Natural Estrogenic Substances, Origins, and Effects

Catherine Bennetau-Pelissero

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C. Bennetau-Pelissero (✉)
Department of Life Science and Health, University of Bordeaux, Bordeaux, France

Department Feed and Food, Bordeaux Sciences Agro, Gradignan, France
e-mail: catherine.bennetau@u-bordeaux.fr; catherine.bennetau@agro-bordeaux.fr

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Abstract

Some natural substances have been scientifically identified as estrogenic since the late 1930s when they were found to be deleterious at high doses for cattle reproduction. Several compounds belonging to different chemical families are considered here: isoflavonoids, coumestans, lignans, and resorcylic acid lactones. This list is not exhaustive. The vegetable sources of these compounds are probably not all identified yet, but all the compounds presented here were shown to act as endocrine disruptors, i.e., modifying the hormonal natural balance, at dietary doses either in human, in cattle, or in other vertebrates.

Estrogenic compounds mimic estradiol activities and can interact nearly with all biological functions in lower and higher vertebrates. Some mollusks are also sensitive to estrogens. The effective dose is crucial to consider since as other endocrine disruptors the natural substances may have opposite effects at low, dietary, and pharmacological concentrations. Different cell pathways are triggered by the natural estrogenic substances, including some that are not influenced by estradiol itself, and this explains why their effect is not a monotonic dose–response line. This questions the classical toxicological approach which considers acute exposure (short and high concentrations) as the key of the toxicity evaluation. The history of human exposure to isoflavones was recently casted on doubt, reinforcing the need for careful study of these compounds' occurrence and effects on humans. It is clear now that the traditional soy food makings were able to remove isoflavones from foodstuffs. This is no longer the case in modern processing, and this means that the exposure to this estrogenic substances has increased markedly in recent times. Estrogens in mammals can have both beneficial and harmful effects which are evoked here.

Keywords

Natural estrogens · Food sources · Modern exposure · Bioavailability · Mechanism of actions · Breast cancer · Bone health · Thyroid · Reproductive disruption

1 Introduction

Nowadays, as the concern on endocrine disruptors is back to front stage, scientists realize that natural estrogenic compounds which were ignored for ages have to be included in the panel of active and potentially deleterious compounds [1]. This is mainly due to the recent discovery that humans were not traditionally exposed to natural estrogenic compounds [1] and that this recent exposure is synchronized with that to other anthropoid endocrine disruptors such as pesticides or wrapping agents [2]. Humans were not traditionally exposed to natural estrogenic compounds because they used to prepare all grain legumes, which are the major sources of estrogenic substances in human diet, by prolonged soaking, cooking, or simmering in water. The water was then discarded, and because natural estrogenic compounds

are mainly under a glycosidic form in plants, they leaked into the water during the cooking steps and were then eliminated with water [3]. Reproductive problems were recently reported in multigenerational toxicological studies in animal models [4] and in humans [5]. They occur at actual dietary exposure levels, although the acute toxicity studies performed classically on short-term durations and with pharmacological concentrations do not show major adverse effects [6]. Since endocrine disruptors were discovered, the toxicology studies dealing with reproductive issues were moved to multigenerational exposure and to low doses. Following this guidance, studies can show hormonal disruption of reproduction including epigenetic effects visible even in generations not exposed per se but issued from exposed genitors [7]. For the compounds studied here, the toxicity studies all converge in claiming an estrogenic activity. The present chapter is therefore focusing on the so-called phytoestrogens taken in their large definition since it included the mycotoxins zearalenone (ZEN) and zearalenol (ZOL) produced on grains by fungi. They also focus on natural estrogenic substances produced by plants or by the human gut after plant-substance absorption, i.e., coumestans, isoflavones, isoflavans, and enterolignans. Flavonoids can also have estrogenic effects, but at nutritional exposure, their effects remain low enough to be neglected here. However, if tomorrow a synergy with other anthropological endocrine disruptors taken at environmental doses is demonstrated, they will also have to be considered. Sources and concentrations, roles in plants, and effects in plant consumers are exposed together with the questions remaining on their beneficial and deleterious effects in human and domestic animal consumers.

2 The Natural Estrogenic Substances and Their Sources

2.1 Estrogenic Effects

The estrogenicity of a substance being anthropogenic or natural relies on two major pillars: its efficiency on estrogen-dependent gene transcription which is approached by its efficient dosage in in vivo and in vitro systems and its bioavailability at the target cell. The estrogenic effects of natural substances are based on their chemical structures which share some similarities with that of the 17β-estradiol (17β-E_2) molecule, which is the most potent estrogen in vertebrate animals. 17β-E_2 is an aromatized C18 steroid with hydroxyl groups at 3- and 17-β-position (Fig. 1a). In humans, it is produced primarily by the cyclic ovaries and the placenta. It is also produced by the brain, the adrenal cortex, and the adipose tissue of men and postmenopausal women. It is also crucial for male tract development [8] and adequate sperm production [9]. As seen in Fig. 1, 17β-E_2 is a three-dimensional molecule with its two hydroxyl groups at a distance of 10 angstroms (Å). 17β-E_2 bears a phenolic ring called cycle A.

The hydroxyl groups form an angle allowing the best interaction with the specific estradiol receptors (ERs) [10]. These receptors are proteins with specific quaternary structures allowing estradiol binding via a ligand-binding domain (LBD) and a

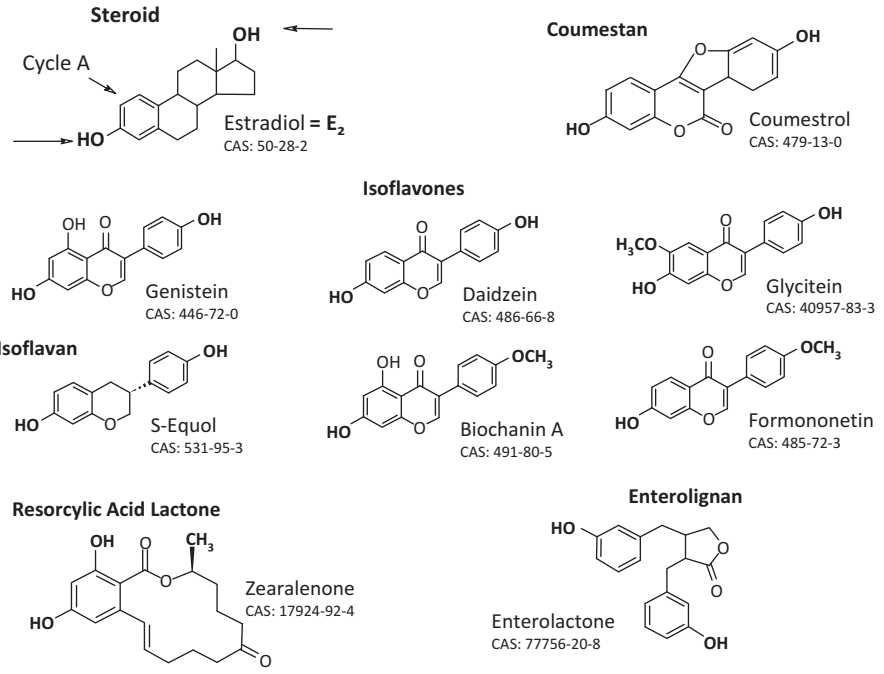

Fig. 1 17β-Estradiol structure together with the structures of the main phytoestrogen families: isoflavones, coumestans lignans, and resorcylic acid lactones

subsequent activation of gene transcription via phosphorylation processes and activation of transcription factors. This transcription phenomenon can either follow the canonical route or be induced via cell phosphorylation pathways. The canonical route involves a direct DNA receptor interaction, and it is known to rely on both ERα and ERβ estradiol receptor subtypes. These two estradiol receptors derive from two different genes: ESR1 located in humans on chromosome 6 and ESR2 located on chromosome 14, respectively [11]. The two receptors derive from a common ancestor but evolved differently, and nowadays they do not exhibit the same primary structure, and their activation by various ligands can be rather different.

These receptors present a LBD with a hydrophobic pocket constituted by the E/F domain (C-terminal domain) of the protein and a part of the A/B domain (N-terminal domain) folded in its vicinity [11]. The E/F domain is constituted of several helix structures, and the spatial position of the H12 helix depends on the chemical structure of the ligand. Estradiol taken as reference is a full agonist of its ERs. Other estrogens can exhibit selective modulating effects depending on the way they fit into the ligand-binding pocket of the ERs. If their structure induces a structural conformation of the H12 helix which does not allow the recruitment of all transcription factors, then the estrogenic effect can be partial. Because the transcription factors are not the same in all cell types, referring to their differentiating history, then some compounds can induce the transcription of estrogen-dependent genes in

certain cell types only. This situation refers to the SERM (selective estrogen receptor modulators) concept [12]. The 17β-E_2 spatial structure is so relevant for the ligand–receptor interaction that the natural 17-α-isomer of estradiol binds only weakly to the ERs and exhibits little estrogenic–genomic activity, considering its natural concentrations, in estrogen-responsive tissues [13]. 17β-E_2 physiological plasma concentrations in women usually vary from 10 to 20 pg/mL (i.e., from 36 to 72 pM) in the metestrous phase of the female cycle to 500–600 pg/mL (i.e., 2 to 4 nM) in the estrous phase of the female cycle. During pregnancy these 17β-E_2 levels can reach values over 30 ng/mL (i.e., over 110 nM). In the meantime, 17β-E_2 levels in men are below 50 pg/mL and below 40 pg/mL in postmenopausal women (i.e., below 200 pM in both cases). Estradiol receptors coded by ESR1 and ESR2 are also present in cell membranes. This is due to the palmitoylation of the ERs at the AA corresponding to the 451 DNA base [14] since a mutation at this point prevents membrane location. The physiological roles of the membrane forms of the ER subtypes are currently under investigation [15]. A role of the membrane ERs as transmembrane channels for steroid hormones is evoked by some authors [16].

Other estradiol receptors were found in cell endoplasmic reticulum and mitochondria membranes, the so-called GPER or GPR-30. This is a completely different molecule belonging to the seven transmembrane G-protein-coupled receptors of the rhodopsin receptor family. These receptors were shown to react with estradiol and with several estrogens, including phytoestrogens [17, 18], and to induce cell signaling pathways and growth factor receptor cross talk [19]. Finally and although these last receptors do not transmit estrogenic effects, the orphan estrogen-related receptors, i.e., ERRα, ERRβ, and ERRγ, were found to bind with different affinities several categories of phytoestrogens [20]. In some cases, the ERRs are involved in physiological processes known to be influenced by estrogens. The ERRs' function is currently under investigation, but they seem to be involved in the cell energy balance and considering an integrative view in metabolic syndrome in humans.

Because of a partial similarity (Fig. 1), the affinities of isoflavones for the ERs are lower than that of 17β-E_2. They range from 1/1000 to 1/10,000 for ERα when genistein (GEN) and kievitone are considered (with the following order of magnitude decrease: GEN > daidzein (DAID) > glycitein > biochanin A > formononetin > phaseolin > kievitone) [11, 21]. For ERβ the affinity of isoflavones is known to be higher, 1/60 and 1/150 for GEN and DAID, respectively. Other compounds like glyceolin were also found to exhibit estrogenic activity. However, these activities are not fully characterized yet [22] even though they are expressed at nutritional doses. Although the affinities of the natural estrogens for the ERs can be low, natural estrogenic substances can be present at mg level in edible plants, and some of them can reach plasma concentrations 10,000 to 100,000 times higher than those of 17β-E_2. Namely, isoflavones which present the highest bioavailability can reach micromolar concentrations in plasma, whereas 17β-E_2 is currently found at picomolar to nanomolar (pregnancy) concentrations in those same plasmas. Cell bioavailability specifically controlled by phase III enzymes (ABC transporters) acting after phase I (CYP) and II (Glut and Sult) enzymes can also play a role in the tissue-specific activities of the natural substances. See [11] for more details.

2.2 Isoflavones

The estrogenic isoflavonoids studied here include GEN, DAID, glycitein, and the DAID metabolite *S*-equol produced by the gut flora and which is an isoflavan (Fig. 1). Two other isoflavones are methylated precursors of estrogenic compounds, the so-called formononetin and biochanin A (Fig. 1). All these compounds are secondary metabolites from legumes and mainly present in Fabaceae. Some of them contain high amounts of isoflavones with estrogenic properties (i.e., up to several hundred mg/100 g of dry matter [23]). The most concentrated Leguminosae are, namely, black beans (a variety of soybean) [21], soy (*Glycine max*) [24], kudzu (*Pueraria lobata* or *tuberosa*) [25], alfalfa (*Medicago* sp.) [23], and clover (*Trifolium* sp.) [26]. Estrogenic isoflavones are also found in a smaller quantity in pulses traditionally edible in Western countries like beans, lentils, broad beans, and chick-peas, but in these vegetable sources, the concentrations are about 500 times lower than in the plants previously cited [27, 28] (Table 1). These compounds were also found in more than 300 different plants, including many species of Leguminosae like *Baptisia*, *Cytisus*, *Dalbergia*, *Genista*, *Lupinus*, *Medicago*, *Phaseolus*, *Teline*, *Trifolium*, and *Ulex* and in several species of Rosaceae like *Prunus* [29, 30]. In Western countries, these plants were traditionally used as sources of antifertility agents [29, 30].

2.3 Coumestans

The main estrogenic substance in the coumestan family is coumestrol (COUM) although it also exists as methylated substances (4′-*O*-methyl and 7-*O*-methyl derivatives) which were found in alfalfa (*Medicago sativa*) [39]. When they reach the liver, the methylated forms can be demethylated in COUM by phase I enzymes. Coumestrol estrogenic potency is by far the highest in vitro especially through ERα [40]. However, its bioavailability appears to be lower than that of isoflavones in rat [41]. No data were found in humans since this compound is considered as toxic, and therefore, pharmacokinetic studies have not been published yet in humans. In addition, this compound is present in significant amounts in alfalfa especially after fungi's attacks (*Pseudopeziza medicaginis*) [42] or in clover sprouts [43]. However, if these pulses can be used for animal feeding, they are only anecdotally used in humans, and in that case, it is essentially as dietary supplements. According to [44], clover sprouts, raw, contain 14.079 mg COUM/100 g; kala chana, mature seeds, raw, contain 6.130 mg COUM/100 g; and pinto beans, mature seeds, raw, contain 1.805 mg COUM/100 g. In addition, alfalfa seeds, sprouted, raw, contain 1.596 mg COUM/100 g; mung beans, mature seeds, sprouted, raw, contain 0.932 mg COUM/100 g; and split peas, mature seeds, raw, contain 0.812 mg/100 g. Still according to [44], soymilk, original and with vanilla, unfortified, can contain 0.807 mg COUM/100 g, and soybeans, mature seeds, sprouted, raw, can contain 0.341 mg COUM/100 g. However, soybean does not seem to be a constant source of COUM. It is likely that COUM synthesis depends on the plant culture conditions and its contamination by fungi.

Table 1 Isoflavones and coumestrol in several edible plants

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	μg/100 g of fresh weight						μg/100 g	
Alfalfa sprout	67	152	3,899	118	na	105	4,341	[31]
Alfalfa sprout	nd	nd	340	nd	na	4,680	5,020	[32]
Alfalfa sprout (freeze-dried)	nd	nd	5,170	nd	na	72,010	77,180	[32]
Almond	25	<1	<1	1	<1	na	26	[33]
Apricot (dry)	tr	nd	nd	tr	na	nd	0	[31]
Asparagus (white)	na	nd	na	nd	na	na	0	[34]
Asparagus (white)	nd	58	nd	tr	na	tr	58	[31]
Asparagus (green)	na	nd	na	nd	na	na	0	[34]
Basilic	na	nd	na	nd	na	na	0	[34]
Beet	na	nd	na	nd	na	na	0	[34]
Black bean freeze-dried	nd	77,440	nd	79,640	na	nd	157,080	[32]
Black bean seeds 1, dry	nd	69,850	nd	61,220	na	nd	131,070	[32]
Black bean seeds 2, boiled	nd	26,950	nd	27,710	na	nd	54,660	[32]
Black trumpet mushrooms	na	nd	na	nd	na	na	0	[34]

(*continued*)

Table 1 (continued)

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	µg/100 g of fresh weight						µg/100 g	
Black-eyed bean seeds, dry	173	nd	nd	nd	na	nd	173	[32]
Brazil nut	13	6	<1	85	<1	na	104	[33]
Broad beans (grilled)	nd	nd	210	129	na	nd	339	[32]
Broccoli	nd	44	nd	nd	na	nd	44	[31]
Broccoli	nd	5	nd	7	na	na	12	[35]
Brocoli sprouts	nd	44	nd	nd	na	na	44	[27]
Brocoli sprouts	nd	44	nd	nd	na	nd	44	[31]
Brussel sprouts	nd	tr	nd	nd	na	nd	0	[31]
Brussel sprouts	na	nd	na	nd	na	na	0	[34]
Capers	na	nd	na	nd	na	na	0	[34]
Carrotts	nd	2	nd	2	na	nd	4	[31]
Carrotts	5	nd	nd	nd	na	na	5	[31]
Cashew nuts	7	nd	2	2	1	na	12	[33]
Celery	na	nd	na	nd	na	na	0	[34]
Ceps	na	nd	na	nd	na	na	0	[34]
Chanterelle	na	nd	na	nd	na	na	0	[34]
Chick pea	126	nd	nd	640	na	6,130	6,896	[32]
Chick pea	2	40	140	60	na	na	242	[35]
Chinese peas, boiled	101	nd	nd	nd	na	nd	101	[32]

Chinese peas, freese-dry	126	nd	nd	nd	na	nd	126	[32]
Clover sprout	440	nd	2,280	350	na	28,060	31,130	[32]
Clover sprout	751	71	4,020	71	na	98	5,011	[31]
Clover sprout freese-dried	8,810	nd	561,140	6,940	na	561,140	1,138,030	[32]
Coconuts	6	nd	nd	nd	4	na	10	[33]
Coconuts powder	na	nd	na	nd	na	na	0	[34]
Codea	nd	50	nd	tr	na	na	50	[31]
Dog Rose Berry	na	nd	na	nd	na	na	0	[34]
Garbanzo bean seeds, dry	152	nd	nd	nd	na	nd	152	[32]
Garbanzo bean seeds, dry	1.52	nd	nd	nd	na	na	1.52	[36]
Garbanzo bean seeds, dry	1,394	nd	52	67	na	na	1,513	[31]
Garbanzo bean seeds, dry	2,822	33	258	82	na	na	3,195	[35]
Garlic	tr	nd	nd	tr	na	nd	0	[31]
Garlic	nd	nd	23	1	na	na	24	[35]
Ginger	na	nd	na	nd	na	na	0	[34]
Grape	na	nd	na	nd	na	na	0	[34]
Grape	nd	tr	nd	tr	na	nd	0	[31]
Grapefruit	tr	36	tr	27	na	50	113	[31]
Grapefruit	na	nd	na	nd	na	na	0	[34]
Great northern bean seeds, dry	60	nd	nd	nd	na	nd	60	[32]

(*continued*)

Table 1 (continued)

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	µg/100 g of fresh weight						µg/100 g	
Green bean freese-dried	nd	nd	211	nd	na	nd	211	[32]
Green bean fresh boiled	tr	nd	tr	nd	na	nd	0	[32]
Green bean fresh raw	nd	nd	15	nd	na	nd	15	[32]
Haricots en grains	838	11	215	73	na	na	1,137	[35]
Hazelnut	12	<1	<1	9	<1	na	21	[33]
Horse radish	na	nd	na	nd	na	na	0	[34]
Kiwi	na	nd	na	nd	na	na	0	[34]
Large lima beans seeds, boiled	nd	nd	10	nd	na	nd	10	[32]
Leek	na	nd	na	nd	na	na	0	[34]
Letuce	na	nd	na	nd	na	na	0	[34]
Licorice	nd	293	1,493	599	na	nd	2,385	[31]
Mungo bean sprout	tr	387	26	424	na	2,000	2,837	[31]
Mungo bean sprout	nd	745	nd	1,902	na	na	2,647	[37]
Mungo bean sprout	nd	nd	tr	nd	na	na	0	[35]

Mungo bean sprout	nd	nd	tr	nd	nd	na	0	[32]
Mungo bean sprout freese-dry	nd	tr	nd	nd	na	na	0	[32]
Mungo Beans	nd	nd	61	nd	na	na	61	[32]
Olive	na	nd	na	nd	na	na	0	[34]
Orange (juices)	tr	tr	tr	tr	na	53	53	[31]
Origan	na	nd	na	nd	na	na	0	[34]
Peach	nd	nd	nd	nd	na	nd	0	[31]
Peanuts (fresh)	8	<1	4	48	10	na	70	[33]
Pear	na	nd	na	nd	na	na	0	[34]
Pecan nut	30	nd	2	2	nd	na	34	[33]
Pine nuts	27	<1	<1	4	<1	na	31	[33]
Pink Bean	nd	nd	105	nd	na	nd	105	[32]
Pinto bean	56	nd	nd	nd	na	361	417	[32]
Plums	nd	nd	nd	nd	na	nd	0	[31]
Red Bean	196	nd	nd	9	na	na	205	[31]
Red Bean	nd	10	nd	5	na	na	15	[35]
Red Bean boiled	41	nd	nd	nd	na	nd	41	[32]
Red Bean dry	560	nd	nd	nd	na	na	560	[36]
Red Bean dry	nd	20	nd	520	na	na	540	[31]
Red Bean dry	4	42	nd	98	na	na	144	[38]
Red Bean dry	nd	nd	nd	310	na	na	310	[32]
Red Bean freese-dried	132	nd	nd	nd	na	nd	132	[32]
round split peas, dry	nd	nd	nd	nd	na	811	811	[32]
Rutabaga	na	nd	na	nd	na	na	0	[34]

(*continued*)

Table 1 (continued)

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	µg/100 g of fresh weight						µg/100 g	
Shitake	na	nd	na	nd	na	na	0	[34]
Small lima bean seeds (dry)	37	nd	55	nd	na	nd	92	[32]
Small white bean seeds, dry	nd	nd	82	74	na	nd	156	[32]
Soft potatoes	nd	nd	nd	tr	na	nd	0	[31]
Sunfolower seeds	nd	nd	nd	nd	na	nd	0	[31]
Turnip	na	nd	na	nd	na	na	0	[34]
Wallnuts	17	1	<1	11	<1	na	29	[33]
Yellow split peas, dry	86	nd	nd	nd	na	nd	86	[32]
Yellow split peas, dry	nd	726	nd	nd	na	nd	726	[32]

2.4 Lignans

Lignans are not estrogenic substances per se, but some compounds in the lignan family (Fig. 2) can be transformed into enterolignans exhibiting low or partial estrogenic effects [45]. As far as it is known, the classical precursors of the estrogenic metabolites enterolactone (ENL) and enterodiol (END) are lariciresinol, matairesinol, medioresinol, pinoresinol, secoisolariciresinol, sesamin, sesamolin, and syringaresinol [46, 47]. Figure 2 gives their chemical formula.

However, in rats it was shown that lignin could be metabolized into enterolignans via its hydrolysis into lignans [48]. The current sources of enterolignan precursors are given in Table 2. However, this list is most likely not exhaustive.

Therefore, the exposure to enterolignan precursors is far to be precise, and in some populations, data are missing. As a consequence, the exposure is more reliably evaluated based on blood or urine measurements of enterolignans. This is particularly true since the ability to form enterolignans varies largely between human subjects [11].

2.5 Resorcylic Acid Lactones

The estrogenic resorcylic acid lactones are the mycotoxins ZEN and ZOL types α and β. All are produced by fungi of the *Fusarium* family developing on maturing corn, wheat, barley, rye oats, soybeans, sorghum, peanuts, and other food and feed crops in the field and in grain during transportation or storage. Zearalenone and ZOL (Fig. 1) are mainly produced by *F. graminearum* and *F. semitectum* [50]. Due to its structural similarity to the naturally occurring estrogens, ZEN is an estrogenic mycotoxin that induces obvious estrogenic effects in animals [51]. Zearalenone and ZOL productions are favored by high-humidity and low-temperature conditions. They can occur simultaneously with other mycotoxins such as deoxynivalenol (DON) and less frequently with aflatoxins [52]. Zearalenone is stable in food under regular cooking temperatures but can be partially eliminated under deep heating [53]. In the human diet, the main sources are grain milling products, for human consumption, e.g., breakfast cereals, breads, and rolls. Cooked pastas only contain small amounts of ZEN, and meat and meat products are always below the detection limits (Table 3).

3 Human Exposure

3.1 Isoflavones

Isoflavones nowadays are present in almost all domestic animal food except when an alternative breeding practice is adopted, namely, if grass or other human residues are used instead of the conventional diet. In most animal breeds, the protein input is brought by pulses. The most nutritionally interesting are soy, alfalfa, or clover. All

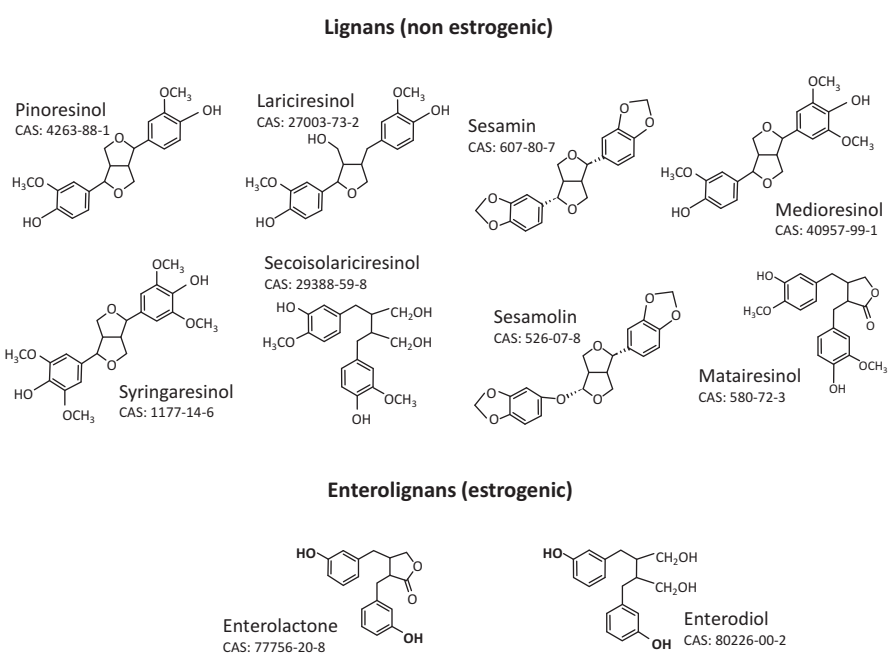

Fig. 2 Chemical structure of enterolignans and of some of their known precursors

three can contain natural estrogenic compounds at high concentrations as previously mentioned. However, estrogenic isoflavones, although having a good bioavailability, have a low distribution volume and are usually eliminated for the most part during the 24 h coming after intake. Because animals are currently fasting for at least 12 h before slaughtering, the amount of isoflavones remaining in their flesh is generally low [55]. Meat or fish, even though they have been fed pulses during their life, are not good isoflavone vectors in human diet.

In human, the exposure is mainly due to soy intake, although clover, alfalfa, and kudzu extracts can be used in food supplements. Until recently, this exposure was thought to be safe assuming that isoflavones had always been part and parcel of the human diet in Asia. However, it was recently shown that all traditional Asian recipes, which were elaborated sometimes over several centuries, included prolonged cooking, simmering, or soaking in water. These steps empirically removed the glycosylated isoflavones which are soluble in water but heat resistant. As an example, for traditional tofu, a rough soy meal is simmered and soaked in water for a total of 2–3 h before curdling. Then, water is squeezed out by pressing to make the soy cheese. In the same way, kudzu, which contains high levels of isoflavones with inverse proportions of GEN and DAID compared to soy, is directly powdered to prepare medicines. It is also boiled in water to be eaten as we could do with potatoes. The boiling step can last over 2 h and removes isoflavones. In 2004, looking at isoflavone intakes in rural Chinese women who are more likely to have kept their

Table 2 Lignan content in several edible fruits and vegetables

Fruits and vegetables	Secoisolariciresinol (CAS 148244-82-0)	Matairesinol (CAS 580-72-3)	Lariciresinol (CAS 27003-73-2)	Pinoresinol (CAS 487-36-5)	Syringaresinol (CAS 21453-69-0)	Medioresinol (CAS 40957-99-1)	Total	Author
	μg/100 g of wet weight						μg/100 g	
Alfalfa sprouts	tr	nd	na	na	na	na	0	[31]
Ananas	7	18	24	3	na	na	52	[49]
Apple	na	3	55	na	na	na	58	[49]
Apricot dry	328.3	tr	na	na	na	na	328.3	[31]
Artichoke	171.45	0	153.76	3,479.71	21.89	56.65	3,883.47	[46]
Asparagus	743	14	92	na	na	na	849	[49]
Asparagus	68	tr	na	na	na	na	68	[31]
Avocado	47	6	31	272	na	na	356	[49]
Baby corn	na	na	25	16	na	na	41	[49]
Baby corn	7	na	14	na	na	na	21	[49]
Bambou sprouts	38	na	na	na	na	na	38	[49]
Banana	nd	17	na	19	na	na	36	[49]
Basilic	546	1.9	na	na	na	na	547.9	[34]
Black trumpet mushrooms	1.3	0.1	na	na	na	na	1.4	[34]
Broad Bean	240.39	34.89	319.23	24.67	57.34	4	681.29	[46]
Broccoli	tr	nd	na	na	na	na	0	[31]
Brussel Sprouts	30	nd	na	na	na	na	30	[31]
Brussel Sprouts	21	0.6	na	na	na	na	21.6	[34]

(*continued*)

Table 2 (continued)

Fruits and vegetables	Secoisolariciresinol (CAS 148244-82-0)	Matairesinol (CAS 580-72-3)	Lariciresinol (CAS 27003-73-2)	Pinoresinol (CAS 487-36-5)	Syringaresinol (CAS 21453-69-0)	Medioresinol (CAS 40957-99-1)	Total	Author
Capers	44.5	15.1	na	na	na	na	59.6	[34]
Carob bean	1,266.51	108.41	1,770.74	294.99	12,965.7	336.08	16,742	[46]
Carrots	38	tr	na	na	na	na	38	[31]
Cauliflower	91	na	36	85	na	na	212	[49]
Celery	12	na	16	43	na	na	71	[49]
Celery	9.9	0	na	na	na	na	9.9	[34]
Ceps	0.6	0.3	na	na	na	na	1	[34]
Chanterelles	3.9	7.3	na	na	na	na	11.2	[34]
Cherry tomato	17	na	43	11	na	na	71	[49]
Cherry tomato	16	na	38	19	na	na	73	[49]
Clover sprouts	nd	nd	na	na	na	na	0	[31]
Coconut (powder)	34	0	na	na	na	na	34	[34]
Cucumber	41	na	65	na	na	na	106	[49]
Dog Rose berry	78.8	2	na	na	na	na	80.8	[34]
Eggplant	8	na	40	51	na	na	99	[49]
Garlic	55	na	84	45	na	na	184	[49]
Garlic	27	37	na	na	na	na	64	[31]
Ginger	na	16	na	15	na	na	31	[49]
Ginger	21.3	0	na	na	na	na	21.3	[34]
Grape	tr	52	na	na	na	na	52	[31]

	µg/100 g de poids frais						µg/100 g	
Grape	2	1.3	na	na	na	na	3.3	[34]
Grapefruit	nd	tr	na	na	na	na	0	[31]
Grapefruit	26.3	0	na	na	na	na	26.3	[34]
Green Asparagus	78.3	3.4	na	na	na	na	81.7	[34]
Green Pepper	5	na	73	6	na	na	84	[49]
Grilled corn	14.46	0	15.33	0	331.55	3.01	364.35	[46]
Horse radish	14	0	na	na	na	na	14	[34]
Horse radish	37	49	219	16	na	na	321	[49]
Kiwi fruit	174.6	1.2	na	na	na	na	175.8	[34]
Kiwi fruit	106	na	20	13	na	na	139	[49]
Leak	11.8	0	na	na	na	na	11.8	[34]
Lentils	1.38	245.17	233.24	86.93	196.63	17	781.1	[46]
Mung bean sprouts	82	1	32	33	na	na	148	[49]
Mung bean sprouts	tr	tr	na	na	na	na	0	[31]
Nashi	7	na	21	na	na	na	28	[49]
Olive	55.9	2.7	na	na	na	na	58.6	[34]
Orange Navelle	14	na	128	24	na	na	166	[49]
Orange Valencia	56	na	193	51	na	na	300	[49]
Oranges (Juice)	tr	tr	na	na	na	na	0	[31]
Oregano (dry)	44.4	1	na	na	na	na	45.4	[34]

(*continued*)

Table 2 (continued)

Fruits and vegetables	Secoisolariciresinol (CAS 148244-82-0)	Matairesinol (CAS 580-72-3)	Lariciresinol (CAS 27003-73-2)	Pinoresinol (CAS 487-36-5)	Syringaresinol (CAS 21453-69-0)	Medioresinol (CAS 40957-99-1)	Total	Author
Oriental Pears	0.28	0	6.9	179.99	23.43	9	220.21	[46]
Paprika	8	79	2	na	na	na	89	[49]
Pea	129	na	9	6	na	na	144	[49]
Pea	na	na	59	50	na	na	109	[49]
Pea	2.73	6.79	150.59	79.81	175.36	24.2	439.48	[46]
Pea	1	98.82	83.73	7.11	1.82	0	192.63	[46]
Pea	2.61	87.75	133.75	8	1.81	0	234.14	[46]
Peach	11	na	38	83	na	na	132	[49]
Peach	28	nd	na	na	na	na	28	[31]
Pear	2	na	34	2	na	na	38	[49]
Pear	9.9	0.7	na	na	na	na	10.6	[34]
Plums	16	na	20	42	na	na	78	[49]
Plums	76	tr	na	na	na	na	76	[31]
Pumpkin	20	na	29	na	na	na	49	[49]
Pumpkin	29	3	58	8	na	na	98	[49]
Radish	na	na	29	43	na	na	72	[49]
Radish	1	15	na	40	na	na	56	[49]
Red bean	nd	nd	na	na	na	na	0	[31]
Red Pepper	9	na	73	1	na	na	83	[49]
Rice Bread	33	4	47	44	na	na	128	[49]
Rice Bread	7	1	18	16	na	na	42	[49]
Rutabaga	3.7	0.6	na	na	na	na	4.3	[34]

Salade (Letuce)	105.6	0.6	na	na	na	na	106.2	[34]
Shitake	0.2	0	na	na	na	na	0.2	[34]
Spanish melon	23	na	69	12	na	na	104	[49]
Spinach	8	na	110	31	na	na	149	[49]
Spinach	13	1	79	15	na	na	108	[49]
Strawberry	51	na	33	21	na	na	105	[49]
Strawberry	61.37	18.3	134	5.21	60.83	3.89	284.49	[46]
Sunflower seed	128	nd	na	na	na	na	128	[31]
Sweet potato	65	2	79	16	na	na	162	[49]
Sweet potato	nd	41	na	na	na	na	41	[31]
Tomato	3	na	11	12	na	na	26	[49]
Turnip	6.3	0	na	na	na	na	6.3	[34]
Watermelon	5	na	67	5	na	na	77	[49]
White Asparagus	28.7	0.6	na	na	na	na	29.3	[34]

Table 3 Occurrence of ZEN in unprocessed grains from EFSA [54]

		>LOD	Concentrations (μg/kg)					
Food group	Nb	%	LB/UB	Mean	P50	P75	P95	Max
Unprocessed grains	9,877	41	LB	33	0.0	15	160	2,969
			UB	40	7.0	27	161	2,969
Wheat	5,318	38	LB	22	0.0	7.0	89	2,969
			UB	27	5.0	20	90	2,969
Barley	1,071	37	LB	10	0.0	5.0	49	775
			UB	13	5.0	10	50	775
Corn	2,460	56	LB	76	16	76	319	2,700
			UB	87	40	78	319	2,700
Oats	596	23	LB	21	0.0	0.0	76	1,590
			UB	23	1.5	5.0	98	1,590
Rice	43	7.0	LB	0.8	0.0	0.0	10	15
			UB	5.5	5.0	5.0	10	15
Sorghum	55	53	LB	96	50	147	450	700
			UB	116	50	147	450	700

N, number of samples; > LOD, indicates the percentage of results above the LOD or LOQ; *LB*, lower-bound; *UB*, upper-bound; (lower-bound, values below the detection limit are extrapolated to 0; upper-bound, values below the detection limit are extrapolated to detection limit)
P50, 50th percentile; P75, 75th percentile; P95, 95th percentile
[a]If $N < 60$, then the calculated P95 should be considered only as an indicative value [54]

traditional tofu making, Liu and co-workers [56] found that 65% of them had an isoflavone intake below 15 mg/day, the majority being below 5 mg/day. Nowadays, in Western countries as in the developed countries of Asia, soy is essentially produced using industrial techniques. In all cases, cooking steps were reduced in time to reduce energy costs, and water is used either in small proportion (steaming) or not used at all. As a consequence, the amounts of isoflavones can be very high. Therefore, the amount of isoflavones in modern products can be high when a portion is considered (Table 4). Soy is also incorporated in processed food as soy flakes (Table 4) which can contain from 90 to 182 mg of isoflavones for 100 g [1]. Therefore, isoflavones can be present in significant amounts in processed food containing soy flakes (Table 4).

Why is there such amount of isoflavones in modern soy preparation? This is because when the industrial processes were set up, in the 1930s, hardly anything was known about isoflavones, and the processes were designed essentially to prevent protein degradation. As an example, defatted soy flakes are treated with hexane which removes fat and concentrates isoflavones in the protein matrix. The matrix is then extruded. Extrusion destroys most of the anti-nutritional factors but not isoflavones. Generally speaking, in the industry, the cooking steps were shortened to save energy and costs. When it was possible to assay isoflavones accurately, in the early 1980s, the assays were performed in several popular areas essentially eating industrial soy-based products produced in mass for city populations. The study by Liu [56] is one of the very few dealing with exposure in remote Chinese areas still

Table 4 Quantities of isoflavones measured in modern dishes based on soy or containing soy as an ingredient

	Genistein (μg/g)	Daidzein (μg/g)	Total (μg/g)	Reasonable portion size	Intake for 1 portion (μg)
Foodstuffs based on soy juice					
Tonyu 1	91.37 (± 6.52)	49.57 (± 3.47)	140.94	1 bowl (350 mL)	49,330
Tonyu 2	51.32 (± 8.16)	39.83 (± 4.39)	91.14	1 bowl (350 mL)	31,899
Yogurts	44.70 (± 3.17)	37.40 (± 3.61)	82.20	1 yogurts	8,220
Soft yogurt	125.00 (± 8.75)	129.19 (± 9.12)	254.31	1 yogurt	30,510
Vanilla soy cream	29.82 (± 2.16)	19.39 (± 1.36)	49.21	1 cup (100 g)	4,921
Caramel soy cream	40.00 (± 2.86)	17.90 (± 0.7)	57.89	1 cup (100 g)	5,789
Soy juice chocolate taste	107.00 (± 7.49)	70.00 (± 4.92)	178.75	1 mug (330 mL)	58,987
Vanilla dessert	159.50 (± 11.16)	63.20 (± 4.42)	224.10	1 cup (100 g)	22,410
Powdered soy "milk"	1,310.00 (± 81.62)	1,070.00 (± 64.96)	2,390.00	1 mug	49,790
Herb cheese made of soy	368.39 (± 25.86)	357.23 (± 22.99)	725.62	50 g	36,280
Cream substitute made of soy	70.08 (± 5.11)	63.16 (± 4.42)	134.95	20 mL	2,700
Foodstuffs based on soy protein					
Sausages made of soy (1)	82.21 (± 5.76)	40.64 (± 2.87)	122.85	2 sausages (90 g)	11,060
Sausages made of soy (2)	134.15 (± 10.55)	66.95 (± 5.53)	201.10	3 sausages (80 g)	16,090
Sausages made of soy (3)	259.50 (± 10.42)	231.00 (± 20.70)	490.50	2 sausages (90 g)	44,145
Soy biscuits with figs	95.38 (± 6.36)	87.74 (± 6.16)	183.12	4 biscuits (80 g)	14,650
Buckwheat pancakes with tofu	228.50 (± 15.86)	154.00 (± 10.35)	382.50	1 pancake (100 g)	38,250
Soy pancakes with tomatoes	202.30 (± 14.16)	116.92 (± 8.56)	319.22	1 pancake (100 g)	31,920
Soy pancakes "provençale"	227.15 (± 15.57)	129.48 (± 11.02)	356.63	1 pancake (100 g)	35,663
Smoked tempeh	165.33 (± 11.56)	112.00 (± 7.84)	277.33	3 slices (50 g)	13,870

(*continued*)

Table 4 (continued)

	Genistein (μg/g)	Daidzein (μg/g)	Total (μg/g)	Reasonable portion size	Intake for 1 portion (μg)
Instant powder for drinks	99.69 (± 6.96)	106.11 (± 7.35)	205.80	3 spoons	8,026
Japanese soft tofu	117.87 (± 10.29)	70.92 (± 2.88)	188.79	100 g	18,879
Natural tofu	225.27 (± 75.14)	117.22 (± 7.43)	342.49	125 g	42,810
Japanese soft tofu	117.87 (± 10.29)	70.92 (± 2.88)	188.79	100 g	18,879
Traditional tofu[a]	224.43 (± 11.79)	100.92 (± 12.48)	325.35 (± 24.26)	100 g	32,535
Whey from traditional tofu[a]	744.36 (± 39.89)	459.93 (± 60.17)	1,204.30 (± 100.06)	60 mL	72,258
Tofu	48.00 (± 3.26)	46.16 (± 3.16)	95.27	1 portion (100 g)	9,530
Breaded tofu (1)	150.33 (± 10.52)	71.22 (± 5.57)	221.55	1 portion (100 g)	22,150
Breaded tofu (2)	289.29 (± 6.04)	188.49 (± 2.00)	477.78	1 portion (100 g)	47,778
Tofu with garlic	216.74 (± 9.60)	138.25 (± 4.09)	354.99	1 portion (80 g)	28,400
Smoked tofu	273.54 (± 15.33)	178.54 (± 5.50)	452.08	1 portion (100 g)	45,210
Legumes mixed with tonyu sauce	98.81 (± 1.90)	62.90 (± 6.35)	161.71	1 dish (300 g)	48,510
Vegan steak tomato and onions	332.44 (± 11.39)	222.65 (± 3.29)	555.09	1 steak (90 g)	49,960
Vegan "Bolognese for pasta"	244.73 (± 81.73)	155.40 (± 8.25)	400.13	120 g	48,020
Conventional soy grain	490.98 (± 6.62)	347.68 (± 78.26)	838.67	100 g	85,421
Toasted soy grain (appetizers)	1,360.00 (± 51.32)	1117.42 (± 119.03)	2,477.42	20 g	49,550
Slimming dish (soup)	223.59 (± 15.46)	135.44 (± 9.24)	359.03	1 pack (50 g)	16,510
Slimming dish (breakfast)	185.03 (± 11.95)	98.36 (± 7.56)	283.39	1 pack (50 g)	13,030
Slimming dish (meal)	287.17 (± 18.53)	193.64 (± 13.22)	480.81	1 pack (50 g)	22,110
Soy lecithin extract (1)	0.17 (± 0.01)	0.68 (± 0.04)	0.97	10 g	9.7

(*continued*)

Table 4 (continued)

	Genistein (μg/g)	Daidzein (μg/g)	Total (μg/g)	Reasonable portion size	Intake for 1 portion (μg)
Soy lecithin extract (2)	0.86 (± 0.06)	2.36 (± 0.16)	3.52	10 g	35.2
Foodstuffs with hidden soy					
Minced beef pie (Parmentier)	4.66 (± 0.32)	1.53 (± 0.11)	6.20	1 portion (300 g)	1,860
Minced beef portions	73.92 (± 5.11)	49.34 (± 3.43)	12.22	1 steak	12,220
Stuffed tomatoes	33.02 (± 2.82)	26.94 (± 1.96)	59.99	2 tomatoes	8,960
Stuffed cabbages	33.04 (± 2.55)	25.48 (± 1.53)	58.48	2 cabbages	9,040
Meatballs (1)	78.14 (± 6.16)	54.23 (± 3.56)	132.37	4 balls (125 g)	16,546
Meatballs (2)	82.55 (± 6.68)	59.60 (± 2.59)	142.15	4 balls (125 g)	17,768
Minced veal (breaded)	55.96 (± 3.86)	35.32 (± 2.45)	91.28	1 steak (100 g)	9,128
Brownies	65.24 (± 4.56)	43.92 (± 3.15)	109.16	3 Pieces (90 g)	9,824

Figures are mean ± SD of three measures performed on three different microtitration plates
[a]The traditional tofu is an industrial product

consuming soy food traditionally cooked. It showed that the largest proportion of rural women was exposed to low doses of isoflavones. Therefore, nowadays and especially in developed Asian countries where soy is traditionally eaten but where the processing has been industrialized, the isoflavone intakes have risen dramatically, sometimes over the 45 mg/day which shown to have an effect on premenopausal menstrual cycle [57]. According to recent studies, the mean human exposure in Japan lies between 45 and 60 mg/day [58], in Korea the highest value recorded was 33.6 mg/day for adults [59], and in China it tends to increase nowadays [60] with 16.2 mg/day for adults and 27 mg/day for adolescents and a large interindividual variation. In Western countries, the exposure to isoflavones also rose recently although it is most likely underestimated. Usually, in Western countries, the isoflavone intake is based on the evaluation of pulse consumption and essentially based on soy which is the major provider [61]. Therefore, many countries consider the intake below 2 mg/day especially because they tend to calculate a mean intake over the total population and taking only soy food intake into account. In the USA, the exposure was estimated to be less than 2.7 mg/day in 2001 [31] and confirmed later by subsequent studies [62, 63]. However, the amount of isoflavones in modern soy-based food is higher than that found in the past in traditional foodstuffs (Table 4). Hence, soy juices which are very popular in the West, together with their side products (yogurts and creams), can be good vectors of isoflavones,

Table 5 Isoflavone intakes by infants fed with soy-based infant formula in different countries

Countries	Isoflavones mg/liter	Daily isoflavone intake (mg/kg bw)	Reference
USA	20.9–47	2.3 to 9.3	[68]
UK	18–46.7	1.7 to 4.4	[68, 69]
Australia	17.2–21.9		[68]
New Zealand	17.1–33	2.9 to 3.8	[68, 70]
Brazil	10–47.4	0.8–1.6 to 6.6	[68, 71, 72]
France	19.4–42.3	2.3 to 6.04	[73]

since in this case, isoflavones are concentrated at the juice filtration step [1]. Soy is also prepared as flakes containing large amount of isoflavones. As already mentioned, flakes are incorporated into many recipes marketed as traditional but made by the food industry. Soy is then incorporated as an ingredient for its nutritional and technological properties or for economic reasons. Most studies have underestimated this exposure, but it is so significant that in recent studies no urine samples were found to be devoid of isoflavones in American men [5]. Isoflavones were also detected in 88% of the urine of American pregnant women [64], in all Israeli adults [65], and in all German adolescents [66] of a study cohort. Finally, it must be pointed out here that the human populations most exposed nowadays are infants or children on soy-based infant formula [67]. These formulas were advised to children with lactose intolerance mainly in the 2000s. In the USA and according to authors, 17–30% of infants received these formulas in the last past years. Table 5 shows data recorded in different countries by several authors.

In their paper [67], Badger and co-workers also showed that infant exposure to soy isoflavones is a modern practice. They explained the exposure to estrogens of infants fed with soy-based infant formula is dramatically different from that of Asian children enjoying traditional Asian soy intake. Traditional soy consumption in Asia was based on solid foodstuffs (tofu, natto, miso, tempeh), which were not suitable for babies. Traditionally, these foodstuffs were prepared following recipes including prolonged cooking, simmering, or soaking in water and most probably contained much less isoflavones than the modern industrial products. Therefore, the isoflavone exposure calculated in [67] was nearly nil before 12 months in Asian children but rose progressively thereafter. By comparison, in Western babies fed with soy-based infant formulas, the exposure rate is high as soon as soy formula intake begins, which rises and continues to rise progressively until food diversification. Exposure levels are currently 10–20 times higher than those inducing an estrogenic effect in women, as shown on breast reactivity [74–76], menstrual cycle lengthening [57, 77], and impaired sperm production [78–80]. The plausible consequences of such exposures will be discussed later.

3.2 Coumestans

Coumestrol as a phytoalexin is essentially produced by pulses following fungal attacks. Therefore, and because human foodstuffs are usually better controlled, this

substance is more likely to be present in animal food rather than in human diet. Although, it is not excluded that it could be present in food supplements such as those based on alfalfa. In England the study of Clarke and co-workers [81] failed to detect any COUM in the samples analyzed. In France, in 2011, according to the EAT2 study [82], the consumer exposure was considered to be at least 1,891 ng/day. However, the authors tend to think that this exposure is underestimated since all the contributors could not be analyzed in the EAT2 study. Recently a protein supplement based on alfalfa was authorized by EFSA although the supplier was able to measure milligrams of COUM in his extracts, i.e., 78 mg/kg [83]. The supplier also showed an estrogenic effect in mice and showed a large variability of phytoestrogen content from one batch to another. If such a supplement is to be used widely by the European consumers as it is proposed, the mean exposure to COUM will probably rise dramatically. However, in South Korea, it was reported in 2009 that the mean level of exposure was 0.3 mg per capita per day due to a local specific foodstuff consumption like soybean sprout and arrowroot [59].

3.3 Lignans

As already mentioned, lignans are not estrogenic in plants. The molecules present in grains and in vegetables are eventual precursors of estrogenic enterolignans depending on the composition and efficiency of the human gut flora. Therefore, the enterolignan exposure is difficult to assess because not all precursor sources are identified yet [49] and because not all consumers are able to produce them after ingestion. According to Zamora-Ros and co-workers [84], in European populations, lignans were the most abundant contributor of phytoestrogen intakes when the global intake is low. This study reports a low lignan intake (1.02 mg/day) in Mediterranean countries and even lower in Italy (0.67 mg/day). It also reported a higher intake (1.26–1.60 mg/day) in non-Mediterranean countries. The study also compared their data with those obtained in the Netherlands (1.24 mg/day), in Sweden (0.50–2.81 mg/day), and Finland (1.22 mg/day). Still according to [84], in other Western countries such as Mexico, the USA, and Canada, lignan intakes were considered to be much lower (from 0.35 to 0.86 mg/day). However, when pharmacokinetic studies are performed in humans, the enterolignan levels recorded in the blood of ENL producers are never null [85]. Moreover, in urine and in serum, of citizen of Western countries, they are of the same range concentrations with isoflavones [86, 87]. As an example according to [86] in the USA (Albany), the urine lignan concentrations range from 0.99 to 1230 ng/mL and were found in 94% of the population. In parallel, still in the same study, isoflavone urine levels range from 1.33 to 1290 ng/mL. The detection rate reached 100% for DAID in women. In the UK and according to [87], the enterolignans in urine sample ranged from 0 to 10,229 μg/mmol creatinine and isoflavones (DAID, GEN and equol) excreted in urine ranged from 0 to 1,442.6 μg/mmol creatinine. This shows that the sources of enterolignan precursors are far from being identified and that the exposure is only approached and most probably underestimated. In addition, this lignan intake cannot

be readily assimilated to a phytoestrogen exposure because the gut metabolism differs between consumers [88].

3.4 Resorcylic Acid Lactones

Because of a careful screening of plant matter, usually the exposure to the mycoestrogens, ZEN, α-ZOL, and β-ZOL in the European populations are considered to be low. More precisely based on a bio-monitoring approach linking the scarce pharmacokinetic data of ZEN in human and data obtained on urinary levels [89], it appeared that zearalenone daily exposure is most probably below the tolerable daily intake (TDI) of 0.25 μg/kg body weight in European study cohorts. However, some individuals from Haiti and in African countries, where corn is a major food source, may have an exposure that exceeds the TDI. Table 6 from [54] gives exposure calculations in different categories of European consumers.

4 Effects in Plants

4.1 Isoflavones

Isoflavones in plants act as phytoalexin [21]. They are antimicrobial and may have a role in plant protection. As an example, the antifungal activity of lupin isoflavones was demonstrated [90]. In soybean cultivars, an increase in isoflavone concentrations was shown to be a specific response to the attack of a saprophytic fungi *Mucor ramosissimus*. In the soybean strains resistant to the fungus, isoflavones (including glyceollins I, II, and III, glycinol, glyceocarpin, GEN, isoformononetin, and *N*-acetyltyramine) were induced by the fungal attack, all compounds possessing antifungal activity with the exception of GEN [91]. A contamination of a soy strain with the fungus *Diaporthe phaseolorum f.* sp. *Meridionalis* induced the accumulation of isoflavones (GEN, DAID), pterocarpans (glyceolins), and flavones (apigenin and luteolin) via a nitric oxide synthase pathway [92]. To go on with interactions of the plants with microorganisms, there is some evidence that the symbiotic relationship between *Rhizobium lupine* and *Lupinus albus* stimulates an increase in the production of prenylated isoflavones in the root nodules [93]. These prenylated isoflavones possess in vivo activities against a number of other *Rhizobium* species. Zhang and Smith [94] also showed that GEN plays a major role in the establishment of the symbiosis between *Bradyrhizobium japonicum*, the arbuscular mycorrhyzas such as *Glomus mosseae*, and their host, i.e., soy (*Glycine max*). Genistein is the soy recognition molecule inducing the greatest plant-to-bacterium signal. In fact, the binding of GEN to *B. japonicum* activates many of the bacteria nod genes.

There is solid proof that farming practices directly influence the levels of isoflavones in soy. As a matter of fact, irrigation was shown to enhance isoflavone content in soybean by as much as 2.5-fold [95]. A deficit in nitrogen fertilizer increases the estrogenic activity of a clover pasture [96]. On the other hand, a

Table 6 Daily exposure of European citizen to zearalenone as calculated by EFSA in ng/kg/b.w [54]

Age class	Summary statistics of exposure to zearalenone (ng/kg/b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants[a]	3.3	87	6.4	87	9.4	88
Toddlers	9.3	51	13	83	23	100
Older children	5.7	29	11	44	22	75
Adolescents	3.6	17	6.1	26	12	42
Adults	2.4	14	4.3	18	7.2	29
Elderly	2.0	13	3.4	16	6.4	26
Very elderly	2.3	12	2.9	16	7.1	29
95th percentile exposure in total population[b]						
Infants	33	[c]	[c]	[c]	[c]	217[d]
Toddlers	24	104	31	182	50	277
Older children	9.9	59	22	80	42	124
Adolescents	7.5	38	15	53	26	76
Adults	4.7	28	9.5	35	14	54
Elderly	3.5	25	7.5	31	12	42
Very elderly	7.0	26	7.7	35	13	47

b.w., body weight; *LB*, lower-bound; *UB*, upper-bound (lower-bound, values below the detection limit are extrapolated to 0; upper-bound, values below the detection limit are extrapolated to detection limit)

[a]Estimates based on only two dietary surveys

[b]The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust [54], and therefore they should not be considered in the risk characterization. Those estimates were not included in this table

[c]Not calculated

[d]Estimates are based on one dietary survey only

supplementation in the same fertilizer decreases the occurrence of isoflavones with estrogenic activity in a clover pasture [96]. A study where soy cultivars (*Glycine max*) were bred for 3 years demonstrated that levels of isoflavones were related to both environmental and genetic characteristics and could be susceptible to selection [97]. The genomic regions implicated in this process have been identified [98]. Vyn and co-workers [99] showed that GEN, DAID, and glycitein contents in soy are correlated with potassium in roots and leaves and with crop management using potassium-rich fertilizers. Lindner in [23] gave an evolutionist theory about the ability of Leguminosae to produce estrogenic isoflavones. Indeed, it appears that isoflavonoid compounds are synthesized by the plants in response to bacterial or fungal attacks or to water stress. Estrogenic isoflavones can then be considered as protective compounds. In the case of overgrazing of a pasture by mammalian predators, the production of great quantities of estrogenic compounds which could impair predator reproduction would result in the reduction of the predator pressure. Finally, because estrogenic isoflavones are also specific attractants

for symbiotic bacteria of the *Rhizobium* gender and of mycorrhizal fungi of the *Glomus* gender, and because these bacteria can fix the atmospheric nitrogen, human selection against the production of isoflavones in pulses would result in the loss of one of their greatest interests in crop management.

5 Effects in Humans and Animals

5.1 Beneficial Effects

5.1.1 Beneficial Effects in Animals

Estrogens are used in cattle as anabolic agents. Therefore, some studies attempted to figure out if a phytoestrogen supplementation can enhance cattle or poultry growth and meat quality [100]. Beside a potential interest in antioxidant content of meat from animal raised on estrogenic food [100], it was seen that a DAID supplementation in the diet increased marbling meat score in steers [101]. If the meat quality was significantly improved considering several parameters, the carcass bone proportion was also greater probably reflecting a positive effect of phytoestrogens on osteoblast function. In another study [102] and at low doses, i.e., 300 and 400 mg/day, DAID was also shown to enhance immune function in late-lactation cows under heat stress. However, the DAID supplementation does not strictly mimic a legume intake since in all the roughages based classically on pulses, DAID or its precursor formononetin is present together with other compounds either estrogenic per se (GEN) or precursors of these estrogenic isoflavones (biochanin A) playing in interaction.

There is no positive effect reported for lignans, COUM, or ZEN on farmed animals although ENL was found in milk of cows fed with flaxseed or forages rich in ENL precursors [103].

5.1.2 Menopause in Women

Isoflavones

Menopausal symptoms encompass hot flushes, night sweats, vaginal dryness, mammary density, and mood fluctuations. Menopause is not a disease. In Western countries, menopausal symptoms were treated for more than 20 years using hormone replacement therapy (HRT). However, several studies including the Women's Health Initiative (WHI) showed that deleterious effects of the US treatments on breast or endometrial cancers and those on cardiovascular diseases may overcome their benefits [104]. More than 543 epidemiological studies were performed to examine if the consumption of soy and isoflavones with estrogenic activities may prevent hot flushes. This effect can be suspected since it is known that hot flushes and night sweats are controlled by the hypothalamus preoptic area implicated in the body core temperature regulation. In addition, the neurones in this region have estradiol membrane receptors involved in body temperature and energy homeostasis [105]. This area induces shiverings or sweatings when it records a body temperature over

a low and an upper threshold. The amplitude of the neutral zone situated between these thresholds is under serotonin and noradrenergic control. 17β-E_2 is known to induce the local synthesis of these two neuromediators as well as their respective receptors [106]. The recent meta-analysis of Chen and co-workers [107] showed a reduction in hot flushes with isoflavone intake. The study is based on 15 randomized clinical trials comparing phytoestrogens vs. placebo for which the mean age of the subjects ranged from 48 to 60.1 years. The number of participants was at least 30 and up to 252. The study duration ranged from 3 to 12 months. The meta-analysis of the 7 studies reporting the Kupperman index gathering 11 menopausal symptoms showed no significant effects of phytoestrogens as compared to placebo. However, the meta-analysis of ten studies showed a significant reducing effect of phytoestrogens on hot flush frequency compared to placebo. The meta-analysis of the five studies that planned to report side effects of phytoestrogens did not show any significant difference between the two groups. Menopausal symptoms are known to be influenced by psychological elements. Therefore, a placebo effect exists. Nevertheless, in accordance with an estrogenic effect in women especially when estradiol receptors are present, the phytoestrogen effect can be observed.

Coumestrol

In women some data exist linking the use of some herbal medicine, some of them containing COUM with other substances with or without estrogenic activities [108]. This is the case for clover or alfalfa preparations. However, menopausal symptoms are influenced by psychological factors [109], and some meta-analyses failed to really correlate isoflavones and/or COUM with a decrease of menopausal symptoms [110]. For COUM its level in the preparation is generally low even if it can also be highly variable. In addition, little is known about its serum bioavailability. Therefore, its effect is very difficult to truly associate to any observation.

Lignans

Only eight randomized, double-blind, placebo-controlled clinical trials were found trying to prove the effectiveness of flaxseed on the management of menopausal symptoms [111–116]. None of them, whatever the amount of flaxseed or secoisolariciresinol diglucoside (SDG) tested, whatever the age and number of volunteers involved, was able to show an effect over placebo treatment. This could be expected at least for two reasons. First, enterolignan precursors are ubiquitously distributed in human diet, and therefore the placebo group also receives estrogenic enterolignans in rather variable amount. See Table 2 and the known sources of lignans. This, of course, reduces the power of the interventional studies. Second, only END or ENL can possibly exert estrogenic effect, which could explain hot flashes relief. However, only a small portion of the population harbors the gut flora able to produce these enterolignans in sufficient amount [117]. Therefore, the enterolignan effect should only be seen if women able to produce enterolignans were compared to women unable to produce them. This would require a selective procedure at the entrance of the clinical studies and enterolignan monitoring in blood to account for the dietary exposure through casual diet. None of the studies done until now did undergo such

protocol. Note that urine measurements may not be precise enough since an eliminated compound is not an active one and that there may be interindividual variations in the elimination pathways of enterolignans.

Zearalenone
Zearalenone and its metabolites α- and β-ZOL as mycotoxins have not been tested in clinical intervention on menopausal symptom relief.

5.1.3 Bone Health in Women

Isoflavones
Two meta-analyses converge in saying that isoflavone from soy may be protective on bone density in menopausal women but only at high doses, i.e., over 80 mg/day. The first one [118] considered only supplementation by soy isoflavone extracts (not soy protein or foods containing isoflavones) on bone mineral density (BMD) in menopausal women. It was based only on randomized controlled trials published in English, Japanese, or Chinese reporting the effects of soy isoflavone extracts on lumbar spine or hip BMD in menopausal women. Only 11 studies in total were found to match the given criteria. The meta-analysis included data from 1,240 menopausal women. It revealed that daily ingestion of an average of 82 mg of soy isoflavones (aglycone) for 6 to 12 months significantly increased spine BMD. Treatment duration, geographic origin, and basal BMD had a major influence on the effect observed. No significant effects on femoral neck, hip total, and trochanter BMD were found, while soy isoflavone extract supplements increased BMD feature that was restricted to the lumbar spine in menopausal women. The second meta-analysis [119] included randomized controlled trials (RCTs) examining the effect of a soy isoflavone supplementation in women for at least 1-year duration. The main outcomes were BMD changes from baseline at the lumbar spine, total hip, and femoral neck. Ten RCTs gathering 896 women were found to be eligible according to the study criteria. A mean dose of 87 mg of soy isoflavones for at least 1 year did not significantly affect BMD changes. However, when doses were stratified, it was shown that only a large dose over 80 mg/day of isoflavone tended to have a weak beneficial effect on spine BMD. The BMD was not increased but preserved.

Coumestrol
*N*o data seem to exist on the effect of COUM on menopausal women bone health. Only one study tested the effect of this compound in rodents [120], showing a preventive effect against bone loss in an ovariectomized rodent model. The estrogenic activity of COUM in vivo on bone cells has also been shown on the prevention of osteoclast differentiation [121] and the enhancement of osteoblast formation [122]. These are classical estrogenic effects.

Lignans
The only study which was found dealing with the association of lignans and bone health is likely to be insufficient to prove any effect [111]. It deals with an exposure

evaluated from a dietary questionnaire, and since median intake of total phytoestrogens was estimated at 876 μg/day, the study most probably underestimated the exposure. Phytoestrogen plasma assays should have been done for this study to really help in determining the enterolignan effects. Nevertheless, the authors said that enterolignans estimated from food intake irrespective of the gut flora efficiency were positively associated with bone density in postmenopausal women. However, this association became nonsignificant when dietary Ca^{2+} was added to the model. In light of this finding, further data are needed before any definitive conclusion was drawn out.

Zearalenone

Because of the toxicity of the molecule and of its metabolites α- and β-ZOL, no study was performed and published in humans. As for COUM, some data can confirm the estrogenic effects of ZEN and metabolites on bone cells in vitro [123] but also in vivo in rodent models [124]. Again the main outcomes are characteristic of estrogenic effects, with a prevention of bone loss [125], although in some studies chromosome aberrations were also reported [126 and publications included].

5.2 Deleterious Effects

Looking at deleterious effects is a challenge since in several occasions it can be seen that the experts do not agree on what is adverse and what is safe. Before the 2010s hormonal changes were not always considered as adverse, and therefore two types of noneffective levels were defined for phytoestrogens: the no observable adverse effect level (NOAEL) and the no observable effect level (NOEL). Conventional toxicology relies upon measures of exposure that induce pharmacological or physiological adverse effects. Hence, the NOEL is the highest dose tested at which there is no measurable effect, and the NOAEL is the highest dose tested at which there is no measurable adverse effect. Then the definition of what is an adverse effect is crucial to consider. Nowadays, the concept of endocrine disruption has emerged, and all hormonal modifications especially those dealing with reproductive hormones can be considered as adverse. However, such adverse effects are usually observed on a long-term basis, and therefore multigenerational studies may sometimes be required to see an effect. For example, if chronic classical toxic effects are considered for GEN, the NOAEL on liver endpoint is 50 mg/kg/day. However, considering hormonal endpoints and according to McClain and co-workers, the NOEL is 5 mg/kg/day [6]. If hormonal effects are seen as endocrine-disrupting effects, then the NOAEL of GEN switches from 50 mg/kg/day to 5 mg/kg/day. This NOAEL is sustained by the recent studies of the National Toxicology Program (NTP) in the USA [4]. However, the NTP study also showed reduced birth weight and reduced weight at weaning of pups at all doses tested including 0.3 mg/kg/day in females and over generations. The authors concluded that if this effect is considered as a toxic effect, then the NOAEL is below 1.2 mg/kg/day in rat. It also showed a reduced anogenital distance reduction in pups of both sexes at various doses and in several

exposure conditions. Litter size was also impaired after several generation of exposure for the highest dose tested. These effects sign a physiological impairment which can have consequences on reproduction which is finely tuned by complex endocrine balances. Therefore, effects which could have been neglected in the past come to the front nowadays because science progresses. NOAEL and LOAEL (lowest observed adverse effect level) can be used to establish mean tolerable daily intake (MTDI). A TDI is an estimate of the amount of a substance that can be taken daily over a lifetime without appreciable health risk. TDIs are calculated on the basis of laboratory toxicity data to which uncertainty factors are applied. According to [127], reasonable uncertainty factors should be applied. When a NOAEL in rat is available in chronic study, this uncertainty should include a factor accounting for the difference of sensitivities among humans and a factor accounting for the relative sensitivities of animals compared to humans. In that case, the MTDI in human is 46 times lower than the NOAEL in rat. When only LOAEL are available, the uncertainty factor should also account for uncertainty in extrapolating from a low-risk level. Therefore, the global uncertainty factor between LOAEL and MTDI increases to 184. Here, the adverse effect can be considered to be a hormonal disruption. As TDIs are regarded as representing a tolerable intake for a lifetime, short-term exposure to levels exceeding the TDI is not a cause for concern, provided the individual's intake averaged over longer periods of time does not appreciably exceed the level set. The uncertainty factors used to establish a TDI provide assurance on the relative safety of the TDI. However, there should be concern if the TDI is substantially exceeded for long time periods. In order to have a quick overview of what can be said about the molecules retained here, we propose Table 7 which gathers NOAEL or LOAEL obtained in rat. It also gives an overview of the estimated exposure and a weight limit under which the exposure is due to overcome the MTDI. This table is partly deduced from that of Hendrich [128].

These data have to be considered with caution, since here again, science evolves and toxicological studies performed more than 10 years ago may not reach the standard of quality actually required. However, if they have not been reproduced recently, they should be considered as valid. The MTDI presented in this table are compared to the mean daily exposure expressed in mg/day. The values given here may be corrupted by many biases when they were estimated from food frequency questionnaires. First these questionnaires may not exactly reflect all population exposure, and second the large variability of the content in the phytoestrogen of interest may not be taken into account. Some food sources can be completely omitted as for isoflavones. Hence, as already mentioned, soy is nowadays ubiquitously present in industrially processed food, and the isoflavone intake is so common that they can be found in a large proportion of consumers' urine samples (100% in the US study by Mumford [5], 100% in the German study by Degen [66], 98% in the Israeli study by Berman [65]). Note that for lignans, no data of exposure were found in Asian consumers. This is due to the fact that many plants including plants used for personal care in Asia are sources of the estrogenic-enterolignan precursors. All these precursors are not known yet, and this has to be combined to the fact that not all the consumers harbor the competent gut flora for ENL production. Therefore, when the

Table 7 Summary of NOAEL or LOAEL of natural estrogenic substances together with the consumer estimated exposures and the limit at which the mean dietary tolerable intake can be overcome

Phytoestrogens	NOAEL in toxicological studies	References	Human MTDI (mg/kg)	Estimated daily mean intake (mg/day)	Country	References	Inferior weight limit for MDTI overcome
Isoflavones	5 mg/kg	[6]	0.11	2.7	USA (general pop)	[31]	24.55
				50	Japan (general pop)	[58]	454.55
				2.257	UK (general pop)	[84]	20.52
				1.12	France (women)	[84]	10.18
				0.1782	Spain (women)	[84]	1.62
				0.22	Greece (women)	[84]	2.00
				0.317	Italy (women)	[84]	2.88
				0.7675	Netherlands (women)	[84]	6.98
				0.46	Denmark (women)	[84]	4.18
				0.893	Sweden (women)	[84]	8.12
				1.285	Norway (women)	[84]	11.68

(*continued*)

Table 7 (continued)

Phytoestrogens	NOAEL in toxicological studies	References	Human MTDI (mg/kg)	Estimated daily mean intake (mg/day)	Country	References	Inferior weight limit for MDTI overcome
Coumestrol	4 mg/kg (LOAEL)	[129]	0.022[a]	0.01243	USA	[130]	0.57
				Trace	China	[60]	–
				0.06259	Europe (men, mean)	[84]	2.85
				0.04348	Europe (women, mean)	[84]	1.98
Lignans	100 mg/kg (LOAEL)	[131]	0.540[a]	0.155	USA	[132]	0.29
				nd	Asia	–	nd
				1.371	Europe (men, mean)	[84]	2.54
				1.207	Europe (women, mean)	[84]	2.24

Zearalenone	0.2 mg/kg	[133]	0.0001	0.000055	Europe mean (UB)	[54]	0.01
				0.000973	New Zealand (mean)	[134]	0.19
				0.000019	Canada (young men)	[135]	0.19
				0.000047	Canada (young children)	[135]	0.19
				0.000098	Canada (adults)	[135]	0.47
				0.0017	USA	[136]	17.00
				0.00153	France (adults UB)	[82]	15.30
				0.001155	France (children UB)	[82]	11.55

The inferior weight limit for MDTI overcome is obtained dividing the estimated daily intake by MTDI
MTDI mean tolerable daily intake
[a]extrapolated from the LOAEL; *nd*, not determined

effect of ENL is analyzed, the substance is assayed in the urine, but the correlation of the urine dose to a dietary intake is far to be simple. Finally, when the dose is low, the detection technique may not be reliable enough to get good results. Therefore, looking at the exposure to lignans in Asia, it was impossible to find any published data. The last column of the table gives a figure which materialized the inferior weight limit for MTDI overcome. It is calculated by dividing the estimated daily intake by the MTDI. As an example, for GEN in the USA, considering a mean daily intake of 2.7 mg in the general population, it appears that all people, whose body weight is lower than 24.55 kg, overcome the MTDI. Because this limit is considered as safe, the concern about this situation can arise only from its chronicity and from the degree of overtaking from the MTDI. The MTDI is a mean of the population exposure, and some people can be exposed to much higher doses. The situation in Japan could be considered as worrying since the calculation indicates that all people under 454.55 kg overcome the MTDI, considering the daily intake estimation. In that case, a fertility problem over generation should be investigated because it is seen in experimentally exposed rats.

5.2.1 Reproduction in Animals

Isoflavones

Isoflavones were considered for many years as estrogenic endocrine disruptors and studied as such by several authors [137–144]. These compounds can be present at high doses in estrogenic pastures based on certain clover species or on alfalfa. In sheep on these pastures, estrogenic isoflavones were shown to induce permanent estrus, heavy vaginal secretion, uterus prolapse, early abortion, reduced prolificity in reproductive ewes, and mammary fluid production in nulliparous ewes and castrated males. Castrated rams also exhibited abnormalities in their reproductive tracts, including enlargement of the prostate, the bulbourethral gland, and the membrane of the *vasa differentia*. In ewes affected by clover diseases, clover isoflavones were shown to delay LH secretion via a GnRH interaction [142]. This LH delay in secretion, which led to progesterone secretion impairment, was considered to be responsible for early abortion. More recently, a renewed interest emerged among scientist for the soy and isoflavone effects on reproduction of domestic animals. This is because it was recently figured out that isoflavone deleterious effects on cattle could have been missed out because soy was progressively incorporated to cattle food while genetic selection was undertook to improve production features including reproduction. Therefore, the antagonistic effects of both processes could have partly masked each other. As an example, it was shown that soy isoflavones can prevent in cows as in rodent the response of the *corpus luteum* to GnRH [145]. This result obtained, in vivo, is confirmed by data recently obtained ex vivo on Prim Holstein cows [146]. In addition, clover isoflavones were shown to block the early progesterone synthesis in Holstein heifers fed with a *Trifolium alexandrinum*-rich roughage after insemination [147]. This disruption was thought to be responsible for lesser ($P = 0.054$) conception rate and the greater ($P = 0.062$) percentage of heifers returning to estrus when compared to the control silage-fed heifers. As demonstrated

for COUM, GEN and DAID are able to increase the secretion of oxytocin by cow *corpus luteum* [148]. This can induce early abortion in these females and reduce global fertility and hence financial incomes to farmers relying either on veal or milk production. Recently, it was shown that isoflavones intake through Egyptian clover (*Trifolium alexandrinum*) as estrogenic roughage exerted a deleterious effect on fertility. Comparing the estrogenic roughage to the control diet based on maize silage, the % of Holstein heifers returning to estrus was 7.70 (1/13) under the control diet, and it was 38.46 (5/13) when cows ate clover roughage. Still in cows, it was shown that soy phytoestrogens increase prostaglandin secretion in cattle during estrous cycle and early pregnancy [149]. The number of cows involved in the trial was low. However, it appeared that soy-derived phytoestrogens and their metabolites disrupt reproductive efficiency and uterus function by increasing the ratio of prostaglandin F_2-alpha (PGF_2-α) to prostaglandin E_2 (PGE_2). Therefore, phytoestrogens led to high, nonphysiological production of luteolytic PGF_2-α in cattle during the estrous cycle and early pregnancy. The consequence of this increased PGF_2-α production is a higher failure rate of pregnancy. In addition, in vitro but at physiological doses, GEN, DAID, and equol were shown to inhibit bovine adrenal 3-beta-hydroxysteroid dehydrogenase directly involved into the synthesis of progesterone [150]. This inhibition seems to be possible in vivo considering the active doses of isoflavones in vivo. On top of the LH disruption, this direct effect may be a way by which isoflavones reduce progesterone secretion in early pregnancy. This can cause early abortion.

The phenomenon is generally enough to have led to endocrine disruption also observed in lower vertebrates of economic importance like birds or fish. Although the isoflavone effect in hens and laying hens does not seem to be deleterious, some long-term effects were recorded in ducks [151]. Noteworthy, ducklings were significantly smaller at hatching when exposed maternally to DAID supplementation. The difference in size compared to control animals disappeared at 4 weeks of age. It was accompanied by changes in the secretion of metabolic hormones and expression of growth-related genes. Although the negative effect of maternal DAID on embryonic growth could be eliminated 4 weeks after hatching, the long-term effect of maternal DAID on reproductive function was noted. Namely, it was an obvious down-regulation of hypothalamic GnRH mRNA expression observed in ducklings maternally exposed to DAID. Fish in fish farms fed with a soy-containing diet can also exhibit altered reproductive performances or at least endocrine-disrupting features [152–154]. These effects were recently confirmed in goldfish considered as a model for other cyprinids [155] and also in other species of commercial interest like catfish or sea bass [156, 157]. Both estrogenic and thyroid functions involved in smoltification were shown to be impaired by soy phytoestrogens in the Atlantic salmon [158].

Coumestrol

Coumestrol was shown to exhibit deleterious effects on grazing animal reproduction [159]. Coumestrol was also involved in the clover disease affecting ewe reproduction in New Zealand in the late 1940s, and these endocrine disruptions still attract

scientific interest [160]. According to Adams [161], cows are more sensitive to COUM than to isoflavones most probably due to differences in metabolism when compared to ewes. As an example, COUM was shown to increase the production of oxytocin by cows decreasing the ratio of progesterone to oxytocin. This endocrine modification induces a higher failure rate to pregnancy. Still in the same study, COUM was shown to decrease PGE_2 secretion. These endocrine-disrupting effects can increase the risk of early abortion in this species [162] and therefore can affect the % of females returning to estrus as well as the calving to calving interval. In mare, COUM exerts endocrine-disrupting effects on chronic exposure leading to ovulation impairment [163]. The authors conclude to a potential infertility syndrome due to estrogenic silage (clover and alfalfa) in mares. The first reports of the deleterious effects of COUM in rat were produced in 1970 [164]. Then the Whitten's group published several coherent data showing the estrogenic effects of dietary COUM on the rat puberty and cycle [165], on uterus development [166], on the embryo implantation [167], and on the pituitary and hypothalamus hormone disruptions [167]. They also pointed out the remaining effects after the end of exposure [168] as well as sexual behavior impairments [169]. Other studies showed reproductive adverse effects on males' semen production which could be explained by a precocious estrogenic effect affecting the pituitary hormones and steroid secretion [170]. According to Hendrich [128], there is no NOAEL for COUM, and the LOAEL is 4 mg/kg/b.w./day.

Lignans

There is few data on the role of estrogenic enterolignans in domestic animals. These lignans can be present in significant amount especially when linseeds are used to modify the meat quality toward a greater proportion of omega-3 polyunsaturated fatty acid (PUFAs). According to [171], ENL concentration is increased in follicular fluid from cows fed with flaxseed-rich diet, although the serum levels are not affected. These increased enterolignan concentrations are correlated with increased concentrations of estradiol locally addressing the question of an effect of ENL on follicular steroid biosynthesis. Still in cattle, [172] showed that ENL deriving from a flaxseed-enriched diet or given alone in parallel of specific mixtures of PUFAs can decrease PGE_2 and $PGF_2\alpha$ concentrations in endometrial, stromal, and epithelial cells. This reduction was associated with lower mRNA abundance of the PG synthase genes in stromal cells. An omega-3-enriched PUFA mixture increased the effect of ENL compared to ENL alone and PUFA mixture rich in omega-3 alone. The authors concluded that considering the known luteolytic properties of $PGF_2\alpha$, a reduction in endometrial $PGF_2\alpha$ secretion would favor the establishment and maintenance of pregnancy. Enterolignans were found in the early 1980s in human and cattle semen. In both cases, their concentrations were shown to be 2.5 times higher than the corresponding plasmas [173]. In rat they were found to alter pregnancy outcome and reproductive development [131].

Resorcylic Acid Lactones

Zearalenone and ZOL are known as endocrine-disrupting agents from fungi since the early 1960s [51]. In cattle, ZEN was shown to lower the conception rate of the heifers [174]. In addition, ZEN contaminating sugar beet pellets was considered to be responsible for a reduced embryo transfer efficiency from a dairy farm experiencing low success rates of embryo transfer [175]. More recently, thanks to better detection techniques, it was shown that low ZEN contamination (below the allowed limit in Japan) was able to modify anti-Mullerian hormone levels in cow showing an effect in the ovarian antral-follicle populations in cows [176]. In swine the deleterious effects of ZEN on reproduction have been studied scientifically as early as in the 1970s [177]. It was later shown that concentrations of 25, 50, or 100 ppm of 95% purified ZEN fed to groups of healthy, multiparous sows during pre-estrus or throughout the gestation period (or both) produced multiple reproductive deficiencies [178]. These disorders included infertility, constant estrus, pseudopregnancy, diminished fertility, reduced litter size, offspring's small size, offspring malformations, juvenile hyperestrogenism, and probably fetal resorption. Sows' reproductive organs exhibited lesions, and their uterus, uterine duct, and cervix showed marked epithelial changes, i.e., squamous metaplasia. Similar features were found in the vagina and mammary glands. Transgenerational effects were also documented in sows [179], and ZEN was shown to reduce the quantity of healthy follicles, which probably lead to premature oocyte depletion in adulthood. In rat toxicity studies were undertook using ZEN (in corn oil) at doses of 0, 1, 2, 4, or 8 mg/kg/b.w. in order to determine the NOAEL for in utero development [180]. The fetal and pups' body weights were decreased in a dose–response manner in both sexes. Fetal development was delayed in all treated groups and linked to maternal toxicity. Zearalenone delayed skeletal ossification at 4 and 8 mg/kg. Fetal anogenital distance was increased in all treated groups, and fetal viability was decreased at 8 mg/kg. The weight of several maternal organs was modified at 4 and 8 mg/kg doses. Gonadotrophins were only marginally affected. Prolactin was significantly increased at 8 mg/kg. Estradiol dose dependently decreases at 2, 4, and 8 mg/kg. Therefore, ZEN was maternally toxic and fetotoxic but not teratogenic. Based on the dose-related maternal and fetal toxicity in all treated groups, the NOAEL for reproductive and teratogenic effects was less than 1 mg/kg.

5.2.2 Reproduction in Human

Isoflavones

Fertility: Unfortunately, as regards the human fertility, no clear-cut data are currently available about the effect of the modern exposure to estrogenic isoflavones. In 2014, an epidemiologic study performed on Adventist populations [181] sorted into six groups of isoflavone consumers showed that in young women (26 years old on average), the highest exposure of >50 mg/day was associated with a significant

decrease in the number of birth. The same isoflavone intake was also correlated to an increased risk in women over 50 years of never being pregnant. Fertility also depends on the ability of men to produce efficient gametes. Noteworthy, sperm production is a long process, starting from spermatogonial recruitment, continuing by spermatogenesis in the testis, and ending by the capacitating steps into *rete testis* and epididymis. In men the first step in the testis lasts for about 74 days [182]. Sperm presence in the *rete testis* and epididymis can vary from 2 days up to 18 days. It is only after sperm has undergone all steps that it can be ejaculated for fertilization and studied for its ability to fecundate. Therefore, the whole process from production to ejaculation can be considered as lasting about 3 months [182]. Consequently, whenever the effects of any endocrine-disrupting compound need to be studied on sperm production in men, exposure to those compounds should exceed 3 months to avoid any effect remaining undetected. As the capacitating process in the epididymis requires adequate thermal conditions, environmental conditions can exert bias on the final sperm quality. It is well-known that sperm production is influenced by both androgens and estrogens and that environmental endocrine disruptors (androgens, antiandrogens, estrogens, antiestrogens) can disturb sperm production in quality and in quantity [183]. As certain interventional studies on sperm production and quality like those of [184] or [185] were, unfortunately, performed over a short duration (less than 50–60 days), those studies cannot be retained when arguing about the effects of isoflavones on full spermatozoa production. Interindividual variation in sperm production and release, over the study period, is also a confounding factor that is not always taken into account and which can explain unexpected results [186]. In addition, the exposing dose should be in the dietary range. The data given in [1] indicate that, if a deleterious effect should be confirmed regarding sperm production and fertility, this should be addressed at a demographic level with data from industrial soy-eating countries and traditional soy-eating countries being treated separately. Noteworthy, the two countries that are most exposed to isoflavones because they have been eating industrial soy since the 1960s are South Korea and Japan. IndexMundi in 2017 indicates that the total fertility rate being the average number of children that would be born to a woman over her lifetime is 1.41 for Japan and 1.25 for Korea. These figures are lower than those recorded in all other developed countries with the exception of China territories. In France the total fertility rate is 2.07, and it is 1.87, 1.44, and 1.89 in the USA, Germany, and the UK, respectively. Noteworthy and still from the same sources, the contraceptive prevalence rate for women between 15 and 49 in Japan is only 33% when it is 80% in South Korea, 88% in France, 74.1% in the USA, 66% in Germany, and 84% in the UK. In addition, in both Asian countries consuming industrial soy, the demography regressed since 1983, i.e., one generation after soy-processing industrialization. This is also concomitant with the massive introduction of plant protection products in crop production all over the world. Moreover, in all studies that did show a correlation between decreased sperm quality and isoflavone intake, it was never possible to definitively exclude the interacting effects of other steroidal estrogens (in the case of obese men [78]) or of other endocrine disruptors like plant protection products [5, 79, 80]. In addition in [5], Mumford showed a positive interaction of

sperm abnormalities with BMI. This indicates that the deleterious action of isoflavones on sperm production is probably influenced by other endocrine disruptors, present or not in the environment, and by endogenous estrogens.

Plausible consequences of early exposure: From a physiological point of view, a significant exposure to estrogens of babies under 6 months of age comes with its inevitable load of consequences [187]. During that period, differentiating processes involving estrogens and androgens are still at work, and steroid receptors are present in the different target tissues. These steroids, which were shown to potentiate isoflavone actions, are well-known to be disturbed in animals [4, 188] or in vitro [189] by isoflavones. The study from Strom et al. [190] showed that early isoflavone exposure via soy-based infant formula led to an endocrine impact with ulterior consequence on menstrual cycle impairment. It should be noted that cycle impairments were noticed in the rats of the NTP study [4] that examined the effects of GEN on the reproductive physiology of rats. However, the subjects enrolled in [190] were too young to allow a fertility impact of early isoflavone exposure to be shown. Noticeably, the sperm quality and production of men exposed to early soy were not assessed either. The number of stillborns in the group of women fed with soy (3 out of 74 births) was not significantly higher than the number of stillborns in the cow milk-fed group (0 out of 149 births), because only a small number of women had given birth at the time of the study. In fact Gilchrist and co-workers [191] discovered that 4-month-old boys fed with soy-based infant formula (S-BIF) from 3 weeks of age had a lower testis volume than that of their counterparts fed with breast milk ($p < 0.03$). Their testes volume was lower but not significantly than that of boys fed with cow's milk. This can be explained by a decrease of LH and testosterone neonatal production due to isoflavone intake. This is sustained by the study of Sharpe et al. on paired twin marmosets fed with either breast milk or soy-based infant formula prepared for human infants [192]. The soy-fed baby marmosets had lower testosterone plasma levels than their twin brother fed with cow's milk formula. In marmosets the exposure was lower than in boys exclusively fed with S-BIF, and no differences were seen in testes histology when the monkeys reached adulthood [193]. Adgent and co-workers reported a masculinization of the play and toy preferences in girls fed with S-BIF from 3 weeks to 6 months of age [194]. The observation was done at 42 months of age and tended to vanish at 57 months apparently due to social pressure. This observation can be linked to the masculinization effects of estrogens on the sexually dimorphic area of the hypothalamus during neonatal exposure (see [11] for further details). In addition and still according to the same team, a subtle acceleration of menarche could be provoked by an early exposure to estrogenic isoflavones via S-BIF [195]. These results although not fully convincing could also be linked to data published by Kim and co-workers [196] associating high serum concentrations of DAID ($P = 0.0202$), GEN ($P = 0.0021$), and total isoflavones ($P = 0.0009$) with central precocious puberty in young South Korean girls. As already mentioned, South Korea is in 2017 the country with the world's lowest total fertility rate [197]. More recently, it was shown that S-BIF can epigenetically modify the promoter of the PRR5L exon 1 gene [198]. The role of this gene is not yet fully understood, but it seems to regulate cell migration via an

interaction with mTORC2 and PKC-delta. The study essentially points out that early exposure to isoflavones can have noticeable consequences later in life. The reason why reproductive deleterious effects linked to early isoflavones intake were not yet observed at population level is essentially because the appropriate studies have not so far been performed [68]. A longitudinal study, which could be the best approach, is unlikely to show significant effects, if it does not involve a huge number of subjects, especially during childhood reproductive dormancy [199]. In [190] Strom et al. were focused on too early a stage, before both adult men and women really want to have children, and the study was unable, therefore, to help in determining whether early soy feeding could induce a fertility problem. One consequence is that, even if no alarming effects have been reported so far, it is not because they do not exist but rather because they may be expressed long after the initial causal exposure. Accordingly, the effect of this initial exposure may be masked by other psychological, social, economic, or even chemical disrupting factors, inducing large interindividual variability. If a reproductive effect really exists, it should be seen in those populations which are largely exposed to isoflavones through industrial modern soy food and not in those consuming traditional soy. Because it is now recognized that there can be interactions with other anthropogenic endocrine disruptors [2] like bisphenol A or glyphosate [200], the effects of isoflavone exposure can be potentiated by other environmental chemicals. Careful monitoring, which would allow these effects to be separated, should be undertaken in order to discover what is really happening. The expected effects could induce lower sperm production in men and an increased incidence of abnormal menstrual cycle, of early or late abortion, and of larger uterine fibroids in women exposed during their infancy [190, 201]. According to [198], a higher risk of uterine cancer may be observed. If the percentage of infants fed with soy-based infant formula in a given population was monitored for many years, then it could be possible to check whether that percentage is maintained or increased in male adults consulting for fertility problems.

Physiological effects of actual exposure: Nutritional doses of modern soy isoflavones (45 mg/day) have estrogenic effects in women, which can lead to a modest lengthening of the menstrual cycle [57, 77]. Noteworthy, when the diet is fully monitored to avoid the presence of hidden isoflavones, the menstrual cycle of American premenopausal women can be significantly lengthened by 2 days [57]. This lengthening is due to a retardation of the LH surge at mid-cycle. The same consequence is also observed in ewes eating clover rich in isoflavones [142]. This phenomenon was also observed in Japanese women. In that case, the mean menstrual cycle length is 30 days as opposed to 28 days in the West [77], with the mean modern isoflavone intake in Japan and Korea currently being evaluated at between 45 and 60 mg [58]. In this context, Nagata and co-workers [77] showed that supplementing the current Japanese diet with 50 mg of extra isoflavones from soy juice can lead to a menstrual cycle length of 32 days. Although this lengthening may possibly be protective against estrogen-dependent cancers, it can also reduce, however, the opportunity of ovulation in fertile women. This may well contribute to reducing the reproductive efficiency of a given population. In addition, as shown in domestic animals, it can be hypothesized that modern dietary intake of isoflavones

can impair embryo implantation and induce early abortion. In women so far, only luteal phase deficiency was associated with isoflavone intake among healthy eumenorrheic women. It is a modest correlation with an adjusted odds ratio (aOR) of 1.38 (95% CI: 0.99, 1.92), $P = 0.06$, because the subpopulation studied was small. Because luteal phase deficiency can lead to miscarriages and impaired fertility, it may be responsible for a progressive demographic regression at the population level. Moreover, according to [202], high soy intake for long durations can have even more significant effects. In this study, the authors reported that high soy intake was responsible for abnormal bleeding in women under norethisterone (contraceptive pills). This means that their large soy intake was able to transform a contraceptive treatment based on progesterone into an estro-progesterone form of contraception. A similar case involving a 32-year-old woman who drank a liter of soy juice, 3 times a week, after her basketball training, was also observed by our team. Her cycle impairment regressed when soy intake was stopped. In [202], the authors also alerted against uterine fibroids and repeated endometriosis features in the three cases they followed. They reported that all the problems regressed when soy intake was stopped.

Vaginal and endometrial health: Both the vaginal mucosa and endometrium develop under estradiol stimulation and therefore bear all the known estradiol receptors: ERα, ERβ, GPER, as well as ERRs [203–205]. The estrogenic effects of isoflavones were first shown on the uterotrophic test and even before on the uterus maturation of the New Zealand ewes on clover pasture. Therefore, negating an effect of estrogenic isoflavones on the endometrium makes no sense. However, in animals, isoflavones effects were reported either in reproductive females or in female freshly ovariectomized. In all cases, this means that estradiol receptors were available to mediate an estrogenic effect of isoflavones. In humans, most of the studies were performed in menopausal women. Most often their distance to menopause was largely variable, and the availability of ERs in the vagina and endometrium, although known to decrease after the arrest of ovarian function [205, 206], was not checked. Therefore, no study showed a significant effect on the endometrium especially in Western postmenopausal women [207], and very few studies were able to show an effect on the vagina in postmenopausal women. Among them is that of Lima and co-workers [208] which showed a significant estrogenic effect of a vaginal gel containing isoflavones on vaginal dryness, on dyspareunia, and on maturing index of vaginal cells. The last endpoint evolves similarly in the isoflavone group and in the conjugated equine estrogen group [208]. Interestingly, a study performed in Japanese students by Watanabe and co-workers [209] dealt with the supplementation on normal Japanese diet with either 20 or 40 mg isoflavones. Considering the mean urinary levels at baseline, the basal isoflavone exposure was most probably close to 25 mg/day. In their study, Watanabe found that extra isoflavone lengthen the menstrual cycle by 2 days and lengthen menstruations and bleedings in a dose-dependent manner. They observed that not all students react equally to the treatment. On the blood samples collected from three volunteers, they did not show any significant modification of steroid or gonadotrophin hormones. This suggests a direct effect of isoflavones on the endometrial mucosa. To conclude, isoflavone effects on

the vagina and uterus of premenopausal women must not be rejected although they require additional work for evidence. In postmenopausal women, a vaginal effect of isoflavones may be achieved, while an endometrial effect has never been clearly shown despite a large number on trials.

Coumestrol

Although there is a great deal of studies reporting the effect of COUM on rodent genital tract, there is little data in women. Only in epidemiology studies COUM is sometimes investigated. Each time the level of intake is usually low (a few μg per day) and can be omitted in front of other phytoestrogens considering its poor availability and its large distribution in human fluid. As an example, in the study by Xia [80], showing a deleterious effect of phytoestrogens in Chinese men presenting idiopathic infertility, the 19th percentile exhibiting the largest impairment presented 619.36, 408.86, and 504.90 μg/g of excreted DAID, equol, and GEN, respectively, while they excreted 4.04 μg/g of COUM. The total excreted isoflavones was then 1533.12 μg/g. This is more than 350 times higher than the dose of COUM.

Lignans

Because not all humans can produce the estrogenic metabolites of dietary lignans, only studies based on either blood or urine measurements can be taken into account while considering the effects of enterolignans on reproduction. Hence, urinary levels reflect an elimination process that reduces the blood bioavailability. Therefore, whenever possible plasma or serum levels should be preferred. Considering these restrictions, the study by Tang and co-workers [210] indicated that when sorted in four quartiles (i.e., 16.54, 85.53, 325.91, 891.7 μg/g of urine) in pregnant women at delivery, birth outcomes were significantly modified. The higher the enterolignans' urinary levels, the lower the gestation length. There is also a tendency toward lower birth weight with END. The decrease in gestation length can be explained by a precocious induction of oxytocin receptor production increasing the action of oxytocin at the time of labor delivery. This has been shown for estrogens, not yet for enterolignans [211]. The smaller birth weight can be linked to a vasoconstriction of placental vessels due to estrogens which are not harvested by the alpha-fetoprotein because their affinity for this protein is lower than that for the estradiol receptor [212]. In addition, it was also shown in American men that urinary END and ENL were associated with subtle deterioration of spermatozoa quality parameters in a dose–response manner [5]. However, it should be kept in mind that lignans are biomarkers associated with grain, vegetable, and fruit consumption. All these items can be contaminated by plant protection products, and this effect may reflect at least a synergistic effect between several endocrine disruptors. Contrarily to isoflavones, enterolignans are associated with better reproductive incomes in humans [213]. Likewise, Mumford and co-workers showed an association of urinary ENL and shorter time to pregnancy. This may be due to the specific effect of enterolignans on the ERα [45].

Zearalenone

There is little convincing information about the effects of ZEN in humans because such an effect could only be recorded on long-lasting exposure if an effect has to be correlated with a serum or urine level. Nevertheless, ZEN was pointed out in the occurrence of precocious puberty, in Italy [214] and Puerto Rico [215], although levels were not actually assessed in any biological fluid. In Hungary [216], an increased incidence of early thelarche was reported in patients with serum ZEN levels of 18.9–103 μg/mL. In 2010 it was published that in Viareggio (Tuscany), the incidence of precocious puberty was 22 to 29 times higher than in the neighboring areas [217]. Among 63 cases, 6 of them had high serum ZEN (933.7 ± 200.3 pg/mL) and α-ZOL (106.5 ± 1.9 pg/mL). But only 5 of 36 patients with early thelarche presented detectable levels of mycoestrogens. In 2003, ZEN was associated with "endemic enlargement disease" in China [218]. All grains analyzed were contaminated by mold, and 34% exhibited *Fusarium* species and COUM. More recently it was found that mycoestrogens were detectable in a large proportion of the urine of the 163 girls, aged 9 and 10 years, participating in the Jersey Girl Study and enrolled in the survey by Bandera et al. [219]. The subjects presenting the highest values were also being significantly of shorter stature and less likely to have reached the onset of breast development. This may sign an estrogenic effect on the pituitary gland with resulting anti-FSH effect. Such effects were reported in rodents [220] and more recently in pigs [221].

6 Questions

6.1 Estrogen-Dependent Cancers

6.1.1 Isoflavones

There has been a long-lasting controversy about the positive or negative effect of isoflavones on breast, endometrial, or prostate cancers all considered being more or less estrogen-dependent. Their incidence is also lower in Asia.

Endometrium

As far as endometrial cancers are concerned, in 2015, the EFSA opinion [222] indicated that no definitive opinion could be given on any deleterious effects of isoflavones on uterine cancer. However, the study only concerns healthy peri- and postmenopausal women, because it was based on the scientific literature in which volunteers are recruited following specific inclusion criteria, and such criteria do not, therefore, exactly reflect the global population. No case studies were performed to see an effect on endometrial cancer, and therefore neither the quality of the population nor its number was designed to demonstrate an effect. According to [222], there are very few studies triggering specifically perimenopausal women, and the number of studies was insufficient to conclude on this specific population. No conclusion could be rigorously drawn out on perimenopausal women because they cannot be assimilated to postmenopausal women. Noteworthy, their production of endogenous

estrogens was still active, and estradiol receptors were still present at the target tissues. On the opposite, there is a progressive decrease in endometrium estradiol-receptor bioavailability in postmenopausal women [206]. Therefore, a lack of action of isoflavones in the uterus of these women is expected, because the estrogenic effects of isoflavones require an interaction with the estradiol receptors. On top of other things, this tissue bears both alpha and beta subtypes of ERs [206], and isoflavones affinity is greater for the beta subtype [223, 224]. As the activation of the ER-beta was shown to negate the proliferative action of the ERα [225], via its AF-1 function [226], normal soy intake is not likely to affect the rate of endometrial cancer of healthy postmenopausal women.

Prostate

For prostate cancer, there is also a controversy. If the incidence of prostate cancer is lower in Asian population, the postmortem tumor detection indicates that the frequency of prostate cancer is equivalent between Asia and the West [227]. To add to the controversy, Nakamura and co-workers [228] recently developed a patient-derived prostate cancer xenograft model. They grafted clinical prostatectomy samples into nude athymic mice fed with 0 or 2 or 10 mg/kg genistein. For these doses, they showed dose-dependent increases in lymph nodes and secondary organ metastases (liver, lungs). They also showed the aggregation of invasive malignant cells in the secondary organs of the genistein-treated groups and not in the control. Their data suggest that the effect of genistein may depend on the kind of prostate tumor considered. A recent meta-analysis [229], performed on 21 case-control and 2 cohort studies, found that GEN and DAID may, in some cases, be associated with decreased risk of prostate cancer. However, when studies were sorted out according to the way isoflavone intakes were assessed, it appeared that the risk was not reduced when isoflavones were assayed in the serum or urine, although it was reduced when GEN and DAID exposure was assessed via food questionnaires. Because this last method is less accurate than the assay in biological fluid, the validity of these results remains questionable.

Breast

Although things are still unclear for endometrial or prostate cancers, there are now enough data to conclude that both positive and negative effects can occur considering isoflavone effects on estrogen-dependent breast cancer. Sufficient data now exist to consider that isoflavone through soy intake may be preventive against the occurrence of breast cancer [230–232] playing its role during the initial phase of cancer development [1]. However, there are also many data indicating that estrogenic isoflavones exert a proliferative effect on breast estrogen-dependent cancer cell lines. These data now exist in vitro [233], in vivo in animals [234], and in women [235].

The mechanism by which cancer prevention occurs is not yet clear and may be related to different mechanisms including early cell differentiation [236, 237], onco-suppressor expression [238], or pro-oncogene depletion [239]. Unfortunately, not all the protective mechanisms were so far recorded in vivo, using plausible dietary

doses. Nevertheless, this preventive effect of isoflavones is in accordance with the demonstrations made by Lamartiniere on rats exposed to dimethylbenz(a)anthracene (DMBA) in which the preventive effect of GEN appears during the initial phase [240]. This protective effect is also sustained by the study of the National Toxicology Program [241] examining the carcinogenic effect of GEN in rats. Hence, they found that females from the first generation exposed to 5 and 100 ppm of GEN from conception exhibited lower incidences of combined mammary adenoma and adenocarcinoma than the control or than the 500 ppm group. The differences were not statistically significant, but they may support the idea that a low exposure from conception would protect against mammary tumor incidence. Of course, reducing the occurrence of cancer cells via inhibiting their occurrence by any pathway during the initial step would result in a decreased incidence of cancer at the population level.

However, most established breast cancers rely on the activation of the alpha estradiol receptor subtype cross talking with the IGF receptor [242, 243]. This means that 17β-E_2 and other estrogens act as growth factors of the tumors [40, 244, 245]. At least three studies showed an estrogenic effect of soy isoflavones at modern dietary doses (45–60 mg/day) on the mammary gland of premenopausal women [74–76]. The three studies were undertaken for short durations and showed a positive impact of the isoflavone dietary intake on estrogenic biomarkers (pS2, nipple aspirate apolipoprotein D, progesterone receptor). In premenopausal women, an interaction of isoflavones with endogenous 17β-E_2 could not be excluded. Therefore, this effect cannot be extrapolated to postmenopausal women. This does not, however, negate the estrogenic effect of 45–60 mg of isoflavones per day in premenopausal women. Such active doses in premenopausal women are sustained by other physiological data concerning the menstrual cycle length duration, as explained before. Unfortunately, when they can exert their estrogenic effect, isoflavones increase cell proliferation speed **on already established** estrogen-dependent cancer cells [233]. This was also shown in vivo in implanted nude mice [234]. This is also sustained by the study of Shike et al. [235] in women with a declared breast cancer. Examining the proliferative effect of soy isoflavones in women diagnosed with breast cancer is both very difficult and ethically disputable, which would explain why only one study [235] has been published so far. The study is not fully conclusive because of a short treatment duration (between 7 and 30 days) and a low compliance with the treatment. Therefore, the biomarkers followed probably reflect a large interindividual response to a large interindividual variation in exposure. Therefore, in [235], the authors relied on isoflavone plasma levels to sort the treated subjects and then went on to perform genomic analysis on proliferation markers. The results they obtained in vivo with plasma forms (i.e., conjugated forms) of isoflavones are consistent with the in vitro data obtained by many other authors, who showed an estrogenic effect of isoflavones on breast cancer cells (i.e., proliferative effect) when used in the μM range (0.2 to 5) [246]. Shike's study indicates that both in vivo and in humans the circulating forms of isoflavones can, as already shown, have estrogenic effects [74–76]. Hence, at modern dietary doses, they can act as growth factors for estrogen-dependent tumors. Although the studies in nude mice implanted with human breast cancer cells cannot help in determining an

active dose, the NTP study [241], which showed a significant increase in adenocarcinomas in the mammary and pituitary gland of rats exposed to 500 ppm GEN from conception to sampling, is particularly important. This classical toxicology study was designed to define the mean tolerable daily intake in humans. In that case, the LOAEL is 100 ppm (5 mg/kg/day for rats), and the NOAEL is 5 ppm in the diet (1.2 mg/kg/day for rats).

Finally, some breast cancer cells can be triple negative for ER, PR, and Her2/neu. They are considered to be resistant to hormone therapy based on antiestrogens or on anti-aromatase and therefore to have particularly negative prognosis [247]. It was shown in some occasions that, in the absence of ER, breast cancer cells such as MDA-MB-231 did not proliferate under isoflavone treatments [248]. This triple-negative cell line only bears trace amounts of GPER [249]. However, some triple-negative cancer cell lines such as HCC1806 were shown to have a large proportion of GPER in their membranes [249]. Because of that although they were first considered as estrogen independent, they can proliferate under estrogen stimulation [250]. GPER can mediate rapid 17β-E_2-induced non-genomic signaling pathways. In addition, by the effect of transactivation of epidermal growth factor (EGF) receptors, GPER induces mobilization of intracellular calcium (Ca^{2+}) stores and activation of mitogen-activated protein kinase and PI3K signaling pathways [247]. Furthermore, when present in triple-negative cancer cells, GPER seems to be with poor clinical outcome. It has been shown to upregulate cyclins and BCL-2 genes favoring cell proliferation and survival. Therefore, GPER possibly plays an important role in the carcinogenesis process. The GPER-induced proliferation can also be blocked in vivo by inactivating GPER [251] with siRNA, using GPER antagonist like G-15 [252] or even better estriol [251], opening a new way for breast cancer therapy. In Egypt, where breast cancers represent 17.5% of all malignant tumors, it was observed that these mammary cancers were more aggressive than that encountered in the West [247]. In this country, it was shown by immunostaining that 65% of archival formalin-fixed paraffin-embedded cases of invasive ductal carcinoma were GPER positive. GPER as a membrane receptor of the rhodopsin-like family is activated by low doses of many xenoestrogens including bisphenol A (BPA) [253]. As mentioned earlier, GEN and DAID are ligands of GPER with affinity 2 to 3 times higher than that of BPA. The IC_{50} for GEN is 133 nM, which is only ten times higher than that of estradiol [253], and GPER pathway activation is achieved using 0.2 μM. This dose can be obtained in human blood with dietary soy intake. Equally, DAID is able to induce GPER pathways at doses as low as 0.1 μM [254]. Therefore, the effects of phytoestrogens on breast cancer should take into account the GPER pathway as another target for proliferation induction. Lastly, data on the effect of isoflavones on breast cancer cell lines through the estrogen-related receptors (ERR) α, β, or γ are presently too scattered to conclude on a clinical effect. When tested, the doses of isoflavones required for the ERR pathway activation do not fit with a dietary isoflavone intake [255].

6.1.2 Coumestrol

Because this substance is only anecdotally present in human food at least in Western developed countries, its effects on estrogen-dependent cancers are only scarcely examined. There is no interventional study that is strictly controlled, and there are a few observational studies mentioning COUM as a parameter which have been taken into account because it was above the assay detection limit in biological samples. In 2006, Hedelin and co-workers showed that COUM together with a precise Snp present in the promoter of the ERβ subtype was correlated with a strong significance to a decreased risk of prostate cancer [256]. However, this correlation should be taken with caution since the COUM urine levels measured are close to the detection limit.

6.1.3 Lignans

Two major problems linked to the exposure evaluation of the estrogenic enterolignans can explain the paucity of clear data gathered on enterolignans and estrogen-dependent diseases. As already mentioned, not all sources of lignans are yet characterized in food [257], and secondly not all human being can efficiently transform lignan precursors into estrogenic enterolignans [258].

Prostate

It is in the late 1980s that lignans were correlated to reduced risk of prostate cancer [259]. Despite this early hope, in 2010, Saarinen and co-workers made a point on population and intervention studies dealing with enterolignans and prostate cancer and found no clear correlations at the population level [260]. In population studies even when ENL was followed in plasma and/or urine, a clear association with prostate cancer incidence, progression, or risk could be pointed out. However, three studies dealing with the administration of flaxseed in men with a diagnosed prostate cancer did show a decreased serum total PSA and proliferation rate of benign epithelium and a significant decrease in total testosterone and free androgen indices. Among men with Gleason score below 6, they reported decreased tumor proliferation index and increased tumor apoptotic scores when comparing the flaxseed group to historic controls. Finally, they also mentioned a significantly reduced tumor proliferation rates with flaxseed supplemented diets. Their conclusion was to say that enterolignans may have a protective effect on prostate cancer but that the normal dietary intake is currently insufficient to induce such protective effect. More recently the study by Azrad and co-workers [261] came to confirm that prostate cancer preoperating treatment with flaxseed supplement was associated with the decrease of several tumor biomarkers. Namely, there was a significant decrease in NFκB, Ki67, and VEGF. The authors conclude that flaxseed supplementation inhibits cancer cell growth and possibly reduces tumor angiogenesis in patients with prostate cancer.

Breast

Enterolignans have a greater affinity for the ERα receptor; however, ENL which is currently the most concentrated in human plasma was shown to better recruit the AF-2 transactivation factors and therefore to reduce the proliferative effects of compounds activating gene transcription through the AF-1 domain [45]. This in vitro mechanism was further confirmed in vivo on nude mice transplanted with MFC-7 cells [262]. In this study, ENL and END were tested alone and together with GEN. They were able to prevent the proliferation induced by GEN. Foods containing the precursors of enterolignans were also tested in the nude mice model [263]. The study showed that secoisolariciresinol diglucoside (SDG), the most classical precursor of enterolignans in the scientific literature, was less efficient than sesamin in protecting against breast tumor proliferation. Sesamin was converted in vivo by the nude mice in unknown compound proportion that was able to increase significantly apoptosis of the cells. If the mice metabolism is involved in this effect, which is most plausible, then the result cannot be translated directly to human. Nevertheless, in some cases, studies showed that a higher intake of lignans was associated with lower risk of developing cancer. In several occasions, the receptor status of cancers was mentioned [264, 265]. The discrepancy between the studies can be explained by at least two factors: (1) not all women can produce enterolignans from their lignan precursors, and if no plasma or urine measurements are available, this induces confusion; (2) not all the sources of lignans are yet characterized, and this reduces the power of the analyses. To illustrate the first point, the study by Buck and co-workers [265] showed a better reduction in the breast cancer risk when data were collected based on an ENL measurement rather than on a lignan dietary intake. When the lignan intake was taken globally, the ER-negative tumors seemed to be more susceptible to a protective effect. Therefore, and because lignan intake was not systematically associated with estrogenic enterolignan production, the action may not be an estrogenic or antiestrogenic effect. Regarding an effect of enterolignans through the GPER pathway, there are too few such data to hypothesize any effect. However, several epidemiological studies gathered in the meta-analysis [266] showed overall that a decreased risk of death by breast cancer could be associated with serum ENL. This study performed on 2,182 patients divided the population into 4 quartiles based on their serum ENL levels. In each case, the highest quartile corresponding to serum levels over 70 nM was associated with lower risks. The endpoints which were monitored were all-cause mortality, breast cancer-specific mortality, distant disease-free survival, and risk of recurrence [266]. In this study and thanks to a large number of subjects, it was possible to see that enterolignans could have a positive effect per se, distinctive of a healthy lifestyle pattern. Finally, as for isoflavone, a preventive effect at the initial phase of breast cancer progression is plausible since it was shown that ENL at a dose of 10 mg/kg/day was able to decrease the occurrence and size of chemo-induced mammary tumors using DMBA in ovariectomized rat [267]. Because DMBA is able to transform a healthy cell in cancer cells, this effect of ENL triggers the initial phase of mammary cancer progression.

6.2 Other Side Effects

6.2.1 Thyroid

Links were made between soy consumption and alterations of fragile thyroid function. In the 1960s when the first soy-based infant formulas were commercialized, hypothyroidism goiters were observed [268] in infants and led to iodine supplementation in these formulas [269]. However, nowadays hypothyroid infants can still develop a goiter under soy-based infant formula, and their thyroid function is still difficult to manage under these soy-based formulas [270–272]. Generally speaking, until now, whenever isoflavone effects were reported on hypothyroidism, it was always in persons with preexisting thyroid impairments [271, 273–275]. In parallel to these data is the case of a woman who was treated with Levothyrox for hypothyroidism. It showed that the intake of soy-based food supplements interacts with the drug absorption [274]. As a result, the doses required to balance her thyroid function had to be increased when soy-based food supplement was taken simultaneously with Levothyrox treatment. Consequently, in Europe, these medications are generally advised avoiding soy consumption. The mechanism of action is not fully characterized, but GEN was found to bind to thyroid hormone receptors [276] and to compete with triiodothyronine (T_3) on its receptor at concentrations of 1 μM which are dietary relevant. In addition, in the presence of iodide ions, genistein and daidzein blocked TPO-catalyzed tyrosine iodination by acting as alternate substrates in vivo [277]. This mechanism would explain the greater need of iodine for correct thyroid function in the presence of soy. Many other studies, however, showed no adverse effects from soy or isoflavones on thyroid function of healthy subjects [278–282]. In addition, in Japan, isoflavones are consumed at the highest rate on earth; until recently [283] no adverse effects were consistently reported. The exception [283] is the case of a 72-year-old woman with preexisting lymphocytic thyroiditis who was admitted to the hospital with acute hypothyroidism. It was due to the consumption of a health drink containing soy. Symptoms progressively disappeared after soy-based health drink arrest. However, the traditional Japanese diet is based on a large variety of seafoods which are rich in iodine. They may usually compensate for the effect of soy and/or isoflavones on an alteration of the thyroid function. Finally, for many years, it was unclear if any soy component or specifically isoflavones were responsible for the hypothyroid effect of soy. The study by Sathyapalan and co-workers [284] finally answered the question. When isoflavones were removed or added to soy, and fed to volunteers with preexisting mild hypothyroidism, the isoflavone supplementation clearly aggravated the hypothyroid symptoms. Taking all these data together, it seems that the adverse effects of soy isoflavones may occur only in persons with previous hypothyroidism. In that case, a balance in the thyroid function is more difficult to achieve medically.

6.2.2 Autoimmune Diseases

Autoimmune diseases occur when the immune system attacks and destroys the organs and tissues of its own host. Several autoimmune diseases occur with a higher

frequency in women than in men [285]. Although links with several factors expressed by the X chromosomes have been related to the occurrence of autoimmune diseases [286], the role played by female sex steroids is currently under investigation. Hence, the incidence of autoimmune diseases is generally higher in premenopausal women than in children (girls) and decreases after menopause [287]. At the cell level, 17β-E_2 was shown to induce the expression of many different factors playing a role in the activation of autoantibodies. As an example, 17β-E_2 at physiological doses increases lymphocyte B and plasma dendritic cell differentiations in vitro [288, 289]. This process is inhibited by the pure antiestrogens ICI 182 780 or by tamoxifen. Plasma dendritic cell activation and IFNα production are enhance in vitro and in vivo by 17β-E_2 and reduced when the ERα receptor is reduced or blocked [289]. Autoimmune processes seem to be reduced during pregnancy, while progesterone is present in the body at high concentrations [290]. Testosterone also seems to exert a preventive effect [291]. For systemic lupus erythematosus (SLE), the sex ratio distortion is one of the highest with currently nine women and one man being hit by the disease in France [292]. In Japan, the ratio seems to be more in favor to men with eight women and two men being sick [287]. In addition, the disease evolves by flares which occurrence is still difficult to prevent because of unknown causes. Among other causes, environmental factors have been suggested to be involved [293, 294]. Dietary phytoestrogens, because of their estrogenic potencies and their mostly unknown sources of exposure in modern diet, are good candidates for further studies. In a transgenic mouse model of SLE, the MRL/Mp-lpr/lpr mouse GEN and DAID at nutritional doses were shown to aggravate the SLE-induced nephropathy and to induce earlier mortality when compared to a diet devoid of soy [295]. Noteworthy, at pharmacological doses, isoflavones seem to have the opposite effect [296]. Fort and co-workers [297] examined the incidence of autoimmune diseases in children fed with soy-based infant formula compared to their counterpart fed with other formulas in their infancy. They found out that the frequency of feedings with soy-based milk formulas in early life was significantly higher in children with autoimmune thyroid diseases (prevalence 31%) as compared with their siblings (prevalence 12% $p < 0.01$) and healthy nonrelated control children (prevalence 13%, $p < 0.02$). More recently working in rodent, Tran and co-workers [298] showed that GEN, DAID, or glycitein and a soy extract suppressed iodine uptake and stimulated the production of autoimmunogen in rat thyrocytes in vitro. However, cells were from rat, and the efficient doses were higher than 1 μM. Therefore, the transposition to the clinical situation should be taken with caution. Finally, Portman and co-workers [299] recently showed an association between soy and the incidence of Kawasaki disease (KD) in children. A significant increased KD risk was observed in children for total isoflavone intake (OR, 2.33; CI 95%, 1.37–3.96) and for genistein intake (OR, 2.46; CI 95%, 1.46–4.16), when comparing high-soy consumers vs. non-consumers. KD risk was also significantly increased in Asian-American children with the highest isoflavone consumption. Hence, total isoflavones were highly significantly correlated to an increased risk (OR, 7.29; CI 95%, 1.73–30.75), and this was also the case for genistein (OR, 8.33;

CI 95%, 1.92–36.24) when compared to white children. The authors concluded that childhood dietary isoflavone consumption, but not maternal isoflavone intake during pregnancy and nursing, strongly relates to KD risk in an ethnically diverse US population. To conclude, the association of autoimmune disease with estrogens is clear, and progesterone and testosterone are most probably protective. Phytoestrogens being estrogenic, antiandrogenic [300], and able to reduce progesterone levels at dietary doses [301] can be good environmental candidate to solve the reason of unexplained autoimmune disease flares. However, additional studies are needed to ascertain this effect and therefore to go toward disease prevention.

7 Conclusion

Natural potent estrogens are ubiquitous in our environment. Resorcylic acid lactones and COUM, because of their strong known effects on rodent reproductive physiology, have been adequately monitored so far. Hence, the human exposure, except in specific circumstances, is not a cause of concern. Lignans, although leading to the eventual production of estrogenic compounds by the gut bacteria, are far less studied, and the exposure to the weak estrogen enterolignans is far from being correctly assessed. A large interindividual response is expected from the exposure to dietary lignans. However, as far as we can approach them with our current tools, these compounds act as SERMs, and their effects appear to be more beneficial than adverse. Their effects on reproduction appear to be positive, and they were shown to reduce the proliferation on estrogen-dependent breast cancer cell lines. Their exposure being associated with grain, fruit, and vegetable intakes does not seem to suppress the benefit of a diet rich in these foodstuffs. Inducing more concerns is the case of isoflavones. Their estrogenic effects in vitro range between ENL on one hand and COUM and ZEN on the other hand. Their bioavailability is the highest of all compounds listed here. Many effects, them being beneficial or having adverse effects, have been reported so far for these compounds. However, based on the assumption that they had always been part and parcel of the human diet, they were, until now, essentially considered as inactive. This discrepancy vanishes if it is admitted that the isoflavone exposure is modern and essentially due to changes in the cooking process of soy. Then the compounds can be considered for what they are, i.e., estrogenic compounds, possibly exerting endocrine-disrupting effects in synergy with other anthropoid compounds recently liberated in the environment. The actual data tend to indicate that their most pronounced deleterious effect is observed on reproduction. Their deleterious effects on already established estrogen-dependent cancers seem to occur at higher doses based on toxicology studies. These effects are counterbalanced by a preventive effect probably due to a favorable interaction at the initial step of breast cancer progression. More mechanistic data are required to ascertain this mechanism. Therefore, although more research is still required to take the best part of these compounds, the question of their endocrine-disrupting effect should be taken into consideration. Because the history shows that isoflavone

can be reduced easily in food, this should be done for both human and domestic animals to reduce the exposure to endocrine disruptors and improve farmers' incomes.

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