Isolated isoflavones are not without risk

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Soy and red clover contain isoflavones. These bioactive plant substances are also called phyto-estrogens because their chemical structure is similar to that of the human hormone, estrogen. They can, therefore, also have a hormone-like effect. There are 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 that is rich in soy. Furthermore, 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. In Germany for some time now food supplements with isolated isoflavones have been available on the market as an alternative to the prescribed hormone replacement therapy for menopausal complaints. The products are claimed to be efficacious natural products without any side effects. Against this backdrop, the Federal Institute for Risk Assessment (BfR) has undertaken a health assessment of isoflavone-containing food supplements.

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

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. It cannot be ruled out that these estrogen-like effects could promote the development of breast cancer. The necessary long-term studies, to prove the safety of isoflavone-containing products, are not available. Nor is 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 is not without risk for this consumer group.

1 Subject matter of the assessment

The Federal Office for Consumer Protection and Food Safety (BVL) has received repeated reports of the adverse effects of soy and red clover-containing products. These products had been placed on the market as foods/food supplements and were recommended above all for post-menopausal women. However, not all the products had been notified pursuant to § 5 Food Supplements Ordinance (NemV) to BVL.

As there have been several incidences of adverse effects of soy and red clover-containing products, the Federal Institute for Risk Assessment (BfR) has undertaken a health assessment of them.

2 Results

The reports about the adverse effects of soy/red clover-containing products do not refer to uniform sets of symptoms and point to allergic reactions and/or other causes, perhaps to the basic symptoms of the menopausal complaints of the persons concerned. The adverse ef-

fects are linked to various food supplements including ones which contain or contained other possible causal substances besides isoflavones, like nicotinic acid - a source of niacin. The daily intake of isolated isoflavones from soybeans was 40, 50 or 80 mg. In one case the daily intake included an additional 250 mg red clover extract.

At the present time, the claimed favourable effects of isolated isoflavones on menopausal complaints as well as other advantageous health effects on the heart, bones and breasts must be deemed to be not sufficiently scientifically substantiated. The potential of isolated isoflavones to trigger adverse health effects (e.g. on the human mammary gland, uterus, thyroid gland) and the dose-dependency of side effects of this kind have not been sufficiently investigated up to now. Post-menopausal women are the main risk group for these adverse effects arising from the consumption of isoflavone-containing products made from soy and red clover. For the time being, BfR therefore advises against the long-term intake of these products given the unproven positive effects and the serious health consequences for meno-pausal women which cannot be ruled out. Bearing in mind the special health sensitivity of this consumer group, BfR comes to the conclusion that the safety of products containing iso-lated isoflavones on a soy or red clover basis, which are placed on the market as food sup-plements or foods for special medical purposes, has not been sufficiently proven. In addition, BfR concludes that there are health risks with low probability from food supplements of this kind for women during and after menopause.

3 Reasons

3.1 Risk assessment

Risk assessment encompasses both the notified adverse effects of soy/red clover-containing products, the biological effects of the isoflavones contained in them, and their toxicity with regard to the food safety requirements applicable to food supplements.

3.1.1 Ingredients

Isoflavones are the main ingredients of soy or red clover-containing products. They belong to the family of phyto-estrogens which, in their capacity as bioactive plant substances, have a similar receptor-relevant subspace to estradiol. This means that they bind to various estro-gen receptors and may trigger estrogen action on the human organism.

Isoflavones made from soybeans (*Glycine hispida max*) are mixtures mainly of daidzein, gen-istein and glycitein or their glycosides daidzin, genistin and glycitin (= binding to sugar mole-cule). Soybeans deliver the isoflavones genistein, daidzein and glycitein roughly on a ratio of 10:8:1 (Wolters/Hahn, 2004). Whole soybeans and non-fermented soy foods mainly contain isoflavones as sugar conjugates in the glycoside form. The glycosylated isoflavones are less active than the free isoflavones (aglucones). Depending on processing the ratio of free to glycosylated may vary. For instance, fermented soy foods (e.g. tempeh) have a higher share of isoflavone aglucones than non-fermented ones like, for instance, tofu (Wang and Murphy, 1994).

Red clover (*Trifolium pratense*) contains many compounds with an isoflavone structure. The main components are formononetin and biochanin A, which is the 4'-methyl ether of daidzein and genistein. Furthermore genistein, daidzein and glycitein as well as the isoflavones irilon, prunetin, pratensein, pseudobaptigenin, calycosin and orobol can also be detected in red clover (Wu *et al.*, 2003).

Isoflavone-containing red clover or soy extracts are not nutrients. It should, therefore, be examined whether isoflavones can be deemed to be other substances with a specific nutritional or other physiological effect subject to the precondition that they can be classified as safe for consumption as foods.

3.1.2 Hazard potential (observed adverse effects)

The hazard potential of isoflavones and their main metabolites is linked to the fact that they can interfere with the hormonal balance which, depending on the dose, can have positive but also adverse effects (Osoki and Kennelly, 2003; Setchell and Cassidy, 1999). Besides the short-term, observed adverse effects, BfR also examined the possible long-term carcinogenic and goitrogenic effects on the thyroid gland. The effects of intakes which are to be expected from food supplements or foods for special medical purposes, must be examined on a case-by-case basis (SKLM, 2005).

Pharmacies reported various products in connection with the observed adverse effects. Isoflavones made from soy or red clover extracts were listed as ingredients on the packaging. The attached reports on side effects from 2003-2005 indicate that the listed adverse effects disappeared in all female patients once they stopped taking them and that none of these products had been prescribed by a doctor.

BfR had already received reports of various adverse effects to soy-containing/red clover-containing products which were placed on the market as foods in the Federal Republic of Germany. Between 2002 and 15 September 2006 a total of 21 reports were received. These were "spontaneous reports" for most of which there were no medical examinations or findings. Some of these reports will probably correlate with the cases of adverse effects mentioned by BVL.

Aside from one case involving soy milk, all the other products consumed are described as food supplements containing isolated fortified isoflavones in which the original food structure is no longer present. The reported adverse effects of soy-containing/red clover-containing products occurred under normal use, involve non-uniform pictures of the complaints and point to allergic reactions, flushing syndrome and/or other causes. They may also point to the basic symptoms of the menopausal complaints which have to be treated.

The adverse effects are mainly described as minor and, in some cases, as moderate. They are: rhinitis, skin tension, swelling not described in more detail, allergies, local oedema, reddening skin/mucosa; itching, flushing, exanthema, swelling skin/mucosa, eczema, myalgia, nausea, dizziness, urticaria, high systolic and diastolic blood pressure, respiratory distress, tingling, abdominal pain, pain not described in more detail, skin rash, tachycardia, circulatory complaints, dizziness, tachypnoae, fertility disorders, exhaustion, coughing, sputum, agitation, indisposition, sweating, anxiety, thorax pain, paraesthesia, precordial pain.

3.1.3 Exposure (sources, incidences)

Soybeans contain approximately 1-3 mg isoflavones per gram raw material, i.e. approximately 0.1-0.3%. Traditional soy-containing foods supply approximately 30 mg isoflavones per portion. The weight of sugar in the glycoside is almost as high as that of the isoflavone itself (around 40% of the total weight of the glycoside). Hence, the actual isoflavone content is correspondingly lower than the content of the isoflavone glycoside. Soybeans have isoflavone levels of 0.2-1.4 mg/g dry weight (Head et al., 1996).

In Asian countries fermented soy products like tempeh, miso or natto are part of a traditional diet. This leads to a daily isoflavone intake of around 15-50 mg, mainly as aglucones (Munro *et al*., 2003; Setchell *et al*., 2001; Messina, 1995). By contrast, in western industrial countries soy products are not conventional foods which means that on average there is a daily intake of less than 2 mg isoflavones (Cassidy, 2005; Kroll *et al*., 2004; Munro *et al*., 2003).

The daily intake of isolated isoflavones from soybeans via food supplements was 40, 50 and 80 mg; in one case the daily intake included an additional 250 mg red clover extract.

3.1.4 Bioavailability, metabolism and excretion

After oral intake free isoflavones (aglucones) appear to be absorbed more quickly than the glycosylated forms which must first be cleaved in the lower small intestine by beta glucosidses. The bioavailability of free isoflavones seems, however, to be slightly lower than of glycosylated isoflavones (Setchell *et al*., 2001). Scientific opinions in the literature disagree about the extent to which the bioavailability of glycoside and aglucone differ and this is the subject matter of further studies (Kroll *et al*., 2004). The level of absorption is said to be around 20-55% (Wolters and Hahn, 2004). Examination of the bioavailability of isoflavones in isolated form as capsules and intake as a soy drink made of soy flour and water revealed comparable results for genistein from both isoflavone sources whereas daidzein had significantly lower bioavailability from the soy drink than from the isolated capsule form (Anupongsanugool *et al*., 2005). In the liver the isoflavones are coupled to glucuronic acid or sulphate. Excretion is mainly renal, a lower proportion via the biliary route. Similar to endogenous estrogens, the enterohepatic circulation of isoflavones excreted in bile is possible. Although this may vary considerably from individual to individual and depending on the colon flora, further metabolisation of non-resorbed isoflavones may take place in the small intestine. For instance daidzein may be metabolised into O-desmethylangolensin or equol or genistein into 6'-hydroxy-O-desmethylangolensin and into other metabolites (Joannou *et al*., 1995; Winter, 1989; Wolters and Hahn, 2004). Furthermore, isoflavones are also substrates for cytochrome-P450-dependent monooxygenases which are mainly localised in the liver. Hence genistein and daidzein are metabolised *in vitro* into a large number of hydroxylated derivatives which in some cases could also be detected in urine following soy consumption (Kulling and Watzl, 2003).

The bioavailability of isoflavones, measured as percentage excretion in faeces and urine, varies from individual to individual and is dependant on various variables like gender, processing of the food, food matrix, intestinal flora, passage time and type and amount of dietary fibre ingested (Adlercreutz, 1990; Morton *et al*., 1994; Tew *et al*., 1996; Xu *et al*., 1995; Przyrembel, 1998; Rowland *et al*., 2003; Munro *et al*., 2003). Equol has a relatively higher estrogen potency than its precursor daidzein. However, not everyone is capable of producing equol and quantitative data on the rate of the equol producers differ (Setchell *et al*., 2001). More recent studies indicate that around 30% of adults are capable of the intestinal conversion of daidzein into equol (Morton *et al*. 1994; Cassidy *et al*., 2006).

In the case of Asians on a traditional diet, i.e. regular consumption of soy-containing foods, concentrations of total isoflavonoids (sum of daidzein, genistein and equol) were measured in the plasma of 870 nmol/l (nanomol per litre) whereas in Europeans because of the lower intakes of isoflavones (< 2 mg/day) on average only approximately 10 nmol/l were found (Adlercreutz *et al*., 1993). Morton *et al*. compared the serum values of a total of 400 people over the age of 40 from Japan and the United Kingdom. The mean values for daidzein and genistein in Japanese men were 282.5 nmol/l and 492.7 nmol/l respectively. Japanese women had very similar values of 246.8 nmol/l and 501.9 nmol/l. In comparison the mean

values for daidzein and genistein in people from the United Kingdom were 17.9 nmol/l and 33.2 nmol/l for men and 12.5 nmol/l and 27.7 nmol/l for women (Morton *et al*., 2002).

3.1.5   Biological effects and toxicity

Isoflavones can trigger various biological effects which are said to contribute to the health advantages of soy-containing foods (Knight and Eden, 1996). The various metabolites of genistein and daidzein differ considerably from one another both in terms of their efficacy and their mechanism of action. Even small differences in chemical structure, for instance an additional hydroxyl or methoxy group or the lack of a covalent bond, can have a major impact on the biological activity of the compounds. The list of biological effects of isoflavones is long and encompasses hormonal and non-hormonal effects. The most well examined is the effect of phyto-estrogens induced by the estrogen receptors. In the body the two ERα and ERβ are distributed differently. In some cases, the data in the scientific literature vary regarding the distrubution of the sub-types of the estrogen receptor system in the individual tissues. There are reports of the receptor binding affinity of isoflavones to both ERα, and ERβ although,a higher binding affinity to ERβ is assumed (Kulling and Watzl, 2003; NAMS, 2000; NAMS, 2004; Gruber *et al.*, 2002; Allred *et al.,* 2004; Harris, 2005).

Regarding the use of isoflavones in food supplements and foods for special medical purposes and the target group of women with menopausal complaints, in the present paper only the biological effects because of their estrogen impact on menopausal complaints should, however, be examined as an alternative to hormone replacement therapy along with the potential anti-carcinogenic and pro-carcinogenic effects of isoflavones on the female mammary glands, uterus and possible adverse effects on the thyroid gland.

3.1.5.1 Effects on women during menopause

Because of their assumed estrogen-agonistic or estrogen-antagonistic effects, phyto-estrogens are said to have numerous tissue-specific effects, probably dependent on the individual amount of endogenous estrogens and the number and type of estrogen receptors. Their affinity to the estrogen receptor is estimated to be between 1,000 and 10,000 times lower than that of estradiol (Przyrembel, 1998; Wolters and Hahn, 2004). Through interaction with the estrogen receptors, isoflavones can imitate or block the physiological effect of the endogenous steroid hormone. Compared with 17ß-estradiol (natural estrogen), their estrogenic effect is – on the one hand - at least 100 times lower. On the other hand, isoflavones in the body may be present in a concentration which is between 100 and 10,000 times higher than the endogenous estrogens. Depending on the level of the endogenous estradiol, phyto-estrogens may have an estrogenic as well as an anti-estrogenic effect. Compared to soy the red clover extract demonstrated a 50 times higher affinity to the estrogen receptor (Beck *et al*., 2003).

Because of the discussions about hormone replacement therapy in menopausal women and the possible side effects of estrogens, interest in alternative treatment methods has grown. This includes isoflavone-containing products which are placed on the market as food supplements or foods for specific medical purposes. On the product packaging, in the enclosed product information or corresponding advertising, isoflavones and, by extension, the corresponding products are frequently described as efficacious, side effect-free natural substances or natural substance products for menopausal complaints or are described as having advantageous health effects for heart, bones and breasts although the claimed effects have not been sufficiently proven. BfR has, therefore, assessed a series of current overviews and meta-analyses from the efficacy angle.

According to a review by Cassidy, 2005, no adverse effects were documented in various, published, clinical trials lasting between one and six months with an intake of between 3 mg and 131 mg isoflavones per day (as aglucone equivalents). However, this study does make reference to substantiated assumptions that isoflavones could have potentially genotoxic and carcinogenic effects and that extrapolations to the human situation only permitted unreliable interpretations. No clear proof was provided of the clinical efficacy of isolated soy isoflavones in conjunction with hot flushes as symptoms of menopause. The question about the possible effects of isoflavones on various organs or organ systems was examined. The author came to the conclusion, "There is a great need for long-term prospective studies and clinical trials to derive empirical proof of the efficacy and safety of isoflavones and to fully explore their potential role in preventative medicine" (Cassidy, 2005).

In a review by Krebs *et al*., 2004, 25 published comparative clinical trials from between 1966 and 2004 were examined with a total of 2,348 participants with menopausal complaints. In studies in which soy-rich foods were examined, the total daily intake of isoflavones was an estimated 34-134 mg. No favourable effect was observed compared to the control group. In studies in which isolated soy isoflavones from various manufacturers were examined in capsule or tablet form against placebo, the daily dose was indicated as 50-150 mg isoflavones. The results were not consistent. In studies in which isoflavones isolated from red clover were examined, there were no statistically significant more favourable effects on the menopausal symptoms compared with the placebo overall. The reported side effects included gastrointestinal complaints, unpleasant taste of the supplements and in one study vaginal spotting. The authors came to the conclusion: "The available evidence suggests that phytoestrogens available as soy foods, soy extracts, and red clover extracts do not improve hot flushes or other menopausal symptoms" (Krebs *et al*., 2004). The authors pick out the study by Tice *et al*., 2003 as being of relatively good quality. Tice and his staff examined isoflavones isolated from red clover in a double blind, placebo-controlled 12-week study involving 252 women with menopausal complaints. Comparable effects were observed in the three groups examined: "Promensil", 82 mg total isoflavones per day, "Rimostil", 57 mg total isoflavones per day and placebo (reduction in hot flushes of between 34% and 41% in the course of the 12 weeks). The authors concluded, "Although the study provides some evidence for a biological effect of Promensil, neither supplement had a clinically significant effect on hot flashes or other menopausal symptoms when compared with placebo" (Tice *et al*., 2003).

In another review from 2003/2004 the authors came to the conclusion that there are some signs of favourable effects of soy isoflavones on vasomotor symptoms in conjunction with the menopause but that because of the heterogeneity of the observed studies no clear statement is possible, "There is some evidence for the efficacy of soy preparations for perimenopausal symptoms. However, the heterogeneity of the studies performed to date means it is difficult to make a definitive statement". Ten randomised, controlled clinical studies from 1995-2002 with soy or isolated soy isoflavones as monotherapy were analysed, not as part of a diet rich in phyto-estrogens. The target criterion was vasomotor symptoms like "hot flushes" or "perimenopausal symptoms". A daily dose of between 34 mg and 134 mg isoflavones was administered. The studies lasted between six and 24 weeks. The main side effects observed were gastrointestinal complaints like nausea, constipation, unpleasant taste, bloatedness as well as allergic reactions to soy. The authors stress that isoflavones do not appear to have any serious side effects in conjunction with short-term use but that risks in conjunction with long-term administration have not yet been examined (Huntley and Ernst, 2004).

The North American Menopause Society (NAMS) concludes in its position paper that the current data situation on soy isoflavones is not sufficient either to advocate their use to treat

mild vasomotor symptoms ("mild hot flashes") or to refute them ("... clinical trial results are insufficient to either support or refute efficacy for soy foods and isoflavone supplements (from either soy or red clover), ... however, no serious side effects have been associated with short-term use of these therapies" (NAMS, 2004). In the 14 controlled clinical trials involving women with vasomotor symptoms in conjunction with the menopause assessed by NAMS, preparations with daily doses of 40-80 mg isoflavones were mainly used. It is stressed that nothing is known about the long-term effects of isolated isoflavones or foods fortified with isoflavones. Nor are there any findings about whether it is safe for women with a (known) mammary carcinoma to use them or whether there is a risk.

The American Heart Association Nutrition Committee comes to the conclusion in its current assessment from 2006 that soy isoflavones were not able to convincingly demonstrate a significantly favourable effect on vasomotor symptoms in conjunction with menopause. In the more than 20 clinical trials assessed, the recognisable improvements in symptoms both in the placebo and control groups as well as in the isoflavone groups over time were frequently between 40% and 60%. Because of the unclarified risks and inadequate data, the Association advises against the consumption of food supplements with isolated isoflavones and foods fortified with isoflavones. The Association stresses that conventional soy-containing foods can generally have a favourable impact on health because they often contain higher amounts of polyunsaturated fatty acids, dietary fibre, vitamins and minerals as well as a lower proportion of saturated fatty acids (Sacks *et al*., 2006).

Nelson *et al*., published a review combined with a meta-analysis of placebo-controlled clinical trials with isoflavones made from red clover extracts (six studies from 1999-2004; daily dose 40-80 mg isoflavones; duration between 12 weeks and 12 months; approximately 600 women with vasomotor complaints like "hot flushes"; all six trials were included in the meta-analysis) and isoflavones from soy extracts (11 trials from 2000-2005, six from 2000-2004 were included in the meta-analysis; daily dose 54-600 mg; duration between four weeks and one year; approximately 600 women with vasomotor symptoms like hot flushes). The authors described the results as follows, "The trials do not support the efficacy of red clover isoflavone extracts and present mixed results for soy isoflavone extracts. ... Hot flash frequency was not reduced when all trials of red clover isoflavone extracts were combined, and results for soy isoflavone extracts were contradictory even among the largest and highest quality trials. These results are consistent with other recent systematic reviews. We also reviewed trials of other forms of soy isoflavones, such as flour, powder, and food items, in another study. However, these trials are difficult to compare because of variability of components and doses. Overall, evidence does not support benefit in relieving hot flashes" (Nelson *et al*., 2006; Tice and Grady, 2006).

The therapy recommendations of the Medicinal Products Committee of German Doctors (Arzneimittelkommission der Deutschen Ärzteschaft) "Hormonal therapy during menopause" 2003, adopts a position on the use of isoflavone extracts, including red clover extracts, soy and soy extracts and comes to the conclusion that preparations of this kind should not be seen as an alternative to estrogen therapy "not just because of the inconsistent study situation but also until sufficient data become available on the long-term efficacy and safety of these products some of which are also commercially available in Germany" (AVP, 2003).

3.1.5.2 Adverse effects and toxicity

3.1.5.2.1   Carcinogenic effects

Global cancer statistics indicate that hormone-dependent cancer diseases like cancer of the breast and prostate occur far less frequently amongst the Asian population for whom soy is of greater importance in their diet than in western countries (Pisani *et al*., 1999). For that reasons various studies examined the effects of soy protein and isoflavones on the development of breast cancer in fully grown adult animals. The data obtained are not completely unequivocal but do generally show that the addition of soy or isoflavones to a standard diet does not markedly reduce the tumour incidence as a rule but does reduce in most cases the amount of tumours (the number of tumours in individual animals) by between 25-50% (Hakkak *et al*., 2000; Zaizen *et al*., 2000; Gotoh *et al*., 1998; Barnes *et al*., 1990). By contrast, the 18 epidemiological studies (12 case studies and six prospective cohort studies) conducted in Asia did not always back the assumption that the consumption of soy by adult Asian women reduces the risk of breast cancer after menopause. The results of the meta-analysis must be interpreted cautiously as the exposure data were referred to "gram soy protein", there is no dose-dependency and the reduction in the breast cancer risk observed in a few studies cannot be attributed to soy intake as the sole factor but to so-called confounders when evaluating this data (Martinez *et al*., 2006; Trock *et al*., 2000; 2006).

According to more recent findings the improved iodine supply of the Asian population compared with the female population in Europe is seen as one such confounder which may explain the lower incidence of breast cancer amongst Asian women. Asian and, above all, Japanese women have a daily intake of around 1 mg iodine mainly from their higher consumption of marine fish and iodine-rich seaweed consumption (Venturi, 2001). Women who develop nodular goitre as a consequence of chronic iodine deficiency have a higher incidence of breast cancer (Turken *et al*., 2003). Iodine lactones, which inhibit the growth of thyroid cells, are also formed in mammary gland cells and thereby inhibit their proliferation or induce apoptosis. When given an iodine-deficient diet young rats develop ductal hyperplasia and perilobular fibrosis of the mammary gland (mastopathy) which can be prevented by sufficient iodine intake (Eskin *et al*., 1995). Animal experiments showed that 5% seaweed in feed can significantly delay the onset of chemically induced mammary carcinoma (Funahashi *et al*., 1999; 2001). The administration of iodine to women with a mastopathy significantly relieved the symptoms (Ghent *et al*., 1993). Improved iodine supply leads to a higher synthesis of iodine lactones in the mammary gland cells which not only reduces the incidence of benign mastopathy but also of breast cancer (Smyth, 2003; Venturi, 2001).

Animal data on the chemopreventive effect of soy isoflavones on breast cancer do not produce a clear picture. In animal experiments (female rats) the occurrence and the growth rate of chemically induced tumours could be considerably reduced when soy or the isolated soy isoflavone genistein was administered prior to the onset of puberty. However, if this substance was not administered until adult age, there was no longer any protective effect (Lamartiniere *et al*., 2002). This could be one possible explanation for the fact that in the epidemiological studies which assessed soy consumption in adults, no effect was observed on the growth of breast cancer.

In other animal experiments (mice with implanted human breast cancer cells) the administration of genistein or a soy protein isolate provoked an accelerated growth of these tumour cells. This experiment was confirmed by studies in cell cultures (Ju *et al*., 2001; 2006). The plasma concentrations reached in these animal experiments with isoflavones of 1-2 μmol/l were in a concentration range which could indeed be achieved through taking food supplements. The effect was recently confirmed by another working group (Power *et al*., 2006; Saarinen *et al*., 2006).

The exposure of rats during pregnancy led in their offspring to a dose-dependent increase in the post-natal induced breast cancer rate (Hilakivi-Clarke *et al*., 1999) caused by the carcinogen DMBA (7,12-dimethyl-benz(a)anthracene). Furthermore, the administration of genistein or a genistein-rich soy extract promoted the induction of precursor tumour stages in the intestines of rats by the carcinogen 1,2-dimethyl hydrazine (Gee *et al*., 2000).

Other animal experiments and *in-vitro* studies showed that low concentrations of isoflavones, like the ones measured in human plasma after consumption of soy products, stimulate the growth of tumour cells whereas the desired growth inhibition was only achieved at high concentrations (Dampier *et al*., 2001; Hsieh *et al*., 1998; Hsu *et al*., 1999). In healthy women with a reduced secretion of hormones from the ovaries, plasma concentrations of 3 and 11 µmol/l of genistein and daidzein respectively were measured after consuming a soy-containing diet (Lu *et al*., 2000). In other healthy volunteers, too, only plasma concentrations < 4 µmol/l were measured after taking large single doses of these two compounds (0.6-0.9 mg/kg body weight) (King and Bursill, 1998). These concentrations can be compared rather with the low plasma concentrations at which *in vivo* stimulation of tumour growth was observed (Zava and Duwe, 1997) than with the high concentrations *in vitro* associated with inhibition of tumour growth (Nakagawa *et al*., 2000; Santell *et al*., 2000; Dees *et al*., 1997; Sathyamoorthy *et al*., 1997; Wang and Kurzer, 1997; Sathyamoorthy and Wang, 1994).

In the literature there are isolated studies on the interaction between isoflavones and tamoxifen. In one animal experiment with ovariectomised athymic mice, into which MCF-7 cells were implanted, tamoxifen inhibited the estradiol-induced proliferation of tumour cells. This effect was negated by the parallel administration of feed fortified with genistein (Ju *et al*., 2002). In a further study with transgenic mice (wild type erbB-2/new) treatment with tamoxifen prevented the development of tumours. This effect was also negated by a low dose of isoflavone fortified feed (Liu *et al*., 2005).

Discussions about the safety assessment of isoflavones look at whether the time of phyto-estrogen exposure in specific development stages of man, for instance in childhood or in puberty, could be a decisive factor when it comes to the question of favourable or adverse effects. The question about any pro-carcinogenic effects of isoflavones cannot be answered definitively at the present time either (Wolters and Hahn, 2004; Sacks *et al*., 2006; Wuttke *et al*., 2002). The Medicinal Products Committee of German Doctors refers in this context to the possible stimulating effects of phyto-estrogenic soy components on the cell proliferation of normal breast tissue and mammary tumours (AVP, 2003; Petrakis *et al*., 1996, McMichael-Phillips *et al*., 1998, De Lemos, 2001; Hargreaves *et al*., 1999; Wuttke *et al*., 2002). Other authors also refer to toxicological studies that provided some indication of a possible unfavourable impact of isoflavones on the proliferation of mammary gland tissue and endometrium (Kroll *et al*., 2004).

Several studies have examined the effects of soy consumption on cell proliferation or of biomarkers on the cell proliferation of mammary gland tissue in women. Hargreaves *et al*., (1999) examined the effect of the 14-day administration of 60 g soy (48 mg total isoflavones) per day on the mammary gland tissue of premenopausal women. Whereas no effects could be observed on the estrogen receptor status, proliferation, apoptosis (programmed cell death) or mitosis of the epithelial mammary gland cells, the concentrations of apolipoprotein D fell significantly and the expression of the gene pS2, which responds significantly to estrogen, increased. This is a sign of a weak estrogen effect. In another randomised clinical trial women (n=48) with benign and malignant breast diseases were given a soy protein-containing food supplement (45 mg isoflavones) daily for a period of 14 days. In biopsy material with the help of the labelled [3H]-thymidine and immunohistochemically labelled prolifera-

tion antigen Ki67, the impact was examined on the proliferation rate of premenopausal, histologically normal mammary gland epithelium and the expression of the progesterone receptor. Soy supplementation led to a significant increase in both the proliferation rate and expression of the progesterone receptor after 14 days. In the opinion of the authors further long-term studies are needed in order to be able to rule out any adverse health effects (McMichael-Phillips *et al.*, 1998).

On the other hand in one study with 23-42 year old women with a regular cycle (n = 10) who were given daily approximately 1 litre soy milk from homogenised whole soybeans with on average 154 mg total isoflavones daily over a period of one month, a drop was observed of 25% in the values circulating in the blood for 17-beta-estradiol and a drop of 45% in the values for progesterone compared with the values measured in conjunction with a normal diet in previous cycles. The soy milk did not manifest any effect on the level of the values for the hormones LH and FSH. Furthermore, under the soy diet the mean cycle length was also unchanged (Lu *et al.*, 2000). The plasma values for daidzein and genistein were 2.89 µg/ml and 0.85 µg/ml respectively equivalent to 11.3 µmol/l and 3.1 µmol/l respectively. From the study the authors concluded that various components of a soy diet – including isoflavones – are capable of directly modulating ovarial hormones without influencing gonadotropins and deduced from this implications for the prevention of breast cancer.

148 women, who were given soy for a period of three to four months during infancy, reported significantly longer menstruation, more severe menstruation complaints and more frequent use of anti-asthmatic and anti-allergic medicines (Strom *et. al.*, 2001). Because the importance of these findings is unclear and because infants fed on soy can reach very high plasma levels of isoflavones (1-4 µmol/l) (Setchell *et al.*, 1998) whose long-term effects cannot be estimated, European, German and Swiss associations for paediatrics and youth medicine recently recommended that soy infant formula should only be used in the case of special indications (Agostoni *et al.*, 2006; Böhles *et al.*, 2006).

In a long-term study the influence of isolated phyto-oestrogens was examined on the endometrium (Unfer *et al.*, 2004). The randomised, double blind, placebo-controlled clinical trial in women during and after menopause observed histologically stimulating effects of isolated soy isoflavones on the endometrium. The verum group (n=179) was given three tablets daily with 50 mg soy isoflavones (150 mg daily dose containing 40-45% genistein, 40-45% daidzein and 10-20% glycitein) for five years whereas the control group (n=197) was given the placebo. Endometrial biopsies were conducted at the beginning of the trial, after 30 months and after five years treatment. 298 women who had received treatment over five years reached the end of the trial. Whereas after 30 months in both groups no hyperplasias were observed in the endometrium, hyperplasias of this nature were observed after five years in six women (3.4%) in the verum group but not in the placebo group. This difference between the treatment groups was statistically significant. Carcinomas were not observed. The study provides some indication of adverse estrogen-like effects on the endometrium in humans following the long-term intake of isolated soy isoflavones. As a consequence the authors of this study have questioned the safety of isoflavones when administered for long periods in conjunction with an increase in endometrium hyperplasias in women during and after menopause (Unfer *et al.*, 2004). Experimental results in rats point in the same direction. Coumestrol, a phyto-oestrogen in the family of isoflavonoids, accelerated growth of the uterus mucosa induced by steroidal estrogen (Whitten *et al.*, 1995).

3.1.5.2.2   Allergenicity of soy

Depending on their manufacture, isolates from soybeans contain different amounts of soy protein which is one of the most well known food allergens. No quantitative data are, however, available. The incidence of a soy allergy is estimated to be 0.3-0.4% of the total population. Foods, to which soy or soy products have been added, must be labelled pursuant to European food law (EFSA, 2004). A threshold dose for the triggering of symptoms in patients allergic to soy cannot be indicated although in the case of immediate IgE-mediated allergies, doses of less than one milligram allergen or 500 mg of soy-containing foods and beverages were sufficient (Binslev-Jensen *et al.*, 2002; Sicherer *et al.*, 2000). There is no information on doses for delayed non-IgE-mediated allergic reactions to soy with clinical symptoms, mostly in the gastrointestinal tract.

Furthermore, it was observed that people with a birch pollen allergy may also have a cross-allergy to soy protein. A specific stress protein from soybeans, which has since been given the name Gly m 4, has a sequence homology of more than 50% with the birch pollen allergen Bet v 1. Hence patients with a birch pollen allergy can manifest immediate allergic reaction after eating soy protein-containing foods (Kleine-Tebbe *et al.*, 2002; Mittag *et al.*, 2004; Süß *et al.*, 2005). The patients concerned who consume soy may suffer symptoms similar to oral allergy syndrome (OAS) such as itching, papules, blisters around the mouth, swollen lips, glottic oedema, dysphagia and dyspnoea. The course of AOS may vary considerably: from mild which are only subjectively unpleasant over rare courses which spread to other organs down to cardiovascular symptoms which can lead, amongst other things, to anaphylactic shock.

3.1.5.2.3   Goitrogenic effects on the thyroid gland

Genistein and daidzein can inhibit the activity of thyroid gland peroxidase, which is essential for the synthesis of thyroid hormones, at concentrations of 1-10 µmol/l *in vitro*. This inhibition is reversible in the presence of iodine (Divi *et al.*, 1997). The half-maximal inhibition (IC_{50}) of thyroidal peroxidase is already reached at a concentration of 1 µm genistein. It is not yet clear to what extent these data obtained from *in vitro* studies are of physiological relevance (Doerge and Sheehan, 2002).

As observed by the DFG Senate Commission on the health assessment of foods (SKLM, 2006), a second point of attack of isoflavones in thyroid hormone metabolisation is inhibition of the sulphotransferase enzymes involved in the inactivation and elimination of thyroid hormones and local recovery of iodine in the human thyroid gland (Ebmeier and Anderson, 2004). A third point of attack of isoflavones in the thyroid hormone axis is transthyretin (TTR, formerly thyroxin-binding prealbumin, TBPA). In serum TTR binds up to 20% of thyroxin (T_4), is involved in the distribution of T_4 in the body and in preventing T_4 excretion in the kidneys. TTR is the most important thyroid hormone-binding protein in cerebral spinal fluid (CSF). In serum and CSF, genistein and related isoflavones are highly efficacious inhibitors of T_4 and T_3 binding to TTR (K_d = 40 nmol/l, equimolar to T_4 binding). They alter the distribution of thyroid hormones in the body (Green *et al.*, 2005; Radovic *et al.*, 2006).

3.1.6   Risk characterisation

3.1.6.1   Adverse effects on hormone balance

The potential hazards of isoflavone-containing products lie above all in their stimulating effect on the growth of breast cancer and the negation of the tumour-inhibiting effect of tamoxifen as an estrogen blocker during the treatment of estrogen-dependent tumours. This is backed

by findings from *in vitro* and animal studies that low concentrations of genistein and daidzein (< 10 µmol/l) promote tumour growth, whereas high concentrations of genistein (> 10 µmol/l) inhibit the growth of breast cancer and reinforce the tumour-inhibiting effect of tamoxifen (Ju *et al*., 2002; Dampier *et al*., 2001; Hsieh *et al*., 1998; De Lemos, 2001; Dees *et al*., 1997; Nakagawa *et al*., 1997; Wang and Kurzer, 1997; Zava and Duwe, 1997; Sathyamoorthy *et al*., 1997; Sathyamoorthy and Wang, 1994). The concentration-dependent biphase effect would seem to indicate that the safety of phyto-estrogens depends on the amount ingested. It is not, however, clear whether the concentrations used *in vitro* can be equated with the concentrations reached *in vivo*. Particularly in the case of women with an elevated risk of breast cancer or an existing mammary carcinoma, the pre-carcinogenic effects of phyto-estrogens could carry more weight (Wolters and Hahn, 2004; Petrakis *et al*., 1996; McMichael-Phillips *et al*., 1998; de Lemos, 2001).

The increase in endometrium hyperplasias in women during and after menopause can be considered a negative effect of isoflavones whereby the risk of neoplasias increases (Unfer *et al*., 2004; Johnson *et al*., 2001).

Here it should be borne in mind that in contrast to a specifically timed, clinically controlled study, possible risk groups could ingest these substances from food supplements for years without any medical supervision. There are no long-term studies which confirm the safety of isoflavone products. Therefore, it is not possible to make any statements about the dose which could still be deemed safe. Hence BfR is of the opinion that women with an estrogen receptor positive mammary carcinoma or endometrium carcinoma or who are at greater risk of these diseases should not use these products. Since women during or after menopause are not aware of this risk, BfR generally advises against the consumption of food supplements based on isoflavones.

Although it is difficult to assess the potential of goitrogenic effects of isoflavones on the thyroid gland, women with increasing age run a higher risk of developing sub-clinical thyroid underfunction. Given the present data situation, it cannot therefore be ruled out that this risk is elevated by taking isoflavone products (SKLM, 2006).

3.1.6.2 Observed adverse effects

The observed intolerance reactions would seem to indicate that the proportion of soy protein in the food supplements is probably responsible for the allergic reaction. The prevalence of food-related allergies in the general population is estimated to be around 1-3% of adults and 4-6% of children (EFSA, 2004). A clinical detection of an existing hypersensitivity reaction to soy was not verified in the reported cases. Overall an assessment of these adverse effects is not possible on the basis of the available indications. Furthermore, no causal relationship can be established particularly as in most cases no medical findings were available.

3.2 Discussion

It is important to distinguish between the intake of phyto-estrogens from food and in isolated form. Phyto-estrogens seem to be able to influence a number of functions in the human body. As they bind to the same receptors as endogenous estrogens, albeit with a far lower hormonal impact, they could theoretically have a favourable impact on carcinogenesis, particularly on breast and prostate cancer and, under certain circumstances, on cardiovascular diseases and osteoporosis. The current level of knowledge about the effects of phyto-estrogens and a number of other bioactive plant substances is not, however, sufficient in order to be able to give intake recommendations for specific individual substances. In popu-

lation studies the consumption of soy products was linked to various health advantages. Hence a diet which is rich in soy products, cereals, vegetables and fruit is recommended. German, Austrian and Swiss nutrition societies advise against taking food supplements containing concentrated bioactive plant substances as it is not known which bioactive plant substances or which amounts of them have a preventive effect (D-A-CH, 2000; Grossklaus, 2000).

Some patients switch to phyto-estrogens because of the adverse effects of hormone replacement therapy (HRT) during menopause. The target group of isoflavone products is mainly women close to / after menopause who are looking for an alternative to hormone replacement therapy. Vasomotor symptoms like hot flushes and sweating in particular are to be relieved. Information that Japanese women suffered less frequently from menopausal complaints than Europeans led to the hypothesis that the high soy consumption in Japan and the related lifelong higher intake of soy isoflavones could be contributory factors. It is not yet clear whether there is a causal relationship or whether soy consumption is simply a marker for a healthier lifestyle.

At the present time isolated isoflavones are ingredients in food supplements and foods for special medical purposes which are targeted above all at women close to/after menopause. Most of the studies that examined whether the additional intake of isolated isoflavones could relieve menopausal symptoms did, however, come to the conclusion that given the strong placebo effect and a time-dependent decrease in menopausal complaints, significant and clinically relevant effects were not detectable in most cases. Placebo-controlled studies frequently show that already under placebo the vasomotor menopausal symptoms could be improved by 30-50%. A comparison of verum with the placebo or control did not reveal any significant superiority of isoflavone administration in the vast majority of cases. Overall the positive effect of isolated isoflavones on menopausal complaints has not been sufficiently scientifically substantiated up to now (Davis, 2001; AMB, 2001).

On the other hand in studies with isoflavones from soybean protein or fermented soy protein products, an impact on the endocrine function in humans could already be demonstrated at doses of 50 mg/day and higher which are in the upper range of Asian countries with a high consumption of soybeans and the customary soy foods found there: in menstruating women estrogen-like inhibition effects on the hypothalamus/pituitary gland with prolongation of the follicle phase of the cycle (Cassidy *et al*., 1995; Cassidy *et al*., 1994); prolongation of menstruation (Watanabe *et al*., 2000) and in women during and after menopause signs of estrogenic effects on the vaginal mucosa (Wilcox *et al*., 1990).

Studies showed that the bioavailability of the ingredients in various food supplements containing isolated isoflavones varies considerably (Setchell *et al*., 2001). Furthermore, daidzein in isolated form seems to have higher bioavailability than in the natural matrix as soy flour (Anupongsanugool *et al*., 2005). The exact composition (total isoflavone content, isoflavone spectrum, food matrix) of food supplements is not generally known or standardised. SKLM (2005; 2006) is, therefore, of the opinion that a safety assessment would have to be undertaken for each product. In Canada the addition of bioactive substances to foods is classified as novel foods and must undergo authorisation and examination in each individual case (Lee, 2006). According to Whereas no. 8 of the Food Supplements Ordinance, specific provisions about nutrients other than vitamins and minerals or about other substances with a nutritional or physiological effect, which are used as ingredients in food supplements, should be specified at a later point in time when sufficient, unbiased data are available on these substances. As national provisions may be used until the issuing of special Community provisions of this nature, BfR recommends another management option which would involve

equating these substances pursuant to § 2 para 3 No. 1 sentence 2 LFGB (Food and Feed Code) with food additives.

Up to now no sufficient safety data for humans, particularly on long-term administration, have been available. Relevant toxicological effects cannot be ruled out and indications of adverse effects can be derived from existing studies. Against this backdrop and because of the insufficiently proven efficacy, BfR is of the opinion that non-critical product advertising is problematic because of the possibility of misleading consumers in terms of food law. Depending on origin, raw material and production the commercial preparations containing isolated isoflavones vary considerably as do the total content, the relations of isoflavones to each other, the forms of the different isoflavones used and the recommended daily doses. This means that the comparability of the various preparations but also the comparability of studies with not exactly defined isoflavone products is not possible or only possible to a very limited degree (Erdman *et al.*, 2004).

The negative effects of isoflavones on the uterus mucosa and the risk of endometrium carcinomas must be viewed very critically. Given the safety concerns prompted by the study by Unfer *et al.* (2004), isolated soy isoflavones in the tested dose (150 mg/day) cannot be recommended particularly as there is no clear proof of advantages and the side effects linked to long-term administration cannot be calculated (a-t, 2004; a-t, 2001; a-t, 2002; a-t, 2003). According to Wolters and Hahn "an oral dose of 50 mg isoflavones daily seems to be safe for the majority of the population" and it could be assumed on the basis of the study data that the short-term administration of soy products was not linked to major adverse effects. Nonetheless, the authors do recommend that women with estrogen receptor positive mammary carcinoma, endometrium carcinoma or an elevated risk of these diseases, should not take isolated isoflavones, for instance as food supplements (Wolters and Hahn, 2004).

The probability of adverse health effects, i.e. that the long-term administration of isoflavone-containing food supplements or supplemented foods for medical purposes could increase the risk of breast cancer, is deemed to be high particularly in the risk group of menopausal women. More particularly women taking tamoxifen as medication to prevent breast cancer or undergoing adjuvant or palliative treatment for breast cancer should be informed of this elevated risk (De Lemos, 2001; This *et al.*, 2001).

In the conclusions of their publication Kroll *et al.* give a critical assessment of the uncertain data situation about the effects of isolated isoflavones on humans as follows, "As far as the physiological effects of isoflavones are concerned, a flood of publications emphasise above all their positive effects. These positive effects are derived on the one hand from the fact that in a number of Asian countries soy products have been a traditional and staple part of the human diet for centuries and that in these countries the risk of specific diseases (hormone-dependent types of cancer… osteoporosis) and menopausal symptoms is lower than in western industrial nations. On the other hand, the positive effects of isoflavones described were established first and foremost for isolated compounds, mostly by drawing on model systems, and in every case from the interpretation of nutritional-epidemiological studies. In many cases the results obtained are seen as evaluation criteria for corresponding food supplements. The negative effects which are also described that may occur in conjunction with comparatively high, long-term ingestion of isoflavones from food supplements, are not taken into account at all in this context. As is the case for most bioactive plant substances, the optimum physiological effects are not known for isoflavones either" (Kroll *et al.*, 2004).

In its assessment from 2003 the British COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment) identified people with thyroid underfunction and

women with oestrogen-dependent diseases of the mammary gland as possible risk groups for phyto-estrogen supplementation. COT comes to the conclusion that overall the available data are not sufficient for more precise recommendations. In conjunction with the effects of soy-based isolated isoflavones on menopausal symptoms, "Studies examining the effect of soy-based products or isoflavones to relieve menopausal symptoms are inconclusive. Some studies have suggested that soy may be beneficial, especially if basal intake is low, or the vasomotor symptoms severe, but the data are equivocal, as positive results are often not statistically significant and strong placebo responses are observed" (COT, 2003).

In the comprehensive meta-analysis by Trock *et al.*, (2006) regarding a possible inverse association between the ingestion of soy foods and the risk of breast cancer, the authors conclude that given the existing uncertainties and animal experiment findings about possible adverse health effects of soy ingredients, it is too early to hand down recommendations for highly dosed isoflavone products for breast cancer prevention (Trock *et al.*, 2006).

Given the difficulty of transferring animal data to humans and the problem that only very few clinical studies are available and their statements are inconsistent, a definitive assessment of the effect of isoflavones on the female breast, particularly regarding the breast cancer risk of women at higher risk or the survival time of breast cancer patients, is not possible at present (Messina *et al.*, 2006, Rice and Whitehead, 2006). Data available up to now from prospective studies on the elevated intake of isoflavone-containing foods do not point to any lowering of the breast cancer rate or possible favourable trends are only interpreted with reservations and constraints and are deemed to be provisional (Trock *et al.*, 2006). The available data do, however, permit the conclusion that isoflavones – particular at high doses – can lead to estrogenic stimulus of mammary gland tissue in women and, by extension, that the growth stimulus of pre-malignant or tumour cells through the ingestion of elevated amounts of isoflavone products cannot be ruled out (SKLM, 2006). In its assessment SKLM comes to the conclusion that the safety of isoflavone products on a soy or red clover basis as food supplements and foods for special medical purposes cannot be established from the traditional administration of soy foods (SKLM, 2006). In its opinion "the potential of isoflavones in products of this kind to trigger adverse health effects and the dose-dependency of these effects have not yet been sufficiently examined". SKLM is of the opinion that what is particularly problematic is that "postmenopausal women (women during and after menopause), who are the main target group for these products, are also a special risk group for adverse effects". Given the current data situation it cannot be ruled out that this group which is already at risk will be at even greater risk of an increased development of pre-carcinogenic changes to the breast or the formation of a sub-clinical thyroid underfunction (SKLM, 2006).

4   Management framework/measures

From the nutritional-medical point of view and according to the latest knowledge available, the claimed positive effects of isoflavones on menopausal symptoms have not been sufficiently substantiated. Bearing in mind the special sensitivity of the consumer group of women during and after menopause, the safety of products containing isolated isoflavones on a soy or red clover basis has not been sufficiently proven. In this context any adverse carcinogenic, goitrogenic effects or other adverse effects on the hormone balance are of particular importance. BfR concludes that there are health risks with low probability from food supplements of this kind and from foods for special medical purposes for women during and after menopause. Therefore, BfR recommends there is a need for action, for instance to raise awareness amongst women with breast cancer or a predisposition for this type of cancer.

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