Institute for Environment and Health

PHYTOESTROGENS IN THE HUMAN DIET

IEH Web Report W3 **OCTOBER 2000**

The Institute for Environment and Health was established by the Medical Research Council at the University of Leicester in 1993. The Institute is partly funded by the Department of the Environment, Transport and the Regions, the Department of Health and other government departments and agencies by way of specific research and consultancy contracts.

This report was prepared by the Institute for Environment and Health for the Ministry of Agriculture, Fisheries and Food and issued to the Ministry in November 1997[*].

The views expressed here do not necessarily represent those of any government department or agency.

Prepared by Charles Humfrey and Philip Holmes

Please cite as:
IEH (2000) *Phytoestrogens in the Human Diet* (Web Report W3), Leicester, UK, Institute for Environment and Health, posted October 2000 at http://www.le.ac.uk/ieh/webpub/webpub.html

MRC Institute for Environment and Health
University of Leicester
94 Regent Road
Leicester
LE1 7DD
UK

[*] Literature search to November 96; supplemented by additional papers to June 97

Contents

Executive summary

Phytoestrogens are naturally occurring plant compounds which have oestrogenic and/or anti-oestrogenic activity. They are present in many human foodstuffs including beans, sprouts, cabbage, spinach, soyabean, grains and hops. The main classes are the isoflavones, coumestans and lignans. Epidemiological studies in humans suggest that they may have a beneficial role in protecting against several hormone-dependent diseases. However, almost no evidence exists to link these effects directly to phytoestrogens.

This review considers specific areas of the published literature on phytoestrogens in the human diet. The areas covered in detail are:

- estimates of phytoestrogen content of foods relevant to human consumption and levels of phytoestrogens found in human plasma, urine and faeces;
- factors during cultivation, harvesting and food processing that may affect the phytoestrogen content of human foodstuffs;
- potency of phytoestrogens relative to endogenous human oestrogens in various *in vitro* and *in vivo* test systems;
- genetic differences and differences in gut microflora between different populations, and
- the possible beneficial effects of phytoestrogens in adults.

The major conclusions covering each of these areas are summarised below.

Presence of phytoestrogens in food and in human plasma, urine and faeces

- The phytoestrogen content of plant-derived foodstuffs is very variable: high concentrations occur in most soya products, alfalfa and linseed.
- Individual exposure to phytoestrogens is significantly affected by dietary practice and short-term dietary regimen change can affect plasma concentration and urinary/faecal excretory rates. Chronic differences may result from the maintenance of particular dietary regimes.
- Both the magnitude and duration of any dietary-induced difference in metabolic profile shows marked interindividual variation.
- Given the variability of data from current studies, use of urinary concentrations of phytoestrogens or their metabolites as markers of total intake is relatively insensitive.
- Future studies must ensure adequate validation and quality assurance procedures, and use appropriate reference samples: use of high performance liquid chromatography (HPLC) and gas chromatography (GC) linked with mass spectroscopy (MS) techniques is recommended.

The influence of agricultural and food processing practices on phytoestrogen content

- Some of the major plant-derived food sources of humans and animals contain oestrogenic chemicals and the total and individual levels show considerable variability.
- Even for a given variety considerable variability occurs depending upon growing condition, stage of life cycle, etc.
- Phytoestrogen content is modifiable by some agricultural factors and post-harvest storage and food processing techniques.
- Soya is a major potential source of human exposure to phytoestrogens. Non-fermented forms retain relatively high concentrations but fermentation can result in significant changes in both levels and composition. Non-traditional foodstuffs containing soya generally contain much lower levels.

Oestrogenic potency of phytoestrogens

- A provisional ranking of the relative potencies of the phytoestrogens and mycoestrogens is zearalenone > coumestrol >isoflavones.
- There is a noticeable absence of data on the potency of the lignans, while the amount of data on the relative binding affinity to sex hormone binding globulin is limited.
- Priority should be given to investigations designed to address the current gaps in knowledge on relative potency and sex hormone binding affinity of the phytoestrogens and to improving understanding of the consequences of these factors *in vivo*.

Differences in gut microflora and interindividual metabolic differences

- Gut microflora play a key role in digestion but the composition is in a dynamic relationship with the individual host.
- The microflora may influence the bioavailability and metabolism of important dietary constituents and affect aspects of the hosts physiology. Given such interrelationships, a particular pattern of microflora may influence disease susceptibility.
- Although each person may possess a unique microflora, certain dietary regimens may be reflected, in general, by certain microfloral compositions.
- There is an outstanding need to investigate the complex interactions between diet, gut microflora and the host's metabolic, absorption and physiological status.

The beneficial effects of phytoestrogens in adults

- On balance, it is concluded that dietary lignans and isoflavones may have a role in the prevention of several types of cancer but that at present the evidence is not sufficient to recommend particular dietary practices or changes.
- For cancer of the breast, prostate, colon, rectum, stomach and lung, the evidence is most consistent for a protective effect resulting from a high intake of plant foods including grains, legumes, fruits and vegetables; it is not possible to identify particular food types or components that may be responsible.
- Epidemiological studies suggest phytoestrogens may have a beneficial role in protecting against cancer of the breast (in women), endometrium, prostate, colon, rectum, stomach and lung and other diseases and conditions such as osteoporosis, postmenopausal symptoms and cardiovascular disease.
- Dietary intervention studies indicate that soya and linseed have effects in women which may decrease the risk of breast cancer and may help to alleviate postmenopausal symptoms.
- For osteoporosis, there is at present only tentative evidence to indicate that naturally-occurring phytoestrogens have similar effects in maintaining bone density to those of the related compound ipriflavone, although emerging evidence suggests these substances are biologically active in experimental models.
- Soya and linseed (as linseed oil) also appear to have beneficial effects on blood lipids which may help to reduce the risk of cardiovascular disease and atherosclerosis.
- Although some epidemiological studies suggest that consumption of foods containing phytoestrogens may have beneficial effects, almost no evidence exists to link these effects directly to phytoestrogens; many other components of soya and linseed are biologically active in various experimental systems and may be responsible for the observed effects in humans.
- Research would be needed to clarify whether consumption of phytoestrogens specifically confers any degree of protection against the numerous diseases in adults described above.

1 Introduction

Phytoestrogens are naturally occurring plant compounds which have oestrogenic and/or anti-oestrogenic activity. They are heterocyclic phenols which bear structural similarity with oestrogenic steroids. Phytoestrogens are constituents of many human foodstuffs including beans, sprouts, cabbage, spinach, soyabean, grains and hops. The main classes of phytoestrogens are the isoflavones, coumestans and lignans. Although their function in plants is not entirely clear, they may act as anti-fungal agents (Naim *et al.*, 1974), plant pigments (Clevenger, 1964) or compounds involved with lignification (Francis & Hume, 1971). It has also been suggested that they may form part of the defensive strategy of plants (Hughes Jr, 1988). Under this hypothesis, chemicals affecting the reproductive system of animals are developed by plants under evolutionary pressure to reduce or control levels of predation by animals and may not, therefore, necessarily have an intrinsic role in the functioning or metabolic processes of the plant. Herbivores would be at particular risk from plant chemicals that interfered with reproductive functioning because of their high exposure and, indeed, there are historical examples of such adverse reproductive effects (e.g. effects of some clovers on sheep and ovine fertility, see below).

A large number of isoflavones have been identified in plants but only relatively few have been shown to have oestrogenic activity including daidzein and genistein, their glucosides daidzin and genistin, and their methyl ether derivatives formononetin and biochanin-A (Price & Fenwick, 1985). Scientific interest in the isoflavones was stimulated in the 1940s by the observation of breeding problems in sheep in Western Australia (Bennetts *et al.*, 1946). The syndrome, termed clover disease, was characterised by a cystic condition of the ovaries, irreversible endometriosis and a failure to conceive and was subsequently shown to be caused by ingestion of subterranean clover (*Trifolium subterraneum*) which contains high levels of isoflavone phytoestrogens (Bradbury & White, 1954). Although not as oestrogenically active as other phytoestrogens present in clover (e.g. biochanin-A and genistein), levels of formononetin correlated with the hormonal activity *in vivo*. Further investigation identified equol, formed in the digestive tract following bacterial metabolism of formononetin, as the ultimate oestrogenic compound responsible (Lindsay & Kelly, 1970).

Coumestans are structurally similar and biosynthetically related to the isoflavones. Although a large number of coumestans have been isolated from plants, only relatively few have been shown to have oestrogenic activity the most important of which are coumestrol and 4'-methoxycoumestrol. These substances have been found in alfalfa, clover and other fodder crops, and soyabeans, with levels being particularly high in fresh sprouts of alfalfa and soyabeans (Price & Fenwick, 1985). As some of these plants and plant products are consumed by man, it is important to consider the oestrogenic coumestans when assessing the total phytoestrogen content of human dietary components and their possible effects on health and disease.

Lignans contain a 2,3-dibenzylbutane structure and are present as minor constituents in many plants where they form the precursors for lignin formation in plant cell walls. They were first identified in humans following observations in monkeys of cycling patterns in the urinary excretion of compounds which had apparent mass spectral similarities to urinary steroid hormone metabolites (Setchell & Adlercreutz, 1988). The major human lignans are enterolactone and enterodiol which are synthesised in the gut from their plant precursors matairesinol and secoisolariciresinol.

Studies in laboratory and other animals have shown that the effects of phytoestrogens vary depending on the timing of exposure. Studies on the developmental effects of phytoestrogens have been limited to rodents, primarily rats, and have indicated mainly adverse effects, including those on sexual differentiation of the brain, maturation of neuroendocrine control of ovulation and puberty and development of the female reproductive tract. However, there are also possible beneficial effects relating to antiproliferative actions on mammary tissue which may be linked to this developmental phase (Chapin *et al*., 1996).

The effects of dietary phytoestrogens during the reproductive period have been investigated in a number of animal species including cattle, cheetahs, mice, quail, rabbits and sheep. The overall effect, which is relatively consistent and more distinct in females than males, is that of depressed fertility. Potential sites of action in females include the genital tract, ovaries, pituitary and the central nervous system. Considering all the available data, Chapin *et al.* (1996) suggested that the effects of phytoestrogens can only be regarded as predominantly adverse with regard to fertility in females of these animal species.

One of the main focuses of this report is the evidence for beneficial effects of phytoestrogens in adults, and epidemiological studies suggest that they may have a beneficial role in protecting against several hormone-dependent diseases including cancer of the breast (in women), endometrium, prostate, colon, rectum, stomach and lung and other endpoints such as osteoporosis, postmenopausal symptoms and cardiovascular disease.

Historically, the incidence of various human cancers and other disease conditions has shown considerable geographical variation (e.g. rates of breast and prostate cancer in Asian countries are significantly lower than in western countries). Such differences must be treated with caution given the different diagnostic and recording practices between nations, however, there is now considerable evidence that such differences are real and that genetic difference is not the principal cause. For example, Armstrong and Doll (1975) demonstrated associations between diet and cancers of the gastrointestinal tract and hormonally responsive tissues, with intake of animal protein or fat apparently playing a key role, while Gray *et al.* (1979) showed that breast cancer incidence/mortality was associated with diet, even after adjustment for differences in height, weight and menarche.

In a study investigating the roles of genetics and lifestyle factors in the incidence of breast cancer, Zeigler *et al.* (1993) reported a case–control study in women from China, Japan or the Philippines living in California or Hawaii. Asian–American women born in the West showed a

relative risk (RR) of 1.6 compared with migrants born in Asia; the risk increased if one or two parents were also born in the West (RR=1.8 and 1.7, respectively) compared to those with parents born in the East (RR 1.5). Risk for Asian–American women born in the West also varied with the number of grandparents born in the West (RR=1.4, 2.2 and 2.9, for none to one, two to three, or all four grandparents born in the West, respectively). For overall relative risk of breast cancer, the urban or rural influence for the time in the East and the number of years subsequently spent in the West were important for women born in the East. For those born in the West, the influence of their grandparents' birthplace was important. This reflected the importance of early lifestyle, since traditional practices would be expected to be maintained where strong pressure was exerted from the grandparents. Lifestyle and environment rather than genetics was, therefore, considered important in the aetiology of this disease.

The changing patterns of disease in Asian or other populations and their descendants following adoption of western-style lifestyles (possibly following migration to western countries) underlines that genetic differences between races are unlikely to be the most significant factor in the differences in disease incidence seen between countries. Although lifestyle factors and altered socio-economic status can be expected to play a role, there is strong evidence that dietary practices exert a significant influence on the occurrence and progression of diseases within a population.

In assessing the evidence for a beneficial effect of phytoestrogens on incidence of the human diseases mentioned above, it is perhaps useful to first consider dietary constituents that contain large amounts of these substances. For example, soyabeans and linseed are rich sources of isoflavone and lignans, respectively. Many epidemiological studies of soya and soya products have been carried out and are reviewed in this report. However, an important point to consider when assessing whether any effect of these materials is directly attributable to their phytoestrogen content is that they may contain other biologically active components which may play a role in these effects. For example, in linseed, active components include linolenic acids and in particular α-linolenic acid, which has been suggested as responsible for the beneficial effects of linseed on blood lipids and platelet aggregation. For soya, other active constituents include protease inhibitors such as Bowman-Birk protease inhibitor (BBI) and Kunitz trypsin inhibitor; the BBI has been shown to inhibit or prevent the development of experimentally-induced colon, oral, lung, liver and oesophageal cancers (Messina & Barnes, 1991). Other biologically active constituents of soya include phytosterols, saponins and inositol hexaphosphate (phytic acid). Phytosterols are structurally similar to cholesterol, and soyabeans are a major contributor of these compounds to the diet, particularly β-sitosterol. Interest in phytosterols and saponins (compounds with surfactant properties), has focused on their ability to inhibit cholesterol absorption, although it has been suggested that they may also have anti-carcinogenic properties (Messina & Barnes, 1991). A typical western diet has been estimated to contain about 80mg/day phytosterols, while Japanese and vegetarian diets may contain approximately 400 and 345mg/day, respectively (Nair *et al.*, 1984; Hirai *et al.*, 1986). Considering the levels of inositol hexaphosphate in cereals, fruits and vegetables, a

negative correlation has been found between this compound and the incidence of colon cancer. Nutritional interest in this compound has focused on the inhibitory effect on mineral absorption. It can chelate metals such as calcium, zinc and iron and this activity, particularly with regard to iron, may explain its action in reducing oxidant activity, inhibiting lipid peroxidation and inhibiting experimentally-induced colon cancer. In addition, the hexaphosphate may be involved with regulating cell differentiation following dephosphorylation to inositol trisphosphate, an important intracellular second messenger.

In spite of the many components of soya mentioned above, only the isoflavones and their metabolites have been detected in urine and blood of individuals from populations having low risks of various diseases, implying that isoflavones may indeed be the active ingredients (Lu *et al.*, 1996). However, it is unclear to what extent, if any, other components of soya are absorbed and therefore whether they contribute to the observed effects.

This review considers specific areas of the published literature on phytoestrogens in the human diet, targeted to areas for which there was either a considerable volume of data, or where information was sparse. The areas covered in detail are as follows:

- estimates of phytoestrogen content of foods relevant to human consumption and levels of phytoestrogens found in human plasma, urine and faeces;
- factors during cultivation, harvesting and food processing that may affect the phytoestrogen content of human foodstuffs;
- potency of phytoestrogens relative to endogenous human oestrogens in various *in vitro* and *in vivo* test systems;
- genetic differences and differences in gut microflora between different populations, and
- the possible beneficial effects of phytoestrogens in adults.

Each of these issues is considered separately in the chapters that follow. Of particular importance, Chapter 6 considers the evidence for beneficial effects of phytoestrogens in adults, divided according to the effects and diseases with which phytoestrogen consumption has been linked. Clearly, in any risk assessment of phytoestrogens in the human diet it is essential to balance beneficial effects against potential adverse effects. However, this report covers only the areas described above; possible adverse effects of these compounds in adults, beneficial or adverse effects on the unborn or in children, and mechanisms of action are not considered in detail.

The initial preparation of texts covering the above topics by staff at the Institute for Environment and Health (IEH) was followed by presentation of a draft document to a group of invited experts[*] at a technical workshop held in Leicester in March 1997. The aim of the workshop was to assess the information contained in the document in order to establish the current state of knowledge, discuss any uncertainties in the data and identify knowledge gaps. Some recommendations for future research were also made. However, the views and

[*] See List of Workshop Participants

judgements expressed in this report are those of IEH and do not necessarily reflect those of the individual scientists who provided material or attended the workshop, nor of the sponsors of this report, the Ministry of Agriculture, Fisheries and Food.

2 Levels of phytoestrogens in foods for human consumption and in human plasma, urine and faeces

2.1 SUMMARY

The phytoestrogen content of plant-derived foodstuffs has been shown to be very variable, with high concentrations being found in most soya products and alfalfa, while high levels of lignans or flavonoids occur in celery, linseed oil and onions. Thus, depending upon the dietary practice of an individual, markedly different exposures to these substances may occur. For example, daily intake of phytoestrogens in Japan has been estimated to be at least 30 times as great as in the UK. In order to assist progress in understanding of the content of foodstuffs and the metabolic fate of the phyto- and mycoestrogens, future chemical analyses should use appropriate reference samples and include rigorous validation and quality assurance procedures; high performance liquid chromatography (HPLC) and gas chromatography (GC) linked with mass spectroscopy (MS) techniques appear to have much to offer.

The analysis of plasma, urine and faecal samples from individuals normally consuming particular diets (e.g. omnivores versus vegetarians), or who were taking part in dietary modification studies, has clearly demonstrated that the dietary regimen can affect the plasma concentrations and the urinary and faecal excretory rates of various phytoestrogens and their metabolites. Both the magnitude and duration of any change does, however, show considerable interindividual variation. For example, supplementation of diets with linseed, soya, soya milk or clover products has been demonstrated to cause a transitory (<24-hour) enhancement of plasma isoflavone and lignan concentrations in humans. Following soya protein ingestion total isoflavone recoveries in urine of between 1.8 and 13% of the total intake have been shown, while if urine and faecal recoveries were combined between 15 and 20% was accounted for. This suggests that urinary isoflavone concentrations may be a relatively insensitive marker of intake. Similarly, urinary concentrations of enterodiol (but not other lignans) increase with high fruit and vegetable diets; however, interindividual variation in urinary excretion again suggests that enterolactone may not be a good marker.

There are limited data to suggest that chronic differences may arise from long-term adoption of particular dietary practices. There are data that suggest that Japanese men have higher plasma concentrations of total, and free and sulphated isoflavones than do Finnish men, and that plasma lignan and isoflavone concentrations (except equol) may be higher in vegetarian than in omnivorous women. Similarly, plasma concentrations of lignans and isoflavones appear high in individuals on macrobiotic diets but low for omnivores. Studies investigating the effects of long-term soya milk ingestion in men have demonstrated no effect on the total amount of isoflavones excreted, but rates of daidzein and genistein excretion are decreased. Similar studies in women demonstrated a reduction in the urinary recovery of daidzein and

genistein, an increase in recovery of equol, and similar effects on the total amounts and peak concentrations of these substances.

2.2 ANALYTICAL METHODOLOGIES

The provision of accurate data on the phytoestrogen content of different foodstuffs and the study of internal exposure to, and the metabolic and excretory fates of, phytoestrogens in the body are clearly of great importance to understanding the potential health effects of these substances. Given the number of known phytoestrogens and the range of chemical forms in which they can occur within various biological matrices, the development of suitable analytical methodologies has proved technically demanding. Fortunately the sensitivity, accuracy and reproducibility of methods have tended to improve with time, in line with the increasing sophistication of available technology and an increased awareness of the need to employ validation techniques, including the use of internal and reference standards.

In some of the early studies the methods employed relatively insensitive and imprecise techniques, such as paper chromatography (PC) with, for example, colorimetric or ultraviolet detection, or thin layer chromatography (TLC) with, for example, fluorimetric detection. The degree of validation and quality assurance included in these designs was also variable. The majority of the data presented herein relates to analyses conducted using high performance liquid chromatography (HPLC). A range of detection systems have been used, e.g. ultraviolet or fluorimetric or mass spectroscopy (MS) detection. In some more recent studies, use has been made of gas chromatography (GC) linked with MS. This latter combination permits the quantification of very low concentrations of substances but is dependent upon development of processes that permit the adequate isolation, extraction and concentration of chemicals from the original matrix. The inclusion of appropriate reference samples and the rigorous validation and quality assurance of the analyses have steadily increased and it must be stressed that all future work should incorporate these essential aspects.

Although it is beyond the scope of this text to review in detail the technical aspects of analytical methods employed in the quantification of phytoestrogen concentrations, it must be stressed that caution is necessary when comparing data from different sources because of the potentially marked effects (e.g. on detection limit, sensitivity, specificity and accuracy) that can arise when using different extraction or analytical procedures. As noted by Price and Fenwick (1985), improving methodologies for the detection and quantification of phytoestrogens in plant material and biological samples has been of great importance in the development of current understanding of their chemical and biological properties. In particular, HPLC techniques have been valuable because of the ability to characterise samples without prior derivatisation and to directly analyse free and conjugated forms (Price & Fenwick, 1985). It should, however, be noted that early studies employing HPLC-techniques may have involved rigorous extraction methods (e.g. involving heating, use of organic solvents and acid or enzymic hydrolysis) which could result in the loss of some conjugated forms. However, these are now easier to detect using, for example, reverse-phase HPLC (Setchell,

1997). Mazur *et al.* (1996) noted the benefits from employing HPLC for foods containing relatively high quantities of the phytoestrogens but considered that GC/MS techniques were preferable when analysing low concentrations, because of their greater specificity and sensitivity despite the greater effort and resource requirements. Setchell (1997) has also noted that the introduction of MS-techniques should enhance understanding of the metabolic fate of phytoestrogens, such as daidzein and genistein, by permitting quantification of intermediates of biotransformation, and has also suggested that investigations of metabolism would be enhanced by improved availability of chemically inert stable-labelled tracers incorporating isotopes of ^{13}C.

Notwithstanding such technical reservations, it is apparent that for many of the foods analysed to date, substantial variations in phytoestrogen content have been reported even within individual studies using a single analytical technique. The data on reported concentrations in food are summarised in Section 2.3 and presented in detail in the Annex to this report. Several reasons for the wide range of values for a given food exist including genetic differences between plant species or varieties, the influence of environmental factors during growth, differences in harvesting or subsequent processing procedures: these aspects are more fully discussed in Section 3. In addition, Section 2.4 presents the available data on phytoestrogen concentrations in human plasma, whilst additional exposure information on the concentrations of these substances and their metabolites in urine is summarised in Section 2.5.

2.3 LEVELS OF PHYTOESTROGENS IN FOOD

The amount of phytoestrogens consumed by an individual will depend upon the types and amounts of food they consume and the composition of the foodstuffs (Dwyer *et al*., 1994). For example, the diet consumed within the UK is different from that of Americans or Asian–Americans (Reinli & Block, 1996). In a total diet study conducted in the UK in 1981 and 1987, isoflavone and coumestan contents of a variety of foods were assessed by HPLC/UV detection, with positive results being quantified by GC (MAFF, 1996). Food groups investigated included carcass meat, offal, meat products, poultry, fish, oils and fats, potatoes, milk, dairy products, eggs, cereals, bread, nuts, sugar and preserves, fruit products, beverages, green vegetables, other vegetables, canned vegetables, and fresh fruit. None was found to contain detectable concentrations of phytoestrogens (limit of detection 0.02 mg/kg), and the maximum expected intake of phytoestrogens for an average person was calculated at less than 1 mg/day. This study was, however, performed before the widespread introduction of soya derivatives into non-traditional food sources (see below) and may, therefore, somewhat underestimate current exposure in the UK. Jones *et al.* (1989), who used HPLC and GC techniques, supplemented the original total diet study data by analysis of additional, commercially available samples of vegetable, fruit, nuts, soya products and alfalfa tablets. For comparison, Cassidy *et al*. (1994) estimated daily isoflavone intake in Japanese at 150–200mg, although Messina (1995) suggested the daily isoflavone intake in Japan was approximately 50 mg and noted that a similar value (32 mg) had been reported for Japanese in

the Okinawa Prefecture by Wilcox *et al*. (1995). Even using the lower figure for Japanese intake, a marked difference from the UK intake is apparent.

The phytoestrogen and mycoestrogen contents of many foodstuffs have been reported (e.g. Reinli & Block, 1996), and are presented in the annex to this report. The principal phytoestrogens found are the isoflavones daidzein, genistein, formononetin and biochanin-A, and the coumestan coumestrol. Daidzein and genistein exist in several forms: as aglycones (daidzein and genistein), 7-*o*-glucosides (daidzin and genistin), 6'-*o*-acetylglucosides and the 6'-*o*-malonylglucosides. Information is also available for a number of other flavonoids (not known to be oestrogenic, e.g. glycitein), and for lignans (which can be metabolised to oestrogenically-active chemicals by mammalian gut microflora). Such data are included for completeness.

Soyabean and the multitude of derived products have probably been the most investigated of the foodstuffs. Soya may be consumed in a wide variety of forms either as the soyabean itself, as traditional food products (such as miso or tofu) or as part of a processed food (e.g. as a replacement for animal protein). Indeed, soya now forms a component of many processed foods in the West; approximately 60% of processed foods in British supermarkets contain soya (ENDS, Report 258, July 1996). The concentrations of phytoestrogens in different varieties of soyabean, in different batches of the same variety and after subsequent processing into the final food product can vary greatly as a result of many factors (see Section 3), however, in summary it is apparent that phytoestrogen concentrations tend to fall as the degree of processing increases. Thus, low concentrations generally occur in fermented products, for example Wang and Murphy (1994a) found only 143µg/g daidzein, 223µg/g genistein and 23µg/g glycitein in fermented soyabean curd, while Murphy (1982) detected no isoflavones in fermented soya sauce. Only low concentrations generally occur in the novel forms of soya, e.g. maximum concentration of any isoflavone in soya-containing cheese was 67µg/g of the 6"-*o*-malonyldaidzin form, while the maximum in tofu yoghurt was 80µg/g for the genistin glucoside form (Wang & Murphy, 1994a). The study of other (non-soya) foods has been less intense than for soya-based products, but it is apparent that many contain significantly lower concentrations of phytoestrogens than does soya.

2.4 LEVEL OF PHYTOESTROGENS IN HUMAN PLASMA

Relatively few data have been published on the concentrations of phytoestrogens and their metabolites in human blood, and many of the available studies relate to short-duration investigations involving dietary modification (generally supplementation).

Xu *et al.* (1994) measured isoflavone concentrations in plasma and urine in women following consumption of soya milk (containing 1.25±0.08mg total isoflavones/g) at three dosages (0.7, 1.3 or 2.0mg total isoflavones/kg bodyweight). Over three-day periods, each separated by two-week washout periods, 12 healthy, omnivorous, adult women (aged 19 to 41 years) received nutritionally complete liquid meals consisting of soya milk, chocolate-flavoured

instant breakfast and whole cow's milk for breakfast, and the latter two only for lunch and dinner.

Blood samples were collected prior to, and at 6.5 and 24 hours after each dose. Urine samples were collected the morning before dosing, over two consecutive 12-hour periods after dosing and at first urination of the second day after dosing. Samples of faeces were collected prior to dosing and up to approximately two days after each dosing period. Samples of plasma and urine were first treated with glucuronidase-sulphatase to generate the parent isoflavone. Samples were analysed by reverse-phase HPLC for total isoflavones, daidzein and genistein. Internal standards (daidzein, genistein and equol) were used to quantify the recoveries achieved. The intakes of isoflavones included in each 'dose' are presented in Table 2.1, while the resultant plasma concentrations are summarised in Table 2.2 (data for urine and faeces are presented in Section 2.5). Plasma isoflavone concentrations had increased by 6.5 hours after dosing but dropped significantly by the 24-hour point.

Table 2.1 Isoflavone content of a single dose of soya milk (mean ± SD)

Soya milk dose (mg/kg bodyweight)	**0.7**	**1.3**	**2.0**
Isoflavone	Amount of isoflavone (mg)		
Daidzein	24.73±3.66	45.93±6.80	70.67±10.45
Genistein	19.29±2.89	36.21±5.36	55.70±6.25
Total isoflavones	44.28±6.61	82.24±12.28	126.50±15.38

Analysis by reverse-phase HPLC
From Xu *et al.* (1994)

Table 2.2 Plasma concentrations of isoflavones (mean±SD; nmol/L) following single isoflavone doses of 0.7, 1.3 or 2.0mg/kg bodyweight

Dosage (mg/kg)	Isoflavone	Time after dosing		
		0	6.5 hours	24 hours
		Plasma isoflavones (nmol/L)		
0.7	Daidzein	ND	790±040	40±80
	Genistein	ND	740±440	70±100
	Total	ND	1530±470	110±170
1.3	Daidzein	ND	1220±670	80±80
	Genistein	ND	1070±630	190±370
	Total	ND	2290±1300	270±450
2.0	Daidzein	ND	2240±1180	120±80
	Genistein	ND	2150±1330	260±220
	Total	ND	4390±2500	380±300

ND None detectable at limit of detection of 2nm/20µL.
Analysis by reverse-phase HPLC
From Xu *et al.* (1994)

Plasma concentrations of phytoestrogens in 14 Japanese men (aged 54.9±12.2 years) consuming a Japanese diet, and 14 Finnish men (aged 54.9±11.5 years) consuming a Finnish diet [no specific details regarding diet were reported], were assessed by Adlercreutz *et al.* (1993a), using an isotope-dilution GC/MS method with internal standards to correct for recovery (Table 2.3). Although the percentage of free or sulphated (presumed biologically active) forms were similar between nationalities, varying between 3 and 54% (geometric mean 19.9%) in Japanese and 8 and 44% in the Finnish men (geometric mean 20.9%), total isoflavone concentrations were low in the Finns while concentrations greater than 100nmol/L were found in eight of the 14 Japanese men. Adlercreutz *et al.* (1993b) also determined concentrations of lignans and isoflavones in plasma from 27 pre- and postmenopausal omnivorous [meat and fish eaters] and vegetarian women [all but one were lactovegetarians who also ate fish] using a similar methodology. Results for this analysis were presented for older and younger women [presumably pre- and postmenopausal but age ranges not defined] (Tables 2.4 and 2.5). For lignans, plasma concentrations were lowest for matairesinol, intermediate for enterodiol and highest for enterolactone, and concentrations were higher in vegetarians than in omnivores. The small number of subjects studied precluded assessment of the significance of the higher concentrations of enterolactone found in older compared with younger women. For the isoflavones, concentrations of daidzein, *o*-desmethylangolensin (a metabolite) and genistein were higher in vegetarians than in omnivores, while concentrations of equol were similar between these groups. The total mean amounts of diphenols in the plasma of these omnivores and vegetarians were 59.5 and 374nmol/L.

Table 2.3 Geometric mean (and 95% confidence interval) plasma concentrations of isoflavones (nmol/L) in Japanese and Finnish men

Isoflavone	Japanese men	Finnish men
Daidzein		
Free + sulphates	12.8 (6.0–27.4)	0.6 (0.4–1.0)
Glucuronides	91.8 (40.4–211)	2.0 (1.1–3.7)
Total	107 (47.4–237)	6.2 (3.9–10.1)
Genistein		
Free + sulphates	7.8 (3.2–19.1)	0.5 (0.2–1.1)
Glucuronides	167 (72.2–388)	5.3 (3.2–8.9)
Total	276 (116–652)	6.3 (3.3–14.6)
***o*-Desmethylangolensin**		
Free + sulphates	1.8 (0.6–5.2)	<0.1*
Glucuronides	0.8 (0.1–5.0)	<0.1*
Total	3.3 (0.9–11.6)	<0.1
Equol		
Free + sulphates	0.6 (0.1–3.0)	0.1 (0–0.2)
Glucuronides	3.9 (0.8–18.2)	0.4 (0.1–1.7)
Total	5.5 (1.4–22.0)	0.8 (0.3–2.2)

Analysis by isotope-dilution GC/MS * most values below limit of detection
Detection level > 1.0 nmol/L (depending on compound)
From Adlercreutz *et al.* (1993a)

Morton *et al.* (1994) measured plasma concentrations of lignans and isoflavones by GC/MS in 29 postmenopausal Australian women in a dietary supplementation study. Following a two-week lead-in period, subjects were divided into six groups who consumed, on a two-week rotation, either clover (red clover sprouts, 45g/day), linseed (25g/day) or soya (full-fat steam-treated soya flour, 45g/day) as a supplement to their regular background diet. Each group received the supplements in a different sequence.

Table 2.4 Plasma concentrations (nmol/L) of lignans in omnivorous and vegetarian women

Lignan	Omnivores			Vegetarians		
	Young [n=10]	Old [n=4]	All	Young [n=10]	Old [n=4]	All
	Mean (range)	Range	Mean	Mean (range)	Range	Mean
Matairesinol						
Free + sulphate [%]	0.1 (0–0.9) [40.9]	All 0	0.1 [43.8]	0.1 (0–0.7) [22.2]	0–1.6	0.2 [48.6]
Glucuronide	0.1 (0–0.8)	All 0	0.1	0.3 (0–1.3)	0–2.2	0.4
Total	0.2 (0–1.7)	All 0	0.2	0.4 (0–1.9)	0–3.7	0.5
Enterodiol						
Free + sulphate [%]	0.2 (0–0.6) [9.2]	0.9–1.6	0.4 [17.3]	3.9 (0–32.1) [22.0]	0.7–17.2	4.3 [24.7]
Glucuronide	1.8 (0–5.6)	0.8–7.0	2.1	13.7 (1.0–108.2)	3.0–30.8	13.0
Total	1.9 (0–5.6)	1.4–8.6	2.5	17.6 (1.1–140.3)	3.8–48.0	17.3
Enterolactone						
Free + sulphate [%]	5.4 (1.4–12.5) [19.5]	2.1–32.3	7.0 [21.0]	29.5 (3.2–195.5) [32.9]	1.7–300.7	52.6 [20.8]
Glucuronide	22.4 (9.0–39.8)	16.5–69.4	26.3	60.2 (10.5–290.9)	29.5–850.2	200.0
Total	27.9 (10.4–48.6)	18.5–74.1	33.3	89.7 (17.9–486.4)	31.2–1078.2	252.6

Analysis by isotope-dilution GC/MS
Detection level > 1.0 nmol/L (depending on compound)
[ ] % of total in free or sulphate form
From Adlercreutz *et al.* (1993b)

Table 2.5 Plasma concentrations [nmol/L] of isoflavones in omnivorous and vegetarian women

Isoflavone	Omnivores			Vegetarians		
	Young [n=10]	Old [n=4]	All	Young [n=10]	Old [n=4]	All
	Mean (range)	Range	Mean	Mean (range)	Range	Mean
Daidzein						
Free + sulphate [%]	0.9 (0.1–2.1) [14.8]	0.8–1.3	1.0 [15.0]	5.7 (0.3–17.0) [9.6]	3.8–6.9	5.6 [11.2]
glucuronide	5.3 (0.3–18.9)	3.5–11.5	5.5	53.7 (0.5–169.3)	6.7–35.2	44.4
total	6.2 (0.6–19.6)	4.9–12.8	6.4	59.4 (0.7–183.7)	10.5–42.0	50.0
o-Desmethylangolensin						
Free + sulphate [%]	0.2 (0–1.2) [29.9]	0–0.4	0.2 [28.4]	3.0 (0–18.3) [28.7]	All 0	2.1 [27.5]
glucuronide	0.5 (0–3.9)	0–0.8	0.5	7.4 (0–46.6)	0.4–1.6	5.6
total	0.8 (0–5.1)	0–0.8	0.7	10.4 (0–64.9)	0.4–1.6	7.7
Equol						
Free + sulphate [%]	0.1 (0–0.6) [3.8]	0–0.9	0.1 [6.4]	0.3 (0–2.5) [20.6]	0–0.5	0.3 [18.1]
glucuronide	1.5 (0–4.2)	0.9–1.9	1.5	1.3 (0.1–3.3)	0–3.2	1.2
total	1.6 (0–4.2)	0.9–2.8	1.6	1.6 (0.1–3.5)	0–3.8	1.5
Genistein						
Free + sulphate [%]	0.7 (0.6–1.0) [7.7]	0.5–1.2	0.7 [9.3]	2.1 (0.7–5.9) [3.5]	0.4–1.3	1.7 [3.8]
glucuronide	8.7 (1.3–36.1)	1.6–4.7	7.0	57.0 (1.5–184.7)	2.8–15.3	43.1
total	9.4 (1.9–37.1)	2.2–5.4	7.7	59.1 (2.3–190.6)	3.4–16.6	44.8

Analysis by isotope-dilution GC/MS
Detection level > 0.2 nmol/L (depending on compound)
[ ] % of total in free or sulphate form
From Adlercreutz *et al.* (1993b)

The six week period of supplementation was followed by a two-week washout period. Blood samples were collected after seven and 14 days of each supplement period and six weeks after the end of the study. Following enzymatic hydrolysis with β-glucuronidase from *Helix pomatia* and ion-exchange chromatography, all samples were analysed by GC/MS using an internal standard to validate the assay. The overall ranges and means of plasma concentrations of daidzein, equol, enterodiol and enterolactone are summarised in Table 2.6.

Table 2.6 Plasma concentrations of isoflavones and lignans following dietary supplementation with linseed, soya and clover sprouts

Analyte	Mean (Range) ng/ml
Equol (soya)	31.1 (1.28–53.4)
Equol (clover)	34.2 (9.5–106.3)
Enterodiol (linseed)	106.3 (1.85–390)
Enterolactone (linseed)	117.5 (41.8–244)
Daidzein (soya)	68.3 (2.74– 138.4)
Daidzein (clover)	49.1 (3.51–153.1)

Analysis by GC/MS
From Morton *et al.* (1994)

Baseline concentrations of enterolactone (normally 0–10ng/ml) were higher than those of enterodiol (normally 0–5ng/ml) by a factor of 5–20, but considerable variation was apparent, probably reflecting variations in the nature of the women's regular diets [which were not recorded]. Following linseed supplementation plasma enterodiol and enterolactone were markedly increased for all subjects, with maximum plasma concentrations of total lignans reaching almost 500ng/ml. No significant increase in plasma lignans occurred following either soya or clover sprout supplementation although these two supplements did lead to marked increases in plasma daidzein and, less frequently, equol concentrations. Both soya and clover sprouts were effective precursors for the formation of daidzein but only four of the 12 subjects were able to metabolise daidzein to equol. Normal plasma concentrations of daidzein and equol were less variable than those for the lignans, possibly reflecting a more uniform intake of their precursors in the western diet. A preliminary semi-quantitative analysis for genistein indicated that soya and clover sprouts contained precursors of this isoflavone. The authors commented that a vegetarian diet appeared to be much more likely to influence concentrations of lignans than isoflavones.

2.5 LEVEL OF PHYTOESTROGENS IN HUMAN URINE AND FAECES

The identification of the lignans enterolactone and enterodiol in human urine was first reported by Setchell *et al.* (1980) and Axelson and Setchell (1980). In the latter paper, daily urine samples were collected from healthy volunteers (aged 6 to 62 years) and analysed for unconjugated (by GC) and conjugated (by GC/MS following enzymatic hydrolysis)

enterodiol and enterolactone. Identification of substances was by GC retention time, full mass spectrogram and partial mass spectra obtained from fragment ion chromatograms. [The dietary practices of these individuals were not reported]; results are summarised in Table 2.7. In a subsequent dietary modification study, Axelson *et al.* (1984) fed 40g of commercial textured soya as a meat substitute for a five-day period to a 34-year old man and a 25-year old woman [no details of normal diets were given] and collected 24-hour urine samples. Excretion of equol (measured by GC/MS) increased by 100- to 1000-fold to concentrations of 4–6mg/24-hour sample, and daidzein was identified as the major precursor of equol.

Table 2.7 Distribution (%) of conjugates of enterolactone and enterodiol and total excretion (µg/24 hours) in human urine

Sex (age)	Unconjugated		Glucuronide		Monosulphate		Disulphate		Total	
	Enl	End	Enl	End	Enl	End	Enl	End	Enl	End
M (6)	0.6	0.6	97.9	90.2	1.2	8.6	0.3	0.6	34.0	16.3
M (25)	0.5	0.9	96.3	93.0	2.8	5.6	0.4	0.5	94.3	81.6
F (12)[1]	0.2	0.9	98.5	94.6	1.2	4.2	0.2	0.3	66.0	33.3
F (33)[2]	0.3	<0.1	98.2	92.6	1.3	6.9	0.2	0.6	394.5	17.5
F (33)[3]	0.2	<0.1	98.4	90.9	1.1	7.8	0.2	1.3	531.1	15.4
F (62)[4]	0.1	<0.1	96.0	87.0	3.6	12.3	0.4	0.7	190.9	27.7
F (28)[5]	0.5	1.1	98.9	94.7	0.4	3.2	0.3	1.1	216.2	9.4

End - enterodiol Enl - enterolactone
[1] Pre-menarche [2] and [3] same woman ([2] Cycle day 4 and [3] cycle day 23)
[4] Postmenopausal [5] Seven weeks pregnant
From Axelson and Setchell (1980)

Adlercreutz *et al.* (1991) collected food intake data over a three-day period and a 48-hour urine sample from each of nine Japanese men (aged 50.4±18.0 years) and 10 Japanese women (aged 46.8±11.5 years) consuming traditional Japanese diets. Concentrations of urinary enterolactone, enterodiol, daidzein, equol and *o*-desmethylangolensin were analysed by GC/MS using internal reference standards (Table 2.8). Enterolactone excretion was relatively low while excretion of isoflavones was very high, although individual variation was large, especially for equol. Correlation of the urinary excretion rates with food components showed total lignans to be weakly correlated with green and yellow vegetables ($p<0.05$), pulses and beans ($p<0.05$), and more highly correlated with boiled soyabeans ($p<0.001$). Both total isoflavones and total diphenols were correlated with pulses and beans ($p<0.01$), algae ($0.05<p<0.01$), soya products except sauce ($p<0.01$) and boiled soyabeans ($p<0.001$).

The biological effects of soya on menstrual cycle was studied for six premenopausal women (aged 21–29 years) by Cassidy *et al.* (1994). Urine samples (24-hour) were collected every third day and analysed by GC/MS for daidzein, genistein and equol, following consumption of 60g/day soya protein (containing 45mg isoflavones) over a one-month study period. Total urinary excretion of isoflavones during a control (non-supplemented) period was low (range

5.6–67.3nmol/24 hours, i.e. 1,400–17 100ng/24 hours). Following dietary supplementation with soya over a complete menstrual cycle, total urinary isoflavone excretion increased by 1000-fold in all subjects, with values ranging from 1400–29 400nmol/24 hours (i.e. 350–7490ng/24 hours). Relative to the total estimated intake of isoflavones, excretion in urine accounted for 1.8 to 12.9% of the total intake. Concentrations of equol in the urine varied markedly from none detectable in one subject, trace in three subjects, to high concentrations in two others. It was noted that those subjects excreting low quantities of equol excreted the highest amounts of daidzein. Conversely, subjects excreting high amounts of equol showed lower urinary concentrations of its precursors daidzein and genistein.

Table 2.8 Urinary excretion of lignans and isoflavones (nmol/24 hours, geometric mean±standard deviation) in Japanese men and women consuming a traditional Japanese diet

Substance	Women (n=10)	Men (n=9)
Enterolactone	1400±1400 [890]	1100±700 [890]
Enterodiol	700±1300 [410]	400±300 [220]
Total lignans	2100±2600 [1380]	1500±900 [1130]
Daidzein	2600±4000 [2550]	2200±2000 [1145]
Equol	2600±4000 [560]	3000±4600 [540]
o-Desmethylangolensin	700±600 [510]	200±300 [110]
Total isoflavones	6900±6800 [4730]	3900±3300 [2570]
Total diphenols	9100±9300 [6700]	5400±4000 [4100]

Analysis by GC/MS
From Adlercreutz *et al.* (1991)

In the study by Xu *et al.* (1994), (see Section 2.4 for details) urine samples were collected from women prior to and for the first two 12-hour periods after diet supplementation with soya milk. A urine sample on the second day after dosing was also collected. Concentrations of daidzein and genistein were measured using a reverse-phase HPLC method (Table 2.9). At each dose, between 15 and 20% of the ingested total dose was excreted, with average urinary recovery of daidzein and genistein in the 24 hours after dosing of approximately 21% and 9%, respectively. No equol was found in any sample, although between 62 and 74% was recovered from spikes. At all doses, urinary excretion of isoflavones in the first 12 hours following dosing was significantly greater than in the second 12-hour period ($p<0.05$) while after 24 hours urinary excretion had almost returned to baseline values. Total urinary excretion of isoflavones increased significantly with dose ($p<0.05$), and at all doses employed the amount of daidzein excreted during the 24-hour period following dosing was significantly greater than that of genistein ($p<0.001$). In this study total faecal excretion was noted to account for only 1–2% of the total ingested dose.

Hutchins *et al.* (1995a) analysed concentrations of lignans and isoflavones in the urine of 17 healthy men (aged 20 to 40 years) following supplementation of the diet with a fermented

soya product (tempeh, 112g/feeding period) or unfermented soya (soyabean pieces, 125g/feeding period) using a randomised crossover designed. Baseline data were collected for five days, followed by two 9-day feeding periods separated by a 12-day washout. Urine samples were collected for 24-hour periods during the last three days of each feeding period and were analysed by isotope dilution GC/MS. Duplicate quality-control urine samples were included in each series of assays to monitor intra-assay variability. The isoflavone content of the food supplements is summarised in Table 2.10, while urinary concentrations of isoflavones and lignans are presented in Table 2.11. Urinary isoflavone concentrations increased on the soya-supplemented diets compared with the self-selected diets ($p<0.05$). Although not statistically significant, urinary recovery of isoflavones was higher when tempeh was used rather than soyabeans, implying that the isoflavones in tempeh may be more readily available than those in soyabean pieces. Again, the five subjects who excreted high amounts of equol tended to excrete less daidzein and *o*-desmethylangolensin than the 12 who excreted low amounts of equol. Urinary excretion of enterolactone and enterodiol was lower when the subjects consumed the soya-supplemented diets than the self-selected diets; concentrations excreted were similar for the two forms of soya supplementation.

In a further study, Hutchins *et al.* (1995b) investigated the influence of vegetables, fruits and legumes on urinary excretion of isoflavones and lignans in seven men and three women, aged 20 to 35 years. Following a four-day period when subjects consumed a basal diet, individuals received the basal diet supplemented with legumes and allium (canned garbanzo beans, onion, garlic and hummus), low vegetable/fruit (four servings including apples, peeled pears, unpeeled potatoes and carrots) or high vegetable/fruit (eight servings of the same examples) in a randomised order. Each diet was consumed for a nine-day period, with washout periods of at least 10 days between each. Urine samples collected over 24-hour periods during the final three days on each diet were analysed for lignans and isoflavones by isotope-dilution GC/MS (Table 2.12).

Urinary excretion of enterodiol was greatest on the high vegetable/fruit diet ($p=0.03$); concentrations on the others were generally similar to each other. Excretion of enterolactone, matairesinol and total lignans did not differ significantly between any of the diets. When subjects consumed the legume/allium diet, significantly greater urinary excretion of genistein ($p=0.0001$), *o*-desmethylangolensin ($p=0.04$) and total isoflavones ($p=0.02$) was noted compared with the other diets. Urinary excretion of equol was greater on the basal and legume/allium diets than on the high vegetable/fruit diet ($p=0.009$) but amounts excreted on the low vegetable/ fruit diet were not significantly different from any other diet.

Table 2.9 Urinary excretion of isoflavones (nmol, mean±SD) in 12 women at 12-hour time intervals after a single dose of soya milk at 0.7, 1.3 or 2.0mg/kg bodyweight

Dose (mg/kg)	Isoflavone	Time after dosing			
		Predose	0–12 hours	12–24 hours	>24 hours
0.7	Daidzein	40±80	14480±7790	4520±3110	310±280
	Genistein	0±40	2700±2960	930±1550	150±150
	Total	40±120	17200±10740	5450±4650	460±420
1.3	Daidzein	80±170	30440±10420	11450±9990	830±1060
	Genistein	ND	10440±6700	3890±6070	440±930
	Total	80±170	40880±17350	15340±17000	1270±1980
2.0	Daidzein	310±670	42210±17350	14200±12000	940±1020
	Genistein	110±190	13770±9880	6180±9770	670±1150
	Total	420±850	55980±27150	20380±21760	1610±2150

ND None detectable at limit of detection of 2nm/20µL.
Analysis by reverse-phase HPLC
From Xu *et al.* (1994)

Table 2.10 Isoflavone content of soya products (µg/daily serving)

Substance	Soyabean pieces	Tempeh
Daidzin	18588	4536
Genistin	28381	16462
6"-*o*-Malonyldaidzin	7395	2859
6"-*o*-Malonylgenistin	23307	12253
6"-*o*-Acetyldaidzin	630	307
6"-*o*-Acetylgenistin	2495	2269
Daidzein	2984	6372
Genistein	4438	9080
Total daidzein	18425	10758
Total genistein	35747	27047

From Hutchins *et al.* (1995a)

Adlercreutz *et al.* (1995) determined the pattern of conjugation of lignans and isoflavones in four 24-hour urine samples from three vegetarian or semi-vegetarian women and in two samples from men [ages not reported]. Samples were analysed using isotope-dilution GC/MS with internal standards (Table 2.13). Two samples were obtained from a woman who had consumed soya products at the time of the first sample, and the amounts of total diphenols were found to be very different for these two samples (1300–5,800nmol/24 hours). The principal conjugate forms were the monoglucuronides which accounted for 85–90% of the total diphenols. For the three lignans a similar pattern was found, with monoglucuronides

accounting for 73–94% and monosulphates accounting for 2–10%, while only very low quantities of free lignans were apparent (0.3–1%). For the isoflavones, 97% of the *o*-desmethylangolensin was in a monoglucuronide form with the remainder divided over the other fractions; equol was found as the monoglucuronide (32–93%), the sulphoglucuronide (0–43%), the monosulphate (0–15%) and the disulphate (0–10%), while daidzein occurred as a monoglucuronide (79–82%) and sulphoglucuronide (6–17%), with the remainder divided between the other fractions. Genistein occurred as monoglucuronide (53–76%), with 12–26% as diglucuronide, 2–15% as sulphoglucuronide and 1–4% as disulphate. The general pattern of conjugation of lignans and isoflavones in urine was considered to be similar to that of endogenous oestrogens.

The effects of dietary supplementation with soya milk were studied by Lu *et al.* (1995) for six healthy non-vegetarian males (aged 21–35 years). During a series of pharmacokinetic study periods (study days 1–4, 16–18 and 30–32), subjects received a basal diet free of identifiable soya products. On days 3, 16, 17, 30 and 31 isoflavone excretion kinetics were studied following ingestion of 36oz of soya milk after overnight fasting. On days 2–4, 16–18 and 30–32 urine was collected for 24 hours before the first soya milk ingestion and for 24 or 48 hours after each dose. Analysis of the soya milk was by GC using flame ionization detection with quantification by comparison against standard curves using authentic standards. Analysis demonstrated that the daily intake of isoflavones when soya milk was consumed was approximately 100mg of daidzin/daidzein and 100mg of genistin/genistein. Urinary recovery of ingested daidzin/daidzein (46.9±15.2%, mean±SD) or genistin/genistein (14.6±9.2%) did not change following prolonged soya ingestion, and peak excretion was apparent at 7.1±1.9 hours for daidzein (n=6), 6.7±2.1 hours for genistein (n=6) and 13.5 hours for equol (n=1). The majority of daidzein and genistein excretion occurred within the first 24 hours (95.73±2.42% and 83.93±4.73%, respectively), although values remained above baseline for up to 48 hours for daidzein and, possibly, genistein.

In an identical study on six healthy non-vegetarian women (Lu *et al.,* 1996), urinary isoflavone excretion was detected 1–2 hours after soya milk ingestion, with peak concentrations occurring after 5–10 hours, although considerable interindividual variation was noted. Urinary recovery of genistein (initially 23.9±17.3% of ingested genistin/genistein), daidzein (initially 66.2±23.5% of ingested daidzin/daidzein) and equol (initially 28% of the ingested precursors daidzin/daidzein in one subject and <1% in 5 subjects) decreased over the four-week period of supplementation with soya milk by 42% for genistein ($p<0.05$) and 31% for daidzein ($p<0.01$), but increased by 3- to 100-fold for equol (four subjects, $p<0.05$). Both total amount and peak concentration were similarly affected.

Table 2.11 Urinary excretion of lignans and isoflavones (nmol/day) following soya supplementation of diet*

Substance	Self-selected diet	Soyabean pieces diet	Tempeh diet
Enterodiol	147±10 (37–850)	114±10 (89–1128)	96±10 (53–748)
Enterolactone	552±45 (201–4022))	328±45 (224–3256))	317±45 (193–2957)
Matairesinol	30±6 (15–456)	16±6 (25–109)	20±6 (20–335)
Equol	175±169 (32–8035)	718±169 (44–14249)	441±169 (59–8657)
o -Desmethyl-angolensin	84±341 (0–906)	2160±337 (135–29121)	1635±323 (0–23543)
Daidzein	315±338 (137–8905)	3875±338 (6760–17847)	3630±338 (301–28721)
Genistein	154±196 (15–4114)	1658±196 (1636–18931)	1719±196 (130–22602)

* Mean ± SD; range in parentheses
From Hutchins *et al.* (1995a)

Table 2.12 Urinary excretion of isoflavones and lignans (nmol/day) following diets supplemented with vegetables, fruit and legumes*

Substance	Basal	Legume/allium	Low veg/fruit	High veg/fruit
Enterodiol	107±40 (43–589)	98±40 (41–327)	154±40 (52–586)	258±40 (65–1155)
Enterolactone	778±141 (356–1867)	713±141 (274–1878)	859±141 (259–1634)	1,092±141 (345–3444)
Matairesinol	33±2 (21–53)	26±2 (16–62)	32±2 (19–63)	30±2 (16–70)
Total lignans	886±177 (-)	811±177 (-)	1013±177 (-)	1350±177 (-)
Equol	101±7 (34–146)	94±7 (37–146)	84±7 (30–434)	66±7 (46–100)
o-Desmethyl-angolensin	63±11 (35–3768)	99±11 (35–254)	63±11 (28–767)	57±11 (35–119)
Daidzein	326±62 (51–3978)	413±62 (97–1038)	252±62 (24–3854)	323±62 (83–1105)
Genistein	145±32 (40–556)	372±32 (175–997)	110±32 (34–669)	139±32 (61–358)
Total isoflavones	635±94 (-)	993±94 (-)	509±94 (-)	584±94 (-)
Total lignans and isoflavones	1521±210 (-)	1744±210 (-)	1521±210 (-)	1935±210 (-)

* Least squares mean±least squares-standard error of the mean, range in parentheses
From Hutchins *et al.* (1995b)

Table 2.13 Lignan and isoflavone conjugates (nmol/24 hours) in urine samples (ranges quoted for females, individual values for males)

	Matairesinol	Enterodiol	Enterolactone	Daidzein	Genistein	*o*-DMA	Equol
Female							
Free	0–0.4	0–115	0–152	168–812	36.2–239	7.7–26.6	0–1.4
Monoglucuronides	11.4–51.8	187–23550	4310–7530	3990–18290	1154–10210	646–2360	58.6–125
Diglucuronides	0	0–52	0–79.8	63.4–305	222–2190	0.3–11.1	0
Sulphoglucuronides	0–1.0	32.9–621	5.2–142	480–2840	116–629	4.2–21.4	0–93.3
Monosulphates	0–8.1	14.6–1250	56.5–279	96.6–1190	45.4–184	2.4–19.8	0–32.4
Disulphates	0	0–38.5	2.8–120	80.5–615	86–396	0.9–5.7	0–21.1
Total	11.4–52.7	249–25627	4375-8342	4879–23070	1786–13519	662–2428	69.7–251
Male							
Free	0, 0	4.0, 0	13.9, 32.2	55.1, 70.4	22.4, 12.6	0, 10.9	6.1, 0
Monoglucuronides	228, 256	328, 627	4771, 1168	3614, 3295	1519, 873	86.4, 1261	402, 65.5
Diglucuronides	0, 0	0, -	0, 34.8	24.5, 24.9	277, 187	0.3, 3.7	5.8, 5.2
Sulphoglucuronides	0, 0	30.7, 243	124, 59.9	490, 712	203, 203	0.6, 12.5	52.2, 21.4
Monosulphates	13.2, 9.6	49.5, 68.3	284, 58.0	182, 96.4	80.9, 30.9	0.6, 10.0	18.6, 12.9
Disulphates	0, 0	4.8, 1.3	146, 78.1	14.8, 25.8	70.9, 57.6	0.2, 0	0, 4.8
Total	269, 238	417, 940	5339, 1431	4380, 4225	2173, 1364	88.1, 1298	485, 110

From Adlercreutz *et al.* (1995)

o-DMA , *o*-Desmethylangolensin

Table 2.14 Excretion of lignans and isoflavones (nmol/day; arithmetic mean* ±SD) in the faeces of women consuming a diet supplemented with linseed

Substance	Basal diet	Supplemented with linseed
Enterodiol	80±80 (60)	2560±3100 (980)
Enterolactone	640±480 (450)	10300±7580 (6820)
Matairesinol	7.33±10.00 (2.01)	11.90±8.06 (9.71)
Total lignans	727±510 (550)	12871±8430 (10337)
Daidzein	120±260 (40)	80±110 (40)
Equol	6066±5.43 (4.63)	14.6±16.7 (7.23)
Genistein	58.5±147 (8.13)	34.4±37.8 (15.3)
o-Desmethylangolensin	55.4±86.6 (12.3)	37.2±57.3 (8.4)
Total isoflavones	241±483 (94.6)	166±188 (98.8)

* Arithmetic mean±SD; geometric mean in parentheses
Analysis by isotope dilution GC/MS
From Kurzer *et al.* (1995)

Kurzer *et al.* (1995) measured faecal concentrations of lignans and isoflavones for 13 premenopausal women (aged 27.8±4.3 years) following dietary supplementation with 10g/day of ground linseed over three menstrual cycles. On days 7–11 of the last menstrual cycle faeces samples were collected into a plastic bag, ascorbic acid immediately added and, after forcing of air from the bag, deep-frozen pending analysis. Analysis was by isotope dilution GC/MS in selective ino-monitoring mode. Samples were analysed in triplicate and quality-control samples were also included to assay recovery. Results are summarised in Table 2.14. Linseed consumption significantly increased the daily faecal concentration of enterolactone (16-fold), enterodiol (32-fold) and matairesinol (1.6-fold), but had no significant effect on faecal isoflavone concentrations. Urinary excretion in the same subjects showed a seven-fold increase in enterolactone and a 26-fold increase in enterodiol with no increase in urinary matairesinol excretion.

3 The influence of agricultural and food processing practices on phytoestrogen and mycoestrogen content

3.1 SUMMARY

Plants containing oestrogenic chemicals form some of the major food sources for humans and animals. Total oestrogenic activity (as assessed by bioassays) and the total and individual concentrations of phytoestrogens (by chemical analysis) often show considerable variability between plant species or varieties. Even for a given variety considerable variability is seen under different growing conditions. Concentrations of oestrogenic substances vary between the tissues of a plant and also change during the life cycle or, in the case of perennial plants, with season.

Although the principal factors influencing the phytoestrogen content of a plant are genetic, content can be modified by external factors (e.g. fertiliser level, temperature, disease status and the frequency/timing of harvest). Post-harvest storage conditions and food processing techniques can also affect the total and individual concentrations of phytoestrogens, and can also affect the mycoestrogen content by influencing the degree of fungal contamination.

In the case of soya products, most traditional forms that do not involve a fermentation stage retain relatively high concentrations of phytoestrogens, but aqueous alcohol treatment (as used to produce some protein concentrates) and fermentation results in a significant loss of isoflavones. The non-traditional forms of soya (e.g. as meat or dairy substitutes) generally contain much lower phytoestrogen concentrations. Indeed, soya oil contains no phytoestrogens, so foodstuffs incorporating only this product from soya will contain no phytoestrogens of soya origin.

3.2 INHERENT VARIABILITY IN OESTROGENIC ACTIVITY OF PLANTS

Examples of reproductive impairment in herbivores following consumption of certain crops (e.g. clover disease in sheep) have historically focused research effort into understanding the variability of oestrogenic activity both between and within species of common fodder crops. In addition, a number of plant species of human relevance have been studied.

Much early work employed bioassays to assess oestrogenic activity. These included rodent and sheep uterotrophic assays. In rodent assays, female rats or mice (either immature or ovariectomised) are either dosed with plant extracts or their diet is supplemented with plant material, after which uterine weight or some other morphological or biochemical marker of uterine function is measured as a marker of oestrogenic stimulation. Similar uterine assays

have been conducted in ewes, while in rodents vaginotrophic assays have also been used, in which the degree of vaginal cornification (assessed microscopically) is assessed as a measure of oestrogenicity. Ewe teat-length assays (increase in teat length indicates increased oestrogenic stimulation and *vice versa*) have also been employed. More recent work has, however, tended to employ a range of analytical techniques to establish the chemical composition of plant material (see Section 2) rather than rely on such bioassays.

Fodder crops

Mouse uterotrophic assays have demonstrated wide variations in oestrogenic activity in various species and varieties of fodder crop, including subterranean clover (*Trifolium subterraneum* L.), red clover (*T. pratense*) (Bickoff *et al.*, 1961), clover hay (*T. alexandrium* variety Fahli) and oat hay (*Avena sativa*) (Adler, 1965). Flux *et al.* (1963) also noted the considerable variability in oestrogenic activity of different plant parts, for example red clover had the highest activity in its leaves. The variation in activity of subterranean clovers has also been demonstrated using ewe uterine assays (Davies & Bennett, 1962; Lloyd Davies & Dudzinski, 1965) while Millington *et al.* (1964a) used an ewe teat-length assay to study subterranean clover and Cyprus medic. Overall, Dwalganup and Mt. Barker strains of subterranean clover have been shown to be particularly oestrogenically active and there was a suggestion that wilting and drying may increase such activity. Little (1976), however, found little oestrogenic activity in a range of tropical legumes (*Desmodium intortum; Lotononis bainsii; Macroptilium atropurpureum; Lablab atropurpureus; T. semipilosum)* with only *Medicago sativa* showing a weak response.

A number of studies have included chemical analysis of fodder crops. Millington *et al.* (1964a) used chromatographic analysis to show that Cyprus medic contained 55ppm coumestrol, a trace of formononetin (approximately 15ppm) but no genistein or biochanin-A. Coumestrol concentrations were 15–20ppm in Geraldton clover, 10ppm in Dwalganup and less than 5ppm in Tarloop or Mt. Barker subterranean clover strains. Millington *et al.* (1964b) also found that formononetin accounted for over 60% of the between-strain variance in total phytoestrogen content, and showed the onset of senescence to associate with falling total isoflavone but increasing coumestrol concentrations. Using a thin layer chromatographic assay, Morley and Francis (1968) found considerable variation in isoflavone concentration between 151 varieties of subterranean clover (*Trifolium subterraneum)* and eight varieties of *T. israeliticum:* The principal factor influencing isoflavone composition was identified as strain, not location. Rossiter and Beck (1966a) found, using thin-layer chromatographic analysis for subterranean clover, that phytoestrogen concentration differed between leaf type and growth stage. Following flower emergence, total isoflavone concentrations increased in leaves of Dwalganup clover (attributed to formononetin, genistein and biochanin-A) then declined because of falling formononetin content. Daidzein reached detectable concentrations only as leaves approached senescence. Kallela *et al.* (1987), using high-performance liquid chromatography (HPLC) noted formononetin and biochanin-A to be the main oestrogens in

red clover and timothy, with only low quantities of genistein and daidzein. Concentrations were highest at spring harvest, declined at mid-summer but then rose.

Human food crops

Bickoff *et al.* (1960a), using a mouse uterine bioassay, detected oestrogenic activity in alfalfa, *Medicago sativa*, a crop used as an animal and human food. Activity varied during the life cycle. Factors affecting the coumestrol content of alfalfa were also investigated using a chromatographic and photofluorometry assay (Hanson *et al.*, 1965); small but significant differences were identified (Table 3.1), but a consistent pattern was not found. Thompson *et al.* (1997) assessed the production of lignan metabolites following incubation of 10 varieties of flaxseed with human faecal innoculum under anaerobic conditions. Concentrations of the metabolites enterolactone, enterodiol and secoisolariciresinol were determined by a GC/MS assay incorporating internal standards. Significant differences attributable to strain, location and, for one strain, year of harvest, were noted.

Table 3.1 Average coumestrol content of alfalfa at 1/10-bloom stage by variety

Variety	Coumestrol concentration (ppm) by year		
	1960	1961	Average
Buffalo	57.7	60.8	59.2
Du Puits	52.9	48.4	50.7
Lahontan	71.9	65.4	68.7
Ranger	62.9	58.0	60.4
Vernal	53.7	52.4	53.0

Analysis by chromatography and photofluorometry
From Hanson *et al*. (1965)

Oestrogenic chemicals were also noted in growing bean (*Phaseolus vulgaris)* plants; maximal concentrations occurred at flower bud development and pod formation. Thin layer chromatographic analysis of different parts of flowering-stage plants showed the highest concentrations (not specifically identified) in the leaves; lower concentrations occurred in stems and roots. Young pods with unripened seeds also had high contents (Kopcewicz, 1971).

Differences in phytoestrogen content have also been noted between varieties of soyabean. Eldridge and Kwolek (1983) attempted to elucidate the influence of location and crop year on isoflavone content, but the picture was complicated by inter-variety differences even if grown at the same location in one season, or for a single variety when grown at different locations. Total and individual glucoside and aglucone concentrations were determined, relative to internal standards, for different varieties of soyabean (including Hardin Corsoy-79, Amcor, Sprite, Century, Amsoy and Tiger) by HPLC. When a number of varieties were grown in a single season at one location, total isoflavone concentrations varied between 116 and 309mg/g

for Hardin and Sprite, respectively, while during a single growing season concentrations for the Hardin strain were shown to vary between 47 and 191mg/g depending upon the location. Wang and Murphy (1994b) analysed, in triplicate, seven varieties of American and three varieties of Japanese soyabean using C_{18} reverse-phase HPLC, including comparison to authentic standards. This showed the Japanese varieties had consistently lower total, conjugated and unconjugated isoflavone concentrations and higher conjugated to unconjugated ratios (Table 3. 2). The Japanese varieties had higher concentrations of 6"-*o*-malonylglycitin and different 6"-*o*-malonyldaidzin:daidzin and 6"-*o*-malonylgenistin:genistin ratios, when compared with American varieties.

In addition to the work described above, Eldridge and Kwolek (1983) investigated the distribution of isoflavones in different tissues of the soyabean. The highest concentrations were located in the hypocotyl (total isoflavone concentration: 1400–1750mg/100g), with lower concentrations occurring in cotyledons (160–320mg/100g) and hulls (10–20mg/100g). The distribution of individual isoflavones varied between plant tissues. In the hypocotyl daidzin and glycitin glycosides predominated, while in the cotyledon there was approximately 20-times as much genistein as in the hypocotyl. Coumestrol occurred mainly in the hull or seed coat. Tsukamoto *et al.* (1995) used an HPLC assay (including the use of purified soyabean isoflavones as standards) to confirm that isoflavone concentrations in the hypocotyl were high (approximately 80–90% of total). In a review of the biological implications of the substances shown to be present in soyabeans, Liener (1994) noted that the phytoestrogen content of soyabean varies with developmental stage, for example, the low coumestrol concentration of mature soyabeans increases 70 to 150 times at germination.

Table 3.2 Isoflavone content of US and Japanese varieties of soyabean

Isoflavone	Isoflavone content (μg/g)						
	US strains (Year: 1989)						
	Pioneer 9111	Pioneer 9202	Prize	HP 204	LS 301	XL 72	Strayer 2233
Total	4216	3806	3886	2053	3551	2201	2344
Conjugated	2554	2460	2141	1426	2438	1397	1384
Un-conjugated	1662	1346	1745	627	1113	804	960
Conjugated: Un-conjugated (C:U) ratio	1.537	1.828	1.227	2.274	2.190	1.738	1.442

	Japanese strains					
	Keburi		Kuro diazu		Raiden	
Year	1991	1992	1991	1992	1991	1992
Total	2343	1411	2041	1261	2305	1417
Conjugated	1974	1098	1773	1035	1824	1115
Un-conjugated	369	313	268	226	481	302
C:U ratio	5.350	3.51	6.616	4.580	3.792	3.692

Values are mean of triplicate assays using high performance liquid chromatography
After Wang and Murphy (1994b)

3.3 AGRICULTURAL PRACTICE AND ENVIRONMENTAL INFLUENCE

Although genetic differences between species or varieties is the principal influence on the oestrogenic activity of a plant, its general environment and the agricultural practices applied can also exert significant influence.

Effect of location and crop year

Different total isoflavone concentrations were found in an American soyabean strain, Vinton 81, grown at different locations within the same year (Eldridge & Kwolek, 1983). The effect of location was, however, less than that of crop year, and the ratio of individual isoflavones remained relatively constant. Differences were also noted year to year in three Japanese varieties (Wang & Murphy, 1994b) although a cause was not established (Table 3.3).

Table 3.3 Total isoflavone concentrations in different crop years for different soyabean strains

Strain	Total isoflavone content (μg/g) for year				
	1989	**1990**	**1991A**	**1991B**	**1991C**
Vinton 81 (American strain)	3309	2776	1176	1563	1749
	1991	**1992**			
Japanese strains: Keburi	2343	1411			
Kuro diazu	2041	1261			
Raiden	2305	1417			

Analyses by HPLC
From Eldridge & Kwolek (1983); Wang & Murphy (1994b)

Tsukamoto *et al.* (1995) analysed (using HPLC) low-isoflavone (Higomusume, Kairyoshirome, Shirosaya 1 and Koganedaizu) and ordinary varieties (Suzuytaka, Fukuyutaka and Lee) of soyabean sown at two sites in Japan (latitudes 33^{o} north and 36^{o} north). Generally, daidzin, genistin, malonyldaidzin and malonylgenistin were detected; aglycone and acetyl forms were present in only trace quantities. At the southern site, for almost all varieties the individual and total isoflavone contents of seeds sown in April/May were markedly lower than for those sown later. Such differences were not, however, seen at the northern site. The aetiology of this difference was not established.

Effect of infestation on phytoestrogen content

The relationship between coumestrol content and degree of defoliation in alfalfa was studied by Hanson *et al.* (1965) using several plant pathogens and pests. Analyses were conducted in duplicate or triplicate, using paper chromatographic and photofluorometric detection

techniques with a coumestrol standard for reference. In general, coumestrol concentrations increased in response to infestation (see Table 3.4). Application of fungicides to control fungal infestation reduced coumestrol concentrations (e.g. values averaged 72.9ppm for unsprayed, infected plants and 28.6ppm for sprayed crops).

Effect of fertiliser application

When Dwalganup subterranean clover was grown using a basal fertiliser mix supplemented with superphosphate at application rates of up to 100g/m^2, the plants' formononetin concentration was 3.5% in the phosphate deficient group but only 1.4% at the highest application rate (Rossiter & Beck, 1966b, using thin-layer chromatography). Genistein concentration also rose from 2.2 to 2.7% but biochanin-A was relatively unchanged (approximately 0.97%). Expressing data as absolute amount per leaf showed isoflavone concentrations only rose to the 25g/m^2 application rate then remained constant. In contrast, in Mt. Barker strain clover, leaf size and absolute genistein and biochanin-A amounts did not vary with superphosphate application but formononetin did. When Mt. Barker strain was grown using increasing amounts of mono-calcium phosphate, biochanin-A and genistein concentrations increased (on a per leaf basis). Formononetin concentrations varied from 40μg/leaf in phosphate-deficient plants to approximately 2μg/leaf at an application rate of 48g/m^3. In addition, using HPLC and fluorometric detectors and internal standards, Kallela *et al.* (1987) showed that nitrogen application reduced the concentrations of phytoestrogens (formononetin, genistein, biochanin-A and daidzein) in red clover. Lloyd-Davies and Dudzinski (1965), however, found cobalt supplementation to have no significant effect on oestrogenic activity (as assessed by ewe uterotrophic assay) in Dwalganup clover.

Table 3.4 Effect of infestation of alfalfa with various plant pests or pathogens

Pest/pathogen	Mean coumestrol content (ppm)	
	Infested	Uninfested
Phoma herbarum var *medicaginis*	219.1	0
Pseudopeziza medicaginis	59.5	0
Leptosphaerulina briosiana	0	0
Stemphylium botryosium	30.0	0
Yellow mosaic virus	32.7(leaf)	0
Yellow mosaic virus	18.8(stem)	0
Pea aphid	125.8	78.8
Spotted alfalfa aphid	201.0	53.8

Analysis by paper chromatography and photofluorometric detection
From Hanson *et al*. (1965)

Effect of temperature

The temperature at which plants grow can affect their phytoestrogen content. For example, the isoflavone content of Yarloop and Mt. Baker strains of subterranean clover seedlings varied depending upon the temperature regimen they grew under (Rossiter & Beck, 1966c). Except for formononetin in Mt. Barker clover, isoflavone concentrations were highest in plants grown at low light/dark temperature ranges (i.e. concentrations increased for groups as light/dark temperatures were reduced from 36/31^{o}C to 15/10^{o}C), but fell at an even lower temperature regimen (9/4^{o}C). Formononetin concentrations in the Mt. Barker strain were generally very low, rising only slightly at high temperatures. Tsukamoto *et al.* (1995) showed a high temperature range (38/28^{o}C) associated with significantly lower isoflavone content in two low-isoflavone varieties and one ordinary variety of soyabean seeds.

3.4 HARVESTING AND FOOD STORAGE

The timing and sequence of harvesting can influence the oestrogenic activity and content of phytoestrogens, particularly in the case of fodder crops subject to multiple harvest.

Using HPLC, Kallela *et al.* (1987) demonstrated that the timing of mowing of pure or mixed swards of red clover influenced phytoestrogen concentrations with, for the same age of plant, concentrations being higher at the earlier mowings. The timing of cutting of alfalfa (*Medicago sativa* variety Rhizoma), but not Ladino clover (*Trifolium repens*), also influenced subsequent oestrogenicity (Kitts *et al.*, 1959). Initial cutting of the crop late in the growth season depressed oestrogenicity when compared with plants left uncut or cut initially early in the growth season.

Once harvested, the oestrogenic activity of crops can be further affected by the conditions used for initial preservation and subsequent storage. For example, oven drying slightly reduced the oestrogenic activity of red clover but increased that of subterranean clover when assessed by a mouse uterotrophic assay (Bickoff *et al.,* 1961). Flux *et al.* (1963) also assessed the oestrogenic activity of red clover after storage for various periods under different conditions (freezing, freeze-drying, preservation with alcohol or acetone, storage in wet or desiccated forms) using a mouse vaginotrophic assay. Activity in freeze-dried, frozen clover was generally stable for 115 days. Acetone-treated and desiccated material lost approximately 50% of potency compared with other storage methods, while the highest activity was preserved by alcohol treatment followed by desiccation.

Bickoff *et al.* (1960b) using a mouse uterotrophic assay, showed drying of alfalfa (*Medicago sativa* var. Chilean) for between 45 minutes and two hours at 70^{o}C increased the loss of oestrogenic activity, but extended drying for up to 24 hours did not result in a significant fall below the amount found after one hour. The coumestrol content of alfalfa forage infested by *Pseudopezia medicaginis* and *Cercospora zebrina* was reduced by storage under aerobic

conditions at high temperature (85°F) for up to 14 days, however, the major factor influencing concentration was degree of infestation of the fresh crop (Hanson *et al.,* 1965).

In addition to changes in the amounts of endogenous oestrogenic chemicals in crops during initial harvesting or storage, fungal contamination may lead to the accumulation of mycoestrogens (or other mycotoxins with the potential to effect animal or human health). Hacking *et al*. (1976) noted that infertility in cattle, reduction in pig litter size and adverse effects in poultry had been attributed to the mycoestrogen zearalenone in stored feed. These authors examined barley grain, after incubation, for fungal contamination. *Fusarium culmorum, F. poae* and *F. graminearum* were identified and cultured on rice for five days followed by analysis for zearalenone content. *F. culmorum* was the most abundant contaminant, with over 50% of the isolates obtained in one crop year producing zearalenone. The authors noted that conventional storage conditions were intended to reduce water content to 14% to prevent fungal growth, but that at 18% water, fungal growth could occur even at 7°C, while mycotoxins could be formed at temperatures only a little above 0°C if fungal infestation was already established. Luo *et al.* (1990) analysed wheat and corn from regions of China which had markedly different human oesophageal cancer incidences, for naturally occurring trichothecenes and zearalenone. Detection of tricothecenes was by gas chromatography and ^{63}Ni electron capture detection method, with external standards of the trichothecenes deoxynivalenol, 15-acetyldeoxynivalenol and nivalenol. The identities of tricothecenes were also confirmed by mass spectrometry. Zearalenone concentrations were determined by HPLC and spectrofluorometry, using an external standard of the mycoestrogen. Linxian County, the part of China showing the highest risk of this cancer, and Shangqui County, a low risk area (approximately 8.4-fold lower incidence) were studied. Marked differences in the occurrence of contamination with mycotoxins (as percentage of contaminated samples) were noted (Table 3.5). Particularly for corn, absolute amounts of the mycotoxins were greatest in samples from Linxian county, which reflected the regional differences noted for cancer incidence.

The effect of water content (expressed as water activity, a_w) and temperature on corn quality during storage was investigated by Montani *et al.* (1988) using samples inoculated with *F. graminearum.* Water activity was controlled by drying samples and then adjusting the water content by adding different amounts of sterile water according to data from water sorption isotherms. Water activity was measured using an electronic hygrometer calibrated against saturated salt solutions. After one week's equilibration at 5°C, samples were inoculated with fungal spores. Zearalenone content was assessed by comparison against a standard solution, using thin layer chromatography. Zearalenone was found to accumulate at a water activity of 0.97 when incubated at either 20 or 15°C; amounts were higher at the high temperature. Maximal levels were reached after six weeks at 20°C or after eight weeks at 15°C; levels subsequently fell. The influence of water activity at constant temperature was complex; toxin accumulation was highest at high water activities for short incubation times, but lowest under these conditions over prolonged storage period. Adjusting temperature and water activity together had a more marked effect than changing these factors independently.

Table 3.5 Mycotoxin concentrations in corn and wheat samples from different counties in China

Crop/ County	% positive samples for TRIC / ZEA	Mean (range) of mycotoxin in positive samples (ng/g)			
		DON	15-ADON	NIV	ZEA
Corn					
Linxian	96.3 / 59.3	574 (17-3505)	274 (44-752)	11 (4-53)	44 (14-169)
Shangqui	40.0 / 5.0	99 (11-612)	104	ND	39
Wheat					
Linxian	46.7 / 40.0	59 (9-309)	ND	ND	Tr
Shangqui	46.7 / 40.0	18 (7-36)	ND	15 (13-21)	Tr

TRIC Tricothecence DON Deoxynivalenol 15-ADON 15-Acetyldeoxynivalenol
NIV Nivalenol ZEA Zearalenone ND None detected Tr <10ng/g.
Analysis by GC/electron capture detector & MS or HPLC/spectrofluorometery, as appropriate.
From Luo *et al.* (1990)

3.5 FOOD PROCESSING EFFECTS

In western societies there is a growing tendency for crops to undergo significant processing before presentation to humans for consumption. The procedures employed are many and varied and may profoundly alter the phytoestrogen profile of the final food product from that of the original plant material. For example, Knuckles *et al.* (1976) used two-dimensional paper chromatography and fluorometric determination incorporating a coumestrol standard to show that the coumestrol content of leaf protein concentrate (LPC) derived from alfalfa was reduced by mildly acidic, but not alkaline, conditions during separation from other plant constituents. Processes used in the production of some common, plant-derived, human foodstuffs appear to remove all oestrogenic activity. For example, Bieber (1986) demonstrated that a range of vegetable oils (commercially-refined corn, safflower and soyabean products) exhibited no oestrogenic activity in a mouse uterotrophic assay when fed at 5 or 20% of the diet.

Soyabeans in particular may be subject to very varied procedures. The different processes result in foodstuffs with markedly different phytoestrogenic compositions. Several forms of soya product involve toasting (a technical term for the live steam treatment of the soyabean). Other methods include boiling in water, dry roasting, extrusion cooking, microwave and gamma- or infrared-irradiation, while many of the traditional processes include a fermentation stage.

To produce tofu (a soya milk curd), protein is precipitated from a hot water extract of soyabeans with calcium magnesium salts (Liener, 1994). Tofu, itself a traditional non-fermented soya product in Japan, can be further processed into sufu by fermentation with a mould (generally of the genus *Mucor* or *Actinomuccor*) for two to seven days, followed by incubation in brine solution and ageing for between one and 12 months (Fukushima, 1985). Fermentation is also used in the production of dishes such as tempeh (*Rhizopus oligosporus* fermented product), miso or natto (Liener, 1994). Different forms of miso exist but the basic process involves cooking rice or barley to make a koji (which contains hydrolytic enzymes) and this is then mixed with soyabeans and fermented with yeast and lactic acid bacteria. The degree of hydrolysis of soyabean protein is less than in soya sauce production. Two forms of natto exist. One, hama-natto, is produced by fermentation with *Aspergillus* species while itohiki-natto (the more popular form) is formed by fermentation with *Bacillus natto.* In both cases, soyabean grain is soaked, cooked and then fermented for a number of days.

Soya sauce may be produced by two methods, involving fermentation or chemical processing. Fermented soya sauce has a very long history as a human food. Historically, manufacture in Japan involves very slow (over six months) hydrolysis of the proteins or carbohydrates under mild conditions at below 30°C. The initial step is production of koji from soyabeans or defatted soyabean flakes or grits by cooking under pressure for approximately two to three days. This is followed by mixing with wheat and inoculation of *Aspergillus oryzae* or *A. sojae*. The koji so formed is subject to further brine fermentation using osmophilic lactic acid bacteria and yeasts over several months at low pH, before the product is finally filtered and pasteurised. In China, the process used is quicker, with defatted soyabean meal and wheat bran used to produce koji over a shorter cooking time, followed by three weeks of brine fermentation and subsequent separation of the finished product. In the chemical process, hydrolysis is achieved over eight to 10 hours using hydrochloric acid at over 80°C. In Asia the chemically produced form is used to extend fermented soya sauce, although in western countries it is sometimes used on its own (Fukushima, 1985).

Although the processes used to defat soyabean flakes do not remove any of the isoflavones or their glycosides (Eldridge & Kwolek, 1983), Eldridge (1982) using HPLC with an internal standard, showed that isoflavone concentrations in soya flours varied considerably between commercial products (178–306mg total isoflavones/100g). The method of manufacture of protein concentrate was also shown to affect composition (aqueous leaching giving concentrations similar to soya flour, while aqueous alcohol treatment gave concentrations of only 16–43 mg/100g). In protein isolates approximately 50% of the total isoflavone content of defatted soyabean meal was lost during separation (103–145mg isoflavones/100g) and the relative ratio of individual isoflavones changed. Glycosides were lost preferentially to aglycones, possibly due to their greater water solubility.

The amounts of daidzein and genistein in tofu vary between 73.0–97.5 and 187.4–215.9µg/g wet weight, respectively, and, in samples of soya-based speciality dietary supplements, values ranged from 0.2–1.4 and 0.6–4.0µg/g wet weight, respectively, when analysed by an isotope dilution GC-MS method using deuterated internal standards (Dwyer *et al,*. 1994).

Genistein content appears the most labile, particularly as processing increases (Murphy, 1982; Leiner, 1994) with particularly large losses occurring during water solvation (e.g. during tofu and soya isolate production). Murphy (1982) presented data for a range of products demonstrating the variability in phytoestrogen content of different foodstuffs (Table 3.6).

Table 3.6 Variations in isoflavone concentrations in different food products

Product	Content as glycoside (ppm)*		Content as free form (ppm)*		
	Genistin	Daidzin	Genistein	Daidzein	Coumestrol
Weber soyabean	1024±55	ND	24±0	22±11	ND
Amsoy soyabean	747±18	117±15	40±11	1±3	ND
Toasted defatted flakes	1601±196	200±13	51±1	1±1	ND
Textured soya protein	882±11	86±8	67±12	30±13	ND
Breakfast patties	37±1	1±0	14±0	ND	ND
Amsoy soya sprouts (day 5)	403±19	92±4	78±2	19±8	7±1
Tofu (Weber)	104±4	ND	29±8	ND	ND
Soya isolate (acid derived)	300±13	10±1	77±11	ND	ND
Soya sauce (fermented)	ND	ND	ND	ND	ND

* Average ± Standard Deviation ND Not detected
Analysed by HPLC and fluorometry, using internal standards
From Murphy (1982)

Data (Table 3.7) generated from analysis of commercially available soya foods in the USA further demonstrated the variability of isoflavone content and led Wang and Murphy (1994a) to propose that soya foods should be classified on the basis of the degree of processing involved in their product. Analysis was by HPLC using authenticated internal standards and values presented in the table are the mean of three replicates. Three food categories were suggested:

Soya ingredients: soya flour, soya granules, textured vegetable protein (TVP), soya isolates and soya concentrates;

Traditional soya foods: roasted soyabeans, instant beverages, tofu, tempeh, bean paste, fermented bean curd and miso; and

Second-generation soya foods: recently developed products incorporating soya in novel food products, as a replacement for animal protein or to reduce fat (e.g. soya hot dog, soya bacon, tempeh burger, tofu yoghurt, soya parmesan and cheddar cheese).

With the exception of soya concentrate, soya ingredients contained large amounts of total isoflavones. Vinton 81 soyabeans, green soyabeans, soya granules, soya flour and TVP had significantly higher concentrations than the other products. Generally 97–98% of the isoflavone content in the whole soyabean or other high soya-protein products was in an esterified form (glucoside, malonylglucoside or acetylglucoside); in whole Vinton 81, green soyabeans and soya flour the predominant forms were 6"-*o*-malonylgenistin and 6"-*o*-

malonyldaidzin. Vinton 81 is a variety used in tofu production while green soyabeans are generally retained for soyabean sprout production. In soya granules, TVP and soya isolates the soyabean isoflavones were genistin and daidzin. The soya granules and TVP also contained appreciable quantities of 6"-*o*-acetylgenistin and 6"-*o*-acetyldaidzin which the authors attributed to the effects of heat treatment during extrusion. In contrast, the total isoflavone content of soya protein isolates was less than half that of Vinton 81 or soya flour. Protein isolates are produced by dilute alkali extraction of soluble protein from defatted soya flakes, neutralisation and drying.

Table 3.7 Isoflavone concentrations in different soya-based food products

PRODUCT	Content (μg/g, as is)			
	Daidzein	Genistein	Glycitein	Total
Vinton 81 soyabeans	600	954	82	1636
Green soyabeans	546	729	79	1354
INGREDIENTS				
Soya flour	226	810	88	1124
Soya granule	549	748	167	1464
TVP	473	707	202	1382
Soya isolate	77	273	115	466
Soya concentrate	trace	13	42	56
TRADITIONAL SOYA FOOD				
Roasted soyabeans	563	869	193	1625
Instant beverage	311	617	109	1037
Tofu	146	162	29	337
Tempeh	273	320	32	625
Bean paste	272	245	77	593
Fermented bean curd	143	224	23	390
Miso	79	177	38	294
SECOND GENERATION FOOD				
Soya hot dog	34	82	34	150
Soya bacon	28	69	24	122
Tempeh burger	64	196	30	289
Tofu yoghurt	57	94	12	164
Soya parmesan	15	8	41	65
Cheddar cheese	2	5	27	34

Analysis by HPLC
From Wang & Murphy (1994a)

The ratio of malonyl to glucoside forms in the protein isolates was significantly lower, and that of acetyl to glucoside forms higher, than in intact beans; differences were attributed to toasting of defatted soya flakes after hexane extraction or heating during the drying of the

isolate. Soya concentrate can be produced by water- or alcohol-washing, with the latter method removing most isoflavones. Of particular interest is the absence of isoflavones from soyabean oil, since this may be included in many novel food products. Non-fermented soya foods, such as roasted soyabeans (1625µg/g) and instant soya beverage powder (1037µg/g) had two- to three-times the isoflavone content of fermented soya food. Among non-fermented forms, tofu contained less isoflavones than roasted soyabeans or soya beverage powder, although when reconstituted, concentrations in the beverage powder would be similar to tofu. The highest amounts of the glucosides genistin and daidzin were noted in non-fermented products (lower concentrations of these glucosides occurred in fermented products where aglycones predominated). In roasted soyabeans, significantly higher concentrations of acetylisoflavones and lower concentrations of malonylglucosides were found, compared with unprocessed products; this was attributed to the heating process. Second-generation soyabean products contained relatively low quantities of isoflavones (6–20% of traditional products/ingredients); this was attributed to most of the matrix in these foods being of non-soya origin. The ratio of individual isoflavones was also significantly affected by processing, with hydrolysis of the malonyl or glucose forms occurring. Overall, heat processing, enzymatic hydrolysis and fermentation significantly altered the distribution of the various isoflavones.

In a recent study, Fukutake *et al.* (1996) analysed genistein and genistin content of soyabeans and a range of soya foods from Japan, using HPLC analysis with UV absorbency monitoring, and comparison against standard curves constructed using authenticated compounds. The data presented in Table 3.8 are mean values of three replicates. Concentrations of the isoflavones were higher in soya nuts than soyabeans, possibly reflecting the later harvest time and lower water content of the nut form. In contrast, concentrations in Fava beans were below the level of detection. Amounts of the ß-glucoside conjugate, genistin, were reduced by tofu and soya milk processing; this was attributed to the filtration stages. Amounts of unconjugated genistein were, however, elevated in miso and natto, suggesting fermentation may unconjugate isoflavones. The low values found in soya sauce were suggested to be due to losses during removal of fat.

In addition to modifying the profile of endogenous oestrogenic chemicals, food processing may influence concentrations of fungal contaminants. Wet-milling of naturally contaminated corn resulted in an increased concentration of zearalenone by 2.2- to 7.6-fold in the gluten fraction; the majority of the remainder occurred in the milling solubles, fibre and germ fractions. With dry-milling, cleaning removed only 3 to 10%; the highest amounts were found in the germ, degermer fines, bran meal, hull and high-fat fractions. The prime product mix (grits, low-fat meal and flour) only contained 10–22.5% of the total. Decomposition during subsequent processing was found to be incomplete; examples included 34–40% losses during bread baking, 48–62% during instant noodle making and 16–27% during biscuit manufacturing. Decomposition products in bread were, however, not oestrogenically active when tested by bioassay. Yellow corn fermentation resulted in little destruction of naturally occurring zearalenone, with concentrations in recovered solids approximately twice those in the corn (Kuiper-Goodman *et al.,* 1987). These authors also referred to studies of beer

production in Zambia in which a significant correlation between mycotoxin concentrations in fermented beer and that in the corn or corn malt used was found. However, ethanol obtained by distillation of fermented corn naturally contaminated with zearalenone did not contain any mycotoxin. Reviewing available methods for detoxification of zearalenone-contaminated corn, the authors noted the experimental use of formaldehyde, ammonia, and bases with cation-active surfactants. The latter method and the use of chlorine were particularly effective. The exposure of stored milled grain to Gasol (a complex mixture of acidic and other chemicals) for 28 days was also effective. Luprosil (proprionic acid) treatment, although not reducing existing mycotoxin concentrations, prevented further growth of the mycelium, *F. graminearum*. Other agents have shown varying success including physical means (e.g. density segregation using water or water/sucrose) and ammonium persulphate or hydrogen peroxide treatment.

Table 3.8 Amounts of genistein and genistin in various foodstuffs from Japan

Product type	Product	Concentration (µg/g original weight of food)	
		Genistein	Genistin
Bean	Soyabean	4.6	200.6
	Soyanuts	11.6	968.1
	Fava bean	<0.1	<0.1
Soyabean product	Soya powder	18.2	464.4
	Soya milk-Brand A	1.9	133.1
	Soya milk-Brand B	11.9	94.8
	Tofu	13.9	137.7
Fermented soya product	Miso-Brand A	229.1	71.7
	Miso-Brand B	56.0	190.2
	Natto-Brand A	38.5	282.8
	Natto-Brand B	64.2	492.8
	Soya sauce Brand A	2.8	20.1
	Soya sauce Brand B	2.5	9.8

Analysed by HPLC Limit of detection 0.01µg/g
From Fukutake *et al*. (1996)

Schoental (1977) suggested that the association between cardiovascular disease and dairy product consumption in different regions of Belgium might be due to mycotoxins (e.g. zearalenone and zearalenol) in milk and milk-products and that, since the mycotoxins were also detected in batches of cereals, an assessment of the contribution of beer consumption to the incidence of cardiovascular disease might be appropriate. However, Prelusky *et al*. (1990) showed, using reverse phase liquid chromatography with fluorescence detection, that measurable amounts of zearalenone in cow's plasma were only achieved when zearalenone was administered at dosages sufficient to cause signs of oestrogenic stimulation or ill-health. The content of milk was assessed by a similar method, which incorporated standards to check

for recovery. This demonstrated that the maximum amounts attainable in milk (2.5ng zearalenone/ml and 3.0ng α-zearalenol/ml) were only reached by administering a dosage of 544.5mg zearalenone/day for 21 days, or by single doses of 1.8 or 6.0g. Calculations showed that protein rations to cows could not contain sufficient zearalenone to provide a dose of 165mg/day (at which no measurable transmission into milk was found experimentally) and, therefore, no human health hazard was considered likely.

4 Oestrogenic potency of phytoestrogenic substances

4.1 SUMMARY

Although information is limited, it is possible to assign a provisional ranking to the potencies of various phytoestrogens and mycoestrogens. Zearalenone appears to be the most potent, followed by coumestrol and then the isoflavones, with the highest activities in this class being found in genistein and daidzein. There is a noticeable absence of data on the potency of the lignans, while data on the relative binding affinity to sex hormone binding globulin are limited. It does, however, appear that binding may be considerably less than for natural steroid hormones and this could influence biological availability *in vivo*. Priority should be given to investigations designed to address the current gaps in knowledge on relative potency and sex hormone binding affinity of the phyto- and mycoestrogens, and to improving understanding of the consequences *in vivo* of these factors.

4.2 INTRODUCTION

A number of types of chemical produced by plants and fungi have been identified as oestrogenically active, including isoflavones, coumestans and lignans and the fungal resorcyclic acid lactones. Evidence relating to the occurrence of such chemicals in different plants and plant products and the factors that may affect their content were discussed in Sections 2 and 3. In this section, the relative oestrogenic potency of these chemicals is compared with the oestrogenic steroids produced by animals and the synthetic oestrogen diethylstilboestrol. In addition, since sex hormone binding globulin plays an important role in the transport of sex steroid hormones around the body of humans and many other animals and is, thus, an important factor in the bioavailability of oestrogens to target tissues, the relative binding affinity to the globulin will also be considered.

In addition to the direct oestrogenic actions of these substances, other mechanisms may be involved in their biological activity. For example, since the major source of oestrogen in postmenopausal woman is the aromatisation (by the enzyme aromatase) of androstenedione to oestrone in peripheral tissues (e.g. adipose tissue), any substance that affects this enzyme may exert an indirect effect upon oestrogen levels. Wang *et al*. (1994) evaluated the potential inhibition of enterolactone, enterodiol, coumestrol and *o*-desmethylangolensin on aromatase activity in a predisposed cell culture system, using the aromatase inhibitor, aminoglutethimide as a positive control. *o*-Desmethylangolensin was inactive; however, enterolactone was moderately active (I_{50}[a] value of 74µM), the other lignan, enterodiol, was a weak inhibitor (I_{50}>100µM) and the flavonoid coumestrol had a I_{50} value of 17µM. By comparison, aminoglutethimide had a I_{50} value of 5µM. It is, however, beyond the scope of this review to

[a] I_{50} Inhibitor concentration blocking 50% of V_{max}

consider in detail the significance of the potency of these substances for this and the other activities (e.g. antioxidant, mutagenicity) they may possess.

Historically, the oestrogenic potency of a chemical has been assessed by bioassay of animals, with the rodent uterotrophic or vaginotrophic assays being most often employed (Verdeal & Ryan, 1979; Price & Fenwick, 1985). Other animal models, used in agricultural research, have included a number of ewe-based assays (see Section 3). Although widely employed bioassays are not without criticism, generally relating to cross-species predictivity and to specificity of action (e.g. Stob, 1983). Over recent decades increasing use has been made of a wide range of *in vitro* models to study specific endpoints of relevance to the detection of oestrogenic activity. These include receptor–chemical interaction studies in cell-free systems and, in human and animal cell models, responses such as cell proliferation, receptor–gene interactions, or genetically-driven receptor or enzymic expression. These cell-based models frequently involve transfection techniques to permit expression of human receptor structures by the cell and reporter gene constructs to facilitate measurement of response.

Over a number of years there has been considerable debate over the relative benefits of using *in vivo* or *in vitro* test systems. Perhaps the greatest criticism of current *in vitro* tests lies in the difficulties in extrapolating from *in vitro* findings to *in vivo* effects because of the many factors that can affect the response in the intact organism (e.g. dosimetry, absorption, metabolism, excretion rate, bioaccumulation potential, pharmacokinetics and pharmacodynamics). In particular, the current absence of a satisfactory metabolising system for use with *in vitro* cell line or cell-free systems limits the usefulness of such receptor mediated assays. In addition, in most *in vitro* systems endpoints are dependent upon interaction with only a single (known) receptor structure or response element, and so may fail to address the potential, but currently unidentified, mechanisms that may exist within the intact organism. At a recent major European workshop on endocrine disruption (EC, 1997), it was agreed that, given the current state of scientific knowledge, oestrogenic activity can only be definitively ascribed on the basis of *in vivo* data. There was, however, general acceptance that *in vitro* assays are of value in identifying chemicals that potentially possess such activity and, in some cases, offer useful measures of relative potency.

4.3 *IN VIVO* ASSESSMENT OF POTENCY

An assessment of the relative oestrogenic potency of phytoestrogens was undertaken by Bickoff *et al.* (1962) using a rodent uterotrophic model in which the dose required to produce a uterine weight of at least 25mg was determined in immature mice. The potencies of coumestrol, coumestrol diacetate, genistein, daidzein, biochanin-A and formononetin were compared to the natural hormone oestrone and the synthetic oestrogen diethylstilboestrol. Coumestrol, although the most active of the phytoestrogens, was 200 and 3000 times less potent in this bioassay than oestrone or diethylstilboestrol, respectively. The isoflavones were found to be between 30 and 100 times less active than coumestrol, with genistein the

most potent of the isoflavones and formononetin the least. Biochanin-A (the 4′-methyl ether of genistein) had a potency approximately half that of genistein (Table 4.1).

Table 4.1. Relative potency of phytoestrogens using a mouse uterotrophic assay

Substance	Potency relative to oestrone *
Diethylstilboestrol	14.49
Oestrone	1
Coumestrol	5.07×10^{-3}
Coumestrol diacetate	3.48×10^{-3}
Genistein	1.45×10^{-4}
Daidzein	1.09×10^{-4}
Biochanin-A	6.67×10^{-5}
Formononetin	3.77×10^{-5}

* Relative potency determined at dose required to induce development of a uterus weighing at least 25 mg
After Bickoff *et al.* (1962)

Further comparative studies using uterotrophic and vaginotrophic assays were performed by Folman and Pope (1966), who compared the agonistic and antagonist activities of the phytoestrogens coumestrol and genistein with those of oestrone, 17β-oestradiol and diethylstilboestrol, and with non-oestrogenic but hormonally active progestins. The results suggested the phytoestrogens to be weak oestrogen agonists that, when given in conjunction with more potent oestrogenic compounds, acted antagonistically.

The literature appears to lack more recent large-scale comparative *in vivo* studies of oestrogenic potency, concentrating instead on studies of the mechanisms of action of individual substances, often using rodent uterotrophic assays. Tang and Adams (1980), using such an assay, demonstrated that equol (a gut floral metabolite of formononetin) had an activity only 10^{-3} that of 17β-oestradiol. Whitten *et al.* (1992) and Galey *et al.* (1993) studied the uterotrophic activity of coumestrol while Rosenblum *et al.* (1993) investigated the oestrogenic potency of bourbon fractions. In these studies the rodents were administered either crude extracts or specific phytoestrogens by incorporation in the diet followed by subsequent measurement of uterine weight. In a different approach, Medlock *et al*. (1995) studied the effects of equol and coumestrol on the development of the uterus in neonatal rats following subcutaneous administration for up to ten-days *post partum:* potency was assessed in terms of effect on uterine weight and expressed relative to diethylstilboestrol. Values of 10^{-3} and 10^{-5} were established for coumestrol and equol, respectively.

The *in vivo* activity of the mycoestrogen zearalenone has also been assessed in a rat model by Kumagai and Shimizu (1982); potency was reported as 0.1% to 10% that of 17β-oestradiol. In addition, using changes in the levels of serum luteinizing and follicle stimulating hormones or as a marker of oestrogenic activity in ovariectomised rhesus monkeys following oral administration of zearalenone, 17ß-oestradiol or diethylstilboestrol, the mycoestrogen was shown to be 80- and 160-times less active than 17β-oestradiol or diethylstilboestrol, respectively (Hobson *et al.*, 1977).

4.4 *IN VITRO* ASSESSMENT OF POTENCY

4.4.1 Oestrogen receptor binding studies

Interaction with the oestrogen receptor is generally considered an important prerequisite for exerting oestrogenic activity, and the assessment of relative binding affinity to this receptor may, therefore, provide an indication of relative potency. Several *in vitro* studies examining this aspect have been summarised by Verdeal and Ryan (1979) and Price and Fenwick (1985), thus permitting a provisional ranking of the substances in terms of binding affinity (see Table 4.2). In addition, Katzenellenbogen *et al*. (1979) found α-zearalenol to be more active than either β-zearalenol or zearalenone, when measured by competitive or direct binding assays using rat uterine oestrogen receptors. The activity of α-zearalenol, when assessed by competitive or direct binding assays, was 13.6% or 15% that of 17β-oestradiol, respectively. From examination of Table 4.2 it is, however, apparent that there are large gaps in knowledge and a need for additional comparative studies.

Table 4.2 Relative affinity of phyto- and mycoestrogens for the oestrogen receptor

Substance	Relative affinity to receptor			
	Rabbit uterine cytosol	Sheep uterine cytosol	Human cell line (MCF-7)	Rat uterine cytosol
17β-oestradiol	100	100	100	100
Coumestrol	1.4	5.0	10	5.0
Zearalenone	-	-	3.3	3.4
Genistein	0.6	-	2	1.3
Equol	-	0.4	-	-
Daidzein	-	0.1	-	0.09
o-Desmethylangolensin	-	0.05	-	-
Angolensin	-	0.03	-	-
Biochanin-A	-	-	-	0.07
Formononetin	-	<0.01	< 0.01	-

Adapted From Verdeal & Ryan (1979); Price & Fenwick (1985)

4.4.2 Oestrogenic potency in established cell lines

Several workers have assessed the potency of phyto- and mycoestrogens in a number of oestrogen-dependent cell line systems, employing a variety of endpoints including simple proliferative responses and reporter gene systems utilising suitable biochemical endpoints.

Welshons *et al.* (1987; 1990) studied the relative potency of phytoestrogens using proliferative responses in MCF-7 and T47D breast cancer cell lines. In the 1987 study, enterolactone and enterodiol and the flavonoid metabolite equol were confirmed as weak oestrogens compared to oestradiol. Half maximal responses were obtained at 10^{-11}M for oestradiol, 10^{-7}M for equol, and 10^{-5}M for enterolactone, while enterodiol was approximately ten times less active than enterolactone. In the 1990 study, relative oestrogenic potencies (expressed as equivalent dry weight of zearalenone) were determined using the MCF-7 cell

proliferative response (see Table 4.3). Zearalenone was markedly less active than either oestradiol or diethylstilboestrol, but was nearly ten times more active than the most active phytoestrogen, coumestrol.

Mayr *et al.* (1992) presented *in vitro* data on the relative oestrogenic potency of zearalenone and several phytoestrogens derived from a number of test systems, and compared these to values obtained from an *in vivo* rodent uterotrophic assay (Table 4.5). Similar orders of relative potency were seen, although the actual activities reported differed markedly from those noted by Welshons *et al*. (1990). It is apparent, however, that the *in vitro* models tended to show higher activities than were found *in vivo*, with the difference being most pronounced for zearalenone and genistein. Further work by Markiewicz *et al.* (1993) supported the order of potencies established by the earlier workers. In this study oestrogenic activity was assessed using a non-proliferative *in vitro* endpoint, namely the oestrogen-specific enhancement of alkaline phosphatase activity of human endometrial adenocarcinoma cells of the Ishikawa-Var I line (Table 4.4).

Table 4.3 Relative activity of phyto- and mycoestrogens in MCF-7 cells

Substance	Relative ability to elicit cell proliferation (by mass)
17ß-oestradiol	117
Diethylstilboestrol	85
Mycoestrogens	
Zearalenol	56
Zearalenone	1
ß-Zearalenol	0.05
Phytoestrogens	
Coumestrol	0.13
Genistein	0.094
Biochanin-A	0.0056
Daidzein	0.00084
Formononetin	0.00047

From Welshons *et al.* (1990)

Table 4.4 Relative potency of phytoestrogens using the Ishikawa-Var I cell line

Substance	EC_{50} (nM) ± SD	Relative potency
Oestradiol	0.0673 + 0.03	100
Coumestrol	33.3 ± 3.9	0.202
Genistein	79.8 ± 11	0.084
Equol	111 ± 17.6	0.061
Daidzein	515 ± 41.1	0.013
Biochanin	>1000	0.006
Formononetin	> 10000	0.0006

[a] Relative potencies: [EC_{50} (E_2) / EC_{50} (isoflavonoid)] × 100
From Markiewicz *et al*. (1993)

Table 4.5 Relative oestrogenic potency of zearalenone and phytoestrogens in *in vivo* and *in vitro* test systems

Substance	Assay system			
	MCF-7 cells[a]		LeC-9 cells[d]	Mouse uterotrophic
	ED_{50} [b]	Relative potency[c]	Relative potency[c]	Relative potency[e]
17β-Oestradiol	$3x10^{-11}$	100	100	100 (DES)
Zearalenone	$6x10^{-10}$	3–5	2.5	0.1
Coumestrol	$1x10^{-7}$	0.03	0.06	0.035
Genistein	$3x10^{-7}$	0.01	0.04	0.001
Daidzein	$1.5x10^{-6}$	0.002	0.003	0.00075
Biochanin-A	$2.5x10^{-6}$	0.0012	0.00053	0.00046
Formononetin	$3x10^{-6}$	0.0010	0.00025	0.00026
Genistin	$6.25x10^{-7}$	0.0048	-	-
Daidzin	$2.5x10^{-6}$	0.0012	-	-

[a] Assay based on induction of an oestrogen-specific exoprotein by MCF-7 cells
[b] ED_{50} corresponds to the concentration (M) resulting in half-maximal effect
[c] Relative to 17ß-oestradiol, expressed as a percentage
[d] Assay based on levels of induction of a CAT, ß-galactosidase reporter system transfected into LeC-9 cells
[e] Oestrogenic potency relative to diethylstilboestrol (DES), expressed as a percentage
From Mayr *et al.* (1992*)*

4.5 *IN VITRO* ASSESSMENT OF RELATIVE BINDING AFFINITY TO SEX HORMONE BINDING GLOBULIN

Sex hormone binding globulin is present in the blood of mammals and is responsible for sequestration of the physiologically present sex hormones, thus restricting the bioavailability and modulating the actions of these hormones. The degree of binding to these globulins shown by the phyto- or mycoestrogens will similarly have direct implications for the relative bioavailability of such xenoestrogens, and as a result would be expected to influence their relative activities in the intact organism. Martin *et al.* (1978) assessed *in vitro* the relative binding affinities of a number of xenoestrogens to human sex hormone binding globulin. Using oestradiol as a reference substance, competitive inhibition was measured at 100-fold excess of each phytoestrogen (Table 4.6) and it was apparent that both phyto- and mycoestrogens had relatively low binding affinities. Clearly such a difference from the endogenous hormones may have important implications *in vivo*, since limited binding to sex hormone binding globulin would be expected to enhance the amount free in plasma and hence available to exert biological activity, and could thereby, to some extent, negate the lower potencies established for these substances relative to endogenous hormones (see above). If binding of these phytoestrogens to the globulin were to prove significant relative to oestradiol, there might also be consequences for the bioavailability of the endogenous hormones.

Arnold *et al* (1996) demonstrated, for a range of substances including the phytoestrogens coumestrol and genistein, that a dose-dependent reduction in oestrogenic activity occurred when sex hormone binding globulin was included in the medium of a Yeast Estrogen Screen (YES) model. This assay comprises transfected yeast expressing a human oestrogen receptor with a reporter gene construct linked to a lac*Z* gene, whose activation expresses a β-galactosidase which can be measured colorimetrically. The maximum inhibition of activity occurred when the globulin was tested with oestradiol, while least reduction occurred with the synthetic compounds diethylstilboestrol and Kepone. A marked effect on the activity of genistein and coumestrol was also noted, indeed the activity of coumestrol was reduced to a similar degree to oestradiol. Clearly this is a highly artificial model, but, taken with the study by Martin *et al.* (1978), suggests that binding globulins may exert a considerable influence on bioavailability and potency of oestrogenic substances *in vivo*.

TABLE 4.6 Relative binding affinity to sex hormone binding globulin

Substance	Relative binding affinity*(%)
Oestradiol	100
Genistein	27
Formononetin	24
Zearalenol (Low melting point isomer)	23
Zearalenol (High melting point isomer)	21
Coumestrol	14
Zearalenone	5

* As a percentage of that shown by oestradiol
From Martin *et al*. (1978)

4.6 OVERVIEW OF RELATIVE POTENCIES

The phyto- and mycoestrogens are structurally similar to 17β-oestradiol and, in addition to eliciting responses characteristic of oestrogens in the intact animal, have been shown to bind to isolated oestrogen receptors and cause proliferative and gene transactivation responses in *in vitro* models. Unsurprisingly, the oestrogenic potency of these substances shows considerable variation depending upon the test model and endpoint employed. The available evidence suggests that some *in vitro* assays can detect substance–receptor interactions or stimulation of cell proliferative or reporter gene endpoints at much lower concentrations than effects can be detected *in vivo*. This difference in sensitivity probably reflects the presence, *in vivo*, of mechanisms such as absorption, distribution, protein binding, metabolism and elimination, which occur only in the intact organism. It must, however, be remembered that the ultimate physiological significance of exposure to a chemical can only be adequately assessed *in vivo*.

Relevant potency data are summarised in Table 4.7. It appears that when potency relative to the steroidal oestrogens is considered, *in vitro* systems generally indicate higher activities for phytoestrogens than are obtained using *in vivo* models. As discussed above, this probably reflects the simplistic nature of the *in vitro* models and the absence of relevant physiological and biochemical protective or modifying mechanisms in such models. Not withstanding this, it is possible to assign a provisional overall ranking to the potencies of the various phytoestrogens and mycoestrogens. The fungal resorcyclic acid lactones, in particular zearalenone, appear to be the most potent, followed by the coumestans of which coumestrol appear most potent. The potencies of the isoflavones are lower, with the highest activity in this class being shown by genistein and daidzein. There is a noticeable absence of data on the potency of the lignans. Similarly, data on relative binding affinity to sex hormone binding globulin are limited, although it appears that the binding of phyto- and mycoestrogens is considerably less than that of the natural steroid hormones. This could influence their biological availability *in vivo*.

TABLE 4.7 Summary of relative potencies of phytoestrogenic and mycoestrogenic chemicals

Substance	Relative binding affinity to SHBG (%)[a]	Relative binding affinity to oestrogen receptor (%)[c]			Relative oestrogenic potency *in vitro* (%)			Relative oestrogenic potency *in vivo* (%)[d]
		MCF7	Rat	Sheep	MCF-7 cells[b]	LeC-9 cells[b]	Ishikawa cells[e]	
Control chemicals								
Diethylstilboestrol								100
17β-Oestradiol	100	100	100	100	100	100	100	100
Oestrone								6.9
Resorcyclic acid lactones								
Zearalenone	5	3.3	3.4		3–5			0.1
Zearalenol	22				2.5			
Coumestans								
Coumestrol	14	10	5	5	0.03	0.06	0.202	0.035
4′-methoxycoumestrol								
Isoflavones								
Daidzein			0.09	0.1	0.002	0.003	0.013	0.00075
Genistein		2	1.3		0.01	0.04	0.084	0.001
Daidzin					0.0012			
Genistin	27				0.0048			
Formononetin	24	<.01	<.01		0.001	.00025	0.0006	0.00026
Biochanin-A			0.07		0.0012	.00053	<0.006	
Equol				0.4			0.061	
Angolensin				0.03				
o-Desmethylangolensin				0.05				
Lignans								
Enterolactone								
Enterodiol								

SHBG Sex hormone binding globulin

[a] Martin *et al*. (1978) [b] Mayr *et al*. (1992) [c] Verdeal & Ryan (1979)
[d] Bickoff *et al*. (1962) [e] Markiewicz *et al*. (1993)

5 Differences in gut microflora and interindividual metabolic differences

5.1 SUMMARY

Gut microflora have been shown to play a key role in digestive processes. However the overall composition of organisms is not static since the population exists in a dynamic relationship with the individual host. Many environmental factors, including diet, affect its composition and metabolic activity. Although each person may possess a unique population with its own metabolic profile, certain dietary regimens (e.g. vegetarian versus omnivorous) may be reflected, in general, by certain microfloral compositions. This has been demonstrated for particular racial or otherwise cohesive groups.

The microflora may in turn influence the bioavailability and metabolism of important, and potentially active, dietary constituents or may affect other aspects of the host's physiological status (e.g. by influencing bile flow). Given such interrelationships, it is possible that a particular pattern of microflora could influence the risk of some diseases. However, it must be stressed that at the individual level many factors are involved and, therefore, it is unsurprising that considerable interindividual variation is seen.

There is an outstanding need to investigate further the complex interactions between diet, gut microflora and host. In particular, the role of phytoestrogens in these processes requires clarification. In order to be effective such future experimental studies will require careful specification of treatment regimens and dosing schedules and improved analytical characterisation of the chemical composition and fate of foodstuffs.

5.2 INTRODUCTION

The potential role of dietary or other risk factors in the aetiology of international differences in disease incidence was noted in Section 1. It is the objective of this section to briefly review the possible influence of diet on the microfloral composition of the gastrointestinal tract, the influence of the individual person on the microflora and the possible consequences of microfloral differences on the metabolic status and disease susceptibility of the host individual.

The key importance of the gut microflora to the digestion and absorption of plant products is firmly established. For example, flavonoids of all subgroups undergo extensive catabolism in the lower intestinal tract before absorption into the host's body, with the metabolic products ultimately being excreted into the urine. Indeed, many plant flavonoids occur as ß-glycosides that require hydrolysis before absorption through the intestinal tract (Kuhnau, 1976). Setchell *et al.* (1981) demonstrated experimentally that the lignans, enterolactone and enterodiol excreted in the urine of humans were formed by gut microflora, and suggested that *Clostrida*

species may be involved. *In vitro* work by Borriello *et al.* (1985) demonstrated that enterodiol is metabolised by gut microflora to enterolactone which is not itself metabolised further. These products can be formed from precursors of plant origin (e.g. secoisolariciresinol and matairesinol) under aerobic and anaerobic conditions, suggesting that the responsible species are facultative. It should be noted that at present it is only possible to account for approximately 30% of ingested phytoestrogens (Setchell, 1997), thus underlining the need for further research.

Gut microflora clearly play a key role in the digestive processes of animals and humans. The microfloral composition is not static but exists in a dynamic relationship with the individual host and is subject to modification in response to changing diet and environment. Thus, any factor influencing the composition or functionality of the gut microflora is likely to be of importance to the health of the host.

5.3 VARIATIONS IN GUT FLORAL COMPOSITION

There is evidence linking dietary practice to gut microfloral composition. For example, in Japanese people migrating to America the gut flora has been shown to change in line with dietary practice. For Japanese–Americans living in Los Angeles, Finegold *et al.* (1974) compared the faecal microflora (under anaerobic conditions) of those adopting a western-style diet with individuals retaining a traditional Japanese diet. In all, 33 Japanese–Americans were investigated. Fifteen had retained a traditional diet (major items included rice, beancurds, noodles, miso soup, Japanese vegetables, raw fish, salted fish, seaweed, bean sprouts and green tea) whilst the remainder had adopted a generally western-style diet. Sex distribution and age ranges were comparable and none had active gastrointestinal disease. Following culturing, numerous differences in bacterial composition of the faecal flora were noted (Table 5.1) with, overall, 220 distinct species, subspecies or groups being found for those on a traditional diet compared with 160 for individuals that had adopted a western diet. Certain Gram-positive non-sporing anaerobic bacilli (*Eubacterium contortum* and *E. lentum)* and some *Peptostreptococcus* species were strongly associated with the Japanese diet while *Bifidobacterium infantis* associated with the western diet.

Modification of hepatic phase I and gut microfloral metabolic processes by alterations in diet regimen has been demonstrated experimentally in animals and humans. Alldrick *et al.* (1988) reported that feeding mice flavonoid (quercetin or rutin) supplemented diets affected caecal mass, caecal bacterial concentration, bacterial ß-glucuronidase and nitrate reductase activities, and hepatic mixed function oxidase and ethylmorphine N-demethylase activities. Holdeman *et al.* (1976) studied changes in faecal flora of humans in response to dietary modification. Serial faecal samples were obtained over a 43-day period from three Caucasians eating a normal western diet and living under normal conditions. Further samples were taken after adoption of the diet used during Skylab missions, while continuing to live under otherwise normal conditions, and a further set again using the Skylab diet while isolated to simulate a space mission (this included reduced atmospheric pressure and increased oxygen level). The Skylab

diet contained a considerable variety of food, but all of it had been subject to canning, freezing, irradiation or dehydration to facilitate long-term storage. In all, 101 species of bacteria were found to account for 98% of the cultivable flora, with four (*Bacteroides fragilis thetaiotaomicron*; *Peptostreptococcus productus* II; *Eubacterium aerofaciens* III & *E. siraeum)* of 15 predominant species showing significant variation between treatments. (It should be noted that the results may have been influenced by initial retention of faecal samples under aerobic conditions and by delays before processing).

Table 5.1 Number of isolates in faeces for predominant species: Statistically significant differences in incidence between different diets

Diet type	Japanese[a]		Western[b]		p value*
Species	No. positive samples	No. organisms (Mean[c]/ Range)	No. positive samples	No. organisms (Mean[c]/ Range)	
Streptococcus faecalis var. *faecalis*	14	9.83 (4–10)	15	8.46 (3–9)	0.038
Other facultative/aerobic	ND	7.20	ND	4.75	<0.01
Eubacterium contortum	5	9.58 (7–10)	0	-	0.033
Eubacterium lentum	16	10.20 (5–10)	10	10.07 (4–10)	0.015
Bifidobacterium infantis, other	0	-	7	10.29 (8–11)	0.009
Peptostreptococcus sp.1	6	10.53 (9–11)	0	-	0.033
Peptostreptococcus sp.1-25	ND	8.29	ND	4.64	0.001

[a] 20 samples from 15 subjects [b] 20 samples from 18 subjects ND Data not presented in paper
[c] mean count $\log_{10}$ no. of organisms/g faeces for positive samples
* by contingency table and Fisher's exact probability statistic
From Finegold *et al*. (1974)

Of the changes related to diet, *E. aerofaciens* III showed a noticeable increase in numbers in two of the subjects while on the special diet. *E. siraeum* level also increased markedly in one man in response to diet.

Moore *et al*. (1981) reported on the faecal floral changes in five individuals in response to changing dietary regimens. Faecal samples were analysed for microbiological composition: 1) while on their normal [i.e. western] diet; 2) three days on a diet of unpolished boiled rice, salt, unsweetened tea or coffee, vitamins and water; 3) two or more weeks after return to normal diet; 4) three days on a non-protein vegetable diet (as dietary regimen 2 above, except with rice replaced by vegetables); 5) two or more weeks after return to a normal diet and 6) after

three days on a diet with lean beef replacing rice or vegetables. On each sampling occasion freshly voided faeces were cultured under oxygen-free conditions for five days, and subsequently 55 colonies randomly selected for characterisation of faecal flora (Table 5.2). Many of the predominant species were unresponsive to dietary change [at least over the study duration] suggesting that their growth may depend on intestinal mucin or other secretions, rather than dietary substrates. Nonetheless, some variations were apparent (*Ruminococcus bromii* and *Eubacterium rectale* II showed reductions for the vegetable and meat diets and *Ruminococcus torgues* for the meat diet).

Table 5.2 Changes in mean number of isolates per faecal specimen (±SEM) in response to diet for most frequent species

Species	Mean no. isolates per specimen (±SEM) for most frequent species for each diet			
	Normal	Rice	Vegetable	Meat
Bacteroides vulgatus	6.4 (2.5)	9.0 (2.5)	6.2 (3.8)	9.4 (4.0)
Ruminococcus bromii	3.5 (0.9)	2.4 (1.6)	0 [c]	0 [c]
Bacteroides uniformis	3.2 (1.0)	1.8 (0.9)	1.6 (0.9)	1.4 (0.7)
Eubacterium rectale II	2.7 (0.7)	1.6 (0.9)	0.2 (0.2) [b]	0.6 (0.6) [b]
Eubacterium aerofaciens	2.5 (0.7)	1.6 (1.1)	2.2 (1.7)	3.0 (2.0)
Eubacterium rectale I	2.5 (0.7)	1.8 (0.8)	1.0 (0.8)	1.0 (1.0)
Bacteroides O	1.8 (0.6)	0.8 (0.6)	3.6 (2.2)	2.8 (1.9)
Coccus DZ	1.8 (0.3)	2.8 (1.5)	0.8 (0.8)	2.4 (1.2)
Fusobacterium prausnitzii	1.7 (0.3)	3.0 (1.2)	3.0 (0.9)	1.6 (1.1)
Peptostreptococcus productus Ib	1.3 (0.6)	1.0 (0.5)	1.2 (0.8)	1.2 (1.0)
Bacteroides 3452A	1.0 (0.6)	0.4 (0.4)	1.6 (1.2)	1.2 (0.7)
Ruminococcus torques	0.8 (0.3)	2.2 (0.7)	2.8 (2.1)	2.6 (1.7) [a]
No. of specimens analysed*	15	5	5	5

* No. specimens taken from 5 people on test
[a] p =0.02 [b] p = 0.005 [c] p =0.0025
From Moore *et al*. (1981)

Moore and Holdeman (1974) and Moore and Moore (1995) have also investigated links between gut microfloral composition, dietary practice and risk of colon cancer. When the predominant microflora species identified in Japanese–Hawaiians taking part in each of these studies were compared, there were similarities but the incidence rankings were different, underlining the potential variability of the gut flora. In the 1995 study a number of populations at different risk of colon cancer were investigated. These comprised high-risk Japanese–Hawaiian polyp patients, moderate- to high-risk Japanese–Hawaiian adults and Caucasians on western diets, and two low-risk groups of rural Japanese natives and rural South African natives, both on traditional diets. Statistical analysis (Lambda analysis with Students *t*-test for comparison of individual species in different populations) showed the flora

of polyp patients (high risk) were similar to those of Japanese–Hawaiians on a western-type diet (moderate to high risk) while the flora in Caucasians on a western diet (moderate to high risk) were not significantly different from Japanese–Hawaiians but were different from those of polyp patients. Although the two low-risk groups had different floral compositions, they both comprised types of bacteria rarely found to associate with colon cancer. Fifteen relatively common species associated with high colon cancer risk and six with low risk. It might be expected that species could occur at higher levels in the high risk groups since they are known to be stimulated by bile flow (which would be expected to be increased where red meat and high fat diets were consumed). However, only two (*Bacteroides vulgatus* and *B. stercoris*) showed such a response, while total concentrations of *Bacteroides* species only showed a slight increase. *Bifidobacterium longum* and *Bifidobacterium angulatum* showed a significant increase in the high risk group. In contrast, *Lactobacillus* and *Eubacterium* species associated with low risk diets. Nonetheless, the authors stressed the important role of the individual in the control and maintenance of a distinctive floral population.

Potentially, differences in gut microflora and the factors controlling species composition could be important modifiers of an individual's exposure to hormonally active food constituents. For example, in a study by Adlercreutz *et al.* (1986) of urinary composition and different habitual diets, the lignan and isoflavone contents of urine taken over 24-hour periods were measured for groups of Finnish and US women (Table 5.3). The method used ion-exchange chromatography with subsequent GC/MS and incorporated deuterated internal standards. As might be expected, marked differences in excretion were noted between the different dietary groups. However, it was also noted that the ratio of urinary daidzein to equol tended to be lower for Finnish women than for Americans, which was suggested to possibly arise from microfloral differences leading to Americans producing the most equol. Such differences in microflora may possibly arise from contamination of the diets with different xenobiotics.

There is thus evidence that particular dietary regimens (e.g. vegetarian versus omnivorous) may, in general, associate with particular microfloral profiles and that this may have consequences for the absorption of potentially hormonally active chemicals.

Table 5.3 Urinary excretion in young women on various habitual diets

Group	Geometric mean urinary excretion rates (nmol/ 24 hours)		
	Omnivores	Lactovegetarians	Macrobiotics
Boston, USA women			
No. of subjects	9	9	12
Enterolactone	2050	4170	17680
Enterodiol	280	740	6260
Daidzein	320	1260	3460
Equol	69	100	868
Daidzein:Equol ratio	4.64	12.60	3.99
Helsinki, Finnish women			
No. of subjects	12	11	Not measured
Enterolactone	2460	3650	
Enterodiol	203	368	
Daidzein	219	275	
Equol	102	64	
Daidzein: Equol ratio	2.15	4.30	

Analysis by ion-exchange chromatography and GC/MS
After Adlercreutz *et al.* (1986)

5.4 INTERINDIVIDUAL DIFFERENCES

The gastrointestinal microfloral composition and metabolism are affected by factors other than diet, with many studies having demonstrated variations between individuals even if given identical diets.

In the study of the effect of Skylab diet on microflora (see Section 5.3), Holdeman *et al.* (1976) noted that seven of the 15 predominant species cultured from faecal samples showed statistically significant person to person variation in composition. One microorganism (*Bacteroides fragalis thetaiotaomicron*) was noted to increase when subjects first began to live under Skylab conditions but then declined. The authors suggested this transitory response might be related to psychological stress, since technical problems had resulted in high stress levels occurring in the subjects during the initial isolation period and similar effects had been observed in previous studies in stressful situations. A suggested mechanism for such an effect was by adrenaline and gastric secretion modifying gut motility, blood flow, gastric and colonic mucin secretion, bile secretion and nutrient- and bile-associated absorption. In the study by Moore *et al.* (1981) discussed above, it was noted that each individual tended to maintain a distinct rank order of microfloral species despite significant changes in diet. A subsequent study investigating differences in microfloral composition between groups at different risk of colon cancer (Moore & Moore, 1995; see above) also showed each individual maintained

distinctive flora even after dietary change. Together these studies suggest that host genetics and physiology is important in the control of the microfloral composition.

Setchell *et al.* (1984) studied the metabolic formation of equol from dietary soya in four men and two premenopausal women by serial analysis of urine samples over a three-day period on a normal diet, followed by a five-day period on a high soya diet (40g of commercially available textured soya per day). Analysis was by gel chromatography and gas chromatography, with detection by flame ionization or selected ion monitoring mass spectroscopy. Authentic standards were included in the assay. Faecal samples were also cultured to confirm the *in vitro* ability of the gut flora to metabolise equol. Basal urinary equol excretion by the individuals was very variable (<5 to 80µg/day) and, following supplementation of the diet with soya, two of the men and both women showed marked increases in excretory rate (by 50- to 1000-fold), with maximal values of equol between 3.5 and 7.0mg/day. Following reversion to the normal diet, urinary equol excretion in these individuals gradually returned to below 100µg/day. However, for two of the men challenge with equol did not elicit a significant response. *In vitro* faecal culture from one of these subjects with soya protein confirmed the intrinsic ability of the microflora to produced equol, so the reason for non-responsiveness *in vivo* is unclear; the authors suggested that intestinal transit times and redox potential may be important factors.

Kelly *et al.* (1995) reported on the variable metabolic response of humans to dietary isoflavones. Six men and six premenopausal women, all Caucasian, were fed a legume-free diet for one week followed by a challenge of 40g of whole soya flour each day for two days (equivalent to approximately 78mg genistein, 64mg daidzein and 24mg glycitein). The profile of urinary phytoestrogens and their metabolites were monitored prechallenge, and for three days following challenge, by GC and GC-MS techniques incorporating the use of reference standard material (Table 5.4).

Urinary concentrations of daidzein, genistein and glycitein increased following challenge, particular on the first day post-challenge. *o*-Desmethylangolensin was similarly affected, being noted in 10/12 individuals prechallenge and in all afterwards. Urinary equol was noted for 9/12 individuals prechallenge and increased in all but one postchallenge; however, the other individual showed no detectable urinary equol prechallenge and only a barely detectable amount (0.04µmol) after challenge. Enterolactone excretion was unaffected by treatment. Overall, higher concentrations of daidzein than genistein were recovered from the urine, despite soya containing higher concentrations of genistein; the reason is unclear. In addition, and more significantly for this discussion, a very high individual variability in metabolic capability was apparent (total excretion rates varied by 4-, 7-, 761- and 17-fold, for daidzein, genistein, equol and *o*-desmethylangolensin, respectively). Variations in peak equol concentrations were even more marked (1527-fold). Thus, although all individuals could metabolise these substances, significant differences in metabolic activity were apparent (Table 5.5).

Table 5.4 Mean (range) of urinary phytoestrogens and metabolites prior to and following soya challenge

Chemical	Urinary concentration (μmol)	
	Prechallenge	Total post challenge (3-day period)
Isoflavones		
Daidzein	3.8 (1.8– 6.6)	20.3 (9.6–40.6)
Genistein	1.1 (0.8–2.0)	11.2 (3.5–23.7)
Glycitein	0.8 0.02–2.4)	4.0 (3.5–23.7)
Isoflavonoid metabolites		
Equol	0.2 (ND–0.7)	22.6 (0.2–152.3)
o-Desmethylangolensin	0.2 (ND–0.5)	14.4 (3.4–58.9)
Lignan		
Enterolactone	11.0 (0.9–35.3)	24.0 (2.5–73.1)

Analysis by GC and GC-MS
From Kelly *et al*. (1995)

5.5 IMPLICATIONS OF GUT FLORA COMPOSITION AND HOST SPECIFIC DIFFERENCES

There is evidence that particular dietary regimens may be associated, in general, with a particular microfloral composition, and that particular microfloral compositions may be associated with altered risk of some disease conditions. It is, however, apparent that individual hosts exert significant influence over their own microfloral composition and the mechanisms involved in this have yet to be fully elucidated.

Associations between faecal microbial flora, colon cancer risk and dietary habit have been reviewed by Finegold *et al.* (1978). The absence of plant bulking agents in diet is known from short-duration experimental studies to decrease stool weight, increase faecal transit time and reduce the diversity of gut microflora. Epidemiological studies have shown differences in flora in subjects from high- and low-risk countries. Higher numbers of steroid nuclear-dehydrogenating clostridia, *Clostridium paraputrificum*, have been reported in the stools of British and American subjects than in populations with a low incidence of bowel cancer. Vegans in England may have lower numbers of Bacteroides (that can dehydroxylate bile acids) than the general population. Fat intake was also suggested to affect floral composition.

Table 5.5 Comparison of individual variation in urinary excretion rates of daidzein and two of its metabolites

Substance	Mean±SD excretion (µmol/ 3 days)	
	Low equol* (n = 8)	High equol * (n = 4)
Daidzein	23.05±12.43	14.95±6.69
Equol	1.53±2.60	64.89±59.23
o-Desmethylangolensin	21.72±17.93	6.97±6.47

* Grouped as either low equol producers (<8 µmol/3 days) or high equol producers (>25 µmol/3 days)
From Kelly *et al*. (1995)

Bokkenheuser *et al.* (1987) showed that, in humans, most of the common dietary flavonoid glycosides (quercetin, rutin and robinin) were hydrolysed by gut flora to mutagenic aglycons, which may be further degraded by bacteria or undergo conjugation by the liver. It has also been shown that bile acids in the intestinal medium may enhance glycosidase-producing bacteria, possibly by inhibiting other species. Such a modification of microflora would be a possible contributory factor to the association between high concentrations of faecal bile acids and increased risk of human colon cancer (Mader & Macdonald, 1985).

Goldin *et al.* (1981) demonstrated that gastrointestinal enzyme levels could be influenced by dietary practice, for example, ß-glucuronidase and nitroreductase activities were lower in lactovegetarians and strict vegetarians than in omnivores, while 7α-dehydrogenase (an enzyme catalysing the conversion of primary to secondary bile acids) were also lower in lactovegetarians. It was also suggested that the activities of the enzymes ß-glucuronidase, nitroreductase and azoreductase (produced by some members of the faecal flora) could influence colon cancer development. Chemicals detoxified by glucoronidation in the host's liver and excreted in the bile might be 'reactivated' in the gut to toxic or carcinogenic metabolites by the action of ß-glucuronidase. Similarly, nitroreductase and azoreductase can catalyse the reduction of nitro- and azo- compounds to mutagenic aromatic amines.

Many factors have thus been suggested to influence the composition of the gut microflora, such that each person may posses a unique microfloral profile. The microflora in turn can have a significant effect on the metabolic and absorptive capabilities of the host organism. There is an outstanding need to investigate further the complex interactions between diet, gut microflora and host. In particular, the role of phytoestrogens in these processes requires clarification. In order to be informative, future experimental studies must incorporate careful specification of treatment regimens and dosing schedules and have improved analytical characterisation of the chemical composition and fate of foodstuffs.

6 The beneficial effects of phytoestrogens in adults

6.1 SUMMARY

The majority of human evidence for the effects of plant food consumption on various diseases originates from ecological, cohort or case–control epidemiological studies. While these provide indications of possible associations, it is often difficult to interpret the significance of any such associations when they are based on information collected from dietary questionnaires which, in general, suffer from inadequate estimations of exposure. In some cases, current dietary intakes have been taken to reflect historical intakes, but this raises questions over accuracy of the data because of possible changes in diet with time. In other studies, current questionnaires have been used prospectively in analyses of subsequent cancer cases. In these cases, the period between questionnaire and case analysis varies from about five years to more than 20 years; without knowledge of the critical window of susceptibility for the particular cancer in question, it cannot be assumed that this reflects the appropriate latency period for the development of the disease.

Despite their limitations, these studies have indicated apparent associations between various dietary factors and the incidence of many of the diseases described above. For cancer of the breast, prostate, colon, rectum, stomach and lung, the evidence is most consistent for a protective effect resulting from a high intake of plant foods including grains, legumes, fruits and vegetables. It has not been possible, however, to identify particular food types or components that may be responsible. In addition to phytoestrogens, food components suggested as possibly contributing to a protective effect include glucosinolates, indoles (e.g. indole-3-carbinol) and possibly phytoalexins in cruciferous vegetables and flavonoids (other than isoflavones) in fruit, vegetables and beverages such as tea and wine.

Dietary intervention studies generally indicate that soya and linseed do have biological activity in men and women, although the effects are not always consistent. There is evidence to suggest that these plant foods have effects in women which may reduce the risk of breast cancer and may help to alleviate postmenopausal symptoms. Both soya and linseed also appear to have beneficial effects on blood lipids, which may help to reduce the risk of cardiovascular disease and atherosclerosis. For endometrial cancer and osteoporosis, there have not been sufficient studies on which to base an evaluation, although there is a suggestion that intake of fruit and vegetables may be protective in the former, and the use of ipriflavone (a synthetic isoflavone derivative) which may help to prevent osteoporosis indicates a worthwhile avenue of research for the natural isoflavones.

It is noteworthy that, although soya and linseed have been shown to be biologically active in humans, there is almost no evidence to link these effects directly to the presence of phytoestrogens. To date, only one study has been identified which compared the effects of

soya both with isoflavones (textured vegetable protein, TVP) and without (Arcon F). Results indicated effects of TVP on length of the menstrual cycle and on hormone levels in premenopausal women which were not observed in the group consuming Arcon F. While this study suggests that isoflavones were responsible, further studies are required to confirm these findings. Many other components of both soya and linseed have been described which have been shown to be biologically active in various experimental systems and these may be responsible for some or all of the observed effects in humans.

In contrast to the observed adverse effects of plant isoflavones on fertility and reproduction in animals, the reproductive capacity of Asian women, who have for centuries consumed large amounts of soya products as part of their staple diet, does not appear to be adversely affected by the high levels of isoflavones present in these foods. In the postreproductive period, only beneficial effects of dietary soya phytoestrogens have been reported in the literature, although it is not possible to be certain whether this is due to a lack of reporting or investigation or a true absence of adverse effects.

Much research is needed to clarify whether consumption of dietary lignans and isoflavonoids have a role in the prevention of several types of cancer described above.

6.2 INTRODUCTION

A number of recent publications have reviewed the evidence for effects of dietary phytoestrogens on various human conditions and diseases (Cassidy, 1996; Knight & Eden, 1995 & 1996). The effects of these substances in various biological systems and during different phases of life have also recently been reviewed (Chapin *et al.*, 1996). As a result of the known variation in endocrine status between individuals, there may be subpopulations of people who are sensitive or resistant to the effects of phytoestrogens. Also, the likelihood of effects may differ according to the period of life, namely the developmental (including pre-pubertal), reproductive and post-reproductive periods. For example, if phytoestrogens act as oestrogens in postmenopausal women, they would be expected to have similar effects to hormone replacement therapy, such as reducing the bone loss associated with osteoporosis, reducing the risk of cardiovascular disease and possibly increasing the risk of breast cancer. Epidemiological studies show that the incidence of cancer of the breast, endometrium, prostate and colon, coronary artery atherosclerosis and osteoporosis differ between Asian populations consuming traditional oriental diets and those consuming a western diet. This suggests a protective effect associated with oriental diets, a major component of which is soya-based food containing phytoestrogens. This section reviews the evidence for the involvement of phytoestrogens in protection against these conditions in adults.

6.3 THE ROLE OF THE DIET IN CANCER RISK

Marked geographical differences exist in the incidences of cancer (e.g. historically, rates of breast and prostate cancer in Asian countries are significantly lower than in western

countries). Early studies established that, across many countries, a wide range of dietary, life-style and socio-economic factors were associated with the incidence of, or mortality from, a variety of cancers and other diseases. However, epidemiological data should be interpreted with caution because of the multiple factors involved, such as stage of cancer detection, screening practices, socio-economic status, reproductive practices and differences in height and weight (Kelsey & Horn-Ross, 1993).

Armstrong and Doll (1975) demonstrated associations between diet and cancers of the gastrointestinal tract and hormonally responsive tissues, with intake of animal protein or fat apparently playing a key role, while Gray *et al.* (1979) showed that diet is associated with variations in breast cancer incidence or mortality or both, even after adjustment for differences in height, weight and menarche. Although oriental women (who consume large amounts of soya products) have historically had a low incidence of breast cancer without apparent adverse effects on reproductive capacity (Messina & Barnes, 1991), Oishi *et al*. (1988) noted that the incidences of lung, breast, colon and prostate cancers in Japan were now rising, and suggested that such changes may reflect the adoption of a more western-style diet.

Epidemiological studies published between 1957 and 1992 suggesting an association between intake of soya products and cancer risk have been summarised recently by Messina *et al.* (1994). The cancer sites reported in these studies included breast, prostate, colon/rectum, lung, stomach, oesophagus, bile duct, liver and pancreas. While the overall data suggested a protective effect of soya against several types of cancer, inconsistencies prevented a stronger conclusion being reached. In the 21 epidemiological studies, which separated unfermented soya products from bean paste and miso soup, 28 cancer sites were investigated and a decreased risk of cancer was shown at 11 (39%) sites, with no association or nonsignificant trends at 16 (57%) sites. An increased risk of oesophageal cancer was reported in one study, which investigated consumption of fried bean curd. No consistent pattern was found for 27 cancer sites in 21 studies of fermented soya foods such as miso, with an increased risk reported at five (18%) sites, inconsistent effects at four (15%) sites, no statistical difference at 14 (52%) sites and decreased risk at four (15%) sites (Table 6.1). The presence of associated dietary variables such as the high sodium content of miso (which may confound any increased risk) or the vegetables often added to miso soup (which may confound any protective effects) may explain the differences in observed effects between studies on miso and other soya products (Messina *et al*., 1994; Persky & Van Horn, 1995).

Lignans have also been proposed as playing a part in human health and disease, particularly in hormone-dependent diseases (Setchell & Adlercreutz, 1988). Such effects, including those of soya are discussed in subsequent sections.

Table 6.1. Numbers of studies reporting effects of unfermented and fermented soya products on cancer risk at various sites*

Cancer site	Unfermented soya products			Fermented soya products		
	Decreased risk	No significant association	Increased risk	Decreased risk	No significant association	Increased risk
Oesophagus		1	1	1[a]		1[b]
Lung	3	1			1	
Breast	1	2		1	1	
Stomach	6	3		2	5	3
Prostate		1			3	
Liver					1	
Gall bladder / bile duct		1			1	
Pancreas					1	
Colon		5			3	
Rectum	1	2			1	1
Cholangio-carcinoma					1	

* Some studies report more than one site. [a] women and [b] men, same study

From Messina *et al.* (1994)

6.4 BREAST CANCER IN WOMEN

This section reviews and assesses the evidence for a protective role of phytoestrogens in breast cancer in women. Breast cancer can occur in either males or females, but only about 1% of all cases occur in men, and male breast cancer is a rare disease in all parts of the world (Sasco *et al*., 1993). Although there appear to be some similar risk factors for breast cancer in males and females, there is no indication in the literature that diet is either a risk or a protective factor for male breast cancer.

Many studies have observed low incidences of hormone-dependent cancers, particularly breast cancer, in Asian compared with western countries. It is becoming increasingly accepted that dietary factors are associated with many of these diseases. The epidemiological correlation in distribution between breast and colon cancer may be due to some populations consuming inadequate amounts of specific fibre-rich foods, such as grains which contain plant lignans and may be protective (Adlercreutz, 1984). The suggestion that lignans may have a role in protecting against breast cancer stemmed from the observation that postmenopausal women with breast cancer excrete significantly lower amounts than do omnivorous or vegetarian controls (Adlercreutz *et al*., 1982). In addition, vegetarians who consume foods rich in fibre have greater quantities of oestrogens in faeces than omnivorous women, due possibly to the inhibitory effects of fibre on biliary oestrogen reabsorption. Fibre-rich foods which contain plant lignans may also have a protective effect on colon cancer (Section 6.10).

While obesity is generally recognised as a risk factor for breast cancer, and case–control studies of fat intake and breast cancer risk have shown a positive association, prospective studies have not confirmed this. A recent study investigating a possible association between diet and breast cancer in two Chinese populations at relatively low risk of the disease reported that dietary fat showed a small but non-significant association with breast cancer risk (Yuan *et al*., 1995).

Studies in populations of women with different diets

Studies in groups of women having different diets (omnivorous, lactovegetarian and macrobiotic) in Boston and Helsinki have demonstrated that urinary excretion of lignans (enterolactone and enterodiol) and isoflavones (daidzein, equol and *o*-desmethylangolensin) is by far the highest in macrobiotics, significantly lower in lactovegetarians and lowest in omnivores (Setchell & Adlercreutz, 1988). The levels of these compounds in urine was between 10 and 1000-fold higher than the levels of endogenous oestrogens in these subjects, depending upon the diet. An earlier investigation had found the urinary excretion of enterolactone to be significantly lower in postmenopausal breast cancer patients (n=7; 1040nmol/24 hours), than in postmenopausal controls eating mixed (n=10; 2300nmol/24 hours) or lactovegetarian (n=10; 3180nmol/24 hours) diets (Adlercreutz *et al.*, 1982). In another area of Finland, North Karelia (where the incidence of breast cancer is lower than in Helsinki or Boston), omnivorous women (n=28) excreted much higher levels of enterolactone (mean 3020nmol/24 hours) than the omnivorous groups in either of the two cities. When levels of lignans and isoflavones were measured in the urine of a group of Japanese subjects, levels of lignans were only slightly higher than for Finnish women, while the level of isoflavones was ten-fold higher. It was concluded that phytoestrogens may be one of the protective dietary factors against hormone-dependent disease in vegetarians and semi-vegetarians (Setchell & Adlercreutz, 1988).

In a study of 27 postmenopausal women (nine lactovegetarians, 10 omnivores and eight surgically treated breast cancer patients) in Boston USA, Adlercreutz *et al*. (1989) recorded food intake over a one-year period and measured levels of plasma androgens and sex hormone binding globulin (SHBG) in these subjects. Levels of androstenedione, testosterone and free testosterone were positively correlated with intakes of protein and fat and negatively correlated with intakes of carbohydrate, grain, total fibre and grain fibre. In breast cancer subjects, levels of androstenedione and testosterone were significantly higher than in vegetarians, and levels of free testosterone and SHBG were significantly higher and lower, respectively, than in control groups. High levels of androstenedione and testosterone and low levels of SHBG in postmenopausal women were associated with a western-type diet and this pattern was shown in the breast cancer patients. Breast cancer patients had the highest protein to carbohydrate intake ratio (0.37), the lowest mean consumption of total fibre and grain fibre and the highest mean ratio of fat intake to grain intake.

Preliminary results of a study of the effects of dietary phytoestrogens in postmenopausal women were reported by Adlercreutz *et al.* (1992). Levels of urinary oestrogens, lignans and isoflavones and plasma SHBG were measured in 11 omnivorous women, 10 vegetarian women and nine apparently healthy women who had undergone mastectomy for breast cancer. Dietary records coinciding with collection of urine and blood samples showed a significantly higher intake of total fibre (22.7g/day) and vegetable fibre (4.5g/day) in the vegetarians compared with the breast cancer patients (16.0g/day and 2.3g/day, respectively) and significantly lower intake of cholesterol in vegetarians compared with omnivores or breast cancer patients. Vegetarians had significantly higher levels of plasma SHBG, and urinary excretion of enterolactone, total lignans, total isoflavones and total diphenols when compared with the other two groups. A significant positive association was demonstrated between plasma SHBG and these compounds in urine even after accounting for the effect of body mass (lower in the vegetarians). A significant negative correlation was found between SHBG and urinary 16α-hydroxyestrone, with the lowest values of SHBG in omnivorous and breast cancer women. An increase in the rate of 16α-hydroxylation and a low rate of 2-hydroxylation has been proposed as a risk factor for breast cancer (Bradlow *et al*., 1995).

Several other studies have investigated the influence of dietary factors on breast cancer risk and the findings of these are summarised in Table 6.2.

A number of investigations have attempted to identify factors, including those relating to diet, associated with the changes in incidence of breast cancer in women who migrated to the USA from countries having a lower cancer incidence. Data on breast cancer collected between 1983 and 1987 indicated that the age-standardised incidence rate of breast cancer in Japan varied from 18 per 100 000 to 28 per 100 000, compared with a rate two- to three-fold higher in Japanese Hawaiians (64 per 100 000), and higher still in white Hawaiians (almost 100 per 100 000) over this period (IARC, 1992). The study by Nomura and co-workers is summarised in Table 6.2. Teas (1981) suggested that foods consumed commonly in Japan but not in the USA, such as seaweed and tea, are important when considering the increases in breast cancer mortality in the offspring of Japanese migrants to the USA. Animal studies and *in vitro* data have indicated several biological properties of seaweed which may possibly have a protective role against breast cancer induction. These include hypocholesterolemic, antioxidant and antibiotic activities and its contribution of trace metals such as selenium to the diet.

Effects on endogenous hormones and the menstrual cycle

In addition to a number of other mechanisms, recent evidence suggests an effect of lignans and isoflavones on the length of the menstrual cycle. Fibre intake has been correlated with a delay in menarche in girls, potentially leading to a reduced breast cancer risk, as an early menarche is considered to be a risk factor (Adlercreutz, 1995). Some epidemiological studies have not found a protective effect of fibre on breast cancer risk when fibre intake was measured as total dietary fibre. However, this may not accurately reflect the lignan content of the diet and so it

is not possible to determine any association between intake of dietary lignans and breast cancer risk from these studies.

Several studies have investigated the effect of soya protein, soya milk, textured vegetable protein (TVP) and linseed on levels of endogenous hormones and the menstrual cycle in premenopausal women. These are summarised in Table 6.3.

Breast cancer summary

A number of epidemiological studies have demonstrated low levels of lignans and isoflavones in the urine (assumed to reflect low intakes) of subjects with breast cancer or at high risk of breast cancer. In contrast, people living in areas having a low risk of hormone-dependent cancers excrete higher levels. Finnish subjects with an intermediate risk of breast and prostate cancer excrete relatively high levels of lignans but low levels of isoflavonoids, while Japanese subjects at low risk excrete high levels of isoflavonoids and have similar levels of biologically active lignans (the free and sulphated forms).

With regard to soya, the epidemiological studies published between 1957 and 1992 in which soya products were mentioned in relation to cancer risk indicate variable results for both fermented and unfermented soya and soya-containing foods. For studies reporting specifically unfermented soya products, one showed a decreased breast cancer risk while two showed no association or a non-significant effect. In two studies reporting the effects of fermented soya products, one found a decreased risk and the other found no association or a non-significant effect (Messina *et al*., 1994). In a study not included in this review, a negative association was found between intake of soya protein and total soya products and breast cancer risk in premenopausal but not postmenopausal women in Singapore (Lee *et al*., 1991). However, in a recent study of two Chinese populations in whom the intake of soya protein was reported to be similar to the population of Lee and co-workers, breast cancer risk was not related to intake of soya protein for either pre- or postmenopausal women (Yuan *et al*., 1995).

Table 6.2 Epidemiological studies investigating the influence of diet on breast cancer risk

Study details	Study type	Findings	Reference
6860 Japanese Hawaiians. Dietary practices of husbands of 86 breast cancer cases compared with remainder	Cohort	Spouses of breast cancer cases consumed American foods more frequently and Japanese foods (e.g. seaweed and green tea) less frequently than did controls	Nomura *et al.* (1978)
200 breast cancer cases in Singapore (46% postmenopausal) 420 controls (51% postmenopausal)	Case–control	Premenopausal: increased risk associated with increased intake of red meat, decreased risk associated with increased intake of polyunsaturated fatty acids, β-carotene, soya protein, total soya products Postmenopausal: no significant effects of diet on risk	Lee *et al.* (1991)
534 cases in Shanghai (aged 20–69), age- and sex-matched community controls	Case–control	Significant inverse association with risk demonstrated for crude fibre, carotene, vitamin C (associated with intake of green vegetables)	Yuan *et al.* (1995)
300 cases in Tiangin (aged 20–55), age- and sex-matched community controls	Case–control	Highest risk in group with lowest tertile of crude fibre intake and highest tertile of fat intake. Findings similar for pre- and postmenopausal women No significant effect of soya protein intake on risk	

While there is limited evidence to suggest that intake of fruit and vegetables is associated with a reduction in risk of breast cancer, the overall epidemiological evidence for a protective effect of phytoestrogens in premenopausal women is inconclusive; there is no evidence for a protective effect in postmenopausal women. The original intention of most of the studies carried out was to look at possible associations between breast cancer risk and general dietary factors and not to investigate the specific effects on risk of foods containing phytoestrogens. As a result, levels of intake in these studies relate to various food items rather than levels of intake of phytoestrogens specifically. Although some estimate of intake could be made from particular food items consumed, it is perhaps not surprising that the latter have not been consistently defined. Even similar food types may differ quite markedly in their levels of phytoestrogens. Other limitations associated with the studies on soya include the small variations in soya intake, the inability to separate soya from other dietary variables and a problem more generally applicable, the inherent difficulty in relating estimated dietary intakes to cancer development several decades later (Persky & Van Horn, 1995).

More direct evidence for an involvement of phytoestrogens in breast cancer risk reduction comes from controlled diet studies in populations of healthy women. Many of these studies have shown that phytoestrogens are biologically active in women, producing a number of effects which have been associated with a reduced breast cancer risk. In premenopausal women, soya has been shown to increase the length of the menstrual cycle and/or delay menstruation, and to reduce the levels of LH, FSH and progesterone at various stages of the cycle. The reported effects of soya on blood levels of 17β-oestradiol have not been consistent, one study reporting a reduced level throughout the cycle (Lu *et al.*, 1996), another reporting an increase in the follicular phase only (Cassidy *et al.*, 1994) and another finding no change in levels (Baird *et al.*, 1995). Based on the studies reviewed, the evidence for changes in levels of the adrenal androgen DHEAS is conflicting, and further investigations would be required in order to clarify these opposing findings. Plasma levels have been shown to vary with energy intake; for example, a recent study in premenopausal women found that for each additional 1MJ (239kcal) consumed, levels of DHEAS decreased by 5.1% (Dorgan *et al.*, 1996). To ensure that energy intake does not confound intervention studies using soya products, it would seem sensible to ensure that all diets investigated are isocaloric.

Both lignans and isoflavones have been reported to increase the levels of plasma SHBG, which may decrease the blood levels of biologically active sex hormones and thus influence cancer risk. Again, however, epidemiological studies are conflicting. Levels were lower in postmenopausal women with breast cancer than in vegetarians or omnivores (Adlercreutz *et al.*, 1989 and 1992), although an earlier study found no difference between postmenopausal breast cancer patients and controls (Bruning *et al.*, 1985). In more informative controlled trials, in which lignans (as linseed) or soya (as textured vegetable protein and miso) was added to the diets of premenopausal women, a small but significant decrease in the level of SHBG was seen only in women consuming the linseed supplement. From these studies, isoflavones do not appear to have any effect on SHBG levels.

Table 6.3 Studies investigating the effect of soya and linseed on endogenous hormone levels and the menstrual cycle

Study details	Findings	Reference
Effect of linseed powder (10g/day) ingestion on menstrual cycle of 18 omnivorous women (aged 20–34 years) for 3 cycles compared with 3 control cycles	Following linseed ingestion, small increase in total fibre intake but variable increases in excretion of enterolactone (13-fold mean increase to 25.4±3.5µmol/day) and enterodiol (55-fold mean increase to 19.7±3.8µmol/day). Mean luteal phase length significantly longer during linseed cycles (12.6±0.4 days) than control cycles (11.4±0.4 days). No effect on mean follicular phase or overall cycle lengths, or levels of oestradiol or oestrone during any stage of the cycle. Luteal phase progesterone/oestradiol ratios significantly increased during linseed ingestion.	Phipps *et al.* (1993)
6 healthy non-vegetarian premenopausal women (aged 21–29). Diet enriched with 60g soya protein per day (containing 45mg isoflavones/day) for 1 month	Follicular phase length and/or delayed menstruation following soya-enriched diet. Other changes included suppression of mid-cycle LH and FSH surges.	Cassidy *et al.* (1994)
15 healthy non-vegetarian premenopausal women. Diet supplemented with 60g TVP/day (n=6), 28g TVP/day (n=6) or 50g miso/day (n=3) for 1 cycle. In a second study, 5 subjects consumed either control diet or diet supplemented with 60g Arcon F/day for 1 cycle.	Follicular phase length significantly increased in 60g TVP group (from 15.0±0.4 to 17.5±0.9 days) and peak progesterone levels delayed, mid-cycle LH and FSH peaks suppressed. Peak progesterone levels significantly delayed in miso group. No effects observed in subjects consuming Arcon F (an isoflavone-free soyabean product)	Cassidy *et al.* (1995)
Measurement of hormone levels in 35 Adventist vegetarian and 40 non-vegetarian adolescent girls (aged 14–18 years).	Significantly higher levels of dehydroepiandrosterone sulphate (DHEAS) in vegetarians compared with non-vegetarians. No differences in levels of testosterone, oestradiol or % free oestradiol. DHEAS is an adrenal androgen reported to be present in lower levels in women with breast cancer or at high risk of developing the disease, particularly in the premenopausal years.	Persky & Van Horn (1995)

Table 6.3 (continued) Studies investigating the effect of soya and linseed on endogenous hormone levels and the menstrual cycle

Study details	Findings	Reference
Effects of soya milk consumption (36oz/day, equivalent to intake of 100mg/day daidzein/daidzin and 100mg/day genistein/genistin) in 6 healthy premenopausal women (aged 22–29 years) for 1 month	Oestradiol levels decreased over early and late follicular and luteal phases by 31, 81 and 49%, respectively, persisting for up to 3 cycles after end of supplementation. During feeding, levels of progesterone and DHEAS fell progressively while menstrual cycle length increased (from 28.3±1.9 to 31.8±5.1 days), returning to normal 5–6 cycles later.	Lu *et al.* (1996)
Effect of soya protein isolate (38g/day, containing 38mg/day genistein) on breast secretion in pre- (n=14) and postmenopausal (n=10) Caucasian women (aged 29–58 years) over 12 months	Premenopausal women: volume of nipple aspirate fluid (NAF) increased 2–6-fold (mean increase from 16.1±16.1µl to 42.3±40.9µl) during soya consumption. No change in length of menstrual cycle. Mean levels of GCDFP-15 (a glycoprotein produced by apocrine metaplastic epithelial breast cells, found in high levels in ducts and breast cysts of benign breast disease and in breast cancer) decreased during soya consumption. Postmenopausal women: minimal or no response. No change in mean levels of GCDFP-15 during soya consumption. For both groups, there were no significant changes in levels of plasma prolactin, progesterone or SHBG.	Petrakis *et al.* (1996)
Effect of linseed (40g/day, providing 27mg/day secoisolariciresinol) on hormone levels in 7 postmenopausal women for 6 weeks Effect of TVP (60g/day, providing 45mg/day isoflavones) on hormone levels in 6 postmenopausal women for 4 weeks	Levels of LH and FSH significantly suppressed (average of 10% and 9%, respectively) at end of supplementation period. No significant changes in oestradiol. Levels of LH significantly reduced but no significant effects on FSH levels.	Cassidy *et al.* (1997)

Although preliminary, the potentially important finding of Petrakis *et al.* (1996) that soya consumption may have an oestrogenic effect by increasing the incidence of hyperplastic epithelial cells in the nipple aspirate fluid of pre- and postmenopausal women, constituting a risk factor for breast cancer, should be the subject of further investigation.

Overall, these studies show that phytoestrogens are biologically active in women and can affect the levels of sex hormones and potentially therefore contribute to a reduced breast cancer risk. The effects produced have not always been consistent between studies, although this may relate to the use of different doses, types of product used, study design or the generally small numbers of women studied. In order to elucidate the potential beneficial effects of phytoestrogens in breast cancer risk reduction, further controlled studies in larger populations of premenopausal women are warranted.

6.5 POSTMENOPAUSAL SYMPTOMS

The reported incidence of hot flushes, one of the most common symptoms of the menopause, varies markedly between different countries, with high levels in Europe (from 70–80% of postmenopausal women), intermediate levels in Malaysia (57%), and low levels in China (18%) and Singapore (14%; Knight & Eden, 1995 and 1996). As a result of dietary differences between these countries, particularly the significant variations in consumption of soya products, several studies have investigated the possible role of these products in modulating these symptoms. It is of interest to note that many plants of the family Leguminosae, which contain high levels of isoflavones and their glucosides, have long been used to treat menopausal symptoms such as hot flushes, sweating and depression by Chinese herbal doctors (Lien & Lien, 1996). Examples include alfalfa, dandelion, red clover and liquorice.

Wilcox *et al.* (1990) showed that consumption by 25 postmenopausal women of a diet supplemented with soya flour (45g/day), red clover sprouts (10g dry seed/day), and linseed (25g/day), each for two weeks, had no effect on LH or FSH levels when analysed after each individual two-week supplement, but had a marginal cumulative effect on FSH levels over the six-week study. No control group was included in this study. However, oestrogenic effects were observed when measured as cytological maturation of the vaginal epithelium. This was not confirmed in subsequent studies by Murkies *et al.* (1995) and Baird *et al.* (1995). In the former study, postmenopausal women with more than 14 hot flushes per week consumed a diet supplemented with either soya flour (45g/day, n=25) or wheat flour (45g/day, n=22) for 12 weeks. No effect on vaginal cell maturation was seen in women consuming either supplement, although hot flushes were significantly reduced at six weeks in the women consuming soya flour and by 40% and 25% at the end of the study in women consuming the soya flour or wheat flour, respectively. A subjective assessment of menopausal symptoms also showed significant reductions in both groups by the end of the study. Urinary levels of daidzein, equol and enterolactone were significantly higher at the end of the study in the soya flour group but not in the wheat flour group (although wheat flour also contains

phytoestrogens). As a result of the decrease in flush frequency over the study period, a placebo effect could not be discounted. In the study of Baird *et al.* (1995), groups of postmenopausal women consumed either a normal diet (control, n=25) or a diet supplemented with soya foods (equivalent to 165mg isoflavones/day, n=66) for four weeks. Despite an average 105-fold increase in urinary excretion of isoflavone phytoestrogens in the soya diet group and an average two-fold increase in the control group (which was not significant), no significant difference in vaginal maturation index was noted between the two groups. In addition to these observations, there were no significant differences in the levels of FSH, LH and SHBG between the two groups or in levels before and after the dietary intervention. A slight decrease in serum oestradiol levels was noted in both groups during the study, but neither was significant. The use of a different sampling technique in this study to the others above may have falsely lowered the estimate of vaginal maturation (Knight & Eden, 1996). However, the lack of measurement of biological dose in this study makes it difficult to determine whether the lack of effect was real or simply due to the dose administered being too low. Measurement of a biological marker of effect, such as a reduction in level of blood cholesterol, would have enabled any effect of the administered dose to be monitored more clearly.

According to Knight & Eden (1996), many studies are being undertaken throughout the world to assess the effectiveness of isoflavones in treating symptoms of the menopause. The current evidence suggests that there may be some beneficial effects but it is not possible to be more categorical at present because of the conflicting observations seen in the limited number of studies measuring hormonal changes and the possibility that placebo effects may explain subjective changes. It is most important in future studies to monitor changes in levels of a biological effect marker of exposure following consumption of phytoestrogens and thus demonstrate that exposure has occurred.

6.6 OSTEOPOROSIS

Osteoporosis is a disease which primarily affects elderly women, and its incidence increases with increasing duration of oestrogen deficiency. Rates of osteoporosis vary between populations living in different geographical areas. Thus the incidence in Asian women is lower than in women from western countries. Also the risk of hip fracture has been found to be lower in Japanese women than in white women and diet is one factor postulated to be involved with these differences (WHO, 1994). However, epidemiological data suggesting a link between osteoporosis and dietary phytoestrogens are scant; evidence suggesting possible beneficial effects in the prevention of osteoporosis comes almost entirely from a small number of animal and *in vitro* studies, which are not discussed here in detail.

A large number of studies have investigated the effect of ipriflavone, a synthetic isoflavone derivative (7-isopropoxyisoflavone), on prevention of osteoporosis in postmenopausal women. This compound appears to function by inhibiting bone resorption, thus improving bone density in these women (Agnusdei *et al.*, 1992; Kovacs, 1994); however, it has been

reported to be devoid of oestrogenic activity (Melis *et al.*, 1992) and its mechanism of action is not thought to involve direct interaction with oestrogen receptors (Petilli *et al.*, 1995). In some countries, ipriflavone has been marketed as a non-hormonal treatment for osteoporosis in women who cannot receive hormone replacement therapy (Magyar, 1995). Ipriflavone has also been used successfully to prevent the rapid bone loss that occurs following induction of hypogonadism in premenopausal women treated with gonadotrophin hormone-releasing hormone agonists (Gambacciani *et al.*, 1994). It is of interest to note that one of the main metabolites of ipriflavone in humans is daidzein, which constitutes approximately 10% of all its metabolites (Brandi, 1992). While these findings cannot be directly extrapolated to natural isoflavones, or indeed other phytoestrogens, they provide a basis for further investigation.

A number of recent reviews have commented on the evidence for a link between osteoporosis and phytoestrogens (Knight & Eden, 1995 and 1996; Clarkson *et al.*, 1995; Cassidy, 1996). Overall, data in humans indicate that the effects of a related compound, ipriflavone, are beneficial in maintaining bone density in postmenopausal women. While there is at present only tentative evidence to indicate that naturally-occurring phytoestrogens have similar effects, emerging evidence suggests these substances are biologically active in experimental model systems. Oestrogen receptors have been demonstrated in osteoblast cells, which are responsible for laying down new bone. Additionally, oestrogens have a negative effect on the activity of osteoclast cells, which are responsible for bone resorption and remodelling, and genistein has been shown to suppress osteoclastic activity both *in vitro* and in rats *in vivo* (Blair *et al.*, 1996). Although the mechanism of action is not clearly understood, the recent discovery of a second human oestrogen receptor (ERβ) may be important (Kuiper & Gustafsson, 1997). Based on the promising effects shown in animal studies, the effects of phytoestrogens on osteoporosis in humans are currently being studied, in particular in the USA, and the results of these investigations are eagerly awaited.

6.7 ENDOMETRIAL CANCER

Many case–control studies have demonstrated that combined oral contraceptives protect against endometrial cancer. During the menopause, however, although oestrogen replacement therapy is effective in relieving menopausal symptoms and preventing the development of postmenopausal osteoporosis, it is well established that unopposed oestrogen therapy in menopausal women can cause endometrial cancer (Vessey, 1984). The risk is related to both the dose and duration of use and declines following cessation of treatment.

In areas which have cancer registries, the incidence of endometrial cancer varies from the highest rates in parts of the USA (25 per 100 000) to the lowest rates in Singapore and Japan (3 per 100 000; (IARC, 1987). Large variations in incidence exist between different ethnic groups within individual countries; age-adjusted rates for white, black and indian Americans between 1978 and 1982 were 23, 12 and 7 per 100 000, respectively, and rates in white and Japanese Hawaiians were 23 and 15 per 100 000, respectively (Parkin, 1989). In England and Wales, endometrial cancer is not a common cancer, the incidence increasing only slightly

between 1962 and 1987 at 0.13% per annum (Dos Santos Silva & Swerdlow, 1995). As with breast cancer, there is an apparent association between the consumption of high quantities of soya products and a low incidence of endometrial cancer.

In a study investigating possible relationships between cancer incidence/mortality and environmental factors, the incidence of endometrial cancer was strongly and positively correlated with intake of meat, eggs, milk, fats and oils, total protein fats and calories (Armstrong & Doll, 1975). Complicating these observations is the fact that protein and fats are correlated with total energy intake, the major determinant of obesity, and obesity is itself a major risk factor for endometrial cancer, probably due to its effect on the hormonal milieu of both pre- and postmenopausal women (La Vecchia, 1989; Hill & Austin, 1996).

No studies appear to have investigated a link between phytoestrogen exposure or intake in humans and risk of endometrial cancer. In a case–control study of endometrial cancer, La Vecchia (1986) found an inverse relationship between cancer risk and reported frequency of intake of green vegetables, fruit and whole-grain foods. However, information was collected on only a limited number of food items; total intake of calories, a known risk factor, was not estimated, making it difficult to comment on the significance of the observed negative association. In addition, the presence of other potentially active compounds in fruits and vegetables would preclude attributing the reduced risk to the presence of phytoestrogens in these foods.

6.8 OVARIAN CANCER

Several case–control studies have investigated the role of dietary factors in the risk of ovarian cancer and suggest that increasing consumption of animal fat and/or meat is associated with an increased risk, while increased consumption of vegetables may be associated with a decreased risk (La Vecchia *et al*., 1987; Engle *et al*., 1991; Fukushima *et al*., 1993; Risch *et al*., 1994). No prospective studies investigating dietary factors and risk of ovarian cancer were identified from the literature and, specifically, no studies appear to have investigated any association or relationship between consumption of phytoestrogens and risk of ovarian cancer.

6.9 BIOLOGICAL EFFECTS IN ADULT MALES

In a study investigating the effects of linseed on sex hormone metabolism, six healthy young men consumed a diet supplemented with linseed (13.5g/day) for six weeks (Schultz *et al.*, 1991). Urinary levels of enterodiol and enterolactone increased 7 to 28-fold over the study period, with 5 to 10-fold greater levels of enterolactone than enterodiol. No significant changes were observed in the plasma levels of total and free testosterone or SHBG. The addition of 40g linseed/day to the diet of a group of middle-aged men produced a significant reduction in the levels of FSH over a four week period (Cassidy *et al.,* 1997).

Cunnane *et al.* (1995) investigated the nutritional effects of linseed (50g/day) in the diet of 10

healthy young adults (five male and five female, aged 25±3 years) for four weeks. Following consumption, plasma levels of n-3 polyunsaturates were increased and α-linolenate was significantly increased in adipose tissue. Total lignan excretion increased more than five-fold, while plasma levels of lipid hydroperoxides and antioxidant vitamins (retinol and α-tocopherol) were unaffected. The number of bowel movements increased by 30% while subjects consumed the linseed supplement and overall results suggested modest beneficial effects.

In a study in middle–aged men, Cassidy *et al.* (1997) assessed the effects of phytoestrogens and lignans in two dietary intervention trials. Addition of linseed (40g/day, providing 27mg/day secoisolariciresinol) to the diet of six men (aged 57–69 years) for four weeks significantly reduced FSH levels and produced a non-significant reduction in LH. Mean levels of serum testosterone and dihydrotestosterone (DHT) showed no significant reduction and the DHT/testosterone ratio did not change. No change in total urinary androgens was observed. In the second study, six men (aged 60–63 years) were given a dietary supplement of 60g TVP (soya)/day, providing 45mg/day isoflavones, for four weeks. No significant changes in levels of LH or FSH were observed. The linseed diet was generally more effective than the TVP diet at suppressing LH and FSH levels in men.

Effects of soya and linseed on blood lipids in men are summarised in the section on coronary heart disease/atherosclerosis.

6.10 PROSTATE CANCER

Studies of migrant populations have shown that when people move from areas of low incidence to areas of high incidence, the incidence of prostate cancer increases, suggesting that environmental factors may be involved aetiologically (Severson *et al.*, 1989). For example, between 1983 and 1987 the age-standardised incidence rate of prostate cancer in different areas of Japan ranged from 6.6 per 100 000 to 10.0 per 100 000, while the rate among Japanese in Hawaii was approximately four-fold higher at 34 per 100 000, but only about half the rate among white Hawaiians (63 per 100 000; IARC, 1992). It has been argued, however, that the incidence in Japan is actually much higher than the figures quoted above due to variation in diagnosis. Adjusting for differences between the USA and Japan in the proportion of latent prostate carcinoma and of localised tumours among all carcinomas of the prostate, Shimizu *et al.* (1991) estimated the incidence rate to be between 25 and 33 per 100 000 population. Clinically significant prostate cancer may be over-diagnosed in the USA and migration may not play as important an effect on prostate cancer risk as previously reported.

Asian men have a low mortality from prostatic cancer and the high amount of isoflavones and lignans in their diets has been suggested to protect them from this disease. Japanese men have small latent carcinomas that infrequently develop to clinical disease. Adlercreutz *et al.* (1993) suggested this may be explained by a life-long high plasma concentration of isoflavones, which may influence the growth of cancer cells, slowing the development of these small latent

carcinomas (Adlercreutz, 1990). In Finland, although the intake of fat is high, the incidence of prostate cancer is much lower than in the USA (though higher than in Japan). Adlercreutz (1995) has suggested that this may be explained by a higher production of lignans in the gut due to the relatively high intake of whole-grain products (particularly rye bread) in the low incidence rural areas.

Messina *et al.* (1994) reviewed the evidence linking consumption of soya with prostate cancer risk from three studies and results are summarised in Table 6.4.

Several studies have investigated the role of dietary factors in prostate cancer risk and although some factors have been tentatively identified, results are not consistent (Table 6.5). Where associations have been demonstrated, these have generally been to various types of food (e.g. meat, fish, eggs, milk and cheese) rather than particular chemical constituents. The apparent association between increased consumption of green vegetables and reduced risk of prostate cancer should be the subject of further investigation. With the limited human data currently available, it is not possible to attribute a direct protective role to phytoestrogens on prostate cancer risk.

Based on experimental evidence, there is some evidence to suggest that genistein and related compounds may be suitable agents for clinical trials in relation to intervention and prevention of prostate cancer in humans (Karp *et al.*, 1996). Indeed, according to Barnes *et al.* (1995), a number of clinical trials were initiated in 1994 to investigate the effect of isolated soya protein on surrogate intermediate endpoint biomarkers of prostate cancer. These studies do not yet appear to have been published; the results are awaited with interest.

Table 6.4 Epidemiological studies relating intake of soya products with risk of prostate cancer*

Type of soya product	Type of study	Risk associated with intake	Estimate of relative risk	Reference
Fermented				
Miso soup	Cohort	No significant effect	0.76	Hirayama (1979)
Miso soup		No significant effect	1.24	Severson *et al.* (1989)
Miso soup	Case–control	No significant effect	0.64	Oishi *et al.* (1988)
Unfermented				
Tofu	Cohort	No significant effect	0.35	Severson *et al.* (1989)

* Adapted from Messina *et al.* (1994)

Table 6.5 Epidemiological studies relating various dietary factors with risk of prostate cancer

Study information	Findings	Reference
Cohort 122 261 Japanese men (63 cases), aged 40 or over	Increased consumption of green-yellow vegetables associated with lower prostate cancer mortality rate in subjects under 74 (not over 75)	Hirayama (1979)
Cohort 7999 Japanese Hawaiian men (174 cases)	Increased consumption of rice associated with decreased risk Increased consumption of seaweeds associated with increased risk No effect of consumption of fish, dairy products or fruit on risk	Severson *et al.* (1989)
Cohort 35 000 non-Hispanic white Seventh-day Adventist men (180 cases)	Increased consumption of beans, lentils, peas, fresh citrus fruit and nuts associated with decreased risk	Mills *et al.* (1989)
Case–control 100 prostate cancer cases, aged 50–79	Factors associated with an increased risk included low intake of bread, spinach and brakenfern, and high intake of mushrooms. No effect of consumption of seaweed or vegetables on risk	Oishi *et al.* (1988)

6.11 CANCER OF THE COLON AND RECTUM

An extensive association between oestrogens and colon cancer has been reported. In women, risk is increased with increasing age at first live birth and decreased with increasing parity (numbers of children). In addition, many colon tumours contain sex hormone receptors and these are thought to play a part in tumour development (Clarkson *et al*., 1995).

Rates of colon cancer vary throughout the world. According to Knight & Eden (1996), the incidence in Japanese males in Hawaii increased from 19 per 100 000 in 1962–1965 to 34 per 100 000 in 1978–1981, while the incidence in native Japanese males is 8.1 per 100 000. Figures for colon cancer in Japanese females between 1978–1981 were 19 per 100 000 in Hawaii and 6.7 per 100 000 in Japan. More recent data between 1981 and 1987 indicate the colon cancer incidence is two- to three-fold higher in Japanese Hawaiians (37 per 100 000 in males; 22 per 100 000 in females) than it is in Japanese in various areas of Japan (14–21 per 100 000 in males; 10–14 per 100 000 in females), with incidence rates as high, or higher, than those in white Hawaiians (32 per 100 000 in males; 24 per 100 000 in females; IARC, 1992). For rectal cancer, the incidence rates in females over this period were similar for native Japanese (6 to 9 per 100 000) and in Hawaii (7 per 100 000), although for males rates were slightly higher in Hawaiian Japanese (20 per 100 000 compared with 11–16 per 100 000 in Japan; IARC, 1992).

The observation that colon cancer incidence is low in countries where the diet is vegetarian or semi-vegetarian has led to suggestions that dietary factors may be important in its aetiology. The changes in incidence in Japanese migrants has been suggested to reflect the consequences of a move towards a more western-type diet, which contains lower levels of soya products and thus phytoestrogens (Knight & Eden, 1996). In recent years, the incidence of and mortality from colorectal cancer in Japan has increased to such an extent that the age-adjusted incidence is approaching that of the white population of the USA (Tamura *et al.*, 1996). This increase has also been attributed to changes in dietary habits towards a more western-type diet, and particularly to the increased amounts of fat and decreased amounts of fibre in the Japanese diet (Furukawa *et al*., 1995).

In a recent review, Marian (1996) examined the factors contributing to the development of colorectal cancer, both hereditary and nutritional. Most observed genetic defects in colon cancer affect growth control. Stimulators of growth are bile acids, 1,2-diglycerides and prostaglandins which stem from consumption of fat. Fruits and vegetables contain substances such as carotinoids, flavonoids and fibre which may inhibit growth. Diet is a complex mixture of constituents, some of which are tumour-enhancing and some of which are tumour inhibiting. Other plant constituents may also be biologically active. Nair *et al.* (1984) found that the risk of colon cancer appeared to be related to the ratio of plant sterols to cholesterol in the diets of non-vegetarians, and vegetarian and non-vegetarian Seventh Day Adventists (a population having a significantly lower standardised mortality ratio compared with the general US population). One of these plant sterols, β-sitosterol, has been shown to protect

against experimentally-induced colon cancer.

Dietary fibre has been shown to have a protective role against colon cancer, possibly reflecting the effects of high concentrations of lignans (Knight & Eden, 1995). Excretion of urinary lignans (assumed to reflect intake) is higher in subjects living in areas of low colon cancer risk (Adlercreutz, 1995). However, it has been suggested that the previously low risk of colon cancer in Japanese may not be explained by a high dietary fibre intake. When data on food consumption for 1959, 1970 and 1979 were used to prepare composite diets, Kuratsune *et al.* (1986) found that average intakes of non-starch polysaccharides did not exceed 13g per day, and were similar to intakes by Scandinavians and the British who have a high colon cancer risk. Jacobs (1986) concluded that if any source of dietary fibre is protective against cancer, it is probably vegetables, although they contain other factors which may also be protective.

In the review by Messina *et al.* (1994), none of the six studies investigating a relationship between soya product consumption and colon cancer risk demonstrated a significant protective effect, although relative risks of less than one were reported in three studies (see Table 6.6) Findings in three studies reporting effects of soya products on the risk of rectal cancer were inconsistent (Table 6.7). In the study by Hu *et al.* (1991), the most important protective factor against colon and rectal cancer was found to be increasing consumption of vegetables, particularly green vegetables (Chinese cabbage, chive, spinach, squash, cucumber, green beans, celery and green pepper). In contrast, Tajima & Tominaga, (1985) found high intakes of green–yellow vegetables (pumpkin, spinach and green pepper) to be associated with an increased risk of both colon and rectal cancer.

In a case–control study of dietary factors in 715 subjects with colorectal cancer and 727 age- and sex-matched controls, Kune *et al.* (1987a) found a significant interaction between total fibre and total vegetables, which showed a protective effect only with a high fibre and high vegetable combination. An independent protective effect by cruciferous vegetables (broccoli, Brussells sprouts, cabbage, cauliflower, swede, turnip and kale) was suggested, possibly due to the presence of indoles in these vegetables. However, intakes of cruciferous and leafy green vegetables were measured with least reliability of all the variables studied and so these data should be interpreted with caution (Kune *et al.*, 1987b).

Steinmetz *et al.* (1994) studied the effects of fruit and vegetable consumption on colon cancer risk in a prospective cohort study of 41 837 postmenopausal women in Iowa, USA from 1986, when a food frequency questionnaire was completed, to 1991, at which point there were 212 colon cancer cases. The majority of associations between consumption of fruit and vegetables and risk were weak and not statistically significant. Weak inverse associations with risk were found for consumption of all vegetables (although no dose–response was shown), legumes (confined to distal colon cancer) and dietary fibre, while a positive association of risk of proximal colon cancer was demonstrated for intake of cruciferous vegetables, attributable largely to cauliflower consumption.

Table 6.6 Epidemiological studies relating intake of soya products with risk of colon cancer*

Type of soya product	Type of study	Risk associated with intake	Estimate of relative risk	Reference
Fermented				
Fermented soyabeans	Case–control	Increased	1.6	Haenszel *et al.* (1973)
Miso soup		No significant effect	0.54	Tajima & Tominaga (1985)
Salted and fermented soya paste		No significant effect	NC	Hu *et al.* (1991)
Unfermented				
Soyabeans	Ecological	No significant effect	-0.08 (correlation)	McKeown-Eyssen & Bright-See (1984)
Soyabeans and bean curd	Case–control	No significant effect	0.63	Watanabe *et al.* (1984)
Tofu		No significant effect	1.08	Tajima & Tominaga (1985)
Tofu/soyabeans		No significant effect	0.53	Poole (1989)
Bean sprouts/bean curd/bean curd products		No significant effect	NC	Hu *et al.* (1991)

Table 6.7 Case–control studies relating intake of soya products with risk of rectal cancer*

Type of soya product	Risk associated with intake	Estimate of relative risk	Reference
Fermented			
Miso soup	Increased	2.05	Tajima & Tominaga (1985)
Salted and fermented soya paste	No significant effect	NC	Hu *et al.* (1991)
Unfermented			
Soyabeans and bean curd	Decreased	0.12	Watanabe *et al.* (1984)
Tofu	No significant effect	1.63	Tajima & Tominaga (1985)
Bean sprouts/bean curd/bean curd products	No significant effect	0.27	Hu *et al.* (1991)

* Adapted from Messina *et al.* (1994); NC - Not calculable from data in original paper

In summary, although there is evidence to suggest that increased consumption of fruits and vegetables are protective against colon and rectal cancer in humans, there is currently no evidence to attribute this effect to the presence of phytoestrogens in these foods. Whilst it is possible that these substances may play a role in protection, other potentially protective components such as fibre, vitamins, glucosinolates, phytic acid (inositol hexaphosphate) and plant sterols have also been described. Studies of soya products and colorectal cancer risk are limited and, in general, results have shown no significant associations.

Animal studies on the effects of phytoestrogens on colon or rectal carcinogenesis indicate that some of these compounds are protective in these systems. For example, linseed has been shown to decrease some early markers of colon carcinogenesis (aberrant crypts and foci), due, in part, to the presence of lignan precursors such as secoisolariciresinol diglycoside (Serraino & Thompson, 1992; Jenab & Thompson, 1996). *In vitro* experiments have shown that genistein, but not daidzein, inhibits inducible nitric oxide synthase activity in human intestinal cell culture; the expression and activity of this enzyme has been shown to be upregulated during inflammation and carcinogenesis in humans (Salzman *et al.*, 1996). While the activities of phytoestrogens demonstrated in these systems are suggestive of a protective role, human data to confirm or refute these observations are not available.

6.12 STOMACH CANCER

Although mortality from stomach cancer has been declining in recent years in Japan (Tominaga, 1995), the incidence in both Japanese men and women is one of the highest in the world (IARC, 1992). Compared with age-standardised rates in the USA, which, between 1983 and 1987 ranged from 6 per 100 000 to 17 per 100 000 in males, and from less than 3 per 100 000 to 8 per 100 000 in females, rates in Japan varied between 74 per 100 000 and 93 per 100 000 in males and 32 per 100 000 and 43 per 100 000 in females. Incidence rates of stomach cancer in Japanese between 1983 and 1987 were lower in those living in Hawaii (24 per 100 000 in males and 11 per 100 000 in females) than in those living in Japan.

In a study of gastric cancer among Japanese in Hawaii, Nomura *et al.* (1995) investigated risk factors in 250 cases diagnosed between 1968 and 1994 out of a total study population of 7972. Dietary information was collected using a 24-hour diet recall questionnaire at the time of induction into the study, between 1965 and 1968. Of the different food groups included, a significant negative association with risk was found only for vegetables (mostly cabbage, lettuce and tomatoes) and fruits (mostly papayas, oranges, apples, mangoes and guavas). An increased risk was associated with prior infection with *Helicobacter pylori*, cigarette smoking and a low serum level of ferritin; no association was found with levels of serum retinol, β-carotene or α-tocopherol.

The review by Messina *et al.* (1994) concluded that the evidence linking soya intake to risk of stomach cancer was inconsistent. Increased risks were noted more often with fermented soya products such as miso soup, while protective effects were observed more frequently with

intake of unfermented products including beans, bean curd (tofu) and soya milk. No studies indicated an increased risk associated with the consumption of unfermented soya products. Results are summarised in Table 6.8.

There is no evidence linking lignans to the risk of stomach cancer in humans, although a large literature exists suggesting that high levels of consumption of fruit and vegetables are associated with a reduced risk of stomach cancer (Palli, 1994). For example, Tsubono *et al.* (1997) investigated differences in dietary practices between five areas of Japan having a three-fold variation in stomach cancer risk. Between 1989 and 1991, 634 men and 373 spouses from the general population completed a food frequency questionnaire relating to consumption patterns over the previous month. When adjusted for sex and smoking, mortality from stomach cancer was positively associated with consumption of rice (rank correlation coefficient r=0.49), pickled vegetables (r=0.36) and bean paste soup (r=0.32) and negatively associated with intake of both green (r=-0.88) and yellow (r=-0.57) vegetables. Intake of fruit was not associated with a reduced risk when comparing the five areas. However, when the area with lowest mortality (also having the lowest intake) was excluded, a strong inverse correlation was observed (r=-0.67).

Lee *et al.* (1995) examined dietary factors and stomach cancer in a case–control study in Korea. A food frequency questionnaire was used to estimate consumption over the three years prior to hospital admission in 213 cases and in an equal number of controls. Foods associated with a significant increased risk included broiled fish ($p<0.01$ for trend), stewed foods (e.g. soyabean paste stew; $p<0.001$ for trend) and hot pepper-soyabean stew ($p<0.05$ for trend) which suggested salt may be an important risk factor. Foods showing apparent protective effects included mung bean pancakes ($p<0.001$ for trend), tofu ($p<0.01$ for trend), spinach ($p<0.001$ for trend) and cabbage ($p<0.05$ for trend). Total consumption of vegetables was not significantly associated with risk of stomach cancer. It was suggested that any protective effect of vegetables may have been masked by prevalent risk factors or a limited variability in vegetable consumption between cases and controls.

In summary, it is uncertain to what extent the apparent protective effects of fruit and vegetable consumption on the risk of stomach cancer may be attributable to the phytoestrogen content of these foods as this does not appear to have been studied directly, and other constituents such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and β-carotene have been suggested as potentially protective components. Migrant studies which indicate that stomach cancer risk is lower in Hawaiian Japanese than in native Japanese suggest that the diet of the latter may represent an increased risk. For soya, however, the evidence for beneficial effects of either fermented or unfermented soya products on stomach cancer risk is inconclusive. In addition, a potential confounder not accounted for in most of the studies reported is infection with *Helicobacter pylori*, a known risk factor and postulated cause of stomach cancer. In the UK, the risk of and mortality from stomach cancer is low. Further research would be necessary to investigate any effect of consumption of soya and also, more specifically, phytoestrogens on stomach cancer risk.

Table 6.8 Epidemiological studies relating intake of soya products with risk of stomach cancer*

Type of soya product	Type of study	Risk associated with intake	Estimate of relative risk	Reference
Fermented				
Fermented soyabeans	Ecological	Increased	NC	Hirayama (1971)
Miso soup		No significant effect	NC	Nagai *et al.* (1982)
Soyabean paste	Cohort	Decreased	0.573	Hirayama (1982)
Miso soup		No significant effect	0.9	Nomura *et al.* (1990)
Miso soup	Case–control	Decreased	0.43-0.55	Segi *et al.* (1957)
Fermented soyabean paste		Increased	10.3	Crane *et al.* (1970)
Miso		No significant effect	NC	Hirayama (1971)
Miso		No significant effect	1.31	Tajima & Tominaga (1985)
Salted and fermented soya paste		No significant effect	1.51	Hu *et al.* (1988)
Miso soup		Increased	1.9	Hoshiyama & Sasaba (1992)
Unfermented				
Tofu	Ecological	Decreased	NC	Hirayama (1971)
Other soya products		No significant effect	NC	Hirayama (1971)
Tofu		Decreased	NC	Nagai *et al.* (1982)
Tofu	Cohort	No significant effect	0.7	Nomura *et al.* (1990)
Tofu	Case–control	Decreased	0.69	Haenszel *et al.* (1972)
Tofu		No significant effect	1.52	Tajima & Tominaga (1985)
Soya milk		Decreased	0.41	Yingnan & Songlin (1986)
Soyabeans		Decreased	0.6	You *et al.* (1988)
Other soya products (excluding miso)		No significant effect	0.9	Hoshiyama & Sasaba (1992)

* Adapted from Messina *et al.* (1994)

NC - Not calculable from data presented in original paper

6.13 LUNG CANCER

In a review of the risk factors associated with lung cancer, Takkouche & GestalOtero (1996) observed that the major risk factor is tobacco use, which almost entirely explains the incidence trends in different parts of the world. Epidemiological evidence from retrospective and prospective observational studies and, more recently from case–control studies (e.g. Sankaranarayanan *et al*., 1994; Lei *et al*., 1996 and Du *et al*., 1996), suggests that consumption of fruit and vegetables in high amounts is protective. However, it is not certain which compounds are responsible and no individual dietary component has been shown to be particularly effective.

The review by Messina *et al.* (1994) included a summary of four case–control studies reporting effects of soya and soya products on the risk of lung cancer. Three of the these reported a significant protective effect of tofu on risk (Swanson *et al.*, 1992; Koo, 1988; Ershow *et al.*, 1990) although one was significant only for adenocarcinomas and large cell tumours among 88 non-smoking cases in Hong Kong (Koo, 1988) and that of Ershow and colleagues was a personal communication to Messina and co-workers. The study by Swanson and co-workers showed a dose–response relationship for increasing tofu consumption and decreasing relative risk of lung cancer in 428 male cases in Yunnan Province, China. The fourth case–control study found no association between consumption of either tofu or fermented soyabean paste with lung cancer risk in a population of 965 female Chinese patients (Wu-Williams *et al.,* 1990).

There are no data available on which to base an evaluation of the effect(s) of lignans on risk of lung cancer. The evidence for a protective effect of soya and soya products on lung cancer risk is limited.

6.14 CARDIOVASCULAR DISEASE/ATHEROSCLEROSIS

In females, the incidence of cardiovascular disease in premenopausal women is lower than in men of similar age, but rises in the postmenopausal years to a level approaching that in males. Elevated concentration of low density lipoprotein (LDL)-cholesterol is thought to be a central cause of coronary artery disease, and plasma LDL may be the primary source of lipid that accumulates in arterial walls as part of the atherosclerotic process (Wilcox & Blumenthal, 1995). Oestrogens, given as hormone replacement therapy, reduce the risk of cardiovascular disease; the mechanism of this is thought to be related to decreasing levels of LDL cholesterol, increasing levels of HDL cholesterol and effects on blood vessels (Knight & Eden, 1995; 1996).

Age-standardised mortality rates for coronary heart disease for men and women aged 40 to 69 years old were approximately 300 per 100 000 and 100 per 100 000, respectively, in the USA and 50 per 100 000 and 15 per 100 000, respectively, in Japan in 1986 (Beaglehole, 1990). Also, the incidence of cardiovascular disease is lower in Asian countries than in

western countries, and lower in vegetarians than in omnivores. These observations suggest that phytoestrogens are cardioprotective (Knight & Eden, 1995). However, it may be important to consider other components of the diet when attempting to identify potentially protective dietary factors. In an epidemiological study of dietary flavonoids (quercetin, kaempferol, myricetin, apigenin and luteolin) and coronary heart disease (CHD) risk, Hertog *et al.* (1993) assessed the flavonoid intake of 805 Dutch men aged 65–84 in 1985, using information from a dietary questionnaire (assessing usual food intake in the previous month) and by measuring levels in various commonly consumed foods. Subjects were followed up to 1990, by which time 43 had died of CHD, and myocardial infarction had occurred in 38 subjects with no previous history. Intake of flavonoids (mean of 25.9mg/day) was significantly negatively associated with mortality from CHD ($p=0.015$ for trend) and showed a non-significant inverse trend with incidence of myocardial infarction ($p=0.08$ for trend). Comparing the highest versus lowest tertile for intake of flavonoids, the relative risk for CHD was 0.42 (95%CI 0.20–0.88) which remained significant after adjustment for dietary variables (vitamins B and C, β-carotene and dietary fibre) and non-dietary risk factors. The major source of flavonoids in this population was not fruit and vegetables but tea, and tea consumption was also found to be negatively associated with CHD mortality. Postulated mechanisms for this effect of flavonoids include effects on platelet function, namely, anti-aggregatory action and inhibition of cyclo-oxygenase activity (which may have a role in reducing thrombosis), free radical scavenging effects through enhancement of the resistance of LDL to oxidation, or, in view of recent findings, a potential effect at the level of the vasculature. A number of *in vitro* studies have shown the protective effects of phytoestrogens against oxidative events. Indeed, the isoflavones, being structurally similar to the flavonoids, can act as phenolic antioxidants, their activity depending on the number of hydroxyl groups and their positions and arrangement in the chemical structure (Ruiz-Larrea *et al.*, 1997).

It is generally agreed that dietary soya protein is anti-atherogenic compared with animal proteins such as casein or lactalbumin, although it is unclear to what extent phytoestrogens are involved in this protective activity (Clarkson *et al.*, 1995). Setchell (1985) proposed that the cardioprotective effects of soya protein-rich diets may be explained by the phytoestrogen content while others have presumed that the amino acid composition is at least partly responsible (Erdman & Fordyce, 1989).

Several reviews of clinical studies investigating the effects of soya protein on cholesterol levels in adults have shown that substitution of soya protein for animal protein in the diet, or addition of soya protein to the diet, lowers total and LDL cholesterol (Carroll, 1991; Anderson *et al.*, 1995; Carroll & Kurowska, 1995; Potter, 1996). These effects are somewhat variable but are generally greater in hypercholesterolemic subjects (changes ranging from -4% to +6%) than in those who are normocholesterolemic (changes ranging from -12% to +1%). Most of the hypocholesterolemic effects of soya protein have been attributed to soya oestrogens (Anderson *et al.*, 1995), although this estimate has recently been questioned (Sirtori *et al.*, 1997). Soya protein diets have also been demonstrated to reduce levels of triglycerides, particularly in individuals with hypertriglyceridemia but they appear to have

little effect on levels of HDL cholesterol. Responses are generally similar between sexes but seem to be greater in younger than older subjects. Table 6.9 summarises data from 38 clinical studies on the effects of soya protein on serum lipids.

Table 6.9 Change in serum lipids and lipoprotein concentrations in subjects ingesting soya-containing diets compared with control diets*

	Number of studies	Number of subjects	Change (mg/dl)(95% Confidence interval)	Percent change
Total cholesterol	38	730	-23.2 (-32.9 to -13.5)	-9.3
LDL cholesterol	31	564	-21.7 (-31.7 to -11.2)	-12.9
HDL cholesterol	30	551	+1.2 (-3.1 to +5.4)	+2.4
VLDL cholesterol	20	255	-0.4 (-4.6 to +3.9)	-2.6
Triglycerides	30	628	-13.3 (-25.7 to -0.3)	-10.5

Net change expressed as change during soya-containing diet minus change during control diet.
VLDL - very-low-density lipoprotein
* Taken from Anderson *et al.* (1995)

More recent studies on the effects of soya, miso, Arcon F (an isoflavone-free soyabean product) and linseed on blood lipids are summarised in Table 6.10.

Experiments carried out *in vitro* suggest that genistein may interfere with many aspects of the coagulation system that are thought to promote vascular lesion development (Wilcox & Blumenthal, 1995). However, consumption of a soya protein powder (containing 80.3mg genistein, 35.6mg daidzein and 15.1mg glycitein) or a casein supplement (60g/day for 28 days) by 20 omnivorous male subjects produced no significant effects on platelet aggregation when assessed *ex vivo* (Gooderham *et al.*, 1996). Similarly, no changes were observed in the composition of plasma polyunsaturated fatty acids or levels of total or HDL cholesterol, although it was noted the subjects were normocholesterolemic at entry into the study. The plasma levels of isoflavones achieved by the soya supplement (907±245nmol/L genistein, 498±102nmol/L daidzein) were higher than those reported in Japanese subjects consuming a traditional diet.

In contrast to soya protein, linseed (as linseed oil) has been shown to have effects on platelet composition and function in humans but this is related to the presence of high concentrations of α-linolenic acid in this oil and not to its lignan content (Allman *et al.*, 1995).

Table 6.10 Summary of recent studies investigating effects of soya (as soya protein or TVP) or linseed on blood lipid levels in men and women

Subjects and dietary information	Effects	Reference
15 healthy non-vegetarian women One control cycle (normal diet) then one cycle on 60g TVP/day (45mg/day conjugated isoflavones, n=6) One control cycle (normal diet) then one cycle on 28g TVP/day (23mg/day conjugated isoflavones, n=6) One control cycle (normal diet) then one cycle on 50g miso/day (25mg/day unconjugated isoflavones, n=3) One control cycle (control diet) then one cycle on diet supplemented with 60g Arcon F (n=5)	 Mean cholesterol levels significantly decreased (by 9%) No significant changes No significant changes Significant increases in LDL, total cholesterol and LDL:HDL ratio*	Cassidy *et al.* (1995)
Pre- (n=14) and postmenopausal (n=10) Caucasian women (aged 29–58) Normal *ad libitum* diet supplemented with 38g soya protein/day (38mg/day genistein) for 6 months	No significant effect on levels of plasma cholesterol, HDL cholesterol or triglyceride	Petrakis *et al.* (1996)
Postmenopausal women (n=6) Diet supplemented with 60g TVP/day (45mg/day isoflavones) for 4 weeks	No significant effect on levels of total cholesterol, HDL or LDL	Cassidy *et al.* (1997)
Middle-aged men (n=6) Diet supplemented with 60g TVP/day (45mg/day isoflavones) for 4 weeks	No significant effect on levels of total cholesterol, HDL or LDL	Cassidy *et al.* (1997)
Healthy young adults (5 male, 5 female, aged 25±3 years) Diet supplemented with 50g linseed/day for 4 weeks	Plasma LDL cholesterol reduced by up to 8%	Cunnane *et al.* (1995)
Postmenopausal women (n=7) Diet supplemented with 40g linseed/day (27mg/day secoisolariciresinol) for 6 weeks	Levels of total cholesterol, LDL and HDL significantly reduced	Cunnane *et al.* (1995)
Middle-aged men (n=6) Diet supplemented with 40g linseed/day (27mg/day secoisolariciresinol) for 4 weeks	Levels of LDL significantly reduced	Cassidy *et al.* (1997)
Middle-aged men Diet supplemented with 40g linseed/day for 4 weeks	Levels of total cholesterol and LDL cholesterol significantly reduced	Hughes *et al.* (1994)

* Possibly attributable to fatty acids in the margarine used to make biscuits containing Arcon F

In summary, it is apparent that soya, some soya products and linseed oil have effects on blood lipid levels, particularly on cholesterol and LDL cholesterol. While the degree of reduction in levels of these blood lipids appears to be largely dependent on a subject's initial serum cholesterol level, the maximum reduction observed is of the order of 10–15%. Although for hyperlipidemic individuals this may not represent a very significant reduction, consumption of soya by the general population is likely to be protective against cardiovascular disease and atherosclerosis. However, components of soya and linseed other than phytoestrogens may contribute to or be responsible for the apparent hypocholesterolemic properties of these products. For example, phytosterols such as β-sitosterol in soya are structurally similar to cholesterol and, although they are poorly absorbed from the intestine, human studies have demonstrated that they reduce levels of serum or plasma total cholesterol and LDL cholesterol (Ling & Jones, 1995). In addition, certain types of dietary fibre have been shown to have a hypolipidemic effect, via their ability to increase faecal excretion of cholesterol and bile acids (Kritchevsky, 1987). However, based on studies in which rhesus monkeys were fed with soya isolates with and without isoflavones (Anthony *et al.*, 1995), it has been suggested that isoflavones may contribute up to 70% of the hypolipidemic effect of soya (Anderson *et al.*, 1995). Recently, this estimate has been questioned, based on the clinical observations of similar reductions in plasma cholesterol in studies using soya flour-derived TVP and soya isolates, the latter of which contains only 25-33% the amount of isoflavones as the former (Sirtori *et al.*, 1997). It is clear that further investigations will be necessary to understand the mechanism of cholesterol reduction by soya proteins.

For linseed, the protective effects against cardiovascular disease may be mediated at least in part by its α-linolenic acid content.

6.15 OTHER HEALTH EFFECTS

From the studies investigating associations between soya and soya-product consumption and cancers of the oesophagus, liver, bile duct and pancreas, the very limited data have generally shown no significant effect. There is currently insufficient information on which to base any further evaluation of the effects of soya on these diseases. It has been suggested that isoflavones may be useful in the treatment of alcohol abuse, based on an animal study showing that interperitoneal. administration of daidzein suppressed the desire for alcohol in ethanol-preferring hamsters (Keung & Vallee, 1993). Until further data become available, it is not possible to assess the relevance of this experimental observation to man.

References

Adler JH (1965) Antioestrogenic activity in fahli clover hay and oat hay. *Acta Endocrin,* 49, 90-96

Adlercreutz H (1984) Does fiber-rich food containing animal lignan precursors protect against both colon and breast cancer? An extension of the "Fiber Hypothesis". *Gastroenterology,* 86, 761-766

Adlercreutz H (1990) Western diet and western diseases: Some hormonal and biochemical mechanisms and associations. *Scand J Clin Lab Invest,* 50, 3-23

Adlercreutz H (1995) Phytoestrogens: Epidemiology and possible role in cancer protection. *Environ Health Perspect,* 103 (suppl 7), 103-112

Adlercreutz H, Fotsis T, Bannwart C, Wakela K, Mäkelä T, Brunow G & Hase T (1986) Determination of urinary lignans and phytoestrogen metabolite, potential antiestrogens and anticarcinogens, in urine of women on various habitual diets. *J Steroid Biochem,* 25, 791-797

Adlercreutz H, Fotsis T, Heikkinen R, Dwyer JT, Woods M, Goldin BR & Gorbach SL (1982) Excretion of lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and in women with breast cancer. *Lancet,* 2, 1295-1298

Adlercreutz H, Fotsis T, Lampe J, Wähälä K, Mäkelä T, Brunow G & Hase T (1993b) Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas chromatography-mass spectrometry. *Scand J Clin Lab Invest,* 53, 5-18

Adlercreutz H, Hämäläinen E, Gorbach lS, Goldin RB, Woods NM & Dwyer TJ (1989) Diet and plasma androgens in postmenopausal vegetarian and omnivorous women and postmenopausal women with breast cancer. *Am J Clin Nutr,* 49, 433-442

Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa T & Okada H (1991) Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutr,* 54, 1093-1100

Adlercreutz H, Markkanen H & Watanabe S (1993a) Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet,* 342, 1209-1210

Adlercreutz H, Mousavi Y, Clark J, Höckerstedt K, Hämäläinen E, Wähälä K, Mäkelä T & Hase T (1992) Dietary phytoestrogens and cancer: *In vitro* and *In vivo* studies. *J Steroid Biochem Mol Biol,* 41, 331-337

Adlercreutz H, van der Wildt J, Kinzel J, Attalla H, Wähälä K, Mäkelä T, Hase T & Fotsis T (1995) Lignan and isoflavonoid conjugates in human urine. *Ster Biochem Molec Biol,* 52, 97-103

Agnusdei D, Adami S, Cervetti R, Crepaldi G, Di Munno O, Fantasia L, Isaia GC, Letizia G, Ortolani S, Passeri M, Serni U, Vecchiet L & Gennari C (1992) Effects of ipriflavone on bone mass and calcium metabolism in postmenopausal osteoporosis. *Bone Miner,* 19, S43-S48

Alldrick AJ, Lake BG, Mallet AK & Rowland IR (1988) Modulation of hepatic phase I and gut-microflora xenobiotic metabolizing enzymes by dietary flavonoids. *Plant Flavonoids in Biology and Medicine II Symposium Proceedings: Biochemical, Cellular and Medicinal Properties,* New York, USA, AR Liss, pp 139-142

Allman MA, Pena MM & Pang D (1995) Supplementation with flaxseed oil versus sunflowerseed oil in healthy young men consuming a low fat diet: Effects on platelet composition and function. *Eur J Clin Nutr,* 49, 169-178

Anderson WJ, Johnstone MB & Cook-Newell EM (1995) Mata-analysis of the effects of soy protein intake on serum lipids. *New Engl J Med,* 333, 276-282

Anthony MS, Clarkson TB, Weddle DL & Wolfe MS (1995) Effects of soy protein phytoestrogens on cardiovascular risk factors in rhesus monkeys. *J Nutr,* 125 (suppl), 803-804

Armstrong B & Doll R (1975) Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int J Cancer,* 15, 617-631

Arnold SF, Collins BM, Robinson MK, Guillette LJ, Jr. & McLachlan JA (1996) Differential interaction of natural and synthetic estrogens with extracellular binding proteins in a yeast estrogen screen. *Steroids,* 61, 642-646

Axelson M & Setchell KDR (1980) Conjugation of lignans in human urine. *Febs Lett,* 122, 49-53

Axelson M, Sjovall J, Gustafsson BE & Setchell KDR (1984) Soya - a dietary source of the non-steroidal oesrtrogen equol in man and animals. *Endocrinology,* 102, 46-56

Baird DD, Umbach DM, Lansdell L, Hughes CL, Setchell KDR, Weinberg CR, Haney AF, Wilcox AJ & McLachlan JA (1995) Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women. *Clin Endocrinol Metab,* 80, 1685-1690

Banyai Z (1995) The place of ipriflavone in the osteoporosis therapy. *Acta Pharm Hunagrica,* 65, 229-232

Barnes S, Peterson G & Coward L (1995) Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. *J Cell Biochem,* 22 (suppl), 181-187

Beaglehole R (1990) International trends in coronary heart disease mortality, morbidity, and risk factors. *Epidemiol Rev,* 12, 1-15

Bennetts HW, Underwood EJ & Shier FL (1946) A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Aust Vet J,* 22, 2-12

Bickoff EM, Booth AN, Livingston AL & Hendrickson AP (1960b) Observations on the effect of drying on estrogenic activity of alfalfa samples of varying maturity. *J Anim Sci,* 19, 745-753

Bickoff EM, Booth AN, Livingston AL & Hendrickson AP (1961) Estrogenic activity of fresh and dried red subterranean clovers. *J Anim Sci,* 20, 133-136

Bickoff EM, Livingston AL, Booth AN, Hendrickson AP & Kohler GO (1960a) Estrogenic activity in dehydrated and suncured forages. *J Anim Sci,* 19, 189-197

Bickoff EM, Livingston AL, Hendrickson AP & Booth AN (1962) Relative potencies of several estrogen like compounds found in forages. *Agric Food Chem,* 10, 410-414

Bieber MA (1986) Lack of oestrogen-like activity in commercially refined vegetable oils. *Food Chem Toxicol,* 24, 251-253

Blair HC, Jordan SE, Peterson TG & Barnes S (1996) Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. *J Cell Biochem,* 61, 629-637

Bokkenheuser VD, Shackleton CHL & Winter J (1987) Hydrolysis of dietary flavonoid glycosides by strains of intestinal *Bacteroides* from humans. *Biochem J,* 248, 953-956

Borriello SP, Setchell KDR, Axelson M & Lawson AM (1985) Production and metabolism of lignans by the human faecal flora. *J Appl Bacteriol,* 58, 37-43

Bradbury RB & White DE (1954) Estrogens and related substances in plants. *Vitamins Hormones,* 12, 207-233

Bradlow HL, Davis DL, Lin G, Sepkovic D & Tiwari R (1995) Effects of pesticides on the ratio of 16 alpha/2-hydroxyestrone: A biologic marker of breast cancer risk. *Environ Health Perspect,* 103 (suppl 7), 147-150

Brandi ML (1992) Flavonoids: Biochemical effects and therapeutic applications. *Bone Miner,* 19 (suppl), S3-S14

Bruning PF, Bonfrer JMG & Hart AAM (1985) Non-protein bound estradiol, sex hormone binding globulin, breast cancer and breast cancer risk. *Brit J Cancer,* 51, 479-484

Carroll KK (1991) Review of clinical studies on cholesterol-lowering response to soy protein. *J Am Diet Assoc,* 91, 820-827

Carroll KK & Kurowska EM (1995) Soy consumption and cholesterol reduction: Review of animal and human studies. *J Nutr,* 125 (suppl 3), 594S-597S

Cassidy A (1996) Breast cancer: weighing up the dietary evidence. *Breast,* 5, 389-397

Cassidy A, Bingham S & Setchell KDR (1994) Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr,* 60, 333-340

Cassidy A, Bingham S & Setchell KDR (1995) Biological effects of isoflavones in young women; importance of the chemical compostition of soyabean products. *Brit J Nutr,* 74, 587-601

Cassidy A, Faughnan M, Hughes R, Fraser C, Cathcart A, Taylor N, Setchell KDR & Bingham S Endocrine modifying effects of phytoestrogens in postmenopausal women and middle aged men. Am J Clin Nutr, (In Press)

Chapin RE, Stevens JT, Hughes CL, Kelce WR, Hess RA & Daston GP (1996) Endocrine modulation of reproduction. *Fundam Appl Toxicol,* 29, 1-17

Clarkson TB, Anthony MS & Hughes CL, Jr. (1995) Estrogenic soybean isoflavones and chronic disease: Risks and benefits. *Trends Endocrinol Metab,* 6, 11-16

Clevenger S (1964) Flower pigments. *Sci Am,* 210, 84-92

Crane PS, Rhee SU & Seel DJ (1970) Experience with 1,079 cases of cancer of the stomach as seen in Korea from 1962 to 1968. *Am J Surg,* 120, 747-751

Cunnane CS, Hamadeh MJ, Liede AC, Thompson LU, Wolever TMS & Jenkins DJA (1995) Nutritional attributes of traditional flaxseed in health young adults. *Am J Clin Nutr,* 61, 62-68

Davies LH & Bennett D (1962) Studies on the oestrogenic potency of subterranean clover *(Trifolium subterraneum L.)* In south-western Australia. *Aust J Agric Res,* 13, 1030-1040

Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, Forman M, Campbell WS, Franz C, Kahle L & Taylor PR (1996) Relation of energy, fat, and fiber intakes to plasma concentrations of estrogens and androgens in premenopausal women. *Am J Clin Nutr,* 64, 25-31

Dos Santos Silva I & Swerdlow AJ (1995) Recent trends in incidence and mortality form breast, ovarian and endometrial cancers in England and Wales and their relation to changing fertility and oral contraceptive use. *Brit J Cancer,* 72, 485-492

Du YX, Cha Q, Chen XW, Chen YZ, Huang LF, Feng ZZ, Wu XF & Wu JM (1996) An epidemiological study of risk factors for lung cancer in Guangzhou, China. *Lung Cancer,* 14 (suppl 1), S9-S37

Dunn JE, Jr (1975) Cancer epidemiology in populations of the United States with emphasis on Hawaii and California and Japan. *Cancer Res,* 35, 3240-3245

ENDS (1996) Supermarkets demand segregation of genetically modified soya. *ENDS Report,* 258, 26

Engle A, Muscat JE & Harris RE (1991) Nutitional risk factors and ovarian cancer. *Nutr Cancer,* 15, 239-247

Erdman WJ & Fordyce JE (1989) Soy products and the human diet. *Am J Clin Nutr,* 49, 725-737

Ershow AG, Gao YI, Zheng W & Blot WJ (1990) Diet effects on lung cancer risk in Chinese men and women. [Abstract] *FASEB J,* 4, A1041

European Commission (1997) *European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife: Report of the Proceedings,* (EUR 17549), Weybridge, UK, European Commission

Finegold MS, Attebery RH & Sutter LV (1974) Effect of diet on human fecal flora: comparison of Japanese and American diets. *Am J Clin Nutr,* 27, 1456-1469

Finegold MS & Sutter LV (1978) Fecal flora in different populations, with special reference to diet. *Am J Clin Nutr,* 31, S116-S122

Flux DS, Munford RE & Wilson GF (1963) Biological estimation of oestrogenic activity in red clover *(Trifolium pratense)*: relative potencies of parts of plant and changes with storage. *J Dairy Res,* 30, 243-249

Folman Y & Pope GS (1966) The interaction in the immature mouse of potent oestrogens with coumestrol, genestein and other utero- vaginotrphic compounds of low potency. *J Endocrinol,* 34, 215-225

Francis CM & Hume ID (1971) The relationship between lignification and flavonoid production in subterranean clover-D. *Aust J Biol Sci,* 24, 1-5

Fukshima M, Mori M, Hara M & Kudo R (1993) Frequent consumption of vegetables and the decreased risk of ovarian cancer. *Tumor Res,* 28, 1-8

Fukushima D (1985) Fermented vegetable protein and related foods of Japan and China. *Food Rev Inter,* 1, 149-209

Furukawa K, Yamamoto I, Tanida N, Tsujiai T, Nishikawa M, Narisawa T & Shimoyama T (1995) The effects of dietary fiber from *Lagenaria scineraria (yugao- melon)* on colonic carcinogenesis in mice. *Cancer,* 75 (suppl), 1508-1515

Galey FD, Mendez L, Whitehead WE, Holstege DM, Plumlee K & Johnson B (1993) Estrogenic activity in forages: Diagnostic use of the classical mouse uterine bioassay. *J Vet Diagn Invest,* 5, 603-608

Gambacciani M, Spinetti A, Piaggesi L, Cappagli B, Taponeco F, Manetti P, Weiss C, Teti GC, Commare P & Facchini V (1994) Ipriflavone prevents the bone mass reduction in premenopausal women treated with gonadotrophin hormone-releasing hormone. *Bone Miner,* 26, 19-26

Goldin BR, Lombardi P, Mayhew J & Gorbach SL (1981) Factors that affect intestinal bacterial activity: Implications for colon carcinogenesis. In: Bruce WR, Correa P, Lipkin M, Tannenbaum SR & Wilkins TD, eds, *Banbury Report Number 7: Gastrointestinal Cancer: Endogenous Factors,* Cold Spring Harbour NY, USA, Cold Spring Harbour Laboratory, pp 41-52

Gooderham MJ, Adlercreutz H, Ojala ST, Wähälä K & Holub BJ (1996) A soy protein isolate rich in genistein and daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men. *J Nutr,* 126, 2000-2006

Gray GE, Pike MC & Henderson BE (1979) Breast-cancer incidence and mortality rates in different countries in relation to known risk factors and dietary practices. *Brit J Cancer,* 39, 1-7

Hacking A, Rosser WR & Dervish MT (1976) Zearalenone-producing species of *Fusarium* on barley seed. *Am Appl Biol,* 84, 7-11

Haenszel W, Berg JW, Segi M, Kurihara M & Locke FB (1973) Large-bowel cancer in Hawaiian Japanese. *J Natl Cancer Inst,* 51, 1765-1779

Haenszel W, Hurihara M, Segi M & Lee RKC (1972) Stomach cancer among Japanese in Hawaii. *J Natl Cancer Inst,* 49, 969-988

Haenszel W & Kurihara M (1968) Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. *J Natl Cancer Inst,* 40, 43-68

Hanson CH, Loper GM, Kohler GO, Bickoff EM, Taylor KW, Kehr WR, Stanford EH, Dudley JW, Pedersen MW, Sorensen EL, Carnahan HL & Wilsie CP (1965) *Variation in Coumestrol Content of Alfalfa: As Related to Location, Variety, Cutting, Year, Stage of Growth and Disease* , Washington DC, USA, United States Department of Agriculture

Henderson BE & Bernstein L (1991) The international variation in breast cancer rates: An epidemiological assessment. *Breast Cancer Res Treat,* 18, S11-S17

Hill H & Austin H (1996) Nutrition and endometrial cancer. *Cancer Causes Control,* 7, 19-32

Hirai K, Shimiazu C, Takezor R & Ozeki Y (1986) Cholesterol, phytosterol and polyunsaturated fatty acid levels in 1982 and 1957 Japanese diets. *J Nutr Sci Vitaminol (Tokyo),* 32, 363-372

Hirayama T (1971) Epidemiology of stomach cancer. *GANN Monogr Cancer Res,* 11, 3-19

Hirayama T (1979) Epidemiology of prostate cancer with special reference to the role of diet. *Natl Cancer Inst Monogr,* 53, 149-155

Hirayama T (1982) Relationship of soybean paste soup intake to gastric cancer risk. *Nutr Cancer,* 3, 223-233

Hobson W, Bailey J, Kowalk A & Fuller G (1977) The hormonal potency of zearalenone in nonhuman primates. In: *Mycotoxins in Human and Animal Health. Proceedings of a Conference,* Park Forest South IL, USA, Pathotox Publishers, pp 365-377

Holdeman VL, Good JI & Moore CEW (1976) Human fecal flora: Variation in bacterial composition within individuals and a possible effect of emotional strees. *Appl Environ Microbiol,* 31, 359-375

Horn Ross PL (1995) Phytoestrogens, body composition, and breast cancer. *Cancer Causes Control,* 6, 567-573

Hoshiyama Y & Sasaba T (1992) A case–control study of stomach cancer and its relation to diet, cigarettes and alcohol comsumption in Saitama Perfecture Japan. *Cancer Causes Control,* 3, 441-448

Hsieh C, Trichopoulos D, Katsouyanni K & Yuasa S (1990) Age at menarche, age at menopause, height and obesity as risk factors for breast cancer: Associations and interactions in an international case–control study. *Int J Cancer,* 46, 796-800

Hu JF, Liu YY, Yu YK, Zhao TZ, Liu SD & Wang QQ (1991) Diet and cancer of the colon and rectum: A case–control study in China. *Int J Epidemiol,* 20, 362-367

Hu JF, Zhang SF, Jia EM, Wang QQ, Liu SD, Liu YY, Wu YP & Cheng YT (1988) Diet and cancer of the stomach: A case–control study in China. *Int J Cancer,* 120, 331-335

Hughes CL, Jr. (1988) Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens. *Environ Health Perspect,* 78, 171-175

Hutchins AM, Lamp JW, Martini MC, Campbell DR & Slavin JL (1995b) Vegetables, fruits and legumes: effect on urinary isoflavonoid phytoestrogen and lignan excretion. *J Am Diet Assoc,* 95, 769-774

IARC (1987) *Cancer incidence in five continents, Volume V,* Lyon, France, International Agency for Research on Cancer

IARC (1992) *Cancer incidence in five continents, Volume VI* (IARC Scientific Publication No 120), Lyon, France, International Agency for Research on Cancer

IEH (1995) *IEH Assessment on Environmental Oestrogens: Consequences to Human Health and Wildlife* (Assessment A1), Leicester, UK, Institute for Environment and Health

Jacobs LR (1986) Relationship between dietary fiber and cancer: Metabolic, physiologic, and cellular mechanisms. *Proc Soc Exper Biol Med,* 183, 299-310

Jenab M & Thompson LU (1996) The influence of flax seed and lignans on colon carcinogenesis and beta-glucuronidase activity. *Carcinogenesis,* 17, 1343-1348

Kallela K, Saastamoinen I & Huokuna E (1987) Variations in the content of plant oestrogens in red clover-timothy-grass during the growing season. *Acta Vet Scand,* 28, 255-262

Karp JE, Chiarodo A, Brawley O & Kelloff GJ (1996) Prostate cancer prevention: Investigational approaches and opportunities. *Cancer Res,* 56, 5547-5556

Katzenellenbogen BS, Katzenellenbogen JA & Mordecain D (1979) Zearelanones: Characterisation of the oestrogenic potencies and receptor interactions of a series of fungal b-resorcylic acid lactones. *Endocrinology,* 105, 33-50

Kelly GE, Joannou GE, Reeder AY, Nelson C & Waring MA (1995) The variable metabolic response to dietary isoflavones in humans. *Proc Soc Exper Biol Med,* 208, 40-43

Kelsey JL & Hore-Ross PL (1993) Breast cancer: Magnitude of the problem and descriptive epidemiology. *Epidemiol Rev,* 15, 7-15

Keung WM & Vallee BL (1993) Daidzin and daidzein suppress free-choice ethanol intake by syrian Golden hamsters. *Proc Soc Nat Acad Sci USA,* 90, 10008-10012

Kitts WD, Swierstra E, Brink VC & Wood AJ (1959) The estrogen-like substances in certain legumes and grasses. *Can J Anim Sci,* 39, 158-163

Knight DC & Eden JA (1995) Phytoestrogens - A short review. *Maturitas,* 22, 167-175

Knight DC & Eden JA (1996) A review of the clinical effects of phytoestrogens. *Obstet Gynecol,* 87, 897-904

Koo LC (1988) Dietary habits and lung cancer risk among Chinese females in Hong Kong who never smoked. *Nutr Cancer,* 11, 155-172

Kopcewicz J (1971) Estrogens in developing bean *(Phaseolus Vulgaris)* plants. *Phytochemistry,* 10, 1423-1427

Kovacs AB (1994) Efficacy of ipriflavone in the prevention and treatment of postmenopausal osteoporosis. *Agents Actions,* 41, 86-87

Kritchevsky D (1987) Dietary fibre and lipid metabolism. *Int J Obesity,* 11 (Suppl 1), 33-43

Kuhnau J (1976) The flavanoids. A class of semi-essential food components: Their role in human nutrition. *Wld Rev Nutr Diet,* 24, 117-191

Kuiper GGJM & Gustafsson JA (1997) The novel estrogen receptor-beta subtype: Potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. *Febs Lett,* 410, 87-90

Kumagai S & Shimizu T (1982) Neonatal exposure to zearalenone causes persistent anovulatory estrus in the rat. *Arch Toxicol,* 50, 279-286

Kune S, Kune GA & Watson LF (1987a) Observation on the reliability and validity of the design and diet history method in the Melbourne colorectal cancer study. *Nutr Cancer,* 9, 5-20

Kune S, Kune GA & Watson LF (1987b) Case–control study of dietary etiological factors: The melbourne colorectal cancer study. *Nutr Cancer,* 9, 21-42

Kuratsune M, Honda T, Englyst HN & Cummings JH (1986) Dietary fiber in the Japanese diet as investigated in connection with colon cancer risk. *Jpn J Cancer Res (Gann),* 77, 736-738

Kurzer MS, Lampe JW, Martini MC & Adlercreutz H (1995) Fecal lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. *Cancer Epidemiol Biomark Prev,* 4, 353-358

La Vecchia C (1989) Nutritional factors and cancers of the breast, endometrium and ovary. *Eur J Cancer Clin Oncol,* 25, 1945-1951

La Vecchia C, Decarli A, Fasoli M & Gentile A (1986) Nutrition and diet in the etiology of endomentrial cancer. *Cancer,* 57, 1248-1253

La Vecchia C, Decarli A, Negri E, Parazzini F, Gentile A, Cecchetti G, Fasoli M & Francheschi S (1987) Dietary factors and the risk of epithelial ovarian cancer. *J Natl Cancer Inst,* 79, 663-669

Lee HP, Gourley L, Duffy SW, Esteve J, Lee J & Day NE (1991) Dietary effects on breast-cancer risk in Singapore. *Lancet,* 337, 1197-1200

Lee J-K, Park B-J, Yoo K-Y & Ahn Y-O (1995) Dietary factors and stomach cancer: A case–control study in Korea. *Int J Epidemiol,* 24, 33-41

Lei YX, Cai WC, Chen YZ & Du YX (1996) Some lifestyle factors in human lung cancer: A case–control study of 792 lung cancer cases. *Lung Cancer,* 14 (suppl 1), S121-S136

Lien LL & Lien EJ (1996) Hormone therapy and phytoestrogens. *J Clin Pharm Ther,* 21, 101-111

Liener IE (1994) Implications of antinutritional components in soybean foods. *Crit Rev Food Sci Nutr,* 34, 31-67

Lindsay DR & Kelly RW (1970) The metabolism of phyto-estrogens in sheep. *Aust Vet J,* 46, 219-222

Ling WH & Jones PJH (1995) Dietary phytosterols: A review of metabolism, benefits and side effects. *Life Sci,* 57, 195-206

Little DA (1976) Assessment of several pasture species, particularly tropical legumes, for oestrogenic activity. *Aust J Agric Res,* 27, 681-686

Lloyd Davies H & Dudzinski ML (1865) Further studies on oestrogenic activity in strains of subterranean clover *(Trifolium subterraneum L.)* in South-western Australia. *Aust J Agric Res,* 16, 937-950

Lu LJW, Anderson KE, Grady JJ & Nagamani M (1996) Effects of soya consumption for one month on steroid hormones in premenopausal women: Implications for breast cancer risk reduction. *Cancer Epidemiol Biomark Prev,* 5, 63-70

Lu LJW, Broemeling LD, Marshall MV & Ramanujam VMS (1995) A simplified method to quantify isoflavones in commercial soybean diets and human urine after legume consumption. *Cancer Epidemiol Biomark Prev,* 4, 497-503

Mader JA & Macdonald IA (1985) Effect of bile acids on formation of the mutagen, quercetin, from two flavonol glycoside precursors by human gut bacterial preparations. *Mutat Res,* 155, 99-104

MAFF (1996) *Natural toxicants in food, Paper No. 2: Inherant natural toxicants in food* (Food Surveillance Paper No. 51), London, UK, HMSO

Marian B (1996) How does diet contribute to colon cancer development? Interaction between alteration and nutrients in the deregulation of growth. *Onkologie,* 19, 132-139

Markiewicz L, Garey J, Aldercreutz H & Gurpide E (1993) *In-vitro* bioassays of non-steroidal phytoestrogens. *J Steroid Biochem Mol Biol,* 45, 399-405

Martin PM, Horwitz KB, Ryan DS & McGuire WL (1978) Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology,* 103, 1860-1867

Mayr U, Butsch A & Schneider S (1992) Validation of two *in vitro* test systems for estrogenic activities with zearalenone, phytoestrogens and cereal extracts. *Toxicology,* 74, 135-149

McKeown-Eyssen GE & Bright-See E (1984) Dietary factors in colon cancer: International relationships. *Nutr Cancer,* 6, 160-170

Medlock KL, Branham WS & Sheehan DM (1995) Effects of coumestrol and equol on the developing reproductive tract of the rat. *Proc Soc Exper Biol Med,* 208, 67-71

Melis GB, Paoleti AM, Cagnacci A, Bufalino L, Spinetti A, Gambacciani M & Fioretti P (1992) Lack of any estrogenic effect of ipriflavone in postmenopausal women. *Endocrinol Invest,* 15, 755-761

Messina M (1995) Isoflavone intake by Japanese were overestimated. *Am J Clin Nutr,* 62, 645

Messina M & Barnes S (1991) The role of soy products in reducing risk of cancer. *J Natl Cancer Inst,* 83, 541-546

Messina MJ, Persky V, Setchell KDR & Barnes S (1994) Soy intake and cancer risk: A review of the *In Vitro* and *In Vivo* data. *Nutr Cancer,* 21, 113-131

Millington AJ, Francis CM & McKeown NR (1964a) Wether bioassay of annual pasture legumes. I. Oestrogenic activity in medicago tribuloides dest. var. Cyprus* relative to four strains of trifolium subterraneum L. *Aust J Agric Res,* 15, 520-526

Millington AJ, Francis CM & McKeown NR (1964b) Wether bioassay of annual pasture legumes. II. The oestrogenic activity of nine strains of *Trifolium subterraneum L.*. *Aust J Agric Res,* 15, 527-536

Mills PK, Beeson WL, Phillips RL & Fraser GE (1989) Cohort study of diet, lifestyle, and prostate cancer in advetist men. *Cancer,* 64, 598-604

Montani ML, Vaamonde G, Resnik SL & Buera P (1988) Influence of water activity and temperature on the accumulation of zearalenone in corn. *Int J Food Microbiol,* 6, 1-8

Moore WEC, Cato EP, Good IJ & Holdeman LV (1981) The effect of diet on human fecal flora. In: Bruce WR, Correa P, Lipkin M, Tannenbaum SR & Wilkins TD, eds, *Banbury Report Number 7: Gastrointestinal Cancer: Endogenous Factors,* Cold Spring Harbour NY, USA, Cold Spring Harbour Laboratory, pp 11-24

Moore WEC & Holdeman LV (1974) Human fecal flora: The normal flora of 20 Japanese-Hawaiians. *Appl Microbiol,* 27, 961-979

Moore WEC & Moore LH (1995) Intestinal floras of populations that have a high risk of colon cancer. *Appl Environ Microbiol,* 61, 3203-3207

Morley FHW & Francis CM (1968) Varietal and environmental variations in isoflavone concentrations in subterranean clover *(Trifolium subterraneum L.)*. *Aust J Agric Res,* 19, 15-26

Morton SM, Wilcox G, Wahlqvist ML & Griffiths K (1994) Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. *Endocrinology,* 142, 251-259

Murkies AL, Lombard C, Strauss BJG, Wilcox G, Burger HG & Morton MS (1995) Dietary flour supplementation decreases post-menopausal hot flushes: Effect of soy and wheat. *Maturitas,* 21, 189-195

Nagai M, Hashimoto T, Yanagawa H, Yokoyama H & Minowa M (1982) Relationship of diet to the incidence of esophageal and stomach cancer in Japan. *Nutr Cancer,* 3, 257-268

Naim H, Gestetrer B, Zilkah S, Bisk Y & Bondi A (1974) Soy bean isoflavones, characterisation, determination and antifungal activity. *J Agric Food Chem,* 22, 806-810

Nair PP, Turjman N, Kessie G, Calkins B, Goodman TG, Davidovitz H & Nimmagadda G (1984) Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. *Am J Clin Nutr,* 40, 927-930

Nomura A, Grove JS, Stemmermann GN & Severson RK (1990) A prospective study of stomach cancer and its relation to diet, cigarettes, and alcohol consumption. *Cancer Res,* 50, 627-631

Nomura A, Henderson BE & Lee J (1978) Breast cancer and diet among the Japanese in Hawaii. *Am J Clin Nutr,* 31, 2020-2025

Nomura AMY, Stemmermann GN & Chyou P-H (1995) Gastric cancer among the Japanese in Hawaii. *Jpn J Cancer Res,* 86, 916-923

Oishi K, Okada K, Yoshida O, Yamabe H, Ohno Y, Hayes RB & Schroeder FH (1988) A case–control study of prostatic cancer with reference to dietary habits. *Prostate,* 12, 179-190

Palli D (1994) Dietary factors. *Eur J Gastroenterol Hepatol,* 6, 1076-1082

Parkin DM (1989) Cancers of the breast, endometrium and ovary: Geographic correlations. *Eur J Cancer Clin Oncol,* 25, 1917-1925

Persky V & Van Horn L (1995) Epidemiology of soy and cancer: Perspectives and directions. *J Nutr,* 125, 709S-712S

Petilli M, Fiorelli G, Benvenuti S, Frediani U, Gori F & Brandi ML (1995) Interactions between ipriflavone and the estrogen receptor. *Calcif Tissue Inter,* 56, 160-165

Petrakis NL, Barnes S, King EB, Lowenstein J, Wiencke J, Lee MM, Miike R, Kirk M & Coward L (1996) Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. *Cancer Epidemiol Biomark Prev,* 5, 785-794

Phipps WR, Martini MC, Lampe JW, Slavin JL & Kurzer MS (1993) Effect of flax seed ingestion on the menstrual cycle. *J Clin Endocrinol Metab,* 77, 1215-1219

Poole C (1989) A case–control study of diet and colon cancer. Boston MA, USA, Harvard School of Public Health,

Potter SM (1996) Soy proteins and serum lipids. *Curr Opin Lipid,* 7, 260-264

Prelusky DB, Scott PM, Trenholm HL & Lawrence GA (1990) Minimal transmission of zearaleone to milk of dairy cows. *J Environ Sci Health - Part B,* 25, 87-103

Price KR & Fenwick GR (1985) Naturally occuring oestrogens on food - a review. *Food Addit contam,* 2, 73-106

Risch HA, Jain M, Marrett LD & Howe GR (1994) Dietary fat intake and risk of epithelial ovarian cancer. *J Natl Cancer Inst,* 86, 1409-1415

Rosenblum ER, Stauber RE, Van Thiel DH, Campbell IM & Gavaler JS (1993) Assessment of the estrogenic activity of phytoestrogens isolated from bourbon and beer. *Alcohol Clin Exper Res,* 17, 1207-1209

Rossiter RC & Beck AB (1966c) Physiological and ecological studies on the oestrogenic isoflavones on subterranean clover *(T. subterraneum L.)*I. Effects of temperature. *Aust J Agric Res,* 17, 29-37

Rossiter RC & Beck AB (1966a) Physiological and ecological studies on the oestrogenic isoflavones on subterranean clover *(T. subterraneum L.)* V. Ontogenetic changes. *Aust J Agric Res,* 18, 561-573

Rossiter RC & Beck AB (1966b) Physiological and ecological studies on the oestrogenic isoflavones on subterranean clover *(T. subterraneum L.)* II. Effects of phosphate supply. *Aust J Agric Res,* 17, 447-456

Ruiz Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP & Rice-Evans CA (1997) Antioxidant activity of phytoestrogenic isoflavones. *Free Radic Res,* 26, 63-70

Saloniemi H, Kallela K & Saastamoinen I (1993) Study of the phytoestrogen content of goat's rue *(Galega orientalis)*, alfalfa *(Madicago sativa)* and white clover *(Trifolium repens)*. *Agric Sci Finl,* 2, 517-524

Salzman AL, Denenberg AG, Ueta I, O'Connor M, Linn SC & Szabo C (1996) Induction and activity of nitric oxide synthase in cultured human intestinal epithelial monolayers. *Am J Physiol Gastro Liver Physiol,* 270, G565-G573

Sankaranarayanan R, Varghese C, Duffy SW, Padmakumary G, Day NE & Nair MK (1994) A case–control study of diet and lung cancer in Kerala, South India. *Int J Cancer,* 58, 644-649

Sasco AJ, Lowenfels AB, Pasker DE & Jong P (1993) Epidemiology of male breast cancer: A meta analysis of published case–control studies and discussion of selected aetiological factors. *Int J Cancer,* 53, 538-549

Schoental R (1977) Health hazards due to oestrogenic mycotoxins in certain foodstuffs. *Int J Environ Studies,* 11, 149-150

Schultz TD, Bonorden WR & Seaman WR (1991) Effect of short-term flaxseed consumption on lignan and sex hormone metabolism in men. *Nutr Res,* 11, 1089-1100

Segi M, Fukushima I, Fujisaku M, Kurihara M & Saito S (1957) An epidemiological study on cancer in Japan. *GANN,* 48 (suppl), 1163

Serraino M & Thompson LU (1992) Flaxseed supplementation and early markers of colon carcinogenesis. *Cancer Lett,* 63, 159-165

Setchell KDR (1985) Naturally occuring non steroidal estrogens of dietary origin. In: McLachlan JA, ed, *Estrogens in the Environment,* New York, USA, Elsevier, pp 69-85

Setchell KDR (1997) Phytoestrogens: Biochemistry, physiology and implications for human health of soy isoflavones. Am J Cancer Nutr, (In Press)

Setchell KDR & Aldercreutz H (1988) Mammalian lignans and phyto-oestrogens recent studies on their formation, metabolism and biological role in health and disease. In: Rowland IR, ed, *Role of the Gut Flora in Toxicity and Cancer,* San Diego, USA, Academic Press Ltd, pp 315-345

Setchell KDR, Borriello SP, Gordon H, Lawson AM, Kirk DN, Harkness R & Morgan DML (1981) Lignan formation in man-microbial involvement and possible roles in relation to cancer. *Lancet,* 2, 4-7

Setchell KDR, Borriello SP, Hulme P, Kirk DN & Axelson M (1984) Nonsteroidal estrogens of dietary origin: Possible roles in hormonal-dependant disease. *Am J Clin Nutr,* 287, 569-578

Setchell KDR, Lawson AM, Mitchell FL, Adlercreutz H, Kirk DN & Axelson M (1980) Lignans in man and in animal species. *Nature,* 287, 740-742

Severson RK, Nomura AMY, Grove JS & Stemmermann GN (1989) A prospective study of demographics, diet, and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer Res,* 49, 1857-1860

Shimizu H, Ross RK & Bernstein L (1991) Possible underestimation of the incidence rate of prostate cancer in Japan. *Jpn J Cancer Res,* 82, 483-485

Sirtori CR, Gianazza E, Manzoni C, Lovati MR & Murphy PA (1997) Role of isoflavones in the cholesterol reduction by soy proteins in the clinic. *Am J Clin Nutr,* 65, 166-171

Steinmetz KA, Kushi LH, Bostick RM, Folsom AR & Potter JD (1994) Vegetables, Fruit, and colon cancer in the lowa women's health study. *Am J Epidemiol,* 139, 1-15

Stob M (1983) Naturally occuring food toxicants: Oestrogens. In: Rechcigl M, Jr, ed, *Handbook of Naturally Ocuuring Food Toxicants,* Boca Raton Fl, USA, CRC Press, pp 81-100

Swanson CA, Mao BL, Li JY, Lubin JH, Yao SX, Wang JZ, Cai SK, Hou Y, Luo QS & Blot WJ (1992) Dietary determinants of lung cancer risk: Results from a case–control study in Yunnan Province, China. *Int J Cancer,* 50, 876-880

Tajima K & Tominaga S (1985) Dietary habits and gastro-intestinal cancers: A Comparative case–control study of stomach and large intestinal cancers in Nagoya, Japan. *Jpn J Cancer Res (Gann),* 76, 705-716

Takkouche B & GestalOtero JJ (1996) The epidemiology of lung cancer: Review of risk factors and Spanish data. *Eur J Epidemiol,* 12, 341-349

Tamura K, Ishiguru S, Munakata A, Yoshida Y, Nakaji S & Sugawara K (1996) Annual changes in colorectal carcinoma incidence in Japan: Analysis of survey data on incidence in Aomori Prefecture. *Cancer,* 78, 1187-1194

Tang BY & Adams NR (1980) The effect of equol on oestrogen receptors and on synthesis of DNA and protein in immature rat uterus. *J Endocrinol,* 85, 291-287

Teas J (1981) The consumption of seaweed as a protective factor in the etiology of breast cancer. *Med Hypoth,* 7, 601-613

Thomas DB & Karagas MR (1987) Cancer in the first and second generation Americans. *Cancer Res,* 47, 5771-5776

Thompson LU, Robb P, Serraino M & Cheung F (1991) Mammalian lignan production from various foods. *Nutr Cancer,* 16, 43-52

Tominaga S (1995) Recent trends in cancer in japan and the world. *Cancer Chemo,* 22, 1-8

Tsubono Y, Kobayashi M & Tsugane S (1997) Food consumption and gastric cancer mortality in five regions of Japan. *Nutr Cancer,* 27, 60-64

Verdeal K & Ryan DS (1979) Naturally occuring estrogens in plant foodstuffs - a review. *J Food Prot,* 42, 577-583

Vessey MP (1984) Exogenous hormones in the aetiology of cancer in women. *J Roy Soc Med,* 77, 542-549

Wang C, Mäkelä T, Hase T, Adlercreutz H & Kurzer MS (1994) Lignans and flavanoids inhibit aromatase enzyme in human preadipocytes. *J Steroid Biochem Mol Biol,* 50, 205-212

Watanabe Y, Tada M, Kawamoto K, Uozumi G, Kajiwara Y, Hayashi K, Yamaguchi K, Murakami K, Misaki F & Akasaki Y (1984) A case–control study of cancer of the rectum and the colon. *Nippon Shokakibyo Gakkai Zasshi,* 81, 185-193

Welshons WV, Murphy CS, Koch R, Calaf G & Jordan VC (1987) Stimulation of breast cancer cells *in-vitro* by the environmental estrogen enterolactone and the phytoestrogen equol. *Breast Cancer Res Treat,* 10, 169-175

Welshons WV, Rottinghaus GE, Nonneman DJ, Dolan-Timpe M & Ross FP (1990) A sensitive bioassay for the detection of dietary estrogens in animal feeds. *J Vet Diagn Invest,* 2, 268-273

Whitten PL, Russell E & Naftolin F (1992) Effects of normal, human-concentration, phytoestrogen diet on rat uterine growth. *Steroids,* 57, 98-106

WHO (1994) *Report of a WHO Study Group Assessment of fracture risk and its application to screening for postmenopausal osteoporosis* (Technical Report Series 843), Geneva, Switzerland, World Health Organization

Wilcox BJ, Fuchigami K & Wilcox DC (1995) Isoflavone intake in Japanese and Japanese-Canadians. [Abstract] *Am J Clin Nutr,* 61, S901

Wilcox G, Wahlqvist ML, Burger HG & Medley G (1990) Oestrogenic effects of plant foods in postmenopausal women. *BMJ,* 301, 905-906

Wilcox JN & Blumenthal BF (1995) Thrombotic mechanisms in atherosclerosis: Potential impact of soy proteins. *J Nutr,* 125 (suppI), S631-S638

Wu-Williams AH, Dai XD, Blot W, Xu ZY, Sun XW, Xiao HP, Stone BJ, Yu SF, Feng YP, Ershow AG, Sun J, Fraumeni JF, Jr & Henderson BE (1990) Lung cancer among women in North-East China. *Brit J Cancer,* 62, 982-987

Xu X, Wang HJ, Murphy PA, Cook L & Hendrich S (1994) Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *J Nutr,* 124, 825-832

Yatani, Chigus I, Akazaki K, Stemmermann GN & Welsh RA (1982) Demographic pathology of latent prostatic carcinoma. *Cancer,* 71, 611-616

Yingnan Y & Songlin Y (1986) A study of etiological factors in gastric cancer in Fuzhou city. *Chin J Epidemiol,* 7, 48-50

You WC, Blot WJ, Chang YS, Ershow AG, Yang ZT, An Q, Henderson B, Xu GW, Fraumeni JF, Jr & Wang TG (1988) Diet and high risk of stomach cancer in Shandong, China. *Cancer Res,* 48, 3518-3523

Yuan JM, Wang QS, Ross RK, Henderson BE & Yu MC (1995) Diet and breast cancer in Shanghai and Tianjin, China. *Brit J Cancer,* 71, 1353-1358

Ziegler RG, Hoover RN, Pike MC, Hildesheim A, Nomura AMY, West DW/, Wu-Williams AH, Kolonel LN, Horn-Ross PL, Rosenthal JF & Hyer MB (1993) Migration patterns and breast cancer risk in Asian–American women. *J Natl Cancer Inst,* 85, 1819-1827

List of workshop participants

PHYTOESTROGENS IN THE HUMAN DIET, LEICESTER, UK ON 21 MARCH 1997

Members

Professor M Ashwell, Ashwell Associates, Ashwell St, Ashwell, Herts, SG7 5PZ

Dr S Bingham, Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH

Mr N Bundred, Department of Surgery, University Hospital of South Manchester, Nell Lane, West Didsbury, Manchester M20 8LR

Dr A Collins, Rowett Research Institute, Green Burn Road, Bucksburn, Aberdeen AB21 9SB

Dr D Ferry, Department of Clinical Oncology, University of Birmingham, Queen Elisabeth Hospital, Birmingham B15 9TH

Professor A Gescher, MRC Toxicology Unit, Hodgkin Building, University of Leicester

Dr M Joffe, Department of Epidemiology and Public Health, Imperial College of Medicine at St. Mary's, Norfolk Place, London W2 1PG

Dr H Makin, Department of Clinical Biochemistry, St. Bartholomew's and The Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, Turner St, London E1 2AD

Professor C Rice-Evans, Division of Biochemistry and Molecular Biology, UMDS Guy's Hospital, London SE1 9RT

Professor K Setchell, Clinical Mass Spectrometry, Department of Paediatrics, Children's Hospital Medical Canter, 3333 Burnet Avenue, Cincinnati, Ohio 45229, USA

Dr A Wilkinson, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA

Representatives

Dr D Aldridge, Department of Health, Room 651c Skipton House, 80 London Road, Elephant and Castle, London SE1

Dr R Burt Food Contaminants Division, MAFF, Room 231, Ergon House, c/o Nobel House, 17 Smith Square, London SW1P 3JR

Dr L Smith, Department of the Environment, Transport and the Regions, Room: A3141, Romney House, 43 Marsham Street, London SW1P 3PY

Dr C Tahourdin, Food Contaminants Division, MAFF, Room 209, Ergon House, c/o Nobel House, 17 Smith Square, London SW1P 3JR

Dr A Zampelas, Chief Scientist's Group (Food and Veterinary Science Division), MAFF, Room 666, St Christopher House, Southwark Street, London SE1 0UD

IEH Secretariat

Professor L Smith (Chairman)
Dr C Humfrey
Mr P Holmes
Dr P Harrison

Annex

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods.

References cited are listed at the end of the Annex

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Non-bean food sources						
Alfalfa	Coumestrol	Saloniemi *et al.* 1995	HPLC	-	25.3-64.8 µg/g dry wt.	Fresh samples of variety Jokionen
		Saloniemi *et al.* 1993	HPLC	3	25.3-33.7 µg/g	Variety Tammisto. Alcohol extraction
		Saloniemi *et al.* 1993	HPLC	4	18.9-71.6 µg/g	Variety Tammisto. Ether extraction
		Barbetti 1995	TLC	-	80-880 µg/g dry wt.	Stems from four strains suffering from foliar diseases. N.B. semi quantitative method
		Barbetti 1995	TLC	-	10-270 µg/g dry wt.	Pods from four strains suffering from foliar diseases. N.B. semi quantitative method
		Knuckles *et al.* 1976	PC	8	18-118 µg/g dry wt.	
		Knuckles *et al.* 1976	PC	8	12-142 µg/g dry wt.	Pressed
		Knuckles *et al.* 1976	PC	4	16-121 µg/g dry wt.	Whole LPC -Process I
		Knuckles *et al.* 1976	PC	4	4-13 µg/g dry wt.	Alfalfa solubles - Process I
		Knuckles *et al.* 1976	PC	4	4-17 µg/g dry wt.	Whole LPC - Process II
		Knuckles *et al.* 1976	PC	4	trace	Alfalfa solubles - Process II

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Alfalfa, cont.	Formononetin	Saloniemi *et al.* 1995	HPLC	-	trace	Fresh samples
	Biochanin-A	Saloniemi *et al.* 1995	HPLC	-	trace	Fresh samples
Alfalfa sprouts	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	46.8 µg/g	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	3.4 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Apples	Quercetin	Hertog *et al.* 1993	HPLC	Av	36 µg/g	Five varieties
	Kaempferol	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	Five varieties
	Myricetin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	Five varieties
	Apigenin	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	Five varieties
	Luteolin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	Five varieties
Apple juice	Quercetin	Hertog *et al.* 1993	HPLC	1	2.8 mg/l	
	Kaempferol	Hertog *et al.* 1993	HPLC	1	<1 mg/l	
	Myricetin	Hertog *et al.* 1993	HPLC	1	<0.5 mg/l	
	Apigenin	Hertog *et al.* 1993	HPLC	1	<1 mg/l	
	Luteolin	Hertog *et al.* 1993	HPLC	1	<0.5 mg/l	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Banana	Zearalenone	Chakrabarti & Ghosal 1986	HPLC	-	17 µg/g	Naturally infected with fungus
Barley	Zearalenone	Kuiper-Goodman *et al.* 1987	-	1/3*	0.006 µg/g	Polished-Japan. From Tanaka *et al.* 1985
Barley flour	Zearalenone	Kuiper-Goodman *et al.* 1987	-	6/6*	0.001-0.004 µg/g	Japanese. From Tanaka *et al.* 1985
Beer	Zearalenone	Kuiper-Goodman *et al.* 1987	-	17/140*	0.3-2 µg/g	Lesotho. From Martin & Keen 1978
Bread	Biochanin-A	Mazur *et al.* 1996	GC/MS		ND	9-grain, US
	Daidzein	Mazur *et al.* 1996	GC/MS		0.076 µg/g	9-grain, US
	Coumestrol	Mazur *et al.* 1996	GC/MS		ND	9-grain, US
	Formononetin	Mazur *et al.* 1996	GC/MS		0.024 µg/g	9-grain, US
	Genistein	Mazur *et al.* 1996	GC/MS		0.105 µg/g	9-grain, US
	Secoiso-lariciresinol	Mazur *et al.* 1996	GC/MS		0.707 µg/g	9-grain, US
Breakfast cereal	Zearalenone	Kuiper-Goodman *et al.* 1987	-	8/56*	<0.051 µg/g	UK. From Norton *et al.* 1982
Bourbon	Biochanin-A	Gavaler *et al.* 1995	GC/MS		Present (not quantified)	
	ß-Sitosterol	Gavaler *et al.* 1995	GC/MS		7-21 µg/ml	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Celery	Quercetin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	
	Kaempferol	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	
	Myricetin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	
	Apigenin	Hertog *et al.* 1993	HPLC	Av	108 µg/g	
	Luteolin	Hertog *et al.* 1993	HPLC	Av	22 µg/g	
Chinese pea, boiled	Biochanin-A	Reinli & Block 1996	HPLC	2-6	91.3 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Corn	Zearalenone	Kuiper-Goodman *et al.* 1987	-	16/55*	0.2-0.75 µg/g	Argentinean. From Lopez & Tapia 1980
	Zearalenone	Luo *et al.* 1990	HPLC	16/27*	44 ng/g	Linxian, China
	Zearalenone	Luo *et al.* 1990	HPLC	1/20*	39 ng/g	Shangqui, China
	Zearalenone	Kuiper-Goodman *et al.* 1987	-	6/26*	0.2-0.5 µg/g	USA. From Eppley *et al.* 1974
	Zearalenone	Kuiper-Goodman *et al.* 1987	-	2/4*	<1.1 µg/g	Transkei. From Marasas *et al.* 1979
	Zearalenone	Kuiper-Goodman *et al.* 1987	-	-	0.29-1.8 µg/g	Zambia. From Lovelace & Nyathi 1977 and Senti 1979

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Corn-beer	Zearalenone	Kuiper-Goodman *et al.* 1987	-	23	0.92 µg/g	From Lovelace & Nyathi 1977
Corn meal-	Zearalenone	Kuiper-Goodman *et al.* 1987	-	9/11*	0.01-0.07 µg/g	USA. From Ware & Thorpe 1978
Corn/corn products-	Zearalenone	Kuiper-Goodman *et al.* 1987	-	24/142*	0.013-0.475 µg/g	Canadian. From Williams 1985
Corn flakes	Zearalenone	Kuiper-Goodman *et al.* 1987	-	-	0.01 µg/g	Canadian. From Scott *et al.* 1978
Crisp bread	Biochanin-A	Mazur *et al.* 1996	GC/MS	3	ND-trace	
	Daidzein	Mazur *et al.* 1996	GC/MS	3	trace-0.131 µg/g	
	Coumestrol	Mazur *et al.* 1996	GC/MS	3	trace	
	Formononetin	Mazur *et al.* 1996	GC/MS	3	trace-0.029 µg/g	
	Genistein	Mazur *et al.* 1996	GC/MS	3	trace-0.098 µg/g	
	Secoiso-lariciresinol	Mazur *et al.* 1996	GC/MS	3	0.277-0.42 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Endive	Quercetin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	
	Kaempferol	Hertog *et al.* 1993	HPLC	Av	46 µg/g	
	Myricetin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	
	Apigenin	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	
	Luteolin	Hertog *et al.* 1993	HPLC	Av	<1µg/g	
Green split pea, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	73 µg/g	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Granola candy bar	Biochanin-A	Mazur *et al.* 1996	GC/MS		0.022 µg/g	USA
	Daidzein	Mazur *et al.* 1996	GC/MS		0.519 µg/g	
	Coumestrol	Mazur *et al.* 1996	GC/MS		trace	
	Formononetin	Mazur *et al.* 1996	GC/MS		0.041 µg/g	
	Genistein	Mazur *et al.* 1996	GC/MS		0.783 µg/g	
	Secoiso-lariciresinol	Mazur *et al.* 1996	GC/MS		0.205 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Human milk	Daidzein	Franke and Custer 1996	HPLC		0-70 nMol/l	After challenge with up to 20 g of roasted soyabeans
	Daidzein	Franke and Custer 1996	HPLC		80-110 nMol/l	Chinese women on traditional diet
	Genistein	Franke and Custer 1996	HPLC		0-60 nMol/l	After challenge with up to 20 g of roasted soyabeans
	Genistein	Franke and Custer 1996	HPLC		30-50 nMol/l	Chinese women on traditional diet
Jacobs tears	Zearalenone	Kuiper-Goodman *et al.* 1987	-	7/7	0.01-0.44 µg/g	Japanese. From Tanaka *et al.* 1985
Kala chana seeds, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	12.6 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	61.3 µg/g	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	6 µg/g	From Franke *et al.* 1994
Lettuce	Quercetin	Hertog *et al.* 1993	HPLC	Av	14 µg/g	
	Kaempferol	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Linseed	Biochanin-A	Mazur *et al.* 1996	GC/MS	-	0 µg/g	
	Daidzein	Mazur *et al.* 1996	GC/MS	-	0 µg/g	
	Coumestrol	Mazur *et al.* 1996	GC/MS	-	0 µg/g	
	Formononetin	Mazur *et al.* 1996	GC/MS	-	0 µg/g	
	Genistein	Mazur *et al.* 1996	GC/MS	-	0 µg/g	
	Matairesinol	Obermeyer *et al.* 1995	HPLC	-	0 µg/g	
	Secoiso-lariciresinol	Mazur *et al.* 1996	GC/MS	-	3699 µg/g	
	Secoiso-lariciresinol	Obermeyer *et al.* 1995	HPLC	-	817 µg/g	
	Secoiso-lariciresinol	Thompson *et al.* 1997	HPLC	28	1.8-4.4 µMol/g	
Linseed meal	Secoiso-lariciresinol	Obermeyer *et al.* 1995	HPLC	-	2260 µg/g	
	Matairesinol	Obermeyer *et al.* 1995	HPLC	-	0 µg/g	
	Myricetin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	
	Apigenin	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	
	Luteolin	Hertog *et al.* 1993	HPLC	Av	<1µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Round split pea, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	81.1 µg/g	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Yellow split pea, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	8.6 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Yellow split pea, dry, cont.	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Rye, polished	Zearalenone	Kuiper-Goodman *et al.* 1987	-	3/5*	0.003-0.004 µg/g	From Lee *et al.* 1985

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Rye bread	Biochanin-A	Mazur *et al.* 1996	GC/MS		ND	
	Daidzein	Mazur *et al.* 1996	GC/MS		trace	
	Coumestrol	Mazur *et al.* 1996	GC/MS		ND	
	Formononetin	Mazur *et al.* 1996	GC/MS		trace	
	Genistein	Mazur *et al.* 1996	GC/MS		trace	
	Secoiso-lariciresinol	Mazur *et al.* 1996	GC/MS		11.52 µg/g	
Onion	Quercetin	Hertog *et al.* 1993	HPLC	Av	347 µg/g	
	Kaempferol	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	
	Myricetin	Hertog *et al.* 1993	HPLC	Av	<1 µg/g	
	Apigenin	Hertog *et al.* 1993	HPLC	Av	<2 µg/g	
	Luteolin	Hertog *et al.* 1993	HPLC	Av	<1µg/g	
	Biochanin-A	Mazur *et al.* 1996	GC/MS		trace	
Sunflower seeds	Daidzein	Mazur *et al.* 1996	GC/MS		trace	
	Coumestrol	Mazur *et al.* 1996	GC/MS		trace	
	Formononetin	Mazur *et al.* 1996	GC/MS		0.26 µg/g	
	Genistein	Mazur *et al.* 1996	GC/MS		trace	
	Secoiso-lariciresinol	Mazur *et al.* 1996	GC/MS		6.095 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tea	Apigenin	Hertog *et al.* 1993	HPLC	Av	<1 mg/l	Black. Five varieties
	Biochanin-A	Mazur *et al.* 1996	GC/MS		0.359 µg/g	Lapacho
	Coumestrol	Mazur *et al.* 1996	GC/MS		ND	Lapacho
	Daidzein	Mazur *et al.* 1996	GC/MS		0.181 µg/g	Lapacho
	Formononetin	Mazur *et al.* 1996	GC/MS		0.075 µg/g	Lapacho
	Genistein	Mazur *et al.* 1996	GC/MS		0.286 µg/g	Lapacho
	Kaempferol	Hertog *et al.* 1993	HPLC	Av	14 mg/l	Black. Five varieties
	Luteolin	Hertog *et al.* 1993	HPLC	Av	<0.5 mg/l	Black. Five varieties
	Myricetin	Hertog *et al.* 1993	HPLC	Av	2.5 mg/l	Black. Five varieties
	Quercetin	Hertog *et al.* 1993	HPLC	Av	20 mg/l	Black. Five varieties
	Secoiso-lariciresinol	Mazur *et al.* 1996	GC/MS		26.7 µg/g	Lapacho
Walnuts	Zearalenone	Kuiper-Goodman *et al.* 1987	-	3/60*	0.05-0.45 µg/g	France. From Jemmali & Mazerand 1980
Wheat	Zearalenone	Luo *et al.* 1990	HPLC	6/15*	trace	Linxian, Chinese
	Zearalenone	Luo *et al.* 1990	HPLC	6/15*	trace	Shangqui, Chinese
	Zearalenone	Kuiper-Goodman *et al.* 1987	-	2/10*	0.008-0.04µg/g	Polished-Korean. From Lee *et al.* 1985

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Wheat flour	Zearalenone	Kuiper-Goodman *et al.* 1987	-	3/27*	0.001-0.006 µg/g	Japanese. From Tanaka *et al.* 1985
Wine, red	Quercetin	Hertog *et al.* 1993	HPLC	Av	11 mg/l	Five varieties
	Kaempferol	Hertog *et al.* 1993	HPLC	Av	<1 mg/l	Five varieties
	Myricetin	Hertog *et al.* 1993	HPLC	Av	9 mg/l	Five varieties
	Apigenin	Hertog *et al.* 1993	HPLC	Av	<1 mg/l	Five varieties
	Luteolin	Hertog *et al.* 1993	HPLC	Av	<0.5 mg/l	Five varieties
Beans (Other than Soya)						
Beans, un-specified	Zearalenone	Kuiper-Goodman *et al.* 1987	-	1/50	0.16 µg/g	Yugoslavian. From Pepeljnjak 1984
Black beans	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Black-eyed beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	17.3 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Broad beans	Apigenin	Hertog *et al.* 1993	HPLC	1	<2 µg/g	
	Kaempferol	Hertog *et al.* 1993	HPLC	1	<2 µg/g	
	Luteolin	Hertog *et al.* 1993	HPLC	1	<1 µg/g	
	Myricetin	Hertog *et al.* 1993	HPLC	1	25 µg/g	
	Quercetin	Hertog *et al.* 1993	HPLC	1	20 µg/g	
Broad beans, fried	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	2.1 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	13 µg/g	From Franke *et al.* 1994
Fava beans	Genistin	Fukutake *et al.* 1996	HPLC	3	<0.1 µg/g	
	Genistein	Fukutake *et al.* 1996	HPLC	3	<0.1 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Garbanzo beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	15.2 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Great northern beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	6.0 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Green beans, raw	Biochanin-A	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	1.5 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Green beans, boiled	Biochanin-A	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Ice bean	Daidzein	Reinli & Block 1996	HPLC	3	35 µg/g	Multiple reference sources
	Genistein	Reinli & Block 1996	HPLC	3	39 µg/g	Multiple reference sources
Kidney beans, cooked	Biochanin-A	Reinli & Block 1996	HPLC	2-6	4.1 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Lima beans, raw	Biochanin-A	Reinli & Block 1996	HPLC	4-12	ND-3.7 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	4-12	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	4-12	ND-14.8 µg/g	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	4-12	trace-5.5 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	4-12	ND	From Franke *et al.* 1994

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Lima beans, boiled	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	0.1 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Mung beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	6.1 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Mung bean sprouts	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Pink beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	10.5 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Pinto beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	5.6 µg/g	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	36.1 µg/g	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Red beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	3 µg/g	From Franke *et al.* 1994
Small white beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	8.2 µg/g	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	7 µg/g	From Franke *et al.* 1994

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
White navy beans, dry	Biochanin-A	Reinli & Block 1996	HPLC	2-6	trace	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Soyabeans and traditional soya products						
Soyabeans	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Coumestrol	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Coumestrol	Murphy 1982	HPLC	Av	ND	Two varieties
	Daidzin	Eldridge & Kwolek 1983	HPLC	Av	142-1244 µg/g	Six varieties
	Daidzin	Murphy 1982	HPLC	Av	ND-117 µg/g	Two varieties
	Daidzin	Wang & Murphy 1994b	HPLC	Av	148-780 µg/g	Seven USA varieties; triplicate assays
	Daidzin	Wang & Murphy 1994b	HPLC	Av	37-115 µg/g	Three Japanese varieties; six assays
	Daidzin	Tsukamoto *et al.* 1995	HPLC	6/variety	28-210 µg/g	Seven varieties

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabeans cont.	Daidzein	Reinli & Block 1996	HPLC	-	22-1915 µg/g	Multiple reference sources
	Daidzein	Eldridge & Kwolek 1983	HPLC	Av	5-35 µg/g	Six varieties
	Daidzein	Murphy 1982	HPLC	Av	1-22 µg/g	Two varieties
	Daidzein	Wang & Murphy 1994b	HPLC	Av	4-38 µg/g	Seven USA varieties; triplicate assays
	Daidzein	Wang & Murphy 1994a	HPLC	Av	7-26 µg/g	Two varieties; triplicate assays
	Daidzein	Wang & Murphy 1994b	HPLC	Av	trace-4 µg/g	Three Japanese varieties; six assays
	Daidzein glucoside	Wang & Murphy 1994a	HPLC	Av	180-690 µg/g	Two varieties; triplicate assays
	Formononetin	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Genistin	Murphy 1982	HPLC	Av	747-1024 µg/g	Two varieties
	Genistin	Eldridge & Kwolek 1983	HPLC	Av	215-2104 µg/g	Six varieties
	Genistin	Fukutake *et al.* 1996	HPLC	3	200.6µg/g	
	Genistin	Wang & Murphy 1994b	HPLC	Av	330-888 µg/g	Seven USA varieties; triplicate assays

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabeans cont.	Daidzein	Reinli & Block 1996	HPLC	-	22-1915 µg/g	Multiple reference sources
	Daidzein	Eldridge & Kwolek 1983	HPLC	Av	5-35 µg/g	Six varieties
	Daidzein	Murphy 1982	HPLC	Av	1-22 µg/g	Two varieties
	Genistin	Wang & Murphy 1994b	HPLC	Av	136-237 µg/g	Three Japanese varieties; six assays
	Genistin	Tsukamoto *et al.* 1995	HPLC	6/variety	ND-149 µg/g	Seven varieties
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	394-852 µg/g	Two varieties; triplicate assays
	Genistein	Reinli & Block 1996	HPLC	-	69-1897 µg/g	Multiple reference sources
	Genistein	Eldridge & Kwolek 1983	HPLC	Av	0.6-46 µg/g	Six varieties
	Genistein	Murphy 1982	HPLC	Av	24-40 µg/g	2 varieties
	Genistein	Fukutake *et al.* 1996	HPLC	3	4.6 µg/g	
	Genistein	Wang & Murphy 1994b	HPLC	Av	15-45 µg/g	Seven USA varieties; triplicate assays
	Genistein	Wang & Murphy 1994b	HPLC	Av	7-11 µg/g	Three Japanese varieties; six assays

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabeans cont.	Genistein	Wang & Murphy 1994a	HPLC	Av	17-29 µg/g	Two varieties; triplicate assays
	Glycitin	Wang & Murphy 1994b	HPLC	Av	60-97 µg/g	Seven USA varieties; triplicate assays
	Glycitin	Wang & Murphy 1994b	HPLC	Av	42-96 µg/g	Three Japanese varieties; six assays
	Glycitin 7ß-glucoside	Eldridge & Kwolek 1983	HPLC	Av	74-255 µg/g	Six varieties
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	53-56 µg/g	Two varieties; triplicate assays
	Glycitein	Eldridge & Kwolek 1983	HPLC	Av	13-32 µg/g	Five varieties
	Glycitein	Wang & Murphy 1994b	HPLC	Av	19-21 µg/g	Seven USA varieties; triplicate assays
	Glycitein	Wang & Murphy 1994b	HPLC	Av	trace-22 µg/g	Three Japanese varieties; six assays
	Glycitein	Wang & Murphy 1994a	HPLC	Av	20 µg/g	Two varieties; triplicate assays
	6"-*o*-malonyldaidzin	Wang & Murphy 1994b	HPLC	Av	198-752 µg/g	Seven USA varieties; triplicate assays

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabeans cont.	6"-*o*-malonyldaidzin	Wang & Murphy 1994b	HPLC	Av	222-562 µg/g	Three Japanese varieties; six assays
	6"-*o*-malonyldaidzin	Tsukamoto *et al.* 1995	HPLC	6/variety	87-1628 µg/g	Seven varieties
	6"-*o*-malonyldaidzin	Wang & Murphy 1994a	HPLC	Av	241-300 µg/g	Two varieties; triplicate assays
	6"-*o*-malonylgenistin	Wang & Murphy 1994b	HPLC	Av	883-1756 µg/g	Seven USA varieties; triplicate assays
	6"-*o*-malonylgenistin	Wang & Murphy 1994b	HPLC	Av	670-1232 µg/g	Three Japanese varieties; six assays
	6"-*o*-malonylgenistin	Wang & Murphy 1994a	HPLC	Av	738-743 µg/g	Two varieties; triplicate assays
	6"-*o*-malonylgenistin	Tsukamoto *et al.* 1995	HPLC	6/variety	61-1546 µg/g	Seven varieties
	6"-*o*-malonylglycitin	Wang & Murphy 1994b	HPLC	Av	72-118 µg/g	Seven USA varieties; triplicate assays

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabeans cont	6"-*o*-malonylglycitin	Wang & Murphy 1994b	HPLC	Av	60-183 µg/g	Three Japanese varieties; six assays
	6"-*o*-malonylglycitin	Wang & Murphy 1994a	HPLC	Av	50-61 µg/g	Two varieties; triplicate assays
	6"-*o*-acetyldaidzin	Wang & Murphy 1994b	HPLC	Av	trace	Seven USA varieties; triplicate assays
	6"-*o*-acetyldaidzin	Wang & Murphy 1994b	HPLC	Av	trace-12 µg/g	Three Japanese varieties; six assays
	6"-*o*-acetyldaidzin	Wang & Murphy 1994a	HPLC	Av	trace-1 µg/g	Two varieties; triplicate assays
	6"-*o*-acetylgenistin	Wang & Murphy 1994b	HPLC	Av	trace-2 µg/g	Seven USA varieties; triplicate assays
	6"-*o*-acetylgenistin	Wang & Murphy 1994b	HPLC	Av	trace-4 µg/g	Three Japanese varieties; six assays
	6"-*o*-acetylgenistin	Wang & Murphy 1994a	HPLC	Av	2-9 µg/g	Two varieties; triplicate assays
	6"-*o*-acetylglycitin	Wang & Murphy 1994b	HPLC	Av	trace-37 µg/g	Seven USA varieties; triplicate assays

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabeans cont	6"-*o*-acetylglycitin	Wang & Murphy 1994b	HPLC	Av	33-41 µg/g	Three Japanese varieties; six assays
	6"-*o*-acetylglycitin	Wang & Murphy 1994a	HPLC	Av	ND-35 µg/g	Two varieties; triplicate assays
Soyabeans dry	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Daidzein	Reinli & Block 1996	HPLC	-	676-1001 µg/g	Multiple reference sources
	Coumestrol	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Formononetin	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Genistein	Reinli & Block 1996	HPLC	-	940-1023 µg/g	Multiple reference sources
Black soyabeans	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	699 µg/g	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
Black soyabeans, boiled	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	612 µg/g	From Franke *et al.* 1994
	Biochanin-A	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Daidzein	Reinli & Block 1996	HPLC	2-6	270 µg/g	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Formononetin	Reinli & Block 1996	HPLC	2-6	ND	From Franke *et al.* 1994
	Genistein	Reinli & Block 1996	HPLC	2-6	277 µg/g	From Franke *et al.* 1994

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Green soyabeans	Daidzein	Wang & Murphy 1994a	HPLC	Av	10 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	16 µg/g	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	18 µg/g	
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	451 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	430 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	48 µg/g	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	515 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	851 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Green soyabeans cont	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	57 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	2 µg/g	
Roasted soyabeans	Biochanin-A	Reinli & Block 1996	HPLC	5-9	ND	From Franke *et al.* 1994
	Coumestrol	Reinli & Block 1996	HPLC	5-9	ND	From Franke *et al.* 1994
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	460 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	397 µg/g	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	45 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	5-9	563-848 µg/g	From Franke *et al.* 1994
	Daidzein	Wang & Murphy 1994a	HPLC	Av	39 µg/g	
	Formononetin	Reinli & Block 1996	HPLC		ND	From Franke *et al.* 1994

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Roast soyabean cont	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	551 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	743 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	63 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	69 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	68 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	102 µg/g	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	72 µg/g	
	Genistein	Reinli & Block 1996	HPLC	5-9	869-1106 µg/g	From Franke *et al.* 1994
	Glycitein	Wang & Murphy 1994a	HPLC	Av	52 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya nuts	Daidzein	Reinli & Block 1996	HPLC	3	575 µg/g	From Coward *et al.* 1993
	Genistin	Fukutake *et al.* 1996	HPLC	3	968.1 µg/g	
	Genistein	Fukutake *et al.* 1996	HPLC	3	11.6 µg/g	
	Genistein	Reinli & Block 1996	HPLC	3	935 µg/g	From Coward *et al.* 1993
Soyabean sprouts	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990
	Coumestrol	Murphy 1982	HPLC	Av	7 µg/g	
	Coumestrol	Reinli & Block 1996	HPLC	-	4.5-9.2 µg/g	From Wang *et al.* 1990
	Coumestrol	Reinli & Block 1996	PC	3	12.1 µg/g	From Knuckles *et al.* 1976
	Daidzin	Murphy 1982	HPLC	Av	92 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	-	138-211 µg/g	From Wang *et al.* 1990
	Daidzein	Murphy 1982	HPLC	Av	19 µg/g	
	Formononetin	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990
	Genistin	Murphy 1982	HPLC	Av	403 µg/g	
	Genistein	Murphy 1982	HPLC	Av	78 µg/g	
	Genistein	Reinli & Block 1996	HPLC	-	112-230 µg/g	From Wang *et al.* 1990

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabean pieces	Coumestrol	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990 & Coward *et al.* 1993
	Daidzin	Hutchins *et al.* 1995	HPLC	3	282 µg/g	
	Genistein	Hutchins *et al.* 1995	HPLC	3	428 µg/g	
	6"-*o*-malonyldaidzin	Hutchins *et al.* 1995	HPLC	3	177 µg/g	
	6"-*o*-acetyl daidzin	Hutchins *et al.* 1995	HPLC	3	13 µg/g	
	6"-*o*-malonyl genistin	Hutchins *et al.* 1995	HPLC	3	550 µg/g	
	6"-*o*-acetyl genistin	Hutchins *et al.* 1995	HPLC	3	33 µg/g	
	Daidzein	Hutchins *et al.* 1995	HPLC	3	1 µg/g	
	Genistein	Hutchins *et al.* 1995	HPLC	3	7 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabean paste	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990 & Coward *et al.* 1993
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-acetyldaidzin	Wang & Murphy 1994a	HPLC	Av	1 µg/g	
	6"-*o*-malonyldaidzin	Wang & Murphy 1994a	HPLC	Av	ND	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	271 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	-	30-224 µg/g	From Wang *et al.* 1990 & Coward *et al.* 1993
	Formononetin	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990 & Coward *et al.* 1993
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	96 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	2 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	ND	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabean paste cont.	Genistein	Wang & Murphy 1994a	HPLC	Av	183 µg/g	
	Genistein	Reinli & Block 1996	HPLC	-	3-300 µg/g	From Wang *et al.* 1990 & Coward *et al.* 1993
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	21 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	19 µg/g	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	54 µg/g	
Soya flakes	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Eldridge & Kwolek 1983
	Daidzin	Jones *et al.* 1989	HPLC	1	1039 µg/g	
	Daidzein	Jones *et al.* 1989	HPLC	1	246 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	-	221-744 µg/g	From Eldridge & Kwolek 1983
	Coumestrol	Reinli & Block 1996	HPLC	-	ND	From Eldridge & Kwolek 1983

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya flakes cont	Formononetin	Reinli & Block 1996	HPLC	-	ND	From Eldridge & Kwolek 1983
	Genistin	Jones *et al.* 1989	HPLC	1	2305 µg/g	
	Genistein	Reinli & Block 1996	HPLC	-	280-1320 µg/g	From Eldridge & Kwolek 1983
	Genistein	Jones *et al.* 1989	HPLC	1	120 µg/g	
Soya flakes, defatted	Daidzein	Reinli & Block 1996	HPLC	-	419-1166 µg/g	Multiple reference sources
	Genistein	Reinli & Block 1996	HPLC	-	1411-1951 µg/g	Multiple reference sources
Soya flakes, toasted/ defatted	Coumestrol	Murphy 1982	HPLC	Av	ND	
	Daidzin	Murphy 1982	HPLC	Av	200 µg/g	
	Genistin	Murphy 1982	HPLC	Av	1601 µg/g	
	Daidzein	Murphy 1982	HPLC	Av	1 µg/g	
	Genistein	Murphy 1982	HPLC	Av	51 µg/g	
Soyabean chips	Daidzein	Reinli & Block 1996	HPLC	3	267 µg/g	From Coward *et al.* 1993
	Genistein	Reinli & Block 1996	HPLC	3	275 µg/g	From Coward *et al.* 1993

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya granules	Daidzein	Wang & Murphy 1994a	HPLC	Av	12 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	27 µg/g	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	22 µg/g	
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	727 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	870 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	132 µg/g	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	106 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	193 µg/g	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	60 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya granules, cont.	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	72 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	135 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	48 µg/g	
Soya powder	Daidzein	Reinli & Block 1996	HPLC	3	355 µg/g	From Coward *et al.* 1993
	Genistein	Reinli & Block 1996	HPLC	3	732 µg/g	From Coward *et al.* 1993
Soyabean meal	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Petterson & Kiessling 1984
	Daidzein	Reinli & Block 1996	HPLC	1	706 µg/g	From Petterson & Kiessling 1984
	Formononetin	Reinli & Block 1996	HPLC	1	ND	From Petterson & Kiessling 1984
.	Genistein	Reinli & Block 1996	HPLC	1	1000 µg/g	From Petterson & Kiessling 1984
Soyabean meal, defatted	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990 & Petterson & Kiessling 1984
	Daidzein	Reinli & Block 1996	HPLC	-	575-616 µg/g	From Wang 1990 & Petterson & Kiessling 1984

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabean meal, defatted cont	Coumestrol	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990 & Petterson & Kiessling 1984
	Formononetin	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990 & Petterson & Kiessling 1984
	Genistein	Reinli & Block 1996	HPLC	-	683-753 µg/g	From Wang *et al.* 1990 & Petterson & Kiessling 1984
Soya fibre	Daidzein	Reinli & Block 1996	HPLC	3	171 µg/g dry wt.	From Coward *et al.* 1993
	Genistein	Reinli & Block 1996	HPLC	3	210 µg/g dry wt.	From Coward *et al.* 1993
Soya flour	Biochanin-A	Reinli & Block 1996	HPLC	8-12	ND	Multiple reference sources
	Biochanin-A	Mazur *et al.* 1996	GC/MS		0.744 µg/g	
	Coumestrol	Reinli & Block 1996	HPLC	8-12	ND	Multiple reference sources
	Daidzin	Eldridge 1982	HPLC	10	480-770 µg/g	Defatted
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	147 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	8-12	355-742 µg/g	Multiple reference sources
	Daidzein	Mazur *et al.* 1996	GC/MS		673.69 µg/g	
	Daidzein	Eldridge 1982	HPLC	10	80-480 µg/g	Defatted
	Daidzein	Wang & Murphy 1994a	HPLC	Av	4 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya flour, cont.	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-malonyldaidzin	Wang & Murphy 1994a	HPLC	Av	261 µg/g	
	Coumestrol	Mazur *et al.* 1996	GC/MS		0 µg/g	
	Formononetin	Reinli & Block 1996	HPLC	8-12	ND	Multiple reference sources
	Formononetin	Mazur *et al.* 1996	GC/MS		µg/g	
	Genistin	Eldridge 1982	HPLC	10	580-1540 µg/g	Defatted
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	407 µg/g	
	Genistein	Reinli & Block 1996	HPLC	8-12	478-1123 µg/g	Multiple reference sources
	Genistein	Mazur *et al.* 1996	GC/MS		969.14 µg/g	
	Genistein	Eldridge 1982	HPLC	10	40-460 µg/g	Defatted
	Genistein	Wang & Murphy 1994a	HPLC	Av	22 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	1 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	1023 µg/g	
	Glycitein	Eldridge 1982	HPLC	10	trace-30 µg/g	Defatted

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya flour, cont.	Glycitein	Wang & Murphy 1994a	HPLC	Av	19 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	41 µg/g	
	Glycitein 7ß-glucoside	Eldridge 1982	HPLC	10	60-220 µg/g	Defatted
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	32 µg/g	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	57 µg/g	
	Secoiso lariciresinol	Mazur *et al.* 1996	GC/MS		1.304 µg/g	
Textured soya protein	Daidzin	Murphy 1982	HPLC	Av	86 µg/g	
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	463-507 µg/g	Two forms
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	187-231 µg/g	Two forms

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Textured soya protein, cont.	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	93-129 g/g	Two forms
	Daidzein	Murphy 1982	HPLC	Av	30 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	8-12 µg/g	Two forms
	Coumestrol	Murphy 1982	HPLC	Av	ND	
	Genistin	Murphy 1982	HPLC	Av	882 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	552-634 µg/g	Two forms
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	320-355 µg/g	Two forms
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	192-256 µg/g	Two forms
	Genistein	Wang & Murphy 1994a	HPLC	Av	22-29 µg/g	Two forms
	Genistein	Murphy 1982	HPLC	Av	67 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Textured soya protein cont.	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	93-146 µg/g	Two forms
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	68-90 µg/g	Two forms
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	58-60 µg/g	Two forms
	Glycitein	Wang & Murphy 1994a	HPLC	Av	25-26 µg/g	Two forms
Soya protein concen-trate	Daidzin	Eldridge 1982	HPLC	5	30-760 µg/g	
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	ND	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	ND	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya protein concen-trate, cont.	Daidzein	Eldridge 1982	HPLC	5	20-200 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	3	43-760 µg/g	From Coward *et al.* 1993
	Genistin	Eldridge 1982	HPLC	5	40-1910 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	18 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	1 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	trace	
	Genistein	Wang & Murphy 1994a	HPLC	Av	ND	
	Genistein	Eldridge 1982	HPLC	5	10-220 µg/g	
	Genistein	Reinli & Block 1996	HPLC	3	58-911µg/g	From Coward *et al.* 1993
	Glycitein	Eldridge 1982	HPLC	5	trace-40 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	31 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya protein concen-trate, cont.	Glycitin 7ß glucoside	Eldridge 1982	HPLC	5	10-220 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	23 µg/g	
Soya protein isolate	Daidzin	Eldridge 1982	HPLC	5	140-300 µg/g	
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	trace-133 µg/g	Three types
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	6-74 µg/g	Three types
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	18-20 µg/g	Three types
	Daidzein	Eldridge 1982	HPLC	5	80-210 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	11-63 µg/g	Three types

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya protein isolate cont.	Daidzein	Reinli & Block 1996	HPLC	5-6	138-271µg/g	From Coward *et al.* 1993 & Seo & Morr 1984
	Genistin	Eldridge 1982	HPLC	5	550-800 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	137-382 µg/g	Three types
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	ND-215 µg/g	Three types
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	88-100 µg/g	Three types
	Genistein	Eldridge 1982	HPLC	5	50-220 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	36-136 µg/g	Three types
	Genistein	Reinli & Block 1996	HPLC	5-6	374-557 µg/g	From Coward *et al.* 1993 & Seo & Morr 1984
	Glycitein 7ß-glucoside	Eldridge 1982	HPLC	5	30-60 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	34-55 µg/g	Three types

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya protein isolate, cont	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	46-33 µg/g	Three types
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	36-39 µg/g	Three types
	Glycitein	Eldridge 1982	HPLC	5	10-30 µg/g	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	22-53 µg/g	Three types
Soya isolate, acid	Coumestrol	Murphy 1982	HPLC	Av	ND	
	Daidzin	Murphy 1982	HPLC	Av	10 µg/g	
	Genistin	Murphy 1982	HPLC	Av	300 µg/g	
	Daidzein	Murphy 1982	HPLC	Av	ND	
	Genistein	Murphy 1982	HPLC	Av	77 µg/g	
Soya milk powder	Daidzein	Reinli & Block 1996	HPLC	3	681 µg/g	From Xu 1995
	Genistein	Reinli & Block 1996	HPLC	3	569 µg/g	From Xu 1995

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya milk, various	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Lu *et al.* 1995
	Daidzin	Jones *et al.* 1989	HPLC	-	7-91	
	Daidzein	Jones *et al.* 1989	HPLC	-	9-16	
	Daidzein	Reinli & Block 1996	HPLC	-	16-74 µg/g	From Lu *et al.* 1995
	Daidzein	Reinli & Block 1996	GC	-	15-37 µg/g	From Lu *et al.* 1995
	Coumestrol	Reinli & Block 1996	HPLC	-	ND	From Lu *et al.* 1995
	Formononetin	Reinli & Block 1996	HPLC	-	ND	From Lu *et al.* 1995
	Genistin	Jones *et al.* 1989	HPLC	-	27-162	
	Genistein	Reinli & Block 1996	HPLC	-	11-88 µg/g	From Lu *et al.* 1995
	Genistein	Jones *et al.* 1989	HPLC	-	2-11 µg/g	
	Genistein	Reinli & Block 1996	GC	-	19-41 µg/g	From Lu *et al.* 1995
Soyabean curd, fermented	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	ND	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soyabean curd, fermented cont	Daidzein	Wang & Murphy 1994a	HPLC	Av	143 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	ND	
	Genistein	Wang & Murphy 1994a	HPLC	Av	223 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	23 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Miso	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	72 µg/g	Honzukuri
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	1 µg/g	Honzukuri
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	ND	Honzukuri
	Daidzein	Wang & Murphy 1994a	HPLC	Av	34 µg/g	Honzukuri
	Daidzein	Reinli & Block 1996	HPLC	9	71-366 µg/g	From Coward *et al.* 1993
	Daidzein	Reinli & Block 1996	GC	3	165 µg/g	From Lu *et al.* 1995
	Genistin	Fukutake *et al.* 1996	HPLC	2	71.7-190.2 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	123 µg/g	Honzukuri
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	11 µg/g	Honzukuri
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	ND	Honzukuri
	Genistein	Reinli & Block 1996	HPLC	9	260-524 µg/g	From Coward *et al.* 1993

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Miso, cont.	Genistein	Reinli & Block 1996	GC	3	228 µg/g	From Lu *et al.* 1995
	Genistein	Fukutake *et al.* 1996	HPLC	2	56.0-229.1 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	93 µg/g	Honzukuri
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	18 µg/g	Honzukuri
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	Honzukuri
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	22 µg/g	Honzukuri
	Glycitein	Wang & Murphy 1994a	HPLC	Av	15 µg/g	Honzukuri
Akamiso soup mix	Daidzein	Reinli & Block 1996	HPLC	3	291 µg/g	From Coward *et al.* 1993
	Genistein	Reinli & Block 1996	HPLC	3	372 µg/g	From Coward *et al.* 1993
Shiromiso soup mix	Daidzein	Reinli & Block 1996	HPLC	3	208 µg/g	From Coward *et al.* 1993
	Genistein	Reinli & Block 1996	HPLC	3	337 µg/g	From Coward *et al.* 1993
Nato	Genistin	Fukutake *et al.* 1996	HPLC	2	282.8-492.8 µg/g	
	Genistein	Fukutake *et al.* 1996	HPLC	2	38.5-64.2 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tempeh	Daidzin	Hutchins *et al.* 1995	HPLC	3	127 µg/g	
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	2 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	11 µg/g	
	6"-*o*-acetyl daidzin	Hutchins *et al.* 1995	HPLC	3	7 µg/g	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	255 µg/g	
	6"-*o*-malonyl daidzin	Hutchins *et al.* 1995	HPLC	3	91 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	3	137 µg/g	From Coward *et al.* 1993
	Daidzein	Wang & Murphy 1994a	HPLC	Av	137 µg/g	
	Daidzein	Hutchins *et al.* 1995	HPLC	3	62 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	65 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tempeh, cont	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-acetyl genistin	Hutchins *et al.* 1995	HPLC	3	24 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	164 µg/g	
	6"-*o*-malonyl genistin	Hutchins *et al.* 1995	HPLC	3	307 µg/g	
	Genistein	Reinli & Block 1996	HPLC	3	235 µg/g	From Coward *et al.* 1993
	Genistein	Wang & Murphy 1994a	HPLC	Av	193 µg/g	
	Genistein	Hutchins *et al.* 1995	HPLC	3	65 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	14 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	24 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tofu	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Biochanin-A	Reinli & Block 1996	GC/MS	-	ND	Multiple reference sources
	Coumestrol	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Coumestrol	Reinli & Block 1996	GC/MS	-	ND	Multiple reference sources
	Coumestrol	Murphy 1982	HPLC	Av	ND	3 brands
	Daidzin	Murphy 1982	HPLC	Av	ND-35 µg/g	3 brands
	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	25 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	8 µg/g	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	159 µg/g	
	Daidzein	Reinli & Block 1996	HPLC	-	ND-253 µg/g	Multiple reference sources
	Daidzein	Reinli & Block 1996	GC/MS	-	57-117 µg/g	Multiple reference sources
	Daidzein	Wang & Murphy 1994a	HPLC	Av	46 µg/g	
	Daidzein	Murphy 1982	HPLC	Av	ND-13 µg/g	3 brands
	Daidzein	Dwyer *et al.* 1994	GC/MS	Av	57.7-117.0 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tofu, cont.	Formononetin	Reinli & Block 1996	HPLC	-	ND	Multiple reference sources
	Formononetin	Reinli & Block 1996	GC/MS	-	ND	Multiple reference sources
	Genistin	Murphy 1982	HPLC	Av	51-104 µg/g	3 brands
	Genistin	Fukutake *et al.* 1996	HPLC		137.7 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	84 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	1 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	108 µg/g	
	Genistein	Reinli & Block 1996	HPLC	-	53-421 µg/g	Multiple reference sources
	Genistein	Reinli & Block 1996	GC/MS	-	159-306 µg/g	Multiple reference sources
	Genistein	Wang & Murphy 1994a	HPLC	Av	52 µg/g	
	Genistein	Murphy 1982	HPLC	Av	29-78 µg/g	3 brands
	Genistein	Fukutake *et al.* 1996	HPLC		13.9 µg/g	
	Genistein	Dwyer *et al.* 1994	GC/MS	Av	158.6-305.6 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	8 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tofu cont	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	29 µg/g	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	12 µg/g	
Tofu, fermented	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990
	Daidzein	Reinli & Block 1996	HPLC	-	36 µg/g	From Wang *et al.* 1990
	Coumestrol	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990
	Formononetin	Reinli & Block 1996	HPLC	-	ND	From Wang *et al.* 1990
	Genistein	Reinli & Block 1996	HPLC	-	40 µg/g	From Wang *et al.* 1990
Tofutti	Daidzein	Reinli & Block 1996	HPLC	3	3 µg/g	From Coward *et al.* 1993
	Genistein	Reinli & Block 1996	HPLC	3	18 µg/g	From Coward *et al.* 1993
Soya sauce	Biochanin-A	Reinli & Block 1996	HPLC	-	ND	From Coward *et al.* 1993 & Wang *et al*. 1990
	Genistin	Fukutake *et al.* 1996	HPLC	2	9.8-20.1 µg/g	
	Genistein	Fukutake *et al.* 1996	HPLC	2	2.5-2.8 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya sauce cont.	Coumestrol	Murphy 1982	HPLC	Av	ND	Fermented
	Coumestrol	Reinli & Block 1996	HPLC	-	ND	From Coward *et al.* 1993 & Wang *et al*. 1990
	Daidzin	Murphy 1982	HPLC	Av	ND	Fermented
	Genistin	Murphy 1982	HPLC	Av	ND	Fermented
	Daidzein	Murphy 1982	HPLC	Av	ND	Fermented
	Daidzein	Reinli & Block 1996	HPLC	-	8-14 µg/g	From Coward *et al.* 1993 & Wang *et al*. 1990
	Formononetin	Reinli & Block 1996	HPLC	-	ND	From Coward *et al.* 1993 & Wang *et al*. 1990
	Genistein	Murphy 1982	HPLC	Av	ND	Fermented
	Genistein	Reinli & Block 1996	HPLC	-	5-9 µg/g	From Coward *et al.* 1993 & Wang *et al*. 1990
Soya sauce, fermented	Coumestrol	Reinli & Block 1996	HPLC	>2	ND	From Murphy 1982
	Genistein	Reinli & Block 1996	HPLC	>2	ND	From Murphy 1982

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Novel soya products						
Soya hot dog	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	35 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	12 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	8 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	67 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	4 µg/g	
.	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	42 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	16 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	15 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya hot dog cont.	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	14 µg/g	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	15 µg/g	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	8 µg/g	
Soya bacon	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	trace	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	26 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	27 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	3 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	5 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya bacon cont.	Genistein	Wang & Murphy 1994a	HPLC	Av	48 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	14 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	12 µg/g	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	9 µg/g	
Tempeh burger	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	36 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	25 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	34 µg/g	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	158 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tempeh burger cont.	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	1 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	ND	
	Genistein	Wang & Murphy 1994a	HPLC	Av	96 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	18 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	18 µg/g	
Soya drink	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	404-525 µg/g	Four types
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	5-12 µg/g	Four types
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	39-98 µg/g	Four types

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya drink cont.	Daidzein	Dwyer *et al.* 1994	GC/MS	Av	6.8-7.2 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	15-30 µg/g	Four types
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	674-775 µg/g	Four types
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	22-27 µg/g	Four types
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	144-259 µg/g	Four types
	Genistein	Wang & Murphy 1994a	HPLC	Av	32-50 µg/g	Four types
	Genistein	Dwyer *et al.* 1994	GC/MS	Av	20.2-21.7 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	68-77 µg/g	Four types
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	33 µg/g	Four types
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	40-44 µg/g	Four types
	Glycitein	Wang & Murphy 1994a	HPLC	Av	20-21 µg/g	Four types

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya-based speciality formulation	Daidzein	Dwyer *et al.* 1994	GC/MS	Av	0.1-1.6 µg/g	Three products
	Genistein	Dwyer *et al.* 1994	GC/MS	Av	0.4-4.9 µg/g	Three products
Soya breakfast patty	Coumestrol	Murphy 1982	HPLC	Av	ND	
	Daidzin	Murphy 1982	HPLC	Av	1 µg/g	
	Genistin	Murphy 1982	HPLC	Av	37 µg/g	
	Daidzein	Murphy 1982	HPLC	Av	ND	
	Genistein	Murphy 1982	HPLC	Av	14 µg/g	
Soya dessert	Daidzin	Jones *et al.* 1989	HPLC	1	329 µg/g	
	Genistin	Jones *et al.* 1989	HPLC	1	708 µg/g	
	Daidzein	Jones *et al.* 1989	HPLC	1	131 µg/g	
	Genistein	Jones *et al.* 1989	HPLC	1	119 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tofu yoghurt	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	42 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	61 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	trace	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	80 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	79 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	3 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	12 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Tofu yoghurt, cont.	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	5 µg/g	
Soya containing cheese	Daidzin glucoside	Wang & Murphy 1994a	HPLC	4	trace-16 µg/g	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	4	ND-trace	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	4	ND-67 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	4	trace	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	4	ND-46 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	4	trace-9 µg/g	
	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	4	trace-7 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	4	4-9 µg/g	

\+ Including reference to source paper for data derived from reviews.
Av Average value quoted
PC Paper chromatography
TLC Thin layer chromatography
ND None detected
* Incidence of positive samples from those analysed.
LPC Leaf protein concentrate
HPLC High performance liquid chromatography
GC/MS Gas chromatography/Mass spectroscopy

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya containing cheese, cont.	Glycitin glucoside	Wang & Murphy 1994a	HPLC	4	12-17 µg/g	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	4	19-27 µg/g	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	4	ND	
	Glycitein	Wang & Murphy 1994a	HPLC	4	8-9 µg/g	
Soya flat noodle	Daidzin glucoside	Wang & Murphy 1994a	HPLC	Av	trace	
	6"-*o*-acetyl daidzin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl daidzin	Wang & Murphy 1994a	HPLC	Av	15 µg/g	
	Daidzein	Wang & Murphy 1994a	HPLC	Av	trace	
	Genistin glucoside	Wang & Murphy 1994a	HPLC	Av	6 µg/g	
	6"-*o*-acetyl genistin	Wang & Murphy 1994a	HPLC	Av	trace	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex continued

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Food type	Chemical	Literature reference	Analyt. method	Samples analysed	Concentrations found	Comments+
Soya flat noodle, cont.	6"-*o*-malonyl genistin	Wang & Murphy 1994a	HPLC	Av	37 µg/g	
	Genistein	Wang & Murphy 1994a	HPLC	Av	13 µg/g	
	Glycitin glucoside	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-acetyl glycitin	Wang & Murphy 1994a	HPLC	Av	ND	
	6"-*o*-malonyl glycitin	Wang & Murphy 1994a	HPLC	Av	37 µg/g	
	Glycitein	Wang & Murphy 1994a	HPLC	Av	19 µg/g	

\+ Including reference to source paper for data derived from reviews.
* Incidence of positive samples from those analysed.
Av Average value quoted
LPC Leaf protein concentrate
PC Paper chromatography
HPLC High performance liquid chromatography
TLC Thin layer chromatography
GC/MS Gas chromatography/Mass spectroscopy
ND None detected

Annex references

Literature values for the phytoestrogen, mycoestrogen and related (non-oestrogenic) flavonoid and phytosterol contents of human foods

Barbetti MJ (1995) Relative resistance, associated yield losses and phyto-estrogen production from fungal foliar diseases in new and old annual Medicago cultivars. *Aust J Agric Res,* 46, 441-450

Chakrabarti DK & Ghosal S (1986) Occurence of free and conjugated 12,13-epoxytrichothecenes and zearalenone in banana fruits infected with *Fusarium moniliforme*. *Appl Environ Microbiol,* 51, 217-219

Dwyer JT, Goldin BR, Saul N, Gualtieri L, Barakat S & Adlercreutz H (1994) Tofu and soy drinks contain phytoestrogens. *J Am Diet Assoc,* 94, 739-743

Eldridge AC (1982) Determination of isoflavones in soybean flours, protein concentrates and isolates. *J Agric Food Chem,* 30, 353-355

Eldridge AC & Kwolek WF (1983) Soybean isoflavones: Effects of environment and variety on composition. *J Agric Food Chem,* 31, 394-396

Franke AA & Custer LJ (1996) Daidzein and genistein concentrations in human milk after soy consumption. *Clin Chem,* 42, 955-964

Fukutake M, Takahasi M, Ishida K, Kawamura H, Sugimura T & Wakabayashi K (1996) Quantification of genistein and genistin in soybeans and soybean products. *Food Chem Toxicol,* 34, 457-461

Gavaler JS, Rosenblum ER, Deal SR & Bowie BT (1995) The phytoestrogen congeners of alcoholic beverages: current status. *Proc Soc Exper Biol Med,* 208, 98-102

Hertog MGL, Hollman PCH, Katan MB & Kromhout D (1993) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. *Nutr Cancer,* 20, 21-29

Hutchins AM, Slavin JL & Lampe JW (1995a) Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *J Am Diet Assoc,* 95, 545-551

Jones AE, Price KR & Fenwick RG (1989) Development and application of a high-performance liquid chromatographic method for the analysis of phytoestrogens. *J Sci Food Agric,* 46, 357-364

Knuckles EB, DeFremery D & Kohler G (1976) Coumestrol content of fractions obtained during wet processing of alfalfa. *Agric Food Chem,* 24, 1177-1180

Kuiper-Goodman T, Scott PM & Watanabe H (1987) Risk assessment of the mycotoxin zearalenone. *Regulat Toxicol Pharmacol,* 7, 253-306

Luo Y, Yoshizawa T & Katayama T (1990) Comparative study on the natural occurrence of *Fusarium* mycotoxins (trichothecenes and zearalenone) in corn and wheat from high - and low-risk areas for human esophageal cancer in China. *Appl Environ Microbiol,* 56, 3723-3726

Mazur W, Fotsis T, Wähälä K, Ojala S, Salakka A & Adlercreutz H (1996) Isotope dilution gas chromatographic - mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. *Anal Biochem,* 233, 169-180

Murphy PA (1982) Phytoestrogen content of processed soybean products. *Food Technol,* 36, 60-64

Obermeyer WR, Musser SM, Betz JM, Casey RE, Pohland AE & Page SW (1995) Chemical studies of phytoestrogens and related compounds in dietary supplements: Flaz and Chaparral. *Proc Soc Exper Biol Med,* 208, 6-12

Reinli K & Block G (1996) Phytoestrogen content of foods - A compendium of literature values. *Nutr Cancer,* 26, 123-148

Saloniemi H, Wähälä K, Nykänen-Kurki P, Kallela K & Saastamoinen I (1995) Phytoestrogen content and estrogenic effect of legume fodder. *Proc Soc Exper Biol Med,* 208, 13-17

Thompson LU, Rickard SE, Cheung F, Kenaschuk EO & Obermeyer WR (1997) Variability in anticancer lignan levels in flaxseed. *Nutr Cancer,* 27, 26-30

Tsukamoto C, Shimada S, Igita K, Kudou S, Kokubun M, Okubo K & Kitamura K (1995) Factors affecting isoflavone content in soy bean seeds: Changes in isoflavone, saponins and composition of fatty acids at different temperatures during seed development. *J Agric Food Chem,* 43, 1184-1192

Wang HJ & Murphy PA (1994a) Isoflavone content in commercial soybean foods. *J Food Agric Chem,* 42, 1666-1673

Wang HJ & Murphy PA (1994b) Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year and location. *J Agric Food Chem,* 42, 1674-1677

In addition, a number of the papers used in the collation of this annex specifically quote other references as the original data source. The original papers are identified below for information:

Coward L, Barnes NC, Setchell KDR & Barnes S (1993) Genestin, Daidzein and their ß-glycoside conjugates: Antitumor isoflavones in Soyabean foods from American and Asian diets. *J Agric Food Chem. 41*, 1961-1967

Eldridge AC & Kwolek WF (1983) Soybean isoflavones: Effect of environment and variety on composition. *J Agric Food Chem. 31*, 394-396

Eppley RM, Stoloff L, Trucksess MW & Chung CW (1974) Survey of corn for *Fusarium* toxins. *J Assoc Off Anal Chem. 57,* 632-635

Franke AA, Custer LJ, Cerna CM & Narala KK (1994) Quantitation of phytoestrogens in legumes by HPLC. *J Agric Food Chem. 42*, 1905-1913

Jemmali M & Mazerand C (1980) Presence de zearalenone ou F2 dans les noix du commerce. *Ann Microbiol (Paris). B131,* 319-321

Knuckles BE, deFremery D & Kohler GO (1976) Coumestrol content of fractions obtained during wet processing of alfalfa. *J Agric Food Chem. 24,* 1177-1180

Lee U-S, Jang H-S, Tanaka T, Hasegawa A, Oh Y-J & Ueno Y (1985) The coexistence of the *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone in Korean cereals harvested in 1983. *Food Addit Contam. 2,* 185-192

Lopez TA & Tapia MO (1980) Identification de la microtoxina zearalenona en la Argentina. *Rev Argent Microbiol. 12*, 29-33

Lovelace CEA & Nyathi CB (1977) Estimation of the fungal toxins, zearalenone and aflatoxin, contaminating opaque maize beer in Zambia. *J Sci Food Agric, 28*, 288-292

Lu LJW, Grady JJ, Marshall MV, Ramanujam VMS & Anderson KE (1995) Altered time course of urinary daidzein and genistein excretion during chronic soya diet in healthy male subjects. *Nutr Cancer. 24,* 311-323

Marasas WFO, van Rensburg SJ & Mirocha CJ (1979) Incidence of *Fusarium* species and the mycotoxins, deoxynivanol and zearalenone, in corn produced in esophageal cancer areas in Transkei. *J Agric Food Chem. 27,* 1108-1112

Martin PMD & Keen P (1978) The occurrence of zearalenone in raw and fermented products from Swaziland and Lesotho. *Sabrouaudia. 16,* 15-22

Norton DM, Toule GM, Cooper SJ, Partington SR & Chapman (1982) In: Pepin GA, Patterson DSP & Gray DE, eds. *Proceedings, Fourth Meeting on Mycotoxins in Animal Disease* Min Agic Fisheries Food, Alnwick, Northumberland, UK, 77-81

Pepeljnjak S (1984) Mycotoxic contamination of harcot beans in nephropathic areas in Yugoslavia. *Microbiol Aliments Nutr. 2*, 331-336

Petterson H & Kiessling KH (1984) Liquid chromatographic determination of the plant estrogens coumestrol and isoflavones in animal feed. *J Assoc Off Anal Chem. 67,* 503-506

Scott PM, Panalakas T, Kanhere S & Miles WF (1978) Determination of zearalenone in cornflakes and other corn-based foods by thin layer chromatography, high pressure liquid chromatography, and gas-liquid chromatography/ high resolution mass spectrometry. *J Assoc Off Anal Chem. 61,* 593-600

Senti FR (1979) Global perspective on mycotoxins. In: *Perspectives on mycotoxins*. FOA, Rome, 15-120

Seo A & Morr CV (1984) Improved high-performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. *J Agric Food Chem. 32,* 530-533

Tanaka T, Hasegawa A, Matsuki Y & Ueno Y (1985) A survey of the occurrence of nivalenol, deoxynivalenol and zearalenone in foodstuffs and health foods in Japan. *Food Addit Contam. 2,* 259-265

Wang G, Kuan SS, Francis OJ, Ware GM, & Carman AL (1990) A simplified HPLC method for the determination of phytoestrogens in soybean in its processed products. *J Agric Food Chem, 38,* 185-190

Ware GM & Thorpe CW (1978) Determination of zearalenone in corn by high pressure liquid chromatography and fluorescence detection. *J Assoc Off Anal; Chem. 61*, 1058-1062

Williams BC (1985) Mycotoxins in foods and foodstuffs. In Scott PM, Trenholm HL & Sutton, eds. *Mycotoxins : A Canadian Perspective.* National Reasearch Council Canada, Ottawa, 49-53

Xu X, Harris KS, Wang HJ, Murphy PA & Hendrich S (1995) Bioavailability of soybean isoflavone depends upon gut microflora in women. *J Nutr. 125*, 2307-2315