Cross-Species and Interassay Comparisons of Phytoestrogen Action

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This paper compiles animal and human data on the biologic effects and exposure levels of phytoestrogens in order to identify areas of research in which direct species comparisons can be made. In vitro and in vivo assays of phytoestrogen action and potency are reviewed and compared to actions, dose–response relationships, and estimates of exposure in human subjects. Binding studies show that the isoflavonoid phytoestrogens are high-affinity ligands for estrogen receptors (ERs), especially ERβ, but have lower potency in whole-cell assays, perhaps because of interactions with binding proteins. Many other enzymatic actions require concentrations higher than those normally seen in plasma. In vivo data show that phytoestrogens have a wide range of biologic effects at doses and plasma concentrations seen with normal human diets. Significant in vivo responses have been observed in animal and human tests for bone, breast, ovary, pituitary, vasculature, prostate, and serum lipids. The doses reported to be biologically active in humans (0.4–10 mg/kg body weight/day) are lower than the doses generally reported to be active in rodents (10–100 mg/kg body weight/day), although some studies have reported rodent responses at lower doses. However, available estimates of bioavailability and peak plasma levels in rodents and humans are more similar. Steroidogenesis and the hypothalamic–pituitary–gonadal axis appear to be important loci of phytoestrogen actions, but these inferences must be tentative because good pharmacokinetic data also are available. The similarity of reported proliferative effects, dose–response data, and multiple end points in assessing phytoestrogen actions precludes their use in clinical trials.

Types of Phytoestrogens and Dietary Sources

A phytoestrogen is defined as any plant substance with hormonal actions, provide an opportunity to examine these relationships more fully. Phytoestrogens exhibit a number of actions that have the potential to alter basic reproductive and developmental processes but also exhibit many potentially beneficial actions (2,7). Phytoestrogen actions and dose–response relationships have been assessed in both in vitro and in vivo studies and can be compared to human clinical and epidemiologic studies. Phytoestrogen exposures have been quantified for human subjects in a number of studies, and some pharmacokinetic data also are available.

This review article represents a first attempt to compile animal and human data on biologic effects and exposure levels of phytoestrogens in order to identify areas of research in which direct species comparisons can be made. In vitro and in vivo assays of phytoestrogen action and potency are reviewed and compared to actions, dose–response relationships, and estimates of exposure in human subjects.

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Concerns about environmental substances with hormonal actions have generated scientific debate about the risks they pose for human and wildlife health. At issue are both the degree to which low potency may limit hormonal effects (1,2) and the difficulty of assigning potencies to compounds with life stage-, cell-, and gene-specific effects (3–6). Evaluating risk requires an accurate assessment of the relation between in vitro and animal tests of endocrine action and human responses. This task is daunting for many environmental substances, as the timing and quantity of human exposure are often uncertain, and suspected toxicity generally precludes their use in clinical trials.

The phytoestrogens, naturally occurring substances with estrogenic actions, provide an opportunity to examine these relationships more fully. Phytoestrogens exhibit a number of actions that have the potential to alter basic reproductive and developmental processes but also exhibit many potentially beneficial actions (2,7). Phytoestrogen actions and dose–response relationships have been assessed in both in vitro and in vivo studies and can be compared to human clinical and epidemiologic studies. Phytoestrogen exposures have been quantified for human subjects in a number of studies, and some pharmacokinetic data also are available.

This review article represents a first attempt to compile animal and human data on biologic effects and exposure levels of phytoestrogens in order to identify areas of research in which direct species comparisons can be made. In vitro and in vivo assays of phytoestrogen action and potency are reviewed and compared to actions, dose–response relationships, and estimates of exposure in human subjects.

Types of Phytoestrogens and Dietary Sources

A phytoestrogen is defined as any plant compound structurally and/or functionally similar to ovarian and placental estrogens and their active metabolites. This definition includes compounds with agonistic, partial agonistic, and antagonistic interactions with estrogen receptors (ERs) and other targets of estrogenic steroids involved in estrogen transport, synthesis, and metabolism. Functions affected by phytoestrogens include the regulation of ovarian cycles and estrus in female mammals and the promotion of growth, differentiation, and physiologic activities of the female genital tract, pituitary, breast, and many other organs and tissues in both sexes. Additional end points may include induction of RNA–protein synthesis and prolactin (PRL) secretion; prevention of bone loss; stimulation of hepatic production of sex hormone-binding globulin (SHBG), thyroid-binding globulin, plasminogen, and blood clotting factors VII–X; inhibition of antithrombin III and low-density lipoprotein (LDL) formation; and many others. Phytoestrogens differ in the combination of these actions they express and can be characterized as anticarcinogens, antioxidants, antiviral agents, and so forth. For example, genistein (GEN) can be characterized as a protein tyrosine kinase inhibitor, in contrast to its inactive chemical analog daidzein (DAI) and chrysin. The latter phytoestrogens are equal or more potent inhibitors of steroid-metabolizing enzymes such as aromatase and 17-hydroxysteroid dehydrogenase (8,9).

The two major classes of phytoestrogens are lignans and isoflavonoids (Table 1). Some structurally related flavonoids also exhibit estrogenic properties. Lignans are minor components of cell walls and fibers of seeds, fruits, berries, vegetables, grains, and nuts. The isoflavonoids are prominent in legumes, especially soybeans, but detectable levels occur in whole-grain products, potatoes, fruits, vegetables, and alcoholic beverages (9,10), and in cows’ milk, meat, and even fish grown with soy-containing food (11). The isoflavonoids are divided into three major classes: isoflavans, isoflavans, and coumestans (Table 1). The best-known isoflavones are GEN and DAI, which are formed from the plant precursors formononin (FRM) and biochanin A, respectively. The most significant isoflavan is equol (EQL) (Table 2), a metabolite of DAI. Coumestrol (COM) is the best-known coumestan and the isoflavonoid with the highest estrogenic potency (12). The mycotoxins are estrogenic fungal products that are not intrinsic components of plants but are found in pasture grasses and legumes infected by the fungal genus Fusarium.

Phytoestrogen Actions

Estrogen Receptor Affinity and Activation

Estrogen receptor binding is the best-documented action of phytoestrogens.

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but only 55% homology in the ligand-binding region. Active competitors for both receptor subtypes isoflavonoids COM and GEN are very similar, with 95% homology in the DNA-binding region. Isoflavonoids COM and GEN are very similar in their action, as they both have a 2-fold higher affinity for ERβ than for ERα, whereas GEN has a 30-fold higher affinity for ERβ (12). A recent study using human hepatoma cells has now shown that DAI also binds to ERβ with a higher affinity than ERα (13). This differential affinity is likely to be of functional significance, as the two receptor subtypes have different distributions across estrogen-responsive tissues (12,14–16) and during development (17). Moreover, as none of the common screening tests for estrogens such as ligand competition assays using rodent uterine cytosol or cell proliferation assays using breast cancer cells [e.g., E-screen (18)] or recombinant cells containing ERα and an estrogen-responsive reporter gene [e.g., recombinant yeast cell bioassay (19)] include significant concentrations of ERβ (18,19), phytoestrogen potency may be underestimated.

Recombinant assays provide evidence that phytoestrogens not only bind to ERs but also initiate gene transcription. These assays are based on recombinant yeast cells containing an ER and one or more estrogen response elements (EREs) linked to genes for reporter proteins like β-galactosidase (β-gal) (20), chloramphenicol acetyltransferase (CAT) (20), or human placental alkaline phosphatase (21). These assays have demonstrated that isoflavonoids, coumestans, and mycoestrogens all induce ERE-linked reporter proteins. Most of these assays are based on ERα, but a recent study has shown that GEN activates reporter gene expression through both ERα and ERβ (22). However, in spite of GEN’s higher affinity for ERβ, it may be a more effective inducer of ERα-mediated actions. Although GEN was slightly more potent via ERβ-linked reporters, it was less efficacious, displaying only partial agonism via ERβ but full agonism via ERα (21). Phytoestrogens can also alter the expression of receptors for hormones other than estrogen, including receptors for progesterone (PR), oxytocin (OTR), and testosterone (AR). In cell culture assays GEN induces PR expression at doses 1,000-fold lower than the concentration required to act as a tyrosine kinase inhibitor (22). GEN also acts as an anti-androgen in a dose-dependent fashion in a breast cancer cell line (23).

Perhaps the most compelling data come from in vivo studies using oral doses of phytoestrogens. Oral doses of DAI too low to be uterotrophic significantly reduced mRNA expression of AR and ER in the rat uterus while upregulating the expression of complement 3, a gene known to be sensitive to estrogen, in a dose-dependent fashion (24). A 0.01% DAI diet also failed to significantly alter uterine weight in female rats but significantly decreased OTR expression in the hypothalamus (25). These studies suggest that phytoestrogen can have a significant impact at the molecular level at doses too low to produce gross physiologic changes.

**Postreceptor Activation**

Some isoflavones and flavones inhibit protein tyrosine kinases (26,27), which play important roles in cell signal transduction and the regulation of the cell cycle (Table 3). GEN is the most potent inhibitor. GEN can also inhibit DNA topoisomerases I and II, enzymes essential for DNA replication (28,29), and is the most powerful antioxidant of the phytoestrogens. It also has the ability to increase the activities of antioxidant enzymes as well as directly inhibit hydrogen peroxide production.

**Hormone Synthesis**

Phytoestrogens have the potential to affect steroid biosynthesis and metabolism through a number of pathways (Table 3). A variety of in vitro assays have now shown that isoflavonoids and lignans are inhibitors of aromatase (30,31) and 5α-reductase (32). A number of phytoestrogens also inhibit 17β-hydroxysteroid dehydrogenase Type I (33).

However, these effects may not be as pronounced in vivo. Male rats fed a diet containing multiple phytoestrogens (224 μg/g DAI, 319 μg/g GEN, and 39.5 μg/g glycitin) for 70 days showed no change in hypothalamic aromatase activity, even though flavonol levels were 8 times higher in the brains of these animals than in the controls (34). Similarly, male rats fed a diet containing 200 μg phytoestrogens for 29 days also showed no significant changes in aromatase activity in either the amygdala or the preoptic area. However, significant changes were seen in both areas in the activity of 5α-reductase (35). The difference between the in vitro and in vivo data may stem from the significant role metabolism and the actions of binding proteins and second messenger systems play in the activity of phytoestrogens.

**Cell Proliferation**

Cell growth assays provide evidence that phytoestrogens can either enhance or suppress proliferation in estrogen-responsive cells, depending on their concentration and relative potency (RP) (Tables 4 and 5). COM, GEN, and α-zearalenol (α-ZEL) exhibit minimal antagonism of E2 in cancer cell or recombinant yeast cell assays and sometimes even augment its action (Table 5). The weaker phytoestrogens, such as biochanin A and enterolactone (ENL), are antagonistic at higher levels, whereas flavonoids like phloretin (PHL), naringenin, kaempferol (KMP), apigenin (APG),
and \( \beta \)-ZEL have been reported to display triphasic activity, inhibiting estradiol at low and high concentrations and augmenting estrogen action at intermediate levels (18).

**Hormone Transport**

Interactions with binding proteins can have important effects on the RP of estrogens. Although serum albumins do not affect the bioavailability and activity of serum hormones, SHBG and \( \alpha \)-fetoprotein differentially reduce sex-steroid availability, resulting in relatively greater bioavailability of estrogens that bind only weakly to these glycoproteins (36). Reports of phytoestrogen interactions with binding globulins are somewhat contradictory. An early study using dilute human serum reported phytoestrogen relative binding affinities of 14–27\% (37), but affinities estimated in more recent studies using partially purified SHBG range from 0.01 to 0.1\% (38,39) (Table 6). The low affinity of isoflavonoids would be expected to result in enhanced bioavailability in the presence of SHBG, and in fact the effective free fraction of COM and GEN in human serum is 45–50\%, whereas only 4\% of E\(_2\) is free (40). Addition of human serum to whole-cell binding assays raises the relative binding affinity of isoflavonoids 10-fold (40), but normal serum concentrations of SHBG (0.002 mg/mL) reduce the activity of COM and GEN in the yeast estrogen screen assay by only 5–10\%, which is similar to the reduction of E\(_2\) activity (5\%) (41). However, pregnancy concentrations of human \( \alpha \)-fetoprotein (40–160 mg/mL) reduce the activity of E\(_2\), COM, and GEN by 50\% (42).

Sequestering steroids is not the only action of binding globulins; binding globulins also contribute to hormone uptake by target cells (42,43–45). Cellular uptake of albumin- and SHBG-bound estrogens precedes their interaction with nuclear receptors and may directly affect the delivery of hormonal signals. Whereas free estrogens and the albumin-bound fraction are internalized into the cytoplasm, SHBG–estrogen complexes bind to membrane receptors, initiating nongenomic influences on cell metabolism through second messenger systems and priming effects on nuclear super-receptor complexes. ATP-binding cassette carriers within cell membranes also may expel estrogens out of cells, preventing their entry into the brain and other tissues. GEN binds to both the multidrug resistance protein (46) and P-glycoprotein (47), but only relatively high concentrations (200–400 \( \mu \)M) have been tested. These transport glycoproteins have low total capacity; ligands compete with one another for the binding sites, with the result dependent on the relative concentrations of binding protein (48–50).
proteins and endogenous and exogenous estrogens (9,48,49). These interactions may play a role in the selectivity of the blood-brain barrier and other tissue-blood barriers and in absorption from the intestine. Although phytoestrogen influences on these processes are not well understood, their affinity for all these binding proteins and relatively high concentrations in plasma provide opportunities for significant effects on these mechanisms of hormone uptake.

In addition to binding competition, phytoestrogens might also influence estrogen availability by raising SHBG synthesis (9). The clinical evidence for this effect is mixed, with some studies demonstrating an increase in SHBG levels in postmenopausal women (51–53) and other studies showing no change in SHBG levels (51–53) or even a decrease (54) in SHBG on soy-based diets.

### Phytostrogen Potencies and Active Concentrations

Estimates of estrogenic potency vary across assays. Because recombinant cells often contain multiple copies of ERs, resulting in E2 sensitivities as low as 0.1–7 pM, these assays can underestimate the in vivo concentrations required for estrogen action (35). Purified receptor preparations also provide unrealistic estimates of binding affinity caused by the absence of binding proteins. Cytosol and whole-cell assays provide more accurate estimates of potency, with effective concentrations in the picomolar to nanomolar range for E2.

Generally, lower concentrations are required for ER-mediated events and for cell proliferation than for other types of responses. The range of estrogen concentrations required for stimulation of cell proliferation varies across assays, but in general, concentrations of 1–100 nM stimulate cell growth by isoflavonoids (Table 4). Relatively high concentrations, ranging from 5 to 100 µM, are generally required for suppression of proliferation (Table 5), and biphasic patterns are frequently seen, with proliferative actions occurring at nanomolar concentrations and inhibition of proliferation occurring at micromolar concentrations. There is evidence that lower concentrations of GEN are required to suppress environmental estrogen-stimulated cell proliferation if other phytoestrogens are also present. However, the concentrations required for these additive effects (25 µM) are still quite high, so it is unclear if the action would occur in vivo (50). Similarly, high micromolar levels are required for estrogen antagonism of cell proliferation, with minimal action by the most potent phytoestrogens.

Inhibition of tyrosine receptor kinases and other targets of phosphorylation occur at micromolar concentrations (Table 3). Micromolar concentrations are required for lignan and isoflavone binding to SHBG (5–50 µM) (38) and α-fetoprotein (Kd 0.5–5 µM) (57,58). Concentrations required for inhibition of most steroid metabolizing enzymes are generally in the micromolar range, with the exception 17β-hydroxysteroid dehydrogenase type 1, which is inhibited by isoflavonoids at concentrations ranging from 120 nM to 1 µM (33,59).

### Phytoestrogen Exposure

#### Dietary Sources

The highest concentrations (1–3 µmol/g) of lignans are found in flaxseed (linsen) products (60,61), which are consumed by some Europeans. Good sources of lignans in the United States and Europe are pumpkin seed (20 mg/100 g), sunflower seed, cranberry, black tea, coffee, garlic, broccoli, brans, and peanuts (62). Green tea is a rich source of lignans and a popular beverage in many Asian countries (63).

Phytostrogenic isoflavonoids are less prevalent than lignans. The highest concentrations are found in legumes, especially in soybeans and soybean-based products, which can contain as much as 0.2–1.6 mg of isoflavones per gram dry weight (64). The isoflavone contents of the basic food types are summarized in Table 7.

#### Plasma Concentrations

**Humans.** Phytoestrogen exposures vary substantially across human populations and individuals (Table 8). Differences across populations are related to dietary variation (9,65), whereas within-population variation may reflect individual differences in the intestinal microflora, local differences in the phytoestrogen content of foods, and dietary effects on phytoestrogen absorption and metabolism (11,66,67). Asian diets are particularly high in soy, resulting in isoflavone consumption as high as 1 mg/kg body weight (bw)/day, whereas vegetarian diets are high in whole grains, vegetables, and legumes, resulting in higher consumption of lignans. These dietary exposures produce plasma isoflavone concentrations as high as 1 µM in Japanese men and women consuming a traditional diet. In Europe and North America, plasma concentrations are generally less than 0.07 µM for omnivores, whereas vegetarians may have levels as high as 0.4 µM isoflavones and 0.8 µM lignans. A recent study of Japanese infants indicates that isoflavonoids gain access to fetal tissues, resulting in concentrations in cord blood and amniotic fluid (0.2–0.3 µM) similar to maternal plasma concentrations (0.2 µM) (68).

Feeding trials provide more detailed data on phytoestrogen bioavailability in adults (Tables 9,10). Single soy meals providing isoflavone doses of 0.06–1.2 mg/kg (4–71 mg) produce peak plasma concentrations of 0.06–2.2 µM. Peak plasma concentrations of GEN and DAI are reached within 4–8 hr, with an absorption half-life of 1–3 hr. The excretion half-life ranges from 3 to 8 hr. DAI, with estimates ranging from 16–66%, appears to be more bioavailable than GEN (5–37%).

The highest human dietary exposures occur in infants fed a soy-based formula.
### Table 4. Estrogenic potency: in vitro range of concentrations for estrogenic actions.

| End point                  | Ref  | E2   | DAI  | GEN  | BIA  | DMA  | EQL  | CDM  | APG  | KMP  | QUC  | IPR  | ENL  | ZLN  | α-ZEL | β-ZEL |
|---------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|-------|
| ER binding                | (12) | 0.2 nM | 4.2 nM | 0.2 nM | 1.3 nM | 0.9 nM | 0.1 nM | 0.02 nM | 0.02 nM | 100 nM | 2 µM | 250 nM | 20 nM | 200 nM | 714 nM | 4 nM | 5.9 nM | 232 nM | 294 nM |
| Uterine cytosol           | (165, 167) | 0.002 nM | 1 µM | 49 nM | > 10 µM | 2 µM | 250 nM | 20 nM | 0.002 nM | 1 µM | 49 nM | > 10 µM | 2 µM | 250 nM | 20 nM | 0.002 nM | 1 µM | 49 nM | > 10 µM | 2 µM | 250 nM | 20 nM |
| Mammary cytosol           | (111) | 200 nM | 200 nM | 1.6 µM | 20 µM | Inactive |
| MCF7 cells                | (104, 114) | 0.2 nM | 61 nM | 200 nM | 200 nM | 1.6 µM | 20 µM | Inactive |
| HER transfected COS7 cells| (169) | 1 nM | 60 µM | > 100 µM | 6 µM | 10 nM | 44 µM | 22 µM | 6 µM | 30 µM |

### Table 5. In vitro concentrations for estrogen antagonist, augmentation, and antiproliferative actions.

| Cell type                  | End point                  | Ref  | DAi  | GEN  | BIA  | DMA  | EQL  | CDM  | PHL  | KMP  | QUC  | NAR  | ENL  | ZLN  | α-ZEL | β-ZEL |
|---------------------------|---------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|-------|
| Brain capillary BBCE      | Antiproliferative E_{50}  | (178) | 90 µM | 5 µM | 50 µM | 30 µM | 50 µM | 30 µM | 50 µM | 30 µM | 50 µM | 30 µM | 50 µM | 30 µM | 50 µM |
| Breast MCF7               | Antiproliferative         | (104, 112) | 50 µM | 50 µM | 50 µM | > 20 µM | 20 µM | 0.1–10 µM | 10 µM | 0.1–10 µM | 10 µM | 0.1–10 µM | 10 µM | 0.1–10 µM | 10 µM | 0.1–10 µM | 10 µM | 0.1–10 µM | 10 µM |
|                           | Inhibit 5 nM E2           | (58) | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM |
|                           | Inhibit 1 nM E2           | (169) | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM |
|                           | Inhibit 10 µM DTT         | (169) | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM |
|                           | Augment 0.3 µM DTT        | (164) | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM | 10–20 µM |
|                           | Inhibit 0.3 nM E2         | (169) | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM | 30–300 µM |
|                           | Augment 0.1 µM E2         | (13) | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM | 1–100 nM |
| Breast MDA48              | Antiproliferative         | (168) | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM |
| Prostate LNCAP            | Antiproliferative         | (168) | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM |
| Prostate DV14S            | Antiproliferative         | (168) | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM | 18–111 µM |
| HER transfected COS7 cells| Inhibit 1 nM E2           | (168) | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM | 25 µM |
|                           | Augment 250 nM DTT        | (164) | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM | 1 µM |

Abbreviations: BBCE, bovine brain capillary endothelial cells; 4-OH-PCB, 4-hydroxy-polychlorinated biphenyl.
Table 6. Phytoestrogen affinities for binding proteins.

| End point                          | Ref | E2  | DAI | FRM | GEN | EQL | COM | KMP | QUC | NAR | ENL | END | ZLN | α-ZEL |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| Human serum RBA, %E2              | (37) | 100 | 24  | 27  | 14  |     |     |     |     |     |     |     |     | 5     |
| SHBG affinity RBA, %E2            | (38) |     |     |     |     |     |     |     |     |     |     |     |     | Inactive |
| Human serum RBA, %E2              | (38) | 100 |     |     |     |     |     |     |     |     |     |     |     | 0.1   |
| Rat α-fetoprotein Ks, %E2         | (58) | 100 | 0.1 |     |     |     |     |     |     |     |     |     |     | 1     |
| Rat α-fetoprotein RBA, %E2        | (38) | 100 | <0.1|     |     |     |     |     |     |     |     |     |     | 1     |
| SHBG comp ED50 (α-ZEL)            | (188)| 0.0031 µM | 4.5 µM | 1.9 µM | 10 µM >100 µM |
| SHBG comp ED50 (E2)               | (38) | 0.02 nM | 100 µM | 30 µM | 20 µM |
| Rat α-fetoprotein Ks (α-ZEL)      | (58) | 5 nM  | 5 µM  | 0.5 µM | 0.5 µM |
| Rat α-fetoprotein Ks (E2)         | (57) | 3 nM  | 6.7 µM | 17 µM | 22 µM |
| P-glycoprotein minimum active     | (47) | 200 µM | 200 µM | 200 µM | 200 µM |
| Multidrug resistance protein      | (48) | 400 µM | 400 µM | 400 µM | 400 µM |

| Abbreviations: END, enterodiol; RBA, relative binding affinity; SHBG comp ED50, SHBG competitive binding affinity, 50% estrogen displacement. |

Table 7. Isoflavone and lignan content of human foods.

| Food            | GEN | DAI | Lignans |
|-----------------|-----|-----|---------|
| Fruit           |     |     |         |
| Vegetables      | 1–3 | 1–3 | 100–400 |
| Cereals         | 100–700 |     |         |
| Flaxseed        | 68,000 |     |         |
| Soy products    | 20–1,100 | 20–900 | 900     |
| Fava beans      | 100 |     |         |
| Indian bread root | 200 |     |         |
| Kudzu root      | 95  |     |         |
| Other nonsoy legumes | 0–80 | 0–10 |         |

Data from Aldercreutz and Mauer (8, Thompson et al. (67), Reinil and Block (1/7), and Kaufman et al. (168).

(SBF). SBF contains high concentrations of isoflavones (100–175 µM), significantly more than breast milk before (18–56 nM) or after (10–70 nM) soy consumption (69) or in bovine based-milk formula (70,71). An infant fed only SBF consumes approximately 6–11 mg/kg/day isoflavone, which is considerably more than the average for adults, even on a soy-rich Asian diet (0.3–1.2 mg/kg/day) (72,73). These high intakes result in markedly higher plasma concentrations (2.4–6.5 µM) in infants fed a soy milk diet than in infants fed either cows’ milk formula (0.03 µM) or breast milk (0.02 µM) (71,74), plasma levels that are 10-fold higher than the average levels reported for Japanese women and equal to those seen at peak plasma levels following soy challenges in adults.

Animal models. Species differences in metabolism may influence bioactive dose. For example, relatively high oral doses are required to produce micromolar concentrations in plasma in sheep, whereas similar peak concentrations can be achieved in cattle and pigs with lower doses (Table 11). However, few studies have examined the pharmacokinetics of isoflavones in rodent models of estrogen action. Estimates of bioavailability of GEN in rats and mice (10–20%) are similar to values reported for humans (Tables 10,11); however, the doses tested in rodents are substantially higher than the doses tested in humans and use free GEN rather than the GEN glycosides present in the soy protein generally used in human trials. Peak plasma levels are achieved in rats within 2 hr and in mice within 3–30 min after oral dosage (Table 11). Single or repeated oral GEN doses of 20–45 mg/kg produce peak plasma concentrations of 2–11 µM in rats. Single oral doses of 50–200 mg/kg GEN produce peak free plasma concentrations of 1–4 µM in mice. These doses are considerably higher than those reported to produce micromolar plasma levels in humans, but data on plasma concentrations resulting from lower dietary doses in rodents will be required to determine whether these dose responses represent species differences.

Tissue Concentrations

Only a few studies have examined phytoestrogen concentrations in tissues and bodily fluids other than plasma and urine (Table 12). Single-dose treatment of female mice with 40 mg/kg/day subcutaneous (sc) FRM produced peak plasma concentrations of 9 µM in 2 hr and peak mammary tissue concentrations of 7 nmo/l/g in 4 hr, with mean half-lives of elimination of 2 and 2.5 hr, respectively (75). In rats, plasma levels of EQL increased from 91 to 450 nM 1 hr following a single sc injection of 4 mg/kg EQL, and the corresponding uterine concentrations were 0.4 and 4 nmo/l/g (76). Chronic oral doses of 8–40 mg/kg GEN resulted in reproductive tissue concentrations of 0.3–1.4 nmo/l/g (77). On a parts per million basis, total isoflavone levels in rodent plasma and mammary gland appear to be quite similar. However, the high proportion of GEN aglycone in reproductive tissues compared to serum suggests that some accumulation occurs (77). A very small sampling of human breast milk (HBM) suggests that isoflavone concentrations are lower in breast milk than in plasma (69,78) (Table 12). On the other hand, a large sample of prostatic fluid concentrations indicates that isoflavone and lignan concentrations are higher on average than mean plasma levels (79,80) (Table 12). Plasma and prostatic fluid concentrations in individual men were well correlated for DAI (r = 0.7) but not for enterolactone (r = 0.2–0.4) (80), suggesting that isoflavones are concentrated in the prostate.

The most illustrative study to date used an oral dose of [14C]GEN (4 mg/kg) in male and female rats (81). The highest concentration of radioactivity was found in the gut; significant levels of radioactivity were also found in a variety of other organs, particularly the liver and reproductive organs, but also the brain, heart, lungs, and kidneys. Interestingly, retention in the liver was sexually dimorphic, with females showing nearly 2.5 times the radioactivity as males 2 and 7 hr after the initial dose was given.

Exposure and Bioactivity

The data reviewed above indicate that total plasma levels of isoflavonoids and lignans in humans range from 10 to 400 nM, of which only about 10% (1–25 nM) is unconjugated (Table 8). Plasma concentrations as high as 2 µM total isoflavonoids may be achieved for a few hours after food consumption. Limited data for tissue concentrations indicate no more than nanomolar levels, even in tissues like the prostate where isoflavonoids may be concentrated relative to plasma. Therefore, overall it appears that average concentrations may be too low to induce most of the reported in vitro actions other than those mediated by ERs (Table 3). Moreover, current data suggest that agonism and cell proliferation are more likely than antagonism and suppression of cell proliferation. However, the latter predictions may be biased by the EKt-based systems from which they were drawn, and a different picture may emerge from assays based on ERβ.

In Vivo Actions

A variety of in vivo actions of phytoestrogens have been reported in animal experiments...
Phytoestrogen actions across species

Although the majority of these actions are in the reproductive tract, there is evidence for effects on functions of the cardiovascular, skeletal, and central nervous systems.

Proliferation of the female reproductive tract is a classic test of estrogenicity that has been demonstrated for a number of phytoestrogens in a variety of animal species (Table 13). More variable results have been achieved using soy treatments. A soy-based diet providing 7 mg/kg/day GEN and 3 mg/kg/day DAi to ovariectomized rhesus macaques did not induce uterine growth or maturation of the vaginal epithelium, although they produced significant changes in cardiovascular risk factors (see below) (82). A similar diet also failed to alter uterine growth or vaginal cytology in ovariectomized rats (83).

More variable results have been obtained with postmenopausal women. Soy protein supplements that provided postmenopausal women with 0.7 mg/kg/day GEN and 2.1 mg/kg/day DAi over a 4-week period did not significantly alter the vaginal maturation index, although there was a trend for an increase in superficial cells (84). On the other hand, increases in the maturation index were observed in an Australian study during 6-week supplementation with a soy flour.

Table 8. Mean plasma isoflavonoid and lignan concentrations in human populations consuming typical diets.

| Locale  | Diet | Sex/age | DAi (nM) | GEN (nM) | DMA (nM) | EQL (nM) | Total (nM) | ENL (nM) | END (nM) | MAT (nM) | Total (nM) | n | Ref  |
|---------|------|---------|----------|----------|----------|----------|------------|----------|----------|----------|------------|---|------|
| Spain   | OM   | M       | 1.3      | 0.4      | 3.9      | 0.4      | 4.3        | 50       |         |          |            |   | (80) |
| United Kingdom | OM | M   | 8.2      | 0.6      | 3.9      |          | 3.9        | 36       |          |          |            |   | (80) |
| Finland | OM   | M       | 6.2      | 6.3      | <0.1     | 0.3      | 12.9       |          |         |          |            |   |      |
| Canada  | OM   | M       | 3.4      | 8.2      |          | 11.6     | 13.9       | 1.9      | 15.0     | 10       | 180        | 14 | (180) |
| Hong Kong | OM   | M       | 31.3     | 3.8      |          |          | 6.2        | 1.7      | 7.9      | 53       | 180        |   |      |
| Japan   | OM   | M       | 107.0    | 276.0    | 3.3      | 5.5      | 391.8      |          |         |          |            | 14 | (189) |
| Finland | OM   | F-PRE   | 4.2      | 4.9      | 0.1      | 0.8      | 10.0       | 28.5     | 1.4      | 0.0      | 314.9      | 14 | (191) |
| Finland | VG   | F-PRE   | 18.5     | 17.1     | 0.8      | 0.7      | 37.1       | 89.1     | 5.6      | 0.1      | 34.6       | 14 | (191) |
| Finland | LV   | F-PRE   | 41.5     | 29.7     | 1.8      | 1.0      | 74.0       | 752.7    | 65.6     | 1.9      | 820.2      | 3  | (191) |
| Finland | OM   | M       | 0.6      | 0.5      | <0.1     | 0.1      | 1.3        |          |         |          |            | 14 | (189) |
| Japan   | OM   | F-PG    |          |          |          |          |            |          |          |          |            | 232|      |
| Japan   | OM   | F-PRE   | 72.5     | 206.1    |          |          |            |          |          |          |            |   | (192) |
| Japan   | OM   | NCB     |          |          |          |          |            |          |          |          |            | 299|      |
| Japan   | OM   | F-PG    |          |          |          |          |            |          |          |          |            | 223|      |
| United States | HBM | Infant | 3600    |          |          |          |            |          |          |          |            |   |      |
| Japan   | OM   | F-PG    |          |          |          |          |            |          |          |          |            | 34 |      |
| Finland | OM   | F-PRE   |          |          |          |          |            |          |          |          |            | 4.9|      |
| Finland | VG   | F-PRE   |          |          |          |          |            |          |          |          |            | 6.9|      |
| Finland | LV   | F-PRE   |          |          |          |          |            |          |          |          |            | 17 |      |
| Japan   | OM   | M       | 12.8     | 7.8      | 1.8      | 0.6      | 23.0       |          |         |          |            | 14 | (189) |
| Finland | OM   | F-PRE   | 0.7      | 0.7      | 0.0      | 0.1      | 1.4        | 4.9      | 0.2      | 0.0      | 290.1      | 14 | (191) |
| Finland | GF   | F-PRE   | 3.2      | 1.3      | 0.1      | 0.2      | 4.8        | 16.8     | 0.7      | 0.0      | 302.5      | 14 | (191) |
| Finland | LV   | F-PRE   | 6.5      | 1.1      | 0.6      | 0.3      | 8.4        | 203.6    | 17.2     | 0.8      | 221.6      | 3  | (191) |

Abbreviations: CMF, cows’ milk formula; END, enterodiol; F, female; LV, lacto vegetarian; M, male; NAF, neonatal amniotic fluid; NCB, neonatal cord blood; OM, omnivorous; PG, pregnant; VG, vegetarian.

Table 9. Human plasma and urinary isoflavone concentrations following a single dietary dose.

| Dose | Dose (mg/kg/day) | Peak plasma (nM) | Urine (µmol/day) | Ref  |
|------|-----------------|------------------|------------------|------|
| 0    | 0               | 3.8              | (193)            |      |
| 0    | 0               | 3.8              | (194)            |      |
| 0    | 0               | 0.3              | (68)             |      |
| 0    | 0               | 0.2              | (53)             |      |
| 0.06 | 0.6             | 50               | (78)             |      |
| 0.10 | 0.1             | 500              | (78)             |      |
| 0.16 | 0.06            | 1,400            | (78)             |      |
| 0.4  | 0.2             | 790              | (190)            |      |
| 0.4  | 0.2             | 1,500            | (190)            |      |
| 0.4  | 0.4             | 22.2             | (53)             |      |
| 0.4  | 0.4             | 424              | (197)            |      |
| 0.4  | 0.4             | 498              | (198)            |      |
| 0.5  | 0.5             | 14.7             | (194)            |      |
| 0.6  | 0.6             | 3.9              | (68)             |      |
| 0.6  | 0.6             | 498              | (190)            |      |
| 0.6  | 0.6             | 1,220            | 41.9             | (196) |
| 1.2  | 1.2             | 2,224            | 56.4             | (196) |
| 1.3  | 1.3             | 14.7             | (190)            |      |

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and human clinical studies. Although the majority of these actions are in the reproductive tract, there is evidence for effects on functions of the cardiovascular, skeletal, and central nervous systems.

Proliferation of Uterine and Vaginal Epithelium

Proliferation of the female reproductive tract is a classic test of estrogenicity that has been demonstrated for a number of phytoestrogens in a variety of animal species (Table 13). More variable results have been achieved using soy treatments. A soy-based diet providing 7 mg/kg/day GEN and 3 mg/kg/day DAI to ovariectomized rhesus macaques did not induce uterine growth or maturation of the vaginal epithelium, although they produced significant changes in cardiovascular risk factors (see below) (82). A similar diet also failed to alter uterine growth or vaginal cytology in ovariectomized rats (83).

More variable results have been obtained with postmenopausal women. Soy protein supplements that provided postmenopausal women with 0.7 mg/kg/day GEN and 2.1 mg/kg/day DAI over a 4-week period did not significantly alter the vaginal maturation index, although there was a trend for an increase in superficial cells (84). On the other hand, increases in the maturation index were observed in an Australian study during 6-week supplementation with a soy flour.
Whitten and Patisaul

(85) estimated to have provided similar isoflavone doses (e.g., about 1–2 mg/kg/day) (84). This study did not include a control group, however, so the results are difficult to separate from the normal waning of menopausal symptoms over time. It may be that the actual dietary content was higher, as another Australian study that provided the same supplement reported much higher urinary excretion (45 µmol/day) of DAI (86) than has been reported in other feeding studies (Table 8); however, the latter study did not observe any changes in vaginal cytology. Two more recent studies did not observe changes in vaginal cytology during soy supplementation (50, 87).

Ovarian Cyclicity

Phytoestrogens could influence ovarian cycles through several pathways. Phytoestrogen interaction with ERs in ovarian granulosa cells might augment or inhibit follicular development, especially in light of the high affinity of isoflavones for ERβ. ERβ is abundant in granulosa cells, although the high intraovarian kilogram body weight per day. The high intraovarian kilogram body weight per day.

For such effects. Phytoestrogen inhibition of ovarian aromatase (88–90) could reduce the availability of endogenous estrogen and limit follicular development. However, the very high concentrations required for inhibition by isoflavones (Table 3) make it unlikely that they would significantly influence estrogen production in the ovary. A more likely route for isoflavone influences on E2 bioavailability would be via inhibition of 17-hydroxysteroid dehydrogenase type I, an effect that occurs at concentrations of 0.1–1 µM (33, 59). In addition, phytoestrogens could augment or inhibit estrogen negative feedback by binding to ERs in the anterior pituitary or hypothalamus and indirectly alter ovarian steroidogenesis (see below).

Natural dietary exposures to phytoestrogens have been associated with cystic ovaries, irregular estrus, and anestrus in cattle (91), and reduced breeding success in California quail (92). Compromised follicular development (91) and reductions in luteal phase plasma progesterone and E2, as well as shortened luteal phases, have been reported in cycling ewes whereas increases in estradiol and cortisol have been reported in pregnant ewes (93, 94). Soy isoflavone diets providing doses of 1 mg/kg/day were associated with infertility in captive cheetahs (95).

Experimental studies of rats suggest that effects on ovarian cycles depend on the hormonal milieu. For example, a 0.01% dietary concentration of COM (a dose of 16 mg/kg bw/day) hastened the onset of ovarian cycles in immature females but suppressed ovarian cycles when provided to cycling adult females (96). Although the suppression of cycles is reversed upon cessation of treatments, prepupal exposure may affect cyclicity later in life. Rats treated prepubertally with 500 mg/kg/day sc GEN spent more time in the estrous stage of vaginal cycles at 50 days of age (97), and rats treated prepubertally with 16 mg/kg/day oral COM exhibited more cycle irregularities at 100 days of age (98).

Several studies have examined the effects of phytoestrogen supplementation on the human menstrual cycle, with variable results (52, 53, 98–101) (Table 14). Isoflavone doses of 0.8–3 mg/kg/day produced significant changes in menstrual cycles, but the direct and type of effect varied considerably across studies. An increased length of the menstrual cycle was the most common outcome, but this change was accompanied by elevations as well as declines in serum E2 and testosterone. The practice of sampling hormonal levels on only a few days of the cycle becomes problematical when cycle length is altered, as the days compared no longer represent comparable stages of the cycle. A recent study reported consistent reductions in urinary estrogens but minimal changes in plasma estrogens, suggesting that urinary steroids may be more sensitive indices of changes in steroid production than plasma concentrations (100, 102). Two studies have reported declines in periiovulatory luteinizing hormone (LH) and follicle-stimulating hormone (52, 100), a response with implications for fertility suggestive of

### Table 10. Estimates of isoflavone bioavailability in human subjects.

| Isoflavone | Source          | Dose (mg/kg/day) | Peak plasma | Time to peak | Absorption half-life | Excretion half-life | Bioavailability (%) | Ref     |
|------------|-----------------|------------------|-------------|--------------|----------------------|---------------------|---------------------|---------|
| GEN        | Soy milk        | 0.3–0.8          | 5–11%       |             |                      |                     |                     | [196]   |
| GEN        | Soy milk        | 0.3–0.8          | 10–17%      |             |                      |                     |                     | [198]   |
| GEN        | Kinako          | 0.4              | 2.4 µM      | 6 hr         |                      | 8.4 hr              | 20%                 | [199]   |
| GEN        | Tofu            | 0.6              |             |             |                      |                     |                     | [199]   |
| GEN        | Soy flour       | 1.0              | 4.1 µM      | 8.4 hr       |                      | 4.7 hr              | 22%                 | [200]   |
| GEN        | Soy milk        | 1.7              |             | 2.2–1.4      |                      | 3.8 hr              | 15%                 | [201]   |
| GEN        | Soy milk        | 1.8              | 2.7–2.0     | 6–8 hr       |                      | 24% to 14%          |                     | [201]   |
| DAI        | Soy milk        | 0.4–1.2          |             |             |                      |                     | 16–32%              | [198]   |
| DAI        | Soy milk        | 0.4–1.2          |             |             |                      |                     | 20–24%              | [198]   |
| DAI        | Tofu            | 0.4              |             |             |                      |                     | 49%                 | [199]   |
| DAI        | Kinako          | 0.4              | 1.8 µM      | 6 hr         |                      | 5.8 hr              | 56%                 | [198]   |
| DAI        | Soy flour       | 0.8              | 3.1 µM      | 7.4 hr       |                      | 5.7 hr              | 62%                 | [200]   |
| DAI        | Soy milk        | 1.5              | 1.6–1.4     | 4–6 hr       |                      | 66% to 45%          |                     | [199]   |
| DAI        | Soy milk        | 1.7              | 1.5–2.5     | 2.9 hr       |                      | 47%                 |                     | [201]   |

*Estimates of isoflavone bioavailability following ingestion of a soy-based product. Dose gives the estimated intake in milligrams per kilogram body weight per day.

### Table 11. Isoflavone absorption and excretion in animals.

| Isoflavone | Species          | Dose (mg/kg/day) | Plasma peak | Time to peak (hr) | Plasma half-life | Excretion half-life | Bioavailability (%) | Ref     |
|------------|------------------|------------------|-------------|------------------|-----------------|---------------------|---------------------|---------|
| GEN, sc    | Rat              | 500              | 4.2 µM      | 2                | 8.8 hr          | 20                  |                     | (170)   |
| GEN, oral  | Rat              | 20               | 11 µM       | 2                | 8.8 hr          | 20                  |                     | (202)   |
| Soy genistein, oral | Rat | 20               | 4.9 µM      | 2                | 8.8 hr          | 18                  |                     | (202)   |
| Soy GEN, oral | Rat     | 20               | 9.5 µM      | 2                | 8.8 hr          | 15                  |                     | (203)   |
| GEN, oral  | Rat              | 45               | 2.2 µM      | 2                |                 |                     |                     | (112)   |
| Soy, oral  | Rat              | 40               | 6–9 µM      | 3–4 hr           | 19               |                     |                     | (203)   |
| Soy daidzein, oral | Rat | 21               | 5 µM        | 2                |                 |                     |                     | (203)   |
| EQL, sc    | Rat              | 5                | 1 hr        | 0.4 µM           |                 |                     |                     | (76)    |
| GEN, iv    | Mouse            | 52               | 237 µM      | 0.6–1.3          |                 |                     |                     | (204)   |
| GEN, oral  | Mouse            | 45               | 2.6 µM free | 0.3              | 4.8 hr          | 20                  |                     | (204)   |
| GEN, oral  | Mouse            | 54–180           | 4.1 µM free | 0.65             | 4.7 hr          | 21                  |                     | (204)   |
| Genistein, oral | Mouse | 50               | 1.5 µM free | 0.5              | 8 hr to 0.4 µM  | 11                  |                     | (204)   |
| FRM, sc    | Mouse            | 40               | 9.2 µM      | 2.5              |                 |                     |                     | (205)   |
| GEN, oral  | Rhesus macaque   | 7                | 55 nm (free + sulfate) | 3.4 hr          | 21               |                     |                     | (82)    |
| DAI, oral  | Rhesus macaque   | 3                | 21 nm (free + sulfate) |                 |                 |                     |                     | (83)    |
| FRM, oral  | Sheep            | 84               | 0.1 µM; 7 µM equal |                 |                 |                     |                     | (205)   |
| FRM, oral  | Cow              | (15 g)           | 0.4 µM; 7 µM equal |                 |                 |                     |                     | (205)   |
| FRM, oral  | Pig              | (0.9 g)          | 3.7 µM; 0.9 µM equal |                 |                 |                     |                     | (205)   |
pituitary or hypothalamic actions of phytoestrogens. However, sample size and duration of treatment limit the conclusions that can be derived from these studies. The effects observed after only a single cycle of treatment may not be representative of the long-term consequences of phytoestrogen consumption. Larger samples of women observed over longer periods of treatment with controlled doses are needed to establish the effects of soy isoflavones on the menstrual cycle.

**Proliferation of Breast Epithelium**

Low rates of breast cancer in populations with high phytoestrogen exposure suggest phytoestrogens may inhibit epithelial cell proliferation (9,65,103–106). On the other hand, the biphasic effects on proliferation observed in *in vitro* studies suggest that both proliferative and antiproliferative effects might be observed, depending on the dose. Results from multiple *in vivo* studies across species have been contradictory. A variety of effects have been noted, ranging from antiproliferation through enhanced proliferation, depending on the tumor cell type, dose, timing of phytoestrogen exposure, and the phytoestrogen given. In general the isoflavones, and particularly GEN (likely due at least partially to its tyrosine kinase-inhibiting properties), produce the most significant results and are thus the most widely explored.

Results seen *in vitro* have been difficult to reproduce *in vivo*. A recent study demonstrated that GEN (20 µM) inhibits cell proliferation in estrogen-independent human breast cancer cells (MDA-MB-231) by as much as 50%. Mice with breast tumors produced by inoculation with these same cells were then placed on a GEN-rich diet (750 µg/g). No significant changes in tumor size or morphology were seen, even when the treatment diet was given before the initial inoculation with the MDA-MB-231 cells (107).

Soy protein and GEN reduce tumor multiplicity, but not incidence, in other animal models of breast cancer (Table 15). Soy protein isolate-containing diets providing oral doses of 2–8 mg/kg/day GEN and 1–3 mg/kg/day DAI reduced the number of mammary tumors in 7,12-dimethylbenz(a)anthracene (DMBA)-treated rats (108). GEN or DAI intraperitoneal (ip) injections that provided doses ranging from 8 mg/kg/day at 35 days of age to 3 mg/kg/day at 215 days of age resulted in an observable but nonsignificant reduction in the number of mammary tumors in *N*-methylnitrosourea (MNU)-treated rats (109). The effect of DAI was delayed compared with that of GEN. No significant changes in protein tyrosine kinase-mediated phosphorylation or topoisomerase II activity were observed in the mammary gland or mammary tumors, even with higher isoflavone doses (40 mg/kg/day), suggesting that the observed chemoprevention was mediated via other mechanisms (109). Three-day sc treatments with a much higher GEN dose (500 mg/kg/day) during the neonatal (110) or prepubertal period (97) resulted in more marked reductions (50%) in tumor number in DMBA-treated rats.

Stimulation of mammary tissue has been seen in FRM-treated mice (111), GEN-treated rats, (97,112), and in cattle grazing on phytoestrogen-rich clover (113) (Table 15). Five-day treatment with sc doses of 40 mg/kg/day FRM, resulting in peak plasma and mammary levels of 9 µM and 4 nmol/g, increased mammary gland proliferation in ovariectomized mice (111). Oral doses of 36 mg/kg/day GEN maintained lobulo-alveolar structure in ovariectomized rats without affecting ducts; no effects were seen in immature rats (112). Transitory increases in mammary gland weight also were produced

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**Table 12. Tissue and fluid concentrations of phytoestrogens.**

| Species                        | Isoflavone | Dose (mg/kg/day) | Peak plasma fluid/tissue (nM) | Concentration Ref |
|-------------------------------|------------|------------------|--------------------------------|-------------------|
| Human - United States         | GEN soy diet | 0.07 100 Prostate | Breast milk 30 nM (78)              |                  |
| Human - United States         | DA1 soy diet | 0.07 60 Breast | 15 nM (78)                   |                  |
| Human - Lisbon                | DA1 Norm diet | 0.1 350 Prostate | Prostatic fluid 18 nM (80)                |                  |
| Human - Hong Kong             | DA1 Norm. diet | 0.1 350 Prostate | Prostatic fluid 275 nM (80)            |                  |
| Human - Hong Kong             | DA1 Norm. diet | 0.1 500 Prostate | Prostatic fluid 709 nM (80)            |                  |
| Human - Australia             | DA1 Unknown | 0.3 1,400 Prostate | Prostate 1.1 nM (210)             |                  |
| Human - Australia             | GEN Unknown | 0.3 1,400 Prostate | Prostate 1.1 nM (210)             |                  |

*Norm diet, normal diet. Human mg/kg/day estimated based on a body weight of 60 kg.*

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**Table 13. Proliferative responses of uterine and vaginal epithelium to phytoestrogen treatments.**

| End point                  | Dose (mg/kg/day)         | Soy isoflavone | FRM | GEN | BIA | IPR | α-ZAL | Ref |
|----------------------------|--------------------------|---------------|-----|-----|-----|-----|------|-----|
| Uterine growth             |                          |               |     |     |     |     |      |     |
| Immature mouse, sc         | 0.1–10                   | 300–800       | 100–400 | 200–800 |     |     |      |     |
| Immature/ovx rat, sc       | 2–10                     | 0.5           | 200  Inactive |      |     |      |     |
| Ovx rat, sc                | 16–160                   | Inactive 58–590 | 10–36 | 1–30 |     |     |      |     |
| Ovx rat, oral              | 0.1–30                   | 10–36         | 1–30 |     |     |     |      |     |
| Ovx heifers, oral          | 0.1–30                   | 10–36         | 1–30 |     |     |     |      |     |
| Vaginal cell maturation    |                           |               |     |     |     |     |      |     |
| Ovx rat, oral              | Inactive 58–590/1,800     | 0.1–30 |     |     |     |     |      |     |
| Ovx longtail macaques      | 10 Inactive              | 10            |     |     |     |     |      |     |
| Human postmenopausal       | 2.9 Inactive             | 2.9           |     |     |     |     |      |     |
| Human postmenopausal       | 1–2                      | 1–2           |     |     |     |     |      |     |

BIA, biochanin A.
with three sc doses of 500 mg/kg/day GEN in prepubertal and neonatal rats (97). Moreover, soy isoflavones or oral GEN actually increased the growth of implanted MAC-33 or MCF7 tumor cells in some recent experiments in rats (114,115). However, soy isoflavones did not increase mammary epithelium proliferation in surgically post-menopausal long-tailed macaques (116).

Early exposure to estrogen also may influence susceptibility to mammary cancer by altering the proliferation and differentiation of epithelial structures that are sensitive to transformation by carcinogens. Neonatal exposure to E2 stimulates the mammary epithelium in rodents, increasing terminal end bud proliferation and reducing the differentiation of terminal end buds during the period from 4 to 16 weeks of age (117). This effect is hypothesized to enhance susceptibility to carcinogenesis, as the terminal end bud is the site for transformation in the rodent mammary gland (118). Perinatal treatment with E2 or diethylstilbestrol (DES) increases the incidence of mammary tumors in mice carrying a mammary tumor virus (119,120). Similar effects have been observed with phytoestrogens. Neonatal treatment with 10 mg/kg/day zearalenone (ZLN) increases the incidence of spontaneous mammary tumors in Wistar rats (121). Increased numbers and reduced differentiation of terminal end buds have been observed in mice after in utero exposure to ZLN (117).

Neonatal or prepubertal exposure to GEN, however, appears to have the opposing effect of enhancing terminal duct differentiation in rats. Neonatal or prepubertal doses of 500 mg/kg/day GEN significantly reduced the number of terminal end buds and cell proliferation indices at 50 days of age (97,110). Transforming growth factor (TGF)-α and epidermal growth factor (EGF) receptor were upregulated in terminal duct structures immediately after treatment, but the EGF signaling pathway was downregulated by 50 days of age (122). These data suggest that high-dose GEN enhances epithelial differentiation. However, in the absence of data on mammary structure beyond 50 days of age, it is difficult to evaluate whether the observed changes represent permanent changes in mammary organization or simply alterations in the timing of developmental events. Moreover, these high-dose treatments induced changes in the reproductive tract that resemble the alterations in sexual differentiation produced by perinatal DES or E2. Both prenatal and neonatal GEN treatments resulted in precocious vaginal opening, and neonatal treatments were associated with reductions in serum progesterone at 50 days of age and follicular abnormalities such as atretic antral follicles and fewer corpora lutea (123).

Only two studies have directly examined isoﬂavone influences on the breast in women (Table 15). Chinese and Japanese women had lower nipple aspirate fluid volume, lower mean levels of gross cystic disease fluid protein, fewer hyperplasic cells, and fewer atypical epithelial cells in their nipple aspirate fluid than American women, an effect that was postulated to be a consequence of consumption of soy isoﬂavones (53). However, supplementation of the diets of American women with soy protein, providing GEN and DAI doses of 0.6 and 0.4 mg/kg/day, respectively, stimulated breast epithelial cell proliferation and 2- to 6-fold increases in nipple aspirate fluid volume in 29% of premenopausal (PRE) women (53). Monthly plasma collections suggested that E2 also was erratically elevated. A second study examined the proliferation rate of breast epithelium in biopsy samples from women with benign or malignant breast disease treated with and without a soy supplement providing an isoﬂavone dose of 0.75 mg/kg/day (124). After 14 days of treatment, the proliferation rate of the lobular epithelium and PR expression were significantly increased when days of menstrual cycle and patient age were taken into account. These unexpected results illustrate the difficulties of predicting human responses and the need for careful clinical trials.

### Table 14. Phytoestrogen influences on the human menstrual cycle.

| Supplement | Dose (mg/kg/day) | Duration | n | Hormonal sampling | Cycle length | E2 | Testosterone | P4 | LH | Ref |
|------------|-----------------|----------|---|-------------------|-------------|----|-------------|----|-----|-----|
| Flax ENL, END | -0.09 | 3 cycles | 18 | Monthly, composite cycle | Prolonged luteal | ↑ | ↓ | ↓ | ↓ | ↓ | [98] |
| Soy GEN, DAI 0.75 | GEN: 0.3 DAI: 0.4 | 1 month | 6 | Daily | Prolonged follicular | ↑ | ↓ | ↓ | [52] |
| Soy GEN, DAI 3 | GEN: 1.5 DAI: 1.5 | 1 month | 6 | Cycle days 7 and 22 | Prolonged 5/6, short 1/6 | ↓ | ↓ | ↓ | ↓ | [99] |
| Soy GEN, DAI 1 | GEN: 0.6; DAI:0.4 | 6 months | 24 | Random | No change | ↑ | ↓ | ↓ | ↓ | [53] |
| Soy GEN, DAI 1 | 2 | 3 cycles | 14 | Every other day | No change | ↓ | ↓ | ↓ | ↓ | [100] |
| Soy milk GEN, DAI 1.5 | | 2 cycles | 31 | Prolonged | E1 ↓ uE2 uE1 | ↓ | ↓ | ↓ | ↓ | [102] |

### Table 15. Isoflavone actions in mammary tissue and breast cancer.

| Species | Focus | Agent | Dose (mg/kg/day) | Age | Effect | Ref |
|---------|-------|-------|------------------|-----|--------|-----|
| Ovx mouse | Mammary epithelium | FRM | 40 sc | Adult | Proliferation | (117) |
| Ovx rat | Mammary lobular alveolar structure | GEN | 36 oral | Adult | Maintained structure | (112) |
| Immature rat | Mammary size | GEN | 500 sc | Adult | Increased weight | (97) |
| Ovx long-tail macaque | Mammary epithelium | Soy isoflavone | 10 oral | Adult | No effect | (119) |
| Human | Mammary epithelium | Soy isoflavone | 1 oral | PRE | Proliferation | (53) |
| Human | Nipple aspirate fluid | Soy isoflavone | 0.75 oral | PRE | Fluid | (124) |
| Rat | DMBA-induced mammary cancer | GEN + DAI | GEN 2–8 DAI 1–3 oral | PND 25–156 | 20–40% ↓ tumor number | (108) |
| Rat | MNU-induced mammary cancer | GEN | 3–8 ip | PND 35–215 | 27% ↓ tumor number, p < 0.07 | (109) |
| Rat | MNU-induced mammary cancer | DAI | 3–8 ip | PND 35–215 | 27% ↓ tumor number, p = 0.26 | (109) |
| Rat | DMBA-induced mammary cancer | GEN | 500 sc | PND 16,18,20 | 50% ↓ tumor number | (97) |
| Rat | DMBA-induced mammary cancer | GEN | 500 sc | 2,4,6 | 50% ↓ tumor number | (118) |
| Rat | Implanted tumor cells | Soy extract | 10 mg/ipl | PND 29 | ↑ tumor growth | (114) |
| Rat | Implanted tumor cells | GEN | 11 oral | PND 28 | ↑ mammary & tumor size | (115) |
| Rat | Spontaneous tumorigenesis | ZLN | 10 sc | PND 7,14 | ↑ tumor incidence | (121) |

**Abbreviations:** P4, progesterone; uE1, urinary estrone; uE2, urinary estradiol.
Phytoestrogen actions across species

Prostate and Testes
Evidence for high expression of ERβ in the prostate suggests that it could be an important target for phytoestrogen action. There is evidence for estrogenic action of isoflavones in the prostate. Isoflavonoids induce c-fos expression in the murine prostate at doses of 0.025–2.5 mg/kg/day sc (GEN) and 5 mg/kg/day (COM, DAI) (125, 126). Ten days of treatment with GEN (2.5 mg/kg/day sc) induced neoplastic transformation in neonatally estrogenized mice, whereas a sc dose of 1.2 mg/kg/day had no effect (125). On the other hand, rats maintained on a soy-free diet for 11 weeks developed prostatitis in particular and serum testosterone, pituitary LH, and prostate weight in adult male rats (126). Isoflavonoids induce c-fos, protein expression in the murine prostate at doses of 0.1–30 mg/kg/day GEN (142), whereas doses of 2 mg/kg/day (but not 6–20 mg/kg/day) preserve bone density in ovariectomized lactating rats (144). A dietary supplement providing approximately 10 mg/kg/day GEN, 17 mg/kg/day FRM, and 19 mg/kg/day biochanin A also had no effect on bone density (142). DAI, in contrast, actually stimulates bone resorption in vitro at concentrations of only 0.01–0.1 nM (145) but has not been tested in vivo. DAI is one of the metabolites of IPR, and its opposing effects may help to explain why such high doses of IPR are required to prevent bone loss.

The role of estrogen receptors in these actions is unclear. Although both ERα and ERβ are expressed in human (146) and rat (147) osteoblasts, there is no convincing evidence for either in osteoclasts (148), and preosteoclastic cells have not been examined. Unlike other isoflavonoids, IPR does not displace E2 binding to MCF7 cells or induce ERα-dependent gene transcription (149). IPR enhances E2 binding to proestrogens, but its binding to the same cells (Kd = 68 nM) is not displaced by E2 (149). Moreover, IPR appears to have minimal or no estrogen action on its own, although it augments the estrogenic effect of E2 and estrone (E1) on uterine weight and thyroid calcitonin release (150).

Cardiovascular Function
Reductions in serum cholesterol were produced in ovariectomized rats by 4 days of oral treatment with α-zearanol (ZAL) (ED50 = 0.2 mg/kg/day), COM (ED50 = 0.4 mg/kg/day), and GEN (ED50 = 0.5 mg/kg) (142). One month of treatment with 100 mg/kg/day IPR also reduced serum cholesterol in ovariectomized rats (153). A soy supplement providing approximately 7 mg/kg/day GEN plus 3 mg/kg/day DAI to ovariectomized female rhesus macaques with diet-induced atherosclerosis produced free plasma concentrations as high as 20 nM GEN and 40 nM DAI (152, 153). The isoflavone diet improved a number of cardiovascular risk factors such as lower total plasma cholesterol, elevated high-density lipoprotein cholesterol, lowered arterial lipid peroxidation, and enhanced dilator response of atherosclerotic arteries (152–154).

Five- to 12-week treatments with soy supplements providing 0.7–1.5 mg/kg/day isoflavones did not alter serum lipids in men and women with average serum cholesterol concentrations (86, 155–157), although reductions in LDL cholesterol have been observed in hypercholesterolemic women following soy isoflavone treatment (158). However, arterial compliance, a measure of arterial elasticity, was improved in menopausal women after 10 weeks of treatment with 0.7–1.2 mg/kg/day clover isoflavones (157).

Hypothalamic and Pituitary Feedback
Several studies have examined the influence of phytoestrogens on basal LH or LH release (Table 16). In ovariectomized rats, a low GEN dose (1 ng/kg iv) enhanced GnRH-induced LH release, whereas higher doses (1–10 µg/kg iv and 0.1–10 mg/kg iv) inhibited LH release (159, 160). Pretreatment with GEN 8 mg/kg sc or ZLN (0.8–8 mg/kg sc) did not inhibit tonic LH release, but these doses did block GnRH-stimulated LH release (161). However, oral doses of 0.1–10 mg/kg GEN, administered by gavage, had no effect on tonic LH (160). In rhesus macaques, ZLN suppressed LH at sc doses of 5–14 µg/kg and oral doses of 400 µg/kg (162).

Table 16. Phytoestrogen effects on anterior pituitary hormones.

| Species          | Phytoestrogen | Dose (mg/kg/day) | Hormone         | Outcome         | Ref     |
|------------------|---------------|------------------|-----------------|-----------------|---------|
| Rhesus macaques  | ZNL           | 0.005–0.014 sc   | Plasma LH       | ↓ LH            | (162)   |
| Ovx rats         | ZNL           | 0.8–8 sc         | Plasma LH       | No effect       | (161)   |
| Ovx rats         | ZNL           | 0.8–8.0 sc       | GnRH-stimulated LH | ↓ LH       | (161)   |
| Ovx rats         | GEN           | 0.1–10 gavage    | GnRH-stimulated LH | No effect   | (161)   |
| Ovx rats         | GEN           | 0.001–0.1 ng/kg  | GnRH-stimulated LH | ↓ LH       | (161)   |
| Ovx rats         | GEN           | 1 ng/kg          | Plasma PRL      | ↑ PRL           | (211)   |
| Postmenopausal women | Soy isoflavones | 0.7–1           | Plasma PRL      | ↑ PRL           | (112)   |
| Postmenopausal women | Soy isoflavones | 0.7–1           | Endogenous LH   | ↓ LH            | (52, 100, 163) |

*Estimated from dietary concentration.
There is some evidence that phytoestrogens can influence human LH secretion. Soy supplements providing 0.7–1 mg/kg/day isoflavones reduced mid-cycle LH (52,100), and suppression of the LH response to GnRH was observed in postmenopausal women with a similar supplement (163). ZLN suppressed LH secretion in postmenopausal women at similar dietary doses (0.4 mg/kg/day) (164).

**Developmental Effects**

The developmental actions of phytoestrogens have been studied mostly in rats and interpreted to have implications for human health. The impact of phytoestrogens on human fetal development is unclear, but the results of DES exposure have shown that early estrogen exposure can have profound effects. Although the immediately observable effects are limited in rodents, prenatal or neonatal exposure to phytoestrogens results in altered prepubertal or adult morphology and/or function in the uterus, vagina, ovary, breast, pituitary, and hypothalamus (Table 17). Reduced responsiveness to estrogen is apparent in the uterus, breast, and prostate, whereas symptoms of hyperestrogenization are apparent in the vaginal tract. Although the phytoestrogens vary in effective dose range and end points effected, COM, GEN, EQL, and ZLN all appear to exhibit some of the developmental actions that have been reported for other more potent nonsteroidal estrogens like DES. Active sc doses in neonates range from 0.005 to 50 mg/kg/day for COM and 10 to 500 mg/kg/day for GEN and ZLN. Effective maternal doses range from 0.2 to 17 mg/kg/day for both oral and sc doses. Although many of these doses are quite high, reports of effects at very low doses, particularly in rat dams, argue for a fuller examination of dose–response relationships.

**Summary and Conclusions**

Binding studies using purified receptor proteins show that the isoflavonoid phytoestrogens are high-affinity ligands for ERs, especially ERβ. In whole-cell assays, however, their RP is significantly lower, probably as a result of interactions with binding proteins and other as yet unidentified factors. As with other endocrine-active compounds, a wide variety of enzymatic actions have been reported for phytoestrogens. However, many of these in vitro actions require concentrations higher than those normally seen in plasma.

In vivo data show that phytoestrogens have a wide range of biologic effects at doses and plasma concentrations seen with normal human diets. Significant in vivo responses have been observed in animal and human tests for bone, breast, ovary, pituitary, vasculature, prostate, and serum lipids. These actions represent a broader range of tissues and processes, including many beneficial outcomes, than the end points generally used to assess health risks posed by exposure to endocrine-active compounds (5). Reports of ERβ in many of the tissues that appear to be responsive to phytoestrogens are intriguing, but currently there are not sufficient data to assess the relative sensitivity of different end points. For some of these end points, similar responses to phytoestrogens have been reported in humans and animal models, although there are currently insufficient data to carry out detailed comparisons.

The biphasic actions of phytoestrogens complicate the process of assigning lowest observed adverse effect levels. In this case, the actions presumed to be more beneficial (e.g., antiproliferative actions) occur at higher in vitro concentrations (micromolar) than the proliferative actions presumed to be more harmful (nanomolar concentrations). This pattern would predict that higher, rather than lower, doses would be more beneficial in vivo. Moreover, human plasma and tissue concentrations are primarily in the nanomolar range, suggesting that proliferative effects should be more likely to be observed.

The in vivo situation is more complex, however. Oral soy isoflavone doses of 3–8 mg/kg/day delay the development of carcinogen-induced breast tumors in rats, whereas GEN doses reported to stimulate growth of breast and uterine epithelia range from 10 to 36 mg/kg/day. However, a recent study has shown that oral GEN doses as low as 11 mg/kg/day enhance the growth of implanted mammary tumors as well as the mammary gland. Moreover, two studies have reported proliferation of breast epithelium in women with oral soy isoflavone doses of 0.7–1 mg/kg/day. The similarity of

**Table 17. Developmental effects of phytoestrogens and mycoestrogens in rodents.**

| Tissue   | Species | Life stage | Phytoestrogen | Dose (mg/kg/day) | Effect                                                                 | Ref     |
|----------|---------|------------|---------------|------------------|----------------------------------------------------------------------|---------|
| Uterus   | Rat     | Neonatal   | COM           | 1–10 sc          | Uterine and gland growth, later ↓ weight, ER ↑                       | 212,213 |
|          |         |            | COM           | 25–50 sc         | Squamous metaplasia                                                  |         |
|          |         |            | EQL           | 10–100 sc        | Later ↓ weight                                                       |         |
|          |         |            | ZNL           | 1–100 sc         | Uterine growth, later                                                |         |
| Vagina   | Mouse   | Neonatal   | COM           | 0.062–50 sc      | Persistent compaction                                               | 214,219 |
|          |         |            | COM           | 25–50 sc         | Cervicovaginal adenosin                                              |         |
|          |         |            | GEN           | 17 sc – dams     | Delayed vaginal opening, normal cycles                               |         |
| Ovary    | Mouse   | Neonatal   | COM           | 10 sc            | Polyovular follicles                                                 | 219     |
|          |         |            | GEN           | 500 sc           | Atretic antral follicles, ↓ corpora lutea                           | 122,123 |
|          |         |            | GEN           | 0.2 oral – dams   | Atretic follicles, cystic reti ovarii                               |         |
| Breast   | Rat     | Neonatal   | GEN           | 500 sc           | Increased terminal duct differentiation                               | 123     |
|          |         |            | ZNL           | 10 sc            | Spontaneous tumors                                                  |         |
| Anogenital| Rat    | Prenatal   | GEN           | 17 sc – dams     | Decreased distance in males                                         | 214     |
| Prostate | Mouse   | Neonatal DES| COM, Dai     | 7 sc – adults     | Increased c-fos                                                     | 217     |
|          |         | Neonatal DES|             | 13% Soy diet [9.4 µM excreted/day] | Prevention of DES lesions                                         |         |
| Pituitary| Rat     | Neonatal   | COM           | 0.01–1 sc        | ↑ Basal LH in females                                               | 218,219 |
|          |         |            | COM           | 1 sc             | ↓ GnRH-stimulated LH                                                |         |
|          |         |            | GEN           | 10 sc            | ↓ GnRH-stimulated LH                                                |         |
|          |         |            | GEN           | 100 sc           | ↓ GnRH-stimulated LH, ↓ basal LH                                   |         |
|          |         |            | ZNL           | 10–100 sc        | ↓ GnRH-stimulated LH, ↓ basal LH                                    |         |
| Hypothalamus | Rat    | Neonatal   | COM           | 7 oral – dams    | Premature anovulation, abnormal sexual behavior in males            | 220     |
| SDN-PDA  | Rat     | Neonatal   | COM           | 0.01–1 sc        | No effect                                                           | 218,219 |
|          |         |            | GEN           | 100 sc           | No effect (10), enlarged in females (100)                           |         |
|          |         |            | ZNL           | 100 sc           | Enlarged in females, 10: no effect                                  |         |

SDN-PDA, sexually dimorphic nucleus of preoptic area.
reported proliferative and antiproliferative doses illustrates the need for fuller examination of dose–response relationships and multiple end points in assessing phytoestrogen actions. The lowest effective concentrations in animals have been reported for induction of c-fos in the prostate and regulation of cholesterol, testosterone, and LH. The low effective doses reported for c-fos suggest that molecular or biochemical end points may be more sensitive indices of phytoestrogen response than the morphologic or functional end points commonly tested. The low doses reported for phytoestrogen effects on hormonal secretion suggest that steroidogenesis and the hypothalamic–pituitary–gonadal axis are important loci of phytoestrogen actions. However, these inferences must be tentative because good dose–response data are not available for many end points and many of the doses may not be the lowest active doses.

The available data indicate that phytoestrogens are biologically active in humans at dietary doses of 0.4–10 mg/kg/day (Table 18). The active isoflavone doses are similar to the daily intakes estimated for adults consuming soy-rich Asian diets, which may be primarily a consequence of attempts to test the hypothesized health benefits of Asian diets. Few human studies have attempted to test a range of doses. Moreover, all of the human studies have used isoflavone mixtures present in extracts of soy or red clover, making it difficult to assess the contributions and activity of individual isoflavones. This issue is particularly important in light of the in vitro and in vivo data showing that GEN and DAI sometimes exhibit opposing effects. Moreover, most studies have relied on manufacturer’s data on isoflavone content to estimate daily intake, so the actual doses used may vary even in studies using the same soy supplements.

The doses reported to be active in humans are lower than the doses usually reported to be active in rodents (10–100 mg/kg/day), although some studies have reported rodent responses at lower doses (0.005–0.2 mg/kg/day). However, the available estimates of bioavailability and peak plasma levels in rodents and humans are more similar. More studies are needed to determine whether lower doses are effective in rodents and humans and whether bioactive doses are associated with similar plasma levels in humans and animal models.

These comparisons illustrate the rich database available on phytoestrogen actions, mechanisms, and metabolism and highlight gaps and discrepancies in the dataset. These findings demonstrate some of the complexities of extrapolating across assays, species, and compounds. As dietary components, phytoestrogens generally have been treated and tested like nutrients rather than pharmacologic agents. As a result, researchers have investigated a number of beneficial properties that would be overlooked in toxicologic studies but also have failed to anticipate some potentially adverse properties. There is currently very little information on the balance of effects in individuals, data that ultimately will be required in order to assess the wisdom of phytoestrogen consumption. More detailed pharmacokinetic data are required to more accurately evaluate the reliability of extrapolating from in vivo to in vitro studies or across species. Such comparisons are likely to provide new insights into the evaluation of other exogenous estrogens.

Table 18. Phytoestrogen actions in humans and animal models.*

| Tissue/End point | Rats | Humans |
|------------------|------|--------|
| **Reproductive** |      |        |
| Uterine endometrium | Proliferation | 10–36 | No effect of soy | 16 | Variable |
| Vaginal cytology | Commination | 16 | 0.7–3 oral | 500 | Untested |
| Ovarian function | Ovarian hormones | 36–500 | 0.75–1 oral | 3–11 | Untested |
| Breast epithelium | Proliferation | 500 | 0.75–1 oral | 2.5 | Untested |
| Terminal ducts | Differentiation | 0.025–5 | Untested | 2.5 | Untested |
| **Breast tumor** | Suppression |                |                   |              |        |
| **Prostate** | Growth |                |                   |              |        |
| c-fos induction | 2.5 | Untested |                   |              |        |
| **Tests** | Reduced testosterone | 2.5 | Untested |              |        |
| **Skeletal** | Increased density | 50–400 variable | 3–10 oral |              |        |
| **Bone** |                |                   |                   |              |        |
| **Cardiovascular** | Reduced LDL cholesterol | 0.2–0.5 | 0.7–1 oral, only in hypercholestermic |              |        |
| **Neuroendocrine** | Reduced LH | 0.1–10 sc | 0.7–1 oral |              |        |
| Hypothalamic, pituitary feedback | Gonadotropin secretion | 0.005–500 sc | Untested |              |        |
| Developmental effects | SON-POA | 0.005–500 sc | Untested |              |        |
| Anogenital distance | exposure, sc |                |                   |              |        |
| Uterine and vaginal development | Maternal |                |                   |              |        |

*Adapted from Whitten and Naftolin (271).

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