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Topic 2.3

Concentration of phytohormones in food and feed and their impact on the human exposure*

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Abstract: Phytohormones, which naturally occur in plants, must be taken into consideration for their contribution to the total human exposure to potential endocrine active substances (EASs). Phytohormones are usually divided into two families: phytoestrogens that mainly occur from soybeans and soy derivatives and phytosterols that occur from vegetables and vegetable oils. The present paper compiles different sources of information about the concentration of phytohormones in foodstuffs in order to assess the current human exposure to those substances via food. Particular attention is given to most exposed groups of consumers, on the one hand, infants and young children fed with soy-based infant formulas for their exposure to phytoestrogens and on the other hand, consumers of fortified foods for their exposure to phytosterols.

Available literature shows that the total dietary intake of isoflavones could reach 20 to 25 mg/day/person for the Japanese adult population. For infants and young children, the quantity of phytoestrogen ingested is likely to be 35–50 mg/day/person corresponding on a body weight basis to an exposure 7 to 11 times higher. Regarding phytosterols, an assessment of the exposure via food was done, considering both their natural occurrence and their potential concentration in fortified foods. Results shows that the "natural" exposure is estimated at 340 ± 440 mg/day/person at the mean and at 1040 mg/day/person at the 95th percentile. Considering the potential exposure via fortified foods, it is estimated at 2700 ± 1200 mg/day/person at the mean and at 4700 mg/day/person at the 95th percentile.

After their ingestion, isoflavones are absorbed from the intestinal tract before being excreted in the urine and feces. The increasing use of phytohormones in human foodstuffs could increase locally their release into the environment. Nevertheless, considering the weak estrogenic potential of phytohormones in relation to synthetic or endogenous steroids, any introduction of these substances into aquatic ecosystems would probably have comparatively minor effects on aquatic organisms.

INTRODUCTION

In men, hypospadias, cryptorchidism, cancer of prostate, testicular cancer, and semen quality and in women, breast cancer, cystic ovaries, and endometriosis have all been suggested as indicators of adverse trends in reproductive health [1]. The idea that these trends are real and are connected with environmental pollution is gaining credence internationally. The effect on human health of chemicals that are mediated through the endocrine system has generated huge interest and investment and even if it was

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postulated that synthetic chemicals in the environment are the prime source of the excessive oestrogenic simulation, it was also recognized that endocrine active substances are potentially present in food as natural phytohormones.

Even if they are a quantitatively less important source than the naturally or industrially produced hormones, phytohormones which naturally occur in plants must be taken into consideration for their contribution to the total human exposure to chemicals having potential hormonal effects. Phytohormones are usually divided in two families: at first, phytoestrogens (daidzein, genistein, coumesterol, formononetin and biochanin A), which are mainly occurring from soybeans and soy derivatives. Secondly, phytosterols (β-sitosterol, campestanol, stigmasterol and dihydrobrassicasterol) which occur from the fat-soluble membrane extracts of plants, including algae. Common known sources of phytosterols are vegetables and vegetable oils.

One of the aims of the present paper is to compile the different sources of information about the concentration of phytohormones in foodstuffs and to assess the current human exposure to those substances via food. It must be noted that when numerous scientists have concerns about endocrine active substances and their possible negative impact on human health, a parallel development of health claims about positive health effects of food fortified by phytohormones can contribute to a major exposure of certain groups of consumers. Therefore, a model of potential human exposure to phytosterols is described in the paper as an example.

DESCRIPTION OF PHYTOHORMONES

Phytoestrogens

A list of 94 plants reported to exhibit estrogenic activity was establish [2] and at the same time, 29 specific compounds were reported to have estrogenic activity. The main known phytoestrogens are the isoflavones daidzein, genistein, formononetin and biochanin A and the coumestan coumesterol. Daidzein and genistein exist in 4 related chemical structures, namely the aglycones, the 7-*O*-glucosides, the 6-*O*-acetylglucosides, and the 6-*O*-malonylglucosides. Formononetin is further metabolized to daidzein and biochanin A is a precursor of genistein.

Phytosterols

Phytosterols are C28 or C29 sterols, structurally very similar to cholesterol (C27), but differing in their nucleus and/or side chain configuration or polar groups. β-Sitosterol contains an ethyl group at C24, campesterol has a methyl group at C24 and stigmasterol is considered to be an unsaturated phytosterol because of the double bond at C22. Chemical saturation of the Δ-5 double bond leads to the 5-α position of the hydrogen atom (i.e., cholestanol, campestanol, sitostanol). Enzyme transformation by gut bacteria leads to epimerization of the H atom at position 5 to the β configuration as represented by coprostanol and its derivatives.

EFFECTS OF PHYTOHORMONES

Effects of phytoestrogens in humans

Phytoestrogens interest in isoflavones has exploded in the past five years after a wealth of scientific data showing that these phytoestrogens possess potent and wide-ranging activities. Most of these results were related to replacement of estrogens in women, which is an established therapy of climacteric symptoms like hot flashes or depressive moods. Soy consumption is suggested to contribute to the prevention of chronic diseases including cardiovascular disorders, osteoporosis, and cancer. Recent animal experiments show that only 3 doses of genistein given to newborn, prepubertal, or perinatal rats can reduce the incidence and number of breast tumors [3]. Beside these beneficial effects, the potential for isoflavones to create steroid hormone imbalances or to compete for the normal steroid, drug, and xenobiotics metabolizing enzymes is presently unknown. Moreover, because these compounds are metabolized differently by different animals (and humans) before binding to the estrogen receptor, the intrinsic estrogenic activity should be viewed with caution.

Effects of phytosterols in humans

Phytosterols in foods were identified as natural cholesterol-lowering agents in the 1950s. They are poorly absorbed in human (5 % for sitosterol, 15 % for campesterol). Emerging evidence associated with use of phytosterols in foods has been focus of both food and pharmaceutical industries seeking to provide the consumer with foods with a health-promoting component. Diets containing phytosterols at levels of 2–3 g/day can reduce total and LDL cholesterol levels to about 10 and 20 %, respectively.

Several animal studies indicated that, when used at high levels, plant sterols, especially sitosterol, might have estrogenic activity. Those studies show a significant decrease in testicular weight and sperm concentrations after long-term treatment in albino male rats [4] and clear estrogenic effects in fish [5,6].

Sources of phytoestrogens

The most important dietary source of isoflavones is soybeans and soy foods [7]. Those plants can contain relatively high amounts of phytoestrogens, up to several mg/g of dry weight [8]. Some other legumes contain very small amounts of phytoestrogens, like snow peas, Brussels sprouts, and spinach leaf [9]. In addition, it was suggested that onion, garlic, potato, cucumber, cabbage and coffee could exhibit estrogenic activity [10] even if the chemical nature of this active compounds remains unclear. There are also reports on alcoholic beverages showing that biochanin A and β-sitosterol were founded at levels between 7 to 21 μg/100 ml in bourbon. Daidzein and genistein were also identified in beer. A recent review of the literature [11] compiles quantitative data available for phytoestrogens naturally occurring in food. Nineteen scientific papers were analyzed; the median results are expressed on a wet weight basis (Table 1).

Table 1 Concentration of phytoestrogens in foodstuffs [11].

Food category	Number of samples	Daidzein μg/g	Genistein μg/g	Coumestrol μg/g	Formononetin μg/g	Biochanin A μg/g
Tofu	15	76	166	nd	nd	nd
Soy sauce	3	8	5	nd	nd	nd
Soy milk	10	18	26	nd	nd	nd
Soy based formula	3	<1	3	nd	nd	nd
Alfafa sprouts	1	nd	nd	47	3	nd
Mung bean sprouts	1	nd	nd	nd	trace	nd
Soybean sprouts	3	138	230	7	nd	nd
Soybean green	1	546	729	na	na	na
Tempeh	3	190	320	na	na	na
Soybean paste	6	159	171	nd	nd	nd
Miso paste	2	266	376	na	na	na
Miso paste (rice or barley)	3	79	260	na	na	na
Soy hot dog, tempeh burger	2	49	139	na	na	na

A special mention must be done about the concentration of phytoestrogens in infant formulas. Those products that have been used for more than 30 years are manufactured from soy protein isolates and contain significant amounts of phytoestrogens of the isoflavone class. The results of recent analytical results are expressed in Table 2.

Table 2 Concentration of phytoestrogens in infant formulas [12].

Food category	Number of samples –	Daidzein μg/g	Genistein μg/g	Total isoflavones (concentrate) mg/g	Total isoflavones (final product) mg/l
Powdered 1	1	7.4 ± 1	6.2 ± 2.1	307 ± 28	46
Powdered 2	1	10.9 ± 2.1	8.7 ± 1.2	317 ± 13	47
Concentrate 1	1	2.2 ± 0.6	1 ± 1	91 ± 18	45
Concentrate 2	1	0.9 ± 0.3	1.2 ± 0.3	64 ± 9	32
Ready to eat	1	1 ± 0.1	1 ± 0.1	43.5 ± 0.7	44

Sources of phytosterols

Phytosterols encompass the entire class of sterols found in the fat-soluble membrane extracts of plants, including algae. These include pure sterols, stanols, their phenolic acids and conjugated glucosides. Phytosterols are not synthesized endogenously in humans. Commonly known sources are vegetables, wood, and vegetable oils. Owing to the lack of substrate specificity of enzymes, plants can operate multiple sterol pathways that give rise to specific end-products. Over 250 naturally occurring sterols have been characterized from plant isolation studies. Despite this diversity, the most frequently occurring phytosterols in nature and thus in human diets include β-sitosterol, campestanol, stigmasterol, and dihydrobrassicasterol.

The concentration of phytosterols in food and feed was reviewed from the scientific literature. It must be stressed that if a considerable number of publications were produced during the last 20 years on the biological properties of phytosterols and on their interest for human health, very few of them provide quantitative estimation of these substances in products of vegetal origin. The most important and reliable source of data is the series of publications by Oka [13–16]. In addition, a compilation of data on the sterol content of foods of plant origin was done by U.S. Department of Agriculture in 1978 [17]. Five food categories were considered relatively to the concentration in phytosterols, namely edible oils, vegetables, fruits, cereals, and pulses. Besides those naturally occurring phytosterols and because of the beneficial effects of these substances in humans, food fortification must be considered as a potential way of exposure. Table 3 shows the mean and maximum concentration of phytosterols both in natural and fortified foods. The phytosterol concentrations of fortified foods are those proposed by food industry, but are not currently accepted (80 mg/g for margarine, 23 mg/g for biscuits, 16 mg/g for cheese and breakfast cereals, 13 mg/g for bread, 5.7 mg/g for yogurt, and 3.2 mg/g for milk).

Table 3 Concentration of phytosterols in foods.

Food category	Number of samples	Mean concentration mg/100 g	SD	Maximum concentration mg/100 g
Edible oils	56	500	982	5400
Vegetables	51	20	40	200
Fruits	22	15	10	60
Cereals	9	250	405	1325
Pulses	10	100	59	220
Spreads & margarines		8000		8000
Biscuits		2300		2300
Milk		320		320
Yogurt		570		570
Cheese		1600		1600
Bread		1300		1300
Breakfast cereals		1600		1600

Dietary exposure to phytohormones

Very few studies are actually available to assess the human exposure to phytoestrogens. In their recent report, WHO and IPCS [18] quoted preliminary results obtained in U.S. adults. Those results show that urinary levels of phytoestrogens did not differ significantly of those reported in the literature from western populations known to consume phytoestrogen supplements. In a recent estimate, Nagata [19] suggests a the total dietary intake of isoflavones of 20 to 25 mg/day/person for the Japanese adult population. Considering the higher amount of soya consumed in Japan, this exposure from naturally occurring phytoestrogen is likely to be higher than those of other countries and particularly of the United States and the European Union. For infants and young children, the most important source of exposure is soya-based infant formula. In case of use of these products, the quantity of phytoestrogen ingested is similar to the one used during postmenopausal substitution, e.g., 35–50 mg/day/person. Related to the respective body weights, the exposure of infants is consequently 7 to 11 times higher [12,20].

Regarding phytosterols, an assessment of the exposure via food was done, considering the available data on the natural occurrence of those substances in five relevant food categories (edible oils, vegetables, fruits, cereals, and pulses). Because only a relatively small number of analytical results were available, the data were included in the model assuming the log normality of the distribution of concentrations in each considered food category. In order to calculate the geometrical standard deviation (GSD), the data were log-transformed and a distribution curve was simulated using the mean and the GSD of the initial data set. Those distribution curves were combined with the intake distribution of these foods in France [21]. Results are shown in Fig. 1. In a second step, the possibility for a consumer to get foods fortified at the levels requested by industry was simulated using the following assumptions:

- The food intake data are from the French household survey (SECODIP panels) recording the purchases of 2759 subjects, consumers only, during one year.
- The phytosterol concentrations are from the industry request (80 mg/g for margarine, 23 mg/g for biscuits, 16 mg/g for cheese and breakfast cereals, 13 mg/g for bread, 5.7 mg/g for yogurt, and 3.2 mg/g for milk).
- All foods were considered fortified at the maximum authorized level.
- The baseline exposure from natural occurring phytosterols was not taken into consideration in the simulation.

Results shows that the “natural” exposure is estimated at 340 ± 440 mg/day/person at the mean and at 1040 mg/day/person at the 95th percentile (Fig. 1). Considering the potential exposure via forti-

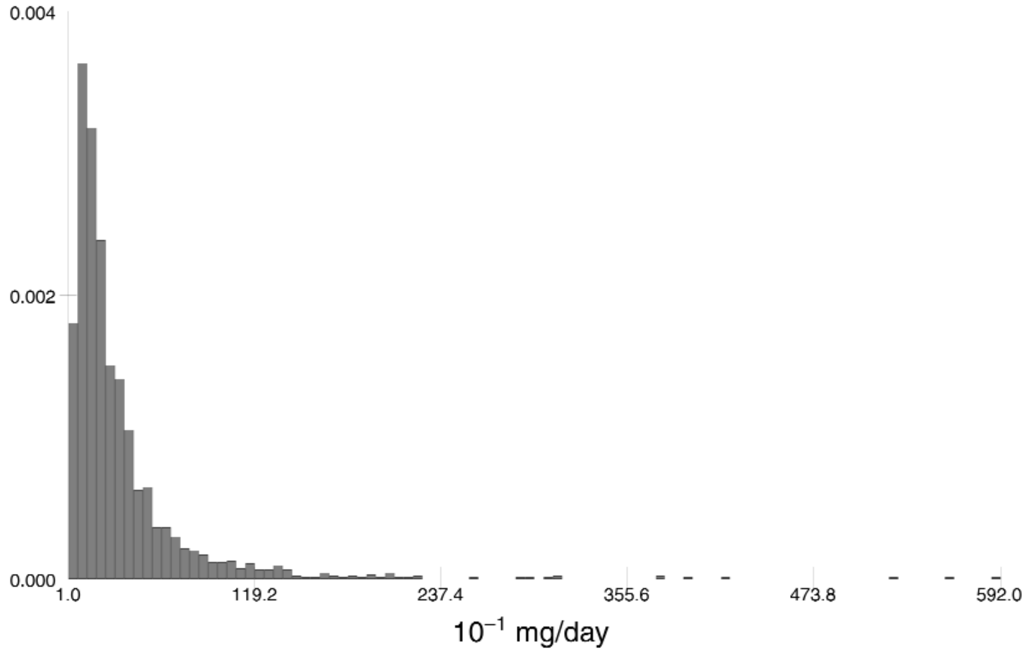

Fig. 1 Simulated exposure to naturally occurring phytosterols in food. (Display distribution as density.)

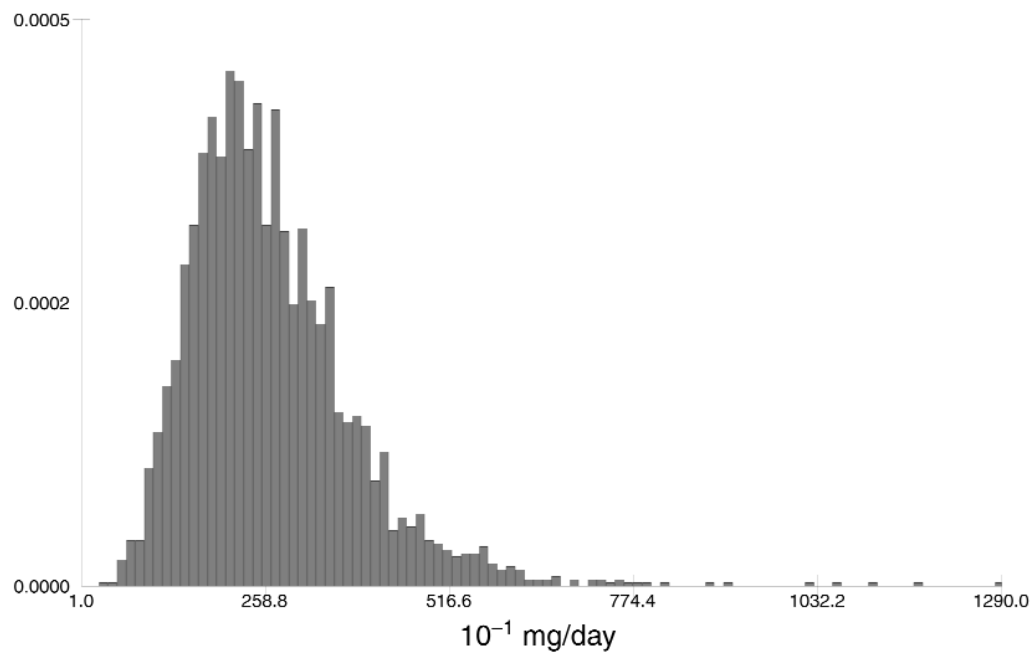

Fig. 2 Simulated distribution of the exposure via fortified foodstuffs. (Display distribution as density.)

fied foods, it is estimated at 2700 ± 1200 mg/day/person at the mean and at 4700 mg/day/person at the 95^{th} percentile (Fig. 2). These results are consistent with a previous study using Australian food intake data [22] and estimating the mean exposure between 1600 and 3900 mg/person/day and the exposure at the 97.5^{th} percentile between 3900 and 8700 mg/person/day.

Fate of phytohormones

Because phytohormones can be metabolized, at least in mammals, it presumably does not accumulate and an exposure of animals is probably limited to particular areas with single-crop farming or animals that are restricted in their choices. Available data indicate that phytohormones are biodegradable. Sufficient information is not available on the occurrence of a relevant exposure of aquatic organisms. In soy processing, phytoestrogens are entering the rinsing water [23,24]. Phytoestrogens are also excreted in the urine and feces of farm animals, and local introductions into surface waters cannot be excluded. Nevertheless, considering the weak estrogenic potential of phytohormones in relation to synthetic or endogenous steroids, any introduction of these substances into aquatic ecosystems would probably have comparatively minor effects on aquatic organisms, if at all [25,26,24].

In humans, after their ingestion, isoflavones are absorbed from the intestinal tract and a peak for plasma concentration is obtained after 5 to 10 h [27] before being excreted in the urine and feces. The increasing use of phytohormones in human foodstuffs because of their beneficial health effects and the elimination of those substances in urine could increase locally their release into the environment. Even if this phenomenon seems quantitatively relatively minor, it could be interesting to get an idea of the order of magnitude of the potential increase of human exposure via fortified foods in order to compare the resulting release into the environment with those from synthetic hormones.

RECOMMENDATIONS

- More specific and sensitive biomarkers should be developed to detect endocrine-mediated effects in individuals and populations.
- Further investigations are needed to assess the risk for aquatic organisms of increased phytohormone exposure.
- Long-term monitoring of wildlife species should be improved.
- More data should be obtained related to the in utero exposure of the fetus to phytohormones from the maternal diet.
- Exposure assessment and epidemiological studies based on specifically exposed groups consuming fortified foods in large amounts should be conducted.
- Investigations on the consequences of high exposure of the human population, including infants consuming soy-based milk substitutes, should be done.
- A full risk assessment vs. benefit of food fortification should be done in order to improve the management of food safety regulation.
- International collaboration and collaborative research should be encouraged.

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