample contamination or unintentional exposure to BPA from environmental sources. No measurements of the endogenous estrogens were conducted to compare relative ER occupancy as a contributor to the observed outcomes and to critically test an estrogenic mode of action for BPA.

Some studies used high doses of BPA which are outside of the linear range of TK or used only non-oral routes of administration (WHO 2011). Different protocols (i.e., different vehicles, individual versus pooled serum samples, different volume of analytical sensitivity and volumes analyzed) introduce uncertainty in the comparison of reported findings. Overall, it has been suggested that interpretation of results from studies using “low dose” BPA may be difficult (Churchwell et al. 2014).

Recent research suggests that BPA can stimulate responses via ERs associated with the cell membrane at very low (ppb) concentrations equivalent in potency to E2 (Quesada et al. 2002, Vom Saal et al. 2007, Wozniak et al. 2005, Zsarnovszky et al. 2005); however, a biologically-plausible explanation for effects that appear at low doses is currently lacking (Chapin et al. 2008). In addition, a definition of “low dose” in relation to BPA exposure is inconsistent. The recent NTP-CERHR (2008) report defined a “low dose” as ≤ 5 mg/kg body weight per day. At the same time, it was also suggested that the “low dose” should be considered as the dose administered to animals which produce blood levels in the range of those measured in human tissues and fluids (0.1–4.0 ng/ml) (Vanderberg et al. 2012) (discussed in Birnbaum et al. 2012).

When administered under high E2 levels, BPA may act as an anti-estrogen (Zsarnovszky et al. 2005). BPA has been shown...
to act differently compared to E2 with the domain of the classical ERs, and in the recruitment of transcriptional coregulators (Routledge et al. 2000), suggesting that interpreting the physiological and toxicological effects of BPA solely within the context of consistency with a classic estrogenic mechanism of action may be simplistic (Chapin et al. 2008) and many critical questions about the mechanism of BPA’s action remain to be answered. Considerable disagreements exist regarding TK at low BPA exposure, identification of molecular mechanisms of actions mediating low dose effects in human and animal tissues and in vivo effects in experimental animals caused by doses within the range of human background exposure to estrogens (Welshons et al. 2006). Whether or not serum concentrations of BPA in humans are sufficiently high to disrupt normal estrogen-related biology is also the subject of intense debate (Teeguarden et al. 2013a, Teeguarden and Hanson-Drury 2013).

The most recent systematic review of low-dose BPA suggests that the generalizability of the observed effects of BPA in in vivo and in vitro studies is limited and has not been assessed in the context of realistic human exposures (Teeguarden et al. 2013a, Teeguarden and Hanson-Drury 2013).

**PBPK model for BPA**

While more than one PBPK model has been developed for BPA (Christensen et al. 2013, Doerge et al. 2011a, 2012, Fisher et al. 2011, Shin et al. 2010, Taylor et al. 2011, Teeguarden et al. 2013, Vom Saal et al. 2014, Yang et al. 2013), we focus here on the human BPA PBPK models developed by Teeguarden et al. (2005), Fisher et al. (2011), and Yang et al. (2013). The work by Teeguarden and colleagues is of interest because it is a modification and extension of the original E2 PBPK model (Plowchalk and Teeguarden 2002). The model contains two sub-models (for BPA and BPAG), as well as enterohepatic recirculation and plasma protein binding, with the uterus as a target tissue (uterine wet weight). This PBPK model is a substrate-specific extension of the E2 model, including the formation of the major, biologically-inactive metabolite BPAG, but with fewer complexities compared to the Ga PBPK model. Inclusion of the uterus allows evaluation of PD (changing uterine weight based on potential ER occupancy). While simulations of BPA concentrations were considered by the model authors to be reliable, those of BPAG concentrations were less adequate, and like the Ga/Ga conjugate simulations, challenges in modeling gut disposition of BPAG were thought to represent a gap in biological understanding.

The work by Fisher and colleagues (Fisher et al. 2011, Yang et al. 2013) builds on the extensive work done in their laboratories on oral and intravenous dosing of nonhuman primates with deuterated BPA (d6-BPA) (Doerge et al. 2010b), as well as data published by Taylor et al. (2011), and data collected in humans by Völkel et al. (2002). These disposition data and modeling exercises have been critical in understanding the role of metabolism of BPA to the conjugated metabolites (BPA-glucuronide [BPAG] and BPA-sulfate [BPAS]), deconjugation, enterohepatic recirculation and a new observation in this discussion: reabsorption from the urinary system (kidney, ureters, and bladder). One of the challenges observed by Fisher et al. (2011) is the use of data without analytical method verification and concerns about contamination (Calafat et al. 2013), and that appeared to obscure model performance across the datasets. Additionally, understanding the role of media in oral absorption as well as gastrointestinal absorption and metabolism continues to represent a challenge (Fisher et al. 2011), however, subsequent work by Yang et al. (2013) has clarified the role of enterohepatic recirculation. Taken together, these models suggest that very little unconjugated BPA circulates in human blood (<1% of the total oral dose) as a consequence of rapid presystemic metabolism, with clearance in the liver being flow limited (Yang et al. 2013). Further, as noted for E2 and Ga PBPK models, data for plasma protein and tissue binding are needed, as is research to characterize the roles of transport processes for influx and efflux of the substrate from the target tissues. In addition, Teeguarden et al. (2005) observed interactions between the BPA and BPAG PBPK models, but the underlying biological processes remain to be elucidated and research to define those mechanisms is needed.

While estrogenicity is thought to reside in BPA with little estrogen activity for BPAG, it will also be important to fully characterize ER binding across all types of ER and/or interactions by both BPA and BPAG, especially given the broad ligand capability of the ER ligand binding site.

**PBPK knowledge gaps**

First pass metabolism of BPA to BPAG involves both intestinal and hepatic glucuronidation. These gut and intestinal processes were included in a non-explicit way in the PBPK model; however, given the potential importance in characterizing metabolism to BPAG as well as the potential for enterohepatic recirculation and reformation of BPA (with its estrogen activity), a fuller characterization of these processes is essential.

**Summary**

The estrogenic chemicals in this review include: E2, EE, CEs, Ga, Da, and S-equol, and BPA (Table 1). These were selected because of clinical interest in understanding ADME and PD as well as clinical and population health interest in potential beneficial and adverse effects associated with exposure to EACs. All of these compounds exhibit some form of estrogen activity, binding to ERs in their various locations (nuclear, cytoplasmic, cell membrane) and producing forms of estrogen responses in vivo and in vitro. Despite the many years of research on these chemicals, there are gaps and uncertainties in the understanding of PK as well as biological responses and health effects. These gaps and uncertainties point to several foundational limitations in our understanding of the “estrogenicity” of chemicals representing three major classes of estrogen-active compounds (endogenous, phytoestrogens, and xenoestrogens). The following are major recurring themes that cut across these chemicals and point to specific research needs:

- Recent research on the TK of BPA and Ga has highlighted the impact of hepatic first pass metabolism in converting these compounds to their estrogically inactive glucuronide forms (Teeguarden and Barton 2004, Teeguarden et al. 2005). However, the lack of robust information on gastrointestinal-hepatic processes limits further understanding and modeling of enterohepatic recirculation.
- There is a need for a better understanding of important determinants of the disposition of E2—and presumably
other EACs—including ER binding, metabolic clearance, plasma protein binding and tissue uptake.

- It is important that researchers develop an improved characterization of plasma protein binding, which is essential to understanding the potential fraction of free chemical available for uptake and pharmacological action.

- There is a lack of sufficient information on gut handling of EACs. Given that internal disposition begins with oral exposure and absorption, it is clear that the complexities of transport through the gut, metabolism by the gut microbiome and brush border enzymes, as well as absorption and elimination from intestinal cells (enterocytes) need substantially more attention.

- Research is needed on the impact on PK and the consequence of exposure to estrogen mixtures in terms of their potential cumulative effects.

- Characterization of the basis for gender differences in PK and PD following exposure to EACs is needed.

- There is an overall gap in our understanding of the effect of low-dose exposure on PK parameters and biologic changes that occur in the range of human exposures or environmentally relevant doses. Further research in this area is needed to better understand the fundamental biology underlying adverse versus non-adverse effects and dose-response relationship for environmentally relevant low-dose exposures.

- Studies of PK parameters need to build on past studies such that difference in study design does not limit our ability to interpret the data.

The PBPK models developed for three general classes of estrogens described in this review (Doerge et al. 2010b, Fisher et al. 2011, Plowchalk and Teeguarden 2002, Teeguarden et al. 2005, Schlosser et al. 2006, Zager et al. 2007) offer insights into our current understanding and identify gaps in knowledge concerning the PK and PD of these chemicals (Archer 2013, Khalil and Laer 2011, Thompson and Beard 2012). PBPK models are of interest in drug development and risk assessment for environmental exposures because they offer the opportunity to characterize internal dosimetry to specific tissues which may be targets for these chemicals (e.g., using PBPK models, the response of reproductive organs to circulating estrogens can be quantified). Because these models are data-rich—requiring substantial information about biological processes involved in ADME—they explicitly highlight data deficiencies in our understanding of PK. The data which underlie these models also can be used to illustrate similarities and differences in the PK process across types of estrogens.

Due to the lack of experimental protocols approved by authoritative bodies for PK studies on EAC as a group (with the exception of regulatory guidelines for therapeutic products), important limitations of the existing human studies include considerable variation in experimental conditions (model selection, dose/concentrations applied, forms and types of the administered compounds, exposures to mixtures of compounds, timing of exposure, lack of data on dietary matrix, sampling strategy, control of co-variables, presence of contaminants, etc.) and as a result available data on PK are inconsistent. Significant inter-study variations together with inter-individual variability further complicate data comparison across studies. Contributions to variability from possible analytical errors could not be excluded, as a variety of methods were used (e.g., colorimetric, HSGC, GC-ECD). Our empirical analysis/hypothesis suggests that although data are suggestive for possible similarities across EACs, variability and uncertainty in estimating total metabolism are substantial across studies. As a result, it is not possible to predict effects of EACs based on only PK. In the future, studies of PK parameters to characterize low-dose effects of putative estrogenic compounds should build on past studies such that difference in study design does not limit our ability to interpret the pharmacokinetic parameters. This will only be possible when there are standard testing paradigms with methodological approaches regarding dose and experimental modes selection, testing regimens and adequate analytical methods that can identify endocrine-related pharmaco- and toxicokinetic changes. These standardized toxico- and pharmacological testing paradigms need to be designed to be able to compare and interpret the data across multiple EACs.

Conclusions

By describing a chemical as “estrogenic”, a certain level of understanding of biological effects and health implications is implied. This is not unreasonable, as the foundational chemical, E2 and its pharmaceutically-derived counterparts have been used and studied for decades. However, despite these decades of research on PK and PD of estrogens used in OCs and HRT, the science even for these relatively well understood agents is still incomplete (Archer 2013). By contrast the EACs reviewed here include natural and synthetic chemicals that are thought to share a common mode of action (Silbergeld et al. 2002) but represent a heterogeneous and less studied group of chemicals. While their common mechanism of action may suggest that they produce or contribute to similar health effects, nevertheless, due to critical differences in basic properties of these chemicals and other related factors (e.g., chemical structure, basic PK parameters, tissue-specific responses arising from differences in the receptor levels, levels of coactivators and corepressors, endogenous E2 metabolism, receptor stability, different target gene estrogen response elements, gene silencing), the possibility for read-across among a group of these chemicals and an opportunity to predict with certainty any potential benefits/adverse health effects in one compound based on the effects observed from another chemical in the same group is, in fact, limited. The reported health benefits of phytoestrogens stand in contrast to the proposed adverse health consequences of the xenoestrogens and further emphasize the importance and complexity of estrogen hormone action and highlight the known capacity of EAC to exert both beneficial and adverse effects via ERβ and ERα, respectively (Ellem and Risbridger 2009).

The following examples illustrate two somewhat unexpected and important gaps in our understanding of the effects of exposure to intensively-studied estrogens and point to the need for caution when drawing conclusions about less well-studied estrogenic compounds. Until recently, the use of HRT in PostM women was thought to protect against development of CVD (Turgeon et al. 2004, 2006). However, two large clinical trials—the Women’s Health Initiative (WHI)
and the Heart and Estrogen/progestin Replacement Study (HERS)—observed that HRT initiated a decade after menopause produced an unexpected increase in CVD, including venous and arterial thrombosis (Gomes and Deitcher 2004, Turgeon et al. 2004, 2006). Given the extensive experience with observational studies on initiation of estrogens in the perimenopause and the protection against CVD, these were surprising findings that have substantially reshaped public health messages concerning the use of HRT for prevention and treatment (Sarrel et al. 2013). More recent analyses of the data as well as application of a more complete consideration of the basic sciences to these observations have suggested that the endocrine status at the time of initiation of therapy and the choice of drug have a potent influence on health benefits and risks. While many women in the WHI initiated treatment a decade or more beyond menopause, earlier studies had focused on women in the immediate perimenopausal period; this difference in age at initiation of treatment has been suggested to be responsible for the difference in benefit-risk profile (Turgeon et al. 2004, 2006). The combination of clinical and basic science information emerging from the WHI highlights the problem that the characterization of a chemical as an “estrogen” is in fact a very general characterization. In terms of understanding the implications of exposure to environmental estrogens, the WHI reinforced the concept that different estrogens are recognized differently by the various ER, in turn producing different responses.

In addition to the newly discovered complexities associated with timing of exposure, attention is now being paid to differential risks from different HRT estrogens. Although there have been decades of clinical care based on the assumption that PostM HRT is beneficial, and that CE with or without gestational agents is the drug of choice, new studies have propelled us to change our assumptions and modify, correct, or completely change our practices (Nabel 2013). This was highlighted by two recent papers with respect to the benefit-risk ratio of CE therapy from the WHI (Manson et al. 2013) and the differential risks of CE and E2 in PostM women (Smith et al. 2013). Results from the 13-year follow-up studies in the WHI illustrate even more clearly the complexity of PD with EACs. In the HRT component of the WHI, treatment was randomly assigned to either CE/medroxyprogesterone acetate (MPA) (in women with a uterus) versus placebo, or CE alone (in women without a uterus) versus placebo to evaluate the effects of HRT on CVD, breast and colon cancer and bone fracture. Both HRT treatment groups experienced increased risk of CVD with an increased risk of heart disease only in the CE/MPA group and not in the CE only treatment group (Manson et al. 2013). In addition, as suggested by others (Turgeon et al. 2004, 2006), the degree of risk was associated with the timing of HRT treatment relative to menopause (Manson et al. 2013).

Further, a recent observational study comparing risks of CVD in women taking CE or E2 as the estrogen in HRT (Smith et al. 2013) found that the risks of venous thrombosis and possibly myocardial infarction were higher in women treated with CE than E2. However, whether E2 is in fact lower risk compared with CEs in such patients must be left to more definitive studies, according to the authors’ opinion. The authors also observed that CE use was associated with higher activated protein C sensitivity, suggesting greater clotting potential with this estrogen in HRT. Finally, recent findings indicate that OC use in adolescents was associated with lower bone mineral density (BMD) than non-users; however, no difference was noted in adult OC users (Scholes et al. 2010, 2011). Whether OC treatment of adolescents and younger women affects the development of optimal bone density when using lower doses of EE (e.g., 20 μg) in newer EE- or E2 based OCs need to be answered (Sørdal et al. 2012).

Clearly, the benefits and risks associated with HRT are complex, and as these two very recent studies illustrate, can vary substantially by endocrine status at the time of initiation of treatment, as well as the estrogen used. The specific HRT estrogens are neither “good” nor “bad”, but rather interact with ER in various locations and as a result can produce different biologic responses. Those responses depend on the estrogen, the endocrine status of the individual, the dose and duration of treatment or exposure and the amount of EAC reaching the receptors in various tissues or organs.

The gaps in our understanding of PD of estrogens used in the clinical setting provide important lessons as we consider the estrogenic effects of phytoestrogens and xenoestrogens. Each estrogenic compound behaves in a unique manner in the body, and responses of specific tissues are dependent on the tissue environment when exposed to the estrogen. These issues are part of the critical characterization of PD that remains limited.

In conclusion, despite our long history of clinical and basic science investigations using E2, EE, and CE, questions are still emerging on PK, PD, and health consequences. It is therefore not surprising that questions remain concerning phytoestrogens and xenoestrogens. The studies reviewed here, combined with our increasing understanding of the basic science of estrogens (PK and PD), highlight core principles that should underlie future investigations of the impact of estrogen exposures:

- Estrogen responses require that the estrogen be absorbed and distributed to the tissue in which it will bind to ERs. Studies of OCs clearly point out that the dose/concentration of the estrogen reaching the receptor determines the response: decreasing doses and circulating concentrations are associated with reduced likelihood of effects.
- While all estrogens bind to ERs, the responses are not all alike. Responses to estrogens are dependent on the estrogen receptor bound (ERα or ERβ) and its location in the cell (nucleus, cytoplasm, or cell membrane).
- The state of the cell at the time an estrogen receptor is bound by an estrogen influences the response observed. Studies of HRT have clearly illustrated that initiation of HRT at the time of the menopausal transition is associated with decreased cardiovascular risks while initiation of treatment a decade beyond menopause is associated with increasing cardiovascular risks.
- Estrogens are potent hormones which act across most organs and systems. Treatment with or exposures to chemicals which act like estrogens require careful study to understand exposure, PK and PD. While the science has advanced for many types of estrogens, there are still substantial gaps in our understanding for therapeutic estrogens, phytoestrogens and xenoestrogens.
- Linking basic science, pharmacology, clinical understanding, and population health perspectives is essential for
developing sound approaches for advancing our understanding of the effects of these pleotropic chemicals.

The gaps in our knowledge about disposition and PD are brought into focus when attempting to develop PBPK models, which require specific biological data for factors related to ADME. All of the PBPK models for the estrogens in this review had clearly defined data gaps in describing PK. Further, for the chemicals reviewed here, the ability to link the PK to tissue dosimetry and subsequent tissue responses remains to be realized.

To summarize, while over the past several decades our ability to describe the properties of estrogenic compounds (agonists, antagonists, selective estrogen receptor modulators, and endocrine disruptors) has grown, there are still substantial knowledge gaps in the understanding of PK as well as biological responses and health impact. Addressing these gaps can inform future research on the health benefits and risks of estrogens.

Declaration of interest

The employment affiliation of the authors is shown on the cover page. The authors have sole responsibility for the writing and content of this paper. LaKind Associates is a private consulting firm specializing in strategic risk management, assessment of human exposures and health risks, biomonitoring, state-of-the-science reviews, and environmental regulatory review; LaKind Associates consults to governmental and private sectors. Risk Sciences International (RSI) is a consulting company, established in partnership with the University of Ottawa, specializing in the assessment, management, and communication of health and environmental risks and their broader impacts on both public and private interests. RSI provides professional services to a wide range of clients, including federal government departments, private sector clients, and international clients. University affiliations of the authors are also shown. All authors consult to both industry and government and conduct research with industry, government and academia. This research was supported by a grant from Cefic (European Chemical Industry Council)-Long-range Research Initiative (LRI) (Grant EMMSG57). Cefic-LRI’s mission is to “identify and fill gaps in [the] understanding of the hazards posed by chemicals and to improve the methods available for assessing the associated risks” and is funded by member organizations, including producers/marketers of chemicals, including phthalates and BPA. Cefic-LRI was not involved in the design, collection, management, analysis, or interpretation of the data; or in the preparation or approval of the manuscript. Cefic-LRI received project updates and had the opportunity to comment on the study progress. The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the views of Cefic-LRI.

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