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## Risk assessment of "other substances" – Isoflavones from soy

**Opinion of the Panel Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety**

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Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact  
with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety  
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# **Risk assessment of "other substances" – Isoflavones from soy**

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The opinion has been assessed and approved by Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Members of the panel are: Inger-Lise Steffensen (Chair), Ellen Bruzell, Berit Granum, Ragna Bogen Hetland, Trine Husøy, Jens Rohloff, Trude Wicklund.

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## **Competence of VKM experts**

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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# Summary

## The request

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has, at the request of the Norwegian Food Safety Authority (NFSA; Mattilsynet), assessed the risk of "other substances" in food supplements and energy drinks sold in Norway. VKM has assessed the risk of doses in food supplements and concentrations in energy drinks given by NFSA. These risk assessments will provide NFSA with the scientific basis while regulating the addition of "other substances" to food supplements and other foods.

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional and/or physiological effect*. It is added mainly to food supplements, but also to energy drinks and other foods. VKM has not in this series of risk assessments of "other substances" evaluated any claimed beneficial effects from these substances, only possible adverse effects.

The present report is a risk assessment of isoflavones from soy, and it is based on previous risk assessments and articles retrieved from literature searches.

According to information from NFSA, isoflavones from soy are ingredients in food supplements sold in Norway. The food supplements on the Norwegian market contain isoflavones isolated from soybeans of the plant *Glycine max* (L.) Merrill by extraction by 80% alcohol and 20% water. It is not known which alcohol is used. NFSA has requested a risk assessment of the intake of 40 and 80 mg isoflavones (as the total of genistein and daidzein) per day from food supplements. No further information was given on the individual isoflavone isomers and their amounts in the extracts in the soy isoflavone supplements on the Norwegian market. The intake of isoflavones from soy in the general population was evaluated for the age groups children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years).

## Phytoestrogens

Phytoestrogens consist of flavonoids and non-flavonoids. The flavonoids are the isoflavones, coumestans and prenyl flavonoids and the non-flavonoids are the lignans. Isoflavones are a class of non-steroidal estrogens that have similarity in chemical structure and properties to estrogen. However, they show conformational binding to the estrogen receptor that classifies them as natural selective estrogen receptor modulators (SERMs) rather than as estrogens, and have estrogenic and anti-estrogenic effects depending on the concentration of endogenous estrogen and amount and type of estrogen receptors in the tissues. Isoflavones have both endocrine and non-endocrine effects. Isoflavones may interact with endocrine pathways and potentially cause negative health effects. In this risk assessment, the focus is on endocrine effects of isoflavones.



## Isoflavones from soy

There are in total 12 different soybean isoflavone isomers. Soy isoflavones comprise three aglycones; genistein, daidzein and glycitein. These also exist as their corresponding  $\beta$ -glycosides; genistin, daidzin and glycitin. In addition, the three  $\beta$ -glycosides can each be esterified with either malonic acid or acetic acid. Most of the isoflavones found in soybeans and non-fermented foods exist as  $\beta$ -glycosides, whereas in fermented soy products, due to fermentation and hydrolysis by microorganisms, much of the isoflavones is present in aglycone form. Typically, there is somewhat more genistein/genistin than daidzein/daidzin in soybeans and soy foods, whereas glycitein/glycitin comprises only 5-10% of the total isoflavone content. Overall, the evidence indicates that whether isoflavones are present as glycosides or aglycones appears not to be critically important with regard to their biological effects, since the glycosides can be hydrolysed *in vivo*.

There is large variation in composition and concentration of isoflavones among different soybean or soy protein products, which is dependent of soy species differences, part of the soy plant used, geographic and environmental growing conditions and cultivating parameters. The extent of industrial processing of the soybeans, including the extraction process, i.e. which mechanical extraction method, solvent type, temperature and time being used, can also affect the composition and concentration of isoflavones.

## Absorption, distribution, metabolism and excretion

A number of factors are influencing the bioavailability of isoflavones, including intestinal microflora, gender, age, food matrix, chemical composition, earlier exposure and background diet.

Isoflavone glycosides genistin, daidzin and glycitin are not absorbed intact across the enterocytes of healthy persons because of their higher hydrophilicity and molecular weights compared with the aglycones. Their bioavailability for uptake into the peripheral circulation requires the conversion of glycosides to aglycones via the action of intestinal  $\beta$ -glycosidase from bacteria that colonize the small intestine. For humans, no data on absolute bioavailability can be given. However, based on the available knowledge it can be deduced that the absolute bioavailability of the two main isoflavones, genistein and daidzein, must be low in humans. After ingestion, soy isoflavones are partially hydrolyzed in the small intestine to release the aglycones genistein, daidzein and glycitein, followed by absorption. The isoflavones appear to be distributed to most organs in the body.

A considerable fraction of isoflavones, which is neither hydrolyzed nor absorbed in the small intestine, reaches the colon, together with an amount of conjugated isoflavones that is excreted into the small intestine through enterohepatic circulation. In the colon, the glycosylated, sulfated and glucuronidated forms of daidzein are deconjugated by bacterial enzymes, and then absorbed or subjected to further metabolism by the intestinal microflora. The main metabolites of genistein and daidzein are the sulfate and glucuronide. Monkeys, rats and mice are described as 100% equol producers, meaning that the microbiotas of

these animals are uniformly able to transform daidzein to a considerable extent to the metabolite *S*-equol. In humans, only one-third to one-half of the population is able to produce *S*-equol. Oxidative phase I metabolites of genistein and daidzein, mainly 6-hydroxy- and 3'-hydroxy-genistein, as well as 6-hydroxy-, 8-hydroxy- and 3'-hydroxy-daidzein, are found in humans, rats and mice, although as minor metabolites.

The extent of metabolism appears to be highly variable among individuals and is influenced by i.a. other components of the diet. Most of the absorbed genistein and daidzein are excreted as phase II conjugates and as phase II conjugates of microbial-derived metabolites in the urine within the first 24 hours after ingestion. Fecal elimination has been found to be a minor route.

Because of the differences in pharmacokinetics between humans and rodent species as described and the abundance of human studies available on isoflavones, toxicity data from experimental animals were not included in this risk assessment, except for *in vivo* genotoxicity data.

### Toxicity

By *in vitro* tests in mammalian cells, genistein, for which a thresholded mechanism of action has been demonstrated, and the two metabolites of daidzein, 3'-hydroxy-daizein and 6-hydroksy-daidzein, are genotoxic. However, this effect has not been reproduced in valid *in vivo* micronucleus tests in rats and mice or in the Comet assay and micronucleus test in human studies. On this basis, the use of isoflavones in food supplements is not of genotoxic concern.

Gastrointestinal symptoms, insomnia or back pain were reported as adverse events/side-effects in peri- and postmenopausal women at the same rate, or in a few studies at higher rate, with isoflavones compared with placebo. The doses of total isoflavones and duration of treatment in these studies were 60 and approximately 120 mg total isoflavones per day for 6 weeks, from 56 to 160 mg/day for 3-6 months and from 52 to 300 mg/day from 9 to 12 months. In addition, one study examined effects of 898 mg/day of total isoflavones for 3 months and another study examined effects of 150 mg/day for 5 years. Based on the available studies from the literature, isoflavones as supplements in these doses and duration of treatment appear to be without significant negative health effects in peri- and post-menopausal women.

The relevance of the few studies that found increased risk of cancer of a very high dose of isoflavone supplements or in occasional comparisons of dietary intake of soy food products in mostly Asian populations is difficult to interpret in relation to intake of isoflavone supplements in Norwegian peri- and post-menopausal women.

EFSA (2015) concluded that doses of treatment used in the intervention studies and their durations could serve as guidance for the dose and duration of use at which no effects have been observed in these three target organs (mammary gland, uterus or thyroid) in peri- and

post-menopausal women from the intake of food supplements. For soy isoflavones/soy extract this dose was 100 mg/day (total isoflavones), 10 months duration of intake. For soy protein, this dose was 99 mg/day (aglycone), 3 months duration of intake. For daidzein-rich isoflavones, this dose was 72 mg/day (total), 6 months duration of intake. For genistein, this dose was 54 mg/day, 36 months duration of intake.

In pre-menopausal women, isoflavones taken for approximately one to three months apparently affected hormone levels and menstruation in doses of 45 to 128 mg/day and 45 to 116.4 mg/day, respectively. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In peri- and post-menopausal women, soy isoflavones are taken with the purpose of decreasing post-menopausal symptoms such as hot flashes, instead of estrogen-based hormone-replacement therapy, whereas pre-menopausal women would not have this need. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy pre-menopausal women. In this risk assessment of isoflavones in healthy pre-menopausal women, VKM considers changes in hormone levels away from the normal range as negative effects.

The most common adverse events/side-effects of isoflavones reported in healthy men were mild gastrointestinal symptoms, or sometimes weight gain, estrogenic effects or reduction in vitamin E. Hormone levels appeared to be affected by doses of isoflavones ranging from 1.64 to 196 mg/day for one to three months. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy men. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In patients with prostate cancer or at high risk for recurrence of prostate cancer and possibly in patients with other types of hormone-related cancers, isoflavones may have a beneficial effect on the progression of the disease by decreasing testosterone. VKM assumes that such therapy with isoflavone administration is given under prescription and medical surveillance, and is outside the scope of this risk assessment. However, useful information can also be found in studies on prostate cancer patients and men with other diagnoses and is therefore included. It was shown that isoflavones in doses of 450 or 900 mg/day for three months could have estrogenic effects in men with prostate cancer, such as breast changes or increased frequency of hot flashes. In healthy men taking supplements without surveillance, isoflavones may potentially also lead to estrogenic effects. In this risk assessment of healthy men, VKM considers changes in hormone levels away from the normal range as negative effects. There is some data indicating that it is soy protein as such rather than the isoflavones that are causing the observed effects on hormone levels, however, this is still uncertain.

### Allergy

Whether supplements with isoflavones from soy may cause food allergy is not known. Soy protein is a well-known food allergen, with a reported incidence of 0.3-0.4% of the total

population in Germany. However, the majority of the cases are presumed to be cross-sensitizations due to primary sensitization to peanut and/or birch pollen.

### Vulnerable groups

Persons with certain polymorphisms in genes affecting estrogenic effects or metabolism of isoflavones may potentially be vulnerable for the effects of isoflavones in supplements. However, polymorphisms associated with decreased health risks rather than increased risk have been reported for isoflavones; in genes affecting estrogenic effects, in phase I and II metabolic enzymes, in genes in the ornithine decarboxylase-polyamine pathway or in genes involved in efflux transport of isoflavone glucuronides. Metabolic enzymes in the gut microflora may determine which metabolites are formed and their levels, such as the daidzein metabolite *S*-equol formed only in a part of the population.

One study on effects of isoflavones on hemodialysis patients concluded that they were not a vulnerable group. Collectively the available data provided little evidence that in euthyroid, iodine-replete individuals, soy foods or isoflavones adversely affect thyroid function. However, individuals with subclinical hypothyroidism may evaluate the need to adjust their dosage of medication, and individuals with inadequate iodine intake may increase their iodine intake further, when consuming soy foods or supplements.

Consumer groups which have an already high dietary intake of isoflavones from a vegetarian or vegan diet, a traditional Asian (i.e. Japanese, Chinese) diet or a diet high in soy-based foods for whatever reasons, or infants eating mainly or solely soy infant formula, may be regarded as high consumers of isoflavones. These groups may be vulnerable to potential negative effects from additional intake of isoflavones from supplements.

### Interaction with medication

Isoflavones may affect some drugs, including prescription medicines, via their modulation of phase I and phase II metabolic enzymes and interaction with drug transporters. It has also been shown that interactions may occur among the various isoflavones.

### Uncertainties in the data

Sources of isoflavones examined in the included studies were reported to be soy protein, soy protein isolate (SPI) or isolated soy protein (ISP), soybeans, soy foods such as soy milk, flour, grits, tofu or tempe, as commercial products or prepared meals, isoflavone concentrate or just referred to as isoflavones. They may also be genistein combined polysaccharide (GCP), which is enriched in aglycone forms of isoflavones, percentages of genistein, daidzein, glycitein, and in some studies also as percentages of genistin, daidzin, glycitin and the malonyl- and acetyl-derivatives. The isoflavones were given in the form of tablets, pills, capsules, powders, extracts or concentrates, sometimes mixed into scones, biscuits, cereal bars, snack bars, cereals, drinks or beverages, or the form was not specified. The chemical form of isoflavones was reported (if stated) as aglycones, aglycone equivalents, glycosides or

as several categories. The ratio of percentages of individual aglycone isoflavones genistein:daidzein:glycitein varied substantially among the studies; 15-95:10-55:1-30. In three studies, the isoflavones were apparently given as high levels of glycosides, with genistin:daidzin:glycitin ratios of 20-52:37.2-50:8.8-30. Five studies reported effects of separate genistein in tablets or capsules.

Based on the included studies, no pattern was obvious regarding type of soy product or isoflavone composition and negative health effects. Therefore, in this risk assessment VKM has used the doses of total isoflavones reported to cause negative effects in studies on the respective gender and age groups for comparisons with 40 and 80 mg/day of total isoflavones in supplements.

In many of the studies used in this risk assessment, the full composition of aglycones vs. glycosides/glycoside esters was not stated, nor were the soybean source and the details of the extraction process. Consequently, there is ambiguity regarding the biologically active amounts of isoflavones in a product even when total amount of isoflavones is indicated. Since the molecular weight of the aglycones is approximately 60% of that of the glycosides, 100 mg isoflavones can actually refer to between approximately 60 and 100 mg of biologically active isoflavones. Therefore, there is considerable uncertainty in relation to the exact substances being studied in the literature and the similarity/dissimilarity with the isoflavone supplements on the Norwegian market regarding their effects (beneficial or adverse).

Many of the studies published on effects of isoflavones are on Asian populations. There is some uncertainty as to whether Asian and non-Asian individuals respond similarly to the effects of isoflavones.

#### Intake/exposure

From the dose of 40 mg/day of isoflavones in supplements, the estimated daily intake of isoflavones were 0.92, 0.65 and 0.57 mg/kg bw per day for children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years), respectively. From the dose of 80 mg/day of isoflavones, the estimated daily intake of isoflavones were 1.84, 1.31 and 1.14 mg/kg bw per day for children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years), respectively.

In addition to isoflavones in supplements, dietary isoflavones from a large number of soy food products are a source of exposure to isoflavones. Genistein and daidzein are used for skin conditioning in cosmetics. However, the total exposure to isoflavones from other sources than food supplements, such as foods and cosmetic products, and from other sources than soy, such as red clover and kudzu root, are not included in this risk assessment.

In Norway, as in other Western countries, the intake of soybeans and soybean-based products is generally low in the average diet. The intake (mean  $\pm$  SE) of isoflavones from dietary soy in women was found to be  $1.474 \pm 0.158$  and  $1.096 \pm 0.178$  mg/day in south

and east of Norway and north and west of Norway, respectively, based on a single 24-hour recall in the EPIC study. Corresponding numbers were  $0.005 \pm 0.000$  and  $0.004 \pm 0.000$  mg/day for the daidzein metabolite *S*-equol. For men, the corresponding intakes of isoflavones were  $0.978 \pm 0.135$  and  $0.721 \pm 0.136$  mg/day, respectively, and for *S*-equol, the numbers were  $0.006 \pm 0.000$  and  $0.007 \pm 0.000$  mg/day, respectively.

The intake of soybeans and soybean-based products may be higher in vegans and persons with milk allergy. The mean intake of soy protein per day has been estimated for persons eating either a vegan menu or a milk-free diet. The mean intake for adults (women or men) was 35 and 19 g soy protein per day, for the vegan and milk allergy scenarios, respectively. In the vegan scenario, the estimated intake was 30 and 41 g soy protein per day for 10-13 year old girls and 14-17 year old boys, respectively. In the milk allergy scenario, the estimated intake was 17 and 23 g soy protein per day for 10-13 year old girls and 14-17 year old boys, respectively. For 10-13 year old boys and 14-17 year old girls, the estimated intake was approximately similar to the intake in adults.

Based on a food frequency questionnaire, among 87800 women in the Norwegian Mother and Child Cohort Study (MoBa), 1.7% used soy milk, and of these only 0.4% drank  $\geq 200$  ml of soy milk per day. Regarding use of soy products for dinner, 4.8% had an occasional intake, and only 12% of these women had soy products for dinner more than once every other week.

## Conclusions

No studies were found addressing both exposure to and effects of isoflavones in adolescents (14 to <18 years). However, there was no evidence in the included literature indicating that adolescents (aged 14 to <18 years, i.e. mostly after puberty) are more sensitive to isoflavones than adults. Therefore, VKM finds that the results for pre-menopausal women and men have validity also for adolescents.

No studies were found on effects of isoflavones from exposure to children (10 to <14 years).

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that

- Isoflavones as supplements in doses of 40 or 80 mg/day taken for several months and even up to several years appear to be without significant negative health effects in peri- and post-menopausal women.
- Isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels and/or menstrual function in pre-menopausal women. These doses do not appear to have other significant negative effects on pre-menopausal women.
- Isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in men. These doses do not appear to have other significant negative effects on men in the general population.

- Isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in adolescents of both genders and/or menstrual function in adolescent women. These doses do not appear to have other significant negative effects on adolescents.
- There is not sufficient data to draw any conclusions on potential adverse effects of isoflavones in supplements in children (aged 10 to <14 years).

## Short summary

According to information from NFSA, isoflavones from soy are ingredients in food supplements purchased in Norway. NFSA has requested a risk assessment of the intake of 40 and 80 mg isoflavones (as the total content of genistein and daidzein) per day from food supplements. No further information was given on which individual isoflavone isomers and in which amounts are found in the extracts in the soy isoflavone supplements on the Norwegian market. The conclusions below are valid given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies. The intake of isoflavones from soy in the general population was evaluated for the age groups children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for several months and even up to several years appear to be without significant negative health effects in peri- and post-menopausal women.

As opposed to in peri- and post-menopausal women, VKM considers changes in hormone levels away from the normal range as negative effects in healthy premenopausal women and in men.

VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels and/or menstrual function in pre-menopausal women. These doses do not appear to have other significant negative effects on pre-menopausal women.

VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in men. These doses do not appear to have other significant negative effects on men in the general population.

VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in adolescents of both genders and/or menstrual function in adolescent women. These doses do not appear to have other significant negative effects on adolescents.

VKM concludes that there is not sufficient data to draw any conclusions on potential adverse effects of isoflavones in supplements in children (aged 10 to <14 years).

**Key words:** Adverse events, aglycones, daidzein, daidzin, food supplement, genistein, genistin, glycitein, glycitin, glycosides, isoflavones, negative health effects, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food Safety, "other substances", risk assessment, soy, VKM



# Sammendrag på norsk

## Oppdraget

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetning av "andre stoffer" i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike doser brukt av kosttilskudd og konsentrasjoner i energidrikker oppgitt fra Mattilsynet. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere "andre stoffer".

"Andre stoffer" er beskrevet i kosttilskuddsdirektivet 2002/46/EC som *stoffer som har en ernæringsmessig og/eller fysiologisk effekt, og som ikke er vitaminer og mineraler*. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke sett på påståtte gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

Denne rapporten er en risikovurdering av isoflavoner fra soya, og den er basert på tidligere risikovurderinger og artikler hentet fra litteratursøk.

I følge informasjon fra Mattilsynet selges det kosttilskudd som inneholder isoflavoner fra soya i Norge. Isoflavonene i disse kosttilskuddene er ekstrahert fra soyabønner fra planten *Glycine max* (L.) Merrill med 80% alkohol og 20% vann. Hvilken type alkohol som brukes er ikke kjent. Oppdraget fra Mattilsynet var å risikovurdere inntak av isoflavoner fra soya (som det totale innholdet av genistein og daidzein) i kosttilskudd i dosene 40 og 80 mg/dag. Det ble ikke gitt ytterligere informasjon om hvilke individuelle isoflavonisoimerer som finnes i hvilke mengder i disse ekstraktene av isoflavoner fra soya i kosttilskudd på det norske markedet. Inntak av isoflavoner fra soya ble vurdert for den generelle befolkningen i aldersgruppene barn (10 til <14 år), ungdom (14 til <18 år) og voksne ( $\geq 18$  år).

## Fytoøstrogener

Fytoøstrogener består av flavonoider og ikke-flavonoider. Flavonoidene er isoflavoner, coumestaner og prenyl flavonoider. Ikke-flavonoidene er lignaner. Isoflavoner er en klasse ikke-steroid østrogener som ligner østrogen i kjemisk struktur og egenskaper. Isoflavoner viser konformasjonsbinding til østrogenreseptorer som klassifiserer dem som naturlige selektive østrogenreseptor modulatorer (SERMs) heller enn som østrogener. De kan ha både østrogene og anti-østrogene effekter avhengig av konsentrasjonen av endogent østrogen og mengde og type av østrogenreseptorer i vevet. Isoflavoner har både endokrine og ikke-endokrine effekter. De kan virke på endokrine signalveier og potensielt gi negative helseeffekter. Denne risikovurderingen fokuserer på endokrine effekter av isoflavoner.

## Isoflavoner fra soya

Det er totalt 12 ulike isomerer av isoflavoner fra soyabønner. Isoflavoner fra soya består av tre aglykoner; genistein, daidzein og glycitein. Isoflavonene finnes også som  $\beta$ -glykosider; genistin, daidzin og glycitin. I tillegg kan de tre  $\beta$ -glykosidene esterifiseres med enten

malonsyre eller eddiksyre. Mesteparten av isoflavonene som finnes i soyabønner og ikke-fermenterte soyamatvarer er  $\beta$ -glykosider. I fermenterte soyaprodukter er mye av isoflavonene aglykoner. Vanligvis er det noe mer genistein/genistin enn daidzein/daidzin i soyabønner og andre matvarer med soya, mens glycitein/glycitin utgjør bare ca. 5-10% av det totale isoflavoninnholdet. Ut i fra det som finnes av data ser det ikke ut til at det er avgjørende for isoflavonenes effekter om de er til stede som glykosider eller aglykoner. Det er fordi glykosidene kan hydrolyseres til aglykoner i kroppen.

Det er stor variasjon i sammensetning og konsentrasjon av isoflavoner i ulike produkter av soyabønner og soyaprotein. Sammensetningen og konsentrasjonen avhenger av arten soyaplante, hvilken del av planten som brukes, geografiske og miljømessige dyrkningsforhold og ulike andre parametre som varierer under dyrkingen. Grad av industriell bearbeiding av soyabønnene, inkludert hvilken ekstraksjonsprosess som brukes, bl.a. hvilken mekanisk ekstraksjonsmetode, type løsemiddel, temperatur og tid for ekstraksjonen, kan også påvirke sammensetning og konsentrasjon av isoflavoner.

#### Opptak, distribusjon, metabolisme og ekskresjon

Flere faktorer påvirker tilgjengeligheten av isoflavoner i kroppen. Disse inkluderer mikroflora i tarm, kjønn, alder, hva slags mat isoflavonene er i, isoflavonenes kjemiske sammensetning, om kroppen har vært eksponert for isoflavoner tidligere og kosten for øvrig.

Glykosidene genistin, daidzin og glycitein absorberes ikke intakte over tarmcellene i friske personer, p.g.a. deres høyere hydrofilisitet og molekylvekt i forhold til aglykonene. Deres biotilgjengelighet for opptak inn i perifer sirkulasjon krever at de omdannes til aglykoner ved hjelp av det bakterielle enzymet  $\beta$ -glukosidase i tynntarmen. For mennesker har man ikke data for absolutt biotilgjengelighet, men dagens kunnskap tyder på at den absolutte biotilgjengeligheten av de to viktigste isoflavonene, genistein og daidzein, er lav. Etter fordøyelse blir isoflavonene delvis hydrolysert i tynntarmen slik at aglykonene genistein, daidzein og glycitein frigjøres og deretter absorberes. Isoflavonene ser ut til å bli distribuert til de fleste organer i kroppen.

En betydelig del av isoflavonene blir verken hydrolysert eller absorbert i tynntarmen, og disse når tykktarmen, sammen med en mengde som skilles ut i tynntarmen via enterohepatisk sirkulasjon. I tykktarmen blir glykosylerte, sulfaterte og glukuroniderte former av daidzein dekonjugert av bakterielle enzymer, og så absorbert og metabolisert videre av tarmfloraen. De viktigste metabolittene er sulfat- og glukuronid-konjugatene av genistein og daidzein. Mikrobiota i tarmen på aper, rotter og mus kan omdanne daidzein i betydelig grad til metabolitten *S*-equol, og disse artene beskrives derfor som 100% equolprodusenter. I mennesker derimot er det kun en tredjedel til halvparten av befolkningen som kan produsere *S*-equol. Oksidative fase I-metabolitter av genistein og daidzein, hovedsakelig 6-hydrokso- og 3'-hydrokso-genistein og 6-hydrokso-, 8-hydrokso- og 3'-hydrokso-daidzein, er også funnet i mennesker, rotter og mus, men i mindre mengder.

Graden av metabolisme er svært variabel blant personer og avhenger bl.a. av andre bestanddeler i kostholdet. Mesteparten av genistein og daidzein skilles ut med urinen i løpet av 24 timer etter inntaket. Relativt lite elimineres via feces.

På grunn av forskjellene i omsetning av stoffene mellom mennesker og gnagere som beskrevet og fordi det var mange tilgjengelige artikler om isoflavoner studert i mennesker, er ikke data på toksisitet av isoflavoner i dyr inkludert i denne risikovurderingen, bortsett fra data på *in vivo* gentoksisitet.

### Toksisitet

Fra *in vitro* studier i pattedyrceller er det rapportert at genistein, som det er vist at har en terskel for effekten, og de to daidzeinmetabolittene 3',4',7-trihydroksyisoflavon og 4',6,7-trihydroksyisoflavon, er gentoksiske. Dette ble ikke bekreftet i *in vivo* mikrokjernetester i rotter og mus eller i Comet-tester og mikrokjernetester i mennesker. På basis av disse resultatene er det ikke grunn til bekymring for at isoflavoner i kosttilskudd er gentoksiske.

Rapporterte negative bivirkninger av isoflavoner hos peri- og post-menopausale kvinner er mage/tarm-symptomer, søvnproblemer og ryggsmertor. Disse bivirkningene ble rapportert med samme frekvens, eller i noen få studier i høyere frekvens, sammenlignet med placebo. De totale dosene av isoflavoner og varigheten av behandlingen i studiene som rapporterte bivirkningene var 60 og ca. 120 mg/dag i 6 uker, fra 56 til 160 mg/dag i 3-6 måneder og fra 52 til 300 mg/dag i 9-12 måneder. I tillegg var det en studie som hadde gitt 898 mg/dag i 3 måneder og en annen studie som hadde gitt 150 mg/dag i 5 år. Basert på de tilgjengelige studiene fra litteraturen ser det ut til at isoflavoner i kosttilskudd i disse dosene og med samme varighet av behandlingen er uten betydelige negative helse-effekter i peri- og postmenopausale kvinner.

I noen få studier ble det funnet økt risiko for kreft med en veldig høy dose av isoflavoner i kosttilskudd eller i enkelte sammenlikninger av daglig inntak av soya-produkter i hovedsaklig asiatiske befolkninger. Relevansen av disse studiene er vanskelig å tolke i forhold til inntak av isoflavoner i kosttilskudd i norske peri- og post-menopausale kvinner.

I sin risikovurdering fra 2015 konkluderte den europeiske myndighet for næringsmiddeltrygghet (EFSA) med at doser og behandlingstid brukt i publiserte intervensjonsstudier kunne være veiledende for hvilke doser og varighet av bruk av kosttilskudd som ikke hadde negative helseeffekter. EFSA vurderte effektene på bryst, livmor og skjoldbruskkjertel i peri- og post-menopausale kvinner. For isoflavoner i soya eller soya-ekstrakter totalt var dosen og varigheten av bruk 100 mg/dag i 10 måneder og for soya-protein (aglykoner) var dette 99 mg/dag i 3 måneder. For totale daidzein-rike isoflavoner var dosen 72 mg/dag i 6 måneder og for genistein alene var dosen 54 mg/dag i 3 år.

I pre-menopausale kvinner ser det ut som at isoflavoner tatt i 1-3 måneder i doser på henholdsvis 45 til 128 mg/dag og 45 til 116,4 mg/dag påvirket hormonnivåer og menstruasjon. Om effektene av eksponering for isoflavoner fra soya på hormonnivåer anses som skadelige eller fordelaktige vil avhenge av målgruppen. Peri- og post-menopausale

kvinner tar isoflavoner fra soya for å behandle symptomer på overgangsalder i stedet for østrogenbasert hormonell erstatningsterapi, mens pre-menopausale kvinner ikke har dette behovet. Få av studiene diskuterer den kliniske betydningen av de observerte forandringene i hormonnivåer i friske pre-menopausale kvinner. I denne risikovurderingen av friske pre-menopausale kvinner, anser VKM forandringer i hormonnivåer bort fra det normale området som negative effekter.

I friske menn er de vanligste rapporterte bivirkningene av isoflavoner mage/tarm-symptomer og noen ganger vektøkning, østrogene effekter eller reduksjon i vitamin E. I tillegg så det ut til at isoflavoner påvirker hormonnivåer (i doser på 1.64 til 196 mg/dag når de ble tatt i en til tre måneder). Få av studiene diskuterer den kliniske betydningen av de observerte forandringene i hormonnivåer i friske menn. Om effektene av eksponering for isoflavoner fra soya på hormonnivåer vil anses som skadelige eller fordelaktige vil avhenge av målgruppen. I pasienter med prostatakreft eller med høy risiko for tilbakefall av prostatakreft og kanskje i pasienter med andre typer av hormonrelatert kreft, kan isoflavoner ha en fordelaktig effekt på forløpet av sykdommen ved å senke nivået av testosteron. VKM antar at slik behandling med isoflavoner gis med resept og under medisinsk overvåking og er utenfor mandatet for denne risikovurderingen. Når slike data likevel er inkludert, er det fordi det er nyttig informasjon også i studier på pasienter med prostata-kreft eller andre diagnoser. Det er vist at isoflavoner i dosene 450 eller 900 mg/dag tatt i tre måneder kan ha østrogene effekter i menn med prostatakreft, slik som brystforandringer og økt frekvens av hetetokter. I friske menn som tar isoflavoner uten legetilsyn kan isoflavoner tenkes å ha østrogene effekter. VKM anser forandringer i hormonnivåer bort fra det normale området som negative effekter i friske menn. Noen data antyder at det er soya-protein som sådan, heller enn isoflavonene, som forårsaker de observerte effektene på hormonnivåer, men dette er fortsatt usikkert.

### Allergi

Det er ikke kjent om kosttilskudd med isoflavoner fra soya kan medføre matallergi. Soyaprotein er et velkjent matallergen, med rapportert insidens på 0,3-0,4% i den tyske befolkningen. Flertallet av tilfellene antas å skyldes kryssreaksjoner etter primær sensibilisering mot peanøtter og/eller bjørk.

### Sårbare grupper

Personer med visse varianter i gener som påvirker østrogene effekter eller metabolisme av isoflavoner, kan være potensielt sårbare for effekter av isoflavoner i kosttilskudd. De fleste genvariantene som er rapportert å påvirke effektene av isoflavoner er imidlertid assosiert med lavere risiko for sykdommer; i gener som påvirker østrogene effekter, i fase I- og fase II-metabolske enzymer, i gener i ornitindekarboksylase-polyamin signalveien eller i gener involvert i transport ut av isoflavonglukuronider. Metabolske enzymer i mikrofloraen i tarmen kan bestemme hvilke metabolitter som dannes og deres nivåer, slik som for daidzeinmetabolitten S-equol som dannes kun i deler av befolkningen.

En studie på effekter av isoflavoner på hemodialysepasienter konkluderte med at de ikke var en sårbar gruppe. Samlet sett tyder tilgjengelige data på at det er få holdepunkter for at

soyamatvarer eller isoflavoner vil ha negative effekter på funksjonen av skjoldbruskkjertelen (thyroidea) i individer med normale nivåer av thyroidhormoner og jod. Men individer med subklinisk hypothyroidisme kan vurdere behovet for å justere sin medikamentdose, og individer med for lavt jodnivå kan øke sitt jodinntak ytterligere, når de spiser soyamatvarer eller tar kosttilskudd med soya.

Forbrukergrupper som allerede har et høyt inntak av isoflavoner fra et vegetarisk eller vegansk kosthold, et tradisjonelt asiatisk (slik som japansk eller kinesisk) kosthold eller et kosthold med høyt inntak av soyabasert mat uansett begrunnelse, eller spedbarn som spiser hovedsakelig eller utelukkende morsmelkerstatning med soya, må anses som høykonsumenter av isoflavoner. Disse gruppene kan derfor være sårbare for potensielle negative effekter fra et tilleggssinntak av isoflavoner fra kosttilskudd.

### Interaksjon med legemidler

Isoflavoner kan påvirke fase I- og fase-II-metabolske enzymer og samvirke med transportproteiner for medikamenter, og kan derfor påvirke effekten til noen legemidler. Det er også vist at interaksjoner kan skje blant ulike isoflavoner.

### Usikkerhet i datagrunnlaget

I de inkluderte studiene i denne risikovurderingen er kildene til isoflavoner rapportert som soyaprotein, soyaprotein isolat (SPI), isolert soyaprotein (ISP), soyabønner, soyamatprodukter som soyamelk, mel, gryn, tofu eller tempe, enten som ferdige kommersielle produkter eller måltider som lages for studien, isoflavonkonsentrater eller de er bare oppgitt som isoflavoner. De kan også være "genistein-kombinert polysakkarid" (GCP), som er anrikt med aglykoner av isoflavoner, eller oppgis som prosentandeler av genistein, daidzein, glycitein, og i noen studier også som prosentandeler av genistin, daidzin, glycitin og deres malonyl- og acetyl-derivater. Isoflavonene kan bli gitt i form av tabletter, piller, kapsler, pulver, ekstrakter eller konsentrater, noen ganger blandet inn i scones, kjeks, cereal-barer, "snack"-barer, cerealer, i drikker og drikkevarer, eller formen ble ikke oppgitt. De kjemiske formene av isoflavoner som ble oppgitt, var som aglykoner, aglykon-ekvivalenter, glykosider eller som flere kategorier. Forholdet mellom prosentandelene av de individuelle aglykonisoflavonene genistein:daidzein:glycitein varierte veldig blant studiene; 15-95:10-55:1-30. I tre studier ble isoflavonene oppgitt som høye andeler av glykosider, med forholdet mellom genistin:daidzin:glycitin på 20-52:37.2-50:8.8-30. Fem studier rapporterte effekter av bare genistein separat, i tabletter eller kapsler.

Ut fra de inkluderte studiene var det ikke noe åpenbart mønster i hvilke typer soyaprodukter eller isoflavonsammensetninger som gav negative helseeffekter. VKM har derfor i denne risikovurderingen brukt de dosene av totale isoflavoner som gav negative helseeffekter i studier på de respektive kjønn- og aldersgruppene som sammenligning med dosene på 40 og 80 mg/dag av totale isoflavoner i kosttilskudd.

I mange av studiene brukt i denne risikovurderingen er ikke den fulle sammensetningen av aglykoner versus glykosider/glykosidestere oppgitt og kilden til soyabønnene eller hvordan

isoflavonene ble ekstrahert fra soya, er heller ikke var alltid oppgitt. Derfor kan det være tvetydighet når det gjelder de biologisk aktive mengdene av isoflavoner i et produkt selv om total mengde av isoflavoner er angitt. Siden molekylvekten av aglykonene er ca. 60% av molekylvekten til glykosidene, kan 100 mg isoflavoner faktisk referere til mellom ca. 60 og 100 mg av biologisk aktive isoflavoner. Det er derfor betydelig usikkerhet med hensyn til de eksakte stoffene som studeres i artiklene og likheter/ulikheter med stoffene som er i kosttilskuddene med isoflavoner på det norske markedet når det gjelder deres effekter (fordelaktige eller skadelige).

Mange av studiene som er publisert på effekter av isoflavoner er gjort på asiatiske befolkninger. Det er usikkerhet når det gjelder om asiatiske og vestlige individer er like med hensyn til effektene av isoflavoner.

### Inntak/eksponering

Fra dosen på 40 mg/dag av totale isoflavoner i kosttilskudd var det beregnede daglige inntaket av isoflavoner henholdsvis 0,92, 0,65 og 0,57 mg/kg kroppsvekt per dag for barn (10 til <14 år), ungdom (14 til <18 år) og voksne ( $\geq 18$  år). Fra dosen på 80 mg/dag av totale isoflavoner i kosttilskudd var det beregnede daglige inntaket av isoflavoner henholdsvis 1,84, 1,31 og 1,14 mg/kg kroppsvekt per dag for barn (10 til <14 år), ungdom (14 til <18 år) og voksne ( $\geq 18$  år).

I tillegg til kosttilskudd med isoflavoner, er et stort antall matvarer med soya kilder til eksponering for isoflavoner. Genistein og daidzein brukes i kosmetiske hudpleieprodukter. Total-eksponering for isoflavoner, fra andre kilder enn kosttilskudd som mat og kosmetikk, og fra andre kilder enn soya som rødkløver og kudzu-rot, er ikke inkludert i denne risikovurderingen.

I Norge, som i andre vestlige land, er inntaket av soyabønner og soyabønne-baserte produkter generelt lavt i det gjennomsnittlige kostholdet. Inntaket (gjennomsnitt  $\pm$  SE) av isoflavoner fra soya i kosten hos kvinner ble rapportert å være  $1,474 \pm 0,158$  og  $1,096 \pm 0,178$  mg/dag i henholdsvis sør og øst i Norge og i nord og vest i Norge, basert på et enkelt 24-timers intervju i EPIC-studien. Tilsvarende tall var  $0,005 \pm 0,000$  og  $0,004 \pm 0,000$  mg/dag for daidzein-metabolitten *S*-equol. For menn var de tilsvarende tallene for inntak av isoflavoner henholdsvis  $0,978 \pm 0,135$  og  $0,721 \pm 0,136$  mg/dag, og for *S*-equol var tallene henholdsvis  $0,006 \pm 0,000$  og  $0,007 \pm 0,000$  mg/dag.

Inntaket av soyabønner og soyabønne-baserte produkter kan være høyere i veganer og personer med melke-allergi. Det gjennomsnittlige inntaket av soyaprotein per dag har blitt beregnet for personer som spiser enten en veganmeny eller en melkefri kost. Det gjennomsnittlige inntaket for voksne (kvinner eller menn) var 35 og 19 g soyaprotein per dag, for henholdsvis veganer og melkeallergikere. For veganer var det gjennomsnittlige inntaket 30 og 41 g soyaprotein per dag for henholdsvis 10-13 år gamle jenter og 14-17 år gamle gutter. For melkeallergikere var det gjennomsnittlige inntaket henholdsvis 17 og 23 g soyaprotein per dag for 10-13 år gamle jenter og 14-17 år gamle gutter. For 10-13 år gamle gutter og 14-17 år gamle jenter var inntaket omtrent likt som for voksne.

Ut fra svarene på et spørreskjema om kosthold, var det blant 87800 kvinner i Den norske mor og barn-undersøkelsen (MoBa), kun 1.7% som drakk soyamelk og av disse drakk kun 0.4%  $\geq 200$  ml av soyamelk per dag. Når det gjaldt bruk av soyaprodukter til middag, oppgav 4.8% at de spiste det av og til, og kun 12% av disse kvinnene hadde soyaprodukter til middag mer enn en gang annenhver uke.

### Konklusjoner

Det ble ikke funnet noen studier av effekter av isoflavoner fra soya etter eksponering av ungdom (14 til  $< 18$  år) som hadde undersøkt effekter også i denne alderen. Det ble heller ikke funnet noen informasjon i den inkluderte litteraturen som tydet på at ungdom (14 til  $< 18$  år, dvs. for de fleste etter puberteten) er mer følsomme for effekter av isoflavoner enn voksne. VKM anser derfor at resultatene for pre-menopausale kvinner og menn er valide også for ungdom.

Det ble ikke funnet noen studier av effekter av isoflavoner fra soya etter eksponering av barn (10 til  $< 14$  år).

Gitt at isoflavonene i kosttilskudd solgt på det norske markedet er sammenlignbare med isoflavonene som ble brukt i de tilgjengelige studiene konkluderer VKM med at

- Isoflavoner i kosttilskudd i doser på 40 eller 80 mg/dag tatt i flere måneder og til og med i flere år synes å være uten negative helseeffekter av betydning i peri- og post-menopausale kvinner.
- Det er en risiko for at dosene på 40 eller 80 mg/dag av isoflavoner i kosttilskudd tatt i en til tre måneder kan påvirke hormonnivåer og/eller menstruell funksjon negativt i pre-menopausale kvinner. Disse dosene synes ikke å ha andre negative effekter i pre-menopausale kvinner av betydning.
- Det er en risiko for at dosene på 40 eller 80 mg/dag av isoflavoner i kosttilskudd tatt i en til tre måneder kan påvirke hormonnivåer negativt i menn. Disse dosene synes ikke å ha andre negative effekter i menn i den generelle befolkningen av betydning.
- Dosene på 40 eller 80 mg/dag av isoflavoner i kosttilskudd tatt i en til tre måneder kan utgjøre en risiko for negativ påvirkning på hormonnivåer i ungdom av begge kjønn og/eller på menstruell funksjon i unge kvinner. Disse dosene synes ikke å ha andre negative effekter i ungdom av betydning.
- Det ikke er tilstrekkelig med data til å kunne trekke noen konklusjoner om mulige negative helseeffekter av isoflavoner fra soya i kosttilskudd for barn (10 til  $< 14$  år).

## Kort sammendrag

I følge informasjon fra Mattilsynet er isoflavoner fra soya ingredienser i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere inntak på 40 og 80 mg/dag av isoflavoner fra soya (som det totale innholdet av genistein og daidzein) i kosttilskudd. Det ble ikke gitt noen ytterligere informasjon om individuelle isoflavonisoimerer og i hvilke

mengder som finnes i disse ekstraktene av isoflavoner fra soya i kosttilskudd på det norske markedet. Konklusjonene under er valide gitt at isoflavonene i kosttilskudd solgt på det norske markedet er sammenlignbare med isoflavonene som ble brukt i de tilgjengelige studiene. Inntak av isoflavoner fra soya ble vurdert for den generelle befolkningen i aldersgruppene barn (10 til <14 år), ungdom (14 til <18 år) og voksne ( $\geq 18$  år).

VKM konkluderer med at isoflavoner i kosttilskudd i doser på 40 eller 80 mg/dag tatt i flere måneder og til og med i flere år synes å være uten negative helseeffekter av betydning i peri- og post-menopausale kvinner.

I motsetning til i peri- og post-menopausale kvinner, anser VKM forandringer i hormonnivåer bort fra det normale området som negative effekter i friske pre-menopausale kvinner og menn.

VKM konkluderer med at det er en risiko for at dosene på 40 eller 80 mg/dag av isoflavoner i kosttilskudd tatt i en til tre måneder kan påvirke hormonnivåer og/eller menstruell funksjon negativt i pre-menopausale kvinner. Disse dosene synes ikke å ha andre negative effekter i pre-menopausale kvinner av betydning.

VKM konkluderer med at det er en risiko for at dosene på 40 eller 80 mg/dag av isoflavoner i kosttilskudd tatt i en til tre måneder kan påvirke hormonnivåer negativt i menn. Disse dosene synes ikke å ha andre negative effekter i menn i den generelle befolkningen av betydning.

VKM konkluderer med at dosene på 40 eller 80 mg/dag av isoflavoner i kosttilskudd tatt i en til tre måneder kan utgjøre en risiko for negativ påvirkning på hormonnivåer i ungdom av begge kjønn og/eller på menstruell funksjon i unge kvinner. Disse dosene synes ikke å ha andre negative effekter i ungdom av betydning.

VKM konkluderer med at det ikke er tilstrekkelig med data til å kunne trekke noen konklusjoner om mulige negative helseeffekter av isoflavoner fra soya i kosttilskudd for barn (10 til <14 år).



# Abbreviations and glossary

## Abbreviations

ABCC2	- multidrug resistance protein
ABCG2	- breast cancer resistance protein
ADME	- absorption, distribution, metabolism, excretion
AE	- adverse event
AFSSA	- French Food Safety Agency [ <i>French</i> : L'Agence Française de Sécurité Sanitaire des Aliments]
AGE	- advanced glycation end product
AIE	- aglycone isoflavone equivalents
ALT	- alanine aminotransferase or alanine transaminase
AMD1	- S-adenosylmethionine decarboxylase 1
ANS	- Panel on Food Additives and Nutrient Sources Added to Food in EFSA
AOPP	- advanced oxidation protein products
Apo	- apolipoprotein
AR	- androgen receptor
AST	- aspartate aminotransferase or aspartate transaminase
AUC	- area under the curve
BC	- bladder cancer
BCRP	- breast cancer resistance protein
BfR	- The Federal Institute for Risk Assessment (in Germany) [ <i>German</i> : Das Bundesinstitut für Risikobewertung]
BMC	- bone mineral content
BMD	- bone mineral density
BMI	- body mass index
BRCA1, BRCA2	- breast cancer type 1 or 2 susceptibility
BRCP	- breast cancer resistance protein
BSAP	- bone-specific alkaline phosphatase
bw	- body weight
CAM	- cell adhesion molecule
CASI	- cognitive abilities screening instrument
CBG	- cytosolic $\beta$ -glycosidase
CI	- confidence interval
CNFDM	- casein and non-fat dry milk
COMT	- catechol O-methyl transferase
CosIng	- EU Cosmetic ingredient database
COT	- Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), Food Standards Agency, UK
CRC	- colorectal cancer
CVD	- cardiovascular disease
CYP	- cytochrome P450

DAI	- daidzein
DHD	- dihydrodaidzein
DHEA	- dehydroepiandrosterone
DHEAS	- dehydroepiandrosterone sulfate
DHT	- dihydrotestosterone
DILI	- drug-induced liver injury
DMA	- <i>O</i> -desmethylangolensin (metabolite of daidzein)
DMBA	- 7,12-dimethylbenz[ <i>a</i> ]anthracene
DSB	- DNA double-strand break
DXA	- dual-energy x-ray absorptiometry
E1	- estrone
E2	- estradiol
E3	- estriol
EC	- European Commission
EDC	- endocrine-disrupting chemicals
EDI	- estimated daily intake
EFSA	- European Food Safety Authority
ELISA	- enzyme-linked immunosorbent assay
EP	- estrogenic potency
ER	- estrogen receptor
E-selectin	- endothelial-leukocyte adhesion molecule-1
FAI	- free androgen index
FFQ	- food frequency questionnaire
FMP	- final menstrual period
FN	- femoral neck
FOSHU	- food for specified health uses
FSH	- follicle-stimulating hormone
GCP	- genistein combined polysaccharide
Ge:D:GI	% - percentage of genistein, daidzein and glycitein
GI	- gastrointestinal
GPOR	- G protein-coupled estrogen receptor
GST	- glutathione <i>S</i> -transferase
HCC	- hepatocellular carcinoma
HDL	- high density lipoprotein
HDL-C	- high density lipoprotein cholesterol
HFSSS	- hot flash symptom severity score
HO	- hydroxy
HOMA-IR	- homeostasis model assessment for insulin resistance
HP	- type 1 collagen alpha1-chain helical peptide
hprt	- hypoxanthine phosphoribosyl transferase
HR	- hazard ratio
H(R)T	- hormone (replacement) therapy
HSD1	- hydroxysteroid dehydrogenase type I
ICAM-1	- intercellular cell adhesion molecule-1

ICE	- isolating <i>in vivo</i> complexes of enzyme to DNA bioassay
IF	- isoflavones
IGF-I	- insulin-like growth factor I
IGFBP 3	- insulin-like growth factor binding protein 3
IQR	- interquartile range
IRR	- incidence rate ratio
ISP	- isolated soy protein
i.v.	- intravenous
KI	- Kupperman index
LDL	- low density lipoprotein
LDL-C	- low density lipoprotein cholesterol
LH	- luteinizing hormone
LS	- lumbar spine
MEKK-1	- mitogen-activated kinase 1
MENQOL	- menopause-specific quality of life
MLL	- myeloid-lymphoid leukemia
MOS	- margin of safety
MPI	- milk protein isolate
MRP	- multidrug resistance associated protein
NAF	- nipple aspirate fluid
NCI	- National Cancer Institute, USA
NFSA	- Norwegian Food Safety Authority [ <i>Norw.</i> : Mattilsynet]
NQO1	- NAD(P)H dehydrogenase quinone 1
n.s	- not statistically significant
NSAIDs	- non-steroidal anti-inflammatory drugs
OAZ2	- ornithine decarboxylase antizyme 2
ODC	- ornithine decarboxylase
ODMA	- <i>O</i> -desmethylangolensin (metabolite of daidzein)
OECD	- Organisation for Economic Co-operation and Development
5-OHmdU	- 5-hydroxymethyldeoxyuridine
OPG	- osteoprotegerin
OR	- odds ratio
OVX	- ovariectomized
PCa/Pca	- prostate cancer
PCE	- polychromatic erythrocyte
PR	- progesterone receptor
PSA	- prostate-specific antigen
Q	- quartile
QOL	- quality of life
RCT	- randomised controlled trial
RIA	- radioimmunoassay
RLP	- remnant-like particles
RR	- relative risk
RUCAM	- Roussel Uclaf Causality Assessment Method

SD	- standard deviation of the mean
SE/SEM	- standard error of the mean
SHBG	- sex hormone-binding globulin
sICAM-1	- soluble intercellular adhesion molecule-1
SILs	- squamous intraepithelial lesions (in cervix)
SPI	- soy protein isolate
(s)VCAM-1	- (soluble) vascular cell/cellular adhesion molecule-1
T3	- triiodothyronine
T4	- thyroxine
TAC	- total antioxidant capacity
TARDIS	- trapped in agarose DNA immunostaining assay
TBARS	- thiobarbituric reactive substances
TBG	- thyroid binding globulin
TC	- total cholesterol
tk	- thymidine kinase
topo	- topoisomerase
TSH	- thyroid stimulating hormone
UDP	- uridine diphospho-
UGT	- UDP-glucuronosyl transferase
VKM	- Norwegian Scientific Committee for Food Safety [ <i>Norw.:</i> Vitenskapskomiteen for Mattrygghet]
VLDL	- very low density lipoprotein
WHO	- World Health Organization
WSB	- whole soybean

## Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (The European Parliament and the Council of the European Union, 2006).

"Negative health effect" and "adverse health effect" are broad terms. VKM uses the definition established by EFSA for "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (EFSA, 2006; WHO, 1994).

# Background as provided by the Norwegian Food Safety Authority

"Other substances" are substances other than vitamins and minerals, with a nutritional and/or physiological effect on the body. "Other substances" are mainly added to food supplements, but these may also be added to other foods and beverages, such as sports products and energy drinks. Ingestion of these substances in high amounts presents a potential risk for consumers.

In Norway, a former practice of classification of medicines had constituted an effective barrier against the sale of potentially harmful "other substances". Ever since this practice was changed in 2009, it has become challenging to regulate and supervise foods with added "other substances". Meanwhile, in the recent years, the Norwegian market has witnessed a marked growth in the sales of products containing "other substances". In 2011, food supplements containing "other substances" constituted more than 50% of the market share.

While within the European Economic Area, these substances fall under the scope of the European Regulation (EC) No. 1925/2006 on the addition of vitamins, minerals and certain "other substances" to foods and the European Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, "other substances" remain largely unregulated. In order to ensure safe use of "other substances" many countries have regulated their use at a national level. For example, Denmark regulates these substances in a positive list, i.e. a list of substances with maximal daily doses, permitted for use in food supplements and other foods (FVM, 2014).

The Norwegian Food Safety Authority (NFSA) is working on the establishment of a regulation on the addition of "other substances" to foods at a national level. The regulation will include a list of substances with permitted maximal doses, based on the substances and doses found in products on the Norwegian market. In preparation for a regulation, NFSA has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of "other substances" found on the Norwegian market. NFSA, in consultation with the industry, has compiled a list of "other substances" found in products marketed in Norway. Only substances with a purity of minimum 50% or concentrated 40 times or more have been included in the list. Substances regulated by other legislations like those for novel foods, food additives, flavourings, foods for special medical purposes etc., have been excluded from the list.

# Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) has requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of isoflavones from soy in food supplements at the following doses: 40 and 80 mg/day.

NFSA requested VKM to assess the safety of "other substances" (in accordance to the guidance document developed in Phase 2) at the doses specified (Phase 3). The safety assessments of "other substances" present in food supplements shall be carried out for a general population, ages 10 years and above.

# Assessment

## 1 Introduction

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional and/or physiological effect*, and may be added to food supplements or e.g. energy drinks.

This risk assessment regards isoflavones from soy *per se*, and no specific products.

VKM has in this series of risk assessments of "other substances" not evaluated documentation of any claimed beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway.

According to information from the Norwegian Food Safety Authority (NFSA), isoflavones from soy are ingredients in food supplements purchased in Norway. The food supplements on the Norwegian market contain isoflavones isolated from soybeans of the plant *Glycine max* (L.) Merrill by extraction by 80% alcohol (which alcohol is unknown) and 20% water. NFSA has requested a risk assessment of the intake of 40 and 80 mg isoflavones (as the total content of genistein and daidzein) per day from food supplements. No further information was given on which individual isoflavone isomers and in which amounts are found in the extracts in the soy isoflavone supplements on the Norwegian market.

The total exposure to isoflavones from other sources than food supplements, such as foods and cosmetic products, and from other sources than soy, such as red clover and kudzu root, are not included in this risk assessment.

Phytoestrogens consist of the flavonoids (isoflavones, coumestans and prenyl flavonoids) and non-flavonoids (lignans) (Bakker, 2004). The flavonoids share a basic structure consisting of two benzene rings (A and B) linked through a heterocyclic C ring (Yuan et al., 2007). Isoflavones constitute a class of non-steroidal estrogens that have similarity in chemical structure and properties to estrogen. However, they show conformational binding to the estrogen receptor that classifies them as natural selective estrogen receptor modulators (SERMs) rather than as estrogens, and have estrogenic and anti-estrogenic effects depending on the concentration of endogenous estrogen and amount and type of estrogen receptors.

Soy isoflavones comprise three aglycones; genistein, daidzein and glycitein (Table 2.2.1-1) (EFSA, 2015; Messina, 2005). These also exist as their corresponding  $\beta$ -glycosides; genistin, daidzin and glycitin. In addition, the three  $\beta$ -glycosides can each be esterified with either malonic acid or acetic acid, forming in total 12 different soybean isoflavone isomers. Typically, there is somewhat more genistein/genistin than daidzein/daidzin in soybeans and

soy foods, whereas glycitein/glycitin comprises only 5-10% of the total isoflavone content (Messina, 2005). Overall, the evidence indicates that whether isoflavones are present as glycosides or aglycones appears not to be critically important with regard to their biological effects, since the glycosides, although not absorbed intact, can be hydrolysed *in vivo* (Messina, 2005).

There is large variation in composition and concentration of isoflavones among different soybean or soy protein products, which is dependent of soy species differences, part of the soy plant used, geographic and environmental growing conditions and cultivating parameters. The extent of industrial processing of the soybeans, including the extraction process, i.e. which mechanical extraction method, solvent type, temperature and time being used, can also affect the composition and concentration of isoflavones (Setchell, 1998).

A number of both internal and external factors are influencing the bioavailability of isoflavones, including intestinal microflora, gender, age, food matrix, chemical composition, earlier exposure and background diet (van de Poll, 2004).

In many of the studies used in this risk assessment, the full composition of aglycones vs. glycosides/glycoside esters was not stated, nor were the soybean source and the details of the extraction process. Consequently, there is ambiguity regarding the biologically active amounts of isoflavones in a product even when total amount of isoflavones is indicated. Since the molecular weight of the aglycones is approximately 60% of that of the glycosides (Table 2.2.1-1), 100 mg isoflavones can actually refer to between approximately 60 and 100 mg of biologically active isoflavones (Messina, 2005). Therefore, there is considerable uncertainty in relation to the exact substances being studied in the literature and the similarity/dissimilarity with the isoflavone supplements regarding their effects (beneficial or adverse).

It is well known that adverse effects can result from high levels of isoflavones fed to animals, such as infertility by red clover in sheep, and thus, there is reason to believe that adverse effects could also occur in humans as results of excessive intakes (Setchell et al., 2001). It is quite unlikely that normal plant-based diets would contain isoflavones in amounts sufficient to induce any severe adverse effects, as they have been a part of human diets for hundreds of years, and there is no historical data on any obvious toxic effects. However, the amounts ingested daily from foods, estimated at 15-50 mg, or even much lower in Western populations, are below the dose promoted by supplements. Therefore, the situation may be different for individuals consuming supplements containing extracts or concentrates with very high isoflavone content.

The adult consumer groups with the highest dietary intake of isoflavones (approximate levels) are consumers taking dietary phytoestrogen containing supplements (40–100 mg/day), vegan breast-feeding women (75 mg/day) and consumers of a traditional South East Asian diet (25-100 mg/day) (Bakker, 2004). The dietary isoflavone intake of average Western consumers and vegetarians is much lower (<1–2 mg/day and 3-12 mg/day, respectively).



Overall background exposure to isoflavones from the diet in the general European population was estimated to be lower than 1 mg/day (0.27-1.43 mg/day in women), whereas in consumers of soy-based foods and vegetarians it could be higher and within the estimated range of exposure to isoflavones from soy food supplements (approximately 0.1-111 mg/day) (EFSA, 2015).

The intake (mean  $\pm$  SE) of isoflavones from dietary soy in women was  $1.474 \pm 0.158$  and  $1.096 \pm 0.178$  mg/day in south and east of Norway and north and west of Norway, respectively, based on a single 24-hour recall in the EPIC study (Zamora-Ros et al., 2012). Corresponding numbers were  $0.005 \pm 0.000$  and  $0.004 \pm 0.000$  mg/day for the daidzein metabolite *S*-equol. For men, the corresponding intake of isoflavones was  $0.978 \pm 0.135$  and  $0.721 \pm 0.136$  mg/day, respectively, and for *S*-equol, the numbers were  $0.006 \pm 0.000$  and  $0.007 \pm 0.000$  mg/day, respectively.

In Norway, as in other Western countries, the intake of soybeans and soybean-based products is generally low in the average diet, but may be higher in vegans and persons with milk allergy. There is no information available on the actual level of dietary exposure to soy isoflavones in vegans or persons with milk allergy in Norway, in any age groups. The mean intake of soy protein per day has been estimated for persons eating either a vegan menu or a milk-free diet, based on weekly menus (VKM, 2015). For the vegan diet, the meat was replaced by soy burgers, soy sausages etc. For the milk-free diet, the milk products were replaced with soy products. The estimated mean intake for an adult was 35 and 19 g soy protein per day, for the vegan and milk allergy scenarios, respectively. For children, the numbers for adults were adjusted for energy requirements, and assuming that milk in coffee and tea was consumed as milk. In the vegan scenario, the estimated intake was 30 and 41 g soy protein per day for 10-13 year old girls and 14-17 year old boys, respectively. In the milk allergy scenario, the estimated intake was 17 and 23 g soy protein per day for 10-13 year old girls and 14-17 year old boys, respectively. For 10-13 year old boys and 14-17 year old girls, the estimated intake was approximately similar to in adults.

There is no information available on the actual level of exposure to soy isoflavones from supplements in Norwegians of any age.

Genistein and daidzein are used for skin conditioning in cosmetics (CosIng, 2015).

In some of the included studies, isoflavone content is expressed as "isoflavone equivalents" or "aglycone equivalents", without any definition being given. However, a definition of "isoflavone aglycone equivalents" (AIE) is given as the maximum amount of bioavailable isoflavone upon ingestion, calculated using the conversion factors presented in Table 4 in (EFSA, 2015).

VKM has based this risk assessment on the information from previous risk assessments and the available literature. Sources of isoflavones examined in the included studies were reported to be soy protein, soy protein isolate (SPI) or isolated soy protein (ISP), soybeans, soy foods such as soy milk, flour, grits, tofu or tempe, as commercial products or prepared

meals, isoflavone concentrate or just referred to as isoflavones, or as genistein combined polysaccharide (GCP), which is enriched in aglycone forms of isoflavones, percentages of genistein, daidzein, glycitein, and in some studies also as percentages of genistin, daidzin, glycitin and the malonyl- and acetyl-derivatives. The ratio of percentage of individual aglycone isoflavones genistein:daidzein:glycitein or glycosides genistin:daidzin:glycitin varied substantially among the studies.

In this opinion, the term "isoflavones" indicates the total isoflavones including glycosides and aglycones from soybean, whereas the term "isolated isoflavones" indicated the individual substances mentioned above or mixtures of them. The generic terms glycoside and aglycone are used unless referring to specific compounds or in quotations of previous publications.

In this risk assessment, the intake of 40 and 80 mg isoflavones from soy per day from food supplements is assessed for the general population.

# 2 Hazard identification and characterisation

## 2.1 Literature

The present risk assessment is based on previous risk assessments of isoflavones from soy and articles retrieved from literature searches. Because of the large number of studies published on isoflavones, including many studies on beneficial effects of isoflavones on various diseases, not all available studies are included in this risk assessment.

### 2.1.1 Previous risk assessments

**The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), Food Standards Agency, UK (1996, 2003, 2012, 2013, 2015) (COT, 1996; COT, 2003; COT, 2012; COT, 2013a; COT, 2013b; COT, 2015)**

In 1996, the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) recommended that research should be undertaken as a matter of high priority to determine whether ingestion of soy based formula carried any risk for infants (COT, 2003). They stated further, that as a result of further research, it might be necessary to consider the potential risk of soy products to other sectors of the population. Further, COT completed an extensive review on phytoestrogens and health in 2003, which included consumers of dietary supplements containing isoflavones (COT, 1996). In this COT (2003) report (the following text is based on EFSA (2015)), some concerns regarding the potential interference between phytoestrogens and thyroxine replacement therapy were noted and further research was recommended to monitor the plasma thyroxine levels of children and adults with hypothyroidism who consume large quantities of dietary phytoestrogens. With respect to the potential effects exerted by phytoestrogens on breast cell proliferation, although no quantitative recommendation was given in the report, COT suggested that, until further research was carried out, women with estrogen-dependent breast disease should be cautious in supplementing their diet with phytoestrogen-rich foods or dietary supplements. Further research was commissioned that included three relevant projects, as follows: (i) a dietary and biomarker prospective study of phytoestrogens in breast and prostate cancer; (ii) an investigation of the phytoestrogen intake of a group of post-menopausal women previously diagnosed with breast cancer; and (iii) a double-blind placebo-controlled parallel trial of soy isoflavones on markers of bone turnover in females in the early menopause. The first two of these were reviewed later (COT, 2012) and the final one was completed in 2014. COT noted that these three studies were based on the recommendation that the programme concentrate on human studies. There was some lack of clarity in the scientific objectives of the study on phytoestrogen exposure in women diagnosed with breast cancer, and COT was disappointed that the results of this study had not been published in the peer-reviewed

literature. COT noted that the analysis of phytoestrogens in a wide range of foods was useful and had allowed robust estimation of short-term dietary exposures to phytoestrogens. While the findings indicated no association between phytoestrogen intake and risk of breast cancer, the data on prostate cancer were inconclusive. COT had considered unpublished results from the final study. The minutes of COT's discussion have been temporarily withheld from publication while a report of the study is submitted for publication in a peer-reviewed scientific journal. COT judged that this delay was acceptable, since the results presented did not indicate any need for action to protect the health of the public.

In 2012, COT published a report summarizing the results from the Phytoestrogen Research programme, which was established to improve the assessment of the risks and benefits from dietary phytoestrogens and the scientific evidence base underpinning advice to consumers (COT, 2012). It was subsequently decided that evidence for claimed benefits was the responsibility on the food industry or retailers and that future work on risks should be incorporated into the Food Standards Agency (FSA) Risk Assessment Research Programme. The majority of the research was reviewed in 2001-2007. In 2011, the Committee was asked to review briefly the final projects, and to consider the overall contribution of this research programme to risk assessment of phytoestrogens.

COT has also published a statement on potential risks from high levels of soy phytoestrogens (isoflavones) in the infant diet, based on the new literature concerning possible health effects from exposure of infants to soy isoflavones, which had come available since the 2003 COT report (COT, 2013a; COT, 2013b). This report stated that epidemiological and clinical studies have produced conflicting results, and while the balance of evidence from the small number of epidemiological studies does not suggest important adverse effects of soy infant formula on reproductive development, they are too limited to provide strong reassurance of safety. Animal studies looking at similar levels of exposure to those reported in infants have suggested developmental and reproductive changes later in life. However, differences in the chemical handling of isoflavones, and in the timing of sexual development, make it difficult to extrapolate findings from animals to humans. The highest potential exposures of infants to isoflavones come from exclusive consumption of soy-based infant formula. While the small number of available epidemiological studies does not suggest that such consumption leads to adverse health effects in humans, the results of animal studies indicate a possible concern, and there is thus some uncertainty about the safety of soy-based infant formula. COT concluded that there is no scientific basis for changing the current government advice – namely, that there is no substantive medical need for, nor health benefit arising from the use of soy-based infant formula, and that it should be used only in exceptional circumstances to ensure adequate nutrition.

COT has also published a statement on the effects of soy phytoestrogen (coumetans, prenylated flavonoids and isoflavones) consumption on thyroid status, based on all evidence which had come available since the 2003 COT report (COT, 2015). This report stated that there are no indications that high intakes of soy impact materially on thyroid function in people in whom thyroid function is not already impaired. However, the evidence that is now

available, although not entirely consistent, suggests that higher intake of soy phytoestrogens, either in food or in dietary supplements, may sometimes precipitate a transition to overt hypothyroidism in people with subclinical, compensated hypothyroidism, and may also affect the dose of thyroxine that is needed in patients who are on treatment for hypothyroidism. This should not have major clinical implications. However, endocrinologists should be made aware of the possibility that consumption of soy phytoestrogens (including in dietary supplements) may affect thyroid function and response to treatment with thyroxine. In view of the persisting uncertainties, there should be continued monitoring of the scientific literature on this topic. However, since any clinical implications are unlikely to be of major importance, further research in this area need not be a priority for future funding by the Food Standards Agency.

COT did not determine a safe upper level of isoflavones.

### **Italian Health Authorities (2002) (Gazzetta Ufficiale Italiana, 2002)**

The Italian Health Authorities have advised the public to maintain daily intake of phytoestrogens as dietary supplements below 80 mg/day, expressed as the total amount of isoflavones isomers) (Gazzetta Ufficiale Italiana, 2002), referenced in Morandi et al. (2005) and Sirtori et al. (2005). This represents a maximal daily intake of about 1 mg/kg bw.

### **AFSSA - L'Agence Française de Sécurité Sanitaire des Aliments (2005) Sécurité et bénéfices des phyto-estrogènes apportés par l'alimentation – Recommendations (in French) (AFFSA, 2005)**

The report indicated 1 mg/kg bw per day of isoflavone aglycone as a safe amount, i.e. 70 mg/day for a person with 70 kg body weight. Also in prepared foods for children containing soy protein as the main component, the amount of phytoestrogen should be limited to 1 mg/l reconstituted formula. In addition, for breast cancer patients or persons with a history of breast cancer in the family, intake should be restricted in consideration of risk of increased tumour growth.

### **Japanese Food Safety Commission, Novel Foods Expert Committee (2006) Fundamental concepts in the safety assessment of foods containing soy isoflavones for the purpose of specified health use (original in Japanese, with a provisional English translation) (Japanese Food Safety Commission, 2006)**

Dietary intakes of isoflavones from soy food products in Japan without any reported health damage over many years of 70 mg/day (95 percentile intake) (64-76 mg/day aglycone equivalent value; pre-menopausal women: 64 mg/day, post-menopausal women: 74 mg/day, men: 76 mg/day)) were considered safe. In addition, based on findings in a human randomized double-blind study of endometrial hyperplasia in 3.8% of post-menopausal women on 150 mg/day of isoflavones as tablets for 5 years (Unfer et al., 2004), compared with none in the control group (significant difference), demonstrating potential for damage to human health, half of this value, 75 mg/day of soy isoflavones, was considered as a safe

upper level for standard daily intake for humans (pre-menopausal and post-menopausal women and men). Based on these two arguments, 70-75 mg/day as soy isoflavones was determined as the upper limit for the safe standard daily intake from food.

Several studies showed changes in serum E2 concentration or menstrual cycle length (decreased or increased for both effects) after use of soy isoflavones as soy milk, soy protein, tablets etc., taken in addition to isoflavones from food. Soy products and soy isoflavones have both estrogenic and anti-estrogenic effects. Based on the minimum effect level of decreases in serum E2 concentration and menstrual cycle length by 57 mg/day in pre-menopausal women (Nagata et al., 1998), roughly half of this dose i.e. 30 mg/day soy isoflavone aglycones (approximately a factor of 0.6 difference between the glycoside and the aglycone after hydrolysis of the sugar by  $\beta$ -glycosidases) was established as the upper limit for safe daily extra dietary intake as a food for specified health uses (FOSHU) (interpreted as similar use as food supplements by VKM) for pre-menopausal women. Similar safe upper limit was extrapolated for post-menopausal women and men, in lack of more specific data. This dose of 30 mg/day is also considered to cover the suggested efficacy on bone calcium of 25 mg/day soy isoflavone aglycone equivalent.

For pre-menopausal women, post-menopausal women and men it was concluded that a normal daily dietary intake of soy isoflavones of 16-22 mg/day (50 percentile, median intake in Japan) and in addition a daily intake from food supplements within the range of 30 mg/day of soy isoflavone aglycones is safe.

Also for the same groups eating 40-45 mg/day (80-85 percentile value of intake in Japan) from food, a daily intake from food supplements within the range of 30 mg/day of soy isoflavone aglycones, was considered safe.

From the reasoning above it is conferred by VKM that for pre-menopausal women, post-menopausal women and men in Japan having a high intake from food (95 percentile) of 64-74 mg/day, an additional intake of isoflavones from supplements cannot be recommended.

For fetuses (covered by the group pregnant women), pregnant women and potentially pregnant women, in consideration of the fact that there are no known benefits concerning intake of soy isoflavones by pregnant women, as well as the inhibition of topoisomerase II (which maintains normal DNA structure) by soy isoflavones, which may potentially cause myeloid-lymphoid leukemia (MLL) gene abnormality in fetuses exposed *in utero*, intake as a food for specified health use for these groups in addition to intake from normal foods cannot be recommended. Based on data suggesting various effects on reproductive function of neonate animals and immature animals through exposure to high concentrations of soy isoflavones and their estrogen-mediated effects, it is considered that intake as a food for specified health use for infants and small children in addition to intake from normal foods cannot be recommended. It is also stated that suggested by animal experiments, sensitivity to exogenous estrogen may possibly be higher in infants than in pre-menopausal women, because the homeostatic mechanisms of infants are not fully developed.

Effects on cancer are also mentioned in this Japanese report. However, apparently cancer was not given weight in this risk assessment. VKM notes that although it is stated that there were no reports directly associating soy isoflavone intake with an increase in incidence of breast cancer in females, it was also mentioned that there was concern regarding the risk of estrogen-sensitive cancers, such as breast cancer, endometrial cancer and prostate cancer. Studies reporting increased cell proliferation or hyperplasia after soy isoflavone exposure were also referred to in this report, both *in vitro*, in animals and in human breast and endometrium tissues.

**BfR - Das Bundesinstitut für Risikobewertung, Isolated isoflavones are not without risk. Updated (29 October 2007) BfR Expert Opinion No. 039/2007, 3 April 2007 (BfR, 2007)**

The Federal Institute for Risk Assessment (BfR) in Germany issued an Expert Opinion on isoflavones from soy and red clover in 2007. The background for this opinion was that there were reports that Asian women who follow a traditional diet and regularly consume soy products scarcely suffer at all from menopausal complaints, however, opinions differ as to whether this can be attributed to a diet rich in soy. In Germany, food supplements with isolated isoflavones had been available on the market as an alternative to the prescribed hormone replacement therapy (HRT) for menopausal complaints. Furthermore, BfR noted that when looking at the assumed effects of isoflavones, a distinction must be made between whether they are ingested naturally from food or in isolated, fortified form via food supplements.

BfR concluded that according to the latest scientific knowledge available, the assumed positive effects of isolated isoflavones on menopausal complaints have not been sufficiently substantiated. The short-term adverse effects reported such as nausea, constipation, swelling and reddening, are possibly caused by allergic reactions to the soy protein contained in these products or other factors. BfR was of the opinion that the toxicological risks regarding the hormonal situation in female users are to be viewed more critically than these short-term, acute, adverse effects.

BfR concluded further that toxicological studies showed that isoflavones, when administered at high doses in isolated or fortified form, impair the functioning of the thyroid gland and can change mammary gland tissue. They said further that it cannot be ruled out that these estrogen-like effects could promote the development of breast cancer. The long-term studies necessary to prove the safety of isoflavone-containing products were not available. Nor was it possible, at the present time, to reliably establish a dose which could be considered safe. As women during and after menopause are at increased risk of breast cancer, the long-term intake of food supplements with a high level of isoflavones was not without risk for this consumer group.

The daily intake of isolated isoflavones from soybeans via food supplements in Germany was 40, 50 or 80 mg.

## **European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) (2015) Risk assessment for peri- and post-menopausal women taking food supplements containing isoflavones (EFSA, 2015)**

The EFSA ANS Panel was asked to deliver a scientific opinion on the possible association between the intake of isoflavones from food supplements and harmful effects on mammary gland, uterus and thyroid in peri- and post-menopausal women. A preparatory report was made to support the risk assessment, identifying data from January 2009 to December 2013 (Tijhuis et al., 2015). In this opinion, isoflavones from soy, red clover and kudzu root were considered. The main isoflavones were genistein, daidzein, glycitein, formononetin, biochanin A and puerarin. Food supplements targeted at peri- and post-menopausal women typically provided a daily dose of isoflavones in the range of 35–150 mg/day. A systematic review was performed to investigate whether an association could be found between intake of isoflavones from food supplements and adverse effects on the three target organs in peri- and post-menopausal women, including literature from 1985 to 23 June 2015.

*Breast:* Four epidemiological studies investigating breast cancer incidence (involving, in total, 2216 isoflavone users), eight interventional controlled studies, measuring mammographic density (741 participants) and two interventional controlled studies, investigating histopathological changes (75 participants), did not suggest an association between exposure to isoflavones-containing food supplements and adverse effects in the mammary gland. In summary, the human data did not support the hypothesis of an increased risk of breast cancer from observational studies nor of an effect on mammographic density nor on proliferation marker Ki-67 expression in interventional studies.

EFSA concentrated on animal studies in ovariectomised animals, which were considered of relevance to the menopausal condition in women. Ten studies in ovariectomised animals were found, investigating breast cell proliferation, and 11 animal studies were identified, investigating histopathological changes in the mammary gland of animals treated with isoflavones. Although in the majority of the studies no effect was noted, a stimulating effect on the mammary gland was observed in two rat studies (genistein 5.4 and 54 mg/kg bw/day and 221 mg/kg bw/day, both studies carried out for 90 days). The findings are consistent with the results from the US National Toxicology Program study conducted in non-ovariectomised animals administered genistein at doses ranging 0.3–44 mg/kg bw/day, in which there was some evidence of carcinogenic activity of genistein in female Sprague–Dawley rats based on an increased incidence of mammary gland adenoma or adenocarcinoma (combined).

*Uterus:* No study was found that investigated the association between intake of isoflavones from food supplements and risk of uterine cancer in the target population. Endometrial thickness was measured in 25 interventional controlled studies (1484 participants) and histopathological investigations of endometrium were carried out in nine interventional controlled studies (677 participants). None of the studies reported statistically significant



changes in endometrial thickness compared with control. In only two studies were some histopathological effects noted. One study was not properly controlled and had further methodological flaws. In the other study, there were no findings after 2.5 years of intervention, whereas after 5 years of intervention only five cases of simple hyperplasia and one case of complex hyperplasia of the endometrium were observed, but no cases of endometrial carcinoma. The findings could indicate an estrogenic effect.

Thirteen studies in animals investigated uterus cell proliferation, among them three in monkeys, and 22 animal studies on uterus histopathological changes, among them four studies in monkeys. An effect of isoflavones was not seen in most of the studies. However, a daidzein-rich soy extract containing daidzein at doses above 40 mg/kg bw/day, caused an increase in cell proliferation of the epithelium and the stroma of the uterus as well as the vaginal epithelium of rats. Racemic equol (36 mg/kg bw/day) administered to rats induced an increase in proliferation of stromal cells of the uterus, whereas an equivocal effect was observed on proliferation of the epithelial cells of the uterus. In monkeys and rats, isoflavones obtained from soy extracts or soy protein isolates did not result in significant changes in endometrial thickness, endometrial hyperplasia, epithelial area or endometrial gland area. Daidzein-rich soy extract and red clover extracts at high doses ( $\geq 125$  mg/kg bw/day) caused a significant increase in endometrial area, endometrial thickness, number of glands and myometrial area in rats. Genistein and daidzein did not induce histopathological changes such as hypertrophy, hyperplasia or squamous metaplasia in the uterus. Racemic equol at a dose of 10 mg/kg bw/day or higher resulted in a significant increase in uterine wall thickness when administered for 90 days, but no such changes were observed after 35 days' administration of the same dose. In summary, no effect was found on endometrial thickness and histopathological changes in the uterus up to 30 months of supplementation with 150 mg/day of soy isoflavones. After 60 months some non-malignant histopathological changes were reported.

*Thyroidea:* Eleven human controlled randomized studies that reported effects of isoflavone administration on thyroid-related endpoints were identified. In total, 925 subjects were allocated to isoflavones. In none of the studies was a clinically relevant effect on the thyroid detected. Although the studies have some flaws (thyroid function not primary end point, sample size calculation not given, low power to detect changes), the Panel's conclusions were that administration of food supplements containing isoflavones was not associated with clinically relevant changes in thyroid function (hypo- or hyperthyroidism) of the population of interest. In summary, thyroid hormones levels were not changed following intake of isoflavones from food supplements.

The background exposure from the diet in the general European population was estimated to be lower than 1 mg/day, whereas in consumers of soy-based foods it could be higher. The Panel concluded that it was not possible to derive a single health-based guidance value or a safe intake level for the different preparations in post-menopausal women. However, the doses of treatment used in the intervention studies and their durations could serve as

guidance for the dose and duration of use at which no effects have been observed in these three target organs from the intake of food supplements.

**The doses that did not cause adverse effects in all three target organs (mammary gland, uterus or thyroid) was identified, as was the minimum duration of the studies, as a conservative approach (Table 30, page 167, in EFSA (2015), of which these levels are of relevance for VKM's risk assessment:**

- For soy isoflavones/soy extract this dose was 100 mg/day (total isoflavones), 10 months duration of intake.
- For soy protein, this dose was 99 mg/day (aglycone), 3 months duration of intake.
- For daidzein-rich isoflavones, this dose was 72 mg/day (total), 6 months duration of intake.
- For genistein, this dose was 54 mg/day, 36 months duration of intake.

**Other conclusions from EFSA (2015) of relevance for VKM's risk assessment:**

- The Panel concluded that the results from this assessment could not be extrapolated to other groups (such as men or children) and other situations in the general population.
- The data on mammary gland and thyroid allowed conclusions applicable to post- and peri-menopausal women.
- Regarding effects on uterus, the database was not sufficient to draw conclusions on peri-menopausal women.
- Based on the evidence reviewed there was no indication for adverse effects on the mammary gland in post-menopausal women from isoflavones when taken in doses and for durations as described above. The opinion could not conclude on the risk of estrogenic isoflavone-based food supplements in post-menopausal women with a current diagnosis or history of estrogen-dependent cancer.
- Based on the evidence reviewed (human and animal studies), the Panel concluded that isoflavones had no adverse effects on the uterus in post-menopausal women when taken in doses and for durations as described above.
- Based on human intervention studies, there were no statistically significant changes to indicate that food supplements containing isoflavones exert a hypothyroid effect in post-menopausal women with normal thyroid function.
- The use of isoflavones in food supplements was not a genotoxic concern.
- The levels of daily intake of soy isoflavones from food supplements may be achieved by consumers of specific soy foods, such as tofu, soy yoghurt, soy milk and drinks.

For more details, please see EFSA (2015).

### **Other risk assessments**

Kwack et al. (2009) conducted a relative risk assessment based upon daily intake levels of soybean-based foods and phytoestrogens in a Korean cohort, and the risks were compared with those posed by estradiol and other chemical endocrine-disrupting chemicals (EDC).

Koreans of approximately 30-49 years of age consume on average a total of 135.2 g/day of soy-based foods including soybean, soybean sauce, soybean paste and soybean oil, and 0.51 mg/kg bw per day of phytoestrogens such as genistein and daidzein. Using estimated daily intakes (EDI) and estrogenic potencies (EP), margins of safety (MOS) (originally called hygiene-based margin of safety (HBMOS) by Bolt et al. (2001) were calculated (as  $MOS = (1/EDI) \times (1/EP)$ ) where 0.05 was found for estradiol (MOS value <1, considered to exert an estrogenic effects). Thus, MOS values of 1.89 for Japanese, 1.96 for Koreans and 5.55 for Americans indicate that consumption of soybean-based foods exerted no apparent estrogenic effects, as all MOS values were higher than 1. For other synthetic EDC used as reference values, MOS values were for dieldrin 27, nonylphenol 250, butyl benzyl phthalate 321, bisphenol A 1000, biochanin A 2203 and coumesterol 2898. The results suggest that dietary exposure to phytoestrogens, such as genistein and daidzein, poses a relative higher health risk for humans than synthetic EDC, although all of their MOS values were greater than 1.

### **2.1.2 Literature searches**

The new and extensive risk assessment from EFSA (2015) covers effects of isoflavones on mammary gland, uterus and thyroid in peri- and post-menopausal women. In the papers obtained by the literature searches, VKM therefore searched for newer publications on effects of isoflavones on these organs in this population subgroup. In addition, VKM also searched for effects on pre-menopausal women, men, children and adolescents, for all potential negative health effects of isoflavones.

Primary literature searches were performed in Embase, Medline, PubMed and Web of Science in order to retrieve publications on negative health effects caused by isoflavones from soy. These databases were chosen to ensure comprehensive study retrieval. The literature searches were performed February 3, 2016. The search strategies are included in Appendix 9.5.1.

#### ***2.1.2.1 Publication selection and data extraction***

The literature searches identified 3063 articles. In the primary screening, titles and abstracts of all publications retrieved were independently screened against the inclusion criteria checklist.

#### **Inclusion criteria checklist:**

- Adverse effects in relation to the substance alone are addressed
- Route of exposure for humans is oral
- Route of exposure for animals is oral, in addition, subcutaneous exposure is included if the toxicokinetic is equal to oral exposure
- Human studies are performed in apparently healthy individuals or patient groups assumed to have normal absorption and metabolism of the assessed substance
- Animal model studies address adverse effects relevant to human health

The inclusion criteria checklist was developed by members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. The primary screening was performed by one person. The full text of articles that passed the primary screening was retrieved for secondary screening. In this screening, the full text articles were reviewed by a second person and compared against the inclusion criteria checklist. The search included also animal studies, however, only human studies on toxicity were retained for the secondary screening.

The secondary screening resulted in 135 full text articles, of which 38 were included among the human studies used for hazard identification and characterisation. When going through these papers, it was realized that quite a high number of papers of interest were missed when including search terms such as adverse, risk, safe, side-effect, hazard, harmful, negative or toxic. Therefore, secondary searches were performed without these search terms on September 19, 2016, and the relevant human studies found were included in the hazard identification and characterisation (Appendix 9.5.2). Additionally, the reference lists in the included studies were screened, and studies from this manual search were included when found relevant. In total, 114 papers reporting human studies were included in the hazard identification and characterisation.

## 2.2 General information

### 2.2.1 Chemistry

Phytoestrogens consist of the flavonoids (isoflavones, coumestans and prenyl flavonoids) and non-flavonoids (lignans) (Bakker, 2004). The flavonoids share a basic structure consisting of two benzene rings, A and B, linked through a heterocyclic C ring (Yuan et al., 2007). Isoflavones are a class of non-steroidal estrogens that have similarity in chemical structure and properties to estrogen. However, they show conformational binding to the estrogen receptor that classifies them as natural selective estrogen receptor modulators (SERMs) rather than as estrogens, and have estrogenic and anti-estrogenic effects depending on the concentration of endogenous estrogen and amount and type of estrogen receptors.

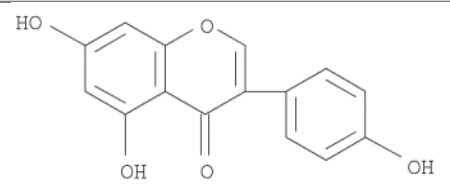
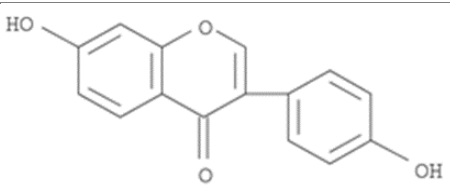
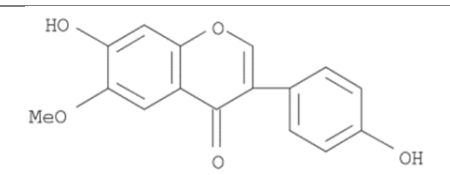
Soy isoflavones comprise three aglycones; genistein, daidzein and glycitein (Table 2.2.1-1) (EFSA, 2015; Messina, 2005). These also exist as their corresponding  $\beta$ -glycosides; genistin, daidzin and glycitin. In addition, the three  $\beta$ -glycosides can each be esterified with either malonic acid or acetic acid to 6''-O-malonylgenistin and 6''-O-acetylgenistin, 6''-O-malonyldaidzin and 6''-O-acetyldaidzin, and 6''-O-malonylglycitin and 6''-O-acetylglycitin, forming in total 12 different soybean isoflavone isomers. Most of the isoflavones found in soybeans and non-fermented foods exist as  $\beta$ -glycosides, whereas in fermented soy products, due to microorganism-induced fermentation and hydrolysis, much of the isoflavones is present in aglycone form (Messina, 2005). Typically, there is somewhat more genistein/genistin than daidzein/daidzin in soybeans and soy foods, whereas glycitein/glycitin comprises only 5-10% of the total isoflavone content (Messina, 2005). Overall, the evidence indicates that whether isoflavones are present as glycosides or aglycones appears not to be critically important with regard to their biological effects, since the glycosides, although not absorbed intact, can be hydrolysed *in vivo* (Messina, 2005).

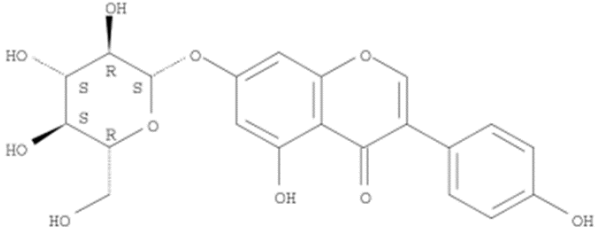
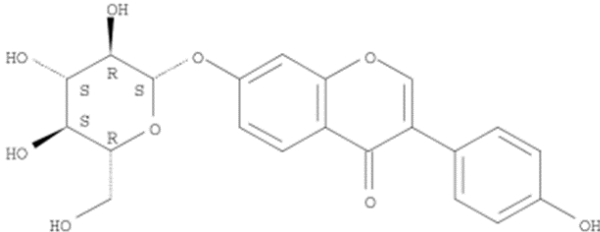
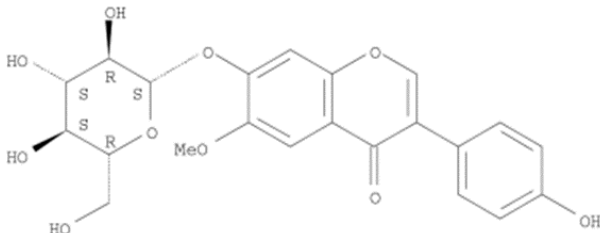
The isoflavone content that is obtained varies with part of the soy plant being used, such as soybean versus whole soybean sprout or cotyledon, hypocotyl or root (Cho et al., 2009). It also depends on the soy cultivar, place of growth, cultivation conditions and harvest time. The extracts obtained from soy typically maintain the naturally occurring glycosylated and derivated forms. However, it also varies depending on type of extraction solvent; the isoflavone content of alcohol-washed soy protein concentrates, the most common type of concentrate, is only 5-10% of the water-washed concentrates (Messina, 2005). The isoflavone forms obtained also depends on the use of ethanol versus methanol, acetonitrile or acetone, the concentration of each solvents and their mixture, temperature under extraction, time for extraction and how many times the extraction process is repeated, as well as what type of mechanical extraction procedure is used (vortexing, shaking, stirring, sonication or Soxhlet) (Achouri et al., 2005; Cho et al., 2009; Luthria et al., 2007; Penalvo et al., 2004). The isoflavones obtained are also dependent on whether acid is included with the alcohol solvent and the amount of protein present in the soy source. Different isoflavones may have different optimal extraction solvents. It was shown that for extraction of glycosidic

isoflavones, the polar mixture of water, acetone and acetonitrile achieved the best extraction, the malonyl-glycosidic forms were better extracted with mixtures of water, acetone and ethanol, the aglycone isoflavones were best extracted with water and acetone, and the best solvents for total isoflavones were water, acetone and ethanol (Yoshiara et al., 2012). Obviously, all these different factors varying in the soybean source and in the extraction process will influence the final composition of isoflavones in a supplement.

Isoflavones in soy foods are rather heat-stable, as normal cooking such as baking or frying, does not reduce the total isoflavone content significantly (Huang et al., 2006; Messina, 2005). Genistein in soy milk showed greater stability to heat treatment than daidzein and glycitein (Huang et al., 2006). Both the daidzein and glycitein contents decreased rapidly during the early stage of heating, but on continued heating the rates of decrease were much slower. Heating may cause an increase or decrease in the genistein content of soy milk depending on the temperature and time used. Upon heating at 95 and 121 °C, there was an increase in the genistein content in the early stage of heating, possibly due the conversion of genistin to genistein. Heating at 140 °C for more than 15 s and prolonged heating at 95 and 121 °C, however, caused a slow decline in the genistein content.

**Table 2.2.1-1** Isoflavones included in this risk assessment, based on EFSA (2015).

Isoflavone	Chemical structure	CAS no.	Chemical formula	Molecular weight	Chemical name ( <i>synonyms</i> )
Aglycones		446-72-0	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	270.24	5,7-Dihydroxy-3-(4-hydroxyphenyl)-4-benzopyrone
		486-66-8	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	254.24	7-Hydroxy-3-(4-hydroxyphenyl)-4-benzopyrone
		40957-83-3	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	284.26	7,4-Dihydroxy-6-methoxyisoflavone

<b>Glycosides</b>	Genistin		529-59-9	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	432.38	4H-1-Benzopyran-4-one, 7-(β-D-glucopyranosyloxy)-5-hydroxy-3-(4-hydroxyphenyl)- ( <i>genistein 7-O-glucoside</i> )
	Daidzin		552-66-9	C <sub>21</sub> H <sub>20</sub> O <sub>9</sub>	416.38	4H-1-Benzopyran-4-one, 7-(β-D-glucopyranosyloxy)-3-(4-hydroxyphenyl)- ( <i>daidzein 7-O-glucoside</i> )
	Glycitin		40246-10-4	C <sub>22</sub> H <sub>22</sub> O <sub>10</sub>	446.40	4H-1-Benzopyran-4-one, 7-(β-D-glucopyranosyloxy)-3-(4-hydroxyphenyl)-6-methoxy- ( <i>glycitein 7-O-glucoside</i> )

EINECS no.: Genistein: 207-174-9, daidzein: 207-635-4.



## 2.2.2 Occurrence and intake

Isoflavones are naturally occurring substances found in, among other sources, soy, red clover and kudzu root (EFSA, 2015). They can therefore be found in foods made from these plant sources, such as tofu, soy yoghurt, soy milk and drinks, as well as in food supplements. In this risk assessment, only isoflavones from soy are considered.

The consumer groups with the highest dietary intake of isoflavones (approximate levels) are consumers taking dietary phytoestrogen containing supplements (40–100 mg/day), vegan breast-feeding women (75 mg/day), soy-based formula fed infants (40 mg/day) and consumers of a traditional South East Asian diet (25-100 mg/day) (Bakker, 2004). The dietary isoflavone intake of average Western consumers and vegetarians is much lower (<1–2 mg/day and 3-12 mg/day, respectively).

Overall background exposure to isoflavones from the diet in the general European population was estimated to be lower than 1 mg/day (0.27-1.43 mg/day in women), whereas in consumers of soy-based foods and vegetarians it could be higher and within the estimated range of exposure to isoflavones from soy food supplements (approximately 0.1-111 mg/day) (EFSA, 2015).

The intake (mean  $\pm$  SE) of isoflavones from dietary soy in women was  $1.474 \pm 0.158$  and  $1.096 \pm 0.178$  mg/day in south and east of Norway and north and west of Norway, respectively, based on a single 24-hour recall in the EPIC study (Zamora-Ros et al., 2012). Corresponding numbers were  $0.005 \pm 0.000$  and  $0.004 \pm 0.000$  mg/day for the daidzein metabolite *S*-equol. For men, the corresponding intake of isoflavones was  $0.978 \pm 0.135$  and  $0.721 \pm 0.136$  mg/day, respectively, and for *S*-equol, the numbers were  $0.006 \pm 0.000$  and  $0.007 \pm 0.000$  mg/day, respectively.

Based on a food frequency questionnaire, among 87800 women in the Norwegian Mother and Child Cohort Study (MoBa), 1.7% used soy milk, and of these only 0.4% drank  $\geq 200$  ml of soy milk per day (unpublished data, personal communication with Margareta Haugen, the Norwegian Institute of Public Health). Regarding use of soy products for dinner, 4.8% had an occasional intake, and only 12% of these women had soy products for dinner more than once every other week.

In Norway, as in other Western countries, the intake of soybeans and soybean-based products is generally low in the average diet, but may be higher in vegans and persons with milk allergy. There is no information available on the actual level of dietary exposure to soy isoflavones in vegans or persons with milk allergy in Norway, in any age groups. The mean intake of soy protein per day has been estimated for persons eating either a vegan menu or a milk-free diet, based on weekly menus (VKM, 2015). For the vegan diet, the meat was replaced by soy burgers, soy sausages etc. For the milk-free diet, the milk products were replaced with soy products. The estimated mean intake for an adult was 35 and 19 g soy protein per day, for the vegan and milk allergy scenarios, respectively. For children, the

numbers for adults were adjusted for energy requirements, and assuming that milk in coffee and tea was consumed as milk. In the vegan scenario, the estimated intake was 18, 24, 30 and 41 g soy protein per day for 2-5 year-olds, 6-9 year-olds, 10-13 year old girls and 14-17 year old boys, respectively. In the milk allergy scenario, the estimated intake was 10, 14, 17 and 23 g soy protein per day for 2-5 year-olds, 6-9 year-olds, 10-13 year old girls and 14-17 year old boys, respectively. For 10-13 year old boys and 14-17 year old girls, the estimated intake is approximately similar to in adults.

There is no information available on the actual level of exposure to soy isoflavones from supplements in Norwegians of any age.

Genistein and daidzein are used for skin conditioning in cosmetics (CosIng, 2015).

## **2.3 Absorption, distribution, metabolism and excretion (ADME)**

Most of this text on ADME is based on EFSA (2015). The studies were identified in a focused literature review (EFSA, 2015). In this review, study results from genotoxicity of isoflavones conducted in numerous *in vitro* and *in vivo* studies were assessed. The available studies focused mainly on genistein and daidzein and at lesser extent on soy extracts. A limited number of studies on daidzein metabolites (*S*-equol, *O*-desmethylangolesin, 3',4',7-trihydroxyisoflavone (3'-HO-DAI) and 4',6,7-trihydroxyisoflavone (6-HO-DAI)) and glycitein were also available.

### **2.3.1 In humans**

#### ***2.3.1.1 Absorption and bioavailability***

The absorption is the percentage of the dose which after oral intake is delivered from the lumen of the gastrointestinal tract into the cells lining the gastrointestinal tract. Absorption is different from bioavailability, which is the percentage of the dose delivered in the systemic circulation; in other words, this is the percentage of the dose which after absorption is escaping pre-systemic elimination in the cells of the gastrointestinal wall and in the liver (EFSA, 2015).

Setchell et al. (2002) showed that the glycosides daidzin and genistin were not found intact in the peripheral blood of healthy adults. According to (Rowland et al., 2003), the glycoside forms present in soy, which are biologically inactive, are not found either in feces or in the blood, indicating that all glycosidic forms are hydrolysed before glucuronidation or sulfation in the gut. They both concluded that isoflavone glycosides are not absorbed intact across the enterocyte but that hydrolysis by intestinal  $\beta$ -glucosidases resulting in the conversion to their corresponding bioactive aglycones is necessary for absorption. In addition, *in vitro* data (Kobayashi et al., 2013) may indicate that genistein is transported through the intestinal barrier by passive diffusion and is a substrate for the efflux protein breast cancer resistance protein (BCRP) after conjugation by phase II enzymes. Daidzein is a substrate for BCRP, multidrug resistance-associated proteins and P-glycoprotein without being conjugated.

Busby et al. (2002) studied pharmacokinetics and safety (see 2.4.2.3.6) of two preparations (formulation A and formulation B) of purified soy isoflavones in capsules containing genistein, daidzein and glycitein given in different proportions to healthy men (n = 30, aged 40-69 years). Each man was administered a single dose of the five doses studied (n = 6, 3 for each formulation). The doses of genistein were 1, 2, 4, 8 and 16 mg/kg bw both in formulation A and B, and the doses of daidzein were 0.11, 0.22, 0.44, 0.89 and 1.8 mg/kg bw in formulation A and 0.49, 0.98, 2.0, 3.9 and 7.8 mg/kg bw in formulation B. Formulation A contained  $\geq 97\%$  total unconjugated isoflavones consisting of  $90 \pm 5\%$  genistein, 10% daidzein and 1% glycitein, whereas formulation B contained 70% unconjugated isoflavones consisting of 43% genistein, 21% daidzein and 2% glycitein. Based on the mean plasma area under the curve (AUC) of unconjugated genistein, the relative bioavailability of genistein formulation A versus genistein formulation B was measured. Formulation A had a relative bioavailability of 60% for the dose of 8 mg genistein (A/B) and of 15% for the dose of 16 mg genistein (A/B), indicating a better bioavailability after intake of formulation B than intake of formulation A. When absorption was calculated based on total isoflavones, formulation B was also found to be better absorbed than formulation A; however, the values were not identical to the values for the bioavailability based on the AUC of unconjugated genistein. In the case of daidzein, data was not sufficient to calculate the AUC of unconjugated daidzein. In urine, total daidzein excretion was lower after ingestion of formulation A than after ingestion of formulation B. No further details were given in the publication. From this study, the concentration at C<sub>max</sub> was used to calculate genistein aglycone as a percentage of total genistein. The value varied between 0.9% and 2.7% for the seven studied doses for which data were available. Likewise, the concentration at C<sub>max</sub> was used to calculate daidzein aglycone as percentage of total daidzein. The value varied between 1.4% and 4.2% for the five studied doses for which data were available.

In a study by Bloedon et al. (2002), both pharmacokinetics and safety (see 2.4.2.1.6) were assessed in healthy post-menopausal women (n = 24, aged 46-68 years). They were administered two preparations (formulation A and formulation B) of purified soy isoflavones in a single dose as capsules containing genistein, daidzein and glycitein in different proportions, which were studied at four doses (n = 6, 3 for each formulation). The doses of genistein were 2, 4, 8 and 16 mg/kg bw (exceeding normal dietary intakes) and the doses of daidzein were varying between 0.28 and 2.2 mg/kg bw in formulation A, and 1.0 and 8.4 mg/kg bw in formulation B. The levels of glycitein varied from 0.014 to 0.11 mg/kg bw in formulation A, and from 0.08 to 0.68 mg/kg bw in formulation B. Formulation A contained 100% total unconjugated isoflavones consisting of 87% genistein, 12% daidzein and 1% glycitein, whereas formulation B contained 70% unconjugated isoflavones consisting of 44% genistein, 23% daidzein and 2% glycitein. Pharmacokinetic studies were performed during the first 24 hours. From this study, the concentration at C<sub>max</sub> was used to calculate genistein aglycone as a percentage of total genistein. The value varied between 0.7% and 2.2% for the eight studied doses for which data were available. Likewise, the concentration at C<sub>max</sub> was used to calculate daidzein aglycone as percentage of total daidzein. The value varied between 1.1% and 2.8% for the six studied doses for which data were available. As

opposed to in men, the estimated bioavailability of both total genistein and total daidzein from each of the two formulations was not significantly different.

In a study by Shelnut et al. (2002), six men (aged 27-52 years) and six women (aged 35-47 years) consumed a soy protein isolate (SPI) drink providing a dose of 1.0 mg genistein (aglycone) equivalents/kg bw and 0.6 mg daidzein (aglycone) equivalents/kg bw. Isoflavones were measured after treating the samples with  $\beta$ -glucuronidase and sulfatase, thus not enabling any statement about the presence or concentration of the conjugated or unconjugated aglycones in plasma. From the amount excreted in urine after 48 hours, a rough estimate could be made about the amount (but not the form) absorbed. In this study the absorption was 35.4% of the dose for genistin, 61.3% for daidzin and 60.4% for glycitin. However, it should be noted that the sampling period could be too short and hence the percentage absorbed could be higher.

In a further study by Setchell et al. (2003a), a single bolus doses of 10, 20 or 40 g of toasted soy nuts containing 16.38, 32.76 or 65.52 mg of total isoflavones as aglycones, respectively, were given to five pre-menopausal and five post-menopausal women on different occasions, 1 month apart. The isoflavone doses contained conjugated forms of 9.8, 19.6 and 39.2 mg equivalents of genistein and 6.6, 13.2 and 26.4 mg equivalents of daidzein. The absorption, calculated from urinary excretion, declined with increasing dose (genistein: 25.2%, 13.4% and 15.8%, and daidzein: 63.2%, 54.4% and 44.0%). There was no difference between pre- and post-menopausal women.

A similar high absorption could be calculated from the data on 12 healthy men (aged 20-29 years) in a randomized two-way cross-over study by (Vergne et al., 2008). After intake of a soy supplement in capsules providing 28.24 mg isoflavone equivalents of daidzein and 6.76 mg as genistein and intake of soy-based cheese providing approximately 15.78 mg isoflavone equivalents of daidzein and 19.22 mg of genistein, roughly 25 mg and 3 mg, respectively (data extrapolated from Figure 3 of the publication), was excreted in the urine over 48 hours from intake of the soy supplement. The resulting absorption from the supplements would be 88.5% for daidzein and 44.3% for genistein. The study indicated that isoflavones in capsules were more bioavailable than those contained in soy-based cheese.

In conclusion, wide variability in absorption was observed. (van der Velpen et al., 2014) found that the dose (intake) of isoflavones from either supplements or soy foods was a poor indicator of internal exposure based on data from 103 post-menopausal women participating in three intervention studies. They found that plasma levels did not increase in proportion to the dose and that interindividual variability was 30–96%. Plasma isoflavone half-lives were short (6-8 hours), therefore, circulating isoflavones are typically biomarkers of recent exposure (Lampe et al., 2007). In humans, there is large interindividual variation in urinary recovery of genistein and daidzein and their metabolites, suggesting that bioavailability may be a critical aspect of exposure that is not captured with dietary report methods, such as food frequency questionnaires (FFQs) (Lampe, 2003).

### **2.3.1.2 Distribution**

In a study by (Rannikko et al., 2006), genistein and daidzein were measured in prostate tissue after two weeks of intervention with an isoflavone-containing clover supplement. The concentrations in the prostate were over two-fold higher than in the plasma. As the analytical method for isoflavones included enzymatic hydrolysis of samples, the results were not representative of the distribution of the individual substances.

Other studies also measured total genistein and the glucuronyl conjugates of genistein and daidzein in breast tissue. For example, Bolca et al. (2010) randomly allocated healthy women (n = 31, aged 18-62 years) to a soy milk (n = 11, containing 16.98 mg genistein and 5.40 mg daidzein aglycone equivalents per dose, three doses per day) group, a soy supplement (n = 10, containing 5.27 mg genistein and 17.56 mg daidzein aglycone equivalents per dose, 3 doses per day) group or a control group (n = 10) for 5 days before surgery for aesthetic breast reduction. In some samples, the aglycones were also measured. In breast tissue, genistein concentration was  $12 \pm 2$  (n = 4) pmol/g tissue and daidzein concentration was  $8 \pm 1$  (n = 6) pmol/g after soy milk, whereas after soy supplement genistein concentration was  $8 \pm 2$  (n = 3) pmol/g tissue and daidzein concentration  $22 \pm 4$  (n = 6) pmol/g tissue.

### **2.3.1.3 Metabolism**

The metabolism of the soy isoflavones genistein and daidzein is summarised in various review papers (Larkin et al., 2008; Mortensen et al., 2009; Rafii, 2015; Yang et al., 2012; Yuan et al., 2007). Based on these reviews, there are no indications that the source of dietary isoflavones has a fundamental impact on the general routes of metabolism. However, the starting material used to manufacture phytoestrogen supplements may have significant effects on the ultimate bioavailability and characteristics of the circulating isoflavone levels. It was demonstrated that two different supplements yielded quite different plasma isoflavone profiles (Setchell et al., 2001). Those with soy germ as starting material resulted in plasma enriched in daidzein and low in genistein. By contrast, with supplements made from extracts of soy proteins, the plasma was enriched to a greater extent in genistein. Therefore, the type of supplement may vary in effect, and this variation may affect the clinical end points studied.

The bioavailability of various isoflavones may be different, since structural differences between them may influence how they are treated by intestinal metabolic enzymes and efflux transporters in the intestines (Wang et al., 2006).

Isoflavones are metabolised by endogenous phase I and phase II enzymes, mainly in the gut and the liver, as well as by the intestinal microbiota. Conjugation as well as microbial transformation reactions are major pathways. Minor metabolites are hydroxylated derivatives formed by the action of cytochrome P450 enzymes.

It is known that in humans, as in other species, biochanin A is mainly demethylated to genistein and formononetin is demethylated to daidzein by phase I hepatic enzymes,

demonstrated when given as tablets of extracts from red clover (*Trifolium pratense*) (Howes et al., 2002; Setchell et al., 2001).

Hosoda et al. (2011) found that the 7-glucuronide-4'-sulfates were the major metabolites of daidzein (53.3%) and genistein (54.0%) in the plasma of 10 healthy Japanese men (n = 5) and women (n = 5) (aged 21-55 years) who ingested kinako (baked soybean flour). In contrast, in the 48-hour urine, daidzein-7-glucuronide constituted, on average, 48.1% of the total daidzein metabolites. In the case of genistein, the excreted amounts of genistein-7-glucuronide and genistein-4'-glucuronide accounted for 28.5% and 27.0%, respectively, of total genistein metabolites.

Soukup et al. (2014) investigated the phase II metabolite profile in plasma and urine of 11 German post-menopausal women after ingestion of a bolus dose of a commercial soy extract (dose 1 mg/kg bw, calculated as isoflavone aglycone equivalents of genistein and daidzein). The result confirmed the findings of Hosoda et al. (2011) that sulfoglucuronides are the major metabolites of genistein and daidzein in the plasma and that the 7-*O*-glucuronides and 4'-*O*-glucuronides are the predominant metabolites in the urine. Interindividual variation regarding the phase II metabolite pattern in humans appears to be low (Hosoda et al., 2011; Soukup et al., 2014). However, studies with a larger number of individuals are needed to confirm this.

Besides glucuronidation and sulfation, transformation reactions catalysed by the intestinal microbiota play a crucial role in the metabolism of isoflavones. Genistein can be reduced to dihydrogenistein and then via ring cleavage to 6'-hydroxy-*O*-desmethylangolensin. A further degradation to 2-(4-hydroxyphenyl)-propionic acid is shown by incubation of genistein with fecal samples (Braune et al., 2010; Coldham et al., 2002). 4-Ethyl-phenol is also mentioned in the literature as a genistein metabolite in humans (Setchell, 1998). However, the relevance of this degradation reaction *in vivo* has so far not been investigated.

Daidzein can be converted to dihydrodaidzein and subsequently to *O*-desmethylangolensin (DMA) and/or *S*-equol. Equol may be further metabolized to 6-hydroxy-equol and 3'-hydroxy-equol (Yuan et al., 2007). Tetrahydrodaidzein, 3'-hydroxy-daidzein, 6-hydroxy-daidzein, 8-hydroxy-daidzein, 3-(4-hydroxyphenyl)-benzopyran-4,7-diol and 2-dehydro-*O*-DMA have also been reported to be metabolites of daidzein (Yuan et al., 2007). The extent of microbial metabolism of genistein and daidzein as well as the resulting microbial metabolite profile varies greatly among individuals. The urinary equol/daidzein ratio of 0.018 with a daidzein threshold of 2 nmol/mg creatinine to identify equol producers has been proposed, a cutoff definition with which equol production was inconsistent over time in 5-30% of both pre-menopausal and post-menopausal women (Franke et al., 2014).

Glycitein was found in high levels in human plasma after ingestion, indicating low biotransformation of this compound (Zhang et al., 1999). Tentative identification of 5'-hydroxy-*O*-desmethylangolensin by urinary analysis suggested that the demethylated glycitein metabolites are present in human urine after soy consumptions (Heinonen et al., 1999). A study in healthy young Caucasian men showed that glycitein was one of the best

absorbed isoflavones after a single intake of a food supplement based on soy germ containing 55.24 mg isoflavones (Shinkaruk et al., 2012). Glycitein plasma bioavailability was similar to that of daidzein and was not affected by equol. Its urinary excretion was significantly higher than that of genistein.

Only approximately one-third to one-half of populations is able to metabolize daidzein to *S*-equol, i.e. in 20-30% of the populations in Western countries and 50-60% of Asian populations consuming soy-containing diets (Rafii, 2015; Yuan et al., 2007). This high variability in *S*-equol production is presumably attributable to interindividual differences in the composition of intestinal microflora. People who have plasma equol concentrations of <40 nmol/l (10 µg/l) are classified as "equol non-producers", whereas they with concentrations >83 nmol/l (20 µg/l) are classified as "equol producers". Equol producers also excrete >1000 nmol/l in the urine. Equol has greater affinity for estrogen receptors, unique antiandrogen properties and superior antioxidant activity compared with other isolated isoflavones.

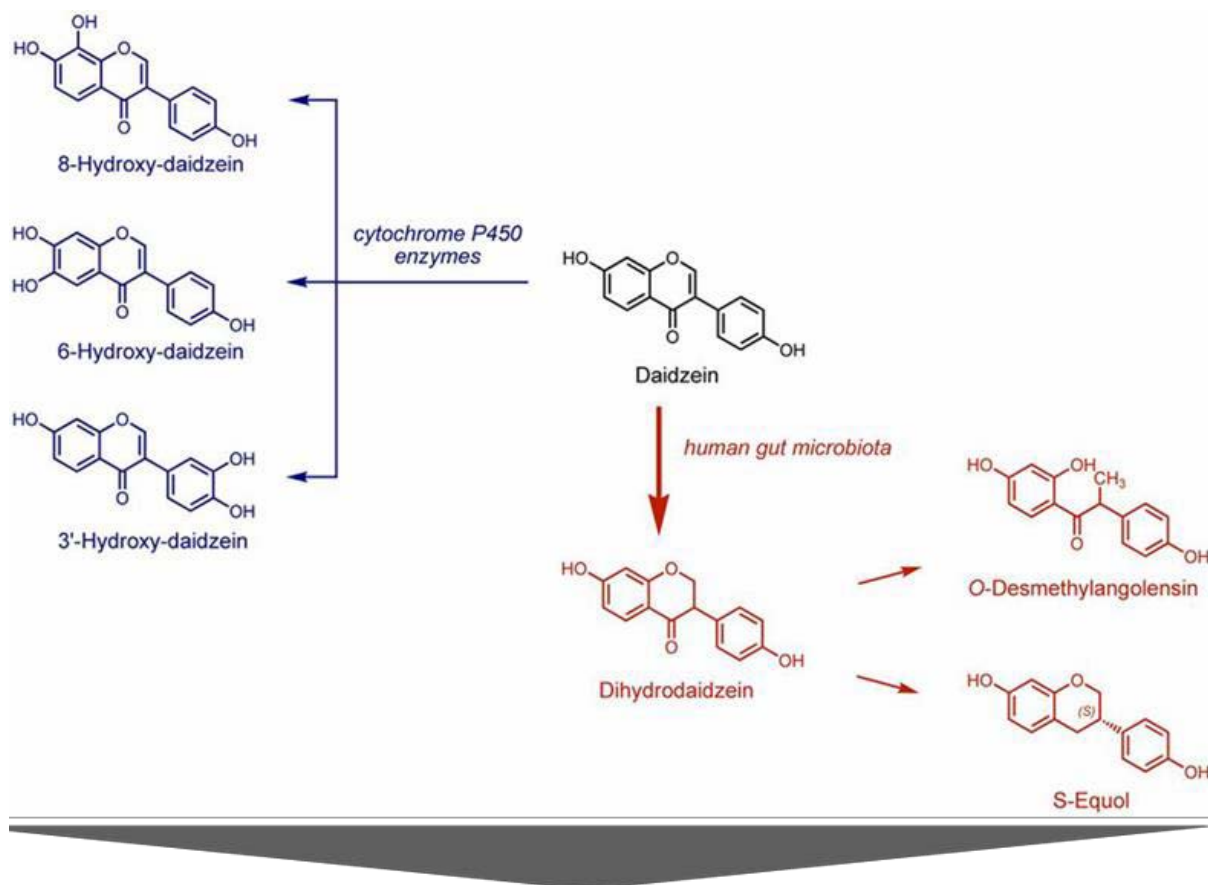
Genistein undergoes enterohepatic recycling (Yang et al., 2012). Genistein is extensively absorbed in enterocytes, converted to conjugates and then enter the portal vein. When the blood circulation passes through the liver, the remaining genistein aglycones are sequestered, converted and the metabolites are excreted into the bile. The bacteria in terminal small intestine and colon can hydrolyze these conjugates, which then may be reabsorbed in the intestine.

Large amounts of genistein conjugated metabolites generated in enterocytes can be excreted back into lumen by efflux transporters expressed at the apical side of enterocytes, i.e. breast cancer resistance protein (BRCP) and multidrug resistance associated protein (MRP)-2. Similarly to enterohepatic recycling, bacteria in small intestine and colon deconjugate these metabolites by hydrolysis and enables them to be reabsorbed in the intestine and conjugated (Yang et al., 2012).

The duo recycling involving both enteric and enterohepatic recycling is the reason for extensive metabolism and prolonged systemic exposure to genistein *in vivo* (Yang et al., 2012).

The food matrix may influence the metabolism of isoflavones. When isoflavonoid profiles in urine were compared between soy bread and soy beverage interventions in women, dihydrodaidzein (DHD) was significantly ( $P = 0.04$ ) higher following soy bread intake, and there was a trend towards higher *S*-equol and *O*-desmethylangolensin (ODMA), both daidzein metabolites, and lower daidzein, after soy bread intake (Ahn-Jarvis et al., 2012). However, this difference was not seen in men.

The biotransformation pathways of daidzein, summarising the metabolites found so far in the plasma or urine of humans or experimental animals (rats, mice, monkeys) are shown (Figure 2.3.1-1) (EFSA, 2015).



**Conjugation to phase-II-metabolites  
(glucuronides, sulfoglucuronides, sulfates)**

**Figure 2.3.1-1** The biotransformation pathways of daidzein, summarising the metabolites found so far in the plasma or urine of humans or experimental animals (rats, mice, monkeys) (EFSA, 2015).

**2.3.1.4 Elimination**

Most absorbed genistein and daidzein is excreted as phase II conjugates and as phase II conjugates of microbial-derived metabolites in the urine. Fecal elimination has been found to be a minor route. Larkin et al. (2008) reported that total fecal excretion of isoflavones in humans accounts for less than 5%, and is predominantly in the unconjugated form, with less than 10% being conjugated.

Urine excretion was investigated in a study conducted by (Xu et al., 1994). In this study, 12 young adult (aged 19-41 years) women ingested defined doses (0.7, 1.3 and 2.0 mg/kg bw) of soy isoflavones as soy milk powder in three meals at defined time points within a 10-hour window on three feeding days, each separated by a two-week washout period. Urine was collected before dosing, in 12-hour fractions for 24 hours after the first dosing and the first urination of the second day after dosing. Depending on the soy isoflavone dose ingested, 85 to 93.5% of the total isoflavone amount excreted in the urine was excreted in the first 24



hours after first dosing (i.e. 14 hours after last dosing), 6 to 14% was excreted in the 24–48 hour urine fraction after first dosing, and after 48 hours (38 hours after last dosing) isoflavone excretion had reached baseline level. This result is consistent with other studies summarised by Larkin et al. (2008), who stated that the majority of urinary excretion of genistein and daidzein occurs within the first 24 hours after ingestion.

The urinary recovery rate was determined in several studies. (Shelnutt et al., 2000) conducted a study in 12 healthy volunteers (six men and six women) who ingested a soy beverage prepared to provide a dose of 1.0 mg/kg bw genistein aglycone equivalents and 0.6 mg/kg bw daidzein aglycone equivalents; 7.2% of the dose of genistein and 27.4% of the dose of daidzein (aglycone plus phase II conjugates) was recovered in the 24-hour urine. Based on urinary dihydrogenistein, dihydrodaidzein, *O*-desmethylangolensin and glycitein, total recovery was 9.3% for genistein, 50.9% for daidzein and 19.0 for glycitein. *S*-equol was not measured in this study and therefore not included in the excretion rates determined over time. The apparent terminal half-lives for genistein and daidzein glucuronides, the main metabolites found in urine, were  $6.0 \pm 0.4$  and  $3.8 \pm 0.4$  hours, respectively, including the microbial metabolites in the calculation made for daidzein. Therefore, circulating isoflavones are typically biomarkers of recent exposure. In other studies, the percentages of a genistein and daidzein dose excreted in the urine were reported to be, respectively, 14.6% and 46.9% (Lu et al., 1995), 17.6% and 35.8% (Watanabe et al., 1998), 22% and 62% (King and Bursill, 1998) and 16% (range 5–42%) and 50% (range 18–95%) (Setchell et al., 2003a). At least part of the variability is certainly based on the fact that not all known microbial metabolites were included in each calculation and that enzymatic hydrolysis, which was used in all studies to quantify the resulting aglycones, might be not complete in every single case. However, the studies consistently showed that the urinary recovery rate is much higher for daidzein than for genistein.

The half-life ( $t_{1/2}$ ) reported in most human studies is the half-life of total genistein and total daidzein. This means that the values were calculated on the basis of a genistein and daidzein measurement after enzymatic hydrolysis of the different phase II conjugates which accounted for >95% of the total genistein and daidzein concentration found in plasma. Reported values for the elimination  $t_{1/2}$  of total genistein are in the range of 5.7–10.8 hours and were determined in intervention studies using different soy foods (soy flour, soy beverage, soy nuts, soybean powder) as isoflavone source. The values are summarised in the review of (Yang et al., 2012). In one study,  $^{13}\text{C}$ -labelled genistein and daidzein were used. The  $t_{1/2}$  values of total genistein and total daidzein were determined to be  $7.41 \pm 0.39$  hours and  $7.18 \pm 0.49$  hours, respectively, for a dose of 0.8 mg genistein/kg bw and 0.8 mg daidzein/kg bw (Setchell et al., 2003b) in healthy pre-menopausal women ( $n = 9$  and  $n = 8$ , for genistein and daidzein, respectively). Chronic soy milk exposure with each meal for a month did not significantly change the absorption half-lives of genistein and daidzein in healthy men (aged 21–35 years), but prolonged the excretion half-lives and thus prolonged the tissue exposure to the isoflavones (Lu et al., 1995).

The pharmacokinetic data suggest that chronic dosing at 12-24 hours intervals would not lead to progressive accumulation of genistein, daidzein or glycitein (Bloedon et al., 2002).

Genis and daidzein were rapidly cleared from plasma and excreted in urine in men with prostate cancer ((Fischer et al., 2004), see 2.4.2.3.6 for safety). Pharmacokinetic data for chronic dose administration (84 days) was similar to single-dose administration for the isoflavones investigated (genistein and daidzein) except that they observed slightly longer circulation time for daidzein. Minimal to no accumulation of isoflavones occurred during daily dosing for 84 days.

Isoflavone bioavailability was found to be influenced by gut bacteria, oral antibiotic treatment and an individual's age and health status (Franke et al., 2014). Apparent bioavailability (a term suggested when urinary data is used for isoflavone exposure) is higher in children than in adults, higher in healthy vs. non-healthy individuals and decreased in children, but increased in adults, during oral antibiotics therapy (Franke et al., 2014).

Daily excretion rates varied little among infants 2-16 weeks of age and the mean values ranged from  $0.15 \pm 0.03$  to  $0.32 \pm 0.04$  mg/kg bw per day for genistein and from  $0.37 \pm 0.03$  to  $0.58 \pm 0.06$  mg/kg bw per day for daidzein (n = 4) (Irvine et al., 1998). The mean percentage of daily isoflavone intake recovered in the urine was  $13.0 \pm 3.0\%$  for genistein and  $38.4 \pm 4\%$  for daidzein, which were similar to values found in adults (Irvine et al., 1998). As adults, infants both excrete relatively more of their intake of daidzein than of genistein. These data indicate that infants as young as 4 weeks of age can digest, absorb and excrete genistein and daidzein from soy-based infant formulas as efficient as do adults consuming soy products.

## **2.3.2 Animal studies**

### ***2.3.2.1 Absorption and bioavailability***

The absorption and absolute bioavailability of genistein and daidzein were measured in female Balb/c mice after intravenous (i.v.) and oral administration by gavage of pure substances in doses of 1.2 mg/kg bw genistein and 0.55 mg/kg bw daidzein or as bolus administration of equimolar doses in a food pellet of soy protein isolate (SPI) (Andrade et al., 2010). The bioavailability of genistein and daidzein was equivalent for the gavage and dietary routes of administration. Absorption, estimated by the comparison of AUCs of the total isoflavones, was nearly complete (>84%). The absolute bioavailability (internal exposure) amounted to 9–14% for genistein and 29–34% for daidzein.

Coldham et al. (2002) determined absorption after oral and intravenous dosing with 4 mg/kg bw (<sup>14</sup>C)-genistein in Wistar rats of both genders (n = 5 per group). Measured by total radioactivity (parent compounds and metabolites), the absorption from the gut was 56% in males and 111% in females. The absolute oral bioavailability of the parent compound genistein was 7% in males and 15% in females.

After oral genistein doses of 6.25 mg/kg bw, 12.5 mg/kg bw and 50.0 mg/kg bw by gavage and an i.v. dose of 12.5 mg/kg bw given to Sprague-Dawley rats (Zhou et al., 2008), the bioavailability at these three doses (estimated from AUCs after oral and i.v. dosing) was 21.9%, 33.5% and 19.0%, respectively.

Yang et al. (2010) investigated the systemic availability after i.v. and oral dosing of 20 mg/kg bw genistein in male FVB mice (8-10 weeks old). The absolute bioavailability as calculated from the AUCs after oral and i.v. administration was 23.4%.

### **2.3.2.2 Distribution**

Three rat studies provided data on the distribution of genistein. In the study of Coldham and Sauer (2000), (<sup>14</sup>C)-genistein was found in every organ of Wistar female and male rats (n = 5) after an oral dose of 4 mg/kg bw. After 2 and 7 hours, the highest levels of radioactivity (>1000 ng genistein-equivalents/g tissue) were in the gastrointestinal tract (stomach and small intestine) and in the liver. Intermediary concentrations (<1000 ng genistein-equivalents/g tissue to 250 ng genistein-equivalents/g tissue) were observed in the kidney and the reproductive organs ovary, uterus, vagina and prostate, but not testis, where most of the radioactivity was claimed to be the parent compound. In testis, the levels were 96-117 ng genistein-equivalents/g tissue. In brain, fat, thymus, spleen, skeletal muscle and bone, the radioactivity concentrations were low (<100 ng genistein-equivalents/g tissue). Using a specific method, Chang et al. (2000) observed high proportions (up to 100%) of genistein aglycone in several tissues, including in the reproductive tissues, of male and female Sprague-Dawley rats after oral administration. Zhou et al. (2008) observed the highest genistein concentrations in the gastrointestinal tract and in the excretory organs, liver and kidney after oral administration of 12.5 mg/kg bw to Sprague-Dawley rats. The concentrations in the reproductive organs were equal to the concentrations in the skeletal muscle and in fat.

### **2.3.2.3 Metabolism**

Allred et al. (2005) investigated the metabolism of genistein and daidzein in ovariectomized (OVX) Balb/c mice using soy products of different degree of processing, including soy flour, soy molasses, soy extract and purified isoflavone fractions. They found that the average fraction of aglycone genistein and daidzein in the blood plasma ranged from 5% to 12% and from 11% to 18%, respectively. The proportion of *S*-equol aglycone was <1% for all feeding groups. In addition, the group investigated the distribution of the conjugated forms. The glucuronides were the main metabolites in the case of genistein and daidzein (53% of total genistein, 44% of total daidzein). In contrast, *S*-equol was present in 78% as sulfate.

Gu et al. (2006) analysed the metabolite profile of genistein and daidzein in adult female cynomolgus monkeys (n = 15) after feeding a diet formulated with SPI containing 4.8, 3.7 and 0.8 mg/kg bw of genistein, daidzein and glycitein as aglycones equivalents, respectively, for 5 weeks. The isoflavones genistein and daidzein as well as the microbial-derived daidzein metabolite *S*-equol were present in the serum of the monkeys predominantly as sulfates

(72.8% of total genistein, 64.9% of total daidzein, 64.2% of total *S*-equol), and to a lower extent as glucuronides (23.8% of total genistein, 34.5% of total daidzein, 29.6% of total *S*-equol). In the blood serum, the proportion of the aglycone was 3.5%, 0.6% and 6.1% for genistein, daidzein and *S*-equol, respectively. *S*-equol represented 52% of the total isoflavones (isoflavones plus metabolites) in the serum of the monkeys. The serum daidzein:*S*-equol ratio was 1:3. Based on these results, monkeys were classified as 100% equol producers. Dihydrogenistein, dihydrodaidzein and *O*-desmethylangolensin were found in the plasma at concentrations considerably lower than *S*-equol. The microbial genistein metabolites 6-hydroxy-desmethylangolensin and 4-ethyl-phenol were not measured.

Gu et al. (2006) determined the metabolite profile in the serum of adult female Sprague–Dawley rats (n = 9) after feeding a diet enriched with SPI containing 13.0, 9.9 and 2.4 mg/kg bw of genistein, daidzein and glycitein as aglycones equivalents, respectively, for 3 days. In the blood serum, the proportion of the aglycones was 3.6, 7.3 and 0.7% for genistein, daidzein and *S*-equol, respectively. *S*-equol was the dominant metabolite, accounting for 77% of total isoflavones (isoflavones plus metabolites) in the serum of the rats. The serum daidzein:*S*-equol ratio was 1:19. Based on these results, rats were classified as 100% equol producers. Dihydrogenistein, dihydrodaidzein and *O*-desmethylangolensin were found in the plasma at concentrations very much lower than *S*-equol. The microbial genistein metabolites 6-hydroxy-desmethylangolensin and 4-ethyl-phenol were not measured.

Yang et al. (2010) investigated the systemic availability after i.v. and oral dosing of 20 mg/kg bw genistein in male FVB mice (8–10 weeks old). More than 80% of genistein was converted to glucuronides and sulfates. In addition to genistein, genistein-7-glucuronide, genistein-4'-glucuronide, genistein-7-sulfate and genistein-4'-sulfate were identified, with average maximum plasma concentration ( $C_{max}$ ) values of 0.71  $\mu\text{M}$ , 0.98  $\mu\text{M}$ , 0.53  $\mu\text{M}$ , 0.65  $\mu\text{M}$  and 0.25  $\mu\text{M}$ , respectively, after oral dosing.

#### **2.3.2.4 Elimination**

In a study comparing the bioavailability of conjugates of genistein and daidzein, male Wistar rats were given a single oral dose by gavage of a soy extract providing 74  $\mu\text{mol}/\text{kg}$  bw of genistein and 77  $\mu\text{mol}/\text{kg}$  bw of daidzein as conjugates (King, 1998). Urinary excretion of genistein was 11.9% of the dose ingested and 17.4% of the dose of daidzein over a 48-hour post-dose period. 4-Ethyl-phenol excretion was 41.9% of the genistein dose and *S*-equol excretion was 5% of the daidzein dose. Fecal genistein accounted for  $3.4 \pm 0.4\%$  and fecal daidzein for  $2.3 \pm 0.5\%$  of the respective doses.

Gu et al. (2006) reported that female monkeys excreted a very high percentage of genistein, daidzein and *S*-equol in the urine as aglycones (89.2, 90.9 and 96.3%, respectively). No information was given on the excretion kinetics of isoflavones.

Gu et al. (2006) found that female Sprague–Dawley rats excreted a high percentage of genistein, daidzein and *S*-equol in the urine as aglycones (46.9, 40.8 and 65.5%,

respectively). Of the original dose, 2.6% of genistein and 3.3% of daidzein was recovered in the 24-hour urine as aglycones plus phase II conjugates. In addition, 17.3% of the daidzein dose was excreted as *S*-equol, compared with only 0.3% and 0.2% which were excreted as *O*-desmethylangolensin and dihydrodaidzein, respectively. Total daidzein (aglycone plus phase II conjugates plus microbial metabolites) recovery in the 24-hour urine was 21.2% of the dose ingested. No information was given on the excretion kinetics of isoflavones in this study.

The excretion of daidzein was measured in male (n = 4) and female (n = 4) Fischer F344 rats after administration of daidzein (100 mg/kg bw, dissolved in corn oil) by gavage (Bayer et al., 2001). For both sexes, 86% of the dose was excreted as unchanged daidzein in the feces within 36 hours after administration, and 8–9% of the dose was excreted in the urine within 24 hours after administration.

## 2.4 Toxicological data/Adverse effects

### 2.4.1 Genotoxicity

The following text on genotoxicity is mainly based on (EFSA, 2015).

#### 2.4.1.1 *In vitro*

In the study by Baechler et al. (2014), daidzein and possible human hydroxylated metabolites, 6-hydroxy-daidzein (4',6,7-trihydroxyisoflavone, 6-HO-DAI) and 8-hydroxy-daidzein (4',7,8-trihydroxyisoflavone, 8-HO-DAI), were assessed for their genotoxicity in the human colon carcinoma (HT29) cell line by means of Comet assay. Potential catalytic inhibition and poisoning of DNA topoisomerase was also investigated. In the Comet assay, cells were treated for either 1 or 24 hours at concentrations ranging from 1 to 50 µM and the results obtained indicated that, in contrast to daidzein, 6-HO-DAI and 8-HO-DAI significantly increased the rate of DNA strand breaks in HT29 cells after 24 hours' treatment and caused a cell cycle delay in S-phase. In addition, the hydroxylated metabolites also suppressed the catalytic activity of topoisomerase II in the decatenation assay but not the level of covalent topoisomerase II–DNA intermediates within HT29 cells by the "isolating *in vivo* complexes of enzyme to DNA" (ICE) bioassay, thus arguing for a catalytic inhibition of topoisomerase II rather than poisoning activity.

The study by Nakayama et al. (2014) showed that genistein at 100 µM in HeLa S3 cells *in vitro* caused abnormal cell division and cleavage furrow regression, resulting in the generation of binucleated cells and hence polyploidy. Moreover, it affected the formation of the central spindle, which is essential for completion of cytokinesis. Its impairment is accompanied by aberrant chromosome segregation, such as a chromosome bridge and lagging chromosomes.

The study by Lepri et al. (2013), which aimed to evaluate the ability of soy isoflavones to prevent micronuclei formation in HTC hepatoma cells, found that genistein and daidzein, when added alone to cell cultures at a single dose level of 10 µM, did not induce significant increases in micronuclei. The EFSA Panel noted that the results obtained were of limited validity since no change in the number of binucleated cells was observed in cultures treated with the test compound or in solvent-treated cultures owing to selection of an inadequate, i.e. not sufficiently high, dose level.

Mizushina et al. (2013) investigated the potential inhibitory activity of three soy isoflavones, genistein, daidzein and glycitein, and their glycosides, genistin, daidzin and glycitin, on purified DNA topoisomerases I and II (topo I and II) from human placenta which generate DNA single- and double-strand breaks, respectively. The catalytic activity of both topo I and II was evaluated by detecting supercoiled plasmid DNA (form I) in its nicked state (form II). The results obtained indicated that none of the six soy isoflavones influenced the topo I nicking activity at concentrations of 100 µM and greater. In contrast, genistein at 100 µM completely inhibited the nicking activity of topo II, while the other compounds were not active, indicating that genistein is a true inhibitor of human DNA topo II. It interacts directly with the enzyme and not through the stabilisation of the "cleavable complex" as indicated by the interaction of genistein with DNA double-strand breaks through its thermal transition. This specificity determines that genistein does not induce DNA double-strand breaks in the same way as classical topo II inhibitors, which stabilise the "cleavable complex". Such inhibitors are adriamycin, amsacrine, ellipticine, saintopin, streptonigrin, terpentecin, etoposide, etc. Instead, genistein induces arrest of cell proliferation. The EFSA Panel noted that the authors' conclusion that genistein is not a topo II poison which stabilises the "cleavable complex" is rather speculative. Indeed, this opinion was mostly based on thermal profiles of the transition of double-stranded DNA to single-stranded DNA with or without genistein. However, the evidence that genistein suppresses growth of HCT116 human colon carcinoma cells in a dose-related way indicated a true cell cycle-inhibitory effect.

The study by Masuda et al. (2012); (Zou et al., 2012) investigated the genotoxic properties of genistein after a nitrite treatment under acidic conditions in the Ames test (*Salmonella typhimurium* strains TA98 and TA100). When genistein was applied alone (without nitrite), no mutagenicity was observed in the Ames test at 10 µM per plate either in the absence or in the presence of S9 metabolic activation. However, the EFSA Panel noted the limited value of the results obtained for genistein alone, which was attributable to a number of shortcomings, including the use of a single dose level, and one that was not sufficiently high (10 µM/plate), and the use of only two *S. typhimurium* tester strains (TA98 and TA100).

The study by Zou et al. (2012) aimed to investigate mutagenic effects of genistein in the mouse lymphoma assay in L5178Y cells. Dose levels of 2.5–20 µg/ml genistein were used to treat cells for 3 or 24 hours in the absence of S9 metabolic activation. The results obtained showed statistically significant and dose-related increases in mutation frequencies from 5 µg/ml at both treatment times. The authors concluded that the induced mutations were mainly of hemizygous type caused by loss of heterozygosity at the thymidine kinase (*tk*)

locus. The EFSA Panel noted that the relative total growth in the solvent control was rather low, thus limiting the strength of the test.

In the study by Schwen et al. (2010), *S*-equol, a metabolite of daidzein, was assessed for its mutagenicity in the reverse mutation assay using *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2 uvrA, in accordance with the method of Ames, and for its clastogenicity in a chromosomal aberration assay in human lymphocytes both in the absence and in the presence of S9 metabolism. In the reverse mutation assay, the pre-incubation and plate incorporation methods both in the absence and in the presence of rat liver S9 metabolism were used. A range of half-log<sub>10</sub> concentrations of *S*-equol up to the standard limit dose of 5000 µg/plate was assayed. In the *in vitro* chromosome aberration assay, cultures were treated with appropriate 2-fold dose intervals up to the dose limit of 10 mM (2422.7 µg/ml) for 4 hours in the absence or presence of S9 mix. In addition, one set of cultures was treated with *S*-equol in the absence of S9 mix for 21 hours without medium change. The results obtained indicated that *S*-equol did not show genotoxic activity in any of the tests employed. The EFSA Panel noted that the studies were adequately performed and met the requirements of the relevant OECD guidelines (TG 471 and TG 473, respectively).

In the study by Ullah et al. (2009), the genotoxicity of genistein and biochanin A was assessed using the alkaline Comet assay in purified human lymphocytes at dose levels ranging from 10 to 50 µM. The test compounds were applied directly onto gelatinised slides. The results obtained indicated that both compounds induced dose-related and statistically significant increases in DNA breakage, as evinced by increased tail length in the comets. The authors concluded that the clastogenic activity of both compounds was caused by their pro-oxidant activity, mediated by copper and not by iron and zinc, as supported by the action of copper chelators. In addition, the authors asserted that these compounds have antioxidant activity. However, the EFSA Panel noted that the pro- and antioxidant activities of genistein and biochanin A claimed by the authors did not appear to be fully supported by the data.

In the study by Lopez-Lazaro et al. (2007), the dietary flavonoids genistein and luteolin were evaluated as topo I and topo II poisons and catalytic inhibitors in human K562 leukaemia cells using the cell-based assay TARDIS ("trapped in agarose DNA immunostaining") at dose levels of 100–300 µM. Both flavonoids induced topo II–DNA complexes with both topo II $\alpha$  and topo II $\beta$ . Genistein, in the same range of concentrations, also decreased the topo II–DNA complexes induced by the topo II poison etoposide, suggestive of a catalytic inhibition of topo II, and luteolin decreased the topo I–DNA complexes induced by the topo I poison camptothecin, indicative of a catalytic inhibition of topo I. However, when using murine transgenic cells lacking topo II $\beta$ , the authors found resistance to genistein-induced cell growth inhibition and cytotoxicity, indicating a key role for genistein in catalytic inhibition of topo II $\beta$ . Since slow-growing tumours contain significant levels of this isoenzyme, genistein might display anticancer effects in these types of tumours. The authors also reported that catalytic inhibition of topo II and topo I occurred at relatively high concentrations *in vitro* (175 and 146 µM genistein and luteolin, respectively). They suggested that it was unlikely that genistein or luteolin inhibited the catalytic activity of topo II and topo I when ingested

via the diet or supplements, considering the pharmacokinetics of dietary flavonoids in humans. The authors further pointed out that the *in vivo* plasma concentrations of genistein after supplementation had been reported to be 0.1–8  $\mu\text{M}$ , a concentration range that encompassed the threshold for genistein induction of topo II–DNA-mediated clastogenicity. This indicated that some flavonoids may exert topo II-mediated toxic and carcinogenic effects when ingested at relatively high concentrations, such as those present in some dietary supplements. On the other hand, accumulating evidence suggested that consumption of flavonoid-containing foods is associated with a reduced cancer risk, indicating that low concentrations of some flavonoids through a diet rich in plant-derived foods may exert anticancer effects via topoisomerase-independent mechanisms, e.g. estrogen receptor  $\beta$ -mediated effects or other effects. To sum up, the authors concluded that some flavonoids such as genistein or luteolin may produce anti-cancer effects at concentrations achievable through a diet rich in plant-derived foods (submicromolar) through topoisomerase-independent mechanisms (e.g. antioxidant, anti-estrogenic). However, at higher concentrations (micromolar, non-cytotoxic), these agents induce topo II-mediated DNA damage that may produce carcinogenic effects. At high concentrations (micromolar, cytotoxic), these dietary agents may produce cancer chemotherapeutic effects through a catalytic inhibition of topo II in the cells, mainly involving topo II $\beta$ .

In the study by McClain et al. (2006), the mutagenicity of genistein was evaluated in the reverse mutation assay using *S. typhimurium* strains TA1535, TA97, TA98, TA100 and TA102, in accordance with the method of Ames, and in mouse lymphoma L5178Y tk<sup>+/-</sup> cells *in vitro*. In the reverse mutation assay, triplicate plates were prepared for each experimental point and treated with a range of concentrations up to 3333  $\mu\text{g}/\text{plate}$  in the first experiment using the plate test methods and with up to 1000  $\mu\text{g}/\text{plate}$  in the second experiment using the pre-incubation method owing to excessive precipitation of genistein at the higher dose levels. Treatments were performed both in the absence and in the presence of rat liver S9 metabolism. In the mouse lymphoma assay, four independent experiments were conducted: two in the absence of S9 (exposure times of 3 and 24 hours) and two in presence of S9 (exposure time 3 hours in both cases). In the absence of S9, cells were treated with up to 60 and 7.5  $\mu\text{g}/\text{ml}$  for 3 and 24 hours, respectively, based on the relative cell survival. In the presence of S9-mix, the experiments were carried out up to a maximum concentration of 6.5 and 7.5  $\mu\text{g}/\text{ml}$ . Genistein did not show any genotoxic activity in the reverse mutation assay (Ames test) using either the standard plate incorporation method or the pre-incubation method and either in the presence or in the absence of metabolic activation. In contrast, in the *in vitro* mouse lymphoma assay, genistein significantly increased resistant mutant colonies both in the absence and in the presence of metabolic activation (S9). These were predominantly small colonies, indicating that genistein acts as a clastogen. This observation was in agreement with published data on the inhibitory action of genistein on topoisomerase II, which is known to lead to chromosomal damage with a threshold dose–response. The EFSA Panel noted that the studies performed were conducted in accordance with the relevant OECD guidelines, TG 471 and TG 476, for the bacterial mutation assay and the *in vitro* mammalian cell gene mutation assay, respectively.



The study by Lehmann et al. (2005) aimed to investigate the genotoxic potential of equol, 3'-hydroxy-daidzein (3',4',7- trihydroxyisoflavone; 3-HO-DAI) and 6-hydroxy-daidzein (4',6,7- trihydroxyisoflavone; 6-HO-DAI), three human metabolites of daidzein, by assessing the induction of micronuclei in cultured human endometrial carcinoma cells (Ishikawa cells). Cells were exposed at concentrations of up to 50  $\mu\text{M}$  for 48 hours, after which time the number of cells with micronuclei was determined. Using fluorescence-labelled anti-kinetochore antibodies, micronuclei containing whole chromosomes (kinetochore-positive micronuclei) could be discriminated from micronuclei containing acentric chromosome fragments (kinetochore-negative micronuclei). The results obtained indicated that at 48 hours sampling time only equol, at concentrations between 5 and 20  $\mu\text{M}$ , induced statistically significant increases in micronuclei (mainly kinetochore negative, thus reflecting a clastogenic activity) compared with the untreated control. However, after an additional 24 hours of compound-free incubation, the number of micronuclei in cells exposed to 3-HO-DAI at concentrations between 5 and 50  $\mu\text{M}$  showed a two-fold increase relative to controls in a dose-dependent way (statistically significant for 10-50  $\mu\text{M}$ ). Again, the induced micronuclei were predominantly kinetochore negative, thus reflecting a clastogenic activity. For 6-HO-DAI, the induction of micronuclei was observed neither immediately after treatment for 48 hours nor after the subsequent compound-free incubation period of 24 hours. However, the EFSA Panel noted that the reliability of this study was limited since the frequency of micronucleated cells in the untreated control was unusually high, possibly reflecting an elevated genomic instability of the cell line employed.

In the study by Di Virgilio et al. (2004), an *in vitro* micronucleus test in Chinese hamster lung fibroblasts (V79 cells) was used to assess the genotoxicity of genistein, daidzein and equol. Cells were treated for 18 hours (1.5 cell cycles) with 5, 10, 18, 25, 50 or 75  $\mu\text{M}$  genistein, 25, 50, 75 or 100  $\mu\text{M}$  daidzein or 5, 10, 18, 25 or 50  $\mu\text{M}$  equol. One solvent (dimethylsulfoxide)-treated control and two positive controls, treated with methylmethanesulfonate at 50  $\mu\text{g}/\text{ml}$  or vincristine at 10 nM, were also employed. In addition, the alkaline version of the Comet assay was also used to detect DNA strand breaks for genistein only. The results showed that genistein at concentrations up to 25  $\mu\text{M}$  induced a statistically significant and dose-related increase in micronucleated cells, with values being more than triple the control values. At higher dose levels, the incidence of micronucleated cells decreased, probably as a result of concomitant cytotoxic effects. In the Comet assay, positive effects in terms of DNA breakage were observed only at dose levels higher than 100  $\mu\text{M}$ , which were confirmed to be cytotoxic. This outcome can be explained by the fact that repair of DNA single- and double-strand breaks proceeds during processing of cells for Comet assay, unless blocked by ice-cold treatment. Consequently, repair of DNA strand breaks, though illegitimate, cannot be seen by the Comet assay. For daidzein, slight, dose-related increases in micronucleated cells were also observed and were statistically significant at concentrations of 50  $\mu\text{M}$  and higher. At 75  $\mu\text{M}$ , the frequency of micronucleated cells was double the control values. Similarly, for equol, slight increases were observed and were statistically significant at concentrations of 50  $\mu\text{M}$  and above. At 75  $\mu\text{M}$ , a maximum 2.5-fold increase compared with the control values was observed. Genistein induced mostly CREST ("stained with anti-kinetochore antibodies") (-) micronuclei, indicating a clastogenic

mechanism of action compatible with the poisoning of topo II, daidzein induced partly CREST(+) and CREST(-) micronuclei, whereas equol induced mostly CREST(+) micronuclei, indicative of an aneugenic action. Overall, genistein proved to be genotoxic in the *in vitro* micronucleus test while the outcome for daidzein and equol should be considered as equivocal.

Schmitt et al. (2003) tested daidzein and four metabolites of daidzein using 5 h exposure in an *in vitro* micronuclei assay in L5178Y mouse lymphoma cells. For each concentration of test compound, three slides with 1000 nuclei per slide were evaluated. No induction of micronuclei was observed with daidzein up to the limit of solubility (100 µM), whereas all four metabolites of daidzein induced micronuclei in a concentration-dependent manner; equol (2.3-fold induction at 100 µM), *O*-desmethylangolensin (6.2-fold induction at 10 µM), 4',6,7-trihydroxyisoflavone (6-HO-DAI) (6.7-fold induction at 100 µM) and 3',4',7-trihydroxyisoflavone (3'-HO-DAI) (8.2-fold at 100 µM). Thus, both reductive and oxidative metabolites of daidzein were genotoxic *in vitro*.

Snyder and Gillies (2003) showed that some flavonoids, such as galangin, fisetin, biochanin, hesperitin, naringenin, and, particularly, daidzein, which is a major constituent of marketed soy products, were catalytic topo II inhibitors (not poisons). In their study, they assessed the topo II catalytic inhibitory activity of these compounds by their ability to antagonise the induction of micronuclei by genistein in Chinese hamster V79 cells at 75 µM. The results obtained indicated that selected compounds were all able to reduce genistein-induced micronuclei by at least 80% when genistein was added to cultures first, thus confirming the topo II catalytic inhibition activity of these compounds.

In the study by Misra et al. (2002), the purified soy isoflavone product PTI G-2535, from Protein Technologies International (St. Louis, MO, USA) containing predominantly genistein (40 to 50%) but also daidzein (18 to 25%), glycitein (1 to 4%) and small quantities of residual lipids and carbohydrates but no proteins, was evaluated for its mutagenicity in the reverse mutation assay using *S. typhimurium* LT2 strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2uvrA in accordance with the method of Ames and in mouse lymphoma L5178Y tk<sup>+/-</sup> cells *in vitro*. In the reverse mutation assay, a range of concentrations up to 625 µg/plate were used in the first experiment based on the toxicity observed in the preliminary toxicity test and up to 1250 µg/plate was used in the second experiment in an attempt to achieve cytotoxicity with *S. typhimurium* TA98 and *E. coli* WP2uvrA, based on the results obtained in experiment 1. Treatments were performed both in the absence and in the presence of rat liver S9 metabolism. In the mouse lymphoma assay, four independent experiments were conducted: two in the absence and two in the presence of S9 metabolic activation with 4 hours' exposure in all cases. In the absence of S9, cells were treated with up to 300 µg/ml in the first experiment and up to 50 µg/ml in the second experiment because of the toxicity observed in the initial experiments. In the presence of S9-mix, the maximum concentration used was 5 µg/ml in the first experiment and 2.5 µg/ml in the second because of the toxicity observed in initial experiments. PTI G-2535 did not show genotoxic activity in the reverse mutation assay (Ames test) either in the

absence or presence of metabolic activation although some increases in the presence of S9 were observed at doses of 156.2 and 312.5 µg/plate in the TA100 strain. However, such increases, which were dose-related and achieved statistical significance, were less than two-fold the control values. In contrast, in the mouse lymphoma assay, statistically significant and dose-related increases in mutation frequency were induced by PTI G-2535 both in the absence and presence of S9 metabolic activation in all the experiments performed and small and large colonies were observed. The EFSA Panel noted that the studies performed were conducted in accordance with the relevant OECD Guidelines, TG 471 and TG 476, for the bacterial mutation assay and the *in vitro* mammalian cell gene mutation assay, respectively.

In the study by Salti et al. (2000), genistein was assessed for its capability to induce DNA breakage by means of the alkaline Comet assay in HT-29 colon cancer cells. Cultures were treated with dose levels ranging from 2 to 200 µM for 1 or 48 hours. The results obtained indicated marked and dose-related increases in DNA breakage from 10 up to 100 µM following 1 hour's treatment. After 48 hours' treatment, marked DNA breakage was only observed at 100–200 µM. The authors concluded that DNA breakage induced by genistein in HT-29 colon cancer cells was the result of poisoning of topo II through the stabilisation of the cleavable complex since aclarubicin, a known catalytic inhibitor of topo II, markedly antagonised the DNA damage induced by both genistein and VP-16 (a representative topo II poison) in HT-29 cells in a similar manner, as determined by the Comet assay.

The clastogenic potential of genistein and daidzein was tested in human peripheral blood lymphocytes *in vitro* by (Kulling et al., 1999). After exposure of the lymphocytes to 25 µM genistein for 6 hours, a clear induction of structural chromosomal aberrations was observed by cytogenetic analysis. The major alterations were chromatid breaks, gaps and chromosome aberrations. In contrast, daidzein did not induce chromosomal aberrations even at 100 µM.

Morris et al. (1998) assessed the ability of genistein to induce micronuclei and gene mutations in the human lymphoblastoid cells AHH-1 *tk*<sup>+/-</sup> (*p53*<sup>+/-</sup>) and L3 (*p53*<sup>+/+</sup>), which differ in the functional status of the tumour suppressor gene, *p53*. The mutant fraction at the thymidine kinase (*tk*) locus was determined by resistance to trifluorothymidine and at the hypoxanthine phosphoribosyl transferase (*hprt*) locus by resistance to 6-thioguanine. The results obtained indicated that genistein induced micronuclei and chromosomal mutations in both cell lines.

### **Summary of *in vitro* data**

Bacterial gene mutation tests (Ames tests) yielded no evidence for the mutagenicity of isoflavones. In contrast, in mammalian cells *in vitro*, genistein proved to be markedly mutagenic (although with a clastogenic mechanism of action) and clastogenic through the induction of micronuclei or DNA breakage as measured by the Comet assay. These effects are attributable to the poisoning of the DNA topoisomerase II through the stabilisation of the "cleavable complex" and generation of DNA double-strand breaks (DSBs) at topoisomerase II–DNA binding sites. Such an indirect effect on DNA is the concept of a threshold for

clastogenicity (Muller and Kasper, 2000; Sofuni et al., 2000). Daidzein, *S*-equol and glycitein did not show significant genotoxic activity. On the other hand, the two oxidative daidzein (DAI) metabolites (3',4',7- trihydroxyisoflavone; 3'-HO-DAI and 4',6,7-trihydroxyisoflavone; 6-HO-DAI) proved to induce micronuclei (Lehmann et al., 2005; Schmitt et al., 2003). This clastogenic effect was attributed to their oxidation to *o*-quinones, which are known to be direct clastogens.

#### 2.4.1.2 *In vivo*

The study by Masuda et al. (2012) investigated the genotoxic properties of genistein after a nitrite treatment under acidic conditions in an *in vivo* micronucleus test in peripheral blood cells of ICR male mice. When genistein was applied alone (without nitrite) to male mice at a single dose of 2 mmol/kg bw by oral gavage, for 24 or 48 hours, no genotoxicity was observed. However, the EFSA Panel noted the use of a single dose level, not sufficiently high to cause any reduction in the ratio of mature to immature erythrocytes. Furthermore, the number of cells scored was low.

In the study by Schwen et al. (2010) *S*-equol, a metabolite of daidzein, was assessed for its genotoxicity in an *in vivo* micronucleus test. Groups of five male and five female adult SD (Hsd:SD) albino outbred rats of Sprague–Dawley origin were treated by oral gavage with a single dose of 500, 1000 or 2000 mg/kg bw *S*-equol, the last dose level being the limit dose for this test. Animals were sacrificed at 24 hours (all dose levels) and at 48 hours (2000 mg/kg only). Bone marrow smears were prepared and stained with acridine orange and 2000 immature erythrocytes per animal were analysed for the presence of micronuclei. *S*-equol did not show any genotoxic activity. The EFSA Panel noted that the study was adequately performed and met the requirements of the relevant OECD guideline, TG 474, with the exception that at the 48-hour sampling time only three females rather than five were analysed.

The study by Pop et al. (2008) was a phase I randomized double-blinded clinical trial that aimed to evaluate changes indicative of estrogenic stimulation and genotoxicity, following administration of a high oral dose of soy isoflavones (genistein, daidzein and glycitein) in 30 healthy post-menopausal women for 84 days. All women were followed until 112 days after study initiation, 28 days after treatment cessation. The genotoxicity was evaluated by means of the alkaline Comet assay and analysis of apurinic/aprimidinic (AP) sites in peripheral blood lymphocytes on day 1 and day 84 following administration of purified isoflavones. The active group (n = 18) received four capsules of the dietary supplement PTI G-2535 from Protein Technologies International, St. Louis, MO, USA, containing approximately 558 mg/day genistein, 296 mg/day daidzein and 44 mg/day glycitein, as unconjugated isoflavones (total isoflavones 898 mg/day), divided into two equal daily doses. The placebo group (n = 12) received the same number of placebo capsules identical in size and color. The mean values obtained in the Comet assay were similar for the isoflavone and placebo groups at baseline (day 1) and at day 84. The mean number of AP sites at day 84 was higher in the placebo group than in the isoflavone group, however, there were no significant

differences between the placebo and isoflavone groups in mean change from day 1 to day 84. The authors concluded that unconjugated soy isoflavones are safe and well tolerated in healthy post-menopausal women at total doses of up to 900 mg per day (see 2.4.2.1.2 for safety). The EFSA Panel noted that the analysis of AP sites was not a standard test and that, in the case of Comet assay, the elapsed time between the last administration and processing of cells was not reported, which partly reduced the strength of the study.

In the study by McClain et al. (2006), genistein was evaluated for its clastogenicity and aneugenicity in three independent micronucleus tests (one test in Moro Albino mice, one in RAIf rats and one in Wistar rats). In the mouse micronucleus test, five male and five female animals were treated by oral gavage with dose levels of 0, 0.2, 2 and 20 mg/kg bw genistein for 14 days and were sacrificed 30 hours after the last administration. Peripheral blood smear slides were prepared and stained with May–Grunwald–Giemsa. The slides were scored for the presence of micronuclei and 2000 polychromatic erythrocytes (PCEs) per animal were analysed. In the rat micronucleus assays, five male and five female rats of each strain were treated by oral gavage once at dose levels of 0, 500, 1000 and 2000 mg/kg bw (RAIf) or 0 and 2000 mg/kg bw (Wistar), the last dose being the limit dose level for this assay. Animals were sacrificed at 24 hours (all dose levels) and at 48 hours (2000 mg/kg only) and bone marrow was collected from femurs. Smears were prepared and stained with May–Grunwald–Giemsa and 2000 (RAIf) or 4000 (Wistar) PCEs per rat were scored for the presence of micronuclei. The results obtained indicated that genistein has no genotoxic activity. The EFSA Panel noted that the studies were conducted in accordance with the relevant OECD guideline, TG 474, with the exception of the micronucleus test in mice. This study showed major shortcomings, including the use of 20 mg/kg bw as the highest dose, selected based on the upper range of human exposure to genistein. Since no signs of toxicity were observed, this dosing regime was considered inadequate for an *in vivo* genotoxicity test. In addition, the sampling time of peripheral blood, 30 hours rather than the 36 hours recommended by the relevant OECD guideline, TG 474, was considered inappropriate. On this basis, the EFSA Panel considered the mouse peripheral blood micronucleus test unreliable.

The study by Manjanatha et al. (2006) aimed to assess ability of genistein and daidzein to potentially protect against 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced *lacI* mutations in the mammary glands of OVX Big Blue rats (n = 5/treatment). When genistein (1 g/kg diet) or daidzein (0.25 g/kg diet) were fed in the diet for 16 weeks to female Big Blue rats homozygous for the *lacI* transgene, no increases in the mutation frequencies were observed compared with the untreated control values. However, when feeding the rats the combination of both isoflavones (1 g/kg/diet of each), a significant reduction in the mutation frequencies was observed. The EFSA Panel acknowledged a limited value of this study for risk assessment since it was not specifically designed as a standard *in vivo* mutagenicity test.

The study by Miltyk et al. (2003) aimed to assess the genotoxicity of purified soy isoflavones (genistein, daidzein and glycitein) from Protein Technologies International, St. Louis, MO, USA, in 20 patients with prostate cancer and 6 healthy volunteers, by means of the alkaline

Comet assay and the cytokinesis-block micronucleus assay in peripheral blood lymphocytes. Chromosome translocation of the myeloid-lymphoid leukaemia (MLL) gene (11q23) was also assessed by using fluorescence *in situ* hybridisation. The ingredients were formulated in capsules which contained each 139.5 mg genistein, 74 mg daidzein and 11 mg glycitein, as unconjugated isoflavones. The patients with prostate cancer were initially administered 300 mg (approximately 4 mg/kg bw) for 28 days. The dose was then escalated to 600 mg (approximately 8 mg/kg bw) given as two divided doses in the morning and evening for an additional 56 days. At the end of the dosing period, fresh blood was collected by venipuncture from each patient, centrifuged to separate mononuclear cells and subsequently processed for Comet assay and cytokinesis-block micronucleus test in accordance with standard procedures. The plasma profiles of genistein, daidzein and glycitein in the patients were measured at 24 hours post dose on days 5, 9, 14, 21 and 28 and 12 hours post dose on days 31, 35, 42 and 56, and the highest concentration of plasma genistein ranged from 4.1 to 27.1  $\mu\text{mol/l}$  (ng/ml). No genotoxic activity was detected in any of the assays performed. The EFSA Panel noted that the studies were accurately and correctly performed. The only limitation observed in the Comet assay was that the elapsed time between last administration and processing of cells was not reported. Chromosome translocation of the MLL gene (11q23) is not a standard assay for chromosomal aberrations.

In the study by Misra et al. (2002), the purified soy isoflavone product PTI G-2535, containing predominantly genistein (40 to 50%) but also daidzein (18 to 25%), glycitein (1 to 4%) and small quantities of residual lipids and carbohydrates but no protein, was evaluated for potential clastogenicity/aneugenicity in the micronucleus test in mice. The dose levels used in the experiments performed were based on genistein concentration. In the mouse micronucleus test, ten 5-week-old male and ten 7-week-old female animals were treated once by oral gavage at dose levels of 0, 500, 1000 and 2000 mg/kg bw, the last dose being the limit dose level for this assay. Animals were sacrificed at 24 and 48 hours after test compound administration and bone marrow was collected from femurs. Bone marrow smear slides were prepared and stained with acridine orange and 2000 polychromatic erythrocytes (PCEs) per animal were scored for the presence of micronuclei. The results obtained indicated that PTI G-2535 had no genotoxic activity, although statistically significant increases in the frequency of micronucleated PCEs were seen in only male mice treated with 500 and 1000 mg/kg bw at 24 hours after treatment. Such increases were small, were not dose-related, and fell within the historical range of micronucleus frequencies for control animals in the laboratory. The EFSA Panel noted that the studies performed were conducted in accordance with the relevant OECD guideline, TG 474.

### **Summary of *in vivo* genotoxicity**

No genotoxic effects were observed for genistein, daidzein, glycitein or *S*-equol when assayed in pivotal mouse and rat micronucleus tests, Comet assays or in humans in phase I randomized double-blinded clinical trials.

## 2.4.2 Human studies from the literature searches

It is well known that adverse effects can result from high levels of isoflavones fed to animals, such as infertility by red clover in sheep Setchell et al. (2001). Phytoestrogens, including isoflavones, can also affect reproductive and endocrine functions in species such as rat, cheetah, mink and fish (Ryokkynen et al., 2006). Thus, there is no reason to believe that adverse effects could not occur in humans as results of excessive intakes. It is quite unlikely that normal plant-based diets would contain isoflavones in amounts sufficient to induce any severe adverse effects, as they have been a part of human diets for hundreds of years, and there is no historical data on any obvious toxic effects. However, the amounts ingested daily from foods, estimated at 15-50 mg, or even much lower in Western populations, are below the dose promoted by supplements. Therefore, the situation may be different for individuals consuming supplements containing extracts or concentrates with very high isoflavone content.

The literature searches by VKM were performed to obtain studies on isoflavones in peri- and post-menopausal women that were published after and/or were on other end points than the studies included in EFSA (2015), as well as to obtain studies of isoflavones on pre-menopausal women, men, children and adolescents, not limited to certain organs or end points.

### 2.4.2.1 *Effects of isoflavones on peri- and post-menopausal women*

The menopausal status of women as peri- and post-menopausal vs. pre-menopausal has been reported in this risk assessment in accordance with the definitions used in the included publications. The included studies on peri- and post-menopausal women are summarized in Table 9.1 in Appendix 9.1.

#### 2.4.2.1.1 Meta-analyses

A meta-analysis examined the efficacy of phytoestrogens for the relief of menopausal symptoms in peri- and post-menopausal women (Chen et al., 2015). Medline, Cochrane, EMBASE and Google Scholar databases were searched until September 30, 2013, using the following key words: vasomotor symptoms, menopausal symptoms, phytoestrogens, isoflavones, coumestrol, soy, red clover. Inclusion criteria were (1) randomized controlled trial (RCT), (2) peri-menopausal or post-menopausal women experiencing menopausal symptoms, (3) intervention with an oral phytoestrogen. Outcome measures included Kupperman index (KI) changes (on 11 menopausal symptoms), daily hot flush frequency and the likelihood of side-effects. Of 543 potentially relevant studies identified, 15 RCTs meeting the inclusion criteria were included. The mean age of the subjects ranged from 49 to 58.3 and 48 to 60.1 years, respectively, in the placebo and phytoestrogen groups. The total number of participants ranged from 30 to 252, from 16 to 167 in the phytoestrogen groups and from 14 to 103 in the placebo groups. The intervention periods ranged from 3 to 12 months. The doses used were: isoflavones (dose not given, one study), isoflavones (25-100

mg/day, 12 studies), equol (40 mg/day, one study) and other phytoestrogens (trifolium, 40 mg/day, one study). Meta-analysis of the seven studies that reported KI data indicated no significant treatment effect of phytoestrogens as compared with placebo (pooled mean difference 6.44,  $P = 0.110$ ). Meta-analysis of the ten studies that reported hot flush data indicated that phytoestrogens resulted in a significantly greater reduction in hot flush frequency compared with placebo (pooled mean difference 0.89,  $P < 0.005$ ). Meta-analysis of the five studies that reported side-effect data showed no significant difference between the two groups ( $P = 0.175$ ). The authors concluded that phytoestrogens appeared to reduce the frequency of hot flushes in menopausal women, without serious side-effects.

A meta-analysis of available epidemiologic studies determined the relationship between soy consumption and colorectal cancer risk in humans (Yan et al., 2010). The women were mostly post-menopausal women judged by their age, but menopausal status was not stated. Publications obtained through a Medline literature search were systematically reviewed and four cohort and seven case-control studies on soy (various soy foods, isoflavones or genistein) and colorectal cancer risk that met the inclusion criteria were identified. The risk estimates (hazard ratio, relative risk or odds ratio) of the highest and the lowest reported categories of intake was extracted from each study and this analysis was conducted using a random-effects model. The analysis did not find that soy consumption was associated with colorectal cancer risk (combined risk estimate 0.90, 95% CI 0.79-1.03) nor did the separate analyses on colon cancer (combined risk estimate 0.88, 95% CI 0.74-1.06) and rectal cancer (combined risk estimate 0.88, 95% CI 0.67-1.14). However, when separately analysed on the basis of gender, it was found that soy was associated with an approximately 21% reduction in colorectal cancer risk in women (combined risk estimate 0.79, 95% CI 0.65-0.97;  $P = 0.026$ ), but not in men (combined risk estimate 1.10, 95% CI 0.90-1.33). The authors concluded that consumption of soy foods may be associated with a reduction in colorectal cancer risk in women, but not in men. However, the analysis also showed that the results may differ for Western vs. Asian populations. The analysis of six studies on isoflavones showed that isoflavone consumption was associated with an approximately 16% reduction in colorectal cancer risk. This significant risk reduction was largely attributable to three studies conducted in Western countries, which was also reflected in the results of the stratified analysis. Of the six studies analysed, two of them assessed isoflavone intakes at low  $\mu\text{g/day}$  to low  $\text{mg/day}$  levels and reported a significant reduction in colorectal cancer risk. In contrast, all three studies conducted in Asian countries reported intakes at levels in the range of several  $\text{mg/day}$  to  $>50 \text{ mg/day}$ , and none of those showed a significant risk reduction. Furthermore, all of the studies conducted in Western countries were case-control studies with relatively small study populations, and none of them were designed to study soy.

In a meta-analysis of randomized clinical trials on post-menopausal women using phytoestrogen supplements for treatment of climacteric syndrome, Tempfer et al. (2009) identified 174 trials of phytoestrogens (isoflavones, lignans and coumestans) of which 92 trials, including cross-over trials, involving 9629 participants (5502 on phytoestrogens and 4806 controls) reported on side-effects. The overall incidence of side-effects in



phytoestrogen and control groups was 36.7% and 38.0%, respectively ( $P = 0.2$ ), with incidence rate ratio (IRR) = 1.01 (95% CI 0.95-1.08). In only 2 of the 92 studies evaluated was there a statistically significant difference in side-effect incidence between treatment group and placebo group (see Unfer et al. (2004) and Albertazzi et al. (2005) in 2.4.2.1.2). Comparing various side-effect categories, significant higher rates of gastrointestinal side-effects among phytoestrogen users ( $P = 0.003$ , IRR = 1.28 (95% CI 1.08-1.50)) were found. Gynecological (IRR = 0.94 (95% CI 0.74-1.20)), musculoskeletal (IRR = 1.20 (95% CI 0.94-1.53)), neurological (IRR = 0.91 (95% CI 0.70-1.19)), and unspecific side-effects (IRR = 0.95 (95% CI 0.88-1.03)) were not significantly different between groups. Within side-effect categories, they found no significantly higher rates of side-effects in women using phytoestrogens. Specifically, the rates of hormone-related side-effects such as endometrial hyperplasia, endometrial cancer and breast cancer were not significantly different between groups. The authors concluded that phytoestrogens supplements had a safe side-effect profile with moderately elevated rates of gastrointestinal side-effects such as abdominal pain, as well as myalgia and sleepiness. There was a regional difference in side-effects with Asian studies showing higher side-effect rates than Western studies. Side-effects were also more common in women over age 55 compared with younger women. Median treatment duration in these trials was 6.2 months. Women using phytoestrogens for longer time periods (cut off points 6, 12 and 24 months) reported fewer side-effects than women enrolled in studies with shorter duration, suggesting no cumulative dose effects over time. However, they could not rule out that rare side-effects may occur in women on long-term treatment with phytoestrogens (>2 years).

#### 2.4.2.1.2 Randomized controlled trials

A double-blind, randomized, placebo-controlled trial compared the effects of a soy-based dietary supplement, low-dose hormone therapy (HT) and placebo on the urogenital system in 60 healthy Brazilian post-menopausal women aged 40 to 60 years (Carmignani et al., 2015). The women were randomized into three groups ( $n = 20$  in each group): a soy dietary supplement group (90 mg/day of isoflavones as soy protein powder (Previna; Sanavita Functional Foods, Piracicaba, Sao Paulo, Brazil, 53 mg as aglycones, with a ratio of genistein:daidzein:glycitein (Ge:D:Gl %) as 57:30:13) and one placebo tablet; mean  $\pm$  SD age,  $52.9 \pm 3.5$  years), a low-dose HT group (a tablet of 1 mg of estradiol plus 0.5 mg of norethisterone acetate, and placebo powder; age  $53.3 \pm 4.5$  years) and a placebo group (as identical appearing tablets and powder (maltodextrin); age  $50.9 \pm 3.4$  years). All supplements were taken twice daily for 16 weeks. Urinary, vaginal and sexual complaints were evaluated using the urogenital subscale of the Menopause Rating Scale. Vaginal maturation value was calculated. Transvaginal sonography was performed to evaluate endometrial thickness. Genital bleeding pattern was assessed. There were no drop-outs during the study. There were no statistically significant differences in the adverse effects evaluated (mastalgia, vaginal bleeding, allergy, headache, nausea, weight gain, water retention and intestinal complaints) between the three treatment groups. Despite the absence of statistical difference, 20% of women in the HT group had genital bleeding. In contrast, in the soy and placebo groups, bleeding occurred in 5% of cases. Vaginal dryness

improved significantly in the soy and HT groups ( $P = 0.04$ ). Urinary and sexual symptoms did not change with treatment in the three groups. After 16 weeks of treatment, there was a significant increase in maturation value only in the HT group ( $P < 0.01$ ). Vaginal pH decreased only in this group ( $P < 0.01$ ). There were no statistically significant differences in endometrial thickness between the three groups. The author concluded that the study showed that a soy-based dietary supplement used for 16 weeks failed to exert estrogenic action on the urogenital tract but improved vaginal dryness.

The safety of high-dose phytoestrogen on coagulation and hematological parameters in healthy post-menopausal women was examined in a 2-year prospective, double-blind, placebo-controlled study (Cheng et al., 2015). This study evaluated the effects of high-dose soy isoflavone (300 mg/day) on blood pressure, hematological parameters and coagulation functions, including circulating microparticles. In total, 431 post-menopausal women (from 3 medical centers in Taiwan) aged 45-65 years (mean  $\pm$  SD age  $57.6 \pm 3.0$  years) were randomly assigned to receive either high-dose oral isoflavone aglycone tablets (Taiwan Biotech Co. Ltd., Taoyuan, Taiwan) or placebo tablets (no further information given) for 2 years. At baseline, 6 months, 1 year and 2 years after treatment, blood pressure, body weight, liver function tests, hematological parameters and lipid profiles were measured. The first year blood specimens of 85 cases of 144 eligible participants (from one of the three centers) were analysed as D-dimer, von Willebrand factor antigen, factor VII, plasminogen activator inhibitor type 1 and circulating cellular microparticles (intact vesicles shed from cell membranes,  $0.2 - 2.0 \mu\text{m}$  in diameter, proposed as a predictive factor for premature coronary calcification), including the measurement of monocyte, platelet and endothelial microparticles. Eighty-two women completed the 1-year follow-up, one and two participants in the isoflavone and placebo groups, respectively, were lost to follow-up. In the isoflavone group, after 1 year, the changes in liver function tests, hematological parameters and coagulation tests were not different from those of the control. Triglyceride levels were significantly lower after 6 months of isoflavone treatment than the placebo group ( $P = 0.034$ ), but the difference did not persist after 1 year. Endothelial microparticles increased steadily in both groups during the 1-year period but the trend was not affected by treatment. There were no significant differences between the groups in systolic or diastolic blood pressure during the overall study period. The authors concluded that the study indicated that high-dose isoflavone treatment (300 mg/day) did not cause hematological abnormalities or activate coagulation factors.

An American randomized controlled clinical trial aimed to examine the potential cognitive benefits of soy isoflavones in patients with Alzheimer's disease, but otherwise free of major medical, neurological and psychiatric illnesses (Gleason et al., 2015). Of the sixty-five participants enrolled over the age of 60, thirty-four (52.3%) were women, and 31 (47.7%) were apolipoprotein E4 (APOE4) positive. Average age was 76.3 (SD = 7.2) years. Fifty-nine (90.8%) subjects completed all study visits. Of the total sixty-five participants, there were 53.1% ( $n = 17$ ) women in the isoflavone group and 51.5% ( $n = 17$ ) in the placebo group and 46.9% ( $n = 15$ ) **men** in the isoflavone group and 46.9% ( $n = 15$ ) in the placebo group. The participants were randomized to receive treatment of 100 mg/day soy isoflavones in

capsules, mainly as glycosides (43.3% genistin, 37.2% daidzin and 8.8% glycitin), less as aglycones (0.4% genistein, 0.8% daidzein and 0.6% glycitein), in addition to malonyl and acetyl glycosides (Novasoy®, Archer Daniels Midland Co., Decatur, IL, USA)) or matching placebo (containing maltodextrin and caramel food colour) capsules for six months.

APOE genotype was determined for all participants. Cognitive outcomes and plasma isoflavone levels were measured at baseline and at two additional time points: three and six months after baseline. Plasma isoflavone levels increased in subjects treated with soy isoflavones compared with baseline and with placebo, although intersubject variability in plasma levels was large. No subjects were discontinued because of abnormal safety laboratory values, and there were no significant differences between isoflavone-treated and controls in average systolic or diastolic blood pressure or in average blood levels of amylase, lipase or phosphate. No significant differences in treatment effects for cognition emerged between treatment groups or genders. Exploratory analyses of associations between changes in cognition and plasma isoflavone levels revealed an association between equal levels and speeded dexterity and verbal fluency. The authors concluded that six months of 100 mg/day treatment with soy isoflavones did not benefit cognition in older women or men with Alzheimer's disease.

A double-blind, randomized, placebo-controlled, 12-week trial conducted at a tertiary care center in USA of 93 healthy, ambulatory, post-menopausal women (mean age 56 years, 80% Caucasian) evaluated the effect of high-dose isoflavones on self-reported quality of life (QOL), cognition, lipoproteins and androgen status (Basaria et al., 2009). Participants were randomly assigned to receive 20 g of soy protein containing 160 mg of total isoflavones (96 mg aglycones, Ge:D:GI %: 40:39:21, from Physicians Pharmaceuticals Inc., Kernersville, NC, USA) vs. taste and appearance-matched placebo (20 g whole milk protein). Concentrations of individual isoflavones were genistein 64 mg, daidzein 63 mg and glycitein 34 mg. Both soy and placebo were provided in the form of a powder to be mixed with beverages. QOL was judged by the Menopause-specific Quality of Life (MENQOL) questionnaire, while cognitive function was assessed with standard instruments. Total, free and bioavailable testosterone, gonadotropins, sex hormone-binding globulin (SHBG) and fasting lipids were measured. Eighty-four women (90%) completed the study (isoflavones; n = 38, placebo; n = 46). Two women were excluded because of non-compliance (both on isoflavones) and 7 women withdrew because of adverse effects (5 because of gastrointestinal upset and 2 because of bad taste). Among these 7 women, 4 were given isoflavones and 3 were given placebo. The majority of the women tolerated the supplement well without any problems. There was a significant improvement in all 4 QOL subscales (vasomotor, psychosexual, physical and sexual) among the women taking isoflavones, while no changes were seen in the placebo group. No significant changes in cognition, serum androgens or plasma lipids were seen within any of the groups over time. However, at the end of the study, a group-by-time interaction was observed such that total testosterone (P = 0.0115) and high density lipoprotein (HDL) (P = 0.0370) levels were significantly lower in the isoflavones compared with placebo groups. The authors concluded that high-dose isoflavones (in slightly higher concentration than consumed by Asian populations, i.e. 50-100 mg/day) was associated with

improved QOL among women who had become menopausal recently. Hence, the timing of isoflavone supplementation with regards to the onset of menopause appeared to be important. The use of isoflavones, as an alternative to estrogen therapy, may be potentially useful and seemingly safe in this group of women who are looking for relief from menopausal symptoms.

A randomized, double-blind, placebo-controlled, parallel, multicenter trial in the Netherlands, Italy and France investigated whether the consumption of isoflavone-enriched foods affected bone mineral density, bone metabolism and hormonal status (Brink et al., 2008). Two hundred thirty-seven healthy early post-menopausal white women with mean  $\pm$  SD age of  $53 \pm 3$  years consumed isoflavone-enriched foods (biscuits and cereal bars) providing a mean daily intake of 110 mg isoflavone aglycones (n = 118) or control products (n = 119) for 1 year while continuing their habitual diet and lifestyle. A soy isoflavone concentrate (Cerestar, Vilvoorde, Belgium) containing 40-50% of isoflavones by weight (60-75% genistein, 25-35% daidzein and 1-5% glycitein) was incorporated into the biscuits and cereal bars. The control biscuits and cereal bars were identical in composition, taste and appearance. Consumption of isoflavone-enriched products did not alter bone mineral density of the lumbar spine and total body or markers of bone formation and bone resorption. Hormone concentrations (estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and sex hormone-binding globulin (SHBG)) did not differ between the isoflavone and control groups. Consumption of isoflavone-enriched products resulted in increased isoflavone concentrations in plasma and urine, whereas control products did not. This finding indicated good compliance with treatment. Subgroup analysis did not support an effect of equol phenotype on bone density (64 equol producers and 54 non-producers). The intervention had no effect on a range of safety variables and reported adverse events (AE). In total, 361 AE were reported by 86 of 118 subjects given isoflavones, and a total of 338 AE were reported by 80 of 119 subjects on control foods. The categories, intensity and number of AE were similar between the two treatment groups. There were no concerns over safety in sustaining this relatively high dosage of 110 mg/day for 1 year, as determined by the measured variables, i.e. reported AE, blood chemistry, hematology, blood lipids and vaginal maturation.

A subcohort (138 patients, not rerandomised) continued therapy for an additional year after 2 years of treatment recruited from a parent randomized, double-blind, placebo-controlled trial to assess the long-term safety of genistein aglycone on breast and endometrium and its effects on bone after 3 years of therapy (Marini et al., 2008). Participants received 54 mg of genistein aglycone (>98% purity) daily (n = 71) or placebo (n = 67). Both treatment arms received calcium and vitamin D<sub>3</sub> in therapeutic doses. Mammographic density was assessed at baseline, 24 and 36 months by visual classification scale and digitized quantification. Breast cancer type 1 susceptibility (BRCA1) and breast cancer type 2 susceptibility (BRCA2), sister chromatid exchange and endometrial thickness were also evaluated. Lumbar spine and femoral neck bone mineral density (BMD) was also assessed. Secondary outcomes were biochemical levels of bone markers. After 36 months, genistein did not significantly change mammographic breast density or endometrial thickness, BRCA1 and BRCA2 expression was

preserved, whereas sister chromatid exchange was reduced compared with placebo. BMD increases were greater with genistein for both femoral neck and lumbar spine compared with placebo. Genistein also significantly reduced pyridinoline, as well as serum carboxy-terminal cross-linking telopeptide and soluble receptor activator of NF- $\kappa$ B ligand while increasing bone-specific alkaline phosphatase, IGF-I and osteoprotegerin levels. Several blood parameters of general safety, such as prothrombin time, partial thromboplastin time, hemoglobin, total serum protein, urinary creatinine, hepatic and pancreatic enzymes, were evaluated using routine methods. They observed a moderate number of gastrointestinal side-effects in the genistein-treated women. These side-effects were also observed in the placebo arm at a lower rate. They also stated that there were no differences in discomfort or adverse events between the groups. The authors stated further in the discussion that it was possible that calcium carbonate may elicit a similar response and that genistein exacerbates the effect. They also mentioned that the findings for safety in this study needed to be viewed in the context of a balanced diet low in saturated fats and high in fruits and vegetables that likely increased the safety and perhaps the beneficial effects of genistein aglycone. The authors concluded that after 3 years of treatment, genistein exhibited a promising safety profile with positive effects on bone formation in a cohort of osteopenic (with reduced bone mass), post-menopausal women.

The study by Pop et al. (2008) was a phase I randomized double-blinded clinical trial in USA that aimed to evaluate changes indicative of estrogenic stimulation and genotoxicity, following administration of a high oral dose of soy isoflavones (genistein, daidzein and glycitein) in 30 healthy post-menopausal women for 84 days. All women (mostly Caucasian, a few African-Americans and Asian-Americans, mostly non-equol producers) were followed until 112 days after study initiation, 28 days after treatment cessation. The genotoxicity was evaluated by means of the alkaline Comet assay and analysis of apurinic/apyrimidinic (AP) sites in peripheral blood lymphocytes on day 1 and day 84 following administration of purified isoflavones (see 2.4.1.2 for results on genotoxicity). The active group (n = 18, mean age  $56.78 \pm 1.25$  years) received four capsules of the dietary supplement PTI G-2535 from Protein Technologies International, St. Louis, MO, USA, containing approximately 558 mg/day genistein, 296 mg/day daidzein and 44 mg/day glycitein, as unconjugated isoflavones, divided into two equal daily doses. The placebo group (n = 12, mean age  $53.50 \pm 1.06$  years) received the same number of placebo capsules identical in size and color. Treatment toxicity was monitored with a panel of blood tests collected on days 1, 14, 28, 84 and 112, consisting of a complete blood count, blood urea nitrogen/creatinine ratio, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase (AST),  $\gamma$ -glutamyl transferase, amylase, lipase and triglycerides. An additional toxicity panel consisting of electrolytes, calcium, magnesium, phosphorus, lactate dehydrogenase, albumin, uric acid, total bilirubin, fibrinogen, prothrombin time, partial prothrombin time, thyroxine (T4), thyroid-stimulating hormone (TSH), triiodothyronine uptake and free thyroxine index was measured on days 1, 28, 84 and 112. Blood pressure measurements were taken on days 1, 2, 7, 14, 28, 56, 84 and 112. Estrogenic/antiestrogenic effects of isoflavones were measured using a self-report questionnaire covering menopausal side-effects (hot flashes, chest pain, shortness of breath, leg cramps or swelling, breast tenderness, breast enlargement, changes

in sex drive, nausea or vomiting) and by measuring serum concentrations of estrogen, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and sex hormone-binding globulin (SHBG) on days 1, 84 and 112. All adverse events were classified by the study physician as mild, moderate or serious, and were graded according to the National Cancer Institute's Common Toxicity Criteria. Moreover, the study physician deemed these events 1) not or unlikely related to the study, 2) unknown, or 3) probably or definitely related to the study using her best clinical judgment. Specifically, if the adverse event was anticipated based on their previous results, or was in the published literature, and was temporally related to isoflavone treatment, then it was deemed as probably study-related. If the association with the event to isoflavone treatment was not conclusively related, but could not be ruled out, then the attribution of the event to the isoflavone treatment was deemed as unknown. Events which were clearly unrelated to isoflavone treatment (for example events of known etiologies or events which temporally preceded the first isoflavone dose) were deemed as unlikely study-related. All subjects in whom adverse events occurred were followed according to Good Clinical Practice guidelines until resolution of the events. Analysis of the laboratory clinical measurements showed no significant change in the mean difference at day 84 from day 1, when comparing isoflavone and placebo groups. Of grade I adverse events possibly related to isoflavones, in the isoflavone group there were two events of increased blood pressure (but no significant changes in mean systolic or diastolic blood pressure) and one event each of increased AST or triglycerides and of decreased T4. In the placebo group, there were four events of decreased T4 and one event each of increased estrogen, increased TSH, decreased FSH/LH and flatulence. By the end of the study, the T4 values were normal in all women, and at no time did they have symptoms of hypothyroidism. Of grade I adverse events probably related to isoflavones, in the isoflavone group there was one event of nausea. In the placebo group, there was one event each of mild swellings in legs and breast tenderness. Of the self-reported estrogenic/antiestrogenic effects possibly related to isoflavones, in the isoflavone group there were six events of increased intensity or frequency of hot flashes, one event of decreased intensity of hot flashes and two events of increased breast size or tenderness. In the placebo group, the corresponding numbers of these events were three, one and zero, respectively. The authors concluded that unconjugated soy isoflavones were safe and well tolerated in healthy post-menopausal women at total doses of up to approximately 900 mg per day when administered for 84 days.

A randomized, double-blind, placebo-controlled trial at three Italian university medical centers assessed the effects of genistein administration (54 mg/day, Laboratori Plants, Messina, Italy) on some predictors of cardiovascular risk in osteopenic (with reduced bone mass), post-menopausal women aged 49-67 years (n = 389) (Atteritano et al., 2007). After a 4-week stabilization on a standard isocaloric, fat-reduced diet, participants were randomly assigned to receive genistein purified from soy (purity  $\geq 98\%$ ) in two tablets per day (mean  $\pm$  SEM age,  $54.7 \pm 0.25$  years, n = 198) or placebo with similar appearance ( $54.2 \pm 0.19$  years, n = 191) daily for 24 months. Both types of tablets also contained 500 mg calcium carbonate and 400 IU vitamin D per tablet. Blood lipid profiles, fasting glucose and insulin, homeostasis model assessment for insulin resistance (HOMA-IR), fibrinogen, soluble

intercellular adhesion molecule-1 (sICAM-1), soluble vascular cellular adhesion molecule-1 (sVCAM-1), F2-isoprostanes and osteoprotegerin (OPG) at baseline and after 12 and 24 months of treatment were measured. Compared with placebo, genistein significantly reduced fasting glucose and insulin as well as HOMA-IR after both 12 and 24 months of treatment. By contrast, genistein administration did not affect blood lipid levels, although fibrinogen, F2-isoprostanes, sICAM-1 and sVCAM-1 decreased significantly compared with placebo after 24 months. Serum OPG was higher in the genistein group compared with placebo. At 24 months, the genistein group showed no change in endometrial thickness compared with placebo. Both genistein and placebo were generally well tolerated and ingested with a high degree of compliance. There were no significant changes in routine biochemistry, liver function or hematology results. Eight placebo recipients and 16 genistein recipients withdrew because of adverse effects in the first 12 months. During year 2, 7 placebo and 21 genistein recipients had adverse effects. In total, 37 (19%) genistein recipients and 15 (8%) placebo recipients discontinued therapy because of adverse events ( $P = 0.002$ ), all of which were moderate gastrointestinal side-effects (abdominal or epigastric pain, dyspepsia, vomiting, constipation). No patient who remained in the study experienced adverse events. The authors concluded that the results suggested that 54 mg genistein plus calcium, vitamin D and a healthy diet was associated with favorable effects on both glycemic control and some cardiovascular risk markers in a cohort of osteopenic post-menopausal women.

Two prospective, randomized, double-blind, placebo-controlled studies evaluated the same patients after 12 months (D'Anna et al., 2007) and 24 months (D'Anna et al., 2009) regarding whether pure administration of the phytoestrogen genistein (54 mg/day) reduced the number and severity of self-reported hot flashes in post-menopausal women with no adverse effect on the endometrium. A total of 389 participants met the main study criteria and were randomly assigned to receive genistein (mean  $\pm$  SD age,  $54.7 \pm 3.5$  years,  $n = 198$ ) or placebo ( $54.2 \pm 2.7$  years,  $n = 191$ ). About 40% of participants in both groups did not suffer from hot flashes, and the evaluation was performed in a subgroup of 247 participants (genistein,  $53.1 \pm 2.3$  years,  $n = 125$ ; placebo,  $53.0 \pm 1.8$  years,  $n = 122$ ) after 12 months and in 236 participants (genistein,  $53.1 \pm 2.3$  years,  $n = 119$ ; placebo,  $53.0 \pm 1.8$  years,  $n = 117$ ) after 24 months. Reductions from baseline in the frequency and severity of hot flashes were the principal criteria of efficacy. Endometrial thickness was evaluated by ultrasonography. The maturation value was also used to determine hormonal action on the vaginal cells. There were no significant differences in age, time since menopause, body mass index and vasomotor symptoms between groups at baseline ( $4.4 \pm 0.33$  hot flashes per day in the genistein group and  $4.2 \pm 0.35$  hot flashes per day in the control group). Genistein was well tolerated and ingested with a high degree of compliance. There were no significant changes in routine biochemistry, liver function or hematology results. Eighty-five participants dropped out in the parent study, 48 in the genistein group and 37 in the placebo group. In the sub-study, 29 participants dropped out, 16 in the genistein group (10 within the first 12 months) and 13 in the placebo group (8 within the first 12 months). Gastrointestinal side-effects were the most common reasons for drop-out; other reasons were the presence of other diseases, loss of some participants to follow-up, and possibly inadequate interaction with the woman's doctor. The effect of genistein was already evident in the first month and

reached its peak after 12 months of genistein therapy (-56.4% reduction in the mean number of hot flushes). Furthermore, there was a significant difference between the two groups at each evaluation time (1, 3, 6 and 12 months). After 24 months, there was no further decrease in the number of hot flushes in both groups. No significant difference was found in mean endometrial thickness and maturation value score between the two groups, either at baseline or after 12 and 24 months. The authors concluded that the phytoestrogen genistein was effective on vasomotor symptoms without adverse effects on the endometrium and vagina, but after the first year, there was no further improvement in the decrease in hot flushes.

The American double-blind, placebo-controlled trial by Fournier et al. (2007) investigated whether soy isoflavones (soy milk and supplement) could improve cognitive functioning in healthy post-menopausal women (no menstruation for the last year) not being on HRT the last 6 months. A total of 79 post-menopausal women, 48-65 years of age, were randomly assigned to one of three experimental groups for 16 weeks after 4 weeks on a diet minimizing isoflavone intake: cow's milk and a placebo supplement (control, n = 27); soy milk and placebo supplement (soy milk, 72 mg isoflavones/day, n = 25; 37 mg genistein, 31 mg daidzein, 4 mg glycitein, Vanilla Silk Soy Milk, White Wave™ Inc.); or cow's milk and isoflavone supplement (isoflavone supplement, 70 mg isoflavones/day, n = 27; 33 mg genistein, 30 mg daidzein, 7 mg glycitein, Novasoy® from Archer Daniels Midland Co., Decatur, IL, USA). Placebo was composed of maltodextrin. Cognitive functioning was assessed using various cognitive tasks before the intervention (baseline) and after the intervention (test). In contrast to predictions, soy isoflavones did not improve selective attention (Stroop task), visual long-term memory (pattern recognition), short-term visuospatial memory (Benton Visual Retention Test) or visuo-spatial working memory (colour match task). Also, the soy milk group showed a decline in verbal working memory (Digit Ordering Task) compared with the soy supplement and control groups. The authors stated that since this was the only significant group difference found among all the cognitive tests, the results may be caused by chance. There was no other information in the paper regarding negative effects of isoflavones. The authors concluded that soy isoflavones consumed as a food (milk) or supplement over a 16-week period did not improve or appreciably affect cognitive functioning in healthy, post-menopausal women.

A 6-month double-blind, randomized, placebo-controlled, parallel group trial by Ho et al. (2007) of healthy Chinese post-menopausal women (at least 12 months since last menstrual cycle) aged 55-76 years living in Hong Kong investigated whether soy-derived isoflavone extract improved performance in cognitive functions and quality of life (QOL). One hundred and ninety one eligible women were randomly assigned to receive a daily oral intake of 80 mg total soy-derived isoflavone powder in capsules (no further information of composition was given) supplied by Acatris Holding B.V., Giessen, The Netherlands, or an identical-appearing placebo containing starch for 6 months. Standardized neuropsychological tests of memory, executive function, attention, motor control, language and visual perception and a global cognitive function assessment were administered face-to-face individually at baseline and at 6-months posttreatment. The validated Chinese version of the Short Form-36 was



used for QOL measurements. Of the participants, 88% (168 women: 80 among the supplementation group and 88 among the placebo group) completed the trial. Intention-to-treat analysis, conducted for 176 participants with 6-month assessment results, revealed no significant differences in outcome measures between treatment groups. Subgroup analysis among the good compliers only (consumed at least 80% of the supplements or placebo; n = 168) and among the age groups younger or older than 65 years also indicated no significant differences for any outcome measures. Types of complaints of adverse events were similar in both treatment groups, i.e. not significantly different in any type, and included mainly gastrointestinal and musculoskeletal problems, and fewer cases of neurological/sensory complaints, gynecological/urinary complaints and non-specific complaints such as headache and hair loss. Among those who completed the study, 103 complaints from 58 participants and 68 complaints from 43 participants were recorded from the isoflavone and placebo group, respectively, which was not significantly different. The authors concluded that this 6-month trial indicated that 80 mg soy-derived isoflavone supplementation did not improve performance on standard neuropsychological tests and overall QOL in generally healthy Chinese post-menopausal women.

In a randomized, double-blind, cross-over, placebo-controlled Italian trial, Casini et al. (2006) investigated the effects of soy isoflavones on mood and cognitive function in 78 healthy post-menopausal women administered 60 mg/day of isoflavones as aglycones (Ge:D:Gl %: 40-45:40-45:10-20) in tablets or placebo (identical appearing tablets) for 6 months. After a washout period of 1 month, the patients who had been treated with phytoestrogens received placebo, and those who previously received placebo were administered phytoestrogens (for 6 months). Total number of subjects; on isoflavones (n = 77) and on placebo (n = 77). Mean age  $\pm$  SD at baseline: group A; 49  $\pm$  4.3 years, group B; 50  $\pm$  3.9 years. One woman in the placebo group dropped out after 2 weeks for personal reasons. One woman dropped out after 2 weeks on isoflavones for the occurrence of a moderate gastralgia. Cognitive performance and mood were assessed by a battery of tests at the end of each treatment period. At the end of the study, the patients were also asked whether they preferred the first or second treatment. The 17 scores on cognitive performance test and the 6 for mood assessments showed an advantage for the treatment with phytoestrogens. Similarly, of the 8 visual analogue scales used to indicate mood, 7 improved significantly after the treatment with phytoestrogens. Moreover, 49 patients preferred phytoestrogens, 9 placebo, and 18 had no preference. The preference was not related to the order of treatment. The authors concluded that the results suggested that isoflavones may have positive effects on post-menopausal women improving cognitive performance and mood. There was no information in the paper regarding negative effects of isoflavones in the women who finished the treatment.

A 12-week cross-over, randomized, placebo-controlled study in UK by Albertazzi et al. (2005) studied effects of genistein on menopausal symptoms and markers of bone turnover in 100 healthy post-menopausal women (aged 44-65 years). The participants were given 90 mg of encapsulated genistein (98.9% purity) per day (n = 50) or placebo (inactive ingredients, n = 50) for 6 weeks, then crossed-over to the other treatment for another 6 weeks. Genistein

reduced menopausal symptoms (severe hot flashes) by 30% ( $P = 0.02$ ). No significant effects were observed on biochemical markers of bone turnover. Significantly higher rates of bloatedness (7 vs. 0 cases,  $P = 0.018$ ) and back pain (10 vs. 2 cases,  $P = 0.03$ ) were reported after genistein treatment. In total, adverse events occurred in 3% of the participants. The other adverse events reported (headache, cold or upper respiratory tract infection, gastroenteritis, joint pain, dizziness and vaginal bleeding) were not significantly different between the genistein and placebo group.

A randomized, controlled, double-blind parallel study examined whether one year daily consumption of soy-containing foods (snack bar, drink mix or cereal, donated by DrSoy Nutrition Irvine, CA, USA), providing daily 25 g soy protein containing 60 mg isoflavones (no further information was given on composition), exerted beneficial effects on bone in post-menopausal American women (Arjmandi et al., 2005). The comparative control regimen was devoid of soy protein and isoflavones. Eighty-seven eligible post-menopausal women <65 years were randomly assigned to consume soy (mean age  $\pm$  SE was  $53 \pm 6$  years) or control foods ( $56 \pm 5$  years) daily for one year. Sixty-two subjects completed the one-year long study; on soy ( $n = 35$ ) and controls ( $n = 27$ ). Twenty-five women dropped out of the study for the following reasons; medical conditions (2 on soy and 1 control), started hormone replacement therapy (1 on soy and 3 controls), non-compliance (2 on soy), dislike of the volume or flavor of the food (3 controls), gastrointestinal side-effects (2 controls), food was causing headaches (1 control), personal reasons (3 on soy and 2 controls), gave no particular reason (5 on soy). Bone mineral density (BMD) and bone mineral content (BMC) of the whole body, lumbar (L1-L4), and total hip were measured using dual energy x-ray absorptiometry at baseline and after one year. Blood and urine markers of bone metabolism were also assessed. The sex hormones follicle-stimulating hormone (FSH),  $17\beta$ -estradiol, estrone and sex hormone-binding globulin (SHBG) were measured. Whole body and lumbar BMD and BMC were significantly decreased in both the soy and control groups. However, there were no significant changes in total hip BMD and BMC irrespective of treatment. Both treatments, control and soy, positively affected markers of bone formation as indicated by increased serum bone-specific alkaline phosphatase (BSAP) activity, insulin-like growth factor-I (IGF-I), and osteocalcin (BSAP 27.8% and 25.8%, IGF-I 12.8% and 26.3%, osteocalcin 95.2% and 103.4%, for control and soy groups, respectively). Neither of the protein supplements had any effect on urinary deoxypyridinoline excretion, a marker of bone resorption, or influenced the sex hormones. The authors concluded that the findings suggested that although one year supplementation of 25 g protein per se positively modulated markers of bone formation, this amount of protein was unable to prevent lumbar and whole body bone loss in post-menopausal women.

In a randomized double-blind, placebo-matched parallel group study in UK on mood, menopausal symptoms and cognition by File et al. (2005), 50 healthy post-menopausal women (aged 51-66 years) not taking other forms of hormone therapy were randomly allocated to receive daily treatment with a soy supplement as capsules (Novasoy, supplied by Archer Daniels Midland Company, Decatur, IL, USA, 60 mg total isoflavone equivalents/day (no further information was given on composition),  $n = 25$ ) or identical-looking placebo

capsules (n = 25). They were tested at baseline before treatment began and after 6 weeks of treatment in tests of attention, memory and frontal lobe function, and completed questionnaires to assess sleepiness, mood and menopausal symptoms. After 6 weeks of treatment, there was a significant ( $P < 0.02$ ) reduction in somatic menopausal symptoms in the group taking soy supplements, but there were no other significant effects of soy on menopausal symptoms or mood. On the test of non-verbal short-term memory, the soy group showed greater improvement than the placebo group ( $P < 0.03$ ), but there were no effects of soy on long-term memory, category generation or sustained attention. However, the soy treatment produced significantly better performance on the two tests of frontal lobe function, those of mental flexibility (simple rule reversal,  $P < 0.05$ ; complex rule reversal,  $P < 0.03$ ) and of planning ability ( $P < 0.05$ ). According to the authors, the results suggested that the main improvement after 6 weeks of soy supplementation was in frontal lobe function. The effects of soy on memory seemed less robust. There was no information in the paper regarding negative effects of isoflavones.

A double-blind randomized cross-over clinical trial was designed to assess the independent effect of soy relative to animal protein and soy-derived isoflavones on circulating estrogen and androgen concentrations in 42 (>50 years) moderately hypercholesterolemic Americans (Goldin et al., 2005). Post-menopausal women (n = 24, mean age 64 years) with low-density lipoprotein cholesterol levels of  $\geq 3.36$  mmol/l were fed each of 4 diets in randomized order for 6 weeks/phase. Study subjects were given each of four diets; soy protein depleted of isoflavones (soy/-), soy protein enriched in isoflavones (soy/+), animal protein with no added isoflavones (animal/-) and animal protein with added isoflavones (animal/+). No information on isoflavone form or composition was given. All food and drink were provided. Diets contained 25 g soy or common sources of animal protein/4.2 MJ containing trace (1.25 mg or not detected) or 46.21 mg or 51.71 mg isoflavones/4.2 MJ, respectively. The mean soy protein intake of the female subjects was  $55 \pm 6$  g/day. The mean isoflavone intake of the female subjects was given as  $108 \pm 12$  mg/day, however, for which group(s) were not specified. For animal/+ and animal/- diets, the variable protein components was contributed by dairy and meat. Isoflavones, in the form of powdered concentrate (Archer Daniels Midland Company, Decatur, IL, USA) were mixed into different food items of the animal/+ diet. For the soy/- and soy/+ diets, the variable protein component was contributed by specially prepared batches of isolated soy protein, one depleted (0.12 mg aglycone/g protein) and one enriched (1.96 mg aglycone/g protein) in isoflavones (Protein Technologies, St. Louis, MO, USA). At the end of each diet phase, concentrations of estrone sulfate, estrone, estradiol, testosterone, androstendione, dihydrotestosterone, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) were measured. In post-menopausal women, concentrations of estrone were higher and its precursor DHEA lower, after consuming soy protein compared with animal protein diets ( $P = 0.0396$  and  $P = 0.0374$ , respectively), independent on isoflavones. There was no significant effect of isoflavones on any of the hormones measured. The authors concluded that the data suggested that relatively large amounts of soy protein or soy-derived isoflavones had modest

and limited sex-specific (see 2.4.2.3.2 for men) effects on circulating hormone levels. No adverse effects were reported in the paper.

A 15-month randomized, double-blind, control pilot trial investigated the effect of soy protein isolate (SPI) with varying concentrations of isoflavones on early post-menopausal bone loss and lipids (Gallagher et al., 2004). A total of 65 women with a mean age of ~55 years were randomized to one of three groups; SPI as powder taken as a single bolus dose (The Solae Company, St. Louis, MO, USA) with 96 mg isoflavones/day, SPI with 52 mg isoflavones/day or SPI without isoflavones (<4 mg isoflavones/day), given as aglycone units adjusted for molecular weight. The daily intakes for the three groups were 52, 28 and 4 mg genistein, and 28, 20 and 0 mg daidzein, respectively. Soy was given for 9 months and then discontinued; participants were followed for an additional 6 months. Fifty women completed the study, whereas 15 women discontinued the soy treatment. Bone mineral density (BMD) and blood lipids were measured during this time. Measurement of serum isoflavones at 3 months showed dose-related increases in the two highest isoflavone groups. There was no significant effect of the soy supplements on BMD of the spine or femoral neck in any of the three groups. BMD increased significantly in the trochanter at 9 months ( $P = 0.0219$ ) and at 15 months ( $P < 0.05$ ) in the group given isoflavone-free soy compared with the other two groups. There was no significant effect of soy on lipid metabolism (cholesterol, triglycerides, HDL and LDL cholesterol, apoprotein A1 and B) at the end of the intervention. There were no differences between equol producers (54%) or non-producers in BMD or blood lipids. Fifteen women discontinued the study for different health-related and other reasons (4 for starting hormone therapy for atrophic vaginitis or hot flashes, 4 for severe constipation or stomach irritation, 1 for chest pain, 1 for hypertension, 1 for breast cancer and 4 for non-compliance). The discontinuation rate was similar across the three treatments ( $P = 0.42$ ). The authors concluded that the study did not find a significant positive effect of soy protein isolate supplemented with isoflavones on BMD and the serum lipid profile in early post-menopausal women.

In a randomized, placebo-controlled clinical trial, a cross-over design was used to test the effect of 6 months of soy isoflavone supplementation on bone formation, bone resorption, bone mineral content (BMC) and bone mineral density (BMD) in post-menopausal women ( $n = 19$ ), mean age  $70.6 \pm 6.3$  years (Harkness et al., 2004). One group was on isoflavones for 6 months, then 6 months on placebo, another group did the opposite. The dose of isoflavones was 110 mg/day, with 98% as conjugated glycosides and 2% as aglycones, with 1.3:1.0:0.22 ratio of genistein:daidzein:glycitein (Ge:D:Gl %: 52:40:9). Both isoflavones (NovaSoy, Archer Daniels Midland Co., Decatur, IL, USA) and placebo was given in capsules, divided in two intakes per day. One subject (of 20) dropped out at month 7 on advice from her physician after passing a kidney stone. There was a 37% decrease in urinary concentrations of type 1 collagen alpha1-chain helical peptide (HP), a marker of bone resorption, during the isoflavone supplementation compared with baseline ( $P < 0.05$ ) and a significant difference in mean ( $\pm$  SE) HP excretion levels when isoflavones were compared with placebo ( $43.4 \pm 5.2$  vs.  $56.3 \pm 7.2$   $\mu\text{g}/\text{mmol}$  creatinine,  $P < 0.05$ ). With isoflavone supplementation, mean spine BMD at L2 and L3 was significantly greater when treatment

was compared with control, with a difference between means of  $0.03 \pm 0.04$  g and  $0.03 \pm 0.04$  g ( $P < 0.05$ ), respectively. There were non-significant increases from baseline for total spine BMC (3.5%), total spine BMD (1%), total hip BMC (3.6%) and total hip BMD (1.3%) with the isoflavone treatment. The authors concluded that soy isoflavone, in isolated form, was effective to significantly decrease bone resorption in post-menopausal women. No information about negative effects was given in the paper.

In a double-blind, randomized, placebo-controlled trial of 202 healthy post-menopausal women aged 60-75 years recruited from a population-based sample in the Netherlands, Kreijkamp-Kaspers et al. (2004) investigated whether soy protein with isoflavones improved cognitive function, bone mineral density and plasma lipids in post-menopausal women. The participants were randomly assigned to receive 25.6 g of soy protein powder containing 99 mg of isoflavones (52 mg genistein, 41 mg daidzein and 6 mg glycitein as aglycones (Ge:D:G1 %: 53:41:6), Solae, Solae Co., St. Louis, MO, USA) or 25.6 g of total milk protein as a powder on a daily basis for 12 months. Cognitive function was assessed using the following instruments: dementia, Mini-Mental State Examination; memory, Rey Auditory Verbal Learning Test, immediate recall, delayed recall and recognition, the Digit Span forward and reversed, and the Doors test; complex attention tasks, Digit Symbol Substitution and Trailmaking, A1, A2, and B; and verbal skills, Verbal Fluency A and N, animals and occupations, and the Boston Naming Task. Bone mineral density of the hip and lumbar spine was assessed using dual-energy x-ray absorptiometry scanning. Lipid assessment included lipoprotein (a), total cholesterol, low-density lipoprotein, high-density lipoprotein and triglycerides. A total of 175 women completed the baseline and at least 1 postintervention analysis and were included in the modified intent-to-treat analysis ( $n = 88$  in the soy protein group and  $n = 87$  in the placebo group). Adherence was good (median plasma genistein levels, 17.2 and 615.1 nmol/L for placebo and soy group, respectively). Forty-nine participants (24%) did not complete the trial for various reasons, the most important being gastrointestinal tract complaints and aversion to the taste of the supplement. There was no difference in drop-out rate between the soy ( $n = 25$ ) or placebo ( $n = 24$ ) groups. The mean number of adverse events per participant was 2.54 in the soy group and 2.56 events in the placebo group. There were also no differences in the types of adverse events between the groups (gastrointestinal complaints, e.g. obstipation and gastric complaints, musculoskeletal complaints, lower and upper airway complaints, including ear, nose and throat, urogenital complaints, e.g. urinary tract infections or vaginal infections, dermatological complaints, e.g. dermatitis or eczema, or miscellaneous). Cognitive function, bone mineral density or plasma lipids did not differ significantly between the groups after one year. The authors concluded that this double-blind randomized trial did not support the hypothesis that the use of soy protein supplement containing isoflavones improves cognitive function, bone mineral density or plasma lipids in healthy post-menopausal women when started at the age of 60 years or later.

In an Italian randomized, double-blind, placebo-controlled study on histological characteristics of the endometrium, Unfer et al. (2004) examined 376 healthy post-menopausal women, of which 298 completed the 5-year treatment. Endometrial biopsies

were performed at baseline (n = 376), after 30 months (n = 369) and after 5 years (n = 319). They reported a significantly (P < 0.05) higher rate of endometrial hyperplasia without atypia in women taking 150 mg of isoflavones per day as soy tablets (n = 179, mean age  $\pm$  SD at start; 49  $\pm$  4.3 years) compared with identically-appearing placebo tablets (n = 197, 50  $\pm$  3.9 years); 6 vs. 0 cases (3.9% vs. 0%), respectively. The formulated percentages of isoflavones were 40-45% genistein, 40-45% daidzein and 10-20% glycitein. Specifically, all 5 cases of simple hyperplasia and 1 case of complex hyperplasia occurred after 5 years of treatment. No cases were observed after 30 months. No cases of endometrial hyperplasia with atypia or endometrial carcinoma were observed at either time point in this study. No information was given in the paper on reasons for drop-out or on other negative effects of isoflavones.

Thirty-three healthy post-menopausal women (50-65 years) not receiving conventional hormone replacement therapy (HRT) in the previous 12 months were randomly allocated in a double-blind parallel UK study to receive a soy supplement (60 mg total isoflavone equivalents/day (no further information was given on composition), Solgen 40 capsules, from Solbar Plant Extracts, Ashdod, Israel) or placebo (colour-matched lactose) for 12 weeks (Duffy et al., 2003). They received a battery of cognitive tests and completed analogue rating scales of mood and sleepiness and a menopausal symptoms questionnaire before the start of treatment and then after 12 weeks of treatment. Those receiving the isoflavone supplement showed significantly greater improvements in recall of pictures and in a sustained attention task. The groups did not differ in their ability to learn rules, but the isoflavone supplement group showed significantly greater improvements in learning rule reversals. They also showed significantly greater improvement in a planning task. There was no effect of treatment on menopausal symptoms, self-ratings of mood, bodily symptoms or sleepiness. The authors concluded that significant cognitive improvements in post-menopausal women can be gained from 12 weeks consumption of a supplement containing soy isoflavones that are independent of any changes in menopausal symptoms, mood or sleepiness. There was no information in the study regarding negative effects of isoflavones.

In a 6-month, double-blind, randomized, placebo-controlled clinical trial in USA by Kritz-Silverstein et al. (2003), 56 healthy women (aged 55-74 years) post-menopausal at least 2 years and not using estrogen replacement therapy, were randomized. Two in the placebo group and one in the active treatment group did not complete the 6-month evaluation, leaving 53 women for analysis, and none withdrew because of adverse effects. Women randomized to active treatment (n = 27) took two pills per day, each containing 55 mg of soy-extracted isoflavones (110 mg total isoflavones per day; Healthy Woman: Soy Menopause Supplement, Personal Products Company, McNeil-PPC Inc., Skillman, NJ, USA). Women assigned to placebo (n = 26) took two identical-appearing pills per day containing inert ingredients (<1 mg isoflavones per day, no further information was given on composition). Cognitive function tests administered at baseline and follow-up included the following: Trails A and B, category fluency, and logical memory and recall (a paragraph recall test assessing immediate and delayed verbal memory). At baseline, all women were cognitively intact; there were no significant differences by treatment assignment in age,

education, depressed mood or cognitive function (all P values >0.10). Compliance was 98% and 97%, respectively, in the placebo and treatment groups; all women took at least 85% of their pills. The women in the treatment group did consistently better, both as compared with their own baseline scores and as compared with the placebo group responses at 6 months. Comparisons of percentage change in cognitive function between baseline and follow-up showed greater improvement in category fluency for women on active treatment as compared with the case of those on placebo (P = 0.02) and showed (non-significantly) greater improvement on the two other tests of verbal memory and Trails B. The author concluded that the results suggested that isoflavone supplementation had a favourable effect on cognitive function, particularly verbal memory, in post-menopausal women. There was no information in the paper regarding negative effects of isoflavones.

An American prospective, double-blinded, randomized, placebo-controlled trial compared the effects of 6 months of dietary phytoestrogen supplementation versus placebo on treatment of menopause-associated symptoms and endometrium histology in healthy post-menopausal women (Balk et al., 2002). Twenty-seven women were randomized (n = 13 in the soy group and n = 14 in the placebo group) and 19 completed the study (n = 7 in the soy group and n = 12 in the placebo group). The isoflavones were given as approximately 100 mg/day of isoflavones (no further information was given on composition) via a soy flour and corn cereal (Nutlettes; Dixie, Houston, TX, USA). A 3/8 cup of Nutlettes contained 92 mg of isoflavones. The subjects were instructed to consume 1/2 cup per day, thus containing approximately 122.7 mg of isoflavones, and to avoid a list of soy and phytoestrogen-containing foods. The placebo was a wheat cereal (Grapenuts, Post, Battle Creek, MI, USA) that was low in isoflavones but similar in appearance and texture to the treatment cereal. The soy and placebo cereals were mixed (3 parts soy to 1 part placebo). A weekly log was used to note side-effects and symptoms. Menopause-associated symptoms (hot flushes, vaginal dryness, night sweats, palpitations, headaches, depression, urinary discomfort, insomnia and decreased libido) were assessed using a questionnaire and a four-point scale. Side-effects assessed included nausea and breast tenderness. Tolerability was also monitored by evaluating gastrointestinal disturbances such as diarrhea and flatus. Endometrium pipelle biopsy was obtained before randomization and after 6 months treatment. Of the 27 women who started the trial, 8 women withdrew from the study during the trial period; 2 from the placebo group (one because of lack of effectiveness and one because of the taste of the cereal) and 6 from the soy group (2 for family reasons, one because of exacerbated hemorrhoids, one for excess flatus, one due to increase in hot flush intensity – which improved after cessation of the treatment) and one was lost to follow-up). All endometrium biopsies after 6 months were consistent with atrophic inactive endometrium. No endometrium proliferation was seen from the soy consumption. The frequencies of menopause symptoms were not different between the groups at baseline. Total 6-month symptom severity scores between soy and placebo groups were not significantly different, except for insomnia which was more frequent in the soy group (P = 0.017). In the placebo group, but not in the soy group, hot flushes, night sweats and vaginal dryness were less severe during the final week than at baseline. All other menopause symptoms did not differ significantly in either group between baseline and the final week. Nausea, breast tenderness,

flatus and diarrhea were monitored in a weekly log. There were no statistical differences in side-effects at baseline between soy and placebo groups. Comparison of baseline and final week means for each group indicated no significant differences in side-effects, although diarrhea and flatus were more common in the soy group.

A randomized double-blind controlled trial determined the effects of 24 weeks of consumption of soy protein isolate with isoflavones given as one-half powder and one-half in a jumbo muffin in attenuating bone loss during the menopausal transition in perimenopausal American women (median age 50.6 years) (Alekel et al., 2000). Perimenopausal subjects (n = 69) were randomly assigned, double-blind, to treatment: isoflavone-rich soy protein (SPI+; 80.4 mg/day as aglycones, n = 24), isoflavone-poor soy protein (SPI-; 4.4 mg/day as aglycones, n = 24) (both from Protein Technologies International, St. Louis, MO, USA), or whey protein (control; n = 21). No further information was given on composition of individual isoflavones. Of 102 women initially recruited, 33 women were dropped because they failed to meet the criteria for follicle-stimulating hormone (FSH) concentration (22), inability to tolerate the treatment (no further information given) (6), death (1), death of family member (1), medical conditions preventing their continuation (2) and non-compliance (1). Sporadically, subjects neglected to consume their powder or muffins because of lack of convenience rather than because of any reported adverse effects. At baseline and post-treatment, lumbar spine bone mineral density (BMD) and bone mineral content (BMC) were measured by using dual-energy X-ray absorptiometry. At baseline, mid-treatment and post-treatment, urinary *N*-telopeptides and serum bone-specific alkaline phosphatase (BAP) were measured. The percentage change in lumbar spine BMD and BMC, respectively, did not differ from zero in the SPI+ or SPI- groups, but loss occurred in the control group (-1.28%, P = 0.0041; -1.73%, P = 0.0037). By regression analysis, SPI+ treatment had a positive effect on change in BMD (5.6%; P = 0.023) and BMC (10.1%; P = 0.0032). Baseline BMD and BMC (P ≤ 0.0001) negatively affected the percentage change in their respective models; baseline body weight (P = 0.0036) and bone-free lean weight (P = 0.016) contributed positively to percentage change in BMD and BMC, respectively. Serum BAP posttreatment was negatively related to percentage change in BMD (P = 0.0016) and BMC (P = 0.019). Contrast coding using analyses of covariance with BMD or BMC as the outcome showed that isoflavones, not soy protein, exerted the effect. The authors concluded that soy isoflavones attenuated bone loss from the lumbar spine in perimenopausal women.

Forty-nine Australian post-menopausal women (age 45-65 years) with elevated fasted total cholesterol (TC) >5.5 mmol/l were recruited for a randomised, double-blind controlled trial of isolated soy protein (ISP) supplement containing 65 mg isoflavones (ISP+) or a soy protein with a low isoflavone content (<4 mg) (ISP-), taken daily for 12 weeks, with 4 weeks on a low fat diet before and for 6 weeks after the soy protein (from Protein Technology International, St. Louis, MI, USA) treatment (Mackey et al., 2000). No further information was given on composition of individual isoflavones. Menopause symptoms in both groups were reduced by 30%. There was an overall reduction after 12 weeks in total cholesterol (TC) (P = 0.0003), low density lipoprotein (LDL)-cholesterol (LDL-C) (P = 0.04), sex



hormone-binding globulin (SHBG) ( $P = 0.002$ ) and luteinizing hormone (LH) ( $P = 0.005$ ). Soy protein had a cholesterol lowering effect in women (see 2.4.2.3.3 for data on men). Since there were no significant differences between the treatment groups in any parameter, the study suggested that this effect was independent of isoflavones. No negative effects were reported.

An Italian double-blind, parallel, multicenter, randomized placebo-controlled trial of 104 post-menopausal women requesting treatment for hot flushes, but otherwise healthy, examined the effects of soy for 12 weeks in addition to a typical Italian diet (Albertazzi et al., 1998). Fifty-one patients (age range 48-61 years) took 60 g of isolated soy protein (Supro Brand; Protein Technologies International Inc., St. Louis, MO, USA) daily and 53 patients (age range 45-62 years) took 60 g of casein (placebo) daily. The 60 g of isolated soy protein contained 40 g of proteins and 76 mg of isoflavones (aglycone units), the major substances being genistein (40 mg) and daidzein (28 mg) (maximum Ge:D %: 53:37), whereas the placebo contained no isoflavones. Both soy and casein were in powder form. The patients were seen at the screening visit and were assessed 4 weeks later, before randomization, and then again at treatment weeks 4, 8 and 12. Forty patients taking soy and 39 patients taking casein completed the 12-week study. Soy was significantly superior to placebo ( $P < 0.01$ ) in reducing the mean number of hot flushes per 24 hours after 4, 8 and 12 weeks of treatment. Data on side-effects were obtained monthly when patients returned to the clinic for their interviews. When a patient reported more than one side-effect, only the one that was considered the most bothersome was taken into account. Gastrointestinal side-effects were the most often reported. Constipation affected approximately half of the women in both groups and was the most common side-effect (48%) that led to premature withdrawal from the study. A total of 25 patients stopped the trial prematurely, 11 in the soy group and 14 in the casein group. Fifteen of the 25 patients who dropped out reached the first month of treatment and then stopped. The remaining patients withdrew within the first month. Gastrointestinal side-effects and food intolerance were the major causes of drop-out for 14 patients, 7 in each group. Lack of efficacy made 3 women in the casein group and one woman in the soy group to leave the study, lack of compliance (complete abandonment of treatment for more than one week) resulted in withdrawal of one patient in each group. Two patients in each group were lost to follow-up and one patient in the casein group stopped for other reasons. However, a large portion (24%) of the 25 patients who withdrew from the study had difficulty with the amount of powder that they were instructed to take and with food intolerance, namely constipation, bloating, nausea and vomiting. These two factors were the most common causes of both discontinuation and lack of compliance. There were no statistically significant differences between the soy and the casein groups when the side-effects were evaluated either as number or as % of complaints.

The effects of soy protein (40 g/day) containing moderate and higher concentrations of isoflavones on blood lipid profiles, mononuclear cell low density lipoprotein (LDL) receptor messenger RNA and bone mineral density and content were investigated in 66 free-living, hypercholesterolemic, post-menopausal American women during a 24-week, parallel-group, double-blind controlled trial with two soy interventions (Potter et al., 1998). After a control

period of two weeks, during which subjects followed a National Cholesterol Education Program Step I low-fat, low-cholesterol diet, all subjects were randomly assigned to 1 of 3 dietary groups for 24 weeks: Step I diet with 40 g protein/day from isolated soy protein containing 1.39 mg isoflavones/g protein (ISP56, 55.6 mg isoflavones/day) or Step I diet with 40 g protein/day from isolated soy protein containing 2.25 mg isoflavones/g protein (ISP90, 90 mg isoflavones/day), from Protein Technologies International, MO, USA, or Step I diet with 40 g protein/day obtained from casein and non-fat dry milk (CNFDM). No further information was given about the composition of the isoflavones. The test proteins were incorporated into food items such as bread, muffins, drinks, milk and soup. Total and regional bone mineral content and density were assessed. Non-HDL cholesterol for both ISP56 and ISP90 groups was reduced compared with the CNFDM group ( $P < 0.05$ ). HDL cholesterol increased in both ISP56 and ISP90 groups ( $P < 0.05$ ). Mononuclear cell LDL receptor mRNA was increased in subjects consuming ISP56 or ISP90 compared with those consuming CNFDM ( $P < 0.05$ ). Significant increases occurred in both bone mineral content and density in the lumbar spine but not elsewhere (proximal femur and total body), for the ISP90 group compared with the control group ( $P < 0.05$ ). The authors concluded that intake of soy protein at both isoflavone concentrations for approximately 6 months decreased the risk factors associated with cardiovascular disease in post-menopausal women, however, only the higher isoflavone-containing product protected against spinal bone loss. The subjects consumed the test proteins without difficulty. No information about drop-outs or adverse effects was given in the paper.

#### 2.4.2.1.3 Prospective (cohort) studies

In a population-based prospective cohort of Japanese women ( $n = 12783$ ) aged 40-69 years, the association between isoflavones (genistein and daidzein) and soy food (three different food types) calculated from a food frequency questionnaire (FFQ) and hepatocellular carcinoma (HCC) was studied (32 cases in women), during 235811 person-years, average 11.8 years of follow-up (both genders, see 2.4.2.3.3 for men) (Kurahashi et al., 2009). The low, middle and high intakes in women were  $<12.2$ ,  $12.2-19.5$ ,  $\geq 19.6$  mg/day for genistein,  $<8.1$ ,  $8.1-12.5$ ,  $\geq 12.6$  mg/day for daidzein and  $<38.2$ ,  $38.2-62.7$  and  $\geq 62.8$  g/day for soy food. In women, genistein and daidzein were dose-dependently associated with an increased risk of HCC, with multivariable hazard ratios (HR) for the highest vs. lowest tertile of 3.19 (95% CI 1.13-9.00,  $P_{trend} = 0.03$ ) and 3.90 (95% CI 1.30-11.69,  $P_{trend} = 0.01$ ), respectively. The HRs for the middle vs. lowest tertile for genistein and daidzein were 2.36 (95% CI 0.85-6.51 (n.s.)) and 3.10 (1.07-8.99). Soy food consumption tended to be associated with HCC in women, but did not reach statistical significance (highest vs. lowest tertile HR 1.74 (95% CI 0.67-4.25)). When analysis was restricted to persons who were either or both anti hepatitis C- or B virus antigen-positive, the HRs between lowest and middle or highest exposure to genistein and daidzein were not statistically significant, only the trends were significant ( $P_{trend} = 0.06$ , for both isoflavones). Only one case of HCC occurred in a pre-menopausal woman, the rest of the cases were in post-menopausal women (their results were similar as for total women).

An international multi-centre, prospective, open-label study (across Australia, Belgium, France and Spain) assessed the effects of an oral soy isoflavone extract (Phytosoya, a specific and standardized isoflavone extract containing 17.5 mg isoflavones per capsule with 20% genistin, 50% daidzin and 30% glycitin (i.a. as glycosides) (producer not given) on endometrium, evaluated by biopsy and ultrasonography, in post-menopausal women aged 45-65 years (n = 395) treated for 12 months (mean study duration was  $364.9 \pm 11.54$  days) (Palacios et al., 2007). The standardized soy isoflavone extract (total of 70 mg/day of isoflavones) was taken as two capsules twice a day. No control group was included in the study. Endometrial biopsy and transvaginal ultrasonography were performed before and after 12 months of treatment according to European guidelines. A total of 301 assessable biopsy specimens were obtained from women treated for 12 months; the results were 99.67% atrophic/inactive endometrium and 0.33% proliferative endometrium. No case of hyperplasia or carcinoma was diagnosed, demonstrating the endometrial safety of this extract (point estimate: 0.0; upper limit of 95% CI: 0.012). Endometrial thickness did not show any increase after 12 months of treatment (2.2 mm at inclusion and 2.12 mm at the end of the study). Of the 395 women included, 19 reported adverse effects (4.8%). The only recurrent adverse effects related to the study product was moderate gastrointestinal disorders, which were reported in 4.6% of the women in the safety set, whereas 68.9% found treatment to be excellent or 25.7% to be good in terms of tolerance. Eight women (all with atrophic endometrium) reported some kind of bleeding (2%) as an adverse event during the study. According to the authors, this is not uncommon from atrophic endometrium after menopause, but is thus far unexplained.

#### 2.4.2.1.4 Retrospective (case-control) studies

A case-control study in Korea aimed to assess the relationship between dietary soy food and isoflavone intake and colorectal cancer risk (Shin et al., 2015). A total of 901 colorectal cancer cases and 2669 controls (up to 3 controls per patient matched by gender and 5 years of age groups) were recruited at the National Cancer Center, Korea. A semi-quantitative food frequency questionnaire (FFQ) was used to assess the usual dietary habits, and the isoflavone intake level was estimated from five soy food items. A high intake of total soy food products was associated with a reduced risk for colorectal cancer in women. The association between high soy food product intake (Q4) and reduced colorectal cancer risk was significant among post-menopausal women (OR: 0.52, 95% CI 0.29-0.95, Ptrend = 0.009), but not in pre-menopausal women. The reduced risk for the highest intake of total soy products persisted for rectal cancer in women when analysed by subsite (Ptrend = 0.006), and was also seen for total isoflavones (Ptrend = 0.035). However, the middle (second and third) quartiles of intake of total soy products were associated with an elevated colon cancer risk in women (Q2: OR: 1.27, 95% CI 0.86-1.88 (n.s.), Q3: OR: 1.37, 95% CI 0.92-2.04 (n.s.)). The same non-significant tendencies of reduced risk associated with Q4 and increased risk associated with Q2 and Q3 vs. Q1 were seen with total isoflavones. The quartiles for total isoflavone intake were: Q1: <8.08, Q2: 8.08-<13.83 mg/day, Q3: 13.83-<22.35, Q4:  $\geq 22.35$  mg/day. The authors concluded that although precaution was required for the probably elevated risk among middle intake quartiles, the results suggested that a

high intake of total soy products or dietary isoflavones was associated with a reduced risk for overall colorectal cancer, and that the association may be more relevant to rectal cancer in women.

A Canadian population-based case-control study by (Anderson et al., 2013) included women aged 25-74 years with pathologically confirmed breast cancer as cases and age-matched women from the same area as controls. They found mostly beneficial effects on breast cancer estrogen (ER) and progesterone (PR) receptor subtypes of intake of total isoflavones (genistein, daidzein, glycitein (soy) and formononetin (non-soy); amounts of each not given), total lignins (secoisolariciresinol, matairesinol, pinoresinol and lariciresinol) and total phytoestrogens (isoflavones, lignans and coumestans) determined by a food frequency questionnaire. However, they found that in post-menopausal women (n = 314) there was a positive association between ER-PR- breast cancer and adult total isoflavone intake ( $\geq 497$   $\mu\text{g}/\text{day}$ ) (highest vs. lowest tertile: OR: 1.50, 95% CI 1.05-2.15, Ptrend = 0.04), indicating increased risk for this breast cancer subtype with total isoflavone intake. They also found that in women not stratified by menopause status there was a positive association between ER-PR- breast cancer (n = 500) and the highest tertile of adult total isoflavone intake ( $\geq 497$   $\mu\text{g}/\text{day}$ ) (OR: 1.38, 95% CI 1.05-1.81, Ptrend = 0.01). For effects of exposure to isoflavones as adolescents in the same study, see 2.4.2.4.2.

#### 2.4.2.1.5 Cross-sectional studies

An Indonesian cross-sectional study on persons of both genders (aged 52-98 years) with a mixed ethnic background found that worse memory, measured with a word learning test sensitive to dementia, was associated with high tofu (unfermented product of boiled soybeans) consumption assessed by a food frequency questionnaire ( $\beta = -0.18$ ,  $P < 0.01$ , 95% CI -0.34 to -0.06) (Hogervorst et al., 2008). However, high intake of tempe, an isoflavone-rich fermented and pressed soybean product, was independently associated with better memory ( $\beta = 0.12$ ,  $P < 0.05$ , 95% CI 0.00 to 0.28). The intake of the soy foods was reported as mean times per week  $\pm$  SD; tofu  $9.3 \pm 6.9$  and tempe  $9.5 \pm 6.8$ , for the total population of men and women (n = 719) of which 65% were women (n = ~467). Amount of tofu or tempe eaten or intake of isoflavones from the foods was not given. Both effects were stronger in persons 68 years or older. The analyses were controlled for age, sex, education, site and intake of other foods. Tempe has higher levels of genistein and increased folate, because of the fermentation, than tofu. Formaldehyde is often added to tofu, but not to tempe as a preservative, and has been suggested to be the reason for the negative effects observed with tofu.

In a Chinese cross-sectional study, women aged  $\geq 65$  years were evaluated for nutrient intake by a 7-day food frequency questionnaire and with screening instruments for cognitive impairment (Community Screening Instrument for Dementia (CSID)-D score  $\leq 28.4$ ) and/or depression (Geriatric Depression Scale (GDS) score  $\geq 8$ ) (Woo et al., 2006). No information on form and composition of isoflavones from the foods was given. There were 604 subjects with cognitive impairment, 372 with depression and 92 with both conditions. For both

cognitive impairment and depression, there were no significant associations with total isoflavone intake; cognitive impairment OR: 0.98 (95% CI 0.76-1.24) and OR: 0.80 (95% CI 0.59-1.09) for 5-18 and 19+ mg of total isoflavones per day vs. OR: 1.0 for isoflavone intake of 0-4 mg per day, and for depression; OR: 0.81 (95% CI 0.59-1.10) and OR: 0.69 (95% CI 0.47-1.01) for 6-18 and 19+ mg of total isoflavones per day vs. OR: 1.0 for isoflavone intake of 0-4 mg per day. However, when adjusting for total calorie intake, total isoflavone intake (0. 2.8-9.7 mg/1000 kcal/day) was inversely associated with depression; OR: 0.73 (95% CI 0.54-1.0),  $P < 0.05$ , whereas no association was apparent for cognitive impairment.

The daily intakes of isoflavones in the diets of 478 post-menopausal Japanese women (44-80 years) who reported soy product consumption were used to evaluate the effects of dietary isoflavones on menopausal symptoms, lipid profiles and bone mineral densities in a cross-sectional study (Somekawa et al., 2001). Serum values of fasting total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and apolipoproteins AI, B and E were recorded. Bone mineral density was measured at the lumbar spine (L2-L4) by dual energy x-ray absorptiometry. Women were assigned to two groups according to years since menopause; early (<5 years menopause,  $n = 269$ ), and late post-menopausal (>5 years since menopause,  $n = 209$ ), and each group was subcategorized into four groups according to dietary isoflavone intake (<35 mg/day, 35-50 mg/day, 50-65 mg/day and >65 mg/day of isoflavones). No information on form and composition of isoflavones from the foods was given. The mean estimated intake of isoflavones among 478 women was  $54.3 \pm 1.0$  mg/day ( $53.3 \pm 1.3$  mg/day in early post-menopausal and  $55.5 \pm 1.6$  mg/day in late menopausal women, not significantly different). The major sources of isoflavones in the soy products were soybean curd (47%), fermented soybeans (30%) and soybean paste (11%), amounting to about 88% of all eight isoflavone sources. With stepwise regression analysis, it was found that weight and years since menopause were significant independent predictors of bone mineral density. Bone mineral densities adjusted to years since menopause and weight were significantly higher in the highest intakes (50-65 and >65 mg/day) compared with the lowest intake (<35 mg/day) category within the early ( $P < 0.001$ ) and late ( $P = 0.01$ ) post-menopausal groups. In the early post-menopausal group, significant lower menopause symptoms scores were found for palpitation ( $P < 0.05$ ) and backaches ( $P < 0.05$ ) in the high versus the low intake categories but were not significant in the late post-menopausal group. Serum values of fasting total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and apolipoproteins AI, B and E were not significantly different between the isoflavone intake groups, either in early or late pre-menopausal women. The authors concluded that high consumption of soy products was associated with increased bone mass in post-menopausal women. No information about adverse effects was given in the paper.

#### 2.4.2.1.6 Other clinical studies

Safety and pharmacokinetics of soy isoflavones in healthy post-menopausal American women ( $n = 24$ , aged 46-68 years, mostly white) were examined by Bloedon et al. (2002) in a clinical study (see 2.3.1.1 for pharmacokinetics). They used two purified isoflavone

preparations (formulation A and formulation B) in capsules containing genistein, daidzein and glycitein in different proportions. Each woman was randomized to receive a single dose of the four doses studied of one of the two preparations (n = 6, 3 for each formulation). No control group was included. The doses of genistein were 2, 4, 8 or 16 mg/kg bw in both formulations (exceeding normal dietary intakes), and the doses of daidzein were 0.28, 0.55, 1.1 and 2.2 mg/kg bw in formulation A and 1.0, 2.1, 4.2 and 8.4 mg/kg bw in formulation B. The levels of glycitein were 0.014, 0.029, 0.057 and 0.11 mg/kg bw in formulation A and 0.08, 0.17, 0.34 and 0.68 mg/kg bw in formulation B. Formulation A contained 100% total unconjugated isoflavones consisting of 87% genistein, 12% daidzein and 1% glycitein, whereas formulation B contained 70% unconjugated isoflavones consisting of 44% genistein, 23% daidzein and 2% glycitein. Clinical laboratory tests and urinalysis were performed at screening and on days 1, 3, 6, 14 and 30. Chest X-rays and electrocardiograms were done at screening and on day 30. In three subjects, rates of apoptosis and tyrosine phosphorylation were measured in lymphocytes within 24 hours after dosing. Four reported adverse events with a grade I (according to National Cancer Institute (NCI)'s Common Toxicity Criteria) were judged to be possibly related to isoflavone administration because similar effects were reported in animals; 2 cases of trace pedal edema (at 2 and 16 mg/kg bw, both with formulation B), 1 case of nausea 1 hour postdose (at 8 mg/kg bw, with formulation B) and 1 case of breast tenderness (at 16 mg/kg bw, with formulation A). There were 12 grade I adverse events in 7 subjects on both formulations (headache, abdominal tenderness, fatigue, diarrhea etc.), which were judged unlikely to be related to isoflavone administration, because they occurred in the 2, 8 and 16 mg/kg bw dose groups. There were 16 grade I adverse events in 12 subjects, which were judged unlikely to be related to isoflavone administration, because they could be explained definitely by other known factors and the events were reported across all dose groups and were evenly distributed between the two formulations. Clinically significant decreases in systolic blood pressure ( $\geq 15$  mmHg) were observed 24 hour postdose with formulation A in 5 women, but only 2 continued to show significant decreases 3 days postdose (at 2, 4 and 8 mg/kg bw). Clinically significant decreases in diastolic blood pressure ( $\geq 15$  mmHg) were observed in various dose groups 24 hour postdose in 8 women, of which 6 were given formulation A, but only 1 continued to show significant decrease 3 days postdose. In two women, both systolic and diastolic blood pressure increased 24 hour postdose at 4 and 16 mg/kg bw with formulation B, but were not elevated at 3 days postdose. Blood variables that showed a change of  $\geq 10\%$  and were statistically significant were examined for differences in dose, formulation and hormone replacement therapy (HRT); neutrophil count, white blood cell counts, alanine transferase, aspartate aminotransferase, triacylglycerol, blood urea nitrogen and lactic dehydrogenase. All changes were at most 15%, except for changes in neutrophil count, which ranged from 20% to 25%. In all cases, the results at the same time point did not differ significantly by formulation. Of the 8 women receiving HRT, the mean change in lipase was increased on day 3 postdose compared with women not on HRT. Despite the observed decrease in tyrosine kinase activity seen in three women, no increase in apoptosis was observed, which may be because of the observed increased activity of Akt-1 kinase. The authors concluded that the administered doses of isoflavones had minimal clinical toxicity.

#### 2.4.2.1.7 Reviews

Zhao and Brinton (2007) reviewed 8 clinical studies published 2000-2007 evaluating the cognitive effects of soy foods or isoflavones, mostly in post-menopausal women. Four of the studies found a positive effect of isoflavones on cognitive functions, providing some reason for optimism about potential cognitive benefits of isoflavones. However, based on 8 different clinical studies evaluated cognitive effects of soy foods or isoflavones mostly in post-menopausal women, it was stated that no conclusions about the relationship between soy and cognition could be made (Messina, 2010a).

#### 2.4.2.1.8 Other articles

Among the many theories and various factors that may be involved in dementia, unfermented soy consumption, especially in the Western-style industrially produced food products, has been raised as a possible contributing factor to the increase in Alzheimer's and other dementia (Roccisano et al., 2014).

#### 2.4.2.1.9 Summary and discussion of effects of isoflavones on peri- and post-menopausal women

The included studies on peri- and post-menopausal women are summarized in Table 9.1 in Appendix 9.1.

##### *Sources of isoflavones*

Sources of isoflavones examined in the studies on peri- and post-menopausal women were reported to be soy protein, soy protein isolate (SPI) or isolated soy protein (ISP), soy foods such as soy milk, flour, tofu or tempe, isoflavone concentrate or just referred to as isoflavones. The isoflavones were given in the form of tablets, pills, capsules, powders or extracts, sometimes mixed into biscuits, cereal bars, snack bars, cereals or drinks, or the form was not specified. The chemical form of isoflavones was reported (if stated) as aglycones as such or as a high percentage of aglycones in most studies. The ratio of percentage of individual isoflavones as aglycones; genistein:daidzein:glycitein, varied substantially among the studies; 20-75:20-50:1-21. In three studies, the isoflavones were apparently given as high levels of glycosides, with genistin:daidzin:glycitin ratios of 43.4:37.2:8.8 (Gleason et al., 2015), 52:40:9 (Harkness et al., 2004) and 20:50:30 (Palacios et al., 2007). A few studies reported effects of separate genistein in tablets or capsules (Albertazzi et al., 2005; Atteritano et al., 2007; D'Anna et al., 2007; D'Anna et al., 2009; Marini et al., 2008). No pattern was obvious regarding type of soy product or isoflavone composition and negative health effects.

##### *Adverse events/side-effects*

Of the available RCTs of isoflavones (n = 28), 2 studies examined doses of 60 and approximately 120 mg total isoflavones per day for 6 weeks, 14 studies used doses from 56 to 160 mg/day for 3-6 months and 5 studies examined 52 to 300 mg/day for 9 to 12

months. In addition, one study examined effects of 898 mg/day of total isoflavones for 3 months (Pop et al., 2008), and another study examined effects of 150 mg/day for 5 years (Unfer et al., 2004). All of these studies found mostly gastrointestinal symptoms at the same rate, or in a few studies at a higher rate, with soy isoflavones compared with control (placebo) treatment. In addition, insomnia as a menopause symptom in healthy women was increased with isoflavones in one RCT after 122.7 mg/day (composition of isoflavones not given) for 6 months (Balk et al., 2002).

In the RCTs (n = 5) studying effects of genistein separate, doses of 54 mg/day for 1-3 years, and 90 mg/day for 6 weeks, reported no other negative effects than moderate gastrointestinal symptoms and back pain significantly different from control treatment.

A meta-analysis of 92 RCTs reporting side-effects on post-menopausal women using phytoestrogen (isoflavones, lignans and coumestans) supplements for treatment of climacteric syndrome (Tempfer et al., 2009), found that in only two of the 92 studies evaluated was there a statistically significant difference in side-effect incidence between treatment group and placebo group (Albertazzi et al., 2005; Unfer et al., 2004). Comparing various side-effect categories, significant higher rates of gastrointestinal side-effects among phytoestrogen users were found. Gynecological, musculoskeletal, neurological and unspecific side-effects were not significantly different between groups. Within side-effect categories, they found no significantly higher rates of side-effects in women using phytoestrogens. Among the 5 studies reporting side-effects in a meta-analysis of in total 15 RCTs on oral intake of phytoestrogens, there was no significant difference in side-effects between phytoestrogen and placebo groups (Chen et al., 2015).

#### *Hormone levels/lipid levels*

One RCT reported no significant changes in serum androgens or plasma lipids within treatment or placebo groups over time in healthy women exposed to 160 mg/day of isoflavones for 12 weeks (Basaria et al., 2009). However, at the end of the study a group-by-time interaction was observed so that total testosterone and HDL levels were significantly lower in the isoflavone group compared with placebo group.

#### *Cancer risk*

Most studied reported that isoflavones seemed to reduce cancer risk. However, a few studies indicated the opposite tendency. In a RCT, a significantly higher rate of endometrial hyperplasia without atypia was reported in healthy Italian women after 150 mg/day of isoflavones in tablets (Ge:D:GI %: 40-45:40-45:10-20) after 5 years (6 vs. 0 cases) (Unfer et al., 2004). However, no cases of endometrial hyperplasia with atypia or endometrial carcinoma were observed. EFSA (2015) mentioned some methodological weaknesses of this study; a considerable number (up to 25%) of specimens of endometrium were neither obtained nor assessable at each time point and that these samples were not consistently obtained from the same participants at each time point, and that the effects observed were indicative of a possible estrogenic but not a carcinogenic effect.



A prospective study reported that genistein and daidzein calculated from a food frequency questionnaire (FFQ) were dose-dependently associated with increased risk of hepatocellular carcinoma in Japanese women, with multivariate hazard ratios for highest vs. lowest tertile of 3.19 (95% CI 1.13-9.00, Ptrend = 0.03) and 3.90 (95% CI 1.30-11.69, Ptrend = 0.01), respectively (Kurahashi et al., 2009).

A retrospective case-control study found that soy food and isoflavone intake from food estimated from FFQ for the highest quartile of intake (Q4) vs. the lowest (Q1) was generally associated with decreased risk of colorectal cancer in Korea (Shin et al., 2015), however, the middle (second and third) quartiles of intake of total soy products were associated with a non-significant elevated colon cancer risk in women (Q2: OR: 1.27, 95% CI 0.86-1.88), Q3: OR: 1.37, 95% CI 0.92-2.04). The same non-significant tendencies of reduced risk associated with Q4 and increased risk associated with Q2 and Q3 vs. Q1 were seen with total isoflavones. However, a meta-analysis of 4 cohort and 7 case-control studies found reduced risk in women or no association in men between soy intake and colorectal cancer (Yan et al., 2010).

Another retrospective case-control study found that post-menopausal Canadian women had a positive association between ER-PR- breast cancer and adult total isoflavone intake from foods ( $\geq 497$   $\mu\text{g}/\text{day}$ ) (highest vs. lowest tertile: OR: 1.50, 95% CI 1.05-2.15, Ptrend = 0.04), indicating increased risk for this breast cancer subtype with total isoflavone intake (Anderson et al., 2013). Also in women not stratified by menopause status was there a positive association between ER-PR- breast cancer and the highest tertile of adult total isoflavone intake ( $\geq 497$   $\mu\text{g}/\text{day}$ ) (OR: 1.38, 95% CI 1.05-1.81, Ptrend = 0.01). However, EFSA (2015) concluded based on a weight of evidence approach that adverse effects on mammary gland have not been seen neither in humans nor in animals.

In the meta-analysis of 92 RCTs on post-menopausal women using phytoestrogen (isoflavones, lignans and coumestans) supplements for treatment of climacteric syndrome, the rates of hormone-related side-effects such as endometrial hyperplasia, endometrial cancer and breast cancer were not significantly different between groups (Tempfer et al., 2009).

To summarize, gastrointestinal symptoms, insomnia or back pain were reported as adverse events/side-effects in peri- and postmenopausal women at the same rate, or in a few studies at higher rate, compared with placebo. The doses of total isoflavones and duration of treatment in these studies were 60 and approximately 120 mg total isoflavones per day for 6 weeks, from 56 to 160 mg/day for 3-6 months and from 52 to 300 mg/day from 9 to 12 months. In addition, one study examined effects of 898 mg/day of total isoflavones for 3 months and another study examined effects of 150 mg/day for 5 years. Based on the available studies from the literature, isoflavones as supplements in these doses and duration of treatment appear to be without significant negative health effects in peri- and post-menopausal women.

The relevance of the few studies that found increased risk of cancer of a very high dose of isoflavone supplements or in occasional comparisons of dietary intake of soy food products in mostly Asian populations is difficult to interpret in relation to intake of isoflavone supplements in Norwegian peri- and post-menopausal women.

#### **2.4.2.2 Effects of isoflavones on pre-menopausal women**

The included studies on pre-menopausal women are summarized in Table 9.2 in Appendix 9.2.

##### **2.4.2.2.1 Randomized controlled trials**

Because soy food consumption may influence breast tissue activity, a study examined its effect on the presence of epithelial cells in nipple aspirate fluid (NAF) in a randomized, cross-over design (Maskarinec et al., 2013). Pre-menopausal American women (n = 82) completed a high-soy and a low-soy diet (consisting of soy milk, tofu and soy nuts, with 2 servings per day and <3 servings per week, respectively) for 6 months each, separated by a 1-month washout period. The population was multi-ethnic and consisted of 58% Caucasians, 17% Asian and 25% other ethnic groups. The mean  $\pm$  SD age at screening was  $40.4 \pm 6.0$  and  $38.3 \pm 6.1$  years, in the women with (n = 36) and without (n = 46) sufficient NAF volume ( $\leq 20 \mu\text{l}$ ) for cytological analysis, respectively (P = 0.12). The women provided NAF samples at baseline, 6 months and 13 months during the midluteal phase of the menstrual cycle. Papanicolaou-stained cytology slides (for 33 women at baseline, 24 at low-soy and 36 at high-soy) were evaluated in women with sufficient NAF. Among the women with sufficient NAF, the high-soy group had a dietary intake of (mean  $\pm$  SD):  $65.0 \pm 29.5$  mg/day of isoflavones, whereas the intake was  $3.8 \pm 5.8$  mg/day in the low-soy group, and the intake at baseline was  $2.2 \pm 1.9$  mg/day. Mixed models evaluated the effect of the high-soy diet on epithelial cytology as compared with baseline and the low-soy diet. At the end of the high-soy diet, cytological subclass had decreased in 8 (24%) and increased in 3 (9%) women compared with baseline, whereas the respective values were 3 (14%) and 6 (29%) for the low-soy diet samples (P = 0.32). Only the change in subclass indicated a trend in lower cytological class (i.e. towards normal cytology, away from malignancy) (P = 0.06), indicating that the soy intake did not have adverse effects on epithelial breast cells and even might have a potential beneficial effect on NAF cytology. However, when restricted to midluteal samples (n = 49), the high-soy diet was not associated with changes in cytological class (P = 0.14). The paper stated that the women reported changes in health status monthly, for monitoring of adverse effects. No specific information about adverse effects was given in the paper. The authors concluded that contrary to an earlier report, the number of NAF samples with hyperplastic epithelial cells did not increase after the soy intervention, suggesting the safety of consuming soy foods in amounts consumed by Asians.

In a controlled, double-blind prospective study, young healthy pre-menopausal American women (Caucasians, except for two with Asian and one with Native American backgrounds), 21 - 25 years of age, were divided into two groups (Anderson et al., 2002). One group was given supplement (n = 15), i.e. soy protein isolate supplement enriched with isoflavones

(approximately 90 mg of total isoflavones/day, 59% as glycones or glycosides, the remainder (41%) as aglycones, with at least 50 mg of genistein in both the glycone and aglycone forms) or given placebo (n = 13), an isoflavone-deficient (by alcohol-extraction) control protein diet containing the same amount of soy protein and other ingredients as the isoflavone-rich diet, for 1 year (both from Protein Technologies International Inc., St. Louis, MO, USA, as powders and as ready to drink chocolate liquid). Assignment of subjects to treatment or control groups alternated in the order of enrolment. Of the original 38 subjects, 10 dropped out before study completion; 4 because of use of disqualifying medications, 3 moved away and 3 left for other unspecified reasons. Dual-energy x-ray absorptiometric (DXA) measurements of bone mineral content (BMC) and bone mineral density (BMD) were made at baseline and at 6 and 12 months. DXA estimates of body composition, including fat mass and lean body mass, were generated from whole-body BMC measurements. BMI was calculated, physical activity was assessed and three-day dietary records were taken at entry (baseline) and at 6 and 12 months. No changes in BMD after 12 months were found in either the isoflavone-treated group or the isoflavone-deficient control group. Other variables also remained essentially constant over the 12-month period, including normal menstrual patterns in both the treatment and control groups. No adverse effects from the supplementation with isoflavones, such as gastrointestinal disturbances, were reported by the subjects. The authors concluded that the isoflavone-rich soy preparation had no effects on BMC and BMD over a 12-month period in young healthy adult females with normal menses.

An American study examined the effects of isoflavone consumption in 14 pre-menopausal women (mean  $\pm$  SD age: 26.5  $\pm$  4.7 years) (Duncan et al., 1999). Isoflavones were consumed in soy protein powders (Supro Brand Isolated Soy Protein, Protein Technologies International, St. Louis, MO, USA) and provided relative to body weight (control diet, 10  $\pm$  1.1 mg/day; low isoflavone diet, 64  $\pm$  9.2 mg/day; high isoflavone diet, 128  $\pm$  16 mg/day, expressed as unconjugated units, Ge:D:G1 %: 55:37:8, with 97% of genistein and daidzein and 91% of glycitein, as glycoside conjugates, for three diet periods, each lasting three menstrual cycles (of approximately 29 days) plus 9 days (approximately 96 days in total) in a randomized cross-over design with approximately 3 weeks wash-out periods between. During the last 6 weeks of each diet period, plasma was collected every other day for analysis of estrogens, progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Dietary effects were assessed during each of four distinctly defined menstrual cycle phases. Plasma from the early follicular phase was analysed for androgens, cortisol, thyroid hormones, insulin, prolactin (PRL) and sex hormone-binding globulin (SHBG). The low isoflavone diet decreased LH (P = 0.009) and FSH (P = 0.04) levels during the periovulatory phase. The high isoflavone diet decreased free T3 (P = 0.02) and dehydroepiandrosterone sulfate (DHEAS) (P = 0.02) levels during the early follicular phase and estrone levels during the midfollicular phase (P = 0.02). No other significant changes were observed in hormone concentrations or in the length of the menstrual cycle, follicular phase or luteal phase. Endometrial biopsies performed in the luteal phase of cycle 3 of each diet period revealed no effect of isoflavone consumption on histological dating. The authors concluded that the results of this study suggested weak hormonal effects of isoflavones in

pre-menopausal women, with uncertain physiological relevance and with no evidence of a dose-dependent effect, and that the data suggested that effects on plasma hormones and the menstrual cycle were not likely to be the primary mechanisms by which isoflavones may prevent cancer in pre-menopausal women. No information about negative effects of isoflavones was given in this paper.

A randomized controlled trial in healthy pre-menopausal Japanese women (mean age about 26 years) determined the effects of soy milk (from Kibun Food, Chemifa Tokyo, Japan) intervention on serum estrogen concentrations (Nagata et al., 1998). The women were randomly assigned to receive either a soy milk-supplemented diet (n = 31) or a normal (control) diet (n = 29). The women in the soy milk-supplemented group were asked to consume about 400 ml of soy milk (containing about 109 mg of isoflavones) daily during a study period that involved three consecutive menstrual cycles. The soy milk (100 g) contained 0.7 mg genistein and 16 mg genistin, and 0.7 mg daidzein and 9.4 mg daidzin. The intake of isoflavones from soy milk at the start and end of the intervention was (mean  $\pm$  SD)  $0.2 \pm 0.9$  mg and  $97.0 \pm 19.2$  mg, respectively. The same numbers were  $0.2 \pm 1.0$  mg and  $0.0$  mg in the control group. In addition to the soy milk, total isoflavones from other dietary sources at the start and end of the intervention were estimated to be  $25.2 \pm 23.4$  mg and  $19.4 \pm 15.0$  mg in the soy milk group, respectively, and  $20.7 \pm 12.9$  mg and  $18.4 \pm 13.4$  mg in the control group, respectively. This gives daily total isoflavone intakes at the start and end of the intervention of approximately 25.4 mg and 116.4 mg in the soy milk group and 20.9 mg and 18.4 mg in the control group, respectively. Follicular-phase blood samples were to be obtained in the menstrual cycles preceding (cycle 1) and following (cycle 3) the 2-month dietary intervention. At the end of the study period, estrone and estradiol levels were decreased by 23% and 27%, respectively, in the soy milk-supplemented group and were increased by 0.6% and 4%, respectively, in the control group. The changes for each hormone between the two groups were not significantly different. In the soy milk-supplemented group, menstrual cycle length was increased by nearly 2 days, and in the control group, it was decreased by approximately 1 day, but the differences were not statistically significant. A subgroup analysis restricted to subjects who provided follicular-phase blood samples on the same day or 1 day apart in menstrual cycles 1 and 3 (n = 21 on soy milk and n = 23 on control diet) showed a reduction in serum estrone levels in the soy milk-supplemented group that was borderline statistically significant (P = 0.07) versus the control group. The authors concluded that high intake of soy milk may modify circulating estrogen concentrations and possibly alter menstrual cycle length, but that much larger studies will be required to confirm the ability of soy to reduce serum estrogen levels. No negative effects were reported in the study.

#### 2.4.2.2.2 Prospective (cohort) studies

To assess the association of total dietary isoflavone intake with ovulatory function, including sporadic anovulation, in healthy pre-menopausal women, a prospective cohort study was performed in a university setting (Filiberto et al., 2013). Participants included 259 healthy regularly menstruating women aged 18-44 years. Serum concentrations of estradiol (E2), free E2, progesterone (P), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and

sex hormone-binding globulin (SHBG) and sporadic anovulation in healthy pre-menopausal women were the main outcomes. Dietary intake of isoflavones was assessed using multiple 24-hour dietary recalls, collected up to four times per menstrual cycle. A database was used to convert dietary exposure to mg of aglycones of genistein, daidzein, glycitein (from soy), biochanin A and formononetin (from red clover), and total isoflavone intake refers to the sum of these. Isoflavone intake was not associated with E2, free E2, P, LH and FSH concentrations. Consumption in the highest quartile (Q4: 1.6-78.8 mg/day) was significantly associated with greater SHBG concentrations ( $\beta = 0.09$ ; 95% CI 0.02-0.16), compared with the first quartile (Q1: 0.0-0.3 mg/day). Isoflavone intake was not associated with sporadic anovulation (Q4 vs. Q1: OR 0.87, 95% CI 0.32-1.66). The authors concluded that dietary isoflavone intake among young pre-menopausal women was not related to sex hormone concentrations or anovulation, but was associated with minimally increased SHBG concentrations, which suggested potential endocrine effects with no subsequent effects on ovulation, easing concerns regarding their impacts on fertility.

#### 2.4.2.2.3 Retrospective (case-control) studies

A case-control survey was undertaken to explore the relationship between environmental exposure and risk of uterine leiomyoma in pre-menopausal Han women aged 30-50 years in Nanjing, China (Shen et al., 2013). The subjects included 600 patients with confirmed uterine leiomyoma and 600 patients with non-uterine leiomyoma or healthy volunteers who presented to the hospital for physical examination during the same period. The results were obtained from a questionnaire and entered into a database and the relationship between risk factors and prevalence of uterine leiomyoma was explored using univariate or multivariate non-conditional logistic regression analysis. The results showed that patients aged 40-45 years had a high prevalence of uterine leiomyoma. The prevalence of uterine leiomyoma in subjects with an education beyond high school was higher than in those with a high school education or less. Exposure to plastic products (odds ratio (OR): 1.481; 95% confidence interval (CI): 1.046-2.097); exposure to cosmetics and other chemicals (OR: 1.954; 95% CI: 1.479-2.582); and dietary consumption of soybean milk (OR: 2.518; 95% CI: 1.894-3.347), food additives, sweeteners and preserved food (OR: 3.166, 95% CI: 2.247-4.461) had a significant effect on the occurrence of uterine leiomyoma ( $P < 0.05$ ). The soybean milk exposed women with no consumption were  $n = 136$  cases and  $n = 216$  controls. Among the women with occasional consumption of soybean milk ( $n = 264$  cases and  $n = 324$  controls), the difference was not significant ( $P = 0.184$ , OR (95% CI) = 1.294 (0.884-1.894)), whereas among the women with frequent consumption ( $n = 200$  cases and  $n = 60$  controls), it was significant ( $P = 0.000$ , OR (95% CI) = 5.294 (3.184-8.803)). No information was given on the composition or levels of isoflavones in the soy milk. The authors concluded that exposure to plastic products, cosmetics and other chemicals, as well as intake of soybean milk, food additives, sweetener and preserved foods, may be risk factors for uterine leiomyoma.

A case-control study on Hawaii examined the association between specific phytoestrogens (genistein, daidzein, glycitein, *O*-desmethylangolensin and equol) and histologically confirmed cervical squamous intraepithelial lesions (SILs) in women aged 18-45 years of mixed ethnicity (39 and 43% white, 13 and 16% Japanese, 16 and 26% Hawaiian, 32% and

15% other, among cases and controls, respectively) (Hernandez et al., 2004). The final study group consisted of 122 cases (mean age  $\pm$  SD: 33.0  $\pm$  10.1 years) and 183 controls (39.0  $\pm$  13.9 years). Menopausal status was not specified. Dietary phytoestrogen intake was obtained by structured dietary survey and plasma phytoestrogens were measured with liquid chromatography photodiode array electrospray mass spectrometry. Plasma level of equol was positively associated with cervical SILs; OR 6.5 (95% CI 1.4-29.2), OR 5.4 (95% CI 1.5-20.0),  $P_{\text{trend}} = 0.02$ , for the highest (>5.9 nM) and next highest (0.6-5.9 nM) relative to the lowest quartile level (0 nM) after adjustment for several factors, including human papilloma virus infection, cigarette smoking, alcohol and number of sexual partners. However, only 24 cases (20%) and 18 controls (10%) had measurable equol levels. Equol was not correlated with any other plasma isoflavones (genistein, daidzein, glycitein and *O*-desmethylangolensin). There were no significant associations between SILs and plasma levels for the other isoflavones. No relationship was observed between consumption of isoflavonoid food sources, including tofu and other soy food products, and SILs risk.

#### 2.4.2.2.4 Cross-sectional studies

A study aimed to examine cross-sectional and longitudinal relations between dietary intake of isoflavones and bone mineral density (BMD) at the lumbar spine (LS) and femoral neck (FN) in black ( $n = 242$ ), white ( $n = 384$ ), Chinese ( $n = 117$ ) and Japanese ( $n = 119$ ) women (total  $n = 862$ ) during the menopausal transition (Greendale et al., 2015). They tested whether tertiles of dietary isoflavone intake were associated with baseline BMD when all women were pre-menopausal or early peri-menopausal. Total dietary isoflavone intake was calculated from the sum of genistein, daidzein, glycitein (from soy), and formononetin (from red clover). Since the isoflavone distribution was strongly bimodal, total isoflavones with separate aggregated Asian (1751 (27-5633), 8851 (5723-15932), 29113 (16147-118252) and aggregated non-Asian (88 (3-161), 286 (161-584), 1230 (584-135102) tertiles of intake (mg/day) were constructed. To analyse whether isoflavone intake was associated with longitudinal BMD, they fitted piecewise linear models to repeated measurements of baseline-normalized LS or FN BMD as functions of time before or after the final menstrual period (FMP) date. Multiply adjusted mean FN BMD values of pre-menopausal Japanese women were monotonically positively related to isoflavone consumption ( $P_{\text{trend}} = 0.0003$ ). Otherwise, no statistically significant baseline associations were observed. During the period of 1 year before the FMP through 5 years after the FMP, all participants lost LS and FN BMD. Loss was unrelated to isoflavone intake, except for Japanese women during the period of 1 year before the FMP to 2 years after the FMP; higher tertiles of isoflavone intake were associated with greater annual LS BMD loss rates ( $P_{\text{trend}} = 0.01$ ) and FN loss rates ( $P_{\text{trend}} = 0.04$ ). The authors concluded that in Japanese women, higher isoflavone intake was associated with higher peak FN BMD, but also with greater rates of LS and FN BMD loss during the menopausal transition. Results for the other racial/ethnic groups did not support a relationship between dietary intake of isoflavones and either peak BMD or BMD loss during the menopausal transition.

A cross-sectional study of 11688 North American Adventist pre-menopausal women aged 30-50 years, of which 54% were vegetarians, found an inverse relationship ( $P = 0.05$ ) between

dietary isoflavone intake and the likelihood of ever having become a mother (Jacobsen et al., 2014). The mean intake of isoflavones was 17.9 mg/day. The isoflavone intake from foods was computed as intake of genistein, daidzein and glycitein as aglycone equivalent weights from a food frequency questionnaire (FFQ). In women (12%) with high ( $\geq 40$  mg/day) isoflavone intake, the adjusted lifetime probability of giving birth to a live child was reduced by approximately 3% (95% CI 0, 7) compared with women with low ( $< 10$  mg/day) intake. No relationship was found between the isoflavone intake and parity or age of first delivery in parous women. A similar inverse relationship ( $P = 0.03$ ) was found between isoflavone intake and the risk of nulligravidity with a 13% (95% CI 2, 26) higher risk of never having been pregnant in women with high ( $\geq 40$  mg/day) isoflavone intake. These relationships were found mainly in women who reported problems with becoming pregnant. The soy intake was estimated with a FFQ validated against 24-hour dietary recalls for intake of nutrients and selected foods ( $r = 0.8$ ) and was also correlated to the total isoflavonoids in urine ( $r = 0.5$ ). The isoflavone intake (mean 17.9 mg/day) was very high in this study compared with other Western populations (typically  $< 3$  mg/day). The women included in this study had very low prevalence of ever smoking (6%) and a low consumption of alcohol; only 33% had ever used alcohol and among the ever-users it was less than monthly. The study indicated that women with a high intake of isoflavones have an increased risk of never becoming pregnant and being childless when they are at an age where they are at the end of their childbearing period (aged 41-50 years).

#### 2.4.2.2.5 Other clinical studies

Short-term effects of soybean intake on oxidative and carbonyl stress were examined in young (age 18-25 years) healthy women ( $n = 55$ ) in Slovakia (Celec et al., 2013). The background for this study was that reduction of oxidative and carbonyl stress has been proposed as the underlying mechanisms of soy-rich diet on reduced risk of cardiovascular diseases and diabetic complications. The test persons were given 2 g (dry weight) of commercially available soybeans (AlfaBio, Bratislava, Slovakia) per kg body weight daily for 7 consecutive days (content and composition of isoflavones were not given). Blood samples were taken before intake of soybeans, after one week of intake and after a wash-out period of another 7 days. Blood was analysed for oxidative stress as levels of thiobarbituric reactive substances (TBARS) - marker for lipid peroxidation, advanced oxidation protein products (AOPP) - marker of protein oxidation and total antioxidant capacity (TAC) - as measure of antioxidant status. Advanced glycation end products (AGEs) are end products of the Maillard reaction between amino groups of macromolecules and free carbonyl compounds, and are markers for carbonyl stress. Total antioxidant capacity was increased by soybean intake in both genders, leading to decreased levels of AOPP in women, but not in men. No effects on carbonyl stress markers were found (AGE-specific fluorescence and fructosamine (one of the Maillard reaction products)). The author concluded that soybean intake had gender-specific effects on oxidative stress in young healthy persons potentially due to divergent action and metabolism of phytoestrogens in men (see 2.4.2.3.6) and women. Whether these effects have any long-term consequences is unknown.

A clinical study in Japan determined the effects of isoflavones on vascular function in healthy pre-menopausal (n = 44) vs. post-menopausal women (n = 11) and between smokers and non-smokers (Hoshida et al., 2011). The women (n = 55, mean age 39 years) were given 50 mg isoflavones per day (0.7 mg/kg bw per day for a 70 kg woman) as black soybean tea (no further information was given on form or composition of isoflavones) for 2 months.

Isoflavone consumption improved vascular endothelial function in both pre-menopausal and post-menopausal non-smokers. Arterial wall stiffness was effectively reduced only in pre-menopausal women, but the effect was noted for both smokers and non-smokers. Thus, the effects of isoflavones on vascular function differed among pre-menopausal and post-menopausal smokers and non-smokers. No adverse events of the isoflavone intake were reported in this study.

Ostatníková et al. (2007) studied cognitive spatial abilities and changes in sex hormones after short-term soybean consumption. They found that after administration of 2 g/kg bw per day of soybeans (no information about isoflavone intake, form or composition was given) for 7 days in healthy adolescent women (n = 54) (age 18-25 years) salivary testosterone and plasma estradiol (E2) levels showed a tendency to decline after soybean intake (the decrease in E2 was statistically significant) and to increase back towards basal levels during the washout period. Mental rotation and spatial visualization were significantly improved by soy.

Soy protein (60 g dry weight containing 45 mg isoflavones (mean intake 0.7 mg/kg bw (mean bw 62 kg)), Protoveg, Direct Foods Ltd., Manchester, UK) was given daily added to meals to six healthy pre-menopausal non-vegetarian UK women (age 21-29 years) for 1 month (Cassidy et al., 1994). No further information was given on the form or composition of the isoflavones. The women were studied over a 9-month period, in which two menstrual cycles were spent on controlled diets. Soy significantly increased follicular phase length (mean  $\pm$  SD: 2.5  $\pm$  1.6 day (P < 0.01) and/or delayed menstruation (by 1-5 days in 5 of 6 subjects). Mid-cycle surges of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were significantly suppressed during the dietary intervention; mean values decreased from 21.2 to 7.1 U/l (P < 0.05) and from 14.6 to 7.8 U/l (P < 0.02), respectively. Plasma estradiol concentrations increased in the follicular phase from 246.2 to 362.5 pmol/l (P < 0.02) and cholesterol concentrations decreased from 4.27 to 3.86 mmol/l (P < 0.05).

#### 2.4.2.2.6 Case reports

A 39-year old nulliparous American women diagnosed with endometrial cancer (grade I endometrioid adenocarcinoma) had a medical history notable for extensive use of numerous supplemental herbs and vitamins over the past two years, including many known phytoestrogens (Johnson et al., 2001). The patient lacked the classically associated risk factors for endometrial cancer, such as obesity (BMI 19) or anovulatory cycles. However, quantifying the amount of phytoestrogens was limited by the lack of such information on the packaging and soy was not specifically mentioned among the herbs.



#### 2.4.2.2.7 Summary and discussion of effects of isoflavones on pre-menopausal women

The included studies on pre-menopausal women are summarized in Table 9.2 in Appendix 9.2.

##### *Sources of isoflavones*

In these studies, isoflavones were given as isolated soy protein powder (Anderson et al., 2002; Cassidy et al., 1994; Duncan et al., 1999) or in the form of soy food products as components of diets (computed from FFQs/24-hour recalls or given as an intervention).

##### *Hormonal effects/menstrual function*

Of 5 RCTs on various end points of isoflavones as soy protein and/or from dietary sources giving isoflavone levels of approximately 64 to 128 mg/day, the only significant negative effects that were detected were effects on hormone levels in one study (Duncan et al., 1999). In this study, the levels of LH and FSH were decreased with 64 mg/day of isoflavones from soy protein powder, whereas free T3, DHEAS and estrone were decreased with 128 mg/day of isoflavones for approximately 14 weeks. The authors stated that this study suggested weak hormonal effects of isoflavones, with uncertain physiological relevance and with no evidence of dose-dependency of the effects. Weaker effects on hormone levels were also found in two other studies. Nagata et al. (1998) reported in a RCT that estrone and estradiol were decreased 23 and 27%, respectively, and that the menstrual cycle length was increased by nearly 2 days after soy milk and dietary soy food intake for approximately 2 months (total intake of 116.4 mg/day of isoflavones), however, both results were not statistically significant. In a clinical study by Ostatnikova et al. (2007), they reported that after intake of soybeans (isoflavones content unknown) for 7 days salivary testosterone and plasma estradiol (E2) levels showed a tendency to decline ( $P < 0.026$  for E2), with a trend to return to basal levels after washout. Another clinical study on soy protein added to meals found that 45 mg/day of isoflavones for one month significantly increased follicular phase length and/or delayed menstruation (by 1-5 days in 5 of 6 subjects), suppressed mid-cycle surges of LH and FSH and increased plasma estradiol concentrations in the follicular phase (Cassidy et al., 1994). A prospective study of dietary intake of isoflavones found that consumption of the highest quartile (1.6-78.8 mg/day of isoflavones) was significantly associated with greater SHBG concentrations compared with the first quartile (0.0-0.3 mg/day) (Filiberto et al., 2013). However, this diet also contained non-soy isoflavones (biochanin A and formononetin).

##### *Effects on uterus/cervix*

Regarding other effects than on hormone levels and menstrual function, a retrospective study found that consumption of soybean milk (isoflavone level and composition unknown) was associated with significantly increased occurrence of uterine leiomyoma in Chinese women, especially among those with frequent consumption (Shen et al., 2013). Plasma equol, highest (>5.9 nM) and next highest (0.6-5.9 nM) quartiles vs. lowest quartile (0.0 nM), was positively associated with cervical squamous intraepithelial lesions (SILs) in Hawaiian women of mixed ethnicity, however, they found no association with plasma levels of other isoflavones, or between SILs and dietary consumption of soy foods (Hernandez et al., 2004).

### *Effects on bone*

A cross-sectional study of bone health in women of various ethnicities, including Caucasians, found greater annual lumbar spine and femoral neck bone mineral density loss rates positively related to dietary isoflavone consumption only in Japanese women (Greendale et al., 2015). However, the Asian populations had much higher aggregated levels of total isoflavones than the non-Asian populations (tertiles of 1751, 8851 and 29113 mg/day vs. 88, 286 and 1230 mg/day, respectively). In addition, a RCT of isoflavones from enriched soy protein isolate on bone mineral density in American (mostly Caucasian) women did not find any effects of approximately 90 mg isoflavones per day (Anderson et al., 2002).

### *Effects on reproduction*

In Adventist women with a unusually high mean dietary isoflavone intake (54% vegetarians) compared with other Western populations, an inverse relationship was found between the likelihood of ever becoming a mother or the risk of nulligravidity and isoflavone intake  $\geq 40$  mg/day (Jacobsen et al., 2014). However, these relationships were mainly in women who reported problems with becoming pregnant.

To summarize, isoflavones apparently affected hormone levels in doses of 45 to 128 mg/day and affected menstruation in doses of 45 to 116.4 mg/day in pre-menopausal women, when taken for approximately one to three months. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In peri- and post-menopausal women, soy isoflavones are taken with the purpose of decreasing post-menopausal symptoms such as hot flashes, instead of estrogen-based hormone-replacement therapy, whereas pre-menopausal women would not have this need. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy pre-menopausal women. In this risk assessment of isoflavones in healthy pre-menopausal women, VKM considers changes in hormone levels away from the normal range as negative effects.

Based on the available studies, isoflavones as supplements in doses of 45 to 128 mg/day taken for approximately one to three months may represent a risk of negative effects on hormone levels and/or menstrual function in pre-menopausal women. These doses do not appear to have other significant negative effects on pre-menopausal women.

### **2.4.2.3 Effects of isoflavones on men**

In theory, phytoestrogens and structurally related compounds could harm the reproductive health of men by acting as antiestrogens by competing for the binding sites of ER or the active site of the estrogen biosynthesising and metabolising enzymes, such as aromatase and estrogen-specific  $17\beta$ -hydroxysteroid oxidoreductase (type 1).

Plasma concentrations of genistein were reported to be 276 (116-652) nmol/l in Japanese men and 6.3 (3.3-14.0) nmol/l in Finnish men (Santti et al., 1998). The corresponding concentrations of daidzein were 107 (47-237) nmol/l and 6.3 (3.9-10.0) nmol/l. Plasma

concentrations of 17 $\beta$ -estradiol were 71.2  $\pm$  3.8 and 81.8  $\pm$  2.7 pmol/l in Japanese and Finnish men, respectively.

The included studies on men are summarized in Table 9.3 in Appendix 9.3.

#### 2.4.2.3.1 Meta-analyses

A meta-analysis of published phytoestrogen consumption data from USA and China was compared with sperm concentration data of healthy men, as a marker of male reproductive health, from the same countries over several years (Lim and Shaw, 2016). Comparisons of isoflavone (genistein, daidzein, equol and desmethylangolensin) intake contained in several soy food products and sperm concentrations trends show that in USA isoflavone intake has increased from 0.8 mg/day in 1993 to 13.7 mg/day in 2005 and sperm concentration is trending down from 137 x 10<sup>6</sup>/ml in 1938 to 64 x 10<sup>6</sup>/ml in 2007 (53% decline, not statistically significant). In China, isoflavone intake has decreased from 64.7 mg/day in 1991 to 15.6 mg/day in 2008, and the sperm concentration increased from 55 x 10<sup>6</sup>/ml in 1999 to 74 x 10<sup>6</sup>/ml in 2008 (34% increase). However, it is possible that the sperm concentration effects in Chinese and American men are also related to changed exposure to myriad other estrogen mimics, as well as other factors, which also have changed over time.

A systematic review and meta-analysis of randomized controlled trials (RCTs) evaluated the evidence on the efficacy and safety of soy/isoflavones in men with prostate cancer (PCa) or with a clinically identified risk of PCa (van Die et al., 2014). MEDLINE, EMBASE, the Allied and Complementary Medicine (AMED), the Cumulative Index to Nursing and Allied Health Literature (CINAHL) and the Cochrane Library databases were searched. RCTs investigating soy/soy isoflavones as dietary supplements or dietary components for the secondary prevention or treatment of PCa in men with PCa or with a clinically identified risk of developing PCa were included. Studies of multi-component formulations were excluded. Six authors were contacted for further information for the meta-analyses. Methodological quality was assessed using the Cochrane Collaboration's risk-of-bias tool. The PRISMA statement for reporting systematic reviews was followed. Of the eight RCTs that met the inclusion criteria, six restricted recruitment to men diagnosed with PCa, while two included men with clinically identified risk of PCa. A large degree of heterogeneity was found with respect to dosages and preparations of soy/isoflavones administered. Most studies had small sample sizes and were of short duration. The risk of bias was assessed as low in all assessed studies except for one, for which the risk of bias was unclear. Meta-analyses of the two studies including men with identified risk of PCa found a significant reduction in PCa diagnosis after administration of soy/soy isoflavones (risk ratio = 0.49, 95% CI 0.26, 0.95). Meta-analyses indicated no significant differences between groups for prostate-specific antigen (PSA) levels or sex steroid endpoints; sex hormone-binding globulin (SHBG), testosterone, free testosterone, dihydrotestosterone and estradiol. The results of a meta-analysis of two studies suggest there may be support for epidemiological findings of a potential role for soy/soy isoflavones in PCa risk reduction; however, a clear understanding of the impact of soy/isoflavones on PSA, total testosterone, free testosterone and SHBG levels in men with, or at identified risk of, PCa could not be derived from these data, given the limitations of

sample size and study duration in individual trials. A good safety profile was shown by this meta-analysis for soy/soy isoflavone supplementation. Seven of the 8 studies reported on adverse events; however, two of these neglected to specify the arm in which the events occurred. Reported adverse events were predominantly mild, although abdominal pain, classified as a grade II (moderate) event, was reported in one study, and one severe (grade III) event in each arm was reported in another (iliac artery stenosis in active group; ileus in placebo group). Mild events included loose stools, diarrhea, constipation, bloating, loss of appetite, dyspepsia, biochemical events (increase in serum lipase, increase of serum bilirubin; grade I elevations in alanine transaminase, marginal elevations in lipase, amylase, phosphorus and calcium (in 1–2 subjects). Overall, only two adverse events resulted in withdrawal (gastrointestinal discomfort and weight gain), however, the treatment group was not specified. In studies reporting on both arms, adverse events occurred with similar frequency in both the treatment and placebo arms. In one study, the incidence of adverse events rated as "probably related" was greater in the placebo arm.

A meta-analysis determined whether isoflavones exerted estrogen-like effects in men by lowering bioavailable testosterone through evaluation of the effects of soy protein or isoflavone intake on testosterone, sex hormone-binding globulin (SHBG), free testosterone and free androgen index (FAI) in men (Hamilton-Reeves et al., 2010). PubMed and CAB Abstracts databases were searched through July 1, 2008, with use of controlled vocabulary specific to the databases, such as soy, isoflavones, genistein, phytoestrogens, red clover, androgen, testosterone and SHBG. Peer-reviewed studies published in English were selected if 1) adult men consumed soy foods (milk, grits, flour or tofu), isolated soy protein (ISP) or isoflavone extracts (from soy or red clover) and 2) circulating testosterone, SHBG, free testosterone or calculated FAI was assessed. Data were extracted by two independent reviewers. Isoflavone exposure was abstracted directly from studies. Fifteen placebo-controlled treatment groups with baseline and ending measures were analysed. In general, isoflavone intake in these studies greatly exceeded typical mean daily dietary Japanese intake of approximately 25-50 mg isoflavones and 6-11 g soy protein and were 20–900 mg/day isoflavones as aglycone equivalents or 0–71 g/day as soy protein. The duration of the studies varied from 1 week to 4 years, but for most trials was less than 6 months (average study duration of about 74 days). Each gram of soy protein in traditional soy foods was associated with approximately 3.5 mg isoflavones (expressed as aglycone weight). In addition, 32 reports involving 36 treatment groups were assessed in simpler models to ascertain the results. No significant effects of soy protein or isoflavone intake on testosterone, SHBG, free testosterone or FAI were detected regardless of statistical model. The authors concluded that the results of this meta-analysis suggested that neither soy foods nor isoflavone supplements altered measures of bioavailable testosterone concentrations in men.

A meta-analysis of available epidemiologic studies determined the relationship between soy consumption and colorectal cancer risk in humans (Yan et al., 2010). The women were mostly post-menopausal women based on age, but menopausal status was not stated. Publications obtained through a Medline literature search were systematically reviewed and

four cohort and seven case-control studies on soy (various soy foods, isoflavones or genistein) and colorectal cancer risk that met the inclusion criteria were identified. The risk estimate (hazard ratio, relative risk or odds ratio) of the highest and the lowest reported categories of intake was extracted from each study and this analysis was conducted using a random-effects model. The analysis did not find that soy consumption was associated with colorectal cancer risk (combined risk estimate 0.90; 95% CI 0.79-1.03) nor did the separate analyses on colon cancer (combined risk estimate 0.88; 95% CI 0.74-1.06) and rectal cancer (combined risk estimate 0.88; 95% CI 0.67-1.14). However, when separately analysed on the basis of gender, it was found that soy was associated with an approximately 21% reduction in colorectal cancer risk in women (combined risk estimate 0.79; 95% CI 0.65-0.97;  $P = 0.026$ ), but not in men (combined risk estimate 1.10; 95% CI 0.90-1.33). The authors concluded that consumption of soy foods may be associated with a reduction in colorectal cancer risk in women, but not in men. However, the analysis also showed that the results may differ for Western vs. Asian populations. The analysis of six studies on isoflavones showed that isoflavone consumption was associated with an approximately 16% reduction in colorectal cancer risk. This significant risk reduction was largely attributable to three studies conducted in Western countries, which was also reflected in the results of the stratified analysis. Of the six studies analysed, two of them assessed isoflavone intakes at low  $\mu\text{g/day}$  to low  $\text{mg/day}$  levels and reported a significant reduction in colorectal cancer risk. In contrast, all three studies conducted in Asian countries reported intakes at levels in the range of several  $\text{mg/day}$  to  $>50 \text{ mg/day}$  and none of those showed a significant risk reduction. Furthermore, all of the studies conducted in Western countries were case-control studies with relatively small study populations and none of them were designed to study soy.

#### 2.4.2.3.2 Randomized controlled trials

A randomized, double-blind trial was conducted from July 1997 to May 2010 at 7 US centres in 177 men at high risk of recurrence after radical prostatectomy for prostate cancer. It was done by comparing daily consumption of a soy protein isolate (SPI) supplement vs. placebo for two years to determine whether this exposure reduced the rate of biochemical recurrence of prostate cancer after radical prostatectomy or delayed such recurrence (Bosland et al., 2013). Supplement intervention was started within 4 months after surgery and continued for up to 2 years, with prostate-specific antigen (PSA) measurements made at 2-month intervals in the first year and every 3 months thereafter. Participants were randomized to receive a daily serving of a beverage powder containing 20 g of protein in the form of either SPI ( $n = 87$ ) or, as placebo, calcium caseinate ( $n = 90$ ). The soy protein beverage powder contained per 1 g of protein 3.67 mg of all forms of isoflavones (aglycones, glycosides and glycoside esters) or 2.13 mg as aglycone equivalents (42.6 mg total isoflavones/day), amounting to (in aglycone equivalents) 1.24 mg of genistein, 0.78 mg of daidzein and 0.11 mg of glycitein (Ge:D:GI %: 58:37:5). The main outcome was biochemical recurrence rate of prostate cancer (defined as development of a PSA level of  $\geq 0.07 \text{ ng/ml}$ ) over the first 2 years following randomization and time to recurrence. However, the trial was stopped early for lack of treatment effects at a planned interim analysis with 81 evaluable participants in the intervention group and 78 in the placebo group. Overall, 28.3% of participants developed

biochemical recurrence within 2 years of entering the trial (close to the a priori predicted recurrence rate of 30%). Among these, 22 (27.2%) occurred in the intervention group and 23 (29.5%) in the placebo group. The resulting hazard ratio for active treatment was 0.96 (95% CI, 0.53-1.72; log-rank P = 0.89). Daily consumption of a beverage powder supplement containing soy protein isolate for 2 years following radical prostatectomy did not reduce biochemical recurrence of prostate cancer in men at high risk of PSA failure. Adherence was greater than 90% and there were no apparent adverse events related to supplementation, i.e. no differences in adverse events (gastrointestinal issues, urinary tract issues, initiation of high cholesterol or hypertension treatments or musculoskeletal pain) between the two groups.

An American double-blinded, randomized, placebo-controlled trial was conducted to examine the effect of soy isoflavone capsules (80 mg/day of total isoflavones, 51 mg/day as aglycone equivalents (15% genistein, 55% daidzein and 30% glycitein), Flav-ein, 3 B'S Ltd., Lenexa, KS, USA) on serum and tissue biomarkers in patients of various ethnicity with localized prostate cancer (Hamilton-Reeves et al., 2013). The placebo capsules contained <0.06 mg total isoflavones aglycone equivalents/day. Eighty-six men were randomized to treatment with isoflavones (n = 42) or placebo (n = 44) for up to six weeks prior to scheduled prostatectomy. Microarray analysis was performed using a targeted cell cycle regulation and apoptosis gene chip (GEArray™). Changes in serum total testosterone, free testosterone, total estrogen, estradiol, prostate-specific antigen (PSA) and total cholesterol were analysed at baseline, mid-point and at the time of radical prostatectomy. In this preliminary analysis, 12 genes involved in cell cycle control and 9 genes involved in apoptosis were down-regulated in the treatment tumour tissues versus the placebo control. Changes in serum total testosterone, free testosterone, total estrogen, estradiol, PSA and total cholesterol in the isoflavone-treated group compared with men receiving placebo were not statistically significant. All adverse events were grade I (mild). In the isoflavone arm, 4 events were recorded; 2 gastrointestinal and 2 general. In the placebo arm, 9 events were recorded; 6 gastrointestinal and 3 general. No patients stopped treatment because of adverse events. The authors concluded that the data suggested that short-term intake of soy isoflavones did not affect serum hormone levels, total cholesterol or PSA.

Hot flashes occur in approximately 80% of androgen-deprived men (treatment to manage and control prostate cancer). Treatment effects on hot flashes was studied in a randomized, double-blind, placebo-controlled, 2 x 2 factorial, multi-center phase III clinical trial in USA (Vitolins et al., 2013). Eligible androgen-deprived men were randomly assigned to one of four daily regimens (2 x 2 factorial design) for 12 weeks: milk protein powder and placebo pill, venlafaxine and milk protein powder, soy protein powder isolate (Revival, Physicians Pharmaceuticals, Kernersville, NC, USA, 160 mg/day of isoflavones, no further information on form or composition) and placebo pill (no further information on content), or venlafaxine and soy protein powder. The primary end point was hot flash symptom severity score (HFSSS), defined as number of hot flashes times severity. The secondary end point was quality of life (QOL), assessed by using the Functional Assessment of Cancer Therapy-Prostate. In all, 120 men aged 46 to 91 years participated. Most were white (78%) and overweight or obese

(83%). In the placebo + soy protein group, the men had median age of 71 years (range 54-85) (n = 30). Toxicity was minimal. Of 19 adverse events reported, 3 were in the soy group. The majority of AE had severity grade 0-I and did not differ significantly among groups. The majority was experienced at baseline and most did not worsen during treatment. Neither venlafaxine nor soy protein alone or in combination had a significant effect on HFSSS. Soy protein, but not venlafaxine, improved measures of QOL. The authors concluded that in androgen-deprived men, neither venlafaxine nor soy proved effective in reducing hot flashes. Interventions that appear effective for decreasing hot flashes in women may not always turn out to be effective in men.

A study aimed at determining whether intake of cow's milk compared with soy beverages prepared from whole soybean (WSB) or soy protein isolate (SPI) would lower soluble cell adhesion molecule (CAM) concentrations as a means of decreasing cardiovascular disease (CVD) risk (Dettmer et al., 2012). Prehypertensive and hypertensive individuals are at increased risk of atherosclerotic CVD, in part because hypertension contributes to endothelial dysfunction and increased cell adhesion molecule expression. Healthy prehypertensive/stage 1 hypertensive American men (n = 60; 18-63 years) and pre-menopausal women (n = 8; 20-48 years) were enrolled. Participants were randomized to 1 of 3 groups for 8 weeks: cow's milk (600 ml/day) from Fareway Stores Inc., soy protein isolate (SPI) beverage (840 ml/day; 30.1 mg total isoflavones/day) or whole soybean (WSB) beverage (840 ml/day; 91.4 mg total isoflavones/day), from WhiteWave Foods Company. No further information on isoflavone composition of the beverages was given. Soluble vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1) and endothelial-leukocyte adhesion molecule-1 (E-selectin) concentrations were measured at baseline and week 8. Soluble CAM concentrations were not altered by treatment and did not differ between prehypertensive and hypertensive participants. However, analysis of variance indicated a treatment × gender interaction (gender effect) for ICAM-1 (P = 0.0037) but not for E-selectin (P = 0.067) or VCAM-1 (P = 0.16). Men had higher concentrations of ICAM-1 and E-selectin, respectively, at baseline (P = 0.0071, P = 0.049) and week 8 (P = 0.0054, P = 0.038) than **women** did. No information was given in the paper about adverse effects. The authors concluded that neither intake of cow's milk nor soy beverage for 8 weeks altered soluble CAM concentrations in prehypertensive/stage 1 hypertensive individuals, suggesting that neither type of beverage diminished atherosclerotic CVD risk in mildly hypertensive individuals by way of improving circulating CAM concentrations.

To examine the prostate cancer risk based on isoflavone intake and equol production, a Japanese phase II, randomized, double-blind, placebo-controlled trial of oral total isoflavone (60 mg/day) for 12 months was carried out (Miyana et al., 2012). The isoflavones tablets contained 0.1% genistein, 0.3% daidzein and 0.3% glycitein, and 5.8% genistin, 31.9% daidzin and 17.3% glycitin, in addition to malonyl and acetyl glycosides. The inclusion criteria were Japanese men between 50 and 75 years of age with a serum prostate-specific antigen level of 2.5–10.0 ng/ml and a single, negative prostate biopsy within 12 months prior to enrollment. The study included 158 men in eight Japanese centers; n = 78 in the isoflavone group and n = 80 in the placebo group. Their median age was 66.0 years, and the numbers

of equol producers and non-producers were 76 (48%) and 82 (52%), respectively. The scheduled intake of tablets was completed by 153 patients (96.8%); 96.2% (75/78) in the isoflavone group and 97.5% (78/80) in the placebo group. Of the 5 who did not complete the treatment, 3 decided themselves to quit taking the tablets. The other two patients had grade III adverse events: one in the isoflavone group suffered iliac artery stenosis, and the other in the placebo group suffered ileus. However, the majority of adverse events were mild or moderate in severity. No significant changes in laboratory data were observed during the study. The prostate-specific antigen value showed no significant difference before and after treatment. Of the 89 patients evaluated by central pathological review, the incidence of biopsy-detectable prostate cancer in the isoflavone and placebo groups showed no significant difference (21.4% vs. 34.0%,  $P = 0.140$ ). However, for the 53 patients aged 65 years or more, the incidence of cancer in the isoflavone group was significantly lower than that in the placebo group (28.0% vs. 57.1%,  $P = 0.031$ ). The authors concluded that these results support the value of isoflavone for prostate cancer risk reduction.

An American randomized, double-blind, cross-over intervention trial determined the effects of consumption of soy protein of varying isoflavone content on parameters of semen quality in healthy young adult men ( $n = 32$ , age 20-40 years, mean  $\pm$  SD age  $27.5 \pm 5.67$  years, body mass index  $25.4 \pm 3.14$  kg/m<sup>2</sup>) (Beaton et al., 2010). The intervention supplements were powders of milk protein isolate (MPI), low-isoflavone soy protein isolate (low-iso SPI; mean  $\pm$  SD:  $1.64 \pm 0.19$  mg isoflavones/day, expressed as total aglycone equivalents) and high-isoflavone soy protein isolate (high-iso SPI;  $61.7 \pm 7.35$  mg isoflavones/day, expressed as aglycone equivalents), from Solae LLC, St. Louis, MO, USA, for three 57-day treatment periods separated by 28-day washout periods. Isoflavone intake was 0.02 and 0.75 mg/kg bw per day for low- and high-iso SPI, respectively. The average percentage intake distribution of genistein, daidzein and glycitein was 78.9%, 12.7% and 8.4% within the low-iso SPI, and 53.3%, 35.6% and 11.1% within the high-iso SPI, respectively. Ten of 32 subjects (31%) were equol excretors. Urinary isoflavones were measured from 24-hour urine samples collected on days 54-56 of each treatment period. Semen quality parameters (semen volume, sperm concentration, sperm count, sperm percent motility, total motile sperm count, sperm morphology) were measured from semen samples collected on days 1 and 57 of each treatment period. Urinary isoflavones were significantly higher after consumption of high-iso SPI compared with the low-iso SPI and MPI. Semen parameters, including semen volume, sperm concentration, sperm count, sperm percent motility, total motile sperm count and sperm morphology were not significantly affected by consumption of either low- or high-iso SPI compared with MPI. The authors concluded that consumption of soy protein of low or high isoflavone content did not adversely affect semen quality in a sample of healthy adult men. Before study completion, seven subjects withdrew (job relocation ( $n = 3$ ), disliked the study treatment powder ( $n = 1$ )) or were excluded (initiated antidepressants ( $n = 1$ ), initiated antibiotics ( $n = 2$ )) from the study. After study completion, data were excluded from four subjects (urinary isoflavone data raised concern about compliance with dietary soy restrictions ( $n = 1$ ) and days of abstinence  $>7$  before collection of  $>1$  of the six semen samples ( $n = 3$ )). No adverse effects were reported in the paper.



A two-part study was conducted in USA over a 12-month period: a double-blind, placebo-controlled, randomized trial from 0 to 6 months and an open-label study from 6 to 12 months (DeVere White et al., 2010). The double-blind, placebo-controlled, randomized trial was conducted in 53 men (of originally 66) with prostate cancer enrolled in an active surveillance program. Of the 13 who discontinued the treatment before 6 months, 7 had diarrhea; 5 in the isoflavone and 2 in the placebo group, and one on isoflavones had a skin rash, the other 5 had non-treatment related reasons for withdrawal. The isoflavone treated men (mean age  $\pm$  SE: 70.5  $\pm$  1.8 years, n = 28) consumed a capsule supplement (5 g/day of an aglycone isoflavone-rich extract; genistein combined polysaccharide (GCP), from Amino Up Chemical Company, Sapporo, Japan) containing 450 mg genistein and 300 mg daidzein daily, for 6 months, whereas the men in the placebo group (mean age  $\pm$  SE: 68.6  $\pm$  1.5 years, n = 25) consumed identical-looking capsules with 5 g/day of inert cellulose. Prostate-specific antigen (PSA) was measured in both groups at baseline, 3 months and 6 months, and serum concentrations of genistein, daidzein and equol were assessed at baseline and 6 months in the treatment group. Following the completion of the 6-month double-blind study, men (n = 17 for isoflavone and n = 18 for placebo administration) were enrolled from the first study in a 6-month open-label trial with the same isoflavone-rich supplement, and PSA was measured at 3 and 6 months. PSA concentrations did not change in either group after 6 months or after 12 months when the open-label study was included. The 6 month serum concentrations of genistein and daidzein (39.85 and 45.59  $\mu$ mol/l, respectively) were significantly greater than baseline values and substantially higher than levels previously reported in other studies. Equol levels did not change. The high intake of aglycone isoflavones was generally well tolerated, with loose stools the most common complaint from a small number of men in the isoflavone group. The authors concluded that although high amounts of aglycone isoflavones may result in significantly elevated serum concentrations of genistein and daidzein, these dietary supplements alone did not lower PSA levels in men with low-volume prostate cancer.

In a phase I dose-finding, randomized, controlled trial, 45 men (mean age 59.48 years, range 45-80 years) with clinically localized prostate cancer were administered 40 (n = 12), 60 (n = 11) or 80 mg (n = 10) mg of isoflavones (Prevastein HC<sup>®</sup>, a soy-based isoflavone concentrate of glycosides with 40% aglycones of genistein, daidzein and glycitein, developed from NovaSoy400 (Archer Daniels Midland Company, Decatur, IL, USA)) daily, or no supplement (n = 11), from biopsy to prostatectomy (Kumar et al., 2010). Forty-four men completed the study with a duration of intervention of 30 ( $\pm$  3 days). One man in the 40 mg isoflavone group dropped out without giving any specific reason. Significant increases in plasma isoflavones were observed after treatment with all doses compared with controls without producing any toxicity. This was observed in plasma daidzein and genistein from baseline to end of the study period in the 40 and 60 mg isoflavone-treated groups, while in the 80 mg isoflavone-treated group there was a significant increase in plasma genistein, but not in daidzein. Significant increases in serum total estradiol were observed in the 40 mg/day groups (P = 0.023) and a significant increase in serum free testosterone was observed in the 60 mg/day group (P = 0.003). Changes in serum SHBG, PSA and cell proliferation (Ki-67) were not statistically significant. All anticipated and unanticipated, Grades I to III,

constitutional, dermatological, gastrointestinal (GI), metabolic and pain symptoms of AE were documented on all subjects throughout the study period (data not shown). Adverse events were Grade I with the exception of one Grade II event reported by one person in the 80 mg isoflavone-treated group, which was determined to be unrelated to the test agent. Grade I metabolic or laboratory changes in serum alanine transaminase (ALT), marginal elevations in lipase, amylase, phosphorus and calcium were observed in 1-2 persons in all groups and were considered to be unrelated to the test agent. One person in the 60 mg and one in the 80 mg isoflavone-treated groups reported Grade I GI events, which were considered possibly related to the study agent. The only group that had a modulation of serum estradiol without a significant increase in serum free testosterone or serum PSA, as well as the least mean percentage of cells in proliferation, with no clinical toxicity, was the 40 mg group.

An American study evaluated the effects of high dose isoflavones, equivalent to that consumed by Asian populations, on the profound hypogonadism due to androgen deprivation therapy for prostate cancer (Sharma et al., 2009). This hypogonadism results in complications such as sexual dysfunction (measured with different variables for libido, erectile function and sexual arousal and satisfaction), poor quality of life, vasomotor symptoms and altered cognition. A total of 33 men undergoing androgen deprivation therapy for prostate cancer were enrolled in this randomized, double-blind, placebo-controlled, 12-week pilot trial. Participants were randomly assigned to receive 20 g soy protein (Revival® as powder) containing 160 mg total isoflavones (n = 17, age mean ± SE; 69.2 ± 2.5 years) vs. taste-matched placebo, that was 20 g whole milk protein (n = 16, age mean ± SE; 69.0 ± 2.2 years). Overall 79% of the men were white, 21% were black. The concentration of individual isoflavones in the soy protein was 64 mg genistein, 63 mg daidzein and 34 mg glycitein (Ge:D:GI %: 40:39:21). At baseline the groups were well matched in demographic parameters, sleep quality, cognition and overall quality of life. However, men in the isoflavone group had a higher baseline prevalence of hot flashes and poor intercourse satisfaction compared with those on placebo. At 12 weeks there were no significant differences between the two groups in any outcome measure. This pilot study of high dose isoflavones in androgen-deprived men showed no significant improvement in cognition, vasomotor symptoms or any other aspect of quality of life measures compared with placebo. Three of the initially enrolled 39 men were excluded from analysis based on screening laboratory values and three withdrew from study due to personal reasons (2) and dislike of the compound taste (1). There were no safety issues during the study and no significant changes in prostate-specific antigen (PSA), weight or BMI in either group. Overall compliance was high at approximately 80%. The authors stated that this study did not rule out any negative sexual effects of isoflavones on healthy eugonadal men.

A Canadian study determined the effects of soy protein isolates of varied isoflavone content on circulating thyroid hormones in healthy young men (Dillingham et al., 2007). Thirty-five healthy men (age mean ± SD: 27.9 ± 5.7 years old) supplemented their habitual diets with milk protein isolate (MPI), low-isoflavone soy protein isolate (low-iso SPI; 1.64 ± 0.19 mg isoflavones/day), and high-isoflavone SPI (high-iso SPI; 61.7 ± 7.4 mg isoflavones/day)

powder (from the Solae Company, St. Louis, MO, USA) expressed as total aglycone isoflavones for 57 days each, separated by 4-week washouts, in three treatments in a randomized cross-over design where the subjects were blinded to the treatment order. The mean percentage distributions of genistein, daidzein and glycitein were 78.9%, 12.7% and 8.4% within the low-iso SPI, and 53.3%, 35.6% and 11.1% within the high-iso SPI, respectively. Blood was collected on days 1, 29 and 57 of each treatment for analysis of total triiodothyronine (T3), free T3, total thyroxine (T4), free T4, thyroid stimulating hormone (TSH) and thyroid binding globulin (TBG). Twenty-four hour urines were collected at the end of each treatment for analysis of isoflavones. The results revealed no significant effects of the low-isoflavone or high-isoflavone SPIs on serum total T3, free T3, total T4, free T4, TSH or TBG when compared with the MPI on either study days 29 or 57. Urinary data revealed that isoflavones were significantly increased by the high-isoflavone SPI relative to the low-isoflavones SPI and MPI. Twelve subjects were categorized as equol excretors (urinary equol >1000 nmol/24 h) and 23 subjects could be categorized as equol non-excretors, however, inclusion of equol excretor status as a covariate in the statistical analyses did not change any of the results. There were a total of eight subjects who dropped out or were excluded from the study, none of these because of adverse effects. The authors concluded that the study demonstrated that soy isoflavones in a protein matrix did not significantly influence circulating thyroid hormones in healthy young men. No further information about negative effects was reported in the paper.

A phase II placebo-controlled, randomized, double-blind clinical trial examined safety and modulation of steroid hormones of 80 mg/day of purified isoflavones, given in two divided doses of 40 mg, in men with early stage, clinically localized prostate cancer (Kumar et al., 2007a; Kumar et al., 2007b). The duration of intervention was 12 weeks and with scheduled follow-up at 4 weeks and 12 weeks. The patients were given a botanical test compound called Prevastein HC®, a soy-based isoflavone concentrate of glycosides with 40% aglycones of genistein, daidzein and glycitein, developed from NovaSoy400 (Archer Daniels Midland Company, Decatur, IL, USA) extracted to assure that the ratio of isoflavones as well as aglycone and glycoside isoforms were maintained as they would be found in soybeans and unfermented soy foods. Significant increases in plasma daidzein, glycitein and genistein were observed from baseline to 4 weeks and from baseline to 12 weeks, in the treatment group compared with the placebo group. The placebo was described as identical to treatment providing inert ingredients (gelatin). Of 53 men (25 in the treatment group, mean age 71.75 years, and 28 in the placebo group, mean age 71.92 years), 50 completed the intervention and provided complete data and analyses at baseline, 4 weeks and 12 weeks. Although significant increases in plasma isoflavones ( $P < 0.001$ ) were observed with no clinical toxicity, the corresponding modulations of serum sex hormone-binding globulin (SHBG), total estradiol and testosterone in the isoflavone-treated group compared with men receiving placebo were non-significant. A total of 3 persons dropped out of the study including 1 from the placebo group and 2 from the isoflavone-treated group. The reasons for dropping out included non-compliance to study agent (1 person) and Grade I to II adverse events (AE) that resulted in two persons not being willing to continue (not specified if they were from the treated group or the placebo group). All anticipated and unanticipated, Grades I to III,

constitutional, dermatological, gastrointestinal (GI), metabolic and pain symptoms of AE were documented on all subjects throughout the study period. Most AE were Grade I and II events in both groups, with 2 events identified as Grade III in the treatment group and determined to be unrelated to the test agent. These grade III events were constitutional symptoms of fever related to viral infection. Anticipated Grade I AE included GI symptoms, such as bloating, loss of appetite, dyspepsia and diarrhea, reported by both groups (5 in the treatment group and 7 in the placebo group). Grade II events were abdominal pain. One person in the treatment group reported a dermatological symptom that was determined to be unrelated to treatment. Grade I metabolic/laboratory changes in serum alanine transaminase (ALT), increases in lipase, amylase, hyperphosphatemia and hypercalcemia were observed in both groups and considered possibly related to study agent. When compared statistically, none of the categories all adverse events, AE by grade, AE by causality, AE by expected or AE by symptom were significantly different between the treated and placebo groups (at the two-sided 0.05 significance level). None of the AE produced clinical toxicity, thus not requiring early stopping or discontinuation of the study agent. According to the authors, unlike earlier reports, no symptoms related to estrogenic effects (such as breast changes, lowered libido and increased frequency of hot flashes) at this dose and period of intervention were observed.

A double-blind randomized cross-over clinical trial was designed to assess the independent effect of soy relative to animal protein and soy-derived isoflavones on circulating estrogen and androgen concentrations in 42 (> 50 years) moderately hypercholesterolemic Americans (Goldin et al., 2005). Older men (n = 18, mean age 61 years) with low-density lipoprotein cholesterol levels of  $\geq 3.36$  mmol/l were fed each of 4 diets in randomized order for 6 weeks/phase. Study subjects were given each of four diets; soy protein depleted of isoflavones (soy/-), soy protein enriched in isoflavones (soy/+), animal protein with no added isoflavones (animal/-) and animal protein with added isoflavones (animal/+). No information on isoflavone form or composition was given. All foods and drinks were provided. Diets contained 25 g soy or common sources of animal protein/4.2 MJ containing trace (1.25 mg or not detected) or 46.21 mg or 51.71 mg isoflavones/4.2 MJ, respectively. The mean soy protein intake of the male subjects was  $71 \pm 18$  g/day. The mean isoflavone intake of the men was  $139 \pm 35$  mg/day, however, for which group(s) were not specified. For animal/+ and animal/- diets, the variable protein components was contributed by dairy and meat. Isoflavones, in the form of powdered concentrate (Archer Daniels Midland Company, Decatur, IL, USA) were mixed into different food items of the animal/+ diet. For the soy/- and soy/+ diets, the variable protein component was contributed by specially prepared batches of isolated soy protein, one depleted (0.12 mg aglycone/g protein) and one enriched (1.96 mg aglycone/g protein) in isoflavones (Protein Technologies, St. Louis, MO, USA). At the end of each diet phase, concentrations of estrone sulfate, estrone, estradiol, testosterone, androstendione, dihydrotestosterone, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) were measured. In the men, DHEAS concentrations were 14% lower after consuming the isoflavone (P = 0.0106) and 8% higher after soy, compared with the animal protein diets (P = 0.0118). The authors concluded that

the data suggested that relatively large amounts of soy protein or soy-derived isoflavones had modest and limited sex-specific (see 2.4.2.1.2 for women) effects on circulating hormone levels. No adverse effects were reported in the paper.

An American controlled, parallel-arm, double-blind intervention study tested the hypothesis that long-term soy isoflavone supplementation decreased circulating insulin-like growth factor I (IGF-I) concentrations (Adams et al., 2003). In total 150 participants of both genders (85% men), 50–80 years old, with adenomatous colorectal polyps found on colonoscopy and therefore of increased risk of colorectal cancer, were randomly assigned to consume a soy beverage powder (DuPont Protein Technologies, later the Solae Company, St. Louis, MO, USA) daily for 12 months. The active treatment group (+ISO) received soy protein containing 83 mg isoflavones daily, whereas the comparison group (-ISO) received soy protein containing 3 mg isoflavones. The daily isoflavone dose of the soy drink in the active intervention (+ISO) was 45.6 mg genistein, 31.7mg daidzein and 5.5 mg glycitein, and the daily isoflavone dose of -ISO was 1.7 mg genistein, 1.0 mg daidzein and <0.1 mg glycitein. The age (mean  $\pm$  SD) was 64.7  $\pm$  7.9 years in the +ISO group and 65.1  $\pm$  7.9 years in the -ISO group. Serum IGF-I and IGF binding protein 3 (IGFBP-3) were measured by enzyme-linked immunosorbent assay (ELISA). Mean change in serum IGF-I concentrations was similar in the two groups (+1.4 nmol/l in +ISO, +1.2 nmol/l in -ISO; P = 0.74, 95% CI -1.1, 1.5 nmol/l for the 0.21 nmol/l difference between groups), indicating no effect of the isoflavone intervention. Similarly, the changes in IGFBP-3 and the IGF-I/IGFBP-3 ratio were similar in both groups, again showing no effect of +ISO treatment. The authors concluded that a 12 months, 83 mg/day soy isoflavone intervention did not modulate serum IGF in an older, mostly male population. At the 12-month clinic visit, 58 participants remained in the +ISO group and 65 in the -ISO group. Sixteen participants dropped out of the +ISO group; reasons obtained from 14 subjects included 7 health- and 7 non-health-related (e.g. logistical) reasons. Eleven participants dropped out of the -ISO group; reasons obtained from 9 subjects included 6 health- and 3 non-health-related reasons (no more information was given about the health effects). However, analyses excluding the drop-outs and those who did not adhere to the protocol did not change the results. In the same population, effects of soy isoflavone supplementation on prostate-specific antigen (PSA) (Adams et al., 2004), colorectal epithelial cell proliferation (Adams et al., 2005) and bone mineral density (Newton et al., 2006), were studied, however, no negative health effects were described in these studies either.

A randomized, double-blind, placebo-controlled, cross-over trial in UK examined the effects on serum sex steroids, lipids and markers of oxidative stress of supplementing the diets of healthy male volunteers with scones made with soy flour (Gardner-Thorpe et al., 2003). Twenty healthy male volunteers ate three scones a day in addition to their normal diet for a period of 6 weeks. After a 6 weeks washout period, the subjects switched to the alternative scone type for a further 6 weeks. The scones were made with either wheat or Nutrisoy soy flour (ADM Europort, The Netherlands, containing 120 mg/day of isoflavones; 45 mg/day of genistein and 75 mg/day of daidzein). However, baseline and final testosterone values for the control group were not presented. Blood was analysed for sex steroids (testosterone,

dihydrotestosterone, estradiol, estrone, sex hormone-binding globulin), albumin and the concentration of non-protein bound sex steroids were calculated, lipid profile (total cholesterol, high density lipoprotein cholesterol and triglycerides) and measures of oxidative stress (hydroperoxides, susceptibility of low density lipoprotein to oxidation with copper and myeloperoxidase) were obtained. The volunteers' mean age was 35.6 (SD 11.2) years. Total serum testosterone fell in volunteers taking the soy scones (19.3-18.2 nmol/l; 95% CI 16.20, 20.44;  $P = 0.03$ ). No significant changes were seen in the concentrations of the other serum sex steroids, albumin or sex hormone-binding globulin throughout the study. Significant improvements in two of the three markers of oxidative stress were seen in volunteers taking soy scones. Lag time for myeloperoxidase rose from 55.0 to 68.0 min. (95% CI -16.0, -3.5;  $P = 0.009$ ) and the presence of hydroperoxides decreased from 2.69 to 2.34  $\mu\text{mol/l}$  (95% CI 0.12, 0.71;  $P = 0.009$ ). There were no changes seen in serum triglycerides or cholesterol. The authors concluded that soy supplements reduced serum testosterone and improved markers of oxidative stress, and that these findings provided a putative mechanism by which soy supplements could protect against prostatic disease and atherosclerosis. Nineteen of the 20 volunteers completed the study, one withdrew for personal reasons. No adverse effects were reported in the paper.

A randomized, placebo-controlled, cross-over UK study examined the effects of supervised high versus low soy diets on attention, memory and frontal lobe function in young healthy adults (student volunteers) of both sexes (15 men and 12 women, mean age 26 years) (File et al., 2001). They were randomly allocated to receive, under supervision, a diet with high soy (100 mg total isoflavones/day) or low soy (0.5 mg total isoflavones/day) for 10 weeks, provided as breakfast, lunch and dinner. Both diets were given to the subjects on a 11-day rotating menu-basis (cross-over design). The two diets were matched for protein, carbohydrate and fat, and the only significant difference was in the soy concentration. No information was given on the form or composition of isoflavones in the diets. They received a battery of cognitive tests at baseline and then after 10 weeks of diet. Those receiving the high soy diet showed significant improvements in short-term (immediate recall of prose and 4-s delayed matching to sample of patterns) and long-term memory (picture recall after 20 min.) and in mental flexibility (rule shifting and reversal). These improvements were found in men and women. In a letter fluency test and in a test of planning (Stockings of Cambridge), the high soy diet improved performance only in women. There was no effect of diet on tests of attention or in a category generation task. Those on the high soy diet rated themselves as more restrained and, after the tests of memory and attention, they became less tense than did those on the control diet. The authors concluded that significant cognitive improvements can arise from a relatively brief dietary intervention, and that the improvements from a high soy diet were not restricted to women or to verbal tasks. There was no information in the paper regarding negative effects of isoflavones.

The effects of soy protein isolate (SPI) intake on remnant-like particles (RLP, which consists mainly of remnants of chylomicron and apoE-rich very low density lipoproteins), lipolytic enzymes, lipid transfer protein, transaminases, sex hormones, iron, calcium and vitamin E were determined in healthy men in Japan (Higashi et al., 2001). In the first randomized,

cross-over experiment, 14 men (mean  $\pm$  SD age: 31  $\pm$  4 years) were given either 20 g per day of SPI (Fuji Oil Co., Osaka, Japan, isoflavone intake, form or composition not given) mixed in milk or yogurt or nothing (control) for each of two 4-week segments, with one to two months washout periods between. After 3 weeks of SPI intake, triglyceride and RLP cholesterol levels were significantly lower than the baseline by 13.4% ( $P < 0.05$ ) and 9.8% ( $P < 0.05$ ), respectively. However, no significant change was found in total and low-density lipoprotein (LDL) cholesterol levels or the activities of lipoprotein lipase, hepatic lipase, cholesteryl ester transfer protein and lecithin cholesterol acyltransferase. Although the levels of transaminases, testosterone, iron and calcium did not change, the vitamin E level was reduced from the baseline by 9.7%, a significant decrease ( $P < 0.01$ ). In the second study, it was attempted to determine the effect of a vitamin E supplement taken with SPI. For each 3-week segment, with one to two months washout periods between, 12 men (mean  $\pm$  SD age: 30  $\pm$  2 years) were given 20 g per day of SPI, either with or without 200 mg per day of vitamin E, in a randomized cross-over design. The vitamin E level was reduced by 9.2%, a significant decrease ( $P < 0.05$ ), after SPI intake for 3 weeks, and vitamin E supplement increased vitamin E level significantly ( $P < 0.05$ ). The authors concluded that the results demonstrated that SPI intake reduced remnant lipoproteins, triglyceride and the plasma level of vitamin E, although vitamin E supplementation compensated for the reduction of vitamin E. Therefore, the supplementation of vitamin E may be required in subjects with long-term and abundant intake of soy protein. No information was given in this study about other negative effects of isoflavones.

A randomized, controlled, dietary intervention study to determine the effects of soy consumption on serum levels of steroid hormones in Japanese men found that estrone concentrations tended to decrease in the soy milk-supplemented group and increase in the control group (Nagata et al., 2001). Thirty-five healthy men (22-50 years old, mean age 32 years) were randomly assigned to either a soy milk-supplemented group or a control group. The men ( $n = 17$ ) in the soy-supplemented group were asked to consume 400 ml (408 g) of soy milk (Kibun Food Chemifa, Tokyo) daily for 8 weeks. The men in the control group ( $n = 17$ ) maintained their usual diet. Blood samples were obtained just before the initiation of the dietary period and thereafter every two weeks for 12 weeks. Changes in hormone concentrations were analysed and compared between the two groups using the mixed linear regression model against weeks from the start of the dietary period. The mean (SD) daily soy milk intake estimated from dietary records during the dietary study period was 342.9 (SD, 74.2) ml in the soymilk-supplemented group, which gave a daily intake of 76.8  $\pm$  16.6 mg of total isoflavones. Hundred grams of soy milk contains 0.6 mg genistein and 13.0 mg genistin, and 0.6 mg daidzein and 7.8 mg daidzin. On the basis of data at 0, 2, 4, 6, 8, 10 and 12 weeks, the interaction term for time and group was statistically significant for estrone ( $P = 0.04$ ), which meant that the slopes for time were significantly different between the two groups; the soy group tended to decrease over time ( $\beta = -0.003352$ , SE = 0.00226) and the control group tended to increase over time ( $\beta = 0.003228$ , SE = 0.00223), based on the difference in slopes. Although not statistically significant, the results were similar at 8 weeks. None of the other hormones measured (estradiol, total and free testosterone or sex hormone-binding globulin) showed any statistical differences between the two groups in

terms of patterns of change. The results of the study indicated that soy milk consumption may modify circulating estrone concentrations in men. No other adverse effects were reported in the paper, except that one participant in the soy milk-group dropped out on the first day of the dietary study period because he felt sick (he was excluded from the data analyses).

An Australian randomised cross-over dietary intervention study was performed to evaluate the effects of replacing meat protein in the diet with a soybean product, tofu, on blood concentrations of testosterone, dihydrotestosterone, androstenediol glucuronide, estradiol, sex hormone-binding globulin (SHBG), and the free androgen index (FAI; total testosterone concentration/SHBG concentration x 100) (Habito et al., 2000). Forty-two healthy adult Caucasian men aged 35-62 years (mean age  $\pm$  SD: 45.7  $\pm$  7.6 years) were studied. Diets were isoenergetic, with either 150 g lean meat or 290 g tofu (35 g soybean protein, Blue Lotus Foods, Kilsyth, Victoria, Australia) daily, providing an equivalent amount of macronutrients, with only the source of protein differing between the two diets. The tofu contained 0.29 mg genistein/g and 0.12 mg daidzein/g, giving 118.9 mg isoflavones daily. Each diet lasted for 4 weeks, with a 2-week washout period before new 4 weeks intervention. Urinary excretion of genistein and daidzein was significantly higher after the tofu diet ( $P < 0.001$ ). Blood concentrations of sex hormones (testosterone, dihydrotestosterone, androstenediol glucuronide and estradiol) did not differ after the two diets, but the mean testosterone:estradiol value was 10% higher ( $P = 0.06$ ) after the meat diet. SHBG was 3% higher ( $P = 0.07$ ), whereas the FAI was 7% lower ( $P = 0.06$ ), after the tofu diet compared with the meat diet. There was a significant correlation between the difference in SHBG and testosterone:estradiol and weight change. Adjusting for weight change revealed SHBG to be 8.8% higher on the tofu diet (mean difference 3 (95% CI 0.7, 5.2) nmol/l;  $P = 0.01$ ) and testosterone:estradiol to be significantly lower,  $P = 0.049$ ). The authors concluded that the replacement of meat protein with soybean protein, such as tofu, may have a minor effect on biologically-active sex hormones, which could influence prostate cancer risk. However, other factors or mechanisms may also be responsible for the different incidence rates in men on different diets. No adverse effects were reported in the paper.

#### 2.4.2.3.3 Prospective (cohort) studies

In a population-based prospective cohort of Japanese men ( $n = 7215$ ) aged 40-69 years, the association between isoflavones (genistein and daidzein) and soy food (three different food types) calculated from a food frequency questionnaire (FFQ) and hepatocellular carcinoma (HCC) was studied (69 cases in men) during 235811 person-years, average 11.8 years, of follow-up (both genders, see 2.4.2.1.3 for women) (Kurahashi et al., 2009). The low, middle and high intakes in men were  $<12.0$ ,  $12.0-19.9$ ,  $\geq 20.0$  mg/day for genistein,  $<8.0$ ,  $8.0-12.7$  and  $\geq 12.8$  mg/day for daidzein and  $<37.6$ ,  $37.6-64.9$  and  $\geq 65.0$  g/day for soy food. When analysis was restricted to persons who were either or both anti hepatitis C- or B virus antigen-positive, the HRs between lowest and middle or highest exposure to genistein and daidzein were not statistically significant, only the trends were significant ( $P_{trend} = 0.06$ , for both isoflavones). Consumption of genistein, daidzein or soy food showed no association with HCC in men.



The association between soy and bladder cancer risk was examined in a prospective cohort study of Chinese men in Shanghai (n = 18244, age 45-64 years) (Sun et al., 2004). The intake data was obtained with a food frequency questionnaire (FFQ). Compared with men consuming soy less than once a week, the RR (95% CI) for those who consumed soy 1-<3 times per week, 3-<7 times a week and daily were 2.05 (0.80-5.29), 2.45 (0.89-6.76) and 4.61 (1.57-13.51), respectively, Ptrend = 0.004) after adjustment for age, cigarette smoking and level of education. For soy isoflavones (mg/1000 Kcal), the RR (95% CI) for intake of  $\leq 1.69$ , 1.70-2.91, 2.92-4.45 and  $\geq 4.46$ , were 1.00, 2.01 (0.90-4.47), 1.03 (0.41-2.60) and 2.78 (1.29-5.98), respectively, Ptrend = 0.02. The soy – bladder cancer risk associations in smokers and non-smokers were comparable. This association became stronger when the analysis was restricted to subjects with longer ( $\geq 2$  years) duration of follow-up. In conclusion, high soy intake was associated with increased risk of bladder cancer after adjustment for potential confounders.

Sun et al. (2002) investigated the association between soy food consumption and subsequent bladder cancer risk in a population-based cohort study of Singapore Chinese (age 49-81 years) of both genders together. The cohort comprised 329 848 person-years of follow-up with 61 cases of bladder cancer (47 in men and 14 in women). Intake of soy food was measured with a validated dietary questionnaire, where total soy intake was the summation of 7 common non-fermented soy foods, expressed in units of plain tofu equivalent. Total soy isoflavone intake was estimated from the summation of genistein, daidzein and glycitein contents of all 7 soy foods. Relative to the lowest quartile of energy-adjusted total soy intake ( $< 36.9$  g/1000 Kcal), the highest quartile ( $\geq 92.5$  g/1000 Kcal as tofu equivalents) was associated with a 2.3-fold increase in bladder cancer risk (95% CI 1.1-5.1) after adjustment for smoking and level of education. For soy protein (% Kcal), the same RR was 2.74 (95% CI 1.18-6.38). For soy isoflavones (mg/1000 Kcal), relative to the lowest quartile of energy-adjusted intake ( $\leq 5.77$  mg/1000 Kcal), the highest quartile (15.43 mg/1000 Kcal as tofu equivalents) was 2.08 and not statistically significant (95% CI 0.94-4.60), whereas the second highest quartile (9.84-15.42 mg/1000 Kcal as tofu equivalents) was 2.47 and statistically significant (95% CI 1.16-5.26). The soy food – bladder cancer risk association did not differ significantly between men and women, and was not explained by other dietary factors. Women reported significantly higher consumption of total soy food compared with men. This association became stronger when the analysis was restricted to subjects with longer ( $\geq 3$  years) duration of follow-up.

Twenty-seven Australian men (mean age was 51.5 years) with elevated fasted total cholesterol (TC)  $> 5.5$  mmol/l were recruited for an open prospective observational pilot study of soy protein containing 65 mg isoflavones (ISP+), taken daily as supplement for 12 weeks, with 4 weeks on a low fat diet before and for 6 weeks after the soy protein powder (from Protein Technology International, St. Louis, MO, USA) treatment (Mackey et al., 2000). No further information on form or composition of isoflavones was given. There was a significant increase in HDL cholesterol (HDL-C) at 6 weeks (P = 0.02) and 12 weeks (P = 0.03), and a reduction in sex hormone-binding globulin (SHBG) at 12 weeks (P = 0.0003). An increase in dehydroepiandrosterone (DHEA) nearly reached significance at 12 weeks (P =

0.06). No significant changes were observed in total cholesterol, LDL cholesterol or triglycerides, or in thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), androstenedione or testosterone. Soy protein had a cholesterol lowering effect in men. The study suggested that this effect was independent of isoflavones (see 2.4.2.1.2 for data on women). No negative effects were reported in this study.

A longitudinal, prospective study begun in 1965 in Japanese men living in Hawaii found that tofu consumption was associated with impaired cognitive function (measured with Cognitive Abilities Screening Instrument (CASI) score <74) (White et al., 2000). Data on tofu intake was available from interviews in 1965-1967 and 1971-1974 and indexed at 4 levels; low-low consumption was fewer than two servings per week at both interviews and no tofu in the prior week in 1971, whereas high-high consumers had two or more servings per week at both interviews. Intermediate or less consistent low and high consumption levels were also defined. No information on amounts of tofu consumed that could be used to estimate isoflavone intake was available. Intake levels of isoflavones per day from tofu or the form and composition of isoflavones was not given. Cognitive functioning was tested at the 1991-1993 examination, when participants were aged 71-93 years (n = 3734). Brain atrophy was assessed by neuroimage (n = 574) and autopsy (n = 290) information. Cognitive function data were also analysed for the wives of a sample of study participants (n = 502). Poor cognitive test performance, enlargement of ventricles and low brain weight were each significantly and independently associated with higher midlife tofu consumption, also in the wives (assuming similar food intake as their husbands). Statistically significant associations were consistently demonstrated in linear and logistic multivariate regression models. Odds ratios comparing endpoints among high-high with low-low consumers were mostly in the range of 1.6-2.0. Weaknesses with this study were that the intake of only 26 foods was assessed and questions about tofu intake were not consistent over the course of the follow-up period. The reasons for the effects of tofu have been suggested to be the presence of formaldehyde in tofu, used as a preservative, which is known to adversely affect memory in rodents and its urinary levels are markedly elevated in dementia patients.

#### 2.4.2.3.4 Retrospective (case-control) studies

A case-control study in Korea aimed to assess the relationship between dietary soy food and isoflavone intake and colorectal cancer risk (Shin et al., 2015). Among men, a total of 624 colorectal cancer cases and 1872 controls (up to 3 controls per patient matched by gender and 5 years of age groups) were recruited at the National Cancer Center, Korea. A semi-quantitative food frequency questionnaire (FFQ) was used to assess the usual dietary habits, and the isoflavone intake level was estimated from five soy food items. A high intake of total soy food products was associated with a reduced risk for colorectal cancer in men (OR 0.67 (95% CI 0.49, 0.92)). However, the middle (second and third) quartiles of intake of total soy products were associated with an elevated colon cancer risk in men (Q2: OR 1.38, 95% CI: 1.05-1.80, Q3: OR 1.40, 95% CI: 1.07-1.83). A high intake of fermented soy paste was also associated with an elevated risk for colorectal cancer in men (Ptrend = 0.012). The quartiles for total isoflavone intake were: Q1: <7.63, Q2: 7.63-<12.56 mg/day, Q3: 12.56-<20.89,

Q4:  $\geq 20.89$  mg/day. The group with the highest intake quartile (Q4) of isoflavones showed a decreased risk for colorectal cancer compared with their counterparts with the lowest intake quartile (Q1) in men (OR: 0.71, 95% CI: 0.52-0.97). However, the middle (second and third) quartiles of intake of total isoflavones were associated with an elevated risk in men (Q2: OR 1.34, 95% CI: 1.02-1.76, Q3: OR 1.37, 95% CI: 1.04-1.79). Ptrend was 0.005. The authors concluded that although precaution was required for the probably elevated risk (statistically significant) among middle intake quartiles (Q2 and Q3), the results suggested that a high intake (Q4) of total soy products or dietary isoflavones was associated with a reduced risk for overall colorectal cancer, and that the association may be more relevant for distal colon cancer.

#### 2.4.2.3.5 Cross-sectional studies

A cross-sectional and observational epidemiological study examined the association of soy foods and isoflavone intake with semen quality parameters (Chavarro et al., 2008). The intake of 15 soy-based foods in the previous 3 months was assessed with a non-validated food frequency questionnaire for 99 male partners of subfertile couples who presented for semen analyses to the Massachusetts General Hospital Fertility Center. The men were primarily Caucasian (90%) with a mean (SD) age of 36.4 (5.0) years. The majority of men were overweight or obese (72%) defined as a BMI  $\geq 25$  kg/m<sup>2</sup>. The mean intake of isoflavones was 5.4 mg/day. The results were evaluated as genistein, daidzein, glycitein, all as mg/day, and soy foods, as servings/day. Linear and quantile regression were used to determine the association of soy foods and isoflavone intake with semen quality parameters while adjusting for personal characteristics. There was an inverse association between soy food intake and sperm concentration that remained significant after accounting for age, abstinence time, body mass index, caffeine and alcohol intake and smoking. In the multivariate-adjusted analyses, men in the highest category of soy food intake ( $\geq 0.30$  servings/day) had 41 million sperm/ml less than men who did not consume soy foods (95% CI -74, -8; Ptrend = 0.02). Results for individual soy isoflavones (genistein, daidzein and glycitein) were similar to the results for soy foods and were strongest for glycitein, but did not reach statistical significance (except for trend for glycitein). In the multivariate-adjusted analyses, men in the highest category of glycitein intake had 35 million sperm/ml less than men who did not consume soy foods (95% CI -73, 2; Ptrend = 0.07, tertiles 0.01-0.08, 0.09-0.28 and  $\geq 0.28$  mg/day). The inverse relation between soy food intake and sperm concentration was more pronounced in the high end of the distribution (90th and 75th percentile) and among overweight or obese men. Soy food and soy isoflavone intake were unrelated to total sperm count, sperm motility, sperm morphology or ejaculate volume. The authors concluded that the data suggested that higher intake of soy foods and soy isoflavones was associated with lower sperm concentration. It was noted that the soy intake had relatively little effects on men with the lowest sperm concentration. Thus, men who were most affected by soy still had a sperm concentration above the level (>20 million/ml) classified by the World Health Organization as oligospermia (WHO, 1999). Also, no information was collected about other dietary factors than soy that may affect sperm quality

and concentration, such as zinc and omega-3 fatty acids. Therefore, the results of this study may not implicate that soy has a negative effect on fertility.

The cross-sectional relationships of soy product intake and serum testosterone, estrone, estradiol, sex hormone-binding globulin and dihydrotestosterone were examined in 69 Japanese men (mean age  $60.5 \pm 10.7$  years) in an epidemiological study (Nagata et al., 2000). Daily soy product intake and isoflavone intake from soy products were estimated from a semiquantitative food frequency questionnaire, and was  $51.0 \pm 21.5$  g and  $21.9 \pm 8.7$  mg, respectively. No information was given on form or composition of isoflavones. Serum estradiol concentration was significantly inversely correlated with soy product intake ( $r = -0.32$ ,  $P = 0.009$ ), and serum estrone concentration was non-significantly inversely correlated with soy product intake ( $r = -0.24$ ,  $P = 0.05$ ) after controlling for age, body mass index, smoking status and ethanol intake. Total and free testosterone concentrations were inversely correlated with soy product intake after controlling for the covariates, but these correlations were of border line significance ( $r = -0.25$ ,  $P = 0.05$  and  $r = -0.25$ ,  $P = 0.06$ , respectively). Similar correlations were observed for these hormones with isoflavone intake from soy products. The authors concluded that the data suggested that soy product intake may be associated with the endogenous hormone levels in Japanese men. No adverse effects were reported in the paper.

#### 2.4.2.3.6 Other clinical studies

Short-term effects of soybean intake on oxidative and carbonyl stress were examined in young (age 18-25 years) healthy men ( $n = 33$ ) in Slovakia (Celec et al., 2013). The background for this study was that reduction of oxidative and carbonyl stress has been proposed as the underlying mechanisms of soy-rich diet on reduced risk of cardiovascular diseases and diabetic complications. The test persons were given 2 g (dry weight) of commercially available soybeans (AlfaBio, Bratislava, Slovakia) per kg body weight daily for 7 consecutive days (content and composition of isoflavones were not given). Blood samples were taken before intake of soybeans, after one week of intake and after a wash-out period of another 7 days. Blood was analysed for oxidative stress as levels of thiobarbituric reactive substances (TBARS) - marker for lipid peroxidation, advanced oxidation protein products (AOPP) - marker of protein oxidation and total antioxidant capacity (TAC) - as measure of antioxidant status. Advanced glycation end products (AGEs) are end products of the Maillard reaction between amino groups of macromolecules and free carbonyl compounds, and are markers for carbonyl stress. Total antioxidant capacity was increased by soybean intake, but did not lead to decreased levels of AOPP in men. On the contrary, in men, soybean intake increased lipid peroxidation. No effects on carbonyl stress markers were found (AGE-specific fluorescence and fructosamine (one of the Maillard reaction products)). The author concluded that soybean intake had gender-specific effects on oxidative stress in young healthy persons potentially due to divergent action and metabolism of phytoestrogens in men and pre-menopausal women (see 2.4.2.2.5). Whether these effects have any long-term consequences is unknown.

A Brazilian study assessed effects of a low daily intake of soybeans (25 grams of roasted soybean flour (Kinako), corresponding to 12.95 g of soy protein and 50 mg isoflavones (content and composition of isoflavones were not given), from Good Soy, Uberaba, Minas Gerais, Brazil) for 90 days on oxidative stress and components of metabolic syndrome (MS) (Bahls et al., 2011). Forty persons (gender distribution unknown) were divided in a soybean-treated group (n = 20) and a control group (n = 20) and matched by sex, age and smoking. There was no difference between the groups at the study start in the various parameters evaluated. The soybean-treated group showed a decrease in fasting glucose and increase in serum high density lipoprotein (HDL) and adiponectin. The authors concluded that low intake of soy protein for 90 days was able to improve several parameters related to pathophysiology of MS. The soybean intake was well tolerated by the MS patients, with complete adherence to the treatment, since no patients withdraw from the study.

A Canadian clinical phase II trial evaluated the tolerability and effect of a daily soy beverage in prostate cancer patients with biochemical failure after radiotherapy (Kwan et al., 2010). Patients (median age 78 years (range 62-85) with rising prostate-specific antigen (PSA) after radical radiation for prostate cancer were instructed to consume 500 ml of soy beverage (SoyaWorld Inc., Burnaby, British Columbia, Canada) daily for 6 months, giving a daily intake of total isoflavones of 65-90 mg. Tolerability of the soy beverage and compliance were assessed. PSA doubling times before and after the consumption of soy were compared. Thirty-four subjects were enrolled; 5 withdrew before 1 month on soy for reasons unrelated to soy consumption. All remaining 29 subjects were included in the analysis. Mean consumption of the assigned soy beverage was 93%. The most common side-effect was mild gastrointestinal upset, such as nausea, flatulence, bloating or constipation, decreasing over time with 31%, 17% and 7% after 1, 3 and 6 months, respectively, not affecting soy consumption. The second most common complaint was weight gain seen in 4 persons (2.7-9.1 kg over 6 months). One person had Grade II side-effects (hives), possibly related to soy. PSA showed a declining trend in 4 patients (13.8%), and there was a >100% prolongation of PSA doubling time in 8 patients (27.6%). However, PSA doubling time also showed a 50% or more shortening in 5 patients (17.2%). The authors concluded that 6 months of a daily soy beverage was well tolerated and was associated with a declining trend or more than two times prolongation of PSA doubling time in 41% of subjects.

In an Italian study presented by A. Serafini and mentioned in a conference report paper by (Messina et al., 2009) (original paper was not found), 20 male volunteers were randomized into three groups receiving 160, 320 or 480 mg/day of isoflavones (form and composition of isoflavones unknown) for 3 months. When compared with baseline, there were no significant differences in ejaculate volume, sperm concentration, sperm count and motility of spermatozoa in men given isoflavones.

A study evaluated the effect of supplementing healthy men with soy isoflavones on the serum levels of sex hormones implicated in prostate cancer development (Tanaka et al., 2009). A total of 28 Japanese healthy volunteers (18 equol producers and 10 equol non-producers) between 30 and 59 years of age were given soy isoflavone (60 mg daily)

supplements as tablets (Isofla A, Fuji Oil Co. Ltd., Osaka, Japan) for 3 months. The changes in their sex hormone levels were investigated at the baseline and after administration. Isoflavone (60 mg) consisted of 0.1 mg genistein, 0.2 mg daidzein and 0.3 mg glycitein, 3.5 mg genistin, 19.1 mg daidzin, 10.4 mg glycitin, 2.2 mg malonyl genistin, 8.1 mg malonyl daidzin, 3.4 mg malonyl glycitin, 1.9 mg acetyl genistin, 7.3 mg acetyl daidzin, 3.6 mg acetyl glycitin. The serum and urine concentrations of genistein, daidzein and the levels of equol in the fasting blood samples and 24-hour stored urine samples were also measured. No significant difference was noticed in the total cholesterol between the mean serum levels at the baseline and the end of study period. The mean high-density lipoprotein (HDL)-cholesterol level at 3 months increased significantly when compared with that of the baseline, whereas the mean low-density lipoprotein (LDL)-cholesterol significantly decreased during 3-month isoflavone administration. No changes in the serum levels of estradiol and total testosterone were detected after 3-month supplementation. The serum levels of sex hormone-binding globulin (SHBG) increased significantly, and the serum levels of free testosterone and dihydrotestosterone (DHT) decreased significantly after 3-month supplementation. Among the 10 equol non-producers, equol became detectable in the serum of two persons after 3-month supplementation. This study revealed that short-term administration of soy isoflavones stimulated the production of serum equol and decreased the serum DHT level in Japanese healthy volunteers. These results suggested the possibility of converting equol non-producers to producers by prolonged and consistent soy isoflavone consumption. All 28 volunteers completed the 3-month supplementation with isoflavone. No statistically significant adverse events were reported by the study participants. Diarrhea was the most frequently reported adverse events and occurred in 3 (11%) of the participants. Two participants had diarrhea of grade I according to the Common Terminology Criteria for Adverse Events (v. 3.0) several times during the consecutive 2 days, and one complained of grade I diarrhea once only. No participants discontinued the study regimen or withdrew from the study because of these adverse events.

An American study evaluated the efficacy of isoflavones in patients with prostate-specific antigen (PSA) recurrent prostate cancer after prior therapy postulating that isoflavone therapy would slow the rate of rise of serum PSA (Pendleton et al., 2008). Twenty patients (median age 73 years, 3 African-American, 17 white) with rising PSA after prior local therapy were enrolled in this open-labelled, phase II, non-blinded, non-randomized trial. Patients were treated with soy milk (Soy Dream Enriched, Original or Vanilla) containing 47 mg of isoflavonoid per 8 oz serving three times per day (141 mg isoflavonoid/day) for 12 months. No further information on composition of isoflavones was given. No control group was included. Serum PSA, testosterone, lipids, isoflavone levels (genistein, daidzein and equol), and quality of life (QOL) were measured at various time points from 0 to 12 months. PSA outcome was evaluated. Of six men who did not complete the study, one (5%) was due to side-effects (diarrhea). No other side-effects were reported. PSA levels were reduced in 6 patients, but increased in 13 patients. Within the mixed regression model, it was estimated that PSA had increased 56% per year before study entry and only increased 20% per year for the 12-month study period ( $P = 0.05$ ). Specifically, the slope of PSA after study entry was significantly lower than that before study entry in 6 patients and significantly higher in 2

patients. For the remaining 12 patients, the change in slope was statistically insignificant. Improvements in PSA doubling times were seen in 14 patients ( $P = 0.044$ ). Free testosterone decreased while on therapy (median 10.3 vs. 9.7 ng/ml,  $P = 0.031$ ), however, neither total testosterone nor cholesterol levels were significantly changed, nor was QOL. Nearly two thirds of the patients were noted to have significant levels of free equol in their serum while on therapy. The authors concluded that dietary intervention with isoflavone supplementation may have biologic activity in men with biochemical recurrent prostate cancer as shown by a decline in the slope of PSA.

A study investigated spatial abilities as cognitive function and sex hormone status of intake of approximately 1.8 g/kg bw per day of soybeans (isoflavone intake, form and composition not given) for 7 days in healthy young men ( $n = 7$ ) (age 18-25 years) (Celec et al., 2007). The treatment improved spatial visualization ( $P = 0.03$ ), but did not change plasma estradiol, total and free testosterone or salivary testosterone and estradiol. The results for mental rotation showed similar dynamics as spatial visualization, but were not significant.

In an American study, 12 healthy male volunteers with mean age of 32.25 years (range 25 to 47) were given two scoops (56 g) of over-the-counter 100% pure soy protein isolate powder (Puritan's Pride, Oakdale, NY, USA) daily for 28 days (Goodin et al., 2007). The isoflavone content or composition of the soy protein isolate powder was not given. No control group was included. Serum testosterone and luteinizing hormone (LH) levels were measured on days -7, 0, 14 and 28 of therapy, and on day 42. A reporter estrogen receptor (ER) assay was used to determine the effect on ER- $\beta$  and ER- $\alpha$  *in vitro*. Serum testosterone decreased 19% ( $\pm 22\%$ ) during the 4-week use of soy protein powder ( $P = 0.021$ ) and increased within 2 weeks after discontinuation of soy protein powder. One subject started the study with a serum testosterone level below the normal reference range (241-827 ng/dl), and it returned to the reference range on day 42; otherwise the serum testosterone did not decrease below the normal reference range in any subjects. Serum LH concentrations decreased during the 4-week use of soy protein powder, then increased within 2 weeks after discontinuation, but the changes did not reach statistical significance ( $P = 0.20$ ). The LH did not decrease below the normal reference range for adult men (1.3 - 13 mIU/ml) in any subjects. Soy protein powder was found to induce agonist activity to ER- $\beta$  using a reporter estrogen receptor assay in yeast. The authors concluded that soy protein powder decreased serum testosterone levels in healthy men and acted as an ER- $\beta$  agonist. There were no negative effects reported by any subjects.

Hamilton-Reeves et al. (2007) determined the effects of soy protein isolate (SPI) supplement consumption on circulating hormone profiles and hormone receptor expression patterns in men at high risk for developing advanced prostate cancer ( $n = 53$ ) or with low grade prostate cancer ( $n = 5$ ). The fifty-eight Caucasian American men (50-85 years old) (20, 20 and 18 per group, respectively, mean age 68 years in all groups) were randomly assigned to consume 1 of 3 protein isolates containing 40 g/day protein: 1) soy protein isolate (SPI+) (107 mg/day of isoflavones); 2) alcohol-washed soy protein isolate (SPI-) (<6 mg/day of isoflavones); or 3) milk protein isolate (MPI) (0 mg/day of isoflavones) (The Solae Company,

St. Louis, MO, USA). The mean distribution of isoflavones was 53% genistein, 35% daidzein and 11% glycitein in SPI+, and 57% genistein, 20% daidzein and 23% glycitein in SPI-. For 6 months, the men consumed the protein isolates in divided doses twice daily as a partial meal replacement. Serum samples collected at 0, 3 and 6 months were analysed for circulating estradiol, estrone, sex hormone-binding globulin, androstenedione, androstanediol glucuronide, dehydroepiandrosterone sulfate, dihydrotestosterone, testosterone and free testosterone concentrations by radioimmunoassay (RIA). Prostate biopsy samples obtained pre- and post-intervention were analysed for androgen receptor (AR) and estrogen receptor-beta expression by immunohistochemistry. At 6 months, consumption of SPI+ significantly suppressed AR expression ( $P = 0.04$ ), but did not alter estrogen receptor- $\beta$  expression or circulating hormones. Consumption of SPI- significantly increased estradiol (approximately 20%) and androstenedione concentrations (approximately 17%), and tended to suppress AR expression compared with the MPI group ( $P = 0.09$ ). According to the authors, although the effects of SPI- consumption on estradiol and androstenedione were difficult to interpret and the clinical relevance was uncertain, these data showed that AR expression in the prostate was suppressed by soy protein isolate consumption, which might be beneficial in preventing prostate cancer. These data suggested that consumption of isoflavone-rich and isoflavone-poor soy protein isolate exerted differing effects on endogenous hormone levels and receptor expression. No other adverse effects were reported in the paper.

Ostatnikova et al. (2007) studied cognitive spatial abilities and changes in sex hormones after short-term soybean consumption. They showed that 2 g/kg bw per day of soybeans (no information about isoflavone intake, form or composition was given) for 7 days in healthy young men ( $n = 32$ ) (age 18-25 years) did not change salivary testosterone and plasma estradiol (E2) levels. However, during the wash-out period of 7 days both parameters showed a tendency to rise non-significantly. The effects of soybean intake on hormonal parameters in men were dependent of basal testosterone levels; it increased significantly after a low basal level, but decreased non-significantly after a high basal level. Mental rotation and spatial visualization were significantly improved by soybean intake.

Inverse associations between soy and prostate cancer and the contribution of hormones to prostate cancer prompted the current study to determine whether soy protein supplement could alter serum hormones in men (Dillingham et al., 2005). Thirty-five healthy Canadian men (age 20-40 years, mean age 27.9 years) consumed milk protein isolate (MPI), low-isoflavone soy protein isolate (SPI) (low-iso SPI;  $1.64 \pm 0.19$  mg isoflavones/day), and high-iso SPI ( $61.7 \pm 7.35$  mg isoflavones/day, expressed as unconjugated units, for 57 days each in a randomized cross-over design (32-week study period consisting of three 57-day treatment periods, each separated by 28-day wash-out periods). The mean percentage distributions of genistein, daidzein and glycitein were 78.9, 12.7, and 8.4% within the low-iso SPI and 53.3, 35.6 and 11.1% within the high-iso SPI, respectively. Twenty-four-hour urine samples indicated that urinary isoflavones were significantly increased by the high-iso SPI relative to the low-iso SPI and MPI. Serum collected on day 1, 29 and 57 of each treatment revealed that dihydrotestosterone (DHT) and DHT/testosterone were significantly decreased by the low-iso SPI (9.4% ( $P = 0.036$ ) and 9.0% ( $P = 0.004$ ), respectively) and



the high-iso SPI (15% (P = 0.047) and 14% (P = 0.013), respectively), compared with the MPI at day 57. Other significant effects included a decrease in testosterone by the low-iso SPI relative to the MPI (P = 0.023) and high-iso SPI (P = 0.020) at day 29; an increase in dehydroepiandrosterone sulfate (DHEAS) by the low-iso SPI relative to the MPI at day 29 (P = 0.001) and relative to the MPI (P = 0.0003) and high-iso SPI (P = 0.005) at day 57; and increases in estradiol and estrone by the low-iso SPI relative to the MPI at day 57 (P = 0.010 and P = 0.005, respectively). The authors concluded that soy protein, regardless of isoflavone content, decreased DHT and DHT/testosterone with minor effects on other hormones, providing evidence for some effects of soy protein as such on hormones. No other negative effects were reported in the paper.

A 6-month, non-randomized, open-label study examined the effects of a genistein-rich extract (genistein combined polysaccharide (GCP), Amino Up Chemical Company, Sapporo, Japan) on men with prostate cancer (deVere White et al., 2004). GCP was prepared by adding soybean extract containing approximately 40% isoflavones (Novasoy, Archer Daniels Midland, Decatur, IL, USA) to a mushroom (*Ganoderma lucidum*) mycelia culture). When the activity of  $\beta$ -glucosidase produced by the mushroom rose to 6.0 IU/l, the soybean extract was introduced and cultured until the glycoside genistin could not be detected in the culture medium. After processing, the final product contained 10% genistein, 6% daidzein, 2% glycitein and 60% nonfibrous carbohydrate (including mushroom polysaccharides), 15% lipid and 5% protein. Intake was 5 g of the GCP extract daily, taken as capsules three times daily, providing a total of 450 mg/day of genistein, plus additional 450 mg/day of other aglycone isoflavones. Of 62 men enrolled (mean age 73.6 years, range 61.4-89.3), 52 men were assessed after 6 months, of these 3 patients discontinued because of adverse effects (diarrhea), which resolved on discontinuation of the supplement, and 7 patients from personal choice. Of the 52 patients, one had a decrease in prostate-specific antigen (PSA) of more than 50%, which was the purpose to examine in this study. An additional 7 patients had PSA reductions less than 50%. Thus, the GCP extract was not an effective treatment for prostate cancer when given alone. The total testosterone level was lowered in one patient, but increased in 5 patients. Clinical chemistry values (alkaline phosphatase, aspartate aminotransferase, total bilirubin, creatinine, cholesterol and gamma-glutamyltransferase) were unaltered during therapy in all men throughout the study.

An American phase I clinical trial was conducted to determine the safety, pharmacokinetic parameters (see 2.3.1.4) and efficacy of orally administered isoflavones (genistein and daidzein) as potential cancer chemotherapeutic agents over a 3-month period in men with prostate neoplasia (Fischer et al., 2004). Aside from prostate cancer, subjects were required to be in otherwise good health. Twenty men, age 40 years and above (mean age  $68.9 \pm 7.3$  years), with stage B, C or D adenocarcinoma of the prostate were treated with a multiple-dose regimen of a soy isoflavone formulation in capsules (Protein Technologies International (PTI), St. Lois, MO, USA) (delivering approximately 300 mg/day genistein and 150 mg/day daidzein for 28 days, then 600 mg/day genistein and 300 mg/day daidzein for the remaining 56 days, i.e. 450 or 900 mg/day isoflavones for each phase (totally 84 days)). Nineteen (95%) of the 20 subjects who completed the study were Caucasian and 1 (5%) was African

American. No placebo group was included. The delivered dose of isoflavones was more than 10-fold higher than that typically taken by prostate cancer patients. In men with prostate cancer, relatively minor side-effects of chronic isoflavone treatment were observed, including some estrogenic effects (breast changes, increased frequency of hot flashes). None of the adverse events were associated with clinically significant organ dysfunction. Nine grade I adverse events (AE) were judged possibly, and 4 probably, related to isoflavones. Four adverse events of grade II were judged possibly related to isoflavones. In one subject with grade II gynecomastia, it was present at baseline and was likely attributed to a herbal preparation with estrogenic effects. In the other subject, it could have been caused by isoflavones and the effect was transient. In a subject with grade II elevated amylase, there were no physical symptoms of pancreatitis detected and the lipase level was normal. One subject with grade I gynecomastia had breast tenderness at baseline and received a drug (Casodex) and his gynecomastia continued after he discontinued isoflavones. All three men with gynecomastia were on the highest dose of isoflavones. Serum dehydroepiandrosterone (DHEA) was decreased by 31.7% ( $P = 0.0004$ ) at the end of treatment. Except for those subjects whose prostate-specific antigen (PSA) values were below 0.4 ng/ml, subjects had a history of increasing PSA levels prior to the trial. This increase continued during the trial both while on soy isoflavones and after treatment was discontinued. On average the rate of rise accelerated after soy isoflavones were discontinued, but that difference did not attain statistical significance.

Seventy-six eligible early stage prostate cancer patients (Gleason score of 6 or below) between 50 and 80 years were supplemented with 60 mg of soy isoflavones (genistein) as a soy beverage per day (Protein Technologies International, St. Louis, MO, USA) or isocaloric placebo daily for 12 weeks (Kumar et al., 2004). Changes in steroid hormones (free and total estradiol, free and total testosterone and sex hormone-binding globulin (SHBG)) and prostate-specific antigen (PSA), implicated in prostate cancer promotion, were analysed at baseline and post-intervention. Fifty-nine patients completed the 12-week intervention. Seventeen of the group of subjects dropped out of the study including 9 from the placebo group and 8 from the isoflavone group. Relevant reasons for dropping out of the study included constipation (8), other medical reasons (1), diarrhea (1), and abdominal bloating (3) (in which group was not specified). Serum free testosterone was reduced or showed no change in 61% of subjects in the isoflavone group compared with 33% in the placebo group. Serum total PSA decreased or was unchanged in 69% of the subjects in the isoflavone treated group compared with 55% in the placebo group. Nineteen percent of subjects receiving soy isoflavones reduced total PSA by two points or more during the intervention period. No increase in SHBG levels was seen. However, none of the mean changes between the two groups were statistically significant. According to the authors, the data suggested that supplementing early stage prostate cancer patients with soy isoflavones, even in a study of short duration (12 weeks), altered surrogate markers of proliferation, such as serum PSA and free testosterone, in a larger number of subjects in the isoflavone supplemented group than the group receiving placebo. Therefore, isoflavones could potentially delay onset of histologic disease in this patient population.

To determine the clinical effects of soy isoflavones on prostate cancer (Pca), an American pilot intervention study was conducted in patients with Pca who had rising serum prostate-specific antigen (PSA) levels (Hussain et al., 2003). Patients with Pca were enrolled in the study if they had either newly diagnosed and untreated disease under watchful waiting with rising PSA (group I) or had increasing serum PSA following local therapy (group II) or while receiving hormone therapy (group III). The study intervention consisted of 100 mg of soy isoflavone (Novasoy®, Archer Daniels Midland Company, Decatur, IL, USA) taken by mouth twice daily (total dose 200 mg/day) for a minimum of 3 or maximum of 6 months (median duration 5.5 months, range 0.8-6 months). The tablets contained 40% glycosylated (conjugated) and 60% aglycones (unconjugated) isoflavones. The ratios of genistein:daidzein:glycitein were 1.1:1:0.2. No control group was included. Forty-one patients were enrolled (4 in group I, 18 in group II and 19 in group III) and had a median PSA level of 13.3 ng/ml. Thirty-nine patients could be assessed for response. Serum genistein and daidzein levels increased during supplementation from 0.11 to 0.65  $\mu\text{M}$  ( $P = 0.00002$ ) and from 0.11 to 0.51  $\mu\text{M}$  ( $P = 0.00001$ ), respectively. A total of 190 patient-months of supplementation were given to the patients enrolled in this study. Soy isoflavone supplement was very well tolerated, with no toxicity attributable specifically to Novasoy treatment. No grade III or worse adverse events were observed. Although nonspecific grade I–II adverse events were reported, their relationship to the study intervention was unclear, and they appeared to be related to preexisting co-morbid conditions. There were no side-effects related to soy isoflavone's estrogenic effects, such as gynecomastia or change in hair growth. There were no digestive effects from study tablets. Although there were no sustained decreases in PSA qualifying for a complete or partial response, stabilization of the PSA occurred in 83% of patients in hormone-sensitive (group II) and 35% of hormone-refractory (group III) patients. There was a decrease in the rate of the rise of serum PSA in the whole group ( $P = 0.01$ ) with rates of rise decreasing from 14 to 6% in group II ( $P = 0.21$ ) and from 31 to 9% in group III ( $P = 0.05$ ) following the soy isoflavone intervention. No significant changes were observed in serum levels of testosterone, insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3) or 5-hydroxymethyldeoxyuridine (5-OHmdU). The authors concluded that the data suggested that soy isoflavones may benefit some patients with Pca.

Safety of soy isoflavones in healthy men ( $n = 30$ , aged 40-69 years) was also examined by Busby et al. (2002) (see 2.3.1.1 for pharmacokinetics) using two preparations (formulation A and formulation B) in capsules containing genistein, daidzein and glycitein in different proportions. Formulation A contained  $\geq 97\%$  total unconjugated isoflavones consisting of  $90 \pm 5\%$  genistein, 10% daidzein and 1% glycitein, whereas formulation B contained 70% unconjugated isoflavones consisting of 43% genistein, 21% daidzein and 2% glycitein. Each man was administered a single dose of the five doses studied ( $n = 6$ , 3 for each formulation). The doses of genistein were 1, 2, 4, 8 and 16 mg/kg bw and the doses of daidzein varied between 0.11 and 1.8 mg/kg bw in formulation A, and between 0.49 and 7.8 mg/kg bw in formulation B. Clinical laboratory tests and urinalysis were performed at screening and on days 1 (predose and 24 h postdose), 3, 6, 14 and 30. Chest X-rays and electrocardiograms were done at screening and on day 30. Four reported adverse events

with a grade I (according to National Cancer Institute (NCI)'s Common Toxicity Criteria) were judged to be possibly related to isoflavone administration because of similar toxicity reported in animals (2 loss of appetite (at 4 and 8 mg/kg bw), 1 pedal edema (at 4 mg/kg bw) and 1 abdominal tenderness (at 8 mg/kg bw), all except one episode of loss of appetite occurred with formulation B. Eight adverse events with grade  $\geq$ II were judged to be possibly related to isoflavone administration because there was no other obvious explanation (2 cases of elevated lipase (at 2 and 8 mg/kg bw, both with formulation A), 1 case of elevated amylase (at 2 mg/kg bw, with formulation A), 1 case of leukopenia (at 16 mg/kg bw, with formulation B) and 4 episodes of hypophosphatemia (1 case at 8 mg/kg bw with formulation A, 2 cases at 4 mg/kg bw, with formulation B, 1 case at 8 mg/kg bw, with formulation B). None of these events were associated with any clinical toxicity. There were 14 adverse events with a grade I (tingling of skin, dry mouth, wheezing, anxiety, headache, nausea, back pain etc.) and 8 events of grade  $\geq$ II (elevated blood lipase, hyperkalemia, leukopenia, elevated bilirubin, associated with other known health conditions in the individuals) that were judged to be unrelated to isoflavone administration. No estrogenic or antiestrogenic symptoms were observed. The authors concluded that no clinically significant behavioral or physical changes were observed after isoflavone administration.

A study in UK determined the effects of a short-term phytoestrogen supplement on semen quality and serum sex steroid and gonadotrophin levels in healthy human men ( $n = 15$ , aged 18-35 years) (Mitchell et al., 2001). Healthy volunteers took a supplement as tablets (Regen™, Novogen Limited, North Ryde; New South Wales, Australia) containing 40 mg of total isoflavones (genistein, daidzein and glycitein, distribution of each not given) daily for 2 months and donated blood and semen samples monthly for 2 months before and 4 months after supplementation. No control group was included. Semen samples were analysed for ejaculate volume, sperm concentration, total sperm count, motility and morphology. Blood samples were analysed for sex hormone and gonadotrophin levels and phytoestrogen concentrations, and testicular volume was measured using an orchidometer. The phytoestrogen supplement increased plasma genistein and daidzein concentrations to approximately 1  $\mu$ M and 0.5  $\mu$ M respectively; yet, there was no observable effect on measurements of estradiol, testosterone, follicle-stimulating hormone (FSH) or luteinizing hormone (LH), or testicular volume or semen parameters (ejaculate volume, sperm concentration, count, motility or movement) over the study period. Seven of the 14 participants were equol producers and reached an average plasma concentration of 0.15  $\mu$ M equol. The authors concluded that the phytoestrogen dose consumed had no effect on semen quality. One subject withdrew from the study midway through the supplementation period and so was excluded from the final analysis, three subjects did not attend for the final post-supplementation assessment. No adverse effects were reported in the paper.

An American study evaluated the amount of soy protein needed to reduce blood lipids in moderately hypercholesterolemic men (Teixeira et al., 2000). Eighty-one men (age mean  $\pm$  SD: from 41.9  $\pm$  12.3 to 49.8  $\pm$  12.9 years in the various groups; not significantly different between groups) with moderate hypercholesterolemia (total cholesterol concentration between 5.70 and 7.70 mmol/l) were studied. After a 3-week lead-in on a Step I diet (<30%

of energy from fat, <10% of energy from saturated fat and <300 mg cholesterol/day), total cholesterol was measured and subjects were randomly divided into 5 groups. For 6 weeks, each group received 50 g protein/day, which included isolated soy protein (ISP, Supro Plus 675HG; Protein Technologies International, St. Louis, MO, USA, with 1.9 mg total isoflavone aglycone units/g protein) and casein (in the form of calcium caseinate (Alanate 391; New Zealand Milk Products, Wellington, New Zealand), respectively, in the following amounts: 50:0 g (95 mg isoflavones/day), 40:10 g (76 mg isoflavones/day), 30:20 g (57 mg isoflavones/day), 20:30 g (38 mg isoflavones/day), and 0:50 g (0 mg isoflavones/day, control group). The content of individual isoflavones was not given. The test proteins were incorporated into a variety of baked products and ready-to-mix beverages (Protein Technologies International), which the subjects received at breakfast 5 days/week. The study was performed with 3 separate cohorts, each of which participated for 9 weeks: cohort 1 (n = 27), cohort 2 (n = 24) and cohort 3 (n = 41). Blood was collected at baseline and weeks 3 and 6 of the intervention. At week 6, significant reductions (P < 0.05) from baseline compared with the control group were found for non-high density lipoprotein (HDL) (i.e. very low density lipoprotein (VLDL) cholesterol and low density lipoprotein (LDL) cholesterol) and total cholesterol and apolipoprotein (apo) B for all ISP groups (except total cholesterol with 40 g ISP), all of which were considered beneficial effects. At week 3, significant reductions (P < 0.05) were found in apo B for the groups that consumed  $\geq 30$  g ISP and in non-HDL cholesterol for the groups that consumed  $\geq 40$  g ISP. HDL-cholesterol, apo A-I, lipoprotein (a), and triacylglycerol concentrations were not significantly affected by dietary treatment. The authors concluded that the findings showed that consuming as little as 20 g soy protein/day instead of animal protein for 6 weeks reduced concentrations of non-HDL cholesterol and apo B by  $\sim 2.6\%$  and  $2.2\%$ , respectively. For cohorts 1, 2 and 3, the numbers of subjects who dropped out were 2, 2 and 4, respectively. Two subjects reported signs of possible allergy to soy protein and the other subjects chose to withdraw from the study. Eighty-four subjects completed the study. Of these, 3 subjects in cohort 1 were excluded from the statistical analyses: 2 failed to comply with the Step I diet and 1 had a weight loss >3 kg. No subjects in cohorts 2 or 3 were excluded from the statistical analyses. No other negative effects were reported.

#### 2.4.2.3.7 Case reports

A case report indicated that a soy protein powder supplement (EAS Sports Nutrition, Abbott, Columbus, OH, USA) may be related to drug-induced liver injury (DILI) in a 48-year-old previously healthy man without a significant past medical history (Pillai and Thapar, 2015). Two months after he began dieting, exercising and taking 20 mg of the soy protein supplement (no information was given on content and composition of isoflavones) daily he noticed urine and stool color changes, right upper quadrant tenderness, fatigue and jaundice. He had no family history of liver disease or colorectal cancer, no history of alcohol or IV drug use and was negative for autoimmune hepatitis, viral hepatitis, Wilson's disease and  $\alpha$ -1 antitrypsin deficiency. Laboratory tests showed increased aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin and direct bilirubin. Liver biopsy demonstrated subacute hepatitis with massive collapse of portal tract and lobules,

inflammatory activity and necrosis in 30% of the hepatic parenchyma. Laboratory tests 105 days after drug discontinuation showed improved liver function tests with lowered values of liver enzymes, and he reported increased energy and was off all diuretic medication. He had a RUCAM (the Roussel Uclaf Causality Assessment Method) score of 10, supporting the conclusion that the liver injury was likely related to the supplement use.

A case report indicated that soy product consumption may be related to hypogonadism and erectile dysfunction in men (Siepmann et al., 2011). A 19 year old man with type 1 diabetes mellitus, but otherwise healthy, experienced sudden onset of loss of libido and erectile dysfunction after the start of ingestion of large quantities of soy-based products in a vegan-style diet equaling 360 mg of isoflavones (no information was given on composition) per day (5.14 mg/kg bw per day based on 70 kg body weight (not stated, but BMI was 22 kg/m<sup>2</sup>)) taken for one year. Blood concentrations of free and total testosterone were initially decreased, whereas dehydroepiandrosterone (DHEA) was increased. These parameters normalized within 1 year after cessation of the vegan diet. Normalization of testosterone and DHEA levels was paralleled by a constant improvement of symptoms; full sexual function was regained one year after cessation of the vegan diet. For comparison, the average dietary intake of isoflavones in four European countries was less than 1 mg/day (van Erp-Baart et al., 2003).

A case report indicated that a 60-year old man developed bilateral gynecomastia (a benign enlargement of the male breast attributable to proliferation of the ductular elements of the breast) and dramatically elevated estrogen levels after daily intake of 3 quarts of soy milk (361 mg isoflavones per day, no information was given on composition) for approximately 1.5 years (Martinez and Lewi, 2008). He was referred to a clinic after 6 month's duration of the gynecomastia. Later, he also took another non-lactose soy product for <1 year. He also reported erectile dysfunction and decreased libido, but no changes in testicular size, no history of testicular trauma and no sexually transmitted diseases. Laboratory tests showed estrone and estradiol concentrations to be 4-fold increased above the upper limit of the reference range. Testicular ultrasonography, computed tomography of the chest, abdomen and pelvis and positron emission tomography were normal. After the patient stopped the intake of soy milk and the other non-lactose soy product, the breast tenderness resolved and his estradiol concentration slowly returned to normal.

A case report involved a 30-year old Italian man with severe oligospermia (10 million/ml) and abnormal sperm motility and morphology who was given soybean isoflavone tablets (80 mg/day, containing 40-45% genistein, 40-45% daidzein and 10-20% glycitein) for 6 months (Casini et al., 2006). He and his partner had been trying to conceive for 3 years, and the woman was healthy at the clinical and endocrinologic examination. No other parameters except sperm count, motility and morphology were altered in the man. During the third month of supplementation, semen parameters improved dramatically (sperm count, 45 million/ml; >50% motility; >30% normal sperm morphology); therefore, intrauterine insemination was performed. This treatment resulted in pregnancy, and a healthy baby weighing 3300 g was born. After 6 months of treatment, sperm parameters maintained their

improvement (sperm count, 50 million/ml; >50% motility; >35% normal sperm morphology). However, 6 months after termination of isoflavone supplementation, sperm parameters had deteriorated (sperm count, 18 million/ml; <20% motility; <10% normal sperm morphology). The authors commented that their results suggested a possible therapeutic role for phytoestrogens in the treatment of oligospermia, but also noted that a randomized controlled trial was needed to confirm their findings. No adverse effects were reported in the paper.

#### 2.4.2.3.8 Other articles

The estrogen-like effects of isoflavones have raised concern that soy foods exert feminizing effects in men, such as causing gynecomastia and reduced circulating testosterone levels and sperm concentration. The totality of evidence, especially the clinical data, indicate that this concern is unwarranted, since feminizing effects are not observed in response to isoflavone exposure equal to or greatly exceeding typical Asian intake regardless of whether exposure occurs via supplements or traditional soy foods (reviewed in (Messina, 2010b)). It was stated in this review that "In contrast to the results of some rodent studies, findings from a recently published metaanalysis and subsequently published studies show that neither isoflavone supplements nor isoflavone-rich soy affect total or free testosterone levels. Similarly, there is essentially no evidence from the nine identified clinical studies that isoflavone exposure affects circulating estrogen levels in men. Clinical evidence also indicates that isoflavones have no effect on sperm or semen parameters, although only three intervention studies were identified and none were longer than 3 months of duration. Finally, findings from animal studies suggesting that isoflavones increase the risk of erectile dysfunction are not applicable to men, because of differences in isoflavone metabolism between rodents and humans and the excessively high amount of isoflavones to which the animals are exposed."

#### 2.4.2.3.9 Summary and discussion of effects of isoflavones on men

The included studies on men are summarized in Table 9.3 in Appendix 9.3.

##### *Sources of isoflavones*

Sources of isoflavones examined in the studies on men were reported to be soy protein, soy protein isolate (SPI) or isolated soy protein (ISP), soybeans, soy foods such as soy milk, flour, grits or tofu, as commercial products or prepared meals, isoflavone concentrate or just referred to as isoflavones, or as genistein combined polysaccharide (GCP), which is enriched in aglycone forms of isoflavones, percentages of genistein, daidzein, glycitein, and in some studies also as percentages of genistin, daidzin, glycitin and the malonyl- and acetyl-derivatives. The isoflavones were given in the form of tablets, pills, capsules, powders or concentrates, sometimes mixed into scones or beverages, or the form was not specified. The chemical form of isoflavones was reported (if stated) as aglycones, aglycone equivalents, glycosides or a mixture. The ratio of percentage of individual isoflavones genistein:daidzein:glycitein varied substantially among the studies; 15-95:10-55:1-30. No

pattern was obvious regarding type of soy product or isoflavone composition and negative health effects.

#### *Adverse events/side effects*

In the studies that gave detailed reports on adverse events/side effects, the most common complaints were mild gastrointestinal effects, such as diarrhea, nausea, flatulence or constipation, or sometimes weight gain or estrogenic effects (breast changes, increased frequency of hot flashes), without clinical toxicity or organ dysfunction. However, Miyanaga et al. (2012) reported one grade III adverse event, i.e. iliac artery stenosis, in an older Japanese man at risk of prostate cancer.

#### *Effects on sperm parameters*

Among the RCTs (n = 8) studying healthy men, one study showed no effects on various sperm parameters and did not report any other negative effects after  $1.64 \pm 0.19$  and  $61.7 \pm 7.35$  mg/day of isoflavones for three periods of 57 days intervention separated by 28 days wash-out periods in American men (Beaton et al., 2010). Mitchell et al. (2001) found no negative effects on semen quality parameters after 2 months exposure to 40 mg/day of isoflavones in a clinical study of men in UK. However, Chavarro et al. (2008) reported in a cross-sectional study on Slovakian men a significant inverse association between soy food intake in the previous 3 months giving a mean intake of 5.4 mg/day of dietary isoflavones and reduced sperm concentrations in a group of men where 72% were overweight/obese and partners in subfertile couples. The same was found to be significant for the trend on glycitein intake, whereas for genistein and daidzein the associations were similar, but not significant. A meta-analysis on healthy men reported an inverse association between changes in sperm concentrations and isoflavone intake in USA (decreased sperm concentrations and increased isoflavone intake) and in China (increased sperm concentrations and decreased isoflavone intake) over time (Lim and Shaw, 2016).

#### *Effects on hormone levels*

One RCT showed no effects on hormone levels and did not report any other negative effects after  $1.64 \pm 0.19$  and  $61.7 \pm 7.35$  mg/day of isoflavones for three periods of 57 days intervention separated by 28 days wash-out periods (Dillingham et al., 2007). Mitchell et al. (2001) found no negative effects on levels of several hormones after 2 months exposure to 40 mg/day of isoflavones in a clinical study. However, three RCTs reported effects of isoflavones on hormone levels. Nagata et al. (2001) reported decreased estrone levels after 8 weeks on  $76.8 \pm 16.6$  mg/day of isoflavones, Habito et al. (2000) found increased levels of SHBG and decreased FAI and testosterone:estradiol ratio after 4 weeks on 118.9 mg/day of isoflavones in tofu, and Gardner-Thorpe et al. (2003) reported decreased total serum testosterone after 6 weeks on 120 mg/day isoflavones. Some non-RCT studies also gave relevant information about doses of isoflavones affecting hormone levels. As opposed to the RCT by Dillingham et al. (2007) mentioned above, the same group reported in a clinical study various changes in hormone levels (decreased DHT and DHT:testosterone ratio and testosterone, increased DHEAS, estradiol and estrone) after both  $1.64 \pm 0.19$  and  $61.7 \pm 7.35$  mg/day of isoflavones from soy protein isolate for three periods of 57 days intervention



separated by 28 days Dillingham et al. (2005). In a cross-sectional study, Nagata et al. (2000) reported that serum estradiol concentrations were significantly inversely correlated with dietary soy product intake giving  $21.9 \pm 8.7$  mg/day of isoflavones, as well as non-significantly inversely associated with serum estrone, total and free testosterone. Three other clinical studies also reported effects of isoflavones on hormone levels. Tanaka et al. (2009) found increased serum SHBG and decreased free testosterone and DHT in men after 3 months on 60 mg/day of isoflavones. Goodin et al. (2007) reported decreased testosterone (although within normal range) after 28 days on 56 g soy protein isolate (i.e. approximately 196 mg/day of isoflavones, assuming that 1 g soy protein gives 3.5 mg of isoflavones, as stated in Hamilton-Reeves et al. (2010)). A clinical study by Ostatnikova et al. (2007) found that intake of soybeans (isoflavone intake unknown) did not change salivary testosterone or plasma estradiol levels. However, they reported that the effects of soy on hormonal parameters were dependent on basal testosterone levels; it increased significantly after a low basal level, but decreased non-significantly after a high basal level. A meta-analysis of 34 papers studying intake of 0-71 g/day soy protein and 20-900 mg/day of isoflavone as aglycones from 1 week to 4 years (average duration 74 days) in men, including prostate cancer patients (16 studies), found no significant effects of soy protein or isoflavone intake on total testosterone, SHBG, free testosterone or FAI (Hamilton-Reeves et al., 2010).

#### *Effects on vitamin E levels and lipoperoxidation*

One RCT reported reduced levels of vitamin E after 3-4 weeks with 20 g/day of soy protein isolate (approximately 70 mg/day of isoflavones, assuming that 1 g soy protein gives 3.5 mg of isoflavones, as stated in (Hamilton-Reeves et al., 2010; Higashi et al., 2001)). Celec et al. (2013) reported that soybeans (isoflavone content unknown) increased lipoperoxidation in men, although total antioxidant capacity was increased.

The two remaining RCTs found reported no negative effects. No negative effects on CAM concentrations and no other negative effects were reported after 8 weeks of 30.1 or 91.4 mg/day of isoflavones (Dettmer et al., 2012). A RCT on cognitive functions in both young men and women did not report any negative effects on cognition or on any other end points after 10 weeks on 0.5 or 100 mg/day of isoflavones (File et al., 2001).

#### *Effects on cancer or other conditions*

A large number of studies were also found on effects of soy food products or isoflavones on non-healthy men, i.e. patients with prostate cancer or with increased PCA at risk for prostate cancer, hepatocellular carcinoma or bladder cancer, men with adenomatous colorectal polyps, hypercholesterolic men and men with metabolic syndrome (see Table 9.3 in Appendix 9.3). Also in these studies, effects on sex hormone levels were reported (considered beneficial in men with or at risk for prostate cancer). Two studies in Chinese men, of uncertain relevance for this risk assessment, reported an increase in relative risk of bladder cancer associated with estimated dietary soy food, soy protein or isoflavone intake (Sun et al., 2002; Sun et al., 2004). Shin et al. (2015) reported a decreased risk of colorectal cancer associated with the highest quartile of dietary soy isoflavone intake (Q4) vs. the lowest quartile (Q1), but an increased risk was found associated with Q2 and Q3. A meta-

analysis of colorectal cancer found no significant association, positive or negative, with soy consumption from food in men (Yan et al., 2010).

To summarize, the most common adverse events/side-effects of isoflavones reported in healthy men were mild gastrointestinal symptoms, or sometimes weight gain, estrogenic effects or reduction in vitamin E levels. Hormone levels appeared to be affected by doses of isoflavones ranging from 1.64 to 196 mg/day for one to three months. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy men. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In patients with prostate cancer or at high risk for recurrence of prostate cancer and possibly in patients with other types of hormone-related cancers, isoflavones may have a beneficial effect on the progression of the disease by decreasing testosterone. VKM assumes that such therapy with isoflavone administration is given under prescription and medical surveillance, and is outside the scope of this risk assessment, which is the risk for the general Norwegian population from isoflavones taken as supplements. Useful information can also be found in studies on prostate cancer patients and men with other diagnoses and is therefore included. It was shown that isoflavones in doses of 450 or 900 mg/day for three months could have estrogenic effects in men with prostate cancer, such as breast changes or increased frequency of hot flashes (Fischer et al., 2004). In healthy men taking supplements without surveillance, isoflavones may potentially also lead to estrogenic effects. In this risk assessment of isoflavones in healthy men, VKM considers changes in hormone levels away from the normal range as negative effects. There is some data indicating that it is soy protein as such rather than the isoflavones that are causing the observed effects on hormone levels (Dillingham et al., 2005), however, this is still uncertain.

Based on the available studies, isoflavones as supplements in doses of 1.64 to 196 mg/day for one to three months may represent a risk of negative effects on hormone levels in men. These doses do not appear to have other significant negative effects on men in the general population.

#### ***2.4.2.4 Effects of isoflavones on children and adolescents***

No studies were found that reported effects of isoflavones on children (aged 10 to <14 years) or on adolescents (aged 14 to <18 years) exposed at these ages to isoflavones from food or supplements. There were some publications available that examined effects of infant isoflavone exposure from soy infant formula or soy milk at 3-12 months of age, adolescence or as young adults, and from dietary exposure to isoflavones as adolescents associated with breast cancer as adults. The studies on children and adolescents are summarized in Table 9.4 in Appendix 9.4.

##### **2.4.2.4.1 Exposure to isoflavones from soy as infants**

Soy formulas are being used as nutrition for infants in many countries. There is a theoretical possibility that the individuals fed endocrine active soy formula during critical stages of

development of reproductive and other organ systems could display reproductive disorders or other negative health effects later in life.

A study investigated if soy formula feeding during infancy disrupted reproductive system development, resulting in altered menstrual bleeding in adulthood (age 23-34 years), in a cohort of 1696 young African American women in USA using enrolment data from the Study of Environment, Lifestyle, & Fibroids (2010-2012) (Upson et al., 2016). Questionnaire data on soy formula feeding (information on isoflavone exposure, content and composition was not given) were available for 1553 participants, 89% based on mother's report. Menstrual bleeding outcomes, including those indicative of heavy menstrual bleeding, were ascertained by interview and compared between participants ever fed (n = 198) and never fed (n = 1355) soy formula. The associations observed indicated a history of heavy menstrual bleeding; ever experiencing heavy, gushing-type bleeding (RR: 1.2, 95% CI: 1.0, 1.4), ever use of non-steroidal anti-inflammatory drugs (NSAIDs) for heavy bleeding (RR: 1.3, 95% CI: 1.0, 1.6) and ever use of a contraceptive method for heavy bleeding (RR: 1.2, 95% CI, 0.9, 1.6). Among the subset of participants with menses in the past year who did not use medication that may alter menstrual flow (n = 762), the data suggested that soy formula feeding was associated with heavier flow and the adverse impact of menstrual bleeding on quality of life, but CIs were wide. The authors concluded that the data suggested that soy formula feeding was associated with heavy menstrual bleeding, and supported the idea that infancy is a susceptible developmental window for female reproductive function.

Age of menarche (beginning of the menstrual function) was studied after early life exposure in subjects that were participants in a prospective, longitudinal study in UK (ALSPAC), where mothers were enrolled during pregnancy and their children were followed prospectively (Adgent et al., 2012). Early life feeding regimes, categorized as primarily breast, early formula, early soy and late soy, were defined using questionnaires administered during infancy. No information on isoflavone exposure, content and composition was given. Age of menarche was assessed through questionnaires administered approximately annually between ages 8 and 14.5 years, to term, singleton, white females. Of 2920 girls, approximately 2% of mothers reported that soy milk or formula were introduced into the infant diet at or before 4 months of age and continued until 6 months (early soy, n = 54), or soy milk or formula were introduced between 5 and 15 months (late soy, n = 111). The control groups were infants who were breast-fed until  $\geq 6$  months, had no soy before 24 months and no other milk/formula before 6 months (primarily breast-fed, n = 631) and infants having any non-soy milk or formula at or before 4 months, continued up to 6 months and no soy before 24 months (early-formula-fed, n = 2124). The median age of menarche (interquartile range (IQR)) in the study sample was 153 months (144-163), approximately 12.8 years. The median age of menarche among the early soy fed girls was 149 months (12.4 years (IQR 140-159)). Compared with girls fed non-soy based infant formula or milk (early formula), early soy fed girls were at 25% higher risk of menarche throughout the course of follow up (Hazard Ratio 1.25, CI 0.92, 1.71). For the remaining characteristics evaluated, early formula and early soy exposure groups were similar. The authors concluded that girls fed soy products in infancy may have an increased risk of menarche specifically in

early adolescence, and further that these findings may be the observable manifestation of mild endocrine disrupting effects of soy isoflavone exposure. However, they also point out that the study was limited by few soy-exposed subjects and was not designed to assess biological mechanisms.

A study examined developmental status (mental, motor and language) of infants fed breast milk, cow's milk or soy formula (no information on isoflavone exposure, content and composition was given) in USA (Andres et al., 2012). They were given soy protein-based formula from 2-12 months, cow's milk formula from 2-12 months or breast milk at least from 2-6 (or preferably up to 12) months. Healthy infants (n = 391) were assessed longitudinally at ages 3, 6, 9 and 12 months. Development was evaluated by using the Bayley Scales of Infant Development and the Preschool Language Scale-3. The study showed that all scores on developmental testing were within established normal ranges and that no significant differences were found between formula-fed infants; milk-based formula versus soy protein-based formula. In addition, the study demonstrated a slight advantage of breast-fed infants on cognitive development compared with both types of formula-fed infants. The soy protein formulas used were Similac Soy Isomil from Abott Nutrition (Columbus, OH, USA) or Enfamil Prosoabee from Mead Johnson (Evansville, IN, USA). The soy protein content of the formulas was not given in the paper, but found on the producers' websites. The Similac soy-protein formula contained 2.45 g protein/100 kcal, and of the ingredients soy protein isolate was 14% (<https://similac.com/baby-formula/similac-soy-isomil>). The Enfamil Prosoabee soy-protein formula contained 2.5 g protein/100 kcal, and of the ingredients soy protein isolate was 14% (<http://www.enfamil.com/products/solutions-feeding-issues/enfamil-prosoabee>).

A retrospective (historical) cohort study in USA among adults aged 20-34 years in 1999, who as infants participated in controlled feeding studies during 1965-1978, examined the association between infant exposure to soy formula and health in young adulthood, with emphasis on reproductive health, i.e. estrogen-related outcomes (Strom et al., 2001). Of the participants, 248 were fed soy formula and 563 were fed cow milk formula during infancy. The isoflavone levels were not measured in the soy formula in this study. The authors estimated the isoflavone content from formula content of soy protein isolate (in 1997), which were 32-47 mg/l, giving isoflavone intake of 4.2-9.4 mg/kg bw per day for the first 16 weeks of life, or isoflavone intake from soy flour at 9-16 mg/kg bw per day. The main outcomes were self-reported pubertal maturation, menstrual and reproductive history, height and usual weight, and current health. No statistically significant differences were observed between groups in either women or men for more than 30 outcomes. However, women who had been fed soy formula reported slightly longer duration of menstrual bleeding (adjusted mean differences 0.37 days; 95% CI 0.06-0.68), with no difference in severity of menstrual flow. They also reported greater discomfort with menstruation (unadjusted relative risk for extreme discomfort vs. no or mild pain 1.77; 95% CI 1.04-3.00). More persons who had used asthma or allergy drugs were seen among adults aged 20-34 years having received soy formula (n = 248) vs. cow milk formula (n = 563) as infants; P = 0.08 for men, but P = 0.047 for women (Strom et al., 2001). The authors concluded that soy formula did not

appear to lead to different general health or reproductive outcomes than exposure to cow milk formula, up to the age studied (20-34 years).

#### 2.4.2.4.2 Exposure to isoflavones from soy as adolescents

There may be a theoretical concern that high consumption of isoflavones from food products or supplements may exert adverse hormonal effects on adolescents under development (Reinwald and Weaver, 2006). However, there is very little hard data to evaluate the impact of isoflavones on adolescents, especially in Western populations.

A population-based prospective cohort study of Chinese women studied mostly beneficial effects on breast cancer of soy intake determined by a validated quantitative food frequency questionnaire (Lee et al., 2009). In pre-menopausal women, there was apparently a reduced risk of breast cancer (305 cases) associated with adolescent (age 13-15 years) isoflavone intake (composition was not given) (RR: 0.89, 95% CI 0.57, 1.40, Ptrend = 0.452), for trend over quintiles, median intakes in Q1 and Q5 were 4.31 and 42.26 mg/day of isoflavones, respectively. However, they found that in post-menopausal women there was a slight positive association between breast cancer (289 cases) and adolescent isoflavone intake (RR: 1.38, 95% CI 1.00, 1.91, P = 0.038), indicating a potentially increased risk of breast cancer associated with adolescent isoflavone intake.

A Canadian population-based case-control study where the cases were women aged 25-74 years with pathologically confirmed breast cancer and the controls were age-matched women from the same area, studied mostly beneficial effects on breast cancer estrogen (ER) and progesterone (PR) receptor subtypes of intake of total isoflavones (genistein, daidzein, glycitein (soy) and formononetin (non-soy); amounts of each not given), total lignins (secoisolariciresinol, matairesinol, pinoresinol and lariciresinol) and total phytoestrogens (isoflavones, lignans and coumestans) determined by a food frequency questionnaire (FFQ) (Anderson et al., 2013). They found that in post-menopausal women there was a negative association between breast cancer and adolescent total isoflavone intake ( $\geq 21$   $\mu\text{g}/\text{day}$ ) for ER+PR+ cases (highest vs. lowest tertile: OR = 0.81, 95% CI 0.67-0.98, Ptrend = 0.09) and for ER+PR- cases (highest vs. lowest tertile: OR = 0.68, 95% CI 0.51-0.90, Ptrend = 0.01), indicating decreased risk for these breast cancer subtypes with adolescent total isoflavone intake. For total phytoestrogens ( $\geq 234$   $\mu\text{g}/\text{day}$ ), the result was similar: for ER+PR+ cases (highest vs. lowest tertile: OR = 0.79, 95% CI 0.65-0.96, Ptrend = 0.04). In pre-menopausal women and in women not stratified by menopause status, the associations with adolescent intake of total isoflavones or total phytoestrogens and breast cancer risk were not statistically significant. Thus, based on results from the same study, adolescent intake of total isoflavones or total phytoestrogens seem to decrease the risk of certain subtypes of breast cancer, whereas adult exposure may seem to increase the risk (see 2.4.2.1.4).

#### 2.4.2.4.3 Summary of effects of isoflavones on children and adolescents

The included studies on children and adolescents are summarized in Table 9.4 in Appendix 9.4.

Exposure of infants to soy formula and/or soy milk containing mostly unknown levels and composition of isoflavones appeared to be associated with heavier menstrual bleeding (Upson et al., 2016) with slightly longer menstruation duration (Strom et al., 2001) in early adulthood and earlier age of menarche at puberty (Adgent et al., 2012). Two studies examined the associations between isoflavone intake in adolescence estimated from FFQs and breast cancer as adults. One of the studies indicated a negative association in post-menopausal women (Anderson et al., 2013), and another study, a slight positive association in post-menopausal, but a negative association in pre-menopausal women (Lee et al., 2009).

No studies were found on effects of isoflavones from exposure to children (10 to <14 years).

No studies were found addressing both exposure to and effects of isoflavones in adolescents (14 to <18 years). However, there was no evidence in the included literature indicating that adolescents (aged 14 to <18 years, i.e. mostly after puberty) are more sensitive to isoflavones than adults. Therefore, VKM finds that the results for pre-menopausal women and men have validity also for adolescents.

Based on the lack of studies on children, there is not sufficient data to draw any conclusions on potential adverse effects of isoflavones in supplements in children (aged 10 to <14 years).

Based on the available studies, isoflavones as supplements in doses of 1.64 to 196 mg/day or in doses of 45 to 116.4 mg/day for one to three months may represent a risk of negative effects on hormone levels in adolescents of both genders and/or menstrual function in adolescent women, respectively. These doses do not appear to have other significant negative effects on adolescents.

#### ***2.4.2.5 Interactions***

It has been shown that interactions may occur among the isoflavones. Flavonoids with catalytic DNA topoisomerase inhibitory activity, such as daidzein, strongly antagonised the clastogenicity of DNA topoisomerase II poisons, such as genistein, in Chinese hamster V79 cells (Snyder and Gillies, 2003).

According to COT (2003), the potential for isoflavone-drug interactions has not been established. However, a recent paper gives an overview of some drugs, including prescription medicines, that may be affected by isoflavones via their modulation of phase I and phase II metabolic enzymes and interaction with drug transporters (Taneja et al., 2016). This is of potential importance for individuals consuming isoflavone supplements while taking prescribed drugs.

#### ***2.4.2.6 Allergic sensitisation (including adjuvant effects)***

Isolates from soybeans contain different amounts of soy protein, which is a well-known food allergen, with a reported incidence of 0.3-0.4% of the total population in Germany (BfR,

2007). It was also observed that people with a birch pollen allergy may have cross-allergy to soy protein. Oral allergy symptoms may range from mild symptoms such as itching and blisters to anaphylactic shock.

Based on the cases of food allergic reactions reported to the National Register of Severe Allergic Reactions to Food at the Norwegian Institute of Public Health during 2000-2015, 199 of the total of 1470 cases reported were analysed with serum-specific IgE to soy (13.5%) (Namork E et al., 2016). The majority of the cases, however, are presumed to be cross-sensitizations due to primary sensitization to birch pollen and/or peanut. Only 2.5% (5 of 199) cases were analysed with high levels of specific IgE to soy alone, and reported with adverse reactions after intake of soy in the food (personal communication with Ellen Namork, Norwegian Institute of Public Health).

In an American study evaluating the amount of soy protein needed to reduce blood lipids in moderately hypercholesterolemic men (Teixeira et al., 2000), two subjects reported signs of possible allergy to soy protein and withdraw from the study.

More persons who had used asthma or allergy medications were seen among adults aged 20-34 years having received soy formula (n = 248) vs. cow milk formula (n = 563) as infants; P = 0.08 for men, but P = 0.047 for women (Strom et al., 2001). This may indicate that soy intake in infancy may lead to increased risk of allergy, requiring a more frequent intake of antihistamine medication in adult life (Sirtori et al., 2005).

Whether supplements with isoflavones from may cause food allergy is not known.

### **2.4.3 Animal studies**

Although it has been shown that phytoestrogens, including isoflavones can affect reproductive and endocrine functions in varied species such as sheep, rat, cheetah, mink and fish (Ryokkynen et al., 2006; Setchell et al., 2001), there are some differences between animals and humans with regard to isoflavones that suggest that caution is warranted when extrapolation animal data to humans in risk assessment (Messina, 2005; Munro et al., 2003). For instance, while the gut flora of rats and mice are able to metabolise large quantities of daidzein to equol, only approximately one-third to one-half of humans is able to metabolize daidzein to equol (Rafii, 2015; Yuan et al., 2007). Degree of processing of the soy in rodent diets may affect the pharmacokinetics and pharmacodynamics of isoflavones from the soy (Allred et al., 2005), and phytoestrogens added to rodent diets are often not standardized in the studies.

Because of the differences in pharmacokinetics between humans and rodent species as described and the abundance of human studies available on isoflavones, toxicity data from experimental animals were not included in this risk assessment, except for *in vivo* genotoxicity data.

#### 2.4.4 *In vitro* studies

Production of thymosin- $\alpha$  1 by murine thymus epithelial cells *in vitro* was significantly inhibited dose-dependently (28-61%) by genistein in a concentration at or above  $3 \times 10^{-9}$  M to  $3 \times 10^{-6}$  M, indicating that genistein may modulate thymus hormone production (Sakabe et al., 1999). Since thymosin(s) are amplifiers of T-cell immunity, this may reduce cell-mediated immunity if it also occurs *in vivo*.

See also section 2.4.5.

#### 2.4.5 Mode of action for effects of isoflavones

##### 2.4.5.1 Endocrine effects

The most active natural physiological form of estrogen is estradiol (E2; 17 $\alpha$ -estradiol and 17 $\beta$ -estradiol), formed from aromatization of testosterone in multiple tissues, but predominantly in the ovaries of pre-menopausal women (Prossnitz and Arterburn, 2015). Additional forms of estrogen are estrone (E1), the least abundant estrogen, derived from aromatization of androstenedione, and estriol (E3), produced primarily during pregnancy from 16-hydroxydehydroepiandrosterone sulfate in the fetal liver and adrenal glands. There are at present at least three major receptors for estrogen, the classical estrogen receptor  $\alpha$  and  $\beta$ , i.e. ER $\alpha$  and ER $\beta$ , and G protein-coupled estrogen receptor (GPER), belonging to two distinct receptor families (Prossnitz and Arterburn, 2015). In addition to the full-length 66-kDa protein, splice variants of estrogen receptors have been described, resulting in shorter proteins. A diverse array of ligands are capable of binding to ERs producing multiple conformational states of the receptor ligand-binding domain that in turn generate multiple protein binding sites for coregulators and other proteins, which, with differential expression in specific tissues, results in very complex physiology of ERs and their ligands. Depending on ligand and tissue, ERs regulate both positively and negatively the expression of thousands of genes, given rise to a multitude of types and sizes of effects (Prossnitz and Arterburn, 2015).

Isoflavones may interact with endocrine pathways and potentially cause negative health effects. Phytoestrogens are considered weak estrogens, since the relative affinities of phytoestrogens to estrogen receptors are more than 100-1000-fold lower than that of estradiol (Albertazzi and Purdie, 2002; Cassidy, 2003). However, even so, some foods and dietary supplements contain comparatively high amounts of these compounds so that plasma levels may exceed endogenous estrogen levels by several orders of magnitude and therefore have the potential to exert biological effects *in vivo* (Cassidy, 2003). Genistein is a prototype ER $\beta$ -selective compound, exhibiting >20 fold higher affinity for ER $\beta$  (7-16 nM) than for ER $\alpha$  (330-910 nM) (Prossnitz and Arterburn, 2015). Genistein also exhibits high binding affinity for GPER (IC<sub>50</sub> = 133 nM), exhibiting a selective binding preference for GPER that is in between the classic ERs. Daidzein can be metabolized to equol in the presence of intestinal bacteria. There are two enantiomeric forms of equol possible, with the 3*S*-enantiomer exhibiting selectivity for ER $\beta$  (Prossnitz and Arterburn, 2015). Since the estrogen receptors



have different tissue distribution, isoflavones estrogenicity can vary markedly from tissue to tissue.

The isoflavones show conformational binding to the estrogen receptor that classifies them as natural selective estrogen receptor modulators (SERMs) rather than as estrogens, and have estrogenic and anti-estrogenic effects depending on the concentration of endogenous estrogen and amount and type of estrogen receptors (Yuan et al., 2007). An *in vitro* study showed that isoflavones can have estrogenic, agonistic or antagonistic activities depending on estrogen concentrations. They act as estrogen antagonists with a pre-menopausal (high) dose of estradiol, whereas they act as estrogen agonists in a low-estrogen environment near the serum level of post-menopausal women (Hwang et al., 2006). Metabolites of genistein (dihydrogenistein and 6'-hydro-*O*-demethylangolensin) and daidzein (dihydrodaidzein, *O*-desmethylangolensin, tetrahydrodaidzein, equol) can have higher or lower biological activity than their precursors. All the metabolites could act as antagonists that weakened the estrogenic actions at physiologic dose of estrogen. This inhibitory effect on estrogen action was more prominent with ER $\beta$  than with ER $\alpha$ . The ER-mediated effects of isoflavones occur at concentrations of 0.1–1  $\mu$ M (EFSA, 2015).

At present, there are work going-on in EU and internationally with the goal to reach consensus on what are to be considered adverse effects of endocrine active substances and to determine whether such effects have thresholds or not.

#### **2.4.5.2 Non-endocrine effects**

There are also hormone-independent actions of isoflavones (EFSA, 2015), including inhibition of tyrosine kinase activity (Akiyama et al., 1987), inhibition of protein kinase C (Osada et al., 1988), inhibition of DNA topoisomerase II (Abe, 1999), inhibition of mitogen-activated kinase 1 (MEKK1) (Sarkar and Li, 2003), antioxidant activity (Djuric et al., 2001; Gyoergy et al., 1964), anti-angiogenic effects (Fotsis et al., 1993) and inhibition of breast cancer resistance protein (BCRP), a cellular efflux protein (Imai et al., 2004). The non-endocrine effects were obtained with isolated compounds *in vitro* at doses typically exceeding 10  $\mu$ M (EFSA, 2015).

### **2.4.6 Potentially vulnerable groups**

#### **2.4.6.1 Polymorphisms in estrogen receptors and in metabolism of estrogens**

There is evidence that cardiovascular risk varies according to Er $\beta$ -AluI polymorphisms, with the AA genotype having a higher risk versus GG or GA genotypes (Mortensen et al., 2009). But it is also this group that benefit from dietary isoflavones in respect to lower plasma soluble vascular cell adhesion molecule (VCAM)-1 because it has been shown that estrogen and genistein may inhibit VCAM-1 expression in endothelial cells stronger in women with AA versus GG or GA genotypes.

A case-control study investigated whether polymorphisms in the estrogen receptors could modify the association between isoflavone intake and risk of histologically confirmed invasive

breast cancer in women aged 20-74 years (Iwasaki et al., 2009). Soy intake was estimated with a validated food frequency questionnaire. No consistent association was found between five single nucleotide polymorphisms in the estrogen receptor  $\alpha$  (rs9340799, rs1913474 and rs2234693) and  $\beta$  (rs4986938 and rs1256049). An inverse association was found between intake of isoflavones and breast cancer risk in post-menopausal Japanese women with the GG genotype of the rs4986938 polymorphism with OR = 0.47,  $P_{trend}$  = 0.01, for highest vs. lowest tertile, Japanese Brazilians with OR = 0.31 for the highest vs. lowest median, and non-Japanese Brazilians OR = 0.37 for consumers vs. non-consumers (P values for interactions were 0.11, 0.08 and 0.21, respectively). The findings suggested that polymorphism in the estrogen receptor  $\beta$  (ESR2 gene) may modify the association between isoflavone intake and breast cancer risk. No associations were found for the other four polymorphisms.

In Chinese women in Shanghai carrying at least one A allele of the rs605059 polymorphism in the 17 $\beta$ -hydroxysteroid dehydrogenase type I (17 $\beta$ -HSD1) gene, catalyzing metabolism of estrone (E1) to the biologically more active estradiol (E2), had a significant 18% reduction in risk of endometrial cancer compared with those without an A allele, primarily restricted to pre-menopausal women (odds ratio was 0.65 (95% CI 0.47-0.88) (Dai et al., 2007). Among these pre-menopausal women, soy isoflavone intake significantly interacted with this polymorphism in relation to endometrial cancer, and this inverse association between soy isoflavone intake and endometrial cancer appeared only among those women with at least one such A allele.

#### ***2.4.6.2 Polymorphisms in enzymes involved in metabolism and/or transport of isoflavones***

There is a vast interindividual variation in isoflavone metabolism, particularly because of differences in gut microflora, but also caused by genetic polymorphisms in metabolic enzymes. These differences may lead to disparate circulating levels of parent isoflavones and their metabolites, for instance >30-fold, 1500-fold and >1000-fold for genistein, daidzein and equol, respectively (Hamilton-Reeves et al., 2010). Approximately one-third to one-half of the population have a microflora that is able to metabolize daidzein to equol, and therefore are high-equol producers (Rafii, 2015; Yuan et al., 2007). They are therefore being exposed to higher levels of equol from daidzein than the non-equol producers, and are potentially vulnerable to additional intake of equol from supplements (COT, 2003). Isoflavones are metabolized by cytochrome P450 (CYP1A1/1A2/1B1, 2E1), catechol *O*-methyltransferase (COMT), glutathione *S*-transferase (GST) and uridine diphospho (UDP)-glucuronosyltransferase (UGT) enzymes and distinct variants acting on the same phytoestrogens produce different metabolites with varying bioactivities. A study finding reduced risk of colorectal cancer (CRC) with phytoestrogen intake (isoflavones and lignans), did not find that the reduction of CRC was markedly modified by polymorphisms in genes of enzymes suspected of being involved in phytoestrogen metabolism (Cotterchio et al., 2006). However, another study showed that women in Shanghai with G/G genotype of the UDP-glucuronosyl transferase UGT1A1 polymorphism rs2070959 had lower risk of endometrial

cancer, especially among women with low levels of endogenous estrogen exposure or with low soy food intake (Deming et al., 2008).

A Korean study examined the associations between genes in the ornithine decarboxylase (ODC)-polyamine pathway and gastric cancer risk, and whether gene-phytoestrogen interactions affected gastric cancer risk (Cho et al., 2015). They found that the NAD(P)H dehydrogenase quinone 1 (NQO1) *rs1800566* polymorphism showed significant decreasing effect on gastric cancer (OR 0.83 (95% CI 0.70-0.995)) and a greater decreased risk at high genistein/daidzein levels (OR 0.36 (95% CI 0.15-0.90) and OR 0.26 (95% CI 0.10-0.64)), respectively. Risk alleles of *S*-adenosylmethionine decarboxylase 1 (AMD1) *rs1279599*, AMD1 *rs7768897* and ornithine decarboxylase antizyme 2 (OAZ2) *rs7403751* had significant gene-phytoestrogen (genistein and daidzein) interaction effect to modify development of gastric cancer. They increased the risk at low isoflavone levels, but decreased the risk at high levels.

A study in UK examined polymorphisms in genes involved in the metabolism and transport of soy isoflavones and 24-hour urine metabolites by RP-HPLC from 100 healthy pre-menopausal women aged 18-50 years (Wakeling and Ford, 2012). Isoflavone glucuronides are substrates for efflux transporters including ABCG2 and ABCC2. The women abstained from consuming foods containing isoflavones for 3 days prior to the study, and were given three capsules of a commercial soy supplement (Soy Life™ complex, Schouten USA Inc.) as a bolus dose after fasting. Each 750 mg capsule contained 23 mg isoflavones, comprising 2.5 mg genistein and genistin, 13.5 mg daidzein and daidzin, and 7.0 mg glycitein and glycitin. The aglycone content comprised 0.03-0.06% of the total isoflavone content. Large differences in isoflavone recovery (mean 39%, eight-fold variation) and metabolites were observed. Genetic variations in UDP-glucuronosyl transferase (UGT) 1A1, cytosolic  $\beta$ -glucosidase (CBG), breast cancer resistance protein (ABCG2) and multidrug resistance protein 2 (ABCC2) influenced isoflavone metabolism.

#### **2.4.6.3 Patients on hemodialysis**

A low-dose intervention study in hemodialysis patients in USA had the purpose of studying safety and antioxidant effects of soy protein (Siefker and DiSilvestro, 2006). Among the 17 patients that completed the treatment (of 20 enrolled in the study), there were 7 men and 10 women, with average age of 50.3 years (range 27-77 years). The patients were randomly and blindly assigned to the two treatment groups. Eight patients were given 25 g soy protein, containing 52 mg of isoflavones as aglycone equivalent weight, 4 times a week for 4 weeks (total intake of 400 g soy protein and 832 mg isoflavones), whereas nine patients were given whey protein as control. Post-treatment isoflavone levels were similar to those reported after a single 20 g soy protein intake by hemodialysis patients. Post-treatment plasma isoflavones in the soy group were (mean  $\pm$  SE) 3.0  $\pm$  0.8  $\mu$ M for genistein and 3.9  $\pm$  1.0  $\mu$ M for daidzein. No significant effects were observed in the comparison of soy with whey treatment groups. Soy protein intake produced no harmful effects based on a battery of routine clinical laboratory blood tests, which included markers for kidney function, liver injury, malnutrition, immune dysfunction, anemia and concentrations of electrolytes. Three

other measures of oxidant stress and/or inflammation were unchanged by the isoflavone intervention. The authors concluded that a 4 week intervention with of a total intake of 400 g soy protein and 832 mg isoflavones produced no obvious harm to the hemodialysis patients, thus, they were not a vulnerable group based on this study.

#### **2.4.6.4 Patients with hypothyroidism**

There have been concerns on effects of isoflavones on thyroid function based primarily on *in vitro* and animal studies involving isolated isoflavones and cases of goiter in the 1960s that were attributed to the use of soy infant formula, which were eliminated when soy formula was fortified with iodine (Messina, 2010a).

Messina and Redmond (2006) reviewed effects of soy protein and soybean isoflavones on thyroid function in healthy adults and hypothyroid patients. In total, 14 trials (none of them with thyroid function as the primary health outcome) were identified in which the effects of soy foods or isoflavones on at least one measure of thyroid function were assessed in presumably healthy subjects; eight involved women only, four involved men, and two both women and men. With only one exception, either no effects or only very modest changes were noted in these trials. Thus, collectively the findings provide little evidence that in euthyroid, iodine-replete individuals, soy foods or isoflavones adversely affect thyroid function. According to the authors, the one Japanese study (Ishizuki et al., 1991) which found marked anti-thyroid and goitrogenic effects, did not include a control group, the description of the soy product was inadequate and the results were not biologically plausible since the low levels of soy proteins (approximately 8 g) and isoflavones (approximately 30 mg) were not likely to have such effects in a population that regularly consumes soy but does not have a high incidence of goiter.

There are two clinical situations where the relationship between soy intake and thyroid function needs caution (Messina, 2010a); individuals with subclinical hypothyroidism (5-10% of the U.S. post-menopausal population), defined as having normal levels of thyroxine and triiodothyronine, but elevated levels of thyroid stimulating hormone, and individuals with inadequate iodine intake. The effects of soy foods should be evaluated in individuals with subclinical hypothyroidism, since they may need to adjust their dosage of medication when consuming soy foods, because of an inhibitory effect of drug absorption, as with other drugs, foods and fiber supplements (Messina, 2010a). It is not necessary for thyroid patients to avoid soy foods, because medication is taken on an empty stomach and dosages can be adjusted to compensate for any effects of soy (Messina, 2010a). Individuals whose iodine intake is inadequate may increase their iodine intake further, not avoid soy foods.

EFSA (2015) evaluated eleven human controlled randomized studies that reported effects of isoflavone administration on thyroid-related end-points including in total 925 subjects. They concluded that administration of food supplements containing isoflavones was not associated with clinically relevant changes in thyroid function (hypo- or hyperthyroidism) of the

population of interest (peri- and post-menopausal women). In summary, thyroid hormones levels were not changed following intake of isoflavones from food supplements.

It is possible that infants with congenital hypothyroidism may be unable to increase free thyroxine production, if they are being lowered by isoflavones, and thus, are a susceptible group (COT, 2003). Therefore, it may be appropriate to monitor thyroxine levels in infants with this condition who are fed soy-based infant formula.

#### ***2.4.6.5 Consumers with high dietary intake of isoflavones***

##### *Vegetarians and vegans*

Consumer groups which have an already high dietary intake of isoflavones from a vegetarian or vegan diet, a traditional Asian (i.e. Japanese, Chinese) diet or a diet high in soy-based foods for whatever reasons may be vulnerable to potential negative effects from additional intake of isoflavones from supplements (COT, 2003).

Plasma isoflavone concentrations were measured in 225 Malaysian subjects according to age (18-34, 35-44 and 45-67 years old) (Hod et al., 2016). In all age groups, vegetarians had a higher concentration of circulating isoflavones compared with non-vegetarians especially in the 45-67 year age group where all isoflavones (from soy: genistein, daidzein, and from red clover: formononetin and biochanin A), but not the daidzein metabolite *S*-equol, were significantly higher in vegetarians compared with omnivores. By contrast, the group of 18-34 year olds had a significantly higher concentration of daidzein in vegetarians. Thus, vegetarians with a high isoflavone intake may potentially be a vulnerable group for adverse effects of additional isoflavones as supplements.

##### *Infants on soy formula*

Infants eating mainly or solely soy infant formula may be regarded as high consumers of isoflavones, and infants and children may also be at a sensitive developmental stage for hormonal influences. They may therefore be vulnerable to potential negative effects from isoflavones, and if exposed to supplements, also to the additional exposure to isoflavones from this source (Bar-El Dadon and Reifen, 2010). The role of isoflavones and other phytoestrogens in infants has been controversial, but few studies have examined their potential biological effects in infants. Although the exposure may be high, no clear correlation between phytoestrogens and negative effects in infants has been observed (Cassidy, 2003; Miniello et al., 2003).

Infant formulas in Norway are subject to regulations which cover the composition, labelling, marketing and distribution of infant formula. The regulations are in line with relevant EU directives. The regulations give minimum and maximum limits for nutrients in infant formulas and include some of the provisions of the WHO Code. Soy protein isolate in infant formula according to EU regulation is minimum 2.25 and maximum 3.0 g/100 kcal according to compositional requirements in regulation on infant formula and follow-on formula (Norwegian Ministry of Health and Care Services, 2008).

Data from the national dietary survey among infants in Norway (Spedkost) showed that at 6 months of age, 43% of the infants had been introduced to infant formula, and 36% used it regularly (Øverby NC et al., 2008). At 1 year of age, 43% of the infants were given infant formula regularly (Øverby NC et al., 2007)). However, there were no data on intake of soy-based formula in Norwegian infants or on isoflavone content in soy-based infant formula used in Norway. Therefore, some data on these issues reported from other countries are included.

Circulating concentrations of isoflavones in soy-formula-fed infants are 13000-22000 times greater (Setchell et al., 1997) than typical infant plasma estradiol concentrations (40-80 pg/ml) (Winter et al., 1976).

Concentrations of total isoflavones in infant soy formulas on the American market have been reported to range from 32-47 mg/l. On the basis of a typical daily intake of 900-1000 ml of a soy-based formula at 4 months of age, total isoflavone intake will be from 4.2 to 9.4 mg/kg bw per day from soy proteins and between 9 and 16 mg/kg bw per day from soy flour (Setchell et al., 1997; Strom et al., 2001). With a daily exposure of a dose of approximately 8 mg/kg bw they may receive 6-11 fold higher levels of phytoestrogens per kg body weight than women receiving phytoestrogens for menopausal complaints or the 0.7 mg/kg bw intake shown to exert significant physiological effects on hormonal regulation of women's menstrual cycle (Cassidy et al., 1994; Sirtori et al., 2005).

The daily intake in infants from human breast milk, predominantly found as glucuronide conjugates, is trivial (0.005-0.01 mg per day) in comparison (Setchell et al., 1998), and for infants fed cow milk formula the plasma concentrations were approximately twice as high as for breast milk. Circulating concentrations of isoflavones in infants fed breast-milk and cow milk formula were <1/200th and 1/100th, respectively, of the concentrations attained in infants fed soy-based formula (Setchell et al., 1998).

Six American infant formula powders contained 126-154 µg and 57-78 µg of total genistein and daidzein per g dry formula, respectively (Murphy et al., 1997).

Total levels of genistein (as genistein, genistin, 6''-O-acetyl- and 6''-O-malonyl-derivatives of genistin) in infant soy formula in UK were 74-170 µg/g of powder, and the total levels of daidzein (daidzein, daidzin, 6''-O-acetyl- and 6''-O-malonyl-derivatives of daidzin) were 39-102 µg/g of powder (Garrett et al., 1999).

In Italy, infant soy formulas contained 121-427 µg/g dry weight of isoflavones, which may give daily intakes of about 2-3 mg/kg bw (Morandi et al., 2005; Sirtori et al., 2005).

Irvine et al. (1998) reported levels of  $87 \pm 6$  µg of total genistein per g and  $49 \pm 6$  µg of total daidzein per g, for four soy infant formulas used in New Zealand.

An Australian study found 17.2-21.9 mg/l of isoflavones in soy-based infant formulas, whereas casein-based infant formulas contained negligible levels of isoflavones (0.001-0.03 mg/l) (Knight et al., 1998).

Infants with milk allergy may use milk-based formulas with partially or extensively broken down proteins (hydrolysed, by heat or enzymes), and not necessarily soy formulas. Of infants aged 3-9 months in Ireland, 5% were fed either a soy-based or hydrolysate formula, and in the Euro-Growth Study group, maximum 7.3% of infants were fed this diet at age 3 months (Food Safety Authority of Ireland, 1999). Hydrolysed infant formula is considered superior to soy-based infant formula for infants with cows' milk allergy (Food Safety Authority of Ireland, 2011).

Isoflavones can cross the placenta after metabolism in the mother. However, it is not known how the fetus metabolises the isoflavones (COT, 2003). Pregnant women may therefore potentially be a vulnerable group. No studies were found on effects of humans after *in utero* exposure to isoflavones.

## **2.5 Summary and discussion of hazard identification and characterisation**

### **2.5.1 Absorption, distribution, metabolism and excretion (ADME)**

A number of both internal and external factors are influencing the phytoestrogen bioavailability, including intestinal microflora, gender, age, food matrix, chemical composition, earlier exposure and background diet (van de Poll, 2004).

This summary/discussion of ADME of isoflavones is mostly based on EFSA (2015).

In humans, calculated from urinary data, the absorption was estimated to be 35.4% for genistin, 61.3% of the dose for daidzin and 60.4% for glycitin (Shelnutt et al., 2002). After 9.8, 19.6 and 39.2 mg of genistein and 6.6, 13.2 and 26.4 mg of daidzein in the glycosylated form, the absorption, calculated from urinary excretion, was found to decline with increasing dose (genistein: 25.2%, 13.4% and 15.8%, daidzein: 63.2%, 54.4% and 44.0%) (Setchell et al., 2003a). In another study, the absorption, calculated from urinary excretion data, was 44.3% for genistein and 88.5% for daidzein (Vergne et al., 2008).

From the study of Busby et al. (2002), who investigated administration of several doses of genistein and daidzein in healthy male volunteers, C<sub>max</sub> for genistein aglycone, as percentage of the total genistein, varied between 0.9% and 2.7% and the C<sub>max</sub> for daidzein aglycone, as percentage of the total daidzein, varied between 1.4% and 4.2%. From the study of (Bloedon et al., 2002), who investigated administration of several doses of genistein and daidzein in healthy female volunteers, C<sub>max</sub> for genistein aglycone, as percentage of the total genistein, varied between 0.7% and 2.2% and the C<sub>max</sub> for daidzein aglycone, as percentage of the total daidzein, varied between 1.1% and 2.8%.

Data on the absorption and absolute bioavailability of isoflavones were available for mice and rats. In mice, the absorption of genistein and daidzein, estimated by the comparison of AUCs of the total isoflavones, was complete. Absolute bioavailability (internal exposure) amounted to 9–14% (Andrade et al., 2010) and 23.4% (Yang et al., 2010) for genistein and to 29–34% for daidzein (Andrade et al., 2010). In rats, genistein absorption from the gut of (<sup>14</sup>C)-genistein at 4 mg/kg bw was 56% in males and 111% in females, and the absolute oral bioavailability of the parent compound genistein was 7% in male rats and 15% in female rats (Coldham et al., 2002). In another rat study, oral genistein doses of 6.25 mg/kg bw, 12.5 mg/kg bw and 50.0 mg/kg bw were given and the bioavailability was 21.9%, 33.5% and 19.0%, respectively (Zhou et al., 2008).

From three rat studies on distribution of oral administered genistein (Chang et al., 2000; Coldham and Sauer, 2000; Zhou et al., 2008), it can be deduced that genistein is found in every organ, mainly in the form of the unconjugated substance.

A study by Setchell et al. (2003a) provided relevant information by comparing the proportion of unconjugated genistein and daidzein (phase II metabolism) in plasma in rats (both genders), various mouse strains and women. Large species differences were found, as the plasma percentages of unconjugated genistein concentrations in Sprague–Dawley rats and C57BL/6, nude and transgenic AngptL4B6 mice were 4.0%, 4.6%, 11.6% and 30.1%, respectively, whereas in humans, the levels were 0.25% and 0.26% in men and women. For daidzein, the corresponding numbers were 8.1%, 7.4%, 16.1% and 32.7% in the animals, and 1.25 and 0.98% in men and women, respectively.

Monkeys, rats and mice are described as 100% equol producers, meaning that the microbiotas of these animals are uniformly able to transform daidzein to a considerable extent to *S*-equol (Andrade et al., 2010; Gu et al., 2006; Setchell et al., 2011). Hampshire/Duroc Cross pigs did not have detectable equol in plasma and excreted isoflavones mainly as glucuronides (>80%), with <10% as aglycones, having an overall metabolic profile closer to women than that of rats and monkeys (Gu et al., 2006). In contrast, the microbial metabolism of daidzein in humans is characterised by a large interindividual variability, and only some of the population are able to produce *S*-equol. As a consequence of this heterogeneity, microbial metabolites other than equol, e.g. dihydrodaidzein or *O*-desmethylangolensin, can be present in human plasma at higher concentrations than *S*-equol.

The unconjugated isoflavones are discussed as they are the biologically most active forms (estrogenic) and are therefore of particular importance (Setchell et al., 2011). According to several intervention studies (Gu et al., 2006; Hosoda et al., 2011; Setchell et al., 2011; Soukup et al., 2014), the mean portion of unconjugated genistein and daidzein in plasma of adults ranges 0.8–1.7% and 1.4–2.1%, respectively, after the intake of various soy foods (soy beverage, kinako, soy nuts, tempe) or soy extract.

The intrinsic estrogenic potency *in vitro* of the glucuronide metabolites of genistein and daidzein was 450–1730 fold lower than the corresponding aglycones in U2OS Era and Erβ



cells, and the proliferative potency of the glucuronides in T47D-wt cells was also 123-525 fold lower than the corresponding aglycones (Islam et al., 2015). Conjugated isoflavones may be deconjugated, as shown *in vitro* in U2OS and T47D cells exposed to 400  $\mu$ M glucuronides, but the degree of deconjugation observed was low (0.2–1.6%), resulting in a final aglycone concentration of about 0.7-6.5  $\mu$ M. Using human breast tissue S9 fraction, the average total deconjugation after 24 hours was 2.5% and 2.0%, respectively, for the 7-*O*-glucuronides of genistein and daidzein, whereas the F344 rat breast tissue S9 fraction could deconjugate 69.3% and 58.3% of these glucuronides, respectively, and therefore was about 30 times more potent (Islam et al., 2015). This is in line with the finding that only low concentrations of total unconjugated daidzein and genistein (aglycones) (20-25 pmol/g breast tissue) were found in breast tissue homogenate from healthy women after the intake of soy milk or a soy supplement (Bolca et al., 2010). The authors estimated overall total glucuronidation of 98% in breast tissue, although not all phase II metabolites were determined.

Compared with daidzein, the microbial degradation of genistein *in vivo* is less well investigated. There is evidence from one study that degradation to 4-ethyl-phenol is the major pathway in the rat (King, 1998) and it is a quantitatively important metabolite in sheep (Setchell, 1998). It has been detected in human plasma and urine, however, the quantitative importance of this metabolite in humans is currently not known (Setchell, 1998). The same applies for 6'-hydroxy-*O*-methylangolensin, a genistein metabolite which is considered more rarely than the analogous daidzein degradation product *O*-desmethylangolensin.

Oxidative phase I metabolites of genistein and daidzein catalyzed by CYP450 enzymes, mainly 6-hydroxy- and 3'-hydroxy-genistein and 6-hydroxy-, 8-hydroxy- and 3'-hydroxy-daidzein, are found in humans, rats and mice (Breinholt et al., 2000; Kulling et al., 2001; Rufer et al., 2008). Although the extent of their formation is described as low, all these minor metabolites bear a catechol structure and might be easily oxidized to form reactive *o*-quinones. Moreover, *o*-quinones are described as reactive metabolites towards nucleophiles. No quantitative data were available on phase I metabolites that would allow a comparison between species.

The main phase II metabolites of genistein and daidzein in human plasma are the 7-glucuronide-4'-sulfates (Hosoda et al., 2011; Soukup et al., 2014), whereas in rats and mice the mono-glucuronides are the predominant conjugates.

## 2.5.2 Genotoxicity

Genistein yielded no evidences for mutagenicity in the *in vitro* bacterial gene mutation test (Ames test) (Masuda et al., 2012; McClain et al., 2006; Misra et al., 2002). In contrast, in mammalian cells *in vitro*, genistein proved to be markedly mutagenic (Kulling et al., 1999; McClain et al., 2006; Misra et al., 2002; Morris et al., 1998; Zou et al., 2012), compatible with a clastogenic mechanism of action as indicated by the partial or complete deletion of the *tk*<sup>+/-</sup> locus following DNA sequencing. It also proved to be clastogenic through the induction of micronuclei (Di Virgilio et al., 2004; Morris et al., 1998; Snyder and Gillies, 2003)

or DNA breakage as measured by the Comet assay (Di Virgilio et al., 2004; Salti et al., 2000; Ullah et al., 2009). It is widely recognised that this genotoxicity arises from poisoning of DNA topoisomerase II through the stabilisation of the "cleavable complex", thus resulting in protein-concealed DNA double-strand breaks at topoisomerase II–DNA binding sites (Lopez-Lazaro et al., 2007; Markovits et al., 1989; Yamashita et al., 1990). However, genistein has also been shown to be a catalytic inhibitor of topo II with the capability to disrupt the enzyme physiology, as reported by Mizushina et al. (2013) and Lopez-Lazaro et al. (2007), who reported that genistein completely inhibited the nicking activity of topo II. An important implication of an indirect (topo II-mediated) effect on DNA is the concept of a threshold for clastogenicity, as demonstrated by Lynch et al. (2003). In this study, a number of topoisomerase type II inhibitors with different clastogenic potencies were investigated and "pragmatic thresholds" for clastogenicity in mouse lymphoma L5178Y cells were defined. For genistein the "pragmatic threshold" was defined at 1 µg/ml. No evidence of *in vivo* genotoxicity was observed for genistein, as shown by negative results obtained in two mouse micronucleus tests (Masuda et al., 2012; Misra et al., 2002) and in two pivotal, limit bone marrow micronucleus tests in RAI and Wistar rats, at 2000 mg/kg bw (McClain et al., 2006). Negative results were obtained in a phase I randomized double-blinded clinical trial in 30 healthy post-menopausal women exposed to approximately 558 mg/day of genistein for 84 days, in which genotoxicity was evaluated by means of the alkaline Comet test and analysis of AP sites in peripheral blood lymphocytes (Pop et al., 2008). Nor were any genotoxic effects observed in an alkaline Comet assay and cytokinesis-block micronucleus assay in peripheral blood lymphocytes in 20 patients with prostate cancer and 6 healthy men in whom genistein 279 mg/day (approximately 4 mg/kg bw/day) was administered for 28 days, increasing to 558 mg/day (approximately 8 mg/kg bw/day) for a further 56 days (Miltyk et al., 2003).

Daidzein (as part of the purified isoflavone product PTI G-2535) was not mutagenic in the bacterial reverse mutation assay (Misra et al., 2002), and no relevant genotoxic effects were generally observed in mammalian cells *in vitro* (Di Virgilio et al., 2004; Kulling et al., 1999; Lehmann et al., 2005; Schmitt et al., 2003). Similarly, negative results were observed *in vivo* in a pivotal limit mouse bone marrow micronucleus test (Misra et al., 2002). The analysis of peripheral blood lymphocytes from 30 healthy post-menopausal women exposed to 296 mg/day of daidzein in 84 days (Pop et al., 2008), and from 20 men with prostate cancer and 6 healthy men given approximately 150 mg/day for 28 days, increasing to approximately 300 mg/day for a further 56 days of daidzein (Miltyk et al., 2003), yielded no evidence of genotoxicity.

In contrast, the two oxidative daidzein metabolites (3'-HO-DAI and 6'-HO-DAI) found after incubation with human hepatic microsomes, and also identified in the urine of volunteers after ingestion of soy food, proved to be clastogenic through the induction of micronuclei in mammalian cells (Schmitt et al., 2003). The clastogenic effect of these two catecholic metabolites may be attributed to oxidation to *o*-quinones, which are known to be clastogens and could represent a potential hazard *in vivo*. However, as soy isoflavones and their metabolites are rapidly conjugated with glucuronic acid and sulfate *in vivo*, it is unlikely that

the high concentrations of the free metabolites required for adverse effects are reached in humans even after ingestion of high levels of isoflavones. Furthermore, the negative outcome observed for daidzein *in vivo* (Miltyk et al., 2003; Misra et al., 2002; Pop et al., 2008) is reassuring about the genotoxicity of these catecholic metabolites.

For the daidzein metabolite *S*-equol, which is produced by intestinal bacteria in the human gut, no genotoxicity was reported in the bacterial reverse mutation assay (Schwen et al., 2010). Equivocal findings in terms of induction of micronuclei were observed in mammalian cells *in vitro* (Di Virgilio et al., 2004; Lehmann et al., 2005; Schmitt et al., 2003), though a negative finding for induction of chromosomal aberrations was observed in a valid study in human lymphocytes *in vitro* (Schwen et al., 2010). *In vivo*, *S*-equol proved to be devoid of any genotoxic activity in a limit rat bone marrow micronucleus test performed in accordance with the relevant OECD guideline, TG 474 (Schwen et al., 2010).

Glycitein (as part of PTI G-2535) (Misra et al., 2002) was not mutagenic in the bacterial reverse mutation assay. Negative results were also observed *in vivo*. Glycitein was negative when peripheral blood lymphocytes from 30 healthy post-menopausal women exposed to 44 mg/day in 84 days (Pop et al., 2008) and 20 patients with prostate cancer and six healthy men given approximately 22 mg/day for 28 days, increasing to approximately 88 mg/day for a further 56 days (Miltyk et al., 2003), were analysed for different genotoxicity endpoints.

The overall conclusion by EFSA (2015) was that the genotoxicity expressed *in vitro* in mammalian cells by the two catecholic oxidative metabolites of daidzein, 3'-HO-DAI and 6'-HO-DAI, and by genistein, for which a thresholded mechanism of action has been demonstrated, has not been reproduced in valid *in vivo* micronucleus tests in rats and mice or in Comet assay and micronucleus test in human studies. On this basis, the use of isoflavones in food supplements is not of genotoxic concern. VKM agrees with EFSA in this statement.

### **2.5.3 Hazard characterization of isoflavones based on human data**

Because of the differences in ADME between humans and rodent species as described and the abundance of human studies available on isoflavones, toxicity data from experimental animals were not included in this risk assessment, except for *in vivo* genotoxicity data.

#### ***2.5.3.1 Sources of isoflavones in the human studies***

Sources of isoflavones examined in the included studies were reported to be soy protein, soy protein isolate (SPI) or isolated soy protein (ISP), soybeans, soy foods such as soy milk, flour, grits, tofu or tempe, as commercial products or prepared meals, isoflavone concentrate or just referred to as isoflavones. They may also be genistein combined polysaccharide (GCP), which is enriched in aglycone forms of isoflavones, percentages of genistein, daidzein, glycitein, and in some studies also as percentages of genistin, daidzin, glycitin and the malonyl- and acetyl-derivatives. The isoflavones were given in the form of tablets, pills,

capsules, powders, extracts or concentrates, sometimes mixed into scones, biscuits, cereal bars, snack bars, cereals, drinks or beverages, or the form was not specified. The chemical form of isoflavones was reported (if stated) as aglycones, aglycone equivalents, glycosides or as several categories. The ratio of percentages of individual aglycone isoflavones genistein:daidzein:glycitein varied substantially among the studies; 15-95:10-55:1-30. In three studies, the isoflavones were apparently given as high levels of glycosides, with genistin:daidzin:glycitin ratios of 20-52:37.2-50:8.8-30. Five studies reported effects of separate genistein in tablets or capsules. No pattern was obvious regarding type of soy product or isoflavone composition and negative health effects.

### ***2.5.3.2 Peri- and post-menopausal women***

The included studies on peri- and postmenopausal women are summarized in Table 9.1 in Appendix 9.1.

#### *Adverse events/side-effects*

Of the available RCTs of isoflavones (n = 28), 2 studies examined doses of 60 and approximately 120 mg total isoflavones per day for 6 weeks, 14 studies used doses from 56 to 160 mg/day for 3-6 months and 5 studies examined 52 to 300 mg/day for 9 to 12 months. In addition, one study examined effects of 898 mg/day of total isoflavones for 3 months (Pop et al., 2008), and another study examined effects of 150 mg/day for 5 years (Unfer et al., 2004). All of these studies found mostly gastrointestinal symptoms at the same rate, or in a few studies at a higher rate, with soy isoflavones compared with control (placebo) treatment. In addition, insomnia as a menopause symptom in healthy women was increased with isoflavones in one RCT after 122.7 mg/day (composition of isoflavones not given) for 6 months (Balk et al., 2002).

In the RCTs (n = 5) studying effects of genistein separate, doses of 54 mg/day for 1-3 years, and 90 mg/day for 6 weeks, reported no other negative effects than moderate gastrointestinal symptoms and back pain significantly different from control treatment.

A meta-analysis of 92 RCTs reporting side-effects on post-menopausal women using phytoestrogen (isoflavones, lignans and coumestans) supplements for treatment of climacteric syndrome (Tempfer et al., 2009), found that in only two of the 92 studies evaluated was there a statistically significant difference in side-effect incidence between treatment group and placebo group (Albertazzi et al., 2005; Unfer et al., 2004). Comparing various side-effect categories, significant higher rates of gastrointestinal side-effects among phytoestrogen users were found. Gynecological, musculoskeletal, neurological and unspecific side-effects were not significantly different between groups. Within side-effect categories, they found no significantly higher rates of side-effects in women using phytoestrogens. Among the 5 studies reporting side-effects in a meta-analysis of in total 15 RCTs on oral intake of phytoestrogens, there was no significant difference in side-effects between phytoestrogen and placebo groups (Chen et al., 2015).

#### *Hormone levels/lipid levels*

One RCT reported no significant changes in serum androgens or plasma lipids within treatment or placebo groups over time in healthy women exposed to 160 mg/day of isoflavones for 12 weeks (Basaria et al., 2009). However, at the end of the study a group-by-time interaction was observed so that total testosterone and HDL levels were significantly lower in the isoflavone group compared with placebo group.

#### *Cancer risk*

Most studies reported that isoflavones seemed to reduce cancer risk. However, a few studies indicated the opposite tendency. In a RCT, a significantly higher rate of endometrial hyperplasia without atypia was reported in healthy Italian women after 150 mg/day of isoflavones in tablets (Ge:D:GI %: 40-45:40-45:10-20) after 5 years (6 vs. 0 cases) (Unfer et al., 2004). However, no cases of endometrial hyperplasia with atypia or endometrial carcinoma were observed. EFSA (2015) mentioned some methodological weaknesses of this study; a considerable number (up to 25%) of specimens of endometrium were neither obtained nor assessable at each time point and that these samples were not consistently obtained from the same participants at each time point, and that the effects observed were indicative of a possible estrogenic but not a carcinogenic effect.

A prospective study reported that genistein and daidzein calculated from a food frequency questionnaire (FFQ) were dose-dependently associated with increased risk of hepatocellular carcinoma in Japanese women, with multivariate hazard ratios for highest vs. lowest tertile of 3.19 (95% CI 1.13-9.00, Ptrend = 0.03) and 3.90 (95% CI 1.30-11.69, Ptrend = 0.01), respectively (Kurahashi et al., 2009).

A retrospective case-control study found that soy food and isoflavone intake from food estimated from FFQ for the highest quartile of intake (Q4) vs. the lowest (Q1) was generally associated with decreased risk of colorectal cancer in Korea (Shin et al., 2015), however, the middle (second and third) quartiles of intake of total soy products were associated with a non-significant elevated colon cancer risk in women (Q2: OR: 1.27, 95% CI 0.86-1.88), Q3: OR: 1.37, 95% CI 0.92-2.04). The same non-significant tendencies of reduced risk associated with Q4 and increased risk associated with Q2 and Q3 vs. Q1 was seen with total isoflavones. However, a meta-analysis of 4 cohort and 7 case-control studies found reduced risk in women or no association in men between soy intake and colorectal cancer (Yan et al., 2010).

Another retrospective case-control study found that post-menopausal Canadian women had a positive association between ER-PR- breast cancer and adult total isoflavone intake from foods ( $\geq 497$   $\mu\text{g}/\text{day}$ ) (highest vs. lowest tertile: OR: 1.50, 95% CI 1.05-2.15, Ptrend = 0.04), indicating increased risk for this breast cancer subtype with total isoflavone intake (Anderson et al., 2013). Also in women not stratified by menopause status was there a positive association between ER-PR- breast cancer and the highest tertile of adult total isoflavone intake ( $\geq 497$   $\mu\text{g}/\text{day}$ ) (OR: 1.38, 95% CI 1.05-1.81, Ptrend = 0.01). However, EFSA (2015) concluded based on a weight of evidence approach that adverse effects on mammary gland have not been seen neither in humans nor in animals.

In the meta-analysis of 92 RCTs on post-menopausal women using phytoestrogen (isoflavones, lignans and coumestans) supplements for treatment of climacteric syndrome (Tempfer et al., 2009), the rates of hormone-related side-effects such as endometrial hyperplasia, endometrial cancer and breast cancer were not significantly different between groups.

To summarize, gastrointestinal symptoms, insomnia or back pain were reported as adverse events/side-effects in peri- and postmenopausal women at the same rate, or in a few studies at higher rate, compared with placebo. The doses of total isoflavones and duration of treatment in these studies were 60 and approximately 120 mg total isoflavones per day for 6 weeks, from 56 to 160 mg/day for 3-6 months and from 52 to 300 mg/day from 9 to 12 months. In addition, one study examined effects of 898 mg/day of total isoflavones for 3 months and another study examined effects of 150 mg/day for 5 years. Based on the available studies from the literature, isoflavones as supplements in these doses and duration of treatment appear to be without significant negative health effects in peri- and post-menopausal women.

The relevance of the few studies that found increased risk of cancer of a very high dose of isoflavone supplements or in occasional comparisons of dietary intake of soy food products in mostly Asian populations is difficult to interpret in relation to intake of isoflavone supplements in Norwegian peri- and post-menopausal women.

### ***2.5.3.3 Pre-menopausal women***

The included studies on pre-menopausal are summarized in Table 9.2 in Appendix 9.2.

#### *Sources of isoflavones*

In these studies, isoflavones were given as soy protein powder (Anderson et al., 2002; Cassidy et al., 1994; Duncan et al., 1999) or in the form of soy food products as components of diets (computed from FFQs/24-hour recalls or given as an intervention).

#### *Hormonal effects/menstrual function*

Of 5 RCTs on various end points of isoflavones as soy protein and/or from dietary sources giving isoflavone levels of approximately 64 to 128 mg/day, the only significant negative effects that were detected were effects on hormone levels in one study (Duncan et al., 1999). In this study, the levels of LH and FSH were decreased with 64 mg/day of isoflavones from soy protein powder, whereas free T3, DHEAS and estrone were decreased with 128 mg/day of isoflavones. The authors stated that this study suggested weak hormonal effects of isoflavones, with uncertain physiological relevance and with no evidence of dose-dependency of the effects. Weaker effects on hormone levels were also found in two other studies. Nagata et al. (1998) reported in a RCT that estrone and estradiol were decreased 23 and 27%, respectively, and that the menstrual cycle length was increased by nearly 2 days after soy milk and dietary soy food intake (total intake of 116.4 mg/day of isoflavones), however, both results were not statistically significant. In a clinical study by Ostatnikova et al. (2007), they reported that after intake of soybeans (isoflavones content unknown) for 7

days salivary testosterone and plasma estradiol (E2) levels showed a tendency to decline ( $P < 0.026$  for E2), with a trend to return to basal levels after washout. Another clinical study on soy protein added to meals found that 45 mg/day of isoflavones significantly increased follicular phase length and/or delayed menstruation, suppressed mid-cycle surges of LH and FSH and increased plasma estradiol concentrations in the follicular phase (Cassidy et al., 1994). A prospective study of dietary intake of isoflavones found that consumption of the highest quartile (1.6-78.8 mg/day of isoflavones) was significantly associated with greater SHBG concentrations compared with the first quartile (0.0-0.3 mg/day) (Filiberto et al., 2013). However, this diet also contained non-soy isoflavones (biochanin A and formononetin).

#### *Effects on uterus/cervix*

Regarding other effects than on hormone levels and menstrual function, a retrospective study found that consumption of soybean milk (isoflavone level and composition unknown) was associated with significantly increased occurrence of uterine leiomyoma in Chinese women, especially among those with frequent consumption (Shen et al., 2013). Plasma equol, highest ( $>5.9$  nM) and next highest (0.6-5.9 nM) quartiles vs. lowest quartile (0.0 nM), was positively associated with cervical squamous intraepithelial lesions (SILs) in Hawaiian women of mixed ethnicity, however, they found no association with plasma levels of other isoflavones, or between SILs and dietary consumption of soy foods (Hernandez et al., 2004).

#### *Effects on bone*

A cross-sectional study of bone health in women of various ethnicities, including Caucasians, found greater annual lumbar spine and femoral neck bone mineral density loss rates positively related to dietary isoflavone consumption only in Japanese women (Greendale et al., 2015). However, the Asian populations had much higher aggregated levels of total isoflavones than the non-Asian populations (tertiles of 1751, 8851 and 29113 mg/day vs. 88, 286 and 1230 mg/day, respectively). In addition, a RCT of isoflavones from enriched soy protein isolate on bone mineral density in American (mostly Caucasian) women did not find any effects of approximately 90 mg isoflavones per day (Anderson et al., 2002).

#### *Effects on reproduction*

In Adventist women with a unusually high mean dietary isoflavone intake (54% vegetarians) compared with other Western populations, an inverse relationship was found between the likelihood of ever becoming a mother or the risk of nulligravidity and isoflavone intake  $\geq 40$  mg/day (Jacobsen et al., 2014). However, these relationships were mainly in women who reported problems with becoming pregnant.

To summarize, isoflavones apparently affected hormone levels in doses of 45 to 128 mg/day and affected menstruation in doses of 45 to 116.4 mg/day in pre-menopausal women, when taken for approximately one to three months. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In peri- and post-menopausal women, soy isoflavones are taken with the

purpose of decreasing post-menopausal symptoms such as hot flashes, instead of estrogen-based hormone-replacement therapy, whereas pre-menopausal women would not have this need. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy pre-menopausal women. In this risk assessment of isoflavones in healthy pre-menopausal women, VKM considers changes in hormone levels away from the normal range as negative effects.

Based on the available studies, isoflavones as supplements in doses of 45 to 128 mg/day taken for approximately one to three months may represent a risk of negative effects on hormone levels and/or menstrual function in pre-menopausal women. These doses do not appear to have other significant negative effects on pre-menopausal women.

#### **2.5.3.4 Men**

The included studies on men are summarized in Table 9.3 in Appendix 9.3.

##### *Sources of isoflavon*

Sources of isoflavones examined in the studies on men were reported to be soy protein, soy protein isolate (SPI) or isolated soy protein (ISP), soybeans, soy foods such as soy milk, flour, grits or tofu, as commercial products or prepared meals, isoflavone concentrate or just referred to as isoflavones, or as genistein combined polysaccharide (GCP), which is enriched in aglycone forms of isoflavones, percentages of genistein, daidzein, glycitein, and in some studies also as percentages of genistin, daidzin, glycitin and the malonyl- and acetyl-derivatives. The isoflavones were given in the form of tablets, pills, capsules, powders or concentrates, sometimes mixed into scones or beverages, or the form was not specified. The chemical form of isoflavones was reported (if stated) as aglycones, aglycone equivalents, glycosides or a mixture. The ratio of percentage of individual isoflavones genistein:daidzein:glycitein varied substantially among the studies; 15-95:10-55:1-30. No pattern was obvious regarding type of soy product or isoflavone composition and negative health effects.

##### *Adverse events/side effects*

In the studies that gave detailed reports on adverse events/side effects, the most common complaints were mild gastrointestinal effects, such as diarrhea, nausea, flatulence or constipation, or sometimes weight gain or estrogenic effects (breast changes, increased frequency of hot flashes), without clinical toxicity or organ dysfunction. However, (Miyanaga et al., 2012) reported one grade III adverse event, i.e. iliac artery stenosis, in an older Japanese man at risk of prostate cancer.

##### *Effects on sperm parameters*

Among the RCTs (n = 8) studying healthy men, one study showed no effects on various sperm parameters and did not report any other negative effects after  $1.64 \pm 0.19$  and  $61.7 \pm 7.35$  mg/day of isoflavones for three periods of 57 days intervention separated by 28 days wash-out periods in American men (Beaton et al., 2010). Mitchell et al. (2001) found no negative effects on semen quality parameters after 2 months exposure to 40 mg/day of



isoflavones in a clinical study of men in UK. However, Chavarro et al. (2008) reported in a cross-sectional study on Slovakian men a significant inverse association between soy food intake in the previous 3 months giving a mean intake of 5.4 mg/day of dietary isoflavones and reduced sperm concentrations in a group of men where 72% were overweight/obese and partners in subfertile couples. The same was found to be significant for the trend on glycitein intake, whereas for genistein and daidzein the associations were similar, but not significant. A meta-analysis on healthy men reported an inverse association between changes in sperm concentrations and isoflavone intake in USA (decreased sperm concentrations and increased isoflavone intake) and in China (increased sperm concentrations and decreased isoflavone intake) over time (Lim and Shaw, 2016).

#### *Effects on hormone levels*

One RCT showed no effects on hormone levels and did not report any other negative effects after  $1.64 \pm 0.19$  and  $61.7 \pm 7.35$  mg/day of isoflavones for three periods of 57 days intervention separated by 28 days wash-out periods (Dillingham et al., 2007). Mitchell et al. (2001) found no negative effects on levels of several hormones after 2 months exposure to 40 mg/day of isoflavones in a clinical study. However, three RCTs reported effects of isoflavones on hormone levels. Nagata et al. (2001) reported decreased estrone levels after 8 weeks on  $76.8 \pm 16.6$  mg/day of isoflavones, Habito et al. (2000) found increased levels of SHBG and decreased FAI and testosterone:estradiol ratio after 4 weeks on 118.9 mg/day of isoflavones in tofu, and Gardner-Thorpe et al. (2003) reported decreased total serum testosterone after 6 weeks on 120 mg/day isoflavones. Some non-RCT studies also gave relevant information about doses of isoflavones affecting hormone levels. As opposed to the RCT by Dillingham et al. (2007) mentioned above, the same group reported in a clinical study various changes in hormone levels (decreased DHT and DHT:testosterone ratio and testosterone, increased DHEAS, estradiol and estrone) after both  $1.64 \pm 0.19$  and  $61.7 \pm 7.35$  mg/day of isoflavones from soy protein isolate for three periods of 57 days intervention separated by 28 days (Dillingham et al., 2005). In a cross-sectional study, Nagata et al. (2000) reported that serum estradiol concentrations were significantly inversely correlated with dietary soy product intake giving  $21.9 \pm 8.7$  mg/day of isoflavones, as well as non-significantly inversely associated with serum estrone, total and free testosterone. Three other clinical studies also reported effects of isoflavones on hormone levels. Tanaka et al. (2009) found increased serum SHBG and decreased free testosterone and DHT in men after 3 months on 60 mg/day of isoflavones. Goodin et al. (2007) reported decreased testosterone (although within normal range) after 28 days on 56 g soy protein isolate (i.e. approximately 196 mg/day of isoflavones, assuming that 1 g soy protein gives 3.5 mg of isoflavones, as stated in Hamilton-Reeves et al. (2010)). A clinical study by Ostatnikova et al. (2007) found that intake of soybeans (isoflavone intake unknown) did not change salivary testosterone or plasma E2 levels. However, they reported that the effects of soy on hormonal parameters were dependent on basal testosterone levels; it increased significantly after a low basal level, but decreased non-significantly after a high basal level. A meta-analysis of 34 papers studying intake of 0-71 g/day soy protein and 20-900 mg/day of isoflavone as aglycones from 1 week to 4 years (average duration 74 days) in men, including prostate cancer

patients (16 studies), found no significant effects of soy protein or isoflavone intake on total testosterone, SHBG, free testosterone or FAI (Hamilton-Reeves et al., 2010).

#### *Effects on vitamin E levels and lipoperoxidation*

One RCT reported reduced levels of vitamin E after 3-4 weeks with 20 g/day of soy protein isolate (approximately 70 mg/day of isoflavones, assuming that 1 g soy protein gives 3.5 mg of isoflavones (Hamilton-Reeves et al., 2010; Higashi et al., 2001). Celec et al. (2013) reported that soybeans (isoflavone content unknown) increased lipoperoxidation in men, although total antioxidant capacity was increased.

The two remaining RCTs found reported no negative effects. No negative effects on CAM concentrations and no other negative effects were reported after 8 weeks of 30.1 or 91.4 mg/day of isoflavones (Dettmer et al., 2012). A RCT on cognitive functions in both young men and women did not report any negative effects on cognition or on any other end points after 10 weeks on 0.5 or 100 mg/day of isoflavones (File et al., 2001).

#### *Effects on cancer or other conditions*

A large number of studies were also found on effects of soy food products or isoflavones on non-healthy men, i.e. patients with prostate cancer or with increased PCA at risk for prostate cancer, hepatocellular carcinoma or bladder cancer, men with adenomatous colorectal polyps, hypercholesterolic men and men with metabolic syndrome (see Table 9.3 in Appendix 9.3). Also in these studies, effects on sex hormone levels were reported (considered beneficial in men with or at risk for prostate cancer). Two studies in Chinese men, of uncertain relevance for this risk assessment, reported an increase in relative risk of bladder cancer associated with estimated dietary soy food, soy protein or isoflavone intake (Sun et al., 2002). Shin et al. (2015) reported a decreased risk of colorectal cancer associated with the highest quartile of dietary soy isoflavone intake (Q4) vs. the lowest quartile (Q1), but an increased risk was found associated with Q2 and Q3. A meta-analysis of colorectal cancer found no significant association, positive or negative, with soy consumption from food in men (Yan et al., 2010).

To summarize, the most common adverse events/side-effects of isoflavones reported in healthy men were mild gastrointestinal symptoms, or sometimes weight gain, estrogenic effects or reduction in vitamin E levels. Hormone levels appeared to be affected by doses of isoflavones ranging from 1.64 to 196 mg/day for one to three months. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy men. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In patients with prostate cancer or at high risk for recurrence of prostate cancer and possibly in patients with other types of hormone-related cancers, isoflavones may have a beneficial effect on the progression of the disease by decreasing testosterone. VKM assumes that such therapy with isoflavone administration is given under prescription and medical surveillance, and is outside the scope of this risk assessment, which is the risk for the general Norwegian population from isoflavones taken as supplements. Useful information can also be found in studies on

prostate cancer patients and men with other diagnoses and is therefore included. It was shown that isoflavones in doses of 450 or 900 mg/day for three months could have estrogenic effects in men with prostate cancer, such as breast changes or increased frequency of hot flashes (Fischer et al., 2004). In healthy men taking supplements without surveillance, isoflavones may potentially also lead to estrogenic effects. In this risk assessment of isoflavones in healthy men, VKM considers changes in hormone levels away from the normal range as negative effects. There is some data indicating that it is soy protein as such rather than the isoflavones that are causing the observed effects on hormone levels (Dillingham et al., 2005), however, this is still uncertain.

Based on the available studies, isoflavones as supplements in doses of 1.64 to 196 mg/day for one to three months may represent a risk of negative effects on hormone levels in men. These doses do not appear to have other significant negative effects on men in the general population.

#### ***2.5.3.5 Children and adolescents***

The included studies on children and adolescents are summarized in Table 9.4 in Appendix 9.4.

Exposure of infants to soy formula and/or soy milk containing mostly unknown levels and composition of isoflavones appeared to be associated with heavier menstrual bleeding (Upson et al., 2016) with slightly longer menstruation duration (Strom et al., 2001) in early adulthood and earlier age of menarche at puberty (Adgent et al., 2012). Two studies examined the associations between isoflavone intake estimated from FFQ in adolescence estimated from FFQs and breast cancer as adults. One of the studies indicated a negative association in post-menopausal women (Anderson et al., 2013), and another study, a slight positive association in post-menopausal, but a negative association in pre-menopausal women (Lee et al., 2009).

No studies were found on effects of isoflavones from exposure to children (10 to <14 years).

No studies were found addressing both exposure to and effects of isoflavones in adolescents (14 to <18 years). However, there was no evidence in the included literature indicating that adolescents (aged 14 to <18 years, i.e. mostly after puberty) are more sensitive to isoflavones than adults. Therefore, VKM finds that the results for pre-menopausal women and men have validity also for adolescents.

Based on the lack of studies on children, there is not sufficient data to draw any conclusions on potential adverse effects of isoflavones in supplements in children (aged 10 to <14 years).

Based on the available studies, isoflavones as supplements in doses of 1.64 to 196 mg/day or in doses of 45 to 116.4 mg/day for one to three months may represent a risk of negative effects on hormone levels in adolescents of both genders and/or menstrual function in

adolescent women, respectively. These doses do not appear to have other significant negative effects on adolescents.

#### **2.5.4 Interactions**

It was shown that flavonoids with catalytic DNA topoisomerase inhibitory activity, such as daidzein, strongly antagonises the clastogenicity of DNA topoisomerase II poisons, such as genistein, in Chinese hamster V79 cells (Snyder and Gillies, 2003).

According to COT (2003), the potential for isoflavone-drug interactions has not been established. However, a recent paper gives an overview of some drugs, including prescription medicines, that may be affected by isoflavones via their modulation of phase I and phase II metabolic enzymes and interaction with drug transporters (Taneja et al., 2016). This is of potential importance for individuals consuming isoflavone supplements while taking prescribed drugs.

#### **2.5.5 Allergic sensitization**

Isolates from soybeans contain different amounts of soy protein, which is a well-known food allergen, with a reported incidence of 0.3-0.4% of the total population in Germany (BfR, 2007). People with a birch pollen allergy may have cross-allergy to soy protein. Oral allergy symptoms may range from mild symptoms such as itching and blisters to anaphylactic shock. The majority of case reactions reported to the National Register of Severe Allergic Reactions to Food at the Norwegian Institute of Public Health are presumed to be cross-sensitizations due to primary sensitization to birch pollen and/or peanut (Namork E et al., 2016). Only 2.5% (5 of 199) cases were analysed with high levels of specific IgE to soy alone, and reported with adverse reactions after intake of soy in the food (personal communication with Ellen Namork, Norwegian Institute of Public Health). One study indicated that receiving soy formula vs. cow milk formula as infant, increased asthma or allergy as adults (Strom et al., 2001). Whether supplements with isoflavones from soy may cause food allergy is not known.

#### **2.5.6 Endocrine effects of isoflavones**

Phytoestrogens are considered weak estrogens, since the relative affinities of phytoestrogens to estrogen receptors are more than 100-1000-fold lower than that of estradiol (Albertazzi and Purdie, 2002; Cassidy, 2003). However, even so, some foods and dietary supplements contain comparatively high amounts of these compounds so that plasma levels may exceed endogenous estrogen levels by several orders of magnitude and therefore have the potential to exert biological effects *in vivo* (Cassidy, 2003). Genistein is a prototype ER $\beta$ -selective compound, exhibiting >20 fold higher affinity for ER $\beta$  (7-16 nM) than for ER $\alpha$  (330-910 nM) (Prossnitz and Arterburn, 2015). They isoflavones show conformational binding to the estrogen receptor that classifies them as natural selective estrogen receptor modulators (SERMs) rather than as estrogens, and have estrogenic and anti-estrogenic effects depending on the concentration of endogenous estrogen and amount and type of estrogen

receptors (Yuan et al., 2007). They act as estrogen antagonists with a pre-menopausal (high) dose of estradiol, whereas they act as estrogen agonists in a low-estrogen environment near the serum level of post-menopausal women (Hwang et al., 2006). Metabolites of genistein and daidzein can have higher or lower biological activity than their precursors. All the metabolites could act as antagonists that weakened the estrogenic actions at physiologic dose of estrogen. This inhibitory effect on estrogen action was more prominent with ER $\beta$  than with ER $\alpha$ . The ER-mediated effects of isoflavones occur at concentrations of 0.1–1  $\mu$ M (EFSA, 2015).

### **2.5.7 Non-endocrine effects of isoflavones**

There are also hormone-independent actions of isoflavones (EFSA, 2015), including inhibition of tyrosine kinase activity (Akiyama et al., 1987), inhibition of protein kinase C (Osada et al., 1988), inhibition of DNA topoisomerase II (Abe, 1999), inhibition of mitogen-activated kinase 1 (MEKK1) (Sarkar and Li, 2003), antioxidant activity (Djuric et al., 2001; Gyoergy et al., 1964), anti-angiogenic effects (Fotsis et al., 1993) and inhibition of breast cancer resistance protein (BCRP), a cellular efflux protein (Imai et al., 2004). The non-endocrine effects were obtained with isolated compounds *in vitro* at doses typically exceeding 10  $\mu$ M (EFSA, 2015).

### **2.5.8 Potentially vulnerable groups**

Persons with certain polymorphisms in genes affecting estrogenic effects or metabolism of isoflavones may potentially be vulnerable for the effects of isoflavones in supplements. However, polymorphisms associated with decreased health risks rather than increased risk have been reported for isoflavones; for genes affecting estrogenic effects (Iwasaki et al., 2009; Mortensen et al., 2009), in phase I and II metabolic enzymes (Cotterchio et al., 2006; Dai et al., 2007; Deming et al., 2008; Wakeling and Ford, 2012), in genes in the ornithine decarboxylase-polyamine pathway (Cho et al., 2015) or in genes involved in efflux transport of isoflavone glucuronides (Wakeling and Ford, 2012). Metabolic enzymes in the gut microflora may determine which metabolites are formed and their levels, such as the daidzein metabolite *S*-equol formed only in a part of the population (Hamilton-Reeves et al., 2010).

One study on effects of isoflavones on hemodialysis patients concluded that they were not a vulnerable group (Siefker and DiSilvestro, 2006). Collectively the available data provided little evidence that in euthyroid, iodine-replete individuals, soy foods or isoflavones adversely affect thyroid function (EFSA, 2015; Messina, 2010a; Messina and Redmond, 2006). However, individuals with subclinical hypothyroidism may evaluate the need to adjust their dosage of medication, and individuals with inadequate iodine intake may increase their iodine intake further, when consuming soy foods or supplements (COT, 2003; Messina, 2010a).

Consumer groups which have an already high dietary intake of isoflavones from a vegetarian or vegan diet, a traditional Asian (i.e. Japanese, Chinese) diet or a diet high in soy-based foods for whatever reasons (COT, 2003; Hod et al., 2016), or infants eating mainly or solely

soy infant formula (Bar-El Dadon and Reifen, 2010), may be regarded as high consumers of isoflavones. These groups may be vulnerable to potential negative effects from additional intake of isoflavones from supplements.

# 3 Exposure / Intake

Exposure to isoflavones from soy from the intake of food supplements was estimated for the age groups children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years). The adults were stratified into pre-menopausal women, peri- and post-menopausal women and men, and evaluated separately.

## 3.1 Food supplements

NFSA requested VKM to perform a risk assessment of 40 and 80 mg/day of isoflavones from soy in food supplement for children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years). The default body weights (bw) for these groups as determined by EFSA were used: 10 to <14 years; 43.4 kg, 14 to <18 years; 61.3 kg and adults; 70.0 kg (EFSA, 2012).

The estimated exposure to soy isoflavones from food supplements for the various age groups is presented in Table 3.1-1.

From the dose of 40 mg/day of isoflavones in supplements, the estimated daily intake of isoflavones were 0.92, 0.65 and 0.57 mg/kg bw per day for children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years), respectively. From the dose of 80 mg/day of isoflavones, the estimated daily intake of isoflavones were 1.84, 1.31 and 1.14 mg/kg bw per day for children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years), respectively.

Table 3.1-1 Estimated daily intake of soy isoflavones (mg/kg bw per day) from food supplements for the various age groups.

Intake (mg/kg bw per day)	Daily doses (mg)	
	40	80
Children (10 to <14 years)	0.92	1.84
Adolescents (14 to <18 years)	0.65	1.31
Adults ( $\geq 18$ years)	0.57	1.14

## 3.2 Other sources

Isoflavone content in various foods can be found in (Bhagwat et al., 2008).

### 3.2.1 From the diet in children and adolescents

Infants fed soy-based formula is a high consumer group with intakes of isoflavones of approximately 40 mg/day (Bakker, 2004). No data was available of intake of isoflavones from foods in Norwegian children and adolescents. However, the mean intake of soy protein

per day has been estimated for persons eating either a vegan menu or a milk-free diet, based on weekly menus (VKM, 2015). For children, the numbers for adults were adjusted for energy requirements, and assuming that milk in coffee and tea was consumed as milk. In the vegan scenario, the estimated intake was 18, 24, 30 and 41 g soy protein per day for 2-5 year-olds, 6-9 year-olds, 10-13 year old girls and 14-17 year old boys, respectively. In the milk allergy scenario, the estimated intake was 10, 14, 17 and 23 g soy protein per day for 2-5 year-olds, 6-9 year-olds, 10-13 year old girls and 14-17 year old boys, respectively. For 10-13 year old boys and 14-17 year old girls, the estimated intake is approximately similar to the intake in adults (see below).

### 3.2.2 From the diet in adults

The adult consumer groups with the highest dietary intake of isoflavones (approximate levels) are consumers taking dietary phytoestrogen containing supplements (40–100 mg/day), vegan breast-feeding women (75 mg/day) and consumers of a traditional South East Asian diet (25-100 mg/day) (Bakker, 2004). The dietary isoflavone intake of average Western consumers and vegetarians is much lower (<1–2 mg/day and 3-12 mg/day, respectively).

Overall background exposure to isoflavones from the diet in the general European population was estimated to be lower than 1 mg/day (0.27-1.43 mg/day in women), whereas in consumers of soy-based foods and vegetarians it could be higher and within the estimated range of exposure to isoflavones from soy food supplements (approximately 0.1-111 mg/day) (EFSA, 2015).

The intake (mean  $\pm$  SE) of isoflavones from dietary soy in women was  $1.474 \pm 0.158$  and  $1.096 \pm 0.178$  mg/day in south and east of Norway and north and west of Norway, respectively, based on a single 24-hour recall in the EPIC study (Zamora-Ros et al., 2012). Corresponding numbers were  $0.005 \pm 0.000$  and  $0.004 \pm 0.000$  mg/day for the daidzein metabolite *S*-equol. For men, the corresponding intakes of isoflavones were  $0.978 \pm 0.135$  and  $0.721 \pm 0.136$  mg/day, respectively, and for *S*-equol, the numbers were  $0.006 \pm 0.000$  and  $0.007 \pm 0.000$  mg/day, respectively.

Based on a food frequency questionnaire, among 87800 women in the Norwegian Mother and Child Cohort Study (MoBa), 1.7% used soy milk, and of these only 0.4% drank  $\geq 200$  ml of soy milk per day (unpublished data, personal communication with Margareta Haugen, the Norwegian Institute of Public Health). Regarding use of soy products for dinner, 4.8% had an occasional intake, and only 12% of these women had soy products for dinner more than once every other week.

In Norway, as in other Western countries, the intake of soybeans and soybean-based products is generally low in the average diet, but may be higher in vegans and persons with milk allergy. The mean intake of soy protein per day has been estimated for persons eating either a vegan menu or a milk-free diet, based on weekly menus (VKM, 2015). For the vegan diet, the meat was replaced by soy burgers, soy sausages etc. For the milk-free diet, the



milk products were replaced with soy products. The mean intake for adults (women or men) was 35 and 19 g soy protein per day, for the vegan and milk allergy scenarios, respectively.

### **3.2.3 From cosmetics**

Genistein and daidzein are used for skin conditioning in cosmetics (CosIng, 2015).

## 4 Risk characterisation

NFSA requested VKM to perform a risk assessment of the intake of 40 and 80 mg isoflavones from soy (as the total of genistein and daidzein) per day from food supplements for the general population, ages 10 years and above.

According to information from NFSA, the food supplements on the Norwegian market contain isoflavones isolated from soybeans of the plant *Glycine max* (L.) Merrill by extraction by 80% alcohol (which alcohol is unknown) and 20% water. No further information was given on the form of isoflavones (aglycones or glycosides), which individual isoflavones are present and in which amounts in the extracts of the soy isoflavone supplements on the Norwegian market.

Based on the included studies, no pattern was obvious regarding type of soy product or isoflavone composition and negative health effects. Therefore, in this risk assessment VKM has used the doses of total isoflavones reported to cause negative effects in studies on the respective gender and age groups for comparisons with 40 and 80 mg/day of total isoflavones in supplements.

### *Peri- and postmenopausal women*

Gastrointestinal symptoms, insomnia or back pain were reported as adverse events/side-effects in peri- and postmenopausal women at the same rate, or in a few studies at higher rate, with isoflavones compared with placebo. The doses of total isoflavones and duration of treatment in these studies were 60 and approximately 120 mg total isoflavones per day for 6 weeks, from 56 to 160 mg/day for 3-6 months and from 52 to 300 mg/day from 9 to 12 months. In addition, one study examined effects of 898 mg/day of total isoflavones for 3 months and another study examined effects of 150 mg/day for 5 years. Based on the available studies from the literature, isoflavones as supplements in these doses and duration of treatment appear to be without significant negative health effects in peri- and postmenopausal women.

The relevance of the few studies that found increased risk of cancer of a very high dose of isoflavone supplements or in occasional comparisons of dietary intake of soy food products in mostly Asian populations is difficult to interpret in relation to intake of isoflavone supplements in Norwegian peri- and post-menopausal women (see 2.4.2.1).

EFSA (2015) concluded that doses of treatment used in the intervention studies and their durations could serve as guidance for a dose and duration of use at which no effects have been observed in these three target organs (mammary gland, uterus or thyroid) in peri- and post-menopausal women from the intake of food supplements. For soy isoflavones/soy extract this dose was 100 mg/day (total isoflavones), 10 months duration of intake. For soy protein, this dose was 99 mg/day (aglycone), 3 months duration of intake. For daidzein-rich isoflavones, this dose was 72 mg/day (total), 6 months duration of intake. For genistein, this dose was 54 mg/day, 36 months duration of intake.

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for several months and even up to several years appear to be without significant negative health effects in peri- and post-menopausal women.

#### *Pre-menopausal women*

Isoflavones apparently affected hormone levels in doses of 45 to 128 mg/day and affected menstruation in doses of 45 to 116.4 mg/day in pre-menopausal women, when taken for approximately one to three months. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In peri- and post-menopausal women, soy isoflavones are taken with the purpose of decreasing post-menopausal symptoms such as hot flashes, instead of estrogen-based hormone-replacement therapy, whereas pre-menopausal women would not have this need. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy pre-menopausal women. In this risk assessment of isoflavones in healthy pre-menopausal women, VKM considers changes in hormone levels away from the normal range as negative effects (see 2.4.2.2).

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels and/or menstrual function in pre-menopausal women. These doses do not appear to have other significant negative effects on pre-menopausal women.

#### *Men*

The most common adverse events/side-effects of isoflavones reported in healthy men were mild gastrointestinal symptoms, or sometimes weight gain, estrogenic effects or reduction in vitamin E levels. Hormone levels appeared to be affected by doses of isoflavones ranging from 1.64 to 196 mg/day for one to three months. Few studies discuss the clinical relevance of the observed changes in hormone levels due to isoflavone exposure in healthy men. Whether the effects of exposure to soy isoflavones affecting hormone levels will be regarded as adverse or beneficial will depend on the target group. In patients with prostate cancer or at high risk for recurrence of prostate cancer and possibly in patients with other types of hormone-related cancers, isoflavones may have a beneficial effect on the progression of the disease by decreasing testosterone. VKM assumes that such therapy with isoflavone administration is given under prescription and medical surveillance, and is outside the scope of this risk assessment, which is the risk for the general Norwegian population from isoflavones taken as supplements. Useful information can also be found in studies on prostate cancer patients and men with other diagnoses and is therefore included. It was shown that isoflavones in doses 450 or 900 mg/day isoflavones for three months could have estrogenic effects in men with prostate cancer, such as breast changes or increased frequency of hot flashes. In healthy men taking supplements without surveillance, isoflavones may potentially also lead to estrogenic effects. In this risk assessment of isoflavones in healthy men, VKM considers changes in hormone levels away from the normal

range as negative effects (see 2.4.2.3). There is some data indicating that it is soy protein as such rather than the isoflavones that are causing the observed effects on hormone levels, however, this is still uncertain.

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in men. These doses do not appear to have other significant negative effects on men in the general population.

#### *Children and adolescents*

No studies were found on effects of isoflavones from exposure to children (10 to <14 years).

Based on the lack of studies on children, VKM concludes that there is not sufficient data to draw any conclusions on potential adverse effects of isoflavones in supplements in children (aged 10 to <14 years).

No studies were found addressing both exposure to and effects of isoflavones in adolescents (14 to <18 years). However, there was no evidence in the included literature indicating that adolescents (aged 14 to <18 years, i.e. mostly after puberty) are more sensitive to isoflavones than adults. Therefore, VKM finds that the results for pre-menopausal women and men have validity also for adolescents (see 2.4.2.4).

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in adolescents of both genders and/or menstrual function in adolescent women. These doses do not appear to have other significant negative effects on adolescents.

# 5 Uncertainties

## 5.1 Hazard identification and characterisation

### 5.1.1 Uncertainty in the included articles from the literature searches

Many of the papers used as information on hazard identification and characterization do not give information about whether the isoflavone content is expressed as aglycones or glycosides, or give information on the composition of the individual isoflavones, i.e. the percentage of genistein, daidzein and glycitein.

Several of the included human studies are RCTs, specifically designed to investigate the positive effects of isoflavones, or more generally of phytoestrogens or soy food products, and not negative effects. Whether negative effects have been looked for, but not observed, or not been looked for, is often not reported.

### 5.1.2 Uncertainty in ADME

A number of both internal and external factors are influencing the phytoestrogen bioavailability, including intestinal microflora, gender, age, food matrix, chemical composition, earlier exposure and background diet (van de Poll, 2004).

There is interindividual variation in metabolism of isoflavones, i.e. for instance in the ability to metabolize daidzein to *S*-equol. This is not always taken into account in the studies on effects of isoflavones.

Because of the variation between animals and humans in ADME of isoflavones, the results from animal studies must be used with caution, and are not included in this risk assessment except for *in vivo* genotoxicity.

The starting material used to manufacture the isoflavone supplements may have significant effects on the ultimate bioavailability and characteristics of the circulating isoflavone levels.

### 5.1.3 Uncertainties related to differences in Western versus Asian populations

Many of the studies published on effects of isoflavones are on Asian populations. There is some uncertainty as to whether Asian and non-Asian individuals respond similarly to the effects of isoflavones. In theory, the long history of exposure, traditional lifestyle factors (including, but not limited to dietary habits), and possible genetic differences in metabolism of isoflavones could limit the ability to extrapolate findings from Asians to non-Asians. There is some evidence indicating that these two population groups have some differences in

isoflavone pharmacokinetics and bioavailability. On acute intake of soy cheese, Asians absorbed soy isoflavones better than Caucasians, regardless of whether the background diet was Western or Asian (Vergne et al., 2009). On chronic ingestions, AUC and  $C_{max}$  values were increased for genistein and daidzein in Caucasians, but not in Asians. Another difference is that Asians are exposed to isoflavones from an early age via the consumption of traditional soy foods, whereas exposure in non-Asians usually begins later in life unless exposed to soy infant formula.

Asian populations have a much higher average isoflavone intake from food than Western populations throughout their lives. Therefore, caution must be used when comparing effects of isoflavone intake on Western populations with Asian populations. The estimated consumption of soy isoflavones ranges from 20-200 mg per day in Japan, and 20-150 mg per day in other Asian populations. Less than 1-5 mg per day is consumed by individuals on a traditional Western diet (Hu, 2004). However, the consumption of soy product such as soy milk is increasing also in countries with a Western diet, such as in USA.

As opposed to a high intake of isoflavones from food, a low prevalence of isoflavone supplementation (<1.6%) was reported in Japanese 55-75 year-olds (Hirayama et al., 2008).

#### **5.1.4 Uncertainties because of lack of data on effects of isoflavones in children and adolescents**

No studies were found on effects of isoflavones from exposure to children (10 to <14 years).

No studies were found addressing both exposure to and effects of isoflavones in adolescents (14 to <18 years). However, there was no evidence in the included literature indicating that adolescents (aged 14 to <18 years, i.e. mostly after puberty) are more sensitive to isoflavones than adults. Therefore, VKM finds that the findings for pre-menopausal women and men have validity also for adolescents. However, not all adolescents may be through their puberty at the age 14 to <18 years.

## **5.2 Exposure characterization**

### **5.2.1 Characterization of the source and type of isoflavones**

Since the isoflavone supplement preparations lack standardization, there is often lack of knowledge of what the exact active ingredients are and of their respective amounts. The content of total isoflavones and the composition of individual isoflavones may vary among different soy products, but also among the same type of products and from batch to batch of the same soy product.

The form (aglycones or glycosides) and composition, i.e. the amounts of each type of isoflavone, genistein, daidzein, glycitein, of the soy isoflavones in supplements sold on the Norwegian market, are unknown to VKM. Therefore, there is a significant uncertainty related

to how relevant the studies used in this risk assessment are to the supplements sold in Norway.

It is still unclear whether the effects (adverse or beneficial) of soy intake resides mainly in the soy protein itself, whereas phytoestrogens, including isoflavones, provide little if any additional effects, or if there is some interaction between the soy protein and isoflavones (Sirtori et al., 2005; Wagner et al., 2001).

There is large variation in composition and concentration among different soybean or soy protein products, which is dependent of soy species differences, part of the soy plant used, geographic and environmental growing conditions and cultivating parameters. The extent of industrial processing of the soybeans, including the extraction process, i.e. which mechanical extraction method, solvent type, temperature and time being used, can also affect composition and concentration of isoflavones (Setchell, 1998).

Striking differences in composition are found between the types of soy proteins commonly used in China, Japan and Indonesia versus those usually used in foods in Western countries (Setchell, 1998). Many of the soy foods consumed in Asia are highly fermented soybean products and many of the bacteria used in their preparation are able to hydrolyse the glycosidic conjugates and modifying the composition to a predominance of aglycones. These compositional differences may be important in regard to metabolism and bioavailability, and thereby for the effects, of isoflavones. These considerations points to uncertainties when using data on isoflavones from soy food intake in Asian populations in a risk assessment of isoflavone supplements in the Norwegian population.

Similarly, the effects of isoflavones may be different in persons having been exposed to high levels of isoflavones in soy formula as infants or from early childhood in foods versus persons not having exposure to isoflavones until adolescence or adulthood. Therefore, both benefit and risk assessments should take into account the developmental stage of the individual at the time of exposure. Both menopausal and prepubescent life stages are characterized by naturally low levels of estrogens and are preceded by life stages where estrogen levels are high and estrogen receptors are abundant as result of the estrogen exposure. It is hypothesized that these periods of low estrogen exposure are potential windows of opportunity for beneficial effects or of susceptibility for negative effects of soy isoflavones, because at these times isoflavones are less opposed by estrogens, enabling them to exert their maximal estrogen receptor-mediated effects (Reinwald and Weaver, 2006). Delayed effects may be unnoticeable at the time of exposure, but become apparent only as temporal endocrinological changes occur later, during puberty, adulthood or pregnancy (Reinwald and Weaver, 2006).

There are too few studies to evaluate whether isoflavone supplements (capsules, pills, powders) have the same effects (positive or negative) as isoflavones taken as soy foods (Wagner et al., 2001). Also the matrix of isoflavones in the supplements, i.e. whether given as a separate powder or mixed into foods, may differently affect adverse events such as constipation and diarrhea.

It has been shown that the type of supplement may vary in effect, and this variation may affect the clinical end points studied (Setchell et al., 2001). For instance, supplements with soy germ as starting material resulted in plasma enriched in daidzein and low in genistein. By contrast, with supplements made from extracts of soy proteins the plasma was enriched to a greater extent in genistein.

### **5.2.2 Intake calculations**

With use of the default (mean) body weight of an age (population) group, the variance in all individuals in the group will not be covered.

## **5.3 Risk characterization**

All the uncertainties mentioned above for both hazard identification and characterization and for exposure characterization, will also bring uncertainties to the risk characterization in this risk assessment of isoflavones in supplements on the Norwegian market.



## 6 Conclusions with answers to the terms of reference

NFSA requested VKM to perform a risk assessment of the intake of 40 and 80 mg isoflavones from soy (as the total of genistein and daidzein) per day from food supplements for the general population, ages 10 years and above.

According to information from NFSA, the food supplements on the Norwegian market contain isoflavones isolated from soybeans of the plant *Glycine max* (L.) Merrill by extraction by 80% alcohol (which alcohol is unknown) and 20% water. No further information was given on the form of isoflavones (aglycones or glycosides), which individual isoflavones are present and in which amounts in the extracts of the soy isoflavone supplements on the Norwegian market.

Based on the included studies, no pattern was obvious regarding type of soy product or isoflavone composition and negative health effects. Therefore, in this risk assessment VKM has used the doses of total isoflavones reported to cause negative effects in studies on the respective gender and age groups for comparisons with 40 and 80 mg/day of total isoflavones in supplements.

### *Peri- and postmenopausal women*

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for several months and even up to several years appear to be without significant negative health effects in peri- and post-menopausal women.

### *Pre-menopausal women*

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels and/or menstrual function in pre-menopausal women. These doses do not appear to have other significant negative effects on pre-menopausal women.

### *Men*

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in men. These doses do not appear to have other significant negative effects on men in the general population.

### *Children and adolescents*

Based on the lack of studies on children, VKM concludes that there is not sufficient data to draw any conclusions on potential adverse effects of isoflavones in supplements in children (aged 10 to <14 years).

Given that the isoflavone supplements sold on the Norwegian market are comparable to the isoflavones used in the available studies, VKM concludes that isoflavones as supplements in doses of 40 or 80 mg/day taken for one to three months may represent a risk of negative effects on hormone levels in adolescents of both genders and/or menstrual function in adolescent women. These doses do not appear to have other significant negative effects on adolescents.

#### *Potentially vulnerable groups*

Persons with certain polymorphisms in genes affecting estrogenic effects or metabolism of isoflavones may potentially be vulnerable for the effects of isoflavones in supplements. However, polymorphisms associated with decreased health risks rather than increased risk have been reported for isoflavones; for genes affecting estrogenic effects, in phase I and II metabolic enzymes, in genes in the ornithine decarboxylase-polyamine pathway or in genes involved in efflux transport of isoflavone glucuronides. Metabolic enzymes in the gut microflora may determine which metabolites are formed and their levels, such as the daidzein metabolite *S*-equol formed only in a part of the population.

One study on effects of isoflavones on hemodialysis patients concluded that they were not a vulnerable group. Collectively the available data provided little evidence that in euthyroid, iodine-replete individuals, soy foods or isoflavones adversely affect thyroid function. However, individuals with subclinical hypothyroidism may evaluate the need to adjust their dosage of medication, and individuals with inadequate iodine intake may increase their iodine intake further, when consuming soy foods or supplements.

Consumer groups which have an already high dietary intake of isoflavones from a vegetarian or vegan diet, a traditional Asian (i.e. Japanese, Chinese) diet or a diet high in soy-based foods for whatever reasons, or infants eating mainly or solely soy infant formula, may be regarded as high consumers of isoflavones. These groups may be vulnerable to potential negative effects from additional intake of isoflavones from supplements.

An overview of the conclusions is presented in Table 6.1 for hormonal effects and in Table 6.2 for all other effects of isoflavones in supplements. Estimated exposures unlikely to cause adverse health effects are shown in green and estimated exposures to isoflavones that may represent a risk of adverse health effects are shown in red.

**Table 6.1** An overview of the conclusions on isoflavones from soy in food supplements related to **hormonal effects and/or menstrual functions**. Green: estimated exposure to isoflavones is unlikely to cause negative health effects. Red: estimated exposure to isoflavones may represent a risk of negative health effects.

Isoflavones		
Doses	40 mg/day	80 mg/day
Age groups		
Children (10 to <14 years)	Cannot conclude because of lack of data	Cannot conclude because of lack of data
Adolescents (14 to <18 years)	Red	Red
Pre-menopausal women (≥18 years)	Red	Red
Peri- and post-menopausal women	Yellow	Yellow
Men (≥18 years)	Red	Red

**Table 6.2** An overview of the conclusions on isoflavones from soy in food supplements **for all other effects than on hormone levels**. Green: estimated exposure to isoflavones is unlikely to cause negative health effects. Red: estimated exposure to isoflavones may represent a risk of negative health effects.

Isoflavones		
Doses	40 mg/day	80 mg/day
Age groups		
Children (10 to <14 years)	Cannot conclude because of lack of data	Cannot conclude because of lack of data
Adolescents (14 to <18 years)		
Pre-menopausal women (≥18 years)		
Peri- and post-menopausal women		
Men (≥18 years)		

## 7 Data gaps

- There is little knowledge on the exact composition of the isoflavone supplements sold on the Norwegian market.
- There are few studies available on the actual level of dietary exposure to soy isoflavones for Norwegians on an average diet.
- There is no information on the actual levels of dietary exposure to soy isoflavones for Norwegians who are vegetarians or vegans of any age.
- There is no information available on the actual level of exposure to soy isoflavones from supplements in Norwegians of any age.
- There is no information available on to which degree, if at all, children and adolescents are exposed to isoflavones from soy as supplements in Norway.
- There is no information available on to which degree, if at all, pre-menopausal women and men are exposed to isoflavones from soy as supplements in Norway.
- There are generally few studies on negative health effects related to isoflavones compared with studies on potentially positive effects.
- In many of the studies on positive effects often it is not mentioned whether negative effects had been looked for at all or were looked for, but not detected.
- There is no information on the distribution of *S*-equol producers and non-producers in the Norwegian population.
- No studies were found on negative health effects of isoflavones from exposure to children (10 to <14 years).
- No studies were found addressing both exposure to and effects of isoflavones in adolescents (14 to <18 years).
- No studies were found on effects of isoflavones in lactating or pregnant women.
- Some data indicate that it is soy protein rather than isoflavones that are affecting hormone levels in men, however, this is still uncertain.

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# 9 Appendix

## 9.1 Human studies on effects of isoflavones on peri- and post-menopausal women

**Table 9.1** An overview of human studies on **peri- and post-menopausal women** investigating health effects of isoflavones (IF).

Study design/ Reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
Meta-analyses								
<b>Chen et al., 2015</b>	Of 543 potentially relevant RCTs with oral intake of phytoestrogens, 15 meeting the inclusion criteria were included in the meta-analysis	Various	n=16-167, mean $\pm$ SD for age: 48 $\pm$ 60.1 years	n=14-103, 49 $\pm$ 58.3 years	Isoflavones (dose not given, one study), isoflavones (25-100 mg/day, 12 studies), equol (40 mg/day, one study) and other phytoestrogens (trifolium, 40 mg/day, one study)	Menopausal symptoms (Kupperman index (KI) or hot flushes) and side-effects	Intervention periods were 3-12 months	Meta-analysis of 7 studies reporting KI data (11 menopausal symptoms) indicated no significant treatment effect of phytoestrogens vs. placebo (pooled mean difference 6.44, P=0.110), 10 studies reporting hot flush data indicated that phytoestrogens gave a significantly greater reduction in hot flush frequency vs. placebo (pooled mean difference 0.89, P<0.005), and 5 studies reporting side-effect data showed no significant difference between the two groups (P=0.175)
<b>Yan et al., 2010</b>	Meta-analysis of 4 cohort and 7 case-control studies on soy and colorectal cancer risk studied in	Various	Soy consumption of various soy foods, isoflavones or		From low $\mu$ g/day to low mg/day in Western studies to from several mg/day to >50 mg/day in the Asian studies	Colorectal cancer, colon cancer, rectal cancer		Soy consumption was not associated with colorectal cancer risk (combined risk estimate 0.90, 95% CI 0.79-1.03) or separate

	men and women (mostly post-menopausal women based on age, but menopausal status was not stated)		genistein (data from FFQ)					analyses on colon cancer (combined risk estimate 0.88, 95% CI 0.74-1.06) and rectal cancer (combined risk estimate 0.88, 95% CI 0.67-1.14). When separately analysed on gender, soy was associated with approximately 21% reduction in colorectal cancer risk in women (combined risk estimate 0.79, 95% CI 0.65-0.97; P=0.026), but not in men (combined risk estimate 1.10, 95% CI 0.90-1.33). When analysing 6 studies on isoflavones, consumption was associated with approximately 16% reduced risk of colorectal cancer, largely attributable to Western case-control studies with low µg to low mg per day isoflavone intakes
<b>Tempfer et al. 2009</b>	92 of 174 trials of post-menopausal women using phytoestrogens (isoflavones, lignans and coumestans) for treatment of climacteric syndrome reported on side-effects	Various	n = 5502	n = 4806	Various	Side-effects	Median treatment duration 6.2 months	Overall incidence of side-effects in phytoestrogen and control groups was 36.7% and 38.0%, respectively (n.s.). <b>In only 2 of 92 studies was there a statistically significant difference in side-effect incidence between treatment and placebo groups (Unfer et al., 2004, and Albertazzi et al., 2005, see below).</b>

								<p><b>Comparing various side-effect categories, significant higher rates of gastrointestinal side-effects among phytoestrogen users (P=0.003, IRR=1.28 (95% CI 1.08-1.50)) were found,</b> whereas for the other categories they were not significantly different between groups. Within side-effect categories, they found no significantly higher rates of side-effects in women using phytoestrogens. Specifically, the rates of hormone-related side-effects such as endometrial hyperplasia, endometrial cancer and breast cancer were not significantly different between groups. The authors concluded that phytoestrogens supplements had a safe side-effect profile with moderately elevated rates of gastrointestinal side-effects such as abdominal pain, myalgia and sleepiness. Women using phytoestrogens for longer time periods (cut off points 6, 12 and 24 months) reported fewer side-effects than women enrolled in studies with shorter duration,</p>
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								suggesting no cumulative dose effects over time
RCTs								
<b>Carmignani et al., 2015</b>	Healthy post-menopausal women, age 40-60 years, (n=60)	Brazil	Soy protein powder and placebo tablet	Placebo tablets and powder (maltodextrin), hormone therapy (HT) (tablets of 1 mg estradiol and 0.5 mg norethisterone acetate, and placebo powder)	90 mg/day IF (53 mg as aglycones, Ge:D:Gl 57:30:13)	Effects on the urogenital system (urinary, vaginal and sexual complaints, vaginal maturation, pH and dryness, endometrium thickness, genital bleeding)	16 weeks	Vaginal dryness improved significantly in the soy and HT groups (P=0.04). Urinary and sexual symptoms did not change with treatment in the three groups. After 16 weeks of treatment, there was a significant increase in maturation value only in the HT group (P<0.01). Vaginal pH decreased only in this group (P<0.01). There were no statistically significant differences in endometrial thickness between the three groups. There were no drop-outs during the study and no statistically significant differences in the adverse effects evaluated (mastalgia, vaginal bleeding, allergy, headache, nausea, weight gain, water retention and intestinal complaints) between the three treatment groups. Despite the absence of statistical difference, 20% of women in the HT group had genital bleeding. In contrast, in the soy and placebo groups, bleeding occurred in 5% of cases
<b>Cheng et al., 2015</b>	Healthy post-menopausal women,	Taiwan	Tablets of IF	Placebo tablets	300 mg/day IF as aglycones (no	Blood pressure, hematological	1 year	In the isoflavone group, after 1 year, the changes



	age 45-65 years, (n=82)				information given on composition)	parameters, coagulation functions including circulating microparticles		in liver function tests, hematological parameters and coagulation tests were not different from controls. Triglyceride levels were significantly lower after 6 months of isoflavone treatment than the placebo group (P=0.034), but the difference did not persist after 1 year. Endothelial microparticles increased steadily in both groups during the 1-year period but the trend was not affected by treatment. There were no significant differences between the groups in systolic or diastolic blood pressure during the overall study period
<b>Gleason et al., 2015</b>	Patients with Alzheimer's disease, but otherwise free of major medical, neurological and psychiatric illnesses, age >60 years, post-menopausal women (n=34), <b>men</b> (n=31)	USA	Soy isoflavone capsules	Placebo (maltodextrin and caramel color) capsules	100 mg isoflavones/day, mostly as glycosides (Ge:D:GI %: 43.4:37.2:8.8), less as aglycones (Ge:D:GI %: 0.4:0.8:0.6), plus minor amounts of malonyl- and acetyl-derivatives of the glycosides	Cognitive outcomes (tests for verbal and visuospatial memory, language, working memory, executive function, visual-motor function) and mood	6 months	No significant differences in treatment effects for cognition emerged between treatment groups or genders. Exploratory analyses of associations between changes in cognition and plasma isoflavone levels revealed an association between equal levels and speeded dexterity and verbal fluency. No subjects were discontinued because of abnormal laboratory values, and there were no significant differences between isoflavone

								treated and controls in average systolic or diastolic blood pressure or in average blood levels of amylase, lipase or phosphate
<b>Basaria et al., 2009</b>	Healthy post-menopausal white women (mean $\pm$ SD age 56 years), 80% Caucasians	USA	Soy protein powder with isoflavones	Placebo powder (whole milk protein)	160 mg total IF (96 mg aglycones, Ge:D:GI %: 40:39:21)	Quality of life, cognition, lipoproteins and androgen status	12 weeks	There was a significant improvement in all 4 QOL subscales (vasomotor, psychosexual, physical and sexual) after isoflavones, with no changes after placebo. No significant changes in cognition, serum androgens or plasma lipids were seen within any groups over time. <b>However, at the end of the study a group-by-time interaction was observed so that total testosterone and HDL levels were significantly lower in the isoflavone compared with placebo group (P=0.0115 and P=0.0370, respectively).</b> 7 women withdrew because of adverse effects; (gastrointestinal upset (5) and bad taste (2)). Among these 7, 4 were given isoflavones and 3 were given placebo. The majority of the women tolerated the supplement well without any problems

<b>Brink et al., 2008</b>	Healthy early post-menopausal white women (mean $\pm$ SD age 53 $\pm$ 3 years)	The Netherlands, Italy and France	Biscuits and cereal bars with isoflavone concentrate	Biscuits and cereal bars without isoflavone concentrate	110 mg IF/day (as aglycone equivalents, Ge:D:GI %: 60-75:25-35:1-5)	Bone mineral density, bone metabolism and hormone status	1 year	The isoflavone-enriched foods did not alter bone mineral density of the lumbar spine and total body or markers of bone formation and bone resorption. Hormone concentrations (estradiol, FSH, LH and SHBG) did not differ between the treatment groups. The categories, intensity and number of AE were similar between the two treatment groups. There were no concerns over safety, as determined by reported AE, blood chemistry, hematology, blood lipids and vaginal maturation
<b>Marini et al., 2008</b>	Osteopenic post-menopausal women (age 49-67 years) (n=138)		Genistein aglycone isolated from soy (purity $\geq$ 98%), in tablets (n=71)	Placebo tablets with similar appearance (n=67)	54 mg/day of genistein	Long-term safety on breast and endometrium and effects on bone	3 years	After 3 years, genistein did not significantly change mammographic breast density or endometrial thickness, BRCA1 and BRCA2 expression was preserved, whereas sister chromatid exchange was reduced compared with placebo. BMD increases were greater with genistein for both femoral neck and lumbar spine compared with placebo. Genistein also significantly reduced pyridinoline, as well as serum carboxy-terminal cross-linking telopeptide and soluble receptor activator of NF- $\kappa$ B ligand

								while increasing bone-specific alkaline phosphatase, IGF-I and osteoprotegerin levels. Several blood parameters of general safety, such as prothrombin time, partial thromboplastin time, hemoglobin, total serum protein, urinary creatinine, hepatic and pancreatic enzymes, were evaluated using routine methods. They observed a moderate number of gastrointestinal side-effects in the genistein-treated women. These side-effects were also observed in the placebo arm at a lower rate, however, there were no differences in discomfort or adverse events between the groups
<b>Pop et al., 2008</b>	Healthy post-menopausal women (n=30)	USA	PTI G-2535 supplement (in capsules), as unconjugated isoflavones, mean age 56.78 ± 1.25 years (n=18), 16 Caucasians, 2 African-Americans, 4 equol producers, 1 intermediate producer and 13 non-producers	Placebo capsules with similar size and color with excipients from the active formulation, mean age 53.50 ± 1.06 years (n=12), 10 Caucasians, 1 African-American and 1 Asian-American, 2 equol	Approximately 558 mg/day genistein, 296 mg/day daidzein and 44 mg/day glycitein (total isoflavones 898 mg/day)	Blood tests: complete blood count, blood urea nitrogen/creatinine ratio, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase (AST), γ-glutamyl transferase, amylase, lipase and triglycerides. Toxicity panel: electrolytes, calcium, magnesium, phosphorus, lactate dehydrogenase, albumin, uric acid, total bilirubin, fibrinogen, prothrombin time, partial prothrombin	84 days exposure, followed until 112 days after study initiation, 28 days after treatment cessation	The clinical tests showed no significant change in the mean difference at day 84 from day 1, comparing isoflavones and placebo. Grade I adverse events possibly related to isoflavones: isoflavones - two events of increased blood pressure (no significant changes in mean systolic or diastolic blood pressure) and one event each of increased AST or triglycerides and of decreased T4, placebo -

				producers, 10 non-producers		time, thyroxine (T4), thyroid-stimulating hormone (TSH), triiodothyronine uptake and free thyroxine index, and blood pressure. Estrogenic/antiestrogenic effects with self-report questionnaire and serum concentrations of estrogen, follicle-stimulating hormone (FSH), luteinizing hormone and sex hormone-binding globulin		four events of decreased T4 and one event each of increased estrogen or TSH, decreased FSH/LH and flatulence. At study end, T4 values were normal in all women and at no time did they have symptoms of hypothyroidism. Grade I adverse events probably related to isoflavones: isoflavones - one event of nausea, placebo - one event each of mild swellings in legs and breast tenderness. Self-reported estrogenic/antiestrogenic effects possibly related to isoflavones: isoflavones - six events of increased intensity or frequency of hot flashes, one event of decreased intensity of hot flashes and two events of increased breast size or tenderness, placebo - the corresponding numbers of events were three, one and zero, respectively. The isoflavones were safe and well tolerated at a total dose of approximately 900 mg/day
<b>Atteritano et al., 2007</b>	Osteopenic post-menopausal women (age 49-67 years) (n=304)	Italy	Genistein isolated from soy (purity ≥98%), in	Placebo tablets with similar appearance (n=154)	54 mg/day of genistein	Blood lipid profiles, fasting glucose and insulin, HOMA-IR, fibrinogen, F2-isoprostanes, sICAM-1,	2 years	Compared with placebo, genistein significantly reduced fasting glucose and insulin as well as HOMA-IR after 12 and 24

			tablets (n=150)			sVCAM-1 and OPG (predictors of cardiovascular risk)		months. By contrast, genistein did not affect blood lipid levels, although fibrinogen, F2-isoprostanes, sICAM-1 and sVCAM-1 decreased significantly compared with placebo after 24 months. Serum OPG was higher on genistein than on placebo. At 24 months, the genistein group showed no change in endometrial thickness compared with placebo. Both genistein and placebo were generally well tolerated and ingested with a high degree of compliance. There were no significant changes in routine biochemistry, liver function or hematology results. <b>In total, 37 (19%) genistein and 15 (8%) placebo recipients discontinued therapy because of adverse events (P=0.002), all of which were moderate gastrointestinal side-effects (abdominal or epigastric pain, dyspepsia, vomiting, constipation)</b>
<b>D'Anna et al., 2007 (12 months);</b>	Osteopenic post-menopausal women (age 50-70 years) (n=389)	Italy	Genistein isolated from soy (purity ≥98%), in tablets	Placebo tablets with similar appearance	54 mg/day of genistein	Hot flushes, thickness and maturation of endometrium	1 year and 2 years	The reducing effect of genistein on hot flushed was seen in the first month and reached its peak after 12 months (-

2009 (24 months)								56.4% in mean number of hot flushes). There was a significant difference between the two groups at 1, 3, 6 and 12 months. After 24 months, there was no further decrease in the number of hot flushes. No significant difference was found in mean endometrial thickness and maturation value score between the two groups at baseline, 12 and 24 months. Genistein was well tolerated and ingested with a high degree of compliance. There were no significant changes in routine biochemistry, liver function or hematology results. In total, 48 dropped-out in the genistein group and 37 in the placebo group in the parent study and 16 in the genistein group and 13 in the placebo group in the substudy on women with hot flushes, mostly because of gastrointestinal effects
<b>Fournier et al., 2007</b>	Healthy post-menopausal women, 48-65 years	USA	1) Soy milk and placebo (maltodextrin) supplement (n=25), or 2) cow's milk and IF supplement (n=27)	Cow's milk and placebo supplement (maltodextrin) (n=27)	1) 72 mg IF/day (Ge:D:GI %: 37:31:4) and 2) 70 mg IF/day (Ge:D:GI %: 33:30:7)	Cognitive functioning (various tests)	4 weeks on adjustment diet of food minimizing IF intake, then 16 weeks treatment	The isoflavones did not improve any of the tested cognitive functions. The soy milk group showed a decline in verbal working memory (Digit Ordering Task) compared with the isoflavone supplement and cow's milk groups.

								There was no other information in the paper regarding negative effects of isoflavones
<b>Ho et al., 2007</b>	Healthy post-menopausal women, 55-76 years	China (Hong Kong)	Soy-derived IF powder in capsules (n=80)	Placebo (starch) (n=88)	80 IF mg/day (no further information about composition was given)	Cognitive functions and quality of life	6 months	Intention-to-treat analysis for participants with 6-month assessment results (n=176), among good compliers only (consumed ≤80% of the supplements or placebo (n=168) and among the age groups younger or older than 65 years indicated no significant differences for any outcome measures. Types of complaints of adverse events were similar in both treatment groups, i.e. not significantly different in any type, and included mainly gastrointestinal and musculoskeletal problems, and fewer cases of neurological/sensory complaints, gynecological/urinary complaints and non-specific complaints such as headache and hair loss. Among those who completed the study, 103 complaints from 58 participants and 68 complaints from 43 participants were recorded from the isoflavone and placebo group, respectively (n.s.)



<b>Casini et al., 2006</b>	Healthy post-menopausal women, mean age $\pm$ SD at baseline: group A; 49 $\pm$ 4.3 years, group B; 50 $\pm$ 3.9 years	Italy	IF as tablets (n=39)	Placebo (identical appearing tablets) (n=39)	60 mg/day IF as aglycones (Ge:D:Gl %: 40-45:40-45:10-20)	Mood and cognitive functions (by various tests)	6 months IF or placebo + 1 month wash-out period, then 6 months placebo or IF (cross-over design), total n=77 for IF and total n=77 for placebo	The results suggested that isoflavones may improve cognitive performance and mood. No information was given in the paper on negative effects of isoflavones
<b>Albertazzi et al., 2005</b>	Healthy post-menopausal Caucasian women, 44-65 years	UK	Capsules of genistein (98% purity) (n=50)	Placebo (inactive ingredients) (n=50)	90 mg/day of genistein	Menopausal symptoms and bone turnover	6 weeks on genistein, then 6 weeks on placebo or opposite (cross-over design)	Genistein reduced menopausal symptoms (severe hot flushes) by 30% (P=0.02). No significant effect was observed on biochemical markers of bone turnover. <b>Significantly higher rates of bloatedness (7 vs. 0 cases, P=0.018) and back pain (10 vs. 2 cases, P=0.03) were seen after treatment.</b> In total, adverse events occurred in 3% of the participants. The other adverse events reported (headache, cold or upper respiratory tract infection, gastroenteritis, joint pain, dizziness and vaginal bleeding) were not significantly different between the genistein and placebo group
<b>Arjmandi et al., 2005</b>	Healthy post-menopausal women, <65 years	USA	Soy protein (25 g/day) from snack bars,	Comparable products without soy protein and IF	60 mg/day IF (no further information given on composition)	Bone mineral density (BMD) and content (BMC), blood and urine markers of bone	1 year	Whole body and lumbar BMD and BMC were significantly decreased in both IF and control

			drink mix or cereal			metabolism and sex hormones		groups. However, there were no significant changes in total hip BMD and BMC irrespective of treatment. Both treatments positively affected markers of bone formation as indicated by increased serum BSAP, IGF-1 and osteocalcin (BSAP: 27.8 and 25.8%, IGF-1: 12.8 and 26.3%, osteocalcin: 95.2 and 103.4% for control and soy groups, respectively). Neither of the protein supplements had any effect on urinary deoxypyridinoline excretion, a marker of bone resorption, or influenced sex hormones. No adverse effects were reported after soy intake
<b>File et al., 2005</b>	Healthy post-menopausal women, 51-66 years	UK	Soy supplement capsules (n=25)	Identical-looking placebo capsules (n=25)	60 mg total IF equivalent/day  (no further information given on composition)	Mood, menopausal symptoms and cognition	6 weeks	The main improvement after 6 weeks of soy supplementation was in frontal lobe function (mental flexibility and planning ability), as well as non-verbal short-term memory. The effects of soy on memory seemed less robust. There was no information in the paper regarding negative effects of isoflavones
<b>Goldin et al., 2005</b>	Moderately hypercholesterolemic postmenopausal women (mean age 64 years) (n=24)	USA	Isolated soy protein depleted of IF (soy/-), soy protein	Animal protein without added IF (animal/-), animal protein with added IF (animal/+)	Mean soy protein intake: 55 ± 6 g/day. Mean daily IF intake 108 ± 12 mg/day (for which group(s) were not specified). Information	Hormonal responses (estrogen and androgen)	Each of 4 diets were fed in randomized order for 6 weeks per	Concentrations of estrone were higher and its precursor DHEA lower, after consuming the soy protein compared with

			enriched in IF (soy/+).  Isoflavone as powdered concentrate mixed in food (soy or animal)		on IF form or composition not given		phase (cross-over design)	animal protein diets (P=0.0396 and P=0.0374, respectively), independent of isoflavone content. There were no significant effects of isoflavones on any of the hormones. No adverse effects were reported in the paper
<b>Gallagher et al., 2004</b>	Healthy early post-menopausal women, 40-62 years	USA	Soy protein isolate (SPI) with two doses of isoflavones	Soy protein isolate (SPI) with very little isoflavones (controls)	96 (Ge:D %: 52:28) or 52 (Ge:D %: 28:20) mg IF/day, or <4 (Ge:D %: 4:0) mg IF/day (controls), as aglycone units adjusted for MW	Bone mineral density and blood lipids	9 months intervention and 6 months follow-up (15 months in total)	There was no significant effect of soy on BMD of the spine or femoral neck in any of the groups. BMD increased significantly in the trochanter at 9 months (P=0.0219) and at 15 months (P<0.05) in the group given isoflavone-free soy. There was no significant effect of soy on lipid metabolism. Fifteen of 65 women discontinued the soy (4 for starting hormone therapy for atrophic vaginitis or hot flashes, 4 for severe constipation or stomach irritation, 1 for chest pain, 1 for hypertension, 1 for breast cancer and 4 for non-compliance). The discontinuation rate was similar across the three treatments (P=0.42)
<b>Harkness et al., 2004</b>	Post-menopausal women (mean age 70.6 ± 6.3 years) (n=19)	USA	Soy IF in capsules	Placebo in capsules	110 mg/day IF (98% conjugated glycosides and 2% aglycones, Ge:D:GI %: 52:40:9)	Bone mineral density and content, and markers of bone turnover	First 6 months on IF, then 6 months on placebo, or the opposite	There was a 37% decrease in urinary concentrations of HP, a marker of bone resorption, during the

							(cross-over design)	isoflavone supplementation compared with baseline (P<0.05) and a significant difference in mean ( $\pm$ SE) HP excretion levels with isoflavones (43.4 $\pm$ 5.2 vs. 56.3 $\pm$ 7.2 $\mu$ g/mmol creatinine, P<0.05). With isoflavones, mean spine BMD at L2 and L3 was significantly greater than control, with a difference between means of 0.03 $\pm$ 0.04 g and 0.03 $\pm$ 0.04 g (P<0.05), respectively. There were non-significant increases from baseline for total spine BMC (3.5%), total spine BMD (1%), total hip BMC (3.6%) and total hip BMD (1.3%) with isoflavones. No information about negative effects was given
<b>Kreijkamp-Kaspers et al., 2004</b>	Healthy post-menopausal women, 60-75 years	The Netherlands	Soy protein powder with isoflavones (n=88)	Milk protein powder (n=87)	99 mg IF/day as aglycones (Ge:D:GI %: 53:41:6)	Cognitive functions (various tests), bone mineral density and plasma lipids	12 months	Cognitive function, bone mineral density or plasma lipids and drop-out rate did not differ significantly between the groups. The mean number of adverse events per participant was 2.54 in the soy group and 2.56 events in the placebo group. There were also no differences in the types of adverse events between

								treatment groups (gastrointestinal complaints, e.g. obstipation and gastric complaints, musculoskeletal complaints, lower and upper airway complaints, including ear, nose and throat, urogenital complaints, e.g. urinary tract infections or vaginal infections, dermatological complaints, e.g. dermatitis or eczema, or miscellaneous)
<b>Unfer et al., 2004</b>	Healthy post-menopausal women	Italy	Isoflavones as soy tablets (n=179), mean age $\pm$ SD at start; 49 $\pm$ 4.3 years	Identical-appearing placebo tablets (n=197), 50 $\pm$ 3.9 years	150 mg IF/day (Ge:D:GI %: 40-45:40-45:10-20)	Histological characteristics of the endometrium	5 years	<b>A significantly (P&lt;0.05) higher rate of endometrial hyperplasia without atypia was seen in women taking isoflavones compared with placebo (6 vs. 0 cases (3.9% vs. 0%), respectively)</b> , all 5 cases of simple hyperplasia and 1 case of complex hyperplasia occurred after 5 years. No cases of endometrial hyperplasia with atypia or endometrial carcinoma were observed. No information was given on reasons for drop-out or on other negative effects of isoflavones

<b>Duffy et al., 2003</b>	Healthy post-menopausal women, 50-65 years	UK	Soy supplement in capsules	Placebo (colour-matched lactose)	60 mg total IF equivalents/day  (no further information given on composition)	Cognitive function, mood, sleepiness and menopause symptoms	12 weeks	Significant cognitive improvements (in recall of pictures, a sustained attention task, learning rule reversals and a planning task) were gained with isoflavones that were independent of any changes in menopausal symptoms, mood or sleepiness. There was no information in the paper regarding negative effects of isoflavones
<b>Kritz-Silverstein et al., 2003</b>	Healthy post-menopausal women, 55-74 years	USA	Soy supplements as pills (n=27)	Identical-appearing pills with inert ingredients (<1 mg IF/day) (n=26)	110 mg total IF/day (no further information given on composition)	Cognitive functions	6 months	Isoflavone supplementation had a favourable effect on cognitive function, particularly in fluency and verbal memory. There was no information in the paper regarding negative effects of isoflavones
<b>Balk et al., 2002</b>	Healthy post-menopausal women, 55-74 years	USA	Soy flour and corn cereal (n=7)	Placebo (wheat cereal) (n=12)	Approximately 100 mg (122.7 mg) IF/day (no further information given on composition)	Menopause-associated symptoms and endometrium histology	6 months	Total symptom severity scores between soy and placebo groups were not significantly different, <b>except for insomnia which was more frequent in the soy group (P=0.017)</b> . In the placebo group, but not in the soy group, hot flushes, night sweats and vaginal dryness were less severe after intervention. All other menopause symptoms did not differ significantly in either groups between baseline

								and final week. No endometrium proliferation was seen from the soy consumption. Comparison of baseline and final week means for each group indicated no significant differences in side-effects (nausea, breast tenderness, flatus and diarrhea), <b>although diarrhea and flatus were more common in the soy group</b>
<b>Alekel et al., 2000</b>	Peri-menopausal women (median age 50.6 years) (n=69)	USA	Soy protein isolate (SPI) with high IF (SPI+, n=24) or low IF (SPI-, n=24)	Whey protein as controls (n=21)	SPI+: 80.4 mg/day IF, SPI-: 4.4 mg/day IF, as aglycones (no further information given on composition)	Bone loss in the lumbar spine	24 weeks	The % change in lumbar spine BMD and BMC, respectively, did not differ from zero in the SPI+ or SPI- groups, but loss occurred in the control group (-1.28%, P=0.0041; -1.73%, P=0.0037). By regression analysis, SPI+ treatment had a positive effect on change in BMD (5.6%; P=0.023) and BMC (10.1%; P=0.0032). Serum BAP posttreatment was negatively related to % change in BMD (P=0.0016) and BMC (P=0.019). Contrast coding using analyses of covariance with BMD or BMC as the outcome showed that isoflavones, not soy protein, exerted the effect. Compliance

								was good, but it was reported that 6 persons left the study because of inability to tolerate the treatment, without any further information
<b>Mackey et al., 2000</b>	Post-menopausal hypercholesterolemic women (age 45-65 years) (n=49)	Australia	Soy protein with high IF (ISP+)	Soy protein with low IF (ISP-)	65 mg isoflavones/day (ISP+), <4 mg isoflavones/day (ISP-) (no further information was given on composition)	Menopause symptoms and plasma lipid levels	12 weeks soy treatment, 4 weeks before and 6 weeks after soy treatment on a low fat diet	Menopause symptoms in both groups were reduced by 30%. There was an overall reduction after 12 weeks in TC (P=0.0003), LDL-C (P=0.04), SHBG (P=0.002) and LH (P=0.005). Soy protein had a cholesterol lowering effect in women. There were no significant differences between the treatment groups in any parameter, therefore, the study suggested that this effect was independent of isoflavones. No negative effects were reported
<b>Albertazzi et al., 1998</b>	Healthy post-menopausal women, 45-62 years	Italy	Soy protein powder (n=40)	Placebo (casein) powder (n=39)	76 mg IF/day as aglycones (maximum Ge:D %: 53:37)	Menopause-associated symptoms (hot flushes)	12 weeks	Soy was significantly superior to placebo (P<0.01) in reducing the mean number of hot flushes per 24 hours after 4, 8 and 12 weeks. A large portion (24%) of the 25 patients who withdrew from the study had difficulty taking the amount of powder and with food intolerance,



								namely constipation, bloating, nausea and vomiting, which were the most common causes of both discontinuation and lack of compliance. There were no statistically significant differences between the soy and the casein groups when the side-were evaluated either as number or as % of complaints
<b>Potter et al., 1998</b>	Hypercholesterolemic post-menopausal women (n=66), mean $\pm$ SD age, 59.8 $\pm$ 9.1, 61.2 $\pm$ 10.3, 61.3 $\pm$ 6.3 years in groups ISP56, ISP90 and CNFDM	USA	Isolated soy protein with moderate or high IF	Casein and non-fat dry milk (CNFDM) controls	ISP56: 55.6 mg/day IF, ISP90: 90 mg/day IF (no further information given on composition)	Blood lipid profiles, mononuclear cell LDL receptor mRNA, bone mineral density and content	2 weeks on basal lead-in diet (low-fat, low-cholesterol), then 24 weeks on soy or control diets	Non-HDL cholesterol for both ISP56 and ISP90 groups was reduced compared with the CNFDM group (P<0.05). HDL cholesterol increased in both ISP56 and ISP90 groups (P<0.05). Mononuclear cell LDL receptor mRNA was increased in subjects consuming ISP56 or ISP90 compared with those consuming CNFDM (P<0.05). Significant increases occurred in both bone mineral content and density in the lumbar spine for the ISP90 group compared with the control group (P<0.05). The subjects consumed the test proteins without difficulty. No information about drop-outs or adverse effects was given in the paper

Prospective (cohort) studies								
<b>Kurahashi et al., 2009</b>	Women with (n=32) or without hepatocellular carcinoma (HCC) (n=12783) (age 40-69 years)	Japan	Consumption of IF (genistein and daidzein) and soy foods calculated from FFQ		Dietary tertiles genistein: <12.2, 12.2-19.5, ≥19.6 mg/day, daidzein: <8.1, 8.1-12.5, ≥12.6 mg/day, soy food: <38.26, 38.2-62.7, ≥62.8 g/day	Risk of hepatocellular carcinoma (HCC)	235811 person-years, average 11.8 years, of follow-up (both genders)	<b>Genistein and daidzein were dose-dependently associated with increased risk of HCC, with multivariable HR for the highest vs. lowest tertile of 3.19 (95% CI 1.13-9.00, Ptrend=0.03) and 3.90 (95% CI 1.30-11.69, Ptrend=0.01), respectively. The HR for the middle vs. lowest tertile for genistein and daidzein were 2.36 (95% CI 0.85-6.51 (n.s.) and 3.10 (1.07-8.99). Soy food consumption tended to be associated with HCC in women, but did not reach statistical significance (HR 1.74, 95% CI 0.67-4.25, for highest vs. lowest tertile)</b>
<b>Palacios et al., 2007</b>	Healthy post-menopausal women (45-65 years) (n=395), mostly Caucasians	International multicenter (n=34) prospective open-label study across Australia, Belgium, France and Spain	A specific and standardized isoflavone extract from soy (Phytosoya, producer not given)	No controls	70 mg/day IF (Genistin:Daidzin:Glycitin %: 20:50:30, as glycosides)	Effects on endometrium evaluated by biopsy and transvaginal ultrasonography (atrophy, proliferation, hyperplasia, carcinoma, endometrium thickness)	12 months	Among 301 endometrium biopsies obtained at 12 months, 99.67% were atrophic/inactive and 0.33% proliferative. No case of hyperplasia or carcinoma was diagnosed, demonstrating the endometrial safety of the

								extract (point estimate: 0.0; upper limit of 95% CI: 0.012). Endometrial thickness showed no increase after 12 months (2.2 mm at inclusion and 2.12 mm at study end). Of the 395 women included, 19 reported adverse effects (4.8%). The only recurrent adverse effects related to the study product were moderate gastrointestinal disorders, which were reported in 4.6% of the women in the safety set, whereas 68.9% found treatment to be excellent or 25.7% to be good in terms of tolerance. Eight women (all with atrophic endometrium) reported some kind of bleeding (2%) as an adverse event
Retrospective (case-control) studies								
<b>Shin et al., 2015</b>	Female colorectal cancer patients (n=298) and matched controls (n=894)	Korea	Total soy food products and subgroups, and total isoflavones, genistein, daidzein and glycitein estimated from FFQ	Up to 3 controls per patient matched by gender and 5 years of age groups	The quartiles for total isoflavone intake were: Q1: <8.08, Q2: 8.08- <13.83 mg/day, Q3: 13.83-<22.35, Q4: ≥22.35 mg/day	Colorectal cancer, colon cancer, rectal cancer		A high intake of total soy food products was associated with a reduced risk for colorectal cancer in women. The association between high soy food product intake (Q4) and reduced colorectal cancer risk was significant among post-menopausal women (OR: 0.52, 95% CI: 0.29-0.95,

								<p>Ptrend=0.009), but not in pre-menopausal women. This association persisted for rectal cancer in women when analysed by subsite (Ptrend=0.006), and was also seen for total isoflavones (Ptrend=0.035).  <b>However, the middle (second and third) quartiles of intake of total soy products were associated with an elevated colon cancer risk in women (Q2: OR: 1.27, 95% CI 0.86-1.88), Q3: OR: 1.37, 95% CI 0.92-2.04) (n.s.).</b> The same non-significant tendencies of reduced risk associated with Q4 and <b>increased risk associated with Q2 and Q3 vs. Q1</b> were seen with total isoflavones</p>
<b>Anderson et al., 2013</b>	Cases: Women with diagnosed breast cancer, stratified by estrogen (ER) and progesterone (PR) receptor subtypes (age 25-74 years), controls: age-matched women from same area	Canada	Adult intake of total isoflavones (genistein, daidzein, glycitein, formononetin (non-soy)) or total phytoestrogens (isoflavones, lignans, coumestans) from foods,		The form and composition of IF was not given	Breast cancer		<p><b>Post-menopausal women (n=314) had a positive association between ER-PR-breast cancer and adult total isoflavone intake (<math>\geq 497</math> <math>\mu\text{g}/\text{day}</math>) (highest vs. lowest tertile: OR: 1.50, 95% CI 1.05-2.15, Ptrend=0.04), indicating increased risk for this breast cancer subtype with</b></p>

			obtained by FFQ					total isoflavone intake. Also in women not stratified by menopause status was there a positive association between ER-PR- breast cancer (n=500) and the highest tertile of adult total isoflavone intake ( $\geq 497 \mu\text{g}/\text{day}$ ) (OR: 1.38, 95% CI 1.05-1.81, Ptrend=0.01)
Cross-sectional studies								
<b>Hogervorst et al., 2008</b>	Women (age 52-98 years)	Indonesian subjects of mixed ethnicity	Tofu and tempe. Intakes were reported as mean times/week $\pm$ SD; tofu $9.3 \pm 6.9$ and tempe $9.5 \pm 6.8$ , for the total population of men and women (n=719) of which 65% were women (n= ~467)		Amounts of IF were not given or could be estimated	Cognitive function (memory)		<b>Worse memory, measured with a word learning test sensitive to dementia, was associated with high tofu consumption assessed by a food frequency questionnaire (<math>\beta = -0.18</math>, <math>P &lt; 0.01</math>, 95% CI -0.34 to -0.06).</b> However, high intake of tempe was independently associated with better memory ( $\beta = 0.12$ , $P < 0.05$ , 95% CI 0.00 to 0.28). Both effects were stronger in persons 68 years or older. Tempe has higher levels of genistein and increased folate than tofu. <b>Formaldehyde is often added to tofu, but not</b>

								<b>to tempe as a preservative, and has been suggested to be the reason for the negative effects of tofu</b>
<b>Woo et al., 2006</b>	Post-menopausal women (≥65 years)	China	0-4, 5-18 or 19+ mg IF/day (cognitive impairment), 0-5, 6-18 or 19+ mg IF/day (depression), from food		No information on form or composition of IF given	Cognitive impairment (n=604), depression (n=372) or both (n=92)		For both cognitive impairment and depression, there were no significant associations with total isoflavone intake; cognitive impairment OR: 0.98 (95% CI 0.76-1.24) and OR: 0.80 (95% CI 0.59-1.09) for 5-18 and 19+ mg of total isoflavones per day vs. OR: 1.0 for isoflavone intake of 0-4 mg per day, and for depression; OR: 0.81 (95% CI 0.59-1.10) and OR: 0.69 (95% CI 0.47-1.01) for 6-18 and 19+ mg of total isoflavones per day vs. OR: 1.0 for isoflavone intake of 0-5 mg per day. However, when adjusting for total calorie intake, total isoflavone intake (0.2.8-9.7 mg/1000 kcal/day) was inversely associated with depression; OR: 0.73 (95% CI 0.54-1.0), P<0.05, whereas no

								association was apparent for cognitive impairment
<b>Somekawa et al., 2001</b>	Post-menopausal women (44-80 years)	Japan	Dietary isoflavones divided in four categories	No control group without IF exposure	Dietary IF intake (<35, 35-50, 50-65 and >65 mg/day), no information given on form or composition of IF	Menopausal symptoms, lipid profiles, bone mineral density		Bone mineral densities adjusted to years since menopause and weight were significantly higher in the highest intakes (50-65 and >65 mg/day) compared with lowest intake (<35 mg/day) within the early (P<0.001) and late (P=0.01) post-menopausal groups. In the early post-menopausal group, significant lower menopause symptoms scores were found for palpitation (P<0.05) and backaches (P<0.05) in the high versus the low intake categories. Serum values of fasting total cholesterol, triglyceride, LDL and HDL cholesterol and apolipoproteins AI, B and E were not significantly different between the isoflavone intake groups, either in early or late pre-menopausal women. No information about adverse effects were given in the paper
Other clinical studies								

<p><b>Bloedon et al., 2002</b></p>	<p>Healthy post-menopausal women (46-68 years), (n=24), mostly white</p>	<p>USA</p>	<p>Two purified IF formulations (A and B) in capsules (n=3 per dose and formulation)</p>	<p>No control group</p>	<p>Formulation A: 2, 4, 8 and 16 mg/kg bw of genistein, 0.28, 0.55, 1.1 and 2.2 mg/kg bw daidzein, 0.014, 0.029, 0.057 and 0.11 mg/kg bw glycitein (as unconjugated IF)</p> <p>Formulation B: 2, 4, 8 and 16 mg/kg bw of genistein, 1.0, 2.1, 4.2 and 8.4 mg/kg bw daidzein, 0.08, 0.17, 0.34 and 0.68 mg/kg bw glycitein (as unconjugated IF)</p>	<p>Safety and pharmacokinetics (see 2.3.1)</p>	<p>Single dose, effects evaluated up to 30 days after administration</p>	<p>Four grade I adverse events were judged to be possibly related to IF (similar effects reported in animals); 2 cases of trace pedal edema (at 2 and 16 mg/kg bw, both with B), 1 case of nausea 1 h postdose (at 8 mg/kg bw, with B) and 1 case of breast tenderness (at 16 mg/kg bw, with A). Clinically significant decreases in systolic blood pressure (<math>\geq 15</math> mmHg) were observed 24 h postdose with A in 5 women, but only 2 continued to show significant decreases 3 days postdose (at 2, 4 and 8 mg/kg bw). Clinically significant decreases in diastolic blood pressure (<math>\geq 15</math> mmHg) were observed in various dose groups 24 h postdose in 8 women, of which 6 were given A, but only 1 continued to show significant decrease 3 days postdose. In two women, both systolic and diastolic blood pressure increased 24 h postdose at 4 and 16 mg/kg bw with B, but were not elevated at 3 days</p>
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									postdose. All changes in blood variables were $\leq 15\%$ , except for changes in neutrophil count, which were 20-25%. In all cases, the results at the same time point did not differ significantly by formulation. The authors concluded that the administered doses of isoflavones had minimal clinical toxicity
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## 9.2 Human studies on effects of isoflavones on pre-menopausal women

**Table 9.2** An overview of human studies on pre-menopausal women investigating health effects of isoflavones (IF).

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
<b>RCTs</b>								
Maskarinec et al., 2013	Intervention study in pre-menopausal women (age 40.4 ± 6.0 years, 58% Caucasians, 17% Asian and 25% other ethnic groups), n=36 with cytology samples	USA	Dietary soy intervention (soy milk and nuts, tofu)	Diet low in soy	After intervention among women with sufficient NAF, high-soy group (mean ± SD): 65.0 ± 29.5 mg/day of isoflavones, low-soy group: 3.8 ± 5.8 mg/day, at baseline: 2.2 ± 1.9 mg/day (no information was given on form or composition of IF in the diets)	Cytology status of epithelial cells from breast nipple aspirate fluid (NAF)	6 months, cross-over design	High-soy diet: cytological subclass had decreased in 8 (24%) and increased in 3 (9%) of women compared with baseline, low-soy diet: 3 (14%) and 6 (29%) (P=0.32). Only change in subclass indicated a trend in lower cytological class (P = 0.06), indicating that the soy intake did not have adverse effects on epithelial breast cells. When restricted to midluteal samples (n = 49), the high-soy diet was not associated with changes in cytological class (P=0.14). The paper stated that the women reported changes in health status monthly, for monitoring of adverse effects. No specific information about adverse effects was given in the paper
Anderson et al., 2002	Healthy pre-menopausal women (age 21-25 years), 12 Caucasians, 2 Asian Americans and 1 Native American (n=28). Assignment	USA	IF enriched soy protein isolate as powder and as ready-to-drink chocolate drink (n=15)	IF deficient soy protein isolate (n=13)	~90 mg IF/day (59% glycones and glycosides, 41% as aglycones, with at least 50 mg of genistein in	Bone mineral content and bone mineral density (BMD)	1 year	No changes in BMD, normal menstrual patterns or other variables after 1 year were found in either group. No adverse effects from the isoflavones, such as gastrointestinal

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
	of subjects to treatment or control groups alternated in the order of enrollment				both the glycone and aglycone forms)			disturbances, were reported by the subjects
File et al., 2001	Healthy pre-menopausal women (n=12), mean age ~26 years	UK	Diet of a wide range of soy-containing foods (commercial products or prepared meals)	Diets of foods free of soy products	High soy: 100 mg total IF/day, Low soy: 0.5 mg total IF/day (no information was given on form or composition of IF in the diets)	Cognitive functions (attention, memory and frontal lobe function)	10 weeks, both diets were given to the subjects on a 11-day rotating menu-basis (cross-over design)	The women on high soy had significant improvements in short-term and long-term memory and in mental flexibility. In a letter fluency test and in a test of planning, the high soy improved performance in women. There was no effect of diet on tests of attention or in category generation. There was no information in the paper regarding negative effects of isoflavones
Duncan et al. 1999	Pre-menopausal women (mean age $\pm$ SD: 26.5 $\pm$ 4.7 years)	USA	Soy protein powder (n=14)		Controls; 10 $\pm$ 1.1 mg/day, low IF; 64 $\pm$ 9.2 mg/day, high IF; 128 $\pm$ 16 mg/day as unconjugated units (Ge:D:Gl %: 55:37:8, 97% of Ge and D, and 91% of Gl, as glycoside conjugates	Hormonal effects, endometrial biopsies	Three dietary periods each lasting three menstrual cycles (of approximately 29 days) plus 9 days (in total 96 days) with a washout period of approximately 3 weeks between (cross-over design)	<b>Low IF decreased LH (P=0.009) and FSH (P=0.04) levels during the periovulatory phase. High IF decreased free T3 (P=0.02) and dehydroepiandrosterone sulfate (P=0.02) levels during the early follicular phase and estrone levels during the midfollicular phase (P=0.02).</b> No other significant changes were observed in hormone concentrations or in the length of the menstrual cycle, follicular phase or

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								luteal phase. Endometrial biopsies performed in the luteal phase of cycle 3 of each diet period revealed no effect of IF on histological dating. The study suggested <b>weak hormonal effects of isoflavones, with uncertain physiological relevance and with no evidence of a dose-dependent effect</b>
Nagata et al., 1998	Intervention study on healthy pre-menopausal, not pregnant, women (mean age about 26 years) (n=60)	Japan	Soy milk (and dietary soy) (n=31)	Normal diet, including some soy foods (n=29)	Postintervention (mean ± SD): 97.0 ± 19.2 mg IF in soy milk group and 0.0 mg in control group. In addition, 19.4 ± 15.0 and 18.4 ± 13.4 mg IF from other dietary soy products, respectively (total IF intake of 116.4 and 18.4 mg/day in the two groups). 100 g soy milk contained 0.7 mg genistein and 16 mg genistin, and 0.7 mg daidzein and 9.4 mg daidzin	Serum estrogen concentrations and menstrual cycle length	3 menstrual cycles; cycle 1 before and cycle 3 after the 2 months dietary intervention	<b>Estrone and estradiol decreased by 23% and 27%, respectively, in the soy milk group</b> and increased 0.6% and 4%, respectively, in the control group, <b>but the groups were not significantly different. In the soy milk group, menstrual cycle length was increased by nearly 2 days</b> , and, in the control group, decreased by approximately 1 day, <b>but the group differences were not significantly different.</b> No negative effects were reported

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
<b>Prospective (cohort) studies</b>								
Filiberto et al., 2013	Healthy regularly menstruating women, age 18-44 years (n=259)	USA	Dietary intake of isoflavones as aglycones of genistein, daidzein, glycitein (from soy) and biochanin A and formononetin (non-soy) (mg) and the sum of these as total isoflavones. Dietary data was collected as 24-hour recalls, up to four times per menstrual cycle			Ovulatory function, including sporadic anovulation and serum concentrations of E2, free E2, P, LH, FSH and SHBG	Follow-up was up to two menstrual cycles	Isoflavone intake was not associated with E2, free E2, P, LH and FSH. <b>Consumption in the highest quartile (Q4: 1.6-78.8 mg/day) was significantly associated with greater SHBG concentrations (<math>\beta=0.09</math>; 95% CI 0.02-0.16), compared with the first quartile (Q1: 0.0-0.3 mg/day).</b> Isoflavone intake was not associated with sporadic anovulation (Q4 vs. Q1: OR 0.87, 95% CI 0.32-1.66, n.s.)
<b>Retrospective (case-control) studies</b>								
Shen al., 2013	Pre-menopausal Han Chinese women with or without uterine leiomyoma, otherwise in good health, 30-50 years	China	Cases: Dietary intake of soybean milk from questionnaire in women with uterine myoleioma. No consumption (n=136), occasional consumption (n=264) and frequent consumption (n=200)	Controls: Intake of soybean milk in women without uterine myoleioma. No consumption (n=216), occasional consumption (n=324) and frequent	No information given on composition or levels of isoflavones	Uterine leiomyoma		<b>Consumption of soybean milk (OR: 2.518; 95% CI: 1.894-3.347) had a significant effect on the occurrence of uterine leiomyoma (P&lt;0.000).</b> Among the women with occasional consumption of soybean milk, the difference was n.s. (P=0.184, OR: 1.294 (95% CI 0.884-1.894)), <b>whereas among the women with frequent consumption, it was significant (P=0.000,</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
				consumption (n=60)				<b>OR: 5.294 (95% CI 3.184-8.803)</b>
Hernandez et al., 2004	Women aged 18-45 years with confirmed cervical squamous intraepithelial lesions (SILs) (menopausal status not specified) of mixed ethnicity (39 and 43% white, 13 and 16% Japanese, 16 and 26% Hawaiian, 32% and 15% other among cases and controls, respectively)	USA (Hawaii)	Dietary survey information and plasma levels of genistein, daidzein, glycitein, <i>O</i> -desmethylangolensin, equol. Cases: mean age $\pm$ SD: 33 $\pm$ 10.1 years, n=122	Controls: mean age $\pm$ SD: 39 $\pm$ 13.9 years, n=183		Cervical squamous intraepithelial lesions (SILs)		<b>Plasma equol level (measured only in 24 cases (20%) and 18 controls (10%)) was positively associated with cervical SILs; OR 6.5 (95% CI 1.4-29.2), OR 5.4 (95% CI 1.5-20.0), P<sub>trend</sub> = 0.02, for the highest (&gt;5.9 nM) and next highest (0.6-5.9 nM) relative to the lowest quartile (0 nM) after adjustment for several factors.</b> No significant associations were seen for other isoflavones. No relationship was observed between consumption of isoflavonoid food sources, including tofu and other soy food products, and SILs risk
<b>Cross-sectional studies</b>								
Greendale et al., 2015	Black (n=242), white (n=384), Chinese (n=117) and Japanese (n=119) women (total n=862) during the menopausal transition (pre-menopausal or early peri-menopausal)	USA	Total dietary isoflavone intake from the sum of genistein, daidzein, glycitein (soy) and formononetin (non-soy)		Total IF with separate aggregated Asian (1751 (27-5633), 8851 (5723-15932), 29113 (16147-118252) and aggregated non-Asian (88 (3-161), 286 (161-584),	Bone mineral density (BMD) at the lumbar spine (LS) and femoral neck (FN)	Longitudinal study	Multiply adjusted mean FN BMD values of pre-menopausal Japanese women were monotonically positively related to isoflavone consumption (P <sub>trend</sub> =0.0003). Otherwise, no statistically significant baseline associations were observed. During the period of 1 year before final menstrual

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
					1230 (584-135102) tertiles of intake (mg/day)			period (FMP) through 5 years after FMP, all participants lost LS and FN BMD. Loss was unrelated to isoflavone intake, <b>except for Japanese women during the period of 1 year before FMP to 2 years after FMP: higher tertiles of isoflavone intake were associated with greater annual LS BMD loss rates (Ptrend=0.01) and FN loss rates (Ptrend=0.04)</b>
Jacobsen et al., 2014	Adventist pre-menopausal women (age 30-50 years), of which 54% were vegetarians, mean IF intake 17.9 mg/day (very high compared with other Western populations), (n=11688)	USA	High dietary IF intake ( $\geq 40$ mg/day), as computed intake of genistein, daidzein and glycitein as aglycone equivalent weights from FFQ	Low dietary IF intake ( $< 10$ mg/day)	$\geq 40$ or $< 10$ mg/day of total IF	Female fertility (risk of nulliparity and nulligravidity)		<b>An inverse relationship (P=0.05) between isoflavone intake and the likelihood of ever having become a mother was found. In women with high isoflavone intake (<math>\geq 40</math> mg/day) the adjusted lifetime probability of giving birth to a live child was reduced by approximately 3% (95% CI 0, 7) compared with women with low intake (<math>&lt; 10</math> mg/day). No relationship was found between the isoflavone intake and parity or age of first delivery in parous women. A similar inverse relationship (P=0.03) was found between isoflavone intake and</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
								<b>the risk of nulligravidity with a 13% (95% CI 2, 26) higher risk of never having been pregnant in women with high isoflavone intake (<math>\geq 40</math> mg/day).</b> These relationships were mainly in women who reported problems with becoming pregnant
<b>Other clinical studies</b>								
Celec et al., 2013	Healthy young women (age 18 – 23 years) (n=55)	Slovakia	2 g soybeans (dry weight) per kg bw		No further information given on form or composition of IF	Oxidative and carbonyl stress	7 days (blood samples taken before intake, after 1-week intake and after 1- week wash-out period)	Total antioxidant capacity was increased by soybean intake, leading to decreased levels of AOPP. No effects on carbonyl stress markers were found (AGE-specific fluorescence and fructosamine)
Hoshida et al., 2011	Healthy pre- menopausal (n=44) and post-menopausal (n=11) women, smokers and non- smokers (n=55, mean age 39 years)	Japan	Isoflavones from black soybean tea		50 mg IF/day (no further information on form or composition of IF was given)	Vascular function (endothelial function, arterial wall stiffness and blood biochemistry)	2 months	Isoflavone consumption improved vascular endothelial function in both pre-menopausal and <b>post- menopausal</b> non- smokers. Arterial wall stiffness was effectively reduced only in pre- menopausal women, but the effect was noted for both smokers and non- smokers. Thus, the effects of isoflavones on vascular function differed among pre-menopausal and <b>post- menopausal</b> smokers and non-smokers. No adverse events of the isoflavone



Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
								intake were reported in this study
Ostatniková et al., 2007	Healthy young women (age 18-25 years) (n=54)	Slovakia	2 g soybeans per kg bw per day		IF intake, form and composition not given	Cognitive abilities and steroid sex hormones	7 days on soybean + 7 days wash-out period	Mental rotation and spatial visualization were significantly improved with soy. <b>Salivary testosterone and plasma estradiol (E2) levels showed a tendency to decline after soybean intake (the decrease in E<sub>2</sub> was statistically significant)</b> and to increase back towards basal levels during the washout period
Cassidy et al., 1994	Healthy pre-menopausal non-vegetarian women (age 21-29 years), (n=6)	UK	Soy protein (60 g/day) added to meals		45 mg IF daily (mean intake 0.7 mg/kg bw per day, mean bw 62 kg). No further information given on form or composition of IF	Hormonal status and regulation of menstrual cycle	1 month exposure, studied over 9 months in which 2 menstrual cycles were spent on soy diet	<b>Soy significantly increased follicular phase length (mean±SD: 2.5±1.6 day, P&lt;0.01) and/or delayed menstruation (by 1-5 days in 5 of 6 subjects). Mid-cycle surges of LH and FSH were significantly suppressed; mean values decreased from 21.2 to 7.1 U/l (P&lt;0.05) and from 14.6 to 7.8 U/l (P&lt;0.02), respectively, on soy. Plasma estradiol concentrations increased in the follicular phase from 246.2 to 362.5 pmol/l (P&lt;0.02) and cholesterol concentrations decreased from 4.27 to 3.86 mmol/l (P&lt;0.05) with soy</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Length of follow-up or duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
<b>Case reports</b>								
Johnson et al., 2001	A nulliparous woman, 39 years old	USA	The patient had a medical history notable for extensive use of numerous supplemental herbs and vitamins over the past two years, including many known phytoestrogens	The patient lacked the classically associated risk factors for endometrial cancer, such as obesity (BMI 19) or anovulatory cycles	Quantifying the amount of phytoestrogens was limited by the lack of such information on the packaging and soy was not specifically mentioned among the herbs			<b>The patient was diagnosed with endometrial cancer (grade 1 endometrioid adenocarcinoma)</b>

## 9.3 Human studies on effects of isoflavones on men

**Table 9.3** An overview of human studies on men investigating health effects of isoflavones (IF).

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
<b>Meta-analyses</b>								
Lim and Shaw, 2016	Healthy men	USA and China	Isoflavone (genistein, daidzein, equol and desmethylangolensin) intake from soy foods			Reproductive health (sperm concentrations)		USA: isoflavone intake increased from 0.8 mg/day in 1993 to 13.7 mg/day in 2005 and sperm concentration decreased from 137 x 10 <sup>6</sup> /ml in 1938 to 64 x 10 <sup>6</sup> /ml in 2007 (53% decline, not statistically significant). China: isoflavone intake has decreased from 64.7 mg/day in 1991 to 15.6 mg/day in 2008 and the sperm concentration increased from 55 x 10 <sup>6</sup> /ml in 1999 to 74 x 10 <sup>6</sup> /ml in 2008 (34% increase)
Van Die et al., 2014	Systematic review and meta-analysis of 8 RCTs on men with prostate cancer (PCa) or with a clinically identified risk of PCa	Various	Dietary soy protein (n=3), isoflavones in tablet form (n=5), 7 different preparations/products	In studies administering tablets, doses ranged from 40 mg to 80 mg/day total isoflavones; in studies administering soy in aglycone form, the range was 16 mg aglycones/day (40 mg isoflavones		Efficacy and safety of soy/isoflavones		Meta-analyses of the two studies including men with identified risk of PCa found a significant reduction in PCa diagnosis after administration of soy/soy isoflavones (risk ratio=0.49, 95% CI 0.26, 0.95). Meta- analyses indicated no significant differences between groups for PSA levels or sex steroid endpoints; SHBG,

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
				containing 40% in aglycone form) to 450 mg genistein aglycone with 300 mg daidzein aglycone as genistein combined polysaccharide (GCP). Soy isoflavones administered as foods ranged from 60 mg genistein to 117 mg total isoflavones daily				testosterone, free testosterone, estradiol and dihydrotestosterone. Seven of 8 studies reported on adverse events. A good safety profile was shown by this meta-analysis for soy/soy isoflavone supplementation
Hamilton-Reeves et al., 2010	Men, including prostate cancer patients (16 of 34 studies) (age 21-74 years)	Various	IF as aglycone equivalents, isolated soy protein (ISP) or soy foods (milk, grits, flour, tofu)		20–900 mg/day IF as aglycone equivalents, 0–71 g/day as soy protein (1 g soy protein in foods = 3.5 mg IF aglycones)	Estrogen-like effects by lowering bioavailable testosterone	1 week to 4 years (1 year data used instead of 4 year data, since they were similar), average study duration about 74 days	No significant effects of soy protein or isoflavone intake on testosterone, SHBG, free testosterone or FAI were detected
Yan et al., 2010	Meta-analysis of four cohort and seven case-control studies on soy and colorectal cancer risk studied in men and women (mostly post-menopausal women based on age, but menopausal status was not stated)	Various	Soy consumption from food as various soy foods, isoflavones or genistein		From low µg/day to low mg/day in Western studies to from several mg/day to >50 mg/day in the Asian studies	Colorectal cancer, colon cancer, rectal cancer		Soy consumption was not associated with colorectal cancer risk (combined risk estimate 0.90; 95% CI 0.79-1.03) or separate analyses on colon cancer (combined risk estimate 0.88; 95% CI 0.74-1.06) and rectal cancer (combined risk estimate 0.88; 95% CI

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
								0.67-1.14). When separately analysed on gender, soy was associated with approximately 21% reduction in colorectal cancer risk in women (combined risk estimate 0.79; 95% CI 0.65-0.97; P=0.026), but not in men (combined risk estimate 1.10; 95% CI 0.90-1.33). When analysing 6 studies on isoflavones, consumption was associated with approximately 16% reduced risk of colorectal cancer, largely attributable to Western studies with low µg to low mg per day isoflavone intakes
<b>RCTs</b>								
Bosland et al., 2013	Men at high risk of recurrence after radical prostatectomy for prostate cancer (n=177)	USA	Soy protein isolate (ISP) as beverage powder (n=87)	Calcium caseinate as placebo (n=90)	2.13 mg IF as aglycone equivalents per 1 g protein x 20 g = 42.6 mg IF/day (Ge:D:Gl %: 58:37:5)	Biochemical recurrence of prostate cancer measured as prostate-specific antigen (PSA)	2 years	The soy treatment did not reduce the biochemical recurrence of prostate cancer. There were no apparent adverse events related to supplementation, i.e. no differences in adverse events (gastrointestinal issues, urinary tract issues, initiation of high cholesterol or hypertension treatments or musculoskeletal pain) between the two groups

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
Hamilton-Reeves et al., 2013	Men with localized prostate cancer, of various ethnicity (n=86)	USA	Soy isoflavone capsules (mean $\pm$ SD age; 62 $\pm$ 7 years) (n=44)	Capsules with <0.06 mg total isoflavones as aglycone equivalents/day (62 $\pm$ 12 years) (n=42)	80 mg/day of total isoflavones, 51 mg/day as aglycone equivalents (Ge:D:Gl %: 15:55:30)	Gene expression, serum hormones, PSA and total cholesterol levels	Up to six weeks	12 genes involved in cell cycle control and 9 genes involved in apoptosis were down-regulated in the treatment tumour tissues versus the placebo control (impact not discussed). Changes in serum total testosterone, free testosterone, total estrogen, estradiol, PSA and total cholesterol in the isoflavone-treated group compared with placebo were not statistically significant. All adverse events (AE) were grade I (mild). In the isoflavone arm, 4 events were recorded; 2 gastrointestinal and 2 general. In the placebo arm, 9 events were recorded; 6 gastrointestinal and 3 general. No patient stopped treatment because of AE
Vitolins et al., 2013	Androgen-deprived (to manage and control prostate cancer) men with hot flashes, aged 46-91 years (n=120)	USA	Soy protein powder and placebo pill (n=30)	Milk protein powder and placebo pill (n=30), venlafaxine and milk protein powder (n=30) and venlafaxine and soy protein powder (n=30)	160 mg/day isoflavones (no information about form or composition given)	Hot flash symptom severity score (HFSSS) and quality of life (QOL)	12 weeks	Neither venlafaxine nor soy protein alone or in combination had a significant effect on HFSSS. Soy protein, but not venlafaxine, improved measures of QOL. Toxicity was minimal. Of 19 adverse events (AE) reported, 3 were in the soy group.

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								The majority of AE had severity grade 0-1, and did not differ significantly among groups. The majority was experience at baseline, and most did not worsen during treatment
Dettmer et al., 2012	Healthy men (age 18-63 years) (n=60), 77.9% white, 16.2% Asian, 2.9% black and 2.9% Hispanic	USA	Soy protein isolate (SPI) beverage or whole soybean (WSB) beverage	Cow's milk	SPI: 30.1 mg total isoflavones/day), WSB: 91.4 mg total isoflavones/day. No information on isoflavone composition was given	Serum cell adhesion molecule concentrations	8 weeks	Soluble CAM concentrations were not altered by treatment (neither cow's milk nor soy beverages) and did not differ between prehypertensive and hypertensive participants. ANOVA indicated a treatment × gender interaction (gender effect) for ICAM-1 (P=0.0037), but not for E-selectin (P=0.067) or VCAM-1 (P=0.16). No information was given about adverse effects
Miyanaga et al., 2012	Men with serum PSA level of 2.5–10.0 ng/ml, and a single, negative prostate biopsy within 12 months prior to enrollment, age 50 and 75 years	Japan	Isoflavone tablets	Placebo tablets	Total isoflavones 60 mg/day, with 0.1% genistein, 0.3% daidzein and 0.3% glycitein, and 5.8% genistin, 31.9% daidzin and 17.3% glycitin, in addition to malonyl and acetyl glycosides	Biopsy-detectable prostate cancer, PSA levels	1 year	PSA showed no significant difference before and after treatment. Of 89 patients evaluated by central pathological review, the incidence of biopsy-detectable prostate cancer in the isoflavone and placebo groups showed no significant difference (21.4% vs. 34.0%,

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								P=0.140). However, for the 53 patients aged $\geq$ 65 years, the incidence of cancer in the isoflavone group was significantly lower than in the placebo group (28.0% vs. 57.1%, P=0.031). The intake of tablets was completed by 96.2% (75/78) on isoflavones and 97.5% (78/80) on placebo. Of 5 who did not complete the treatment, 3 decided themselves to quit taking the tablets. <b>The other two had grade III adverse events: one on isoflavones suffered iliac artery stenosis</b> , and the other on placebo suffered ileus. However, the majority of adverse events were mild or moderate in severity. No significant changes in laboratory data were observed during the study
Beaton et al., 2010	Healthy men (age 20-40 years) (n=32), 10 of 32 (31%) were equol secretors	USA	Low isoflavone soy protein isolate (SPI), high isoflavone SPI, as powder	Milk protein isolate (MPI)	Low iso SPI (mean $\pm$ SD): 1.64 $\pm$ 0.19 mg/day of IF as total aglycone equivalents (0.02 mg/kg bw per day), Ge:D:Gl %:	Parameters of semen quality	Three 57-day treatment periods separated by 28-day washout periods (cross-over design)	Semen volume, sperm concentration, sperm count, sperm percent motility, total motile sperm count and sperm morphology were not significantly affected by consumption of either low- or high-iso SPI



Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
					79:13:8, high iso SPI: 61.7 ± 7.35 mg/day of IF (0.75 mg/kg bw per day), (Ge:D:Gl %: 53:36:11)			compared with MPI. No adverse effects were reported
deVere White et al., 2010	Men with prostate cancer enrolled in an active surveillance program. 1) Randomized, double-blind placebo-controlled study in months 0-6 (n=53), 2) Open-label study in months 6-12 with some of the same men as in 1) (n=35)	USA	Aglycone isoflavone- rich extract; genistein combined polysaccharide (GCP) in capsules, mean age ± SE: 70.5 ± 1.8 years, 1) n=28, 2) n=17	Identical-looking placebo capsules with inert cellulose, mean age ± SE: 68.6 ± 1.5 years, 1) n=25, 2) n=18	450 mg genistein and 300 mg daidzein	Serum PSA levels	1) 6 months, 2) 6 months (totally 12 months)	PSA concentrations did not change in either group after 6 or 12 months. 6 month serum concentrations of genistein and daidzein (39.85 and 45.59 µmol/l, respectively) were significantly greater than at baseline. Equol levels did not change. The high intake of aglycone isoflavones was generally well tolerated, with loose stools <b>(diarrhea)</b> the most common complaint from a small number of men and <b>skin rash</b> in one man in the isoflavone group
Kumar et al., 2010	Men with clinically localized prostate cancer (age 45-80 years) (n=44)	USA	Soy-based IF concentrate (glycosides) as tablets	No IF concentrate	0 (n=11), 40 (n=12), 60 (n=11) or 80 mg (n=10) IF/day, as glycosides of genistein, daidzein and glycitein with 40% aglycones	Phase I-dose- finding trial to evaluate the safe and effective dose to be used in future trials for prostate cancer prevention	30 ± 3 days intervention	<b>Significant increases in serum total estradiol were observed with 40 mg/day isoflavone concentrate (P=0.23), and a significant increase in serum free testosterone was observed with 60 mg/day (P=0.003).</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								Changes in serum SHBG, PSA and cell proliferation (Ki-67) were not statistically significant. One person in the 60 mg and one in the 80 mg isoflavone-treated groups reported Grade I GI events, which were considered possibly related to the study agent. The only group that had a modulation of serum estradiol without a significant increase in serum free testosterone or serum PSA, as well as the least mean % of cells in proliferation, with no clinical toxicity, was the 40 mg group
Sharma et al., 2009	Men undergoing androgen deprivation therapy for prostate cancer (n=33, 79% white, 21% black)	USA	Soy protein powder (n=17)	Whole milk protein as placebo (n=16)	160 mg total IF (Ge:D:GI %: 40:39:21)	Cognition, vasomotor symptoms, sexual dysfunction or quality of life	12 weeks	There were no significant differences between the two groups in any outcome measure. No significant improvement was found in cognition, vasomotor symptoms or any other aspect of quality of life compared with placebo. There were no safety issues during the study and no significant changes in PSA, weight or BMI in either group
Dillingham et al., 2007	Healthy men (age 20-40 years), (n=35), 12 were equol excretors (34%)	Canada	Low isoflavone soy protein isolate (SPI), high isoflavone SPI as supplement	Milk protein isolate (MPI)	Low iso SPI (mean ± SD): 1.64 ± 0.19 mg/day of IF as	Parameters of semen quality	Three 57-day treatment periods separated by 4-	No significant effects were found of low- or high-isoflavone SPIs on serum total T3, free T3,

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
					total aglycones (0.02 mg/kg bw per day), Ge:D:Gl %: 79:13:8, high iso SPI: 61.7 ± 7.4 mg/day of IF (0.75 mg/kg bw per day), Ge:D:Gl %: 53:36:11		week washout periods (cross- over design)	total T4, free T4, TSH or TBG when compared with the MPI. No negative effects were reported
Kumar et al., 2007a,b	Men with early stage, clinically localized prostate cancer (n=50), about 92- 96% white, 2% black and 1% unknown	USA	Soy-based IF concentrate (glycosides) as tablets (n=23)	Inert ingredients as placebo (gelatin) (n=27)	80 mg IF/day (as 2 X 40 mg/day), as glycosides of genistein, daidzein and glycitein with 40% aglycones	Safety	12 weeks	No categories of adverse events (AE); all, by grade, by causality, by expected or by symptom, were significantly different between the treated and placebo groups. None of the AE produced clinical toxicity. Modulation of SHBG, total estradiol and testosterone in the isoflavone-treated group compared with placebo was non-significant. No symptoms related to estrogenic effects (such as breast changes, lowered libido and increased frequency of hot flashes) were seen
Goldin et al., 2005	Moderately hypercholesterolemic older men (mean age 61 years) (n=18)	USA	Isolated soy protein depleted of IF (soy/-), soy protein enriched in IF (soy/+). Isoflavone as powdered	Animal protein without added IF (animal/-), animal protein with added IF (animal/+)	Mean soy protein intake: 71 ± 18 g/day. Mean daily IF intake 139 ± 35 mg/day (for which group(s) were not	Hormonal responses (estrogen and androgen)	Each of 4 diets were fed in randomized order for 6 weeks per phase (cross- over design)	<b>DHEAS concentrations were 14% lower after consuming the isoflavone (P=0.0106) and 8% higher after soy, compared with the</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
			concentrate mixed in food (soy or animal)		specified). Information on IF form or composition not given			<b>animal protein diets (P=0.0118)</b> . Relatively large amounts of soy protein or soy-derived isoflavones had modest and limited sex-specific effects on circulating hormone levels. No adverse effects were reported in the paper
Adams et al., 2003 (+ other studies on the same population, with other end points: Adams et al., 2004, Adams et al., 2005, and Newton et al., 2006)	Older men (50-80 years) with adenomatous colorectal polyps, (n=128)	USA	Soy protein beverage powder (+ISO)	Ethanol extract of +ISO	+ISO: 83 mg IF/day, daily IF dose 45.6 mg genistein, 31.7 mg daidzein and 5.5 mg glycitein, -ISO: 3 mg IF/day, daily IF dose 1.7 mg genistein, 1.0 mg daidzein and <0.1 mg glycitein	Effects on circulating insulin-like growth factor (IGF)-I concentrations	12 months	Mean change in serum IGF-I concentrations was similar in the two groups (+1.4 nmol/l in +ISO, +1.2 nmol/l in -ISO; P=0.74, 95% CI -1.1, 1.5), indicating no effect of the isoflavone intervention. Similarly, the changes in IGFBP-3 and the IGF-I/IGFBP-3 ratio were similar in both groups. No negative effects were reported in this study (or in three other studies on the same population)
Gardner-Thorpe et al., 2003	Healthy men, average age $\pm$ SD: 35.6 $\pm$ 11.2 years (n=19)	UK	Scones baked with soy flour (3 per day)	Scones baked with wheat flour (3 per day)	120 mg IF/day (genistein 45 mg/day and daidzein 75 mg/day)	Effects on serum sex steroids, lipids and markers of oxidative stress	6 weeks on one types of scones, then 6 weeks washout period, before 6 weeks on the other type (cross-over design)	Significant improvements in 2 of 3 markers of oxidative stress were seen in the soy group. Lag time for myeloperoxidase rose from 55.0 to 68.0 min. (95% CI -16.0, -3.5; P=0.009) and presence of hydroperoxides decreased from 2.69 to 2.34 $\mu$ mol/l (95% CI 0.12, 0.71; P=0.009).

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								<b>Total serum testosterone fell in the soy group (19.3-18.2 nmol/l; 95% CI 16.20, 20.44; P=0.03).</b> No significant changes were seen in the concentrations of the other serum sex steroids, albumin or SHBG. There were no changes seen in serum triglycerides or cholesterol, and no negative effects were reported
File et al., 2001	Healthy men (n=15), mean age ~26 years	UK	Diet of a wide range of soy-containing foods (commercial products or prepared meals)	Diets of foods free of soy products	High soy: 100 mg total IF/day, Low soy: 0.5 mg total IF/day (no information was given on form or composition of IF in the diets)	Cognitive functions (attention, memory and frontal lobe function)	10 weeks, both diets were given to the subjects on a 11-day rotating menu-basis (cross-over design)	The men on high soy had significant improvements in short-term and long-term memory and in mental flexibility. There was no effect of diet on tests of attention or in category generation. There was no information in the paper regarding negative effects of isoflavones
Higashi et al., 2001	Healthy men 1) mean age $\pm$ SD: 31 $\pm$ 4 years, (n=14) 2) 30 $\pm$ 2 years, (n=12)	Japan	1) 20 g/day soy protein isolate (ISP), 2) 20 g/day ISP +/- 200 mg vitamin E, SPI mixed in milk or yogurt	1) No ISP	IF intake, form or composition not given	Remnant-like particles, lipolytic enzymes, lipid transfer protein, transaminases, sex hormones, Fe, Ca, vitamin E	1) +ISO or -ISP for each of 2 4-week periods with 1-2 months wash-out period between, 2) ISO +/- vitamin E for 3-weeks with 1-2 months wash-out period	1) Triglyceride and RLP cholesterol levels were significantly lower than baseline by 13.4% (P<0.05) and 9.8% (P<0.05), respectively. No significant changes were found in total and LDL cholesterol or the activities of lipoprotein lipase, hepatic lipase, cholesteryl ester transfer

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
							between (cross-over design)	protein and lecithin cholesterol acyltransferase. Although the levels of transaminases, testosterone, Fe and Ca did not change, <b>the vitamin E level was significantly reduced from baseline by 9.7% (P&lt;0.01)</b> . 2) <b>Vitamin E was significantly reduced 9.2% (P&lt;0.05)</b> , and vitamin E supplement increased vitamin E level significantly (P<0.05). No negative effects were reported in study 1) or 2)
Nagata et al., 2001	Healthy men (22-50 years) (n=34)	Japan	Soy milk as supplement (n=17)	No soy milk (n=17)	76.8 ± 16.6 mg total IF/day after the intervention. 100 g soy milk contains 0.6 mg genistein and 13.0 mg genistin, and 0.6 mg daidzein and 7.8 mg daidzin	Serum levels of steroid hormones	8 weeks on soy milk (blood samples taken from initiation and every 2 weeks until 12 weeks)	<b>There was a significant difference in time (0-12 weeks) X group interaction (P=0.04) between the groups in changes in estrone concentrations, which tended to decrease in the soy group (<math>\beta=-0.003352</math>, SE=0.00226) and increase in the control group over time (<math>\beta=0.003228</math>, SE=0.00223), for the difference in slopes.</b> None of the other hormones measured (estradiol, total and free

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								testosterone or SHBG) showed any statistical difference between the two groups. No adverse events were reported, except <b>that one person dropped out of the soy group for feeling sick</b>
Habito et al., 2000	Healthy men (age 35-62 years) (n=42)	Australia	Tofu (290 g/day)	Lean meat protein (150 g/day)	118.9 mg IF/day, 0.29 mg genistein/g and 0.12 mg daidzein/g	Sex hormone concentrations	4-week intervention, 2-week washout period, then 4-week intervention (cross-over design)	Blood concentrations of sex hormones (testosterone, dihydrotestosterone, androstenediol glucuronide and estradiol) did not differ after the two diets, but the mean testosterone:estradiol value was 10% higher (P=0.06) after the meat diet. <b>SHBG was 3% higher (P=0.07), whereas FAI was 7% lower (P=0.06), after tofu compared with meat.</b> There was a significant correlation between the difference in SHBG and testosterone:estradiol and weight change. <b>Adjusting for weight change revealed SHBG to be 8.8% higher on tofu (mean difference 3 (95% CI 0.7, 5.2) nmol/l; P=0.01) and testosterone:estradiol</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								<b>to be significantly lower, P=0.049</b> ). No negative effects were reported
<b>Prospective (cohort) studies</b>								
Kurahashi et al., 2009	Men with (n=69) or without hepatocellular carcinoma (HCC) (n=7215) (age 40-69 years)	Japan	Consumption of IF (genistein and daidzein) and soy foods calculated from FFQ		Dietary tertiles genistein: <12.0, 12.0-19.9, ≥20.0 mg/day, daidzein: <8.0, 8.0-12.7, ≥12.8 mg/day, soy food: <37.6, 37.6-64.9, ≥65.0 g/day	Hepatocellular carcinoma (HCC)	Average 11.8 years of follow-up	Consumption of genistein, daidzein or soy food showed no association with HCC in men
Sun et al., 2004	Men with bladder cancer (BC) (n=61) or without (age 45-64 years) (n=18244)	Chinese in Shanghai	Dietary soy IF, from FFQ		Dietary quartiles of soy IF (mg/1000 Kcal): ≤1.69, 1.70-2.91, 2.92-4.45 and ≥4.46	Bladder cancer (BC)	Median time interval from baseline to diagnosis was 8.3 years, 233482 person-years of follow-up	<b>Compared with soy intake less than once a week, the RR (95% CI) for BC for intake 1-&lt;3 times per week, 3-&lt;7 times a week and daily were 2.05 (0.80-5.29), 2.45 (0.89-6.76) and 4.61 (1.57-13.51), respectively, Ptrend = 0.004 (adjusted). For soy isoflavones (mg/1000 Kcal), the RR (95% CI) for BC for intake of ≤1.69, 1.70-2.91, 2.92-4.45 and ≥4.46, were 1.00, 2.01 (0.90-4.47), 1.03 (0.41-2.60) and 2.78 (1.29-5.98), respectively, Ptrend = 0.02</b>



Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
Sun et al., 2002	Men (n=47) <b>and women (n=14)</b> (total n=61) with bladder cancer (BC) or without (age 49-81 years)	Chinese in Singapore	Total soy intake from 7 soy foods (as tofu equivalents), from FFQ		Dietary quartiles of soy IF (mg/1000 Kcal): ≤5.77, 5.78-9.83, 9.84-15.42, 15.43+	Bladder cancer (BC)	329848 person-years of follow-up (both genders)	<b>Relative to lowest quartile of total soy intake (&lt;36.9 g/1000 Kcal), the highest quartile (≥92.5 g/1000 Kcal) was associated with a 2.3-fold increase in BC (95% CI 1.1-5.1) (adjusted). For soy protein, the same RR was 2.74 (95% CI 1.18-6.38). For soy isoflavones (mg/1000 Kcal), relative to lowest quartile intake (≤ 5.77 mg/1000 Kcal), the highest quartile (15.43 mg/1000 Kcal) was 2.08 (n.s.) (95% CI 0.94-4.60), whereas the second highest quartile (9.84-15.42 mg/1000 Kcal) was 2.47 and statistically significant (95% CI 1.16-5.26)</b>
Mackey et al., 2000	Hypercholesterolemic men (mean age was 51.5 years) (n=27)	Australia	Soy protein powder with high IF (ISP+), as supplement	Soy protein powder with low IF (ISP-)	65 mg isoflavones/day (ISP+), <4 mg isoflavones/day (ISP-). No further information on form or composition of IF given	Reduction of plasma lipids and hormones	12 weeks soy treatment, with a low fat diet 4 weeks before and 6 weeks after soy treatment	There was a significant increase in HDL-cholesterol at 6 (P=0.02) and 12 weeks (P=0.03), <b>and a reduction in SHBG at 12 weeks (P=0.0003) with SPI +. An increase in DHEA nearly reached significance at 12 weeks (P=0.06)</b> . No significant changes were

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
								observed in in total cholesterol, LDL-cholesterol, triglycerides, TSH, FSH, LH, androstenedione or testosterone. Soy protein had a cholesterol lowering effect in men, probably independent of isoflavones. No negative effects were reported
White et al., 2000	Men (n=3734, 71-93 years, and <b>their wives</b> (n=502)	Japanese men and women living in Hawaii	Tofu (amount eaten was not given). Intake in <b>women</b> was assumed to be similar to their husbands who were interviewed. Intake was indexed at 4 levels; low-low (<2 servings/week at both interviews and no tofu in the prior week in 1971, high-high (≥2 servings/week at both interviews and defined intermediate or less consistent low or high intake levels	Intake levels of IF/day from tofu or the form and composition of IF was not given		Cognitive functions	Data on tofu intake was from interviews in 1965-1967 and 1971-1974, cognitive functions were tested in 1991-1993	<b>Poor cognitive test performance, enlargement of ventricles and low brain weight were each significantly and independently associated with higher midlife tofu consumption.</b> Statistically significant associations were consistently demonstrated in linear and logistic multivariate regression models. Odds ratios comparing endpoints among high-high with low-low consumers were mostly in the range of 1.6-2.0. Study weaknesses: the intake of only 26 foods was assessed and questions about tofu intake were not consistent over the course of the follow-up period. <b>The reasons</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								<b>for the effects of tofu have been suggested to be formaldehyde used as preservative, which adversely affects memory in rodents and is markedly elevated in urine of dementia patients</b>
<b>Retrospective (case-control) studies</b>								
Shin et al., 2015	Male colorectal cancer patients (n=624) and matched controls (n=1872)	Korea	Total soy food products and subgroups, and total isoflavones, genistein, daidzein and glycitein estimated from FFQ	Up to 3 controls per patient matched by gender and 5 years of age groups	The quartiles for total isoflavone intake were: Q1: <7.63, Q2: 7.63- <12.56 mg/day, Q3: 12.56- <20.89, Q4: ≥ 20.89 mg/day			The group with the highest intake quartile (Q4) of total isoflavones showed a decreased risk for colorectal cancer compared with their counterparts with the lowest intake quartile (Q1) in men (OR 0.71, 95% CI: 0.52-0.97). <b>However, Q2 and Q3 of total isoflavones were associated with an elevated risk in men (Q2: OR: 1.34, 95% CI 1.02-1.76, Q3: OR: 1.37, 95% CI 1.04-1.79. Ptrend=0.005</b>
<b>Cross-sectional studies</b>								
Chavarro et al., 2008	Healthy male partners of subfertile couples, age 18-25 years (n=33), 90% Caucasian, 72% overweight/obese	Slovakia	15 soy-based foods eaten in previous 3 months, from FFQ		Mean intake of IF 5.4 mg/day. Results evaluated as genistein, daidzein, glycitein, all as	Semen quality parameters	3 months	<b>There was a significant inverse association between soy food intake and sperm concentration (adjusted). In multivariate-adjusted</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
					mg/day, and soy foods, as servings/day			<p>analyses, men in the highest category of soy food intake (<math>\geq 0.30</math> servings/day) had 41 million sperm/ml less than men who did not consume soy foods (95% CI -74, -8; Ptrend=0.02). Results for individual soy isoflavones (genistein, daidzein and glycitein) were similar to the results for soy foods and strongest for glycitein, but not statistically significant (except for trend for glycitein). In the multivariate-adjusted analyses, men in the highest category of glycitein intake was 35 million sperm/ml less than non-consumers of soy foods (95% CI -73, 2; Ptrend=0.07, tertiles 0.01-0.08, 0.09-0.28 and <math>\geq 0.28</math> mg/day). Soy food and soy isoflavone intake were unrelated to total sperm count, sperm motility, sperm morphology or ejaculate volume</p>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
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Nagata et al., 2000	Healthy men (mean age $\pm$ SD: 60.5 $\pm$ 10.7 years) (n=69)	Japan	Daily soy product intake: 51.0 $\pm$ 21.5 g, daily IF intake: 21.9 $\pm$ 8.7 mg, from FFQ		No information given on form or composition of IF			<b>Serum estradiol concentration was significantly inversely correlated with soy product intake (r=-0.32, P=0.009), and serum estrone concentration was non-significantly inversely correlated with soy product intake (r=-0.24, P=0.05) (adjusted). Total and free testosterone concentrations were inversely correlated with soy product intake after controlling for the covariates, but these correlations were of border line significance (r=-0.25, P=0.05 and r=-0.25, P=0.06, respectively). Similar correlations were observed for these hormones with isoflavone intake from soy products</b>
<b>Other clinical studies</b>								
Celec et al., 2013	Healthy young men (age 18-25 years) (n=33)	Slovakia	2 g soybeans (dry weight)/kg bw per day		No information on IF content or composition	Oxidative and carbonyl stress	7 days (blood samples taken before intake, after 1-week intake and after 1-week wash-out period)	Total antioxidant capacity was increased by soybean intake, but did not decrease levels of AOPP. <b>Soybean intake increased lipoperoxidation in</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
								<b>men.</b> No effects on carbonyl stress markers were found (AGE and fructosamine)
Bahls et al., 2011	Patients of both genders with metabolic syndrome (gender distribution unknown) (n=40)	Brazil	25 g of roasted soybean flour daily (n=20)	Controls (n=29)	12.95 g soy protein and 50 mg IF daily (no information about composition of IF given)	Oxidative stress and components of metabolic syndrome	90 days	The soybean-treated group showed a decrease in fasting glucose and increase in serum HDL and adiponectin. Treatment was well tolerated by the patients
Kwan et al., 2010	Patients with rising prostate-specific antigen (PSA) after radical radiation for prostate cancer, median age 78 years (range 62-85), n=29	Canada	Soy isoflavone beverage	No controls	Total isoflavones of 65-90 mg/day	PSA doubling time and tolerability	6 months	PSA showed a declining trend in 4 patients (13.8%) and there was a >100% prolongation of PSA doubling time in 8 patients (27.6%). <b>However, PSA doubling time also showed a 50% or more shortening in 5 patients (17.2%).</b> The commonest side-effect was mild gastrointestinal upset, such as nausea, flatulence, bloating or constipation, decreasing over time. The second most common complaint was weight gain seen in 4 persons (2.7-9.1 kg over 6 months). One person had Grade II side-effects (hives) possibly related to soy
Serafina A. (paper not found), cited in Messina et al., 2009	Healthy men (age unknown) (n=20)	Italy	Form and composition of IF unknown		160, 320 or 480 mg IF/day	Male fertility parameters	3 months	Compared with baseline, no significant differences in ejaculate volume, sperm concentration,

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
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								sperm count or motility of spermatozoa were seen after isoflavone intake
Tanaka et al., 2009	Healthy men (age 30-59 years) (n=28), 18 equol producers, 10 non-producers	Japan	Soy isoflavone supplement as tablets		60 mg IF/day (0.1 mg genistein, 0.2 mg daidzein and 0.3 mg glycitein, 3.5 mg genistin, 19.1 mg daidzin, 10.4 mg glycitin, 2.2 mg malonyl genistin, 8.1 mg malonyl daidzin, 3.4 mg malonyl glycitin, 1.9 mg acetyl genistin, 7.3 mg acetyl daidzin, 3.6 mg acetyl glycitin)	Serum levels of sex hormones implicated in prostate cancer and cholesterol	3 months	No significant difference was seen in mean total cholesterol between baseline and end of study. The mean HDL-cholesterol increased significantly, whereas the mean LDL-cholesterol decreased significantly. No changes in serum estradiol and total testosterone were detected. <b>Serum SHBG increased significantly, and free testosterone and DHT decreased significantly.</b> Equol production was stimulated. <b>Diarrhea was reported in 3 persons.</b> No statistically significant adverse events were reported
Pendleton et al., 2008	Men with PSA recurrent prostate cancer after radiation therapy or radical prostatectomy (median age 73 years, 3 African-American, 17 white), (n=20)	USA	Soy milk	No controls	141 mg isoflavones/day (no information on form or composition was given)	Serum PSA levels	1 year	PSA had increased 56% per year before study entry and only increased 20% per year for the 12-month study period (P=0.05). The slope of PSA after study entry was significantly lower than that before study entry in 6 patients <b>and significantly higher in 2 patients.</b> For 12

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
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								patients, the change in slope was statistically insignificant. Improvements in PSA doubling times were seen in 14 patients (P = 0.044). <b>Free testosterone decreased while on therapy (median 10.3 vs. 9.7 ng/ml, P=0.031)</b> . Total testosterone, cholesterol and QOL were not significantly changed. Of 6 men who did not complete the study, one (5%) was due to side-effects ( <b>diarrhea</b> ). No other side-effects were reported
Celec et al., 2007	Healthy young men (age 18-25 years) (n=7)	Slovakia	About 1.8 g soybeans per kg bw per day		IF intake, form and composition not given	Spatial abilities as cognitive function and sex hormone status	7 days	Spatial visualization as cognitive performance were improved with soy (P=0.03). Soy did not change plasma estradiol, total and free testosterone or salivary testosterone and estradiol
Goodin et al., 2007	Healthy men (age 25-47), (n=12)	USA	56 g 100% pure soy protein isolate powder daily		IF intake, form and composition not given	Biological activity <i>in vivo</i> and <i>in vitro</i> (estrogen receptor assay)	28 days	<b>Serum testosterone decreased 19% (<math>\pm 22\%</math>) on soy (P=0.021) (still within normal reference range)</b> and increased 2 weeks after discontinuation. <b>Serum LH concentrations decreased (n.s.) on</b>



Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
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								soy (still within normal reference range), then increased 2 weeks after discontinuation. No negative effects were reported
Hamilton-Reeves et al., 2007	Men (age 50-85 years) at high risk for developing advanced prostate cancer (n=53) or with low grade prostate cancer (n=5), Caucasian	USA	1) Soy protein isolate (SPI+), 2) alcohol-washed soy protein isolate (SPI-), as supplement	Milk protein isolate (MPI)	SPI+: 107 mg IF/day, Ge:D:Gl %: 53:35:11, SPI-: <6 mg IF/day, Ge:D:Gl %: 57:20:23, MPI: 0 mg IF/day	Circulating hormone profiles and hormone receptor expression patterns	6 months	<b>SPI+ significantly suppressed AR expression (P=0.04)</b> , but not ERβ expression or circulating hormones. <b>SPI- significantly increased estradiol (~20%) and androstenedione concentrations (~17%), and tended to suppress AR expression compared with MPI (P=0.09)</b> . No negative effects were reported
Ostatníková et al., 2007	Healthy young men (age 18-25 years) (n=32)	Slovakia	2 g soybeans per kg bw per day		IF intake, form and composition not given	Cognitive abilities and steroid sex hormones	7 days on soybean + 7 days wash-out period	Mental rotation and spatial visualization were significantly improved with soy. Soy did not change salivary testosterone and plasma estradiol (E2) levels, but during the wash-out period both parameters showed a tendency to rise (n.s.). The effect of soy on hormonal parameters was dependent of basal testosterone levels; it increased significantly after a low basal level,

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								but decreased non-significantly after a high basal level
Dillingham et al., 2005	Healthy men (age 20-40 years) (n=35)	Canada	Low IF soy protein isolate (low-iso SPI), high IF SPI (high-iso SPI), as supplement	Milk protein isolate (MPI)	Low-iso SPI: 1.64 ± 0.19 mg IF/day, as unconjugated units, Ge:D:Gl %: 79:13:8, High-iso SPI: 61.7 ± 7.35 mg IF/day, Ge:D:Gl %: 53:36:11	Circulating hormone profiles and hormone receptor expression patterns	32-week study period consisting of three 57-day treatments, each separated by 28-day washout	<b>DHT and DHT/testosterone were significantly decreased by the low-iso SPI (9.4% (P=0.036) and 9.0% (P=0.004), respectively) and the high-iso SPI (15% (P=0.047) and 14% (P=0.013), respectively), compared with the MPI. Other significant effects included a decrease in testosterone by low-iso SPI relative to MPI (P=0.023) and high-iso SPI (P=0.020) at day 29; an increase in DHEAS by low-iso SPI relative to MPI at day 29 (P=0.001) and relative to MPI (P=0.0003) and high-iso SPI (P=0.005) at day 57; and increases in estradiol and estrone by low-iso SPI relative to MPI at day 57 (P=0.010 and P=0.005, respectively).</b> No negative effects were reported

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
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deVere White et al., 2004	Men with prostate cancer (age 61-89 years) (n=52)	USA	5 g/day of a genistein-rich extract, genistein-combined polysaccharide (GSP), in capsules	No controls	450 mg/day genistein plus 450 mg/day of other aglycone IF, 10% genistein, 6% daidzein, 2% glycitein	50% reduction in prostate-specific antigen (PSA)	6 months	One patient had a decrease in PSA >50%, and 7 patients had PSA reductions <50%. Thus, the GCP extract was not an effective treatment for prostate cancer. <b>The total testosterone level was lowered in 1 patient, but increased in 5 patients.</b> Clinical chemistry values (alkaline phosphatase, aspartate aminotransferase, total bilirubin, creatinine, cholesterol and gamma-glutamyltransferase) were unaltered in all men. Three patients dropped out because of <b>diarrhea</b> , which resolved on discontinuation of the supplement
Fischer et al., 2004	Men with stage B, C or D prostate adenocarcinoma, but otherwise in good health (age >40 years) (n=20), 19 (95%) Caucasian and 1 (5%) African American	USA	Soy IF formulation in capsules	No controls	About 300 mg/day genistein and 150 mg/day daidzein for 28 days, then 600 mg/day genistein and 300 mg/day daidzein for the remaining 56 days, i.e. 450 or 900 mg/day IF (totally 84 days)	Safety, pharmacokinetics and efficacy of IF as potential cancer chemotherapeutic agent	3 months	<b>Serum DHEA was decreased by 31.7% (P=0.0004) and PSA increased during the trial both while on isoflavones and after.</b> Relatively minor side-effects were observed, including some <b>estrogenic effects (breast changes, increased frequency of hot flashes)</b> . None of the adverse events

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
								were associated with clinically significant organ dysfunction. Nine grade I adverse events (AE) were judged possibly, and 4 probably, related to IF, and there were 4 AE of grade II (constipation, gynecomastia, elevated amylase, hypocalcemia)
Kumar et al., 2004	Men with early stage prostate cancer (age 50-80 years) (n=59)	USA	Soy protein as beverage	Placebo (isocaloric)	60 mg IF (genistein)/day	Changes in steroid hormones and PSA	12 weeks	<b>Serum free testosterone was reduced</b> or showed no <b>change in 61% of subjects in the isoflavone group compared with 33% in the placebo group.</b> Serum total PSA decreased or was unchanged in 69% of the subjects in the isoflavone group compared with 55% in the placebo group. 19% of subjects receiving soy isoflavones reduced total PSA by $\geq 2$ points during intervention. No increase in SHBG was seen. Drop-outs: 8 from isoflavone group and 9 from placebo group. However, none of the mean changes between the two groups were statistically significant
Hussain et al., 2003	Men either newly diagnosed and with untreated disease	USA	Soy isoflavone tablets, 40%	No controls	200 mg/day of total isoflavones	Serum PSA, testosterone,	3-6 months (median	Although there were no sustained decreases in

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
	under watchful waiting with rising PSA (group I) or had increasing serum PSA following local therapy (group II) or while receiving hormone therapy (group III), mean age 73 years (range 55-82), (n=41) (19 Caucasian, 21 African-American, 1 Asian)		glycocides, 60% aglycones, Ge:D:GI ratios: 1.1:1:0.2			IGF-1, IGFBP-3 and 5-OHmdU	duration 5.5 months , range 0.8-6 months), a total of 190 patient-months of supplementation	PSA, stabilization occurred in 83% of hormone-sensitive and 35% of hormone-refractory patients. There was a decrease in the rate of rise of serum PSA in the whole group (P=0.01) with rates of rise decreasing from 14 to 6% in hormone-sensitive (P=0.21) and from 31 to 9% in hormone-refractory patients (P=0.05) on isoflavones. No significant changes were observed in serum levels of testosterone, IGF-1, IGFBP-3 or 5-OHmdU. Soy isoflavone supplement was very well tolerated, with no toxicity attributable specifically to treatment. No grade III or worse adverse events were observed. There were no side-effects related to soy isoflavone's estrogenic effects and no digestive effects
Busby et al., 2002	Healthy men (age 40-69 years) (n=30)	USA	Single dose of 1 of 2 soy IF preparations (Formulation A: ≥97% unconjugated IF, formulation B: 70% unconjugated IF)	No controls	Genistein doses: 1, 2, 4, 8 and 16 mg/kg bw i A and B, daidzein doses: 0.11, 0.22, 0.44, 0.89 and 1.8 mg/kg bw i A and 0.49,	Safety and pharmacokinetics	Study period 26-30 h of inpatient admission after a 10-h fast at home	Adverse events (AE) judged to be possibly related to IF: Four grade I (2 loss of appetite, 1 pedal edema and 1 abdominal tenderness), eight grade ≥II (2 elevated lipase, 1

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in <b>bold</b> )
			Isoflavones	Control				
					0.98, 2.0, 3.9 and 7.8 mg/kg bw in B. A Ge:D:GI %: 90 ± 5:10:1, B Ge:D:GI %: 43:21:2. n=6 per dose group			elevated amylase, 1 leukopenia and 4 hypophosphatemia). None AE were associated with any clinical toxicity. No estrogenic or antiestrogenic symptoms were observed
Mitchell et al., 2001	Healthy men (age 18-35 years) (n=15)	UK	Soy extract in tablets	No controls	40 mg total IF/day (genistein, daidzein and glycitein, distribution of each not given)	Semen quality and serum sex steroid and gonadotrophin levels	2 months exposure, blood and semen sampled monthly for 2 months before and 4 months after exposure	No observable effects on estradiol, testosterone, FSH or LH, or testicular volume or semen parameters (ejaculate volume, sperm concentration, count, motility or movement), were found. No negative effects were reported
Teixeira et al., 2000	Moderately hypercholesterolemic men (age 23-74 years) (n=81), study started with 92 men, performed in 3 cohorts, each for 9 weeks (n=27, 24 and 41)	USA	50 g protein/day, in combinations of isolated soy protein with 1.9 mg total IF aglycone units/g protein or casein (calcium caseinate) or both, in baked products and ready- to-mix beverages 5 days/week	0:50 g (0 mg IF/day)	Soy protein: casein: 50:0 g (95 mg IF/day), 40:10 g (76 mg IF/day), 30:20 g (57 mg IF/day), 20:30 g (38 mg IF/day). Content of individual IF not given	Reduction of blood lipids	3-week lead-in period on specified diet and 6 weeks IF exposure (total 9 weeks)	At week 6, significant reductions (P<0.05) from baseline compared with controls were found for non-HDL, total cholesterol and apo B for all ISP groups (except total cholesterol with 40 g ISP). At week 3, significant reductions (P<0.05) were found in apo B for the groups on ≥30 g ISP and in non- HDL cholesterol for the groups on ≥40 g ISP. HDL-cholesterol, apo A- I, lipoprotein (a), and triacylglycerol concentrations were not significantly affected. <b>Two subjects</b>

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								reported signs of possible allergy to soy. No other negative effects were reported
<b>Case reports</b>								
Pillai and Thapar, 2015	Previously healthy man, without significant past medical history (48 years old)	USA	20 mg soy protein powder supplement/day	No family history of liver disease or colorectal cancer, no history of alcohol or IV drug use and he was negative for autoimmune hepatitis, viral hepatitis, Wilson's disease and $\alpha$ -1 antitrypsin deficiency	No information was given on content and composition of IF		2 months	<b>Symptoms: urine and stool color changes, right upper quadrant tenderness, fatigue and jaundice. Laboratory tests showed increased AST, ALT, total bilirubin and direct bilirubin. Liver biopsy demonstrated subacute hepatitis with massive collapse of portal tract and lobules, inflammatory activity and necrosis in 30% of the hepatic parenchyma.</b> Laboratory tests 105 days after drug discontinuation showed improved liver function tests with lowered values of liver enzymes, and he reported increased energy and was off all diuretic medication. He had a RUCAM score of 10, supporting the conclusion that the liver injury was likely related to supplement use

Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
Siepmann et al., 2011	A diabetic, but otherwise healthy, man (19 years old)	USA	Soy-based food products in vegan-style diet		360 mg IF/day (no information was given on composition of IF)		1 year	<b>Symptoms: sudden onset of loss of libido and erectile dysfunction. Blood concentrations of free and total testosterone were initially decreased, whereas DHEA was increased.</b> These parameters normalized within 1 year after cessation of the vegan diet, paralleled by a constant improvement of symptoms; full sexual function was regained 1 year after cessation of the vegan diet
Martinez and Lewi, 2008	A healthy man (60 years old)	USA	3 quarts of soy milk daily, and later another non-lactose soy product	No changes in testicular size, no history of testicular trauma and no sexually transmitted diseases. Testicular ultrasonography, computed tomography of the chest, abdomen and pelvis and positron emission tomography were normal	361 mg IF/day from soy milk, also another non-lactose soy product (no information on composition of IF)		Soy milk for approximately 1.5 years, another non-lactose soy product for < 1 year	<b>Symptoms: bilateral gynecomastia, dramatically elevated estrogen levels (estrone and estradiol concentrations 4-fold increased above the upper limit of the reference range), erectile dysfunction and decreased libido.</b> After he stopped the intake of soy milk, and another non-lactose soy product, the breast tenderness resolved and his estradiol concentration slowly returned to normal



Study design/ Reference	Participant characteristics	Country	Number in treatment group		Dose(s)	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
Casini et al., 2006	A man with severe oligospermia (10 million/ml) and abnormal sperm motility and morphology (30 years old)	Italy	Soybean IF tablets	A couple had tried to conceive for 3 years, and the woman was healthy at the clinical and endocrinologic examination	80 mg IF/day (Ge:D:Gl %: 40-45:40-45:10-12)		6 months	No other parameters except sperm count, motility and morphology were altered in the man. During the 3. month on IF, semen parameters improved dramatically (sperm count, 45 million/ml; >50% motility; >30% normal sperm morphology); therefore, intrauterine insemination was performed, which resulted in a healthy baby. After 6 months on IF, sperm parameters maintained their improvement (sperm count, 50 million/ml; >50% motility; >35% normal sperm morphology). However, 6 months after termination of IF, sperm parameters had deteriorated (sperm count, 18 million/ml; <20% motility; <10% normal sperm morphology). No adverse effects were reported

## 9.4 Human studies on effects of isoflavones on children and adolescents

**Table 9.4** An overview of human studies on children and adolescents investigating health effects of isoflavones (IF).

Age group/ Reference	Study design/ Participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
<b>Infants</b>								
Upson et al., 2016	Prospective (cohort) study. Effects of soy-formula feeding during infancy evaluated in young (age 23-34 years) African American women (n=1553)	USA	Participants ever fed soy formula (n=198)	Participants never fed soy formula (n=1355)	IF exposure, content and composition not given	Altered menstrual bleeding in adulthood (age 23-34 years)		Associations were found between soy formula feeding and variables indicating <b>heavy menstrual bleeding, including ever experiencing heavy, gushing-type bleeding (RR: 1.2, 95% CI: 1.0, 1.4), ever use of NSAIDs for heavy bleeding (RR: 1.3, 95% CI: 1.0, 1.6), and ever use of a contraceptive method for heavy bleeding (RR: 1.2, 95% CI, 0.9, 1.6).</b> Among the subset of participants with menses in the past year who did not use medication that may alter menstrual flow (n=762), the data suggested <b>that</b>

Age group/ Reference	Study design/ Participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								soy formula feeding was associated with heavier flow and adverse impact on quality of life, but CIs were wide
Adgent et al., 2012	Longitudinal prospective (cohort) study where mothers were enrolled during pregnancy and their children were followed prospectively (n=2920 girls)	UK	Soy milk or formula in infant diet at or before 4 months and until 6 months (early soy, n=54), soy milk or formula between 5 and 15 months (late soy, n=111)	Breast-fed until ≥6 months, no soy before 24 months, and no other milk/formula before 6 months (primarily breast-fed, n=631), any non-soy milk or formula at or before 4 months, continued up to 6 months and no soy before 24 months (early-formula-fed, n=2124)	IF exposure, content and composition not given	Age of menarche reported between age 8 and 14.5 years		<b>The median age of menarche (interquartile range (IQR)) in the study was 153 months (144-163), ~12.8 years, vs. 149 months (12.4 years (IQR 140-159) among the early soy fed girls. Compared with girls fed non-soy based infant formula or milk (early formula), early soy fed girls were at 25% higher risk of menarche throughout the course of follow up (Hazard Ratio 1.25, CI 0.92, 1.71). For the remaining characteristics evaluated, early formula and early soy exposure</b>

Age group/ Reference	Study design/ Participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								groups were similar
Andres et al., 2012	Longitudinal prospective (cohort) study. Healthy infants (n=391), assessed at age 3, 6, 9 and 12 months	USA	Soy protein-based formula from 2-12 months	Cow's milk formula from 2-12 months, or breast milk at least from 2-6 (or up to 12) months	IF exposure, content and composition not given	Developmental status (mental, motor and language) from 3-12 months of age		All scores on developmental testing were within established normal ranges, with no significant differences between formula-fed infants; milk-based formula versus soy protein-based formula
Strom et al., 2001	Retrospective (historical) cohort study of young adults (age 20-34 years) being exposed as infants	USA	Soy infant formula (n=248)	Cow milk formula (n=563)	IF levels were not measured in the soy formula. Estimated IF from formula content of SPI (1997): 32-47 mg/l, 4.2-9.4 mg/kg bw per day for the first 16 weeks of life, or IF from soy flour: 9-16 mg/kg bw per day	Self-reported pubertal maturation, menstrual and reproductive history, height and usual weight, and current health		No statistically significant differences were observed between groups in either women or men aged 20-34 years for more than 30 outcomes. <b>However, women who had been fed soy formula reported slightly longer duration of menstrual bleeding (adjusted mean differences 0.37 days; 95% CI= 0.06-0.68), with no difference in severity of menstrual flow, and greater discomfort with</b>

Age group/ Reference	Study design/ Participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								menstruation (unadjusted relative risk for extreme discomfort vs. no or mild pain 1.77; 95% CI 1.04-3.00). More users of asthma or allergy drugs were seen in those on soy vs. cow milk formula, P=0.08 for men, P=0.047 for women
<b>Adolescents</b>								
Lee et al., 2009	A population-based prospective (cohort) study of women (n=72223), with breast cancer (n=592)	China	Adolescent (13-15 years) soy food intake, evaluated as soy protein or IF intake from a validated quantitative FFQ		Composition of IF was not given	Breast cancer	Mean follow-up of 7.4 years	In pre-menopausal women there was a reduced risk of breast cancer (305 cases) associated with adolescent isoflavone intake (RR: 0.89, 95% CI 0.57, 1.40, P <sub>trend</sub> =0.452), for trend over quintiles, median intakes in Q1 and Q5 were 4.31 and 42.26 mg/day of IF, respectively). <b>In post-menopausal women there was a slight</b>

Age group/ Reference	Study design/ Participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								<b>positive association between breast cancer (289 cases) and adolescent isoflavone intake (RR: 1.38 95% CI 1.00, 1.91, (Ptrend=0.038))</b>
Anderson et al., 2013	Retrospective (case-control) study. Cases: Women with diagnosed breast cancer, stratified by estrogen (ER) and progesterone (PR) receptor subtypes (age 25-74 years), controls: age-matched women from same area	Canada	Adolescent (age not given) intake of total isoflavones (genistein, daidzein, glycitein, formononetin) or total phytoestrogens (isoflavones, lignans, coumestans) from FFQ		IF form and composition was not given	Breast cancer		Post-menopausal women had a negative association between breast cancer and adolescent total isoflavone intake ( $\geq 21$ $\mu\text{g}/\text{day}$ ) (highest vs. lowest tertile: OR=0.81, 95% CI 0.67-0.98, Ptrend=0.09) for ER+PR+ cases, and (highest vs. lowest tertile: OR=0.68, 95% CI 0.51-0.90, Ptrend=0.01) for ER+PR- cases, indicating decreased risk for these breast cancer subtypes with adolescent total isoflavone intake. For total phytoestrogens ( $\geq$

Age group/ Reference	Study design/ Participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Duration of the study	Observed effects (negative effects in bold)
			Isoflavones	Control				
								234 µg/day), the result was similar: (highest vs. lowest tertile: OR=0.79, 95% CI 0.65-0.96, Ptrend=0.04) for ER+PR+ cases

## 9.5 Literature searches

### 9.5.1 Primary searches

**Database:** Embase 1974 to 2016 February 02, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present

**Date:** 03.02.2016

**Total result:** 2704

#	Searches	Results
1	(isoflavone* or soybean* or soy bean* or soyabean* or soya or soy or daidzein or genistein or glycitein or daidzin or genistin or glycitin or phytoestrogen* or phytoestrogen*).ti.	45678
2	(risk* or safe* or adverse or side-effect*1 or hazard* or harm or harmful or negative or contraindicat* or contra-indicat* or toxicity or toxic).tw,kw.	7964254
3	1 and 2	7108
4	(animal* or rat or rats or mouse or mice or nonhuman).mp.	13517361
5	(human or humans or woman or women or man or men or child* or adolescen* or youth* or teen* or adult* or middle aged or aged or elder* or senior citizen*).mp.	34896511
6	4 not (4 and 5)	8778190
7	3 not 6	5547
8	(conference abstract* or letter* or editorial*).pt.	4849404
9	7 not 8	5121
10	limit 9 to (danish or english or norwegian or swedish)	4797
11	limit 10 to yr="1985 -Current"	4691
12	remove duplicates from 11	2704

**Database:** PubMed

**Date:** 03.02.2016

**Total result:** 2

Search	Query	Items found
<u>#10</u>	Search (Pubstatusaheadofprint AND #9)	<u>2</u>



<u>#9</u>	Search #7 AND 8	<u>224</u>
<u>#8</u>	Search (danish[Language] OR english[Language] OR norwegian[Language] OR swedish[Language])	<u>21323924</u>
<u>#7</u>	Search #5 NOT #6	<u>1006</u>
<u>#6</u>	Search (conference abstract*[Publication Type] OR letter*[Publication Type] OR editorial*[Publication Type])	<u>1296852</u>
<u>#5</u>	Search #1 AND #4	<u>1017</u>
<u>#4</u>	Search #2 NOT (#2 AND #3)	<u>3747829</u>
<u>#3</u>	Search (human or humans or woman or women or man or men or child* or adolescen* or youth* or young* or teen* or adult* or middle aged or aged or elder* or senior citizen*)	<u>16854680</u>
<u>#2</u>	Search (animal* or rat or rats or mouse or mice or nonhuman)	<u>6088387</u>
<u>#1</u>	Search ((isoflavone*[Title] OR soybean*[Title] OR soy bean*[Title] OR soyabean*[Title] OR soya[Title] OR soy[Title] OR daidzein[Title] OR genistein[Title] OR glycitein[Title] OR daidzin[Title] OR genistin[Title] OR glycitin[Title] OR phytoestrogen*[Title] OR phyto-estrogen*[Title])) AND (risk* or safe* or adverse or side-effect* or hazard* or harm or harmful or negative or contraindicat* or contra-indicat* or toxicity or toxic)	<u>4505</u>

**Database:** Web of Science [Core Collection]

**Date:** 03.02.2016

**Total result:** 690

# 6	690	(#1 NOT #4) <b>AND LANGUAGE:</b> (English OR Danish OR Norwegian OR Swedish) <b>AND DOCUMENT TYPES:</b> (Article OR Book OR Book Chapter OR Review) Refined by: RESEARCH AREAS: ( FOOD SCIENCE TECHNOLOGY ) <i>Indexes=SCI-EXPANDED, SSCI, A&amp;HCI, ESCI Timespan=1987-2016</i>
# 5	<b>4,631</b>	(#1 NOT #4) <b>AND LANGUAGE:</b> (English OR Danish OR Norwegian OR Swedish) <b>AND DOCUMENT TYPES:</b> (Article OR Book OR Book Chapter OR Review) <i>Indexes=SCI-EXPANDED, SSCI, A&amp;HCI, ESCI Timespan=1987-2016</i>
# 4	<b>1,429,660</b>	(#2 NOT (#2 AND #3)) <b>AND LANGUAGE:</b> (English OR Danish OR Norwegian OR Swedish) <b>AND DOCUMENT TYPES:</b> (Article OR Book OR Book Chapter OR Review) <i>Indexes=SCI-EXPANDED, SSCI, A&amp;HCI, ESCI Timespan=1987-2016</i>
# 3	<b>5,606,686</b>	(TS=(human or humans or woman or women or man or men or child* or adolescen* or youth* or teen* or adult* or "middle aged" or aged or elder* or "senior citizen*")) <b>AND LANGUAGE:</b> (English OR Danish OR Norwegian OR Swedish) <b>AND DOCUMENT TYPES:</b> (Article OR Book OR Book Chapter OR Review) <i>Indexes=SCI-EXPANDED, SSCI, A&amp;HCI, ESCI Timespan=1987-2016</i>
# 2	<b>2,330,167</b>	(TS=(animal* or rat or rats or mouse or mice or nonhuman)) <b>AND LANGUAGE:</b> (English OR Danish OR Norwegian OR Swedish) <b>AND DOCUMENT TYPES:</b> (Article OR Book OR Book Chapter OR Review)

		<i>Indexes=SCI-EXPANDED, SSCI, A&amp;HCI, ESCI Timespan=1987-2016</i>
# 1	<b>5,122</b>	(TI=(isoflavone* or soybean* or "soy bean*" or soyabean* or soya or soy or daidzein or genistein or glycitein or daidzin or genistin or glycitin or phytoestrogen* or "phyto-estrogen*") AND TS=(risk* or safe* or adverse or "side-effect*" or hazard* or harm or harmful or negative or contraindicat* or "contra-indicat*" or toxicity or toxic)) AND LANGUAGE: (English OR Danish OR Norwegian OR Swedish) AND DOCUMENT TYPES: (Article OR Book OR Book Chapter OR Review) <i>Indexes=SCI-EXPANDED, SSCI, A&amp;HCI, ESCI Timespan=1987-2016</i>

## 9.5.2 Secondary searches

Database(s): Ovid MEDLINE(R) 2012 to September Week 1 2016

Date: 19.09.16

Total result: 39

#	Searches	Results
1	(isoflavone or soybean or soyabean or soya or soy or daidzein or genistein or glycitein or daidzin or genistin or glycitin of phytoestrogen or phyto-estrogen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	9641
2	limit 1 to (english language and humans)	2887
3	(perimenopausal or postmenopausal).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	500

4	limit 3 to (english language and humans and yr="2015 - Current"))	113
5	2 and 4	3
6	premenopausal.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	2648
7	limit 6 to (english language and humans and yr="2015 - Current"))	625
8	2 and 7	6
9	men.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	80263
10	limit 9 to (english language and humans and yr="2015 - 2016"))	19059
11	2 and 10	34
12	limit 9 to (english language and humans	74797
13	2 and 12	137
14	limit 6 to (english language and humans)	2531
15	2 and 14	39