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Isoflavones as phytoestrogens in food supplements and dietary foods for special medical purposes

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Isoflavones with estrogen-like activities are used as ingredients in food supplements and dietary foods for special medical purposes, advertised primarily to women in menopause. The DFG Senate Commission on Food Safety (SKLM) has evaluated the safety of phytoestrogenic isoflavones in food supplements and in supplementary balanced diets together with external experts. Data on their content in foods and food supplements, dietary intake, bioavailability, metabolism, biological effects and toxicity have been considered as well. The German opinion was adopted on 10th November 2006, the English version was accepted on 23rd May 2007.

Isoflavones as phytoestrogens in food supplements and dietary foods for special medical purposes

Preamble

The Senate Commission on Food Safety (SKLM) has addressed issues concerning potential adverse effects caused by polyphenols/flavonoids used in an isolated or enriched form in the past and has adopted a corresponding opinion (SKLM, 2003). In addition, criteria for the evaluation of functional foods (SKLM, 2005) as well as an opinion on the evaluation of food supplements with constituents other than vitamins and minerals (SKLM, 2006) have been published.

Food supplements and dietary foods based on soy or red clover are frequently rich in isoflavones that may have an estrogenic effect. This has prompted the SKLM to reevaluate the safety of this group of substances. Not included in this opinion are soy protein-based infant formulae that are retailed in Germany as “dietary food for special medical purposes”. European, German and Swiss scientific bodies for pediatric medicine have recently published statements on soy protein-based infant formulae that only recommend their use for particular indications since there are hints that adverse effects might occur as a consequence of taking up rather high amounts of isoflavones with such formulae (Agostoni et al., 2006; Böhles et al., 2006).

Soy extracts are marketed as food supplements as well as "dietary foods for special medical purposes", whereas in most cases red clover extracts have hitherto been marketed as food supplements. Depending on the manufacturer, these preparations are freely available in pharmacies, health food shops, supermarkets and/or on the internet and are frequently used for self-medication.

The main target group for isoflavone containing products consists of menopausal women looking for an alternative to hormone replacement therapy. Claims are frequently made on the product packaging, the enclosed product information leaflet or in advertisements, stating that isoflavones and thus the corresponding products by themselves are effective natural substances or preparations without side-effects, which can be used to treat menopausal symptoms or are claimed to be beneficial to heart, bone and breast tissues, although the claimed effects have not been adequately substantiated (Krebs *et al*., 2004). Sporadically, advertisements relating their use to the treatment and prevention of certain types of cancer, such as breast cancer in women and prostate cancer in men, have also appeared.

1. Classification, occurrence and contents

The term phytoestrogens refers to substances produced by plants that may have an estrogenic effect on the human organism. Substances that are classified as phytoestrogens include, in particular, the isoflavones and lignans. At present, lignans are not retailed in an isolated or enriched form in corresponding products and are thus not included in this opinion. Isoflavones belong to the polyphenol group and are present in significant concentrations in certain plants, primarily in soy and foods made from it as well as in red clover (*Trifolium pratense*) (Tab. 1a and b). The main isoflavones present in soy are daidzein, genistein and glycitein (Fig. 1). In contrast, red clover contains numerous isoflavones (Wu *et al*., 2003). The major constituents are formononetin and biochanin A, which differ from daidzein and genistein only in the methoxy group instead of the hydroxy group at C4' (Fig. 1). Red clover has also been found to contain genistein, daidzein and glycitein as well as the isoflavones irilone, prunetin, pratensein, pseudobaptigenin, calycosin and orobol. The chemical structures of these compounds are shown in Fig. 2. These compounds are frequently present in the plant in form of the corresponding glucosides or esterified glucosides (particularly malonyl glucosides, Fig. 1).

Soy-based food supplements and "dietary foods for special medical purposes" contain the biologically active isoflavones genistein, daidzein and glyciteinin differing ratios. The compounds may be present as glucosides or esterified glucosides or even in their free form as aglycons. Because soy extracts are not produced according to standardised production processes, it must be assumed that the preparations also differ with respect to their associated matrix (e.g. further bioactive components, such as lysophosphatides and saponines) (Fang *et al*., 2004). Independently of the raw material and the manufacturing process, the preparations also differ with respect to their formulation and added ingredients.

New preparations recently launched on the market include, in particular, high-dose soy products in a retard or long-term formulation. The isoflavone content in soy preparations is frequently declared by the manufacturer as the sum of the three isoflavones daidzein, genistein and glycitein; in some cases quantitative information on the content of individual isoflavones is also given.

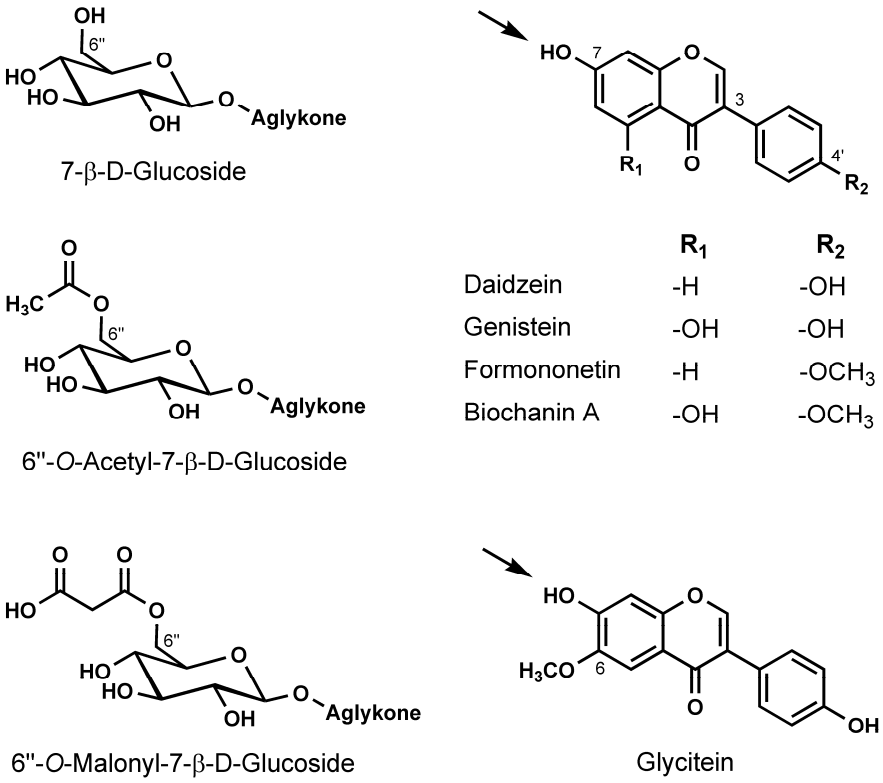

Fig. 1: Chemical structures of isoflavones in soy and red clover and their sugar conjugates. The 7-*O*-ß-D-glucosides of daidzein, genistein and glycitein are known as daidzin, genistin and glycitin. The arrow in the Figure shows the position of sugar conjugation.

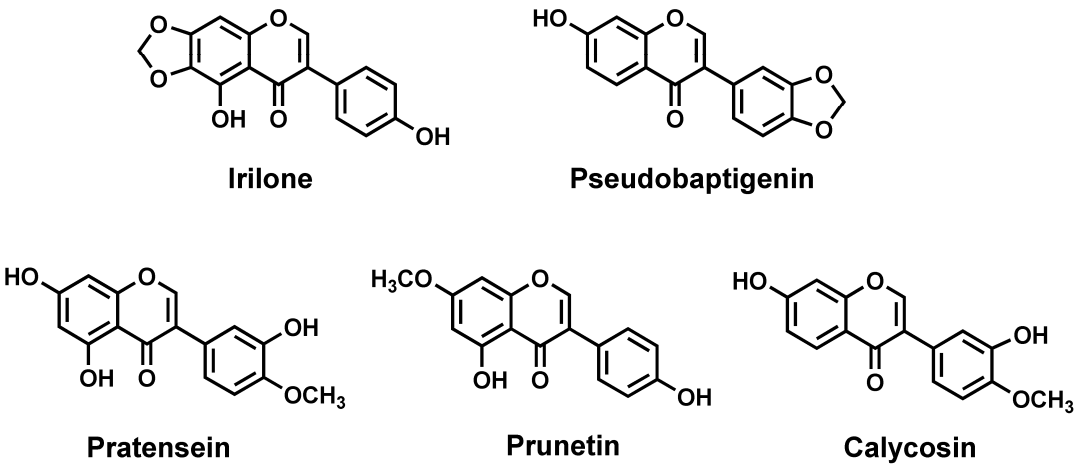

Fig. 2: Chemical structures of further isoflavones present in red clover. The aglycon moiety is shown.

Tab. 1a: Isoflavone contents in foods (µg/g fresh weight)

(Wang und Murphy, 1994 ; Franke et al., 1995 ; Mazur et al., 1998 ; Franke et al., 1999 ; Murphy et al., 1999 ; Horn-Ross *et al*., 2000 ; Liggins *et al*., 2000a; Liggins *et al*., 2000b; Nakamura *et al*., 2000; Rupp *et al*., 2000; USDA, 2002)

Type of Food	Genistein	Daidzein	Glycitein	Biochanin A	Formononetin
Soybeans	335 - 1201	452 - 1138	37 - 145	< 1	< 1
Soy milk [µg/mL]	52 - 168	26 - 126	2 - 16	n.d.	n.d.
Tofu	111 - 304	73 - 191	15 - 39	n.d.	n.d.
Miso	51 - 398	35 - 363	4-53	n.i.	n.i.
Soy oil	n.d. - 3	n.d. - 1	n.d.	n.i.	n.i.
Soy sauce [µg/mL]	n.d. - 3	n.d. - 9	n.d. - 5	n.d.	n.d.
Soy flour	876 - 1155	715 - 1496	306 - 593	< 1	< 1
Soy protein isolate	272 - 1106	77 - 689	54 - 264	n.i.	n.i.
Tempeh	316 - 320	193 - 273	22 - 32	n.i.	n.i.
Natto	215 - 425	160 - 342	37 - 130	n.i.	n.i.
Soy cheese	3 - 150	3-98	3 - 53	n.i.	n.i.
Soy noodles	37 - 58	9 - 36	39	n.i.	n.i.
Soy bean sprouts	20	24	n.i.	n.d.	2
Clover sprouts	< 1	< 1	n.i.	8	40
Beans [a)]	n.d. - 7	n.d. - 0.2	n.d.	n.d.	n.d.
Garbanzo beans	< 1	n.d.	n.i.	14	0.5
Peas [a)]	n.d. - 53	n.d. - 73	n.i.	n.d.	n.d. - 93
Fruit, vegetables, nuts	0 -2 [b]		n.d.	n.d.	n.d.

[a)] dry seeds, different varieties; [b)] total value;
n.d = below the given limit of detection; n.i. = no information;

Tab. 1b: Isoflavone contents in red clover, *Trifolium pratense*, in µg per g dry weight (Wu *et al*., 2003, Tsao *et al*., 2006)

Part of the plant	Total isoflavone content [µg/g dw] according to	
	Wu et al., 2003	Tsao et al., 2006 [a)]
Leaf	17400 - 22700	20390 - 27780
Stem	7400 - 18500	12080 - 17340
Leaf stalk	n.i.	12300 - 14690
Root	13600 - 28500	n.i.
Flower	3000 - 6300	2380

[a)] 13 different varieties in early and late stages of flowering; n.i. = no information;

In recent years, there has been an increasing number of red clover extracts retailed as food supplements. In contrast to most soy preparations, red clover products frequently only state the amount of the extract contained in each capsule, dragée or tablet. For those red clover preparations that do give details on their isoflavone content, the complexity of the composition (11 different isoflavone aglycons have been detected up to date) leads to the question as to which isoflavone compounds were determined analytically and which were used to calculate the isoflavone content. The available literature data show that in contrast to soy preparations isoflavones in red clover products are mainly present in the aglycon form, (Setchell *et al*., 2001; Maul *et al*., 2005), although the corresponding glucosides dominate in the red clover plant as well (Toebes *et al*., 2005).

The dosage recommended by the manufacturers varies greatly from product to product; however, it usually lies between 20 and 80 mg isoflavone/day. Investigations carried out by a number of research groups on the isoflavone content in food supplements made from soy and red clover that are retailed in the USA, Great Britain, Finland and Germany, showed that in the majority of the preparations there were considerable differences between the isoflavone content declared by the manufacturer and the analysed values (Setchell *et al*., 2001; Nurmi *et al*., 2002; Howes & Howes, 2002, Maul *et al.*, 2005). An additional problem is the often imprecise data given for the isoflavone content. For example, in some preparations it is not clear whether the content given by the manufacturer refers to the sugar conjugate or to the free isoflavone aglycon. Furthermore, although studies have shown that the same product from one manufacturer may show relatively constant total isoflavone contents for different batches, this did not hold true, however, for the ratio between the individual isoflavones (Chua *et al*., 2004).

2. Dietary intakes

In Asian countries, fermented soy products such as tempeh, miso or natto are part of the traditional diet. This leads to a daily isoflavone intake of about 15-50 mg, predominantly as aglycons. In contrast, soy products are not traditional foods in western industrial countries, and the average daily intake is less than 2 mg isoflavones/day. Table 2 summarises the isoflavone intake in various countries.

Tab. 2: Average daily isoflavone intake in various countries

Average isoflavone intake per day in mg	n	Population group	Country	Reference
<1	>1000	cross-section	Netherlands, UK, Italy, Ireland, Finland	Van Erp-Baart *et al*. 2003
0.76	964	post-menopausal women	USA	De Kleijn *et al*. 2001
1.78	111526	women (21-103 years)	USA	Horn-Ross *et al*. 2002
12	25	vegetarians	United Kingdom	Clarke *et al*. 2003
14.88	3224 m 3475 f	cross-section	Korea	Kim & Kwon, 2001
25.4	650	women (19-86 years)	China	Mei *et al*. 2001
31.5-51.4	1274	cross-section	Japan	Wakai *et al*. 1999
39.5	50	women	Japan	Kimira *et al*. 1998
47.2	115	women (29-78 years)	Japan	Arai et al., 2000
61	76 m 71 f	cross-section	Singapore	Seow *et al*., 1998

f = female, m = male.

3. Bioavailability, kinetics and metabolism

Previous studies on the bioavailability of isoflavones focused on daidzein and genistein. Compared to many other secondary plant metabolites and also to the group of flavonoids, the bioavailability of isoflavones is regarded as being comparatively high. The bioavailability is not only affected by the food matrix, but in particular also by the sugar conjugation.

Depending on the type of soy product, the isoflavones are consumed in form of glucosides (consumption of native soy products such as tofu and soy milk) or predominantly as algycons (consumption of fermented soy products). According to currently available information, if they are consumed as glucosides, the aglycons are cleaved by ß-glucosidases of the small intestine or of the gut microflora. They are metabolised in the intestinal tract as well as in the liver. The intestinal flora metabolically reduces daidzein to dihydrodaidzein, *O*-demethylangolensin and equol; genistein is mainly metabolised to dihydrogenistein and 6'-*O*-demethylangolensin (Fig. 3 and 4). To a lesser extent, daidzein and genistein are monohydroxylated in the liver to produce the catechol metabolites 6-, 8- and 3'-hydroxy-daidzein and 6-, 8- and 3'-hydroxy-genistein, respectively (Kulling *et al*., 2002; Rüfer, 2005). Isoflavones are mainly present in plasma as phase-II conjugates in the form of monoglucuronides (Shellnut *et al*., 2002). The fraction of free aglycons in the blood is given as < 3 % in most studies. However, in a few studies the fraction of non-conjugated compounds was found to be as high as 20 % (Zhang *et al*., 2003). Pharmacokinetic studies have shown that after consumption of isoflavones the aglycon fraction initially increases and subsequently decreases, so that in addition to differences in the activity of the phase-II enzymes due to genetic variations the timepoint of the analytical determination is also an important factor (Rüfer, 2005).

Biochanin A and formononetin, the major isoflavones in red clover, are mainly metabolised by a demethylation pathway to genistein and daidzein (Setchell *et al*., 2002; Kulling *et al*., 2003; Heinonen *et al*., 2004). Furthermore, minor amounts of hydroxylated derivatives and reduction products of the primary metabolites daidzein and genistein as well as of the parent compounds, formononetin and biochanin A, occur (Heinonen *et al*., 2004). The metabolism of other isoflavones in red clover, such as irilone, pseudobaptigenin, prunetin, pratensein and calycosin (Fig. 2) has not yet been studied. In addition, there is no data available on the bioavailability of these compounds.

There may be large variations in the metabolism of isoflavones between different individuals, particularly with respect to the formation of equol. These variations depend on the diet, which

influences both, the intestinal microflora as well as the intestinal transit time (Fig. 3). More recent studies suggest that equol is a key metabolite with regard to the biological effect of isoflavones. However, this metabolite is formed from daidzein in the intestinal tract by only about one third of the population. The remaining two thirds metabolise daidzein to dihydrodaidzein and *O*-demethylangolensin (Fig. 3). The formation of different metabolites might lead to substantial differences with respect to biological effects. In addition to the diet, further factors that may influence biotransformation are age, gender as well as the frequency and duration of the intake.

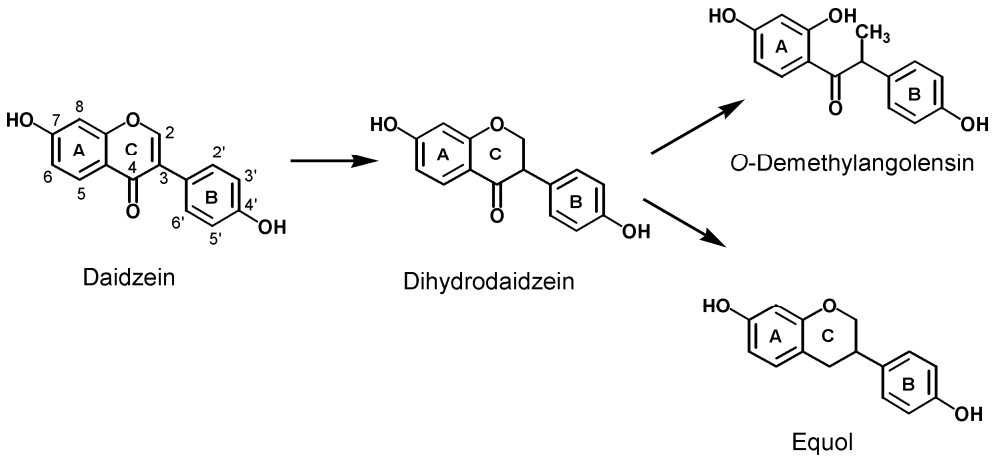

Fig. 3: Metabolism of daidzein by intestinal microflora

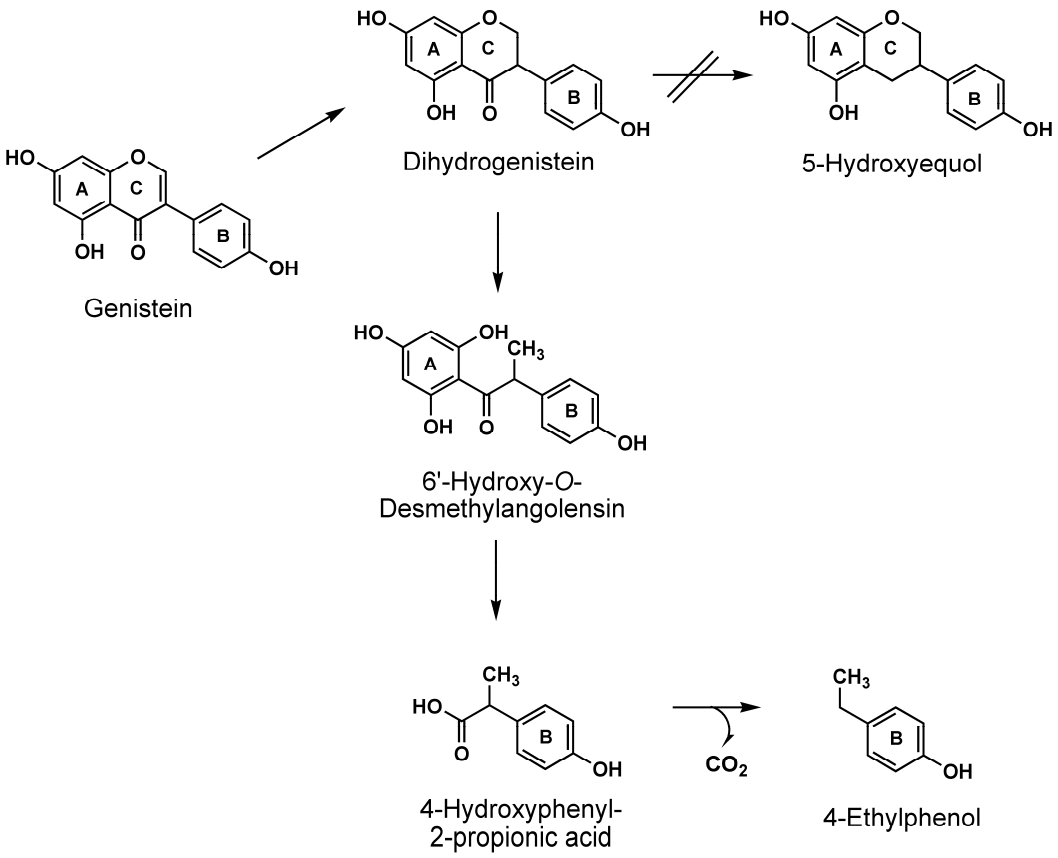

Fig. 4: Metabolism of genistein by intestinal microflora

Plasma levels

A "standard" western diet, which usually includes only minor amounts of isoflavones (< 2 mg/day) leads to low total isoflavone plasma concentrations (sum of daidzein, genistein and equol), with an average of approx. 10 nM (Adlercreutz *et al*., 1993). In contrast, in certain Asian population groups with a traditional diet, i.e. regularly consuming soy-based foods, the total isoflavone plasma concentrations are on average 870 nM (Adlercreutz *et al*., 1993). A further study obtained comparable results (Morton *et al*., 2002).

Isoflavone contents have only rarely been determined in human tissues. Isoflavones were detected in the breast tissue of premenopausal women and in the prostate fluid of men after supplementing the test subject's diet with soy products. The concentrations of genistein and daidzein in the breast tissue were comparable to those found in plasma; however, the equol concentrations were higher (Hargreaves *et al*., 1999; Maubach *et al*., 2003). The prostate fluid was found to contain higher concentrations of isoflavones than plasma (Morton *et al*., 1997; Hedlund *et al*., 2005).

Rats administered doses of ^{14}C-labelled genistein exhibited a much higher fraction of free genistein in tissues as compared to plasma levels (Coldham *et al*., 2002). The fraction of free genistein was 49% in the mammary gland tissue of the females, and even as much as 80 or 100 % in the ovaries and the uterus, respectively, compared to less than 5% in the plasma. For humans, it is not known whether the measured plasma concentrations and the distribution of metabolites in the plasma correlate with the amount of the compounds in individual organs, particularly in potential target organs such as breast, prostate, and thyroid. There are no comprehensive data in the literature that give details about isoflavone plasma levels reached after consumption of isoflavone-containing food supplements. In particular, there is frequently a lack of information on the concentration of quantitatively important metabolites. Differences in the preparations with respect to production, starting material and formulation render estimations of the resulting plasma concentrations difficult. Moreover, the ratio between the aglycon and the glycoside fractions is frequently not given. Tables 3 and 4 (see the Annex) summarise the results from individual studies, in which isoflavones were used in a concentrated form as soy or red clover extracts (Tab. 3) or in a highly purified or pure form (Tab. 4). The average values of the maximum plasma concentrations (C_{max}) are given for each isoflavone, calculated as the aglycon equivalent. However, it must be emphasised once again that a large fraction of the isoflavones are present in the plasma as phase-II conjugates and not in a free form.

The studies, in which isoflavone extracts were used, are summarised in Tab. 3. They show that the intake of comparable amounts of isoflavones may lead to marked differences in the maximum plasma concentrations. On the one hand, this may be caused by interindividual differences among the test subjects. On the other hand, different formulations and influences of the associated matrix may also contribute.

Studies with highly purified or pure isoflavones indicate that isoflavones consumed in the glucoside form have a higher bioavailability (Tab. 4). However, this point is still a matter of debate in the literature and is not consistent, for example, with results obtained by Zubik & Meydani (2003, Tab. 3, Nos. 4 and 5). The results of the study by Rüfer (2005) show that metabolites are also present in relevant concentrations and the biological effects mediated by the metabolites must therefore also be taken into account. Furthermore, the findings by Rüfer (2005) show that there were large variations in the concentration of bacterial metabolites (dihydrodaidzein, *O*-demethylangolensin) in the plasma of the test subjects. This indicates that the interindividual variability of the intestinal flora also influences metabolism.

4. Biological effect and toxicity

An adequate assessment of the product safety is hampered by the fact that isoflavones can trigger a variety of biological effects that are mediated by completely different cellular targets. Further details are given in the SKLM Opinion “Aspects of Potentially Adverse Effects of Polyphenols/Flavonoids Used in Isolated or Enriched Form" (SKLM, 2005) and in the report by the British Committee on Toxicity of the Food Standards Agency (COT, 2003).

Most studies have focused on the soy isoflavones daidzein and genistein. In some cases their main metabolites have also been investigated. Genistein and daidzein can interfere with hormone homeostasis by interacting with various transport proteins, enzymes and receptors that are directly or indirectly involved in the mediation of hormonal signals. Which effects are triggered by intake levels that are to be expected from consuming food supplements and balanced diets must therefore be studied on a case-by-case basis (SKLM, 2005).

Some of the effects of isoflavones that are described in the literature are discussed in more detail below.

4.1 Investigations of the effects of isoflavones on the female breast

Animal studies have shown that the effects of isoflavones on the female breast greatly depend on the stage of development and the degree of differentiation of the mammary gland tissue (Lamartiniere *et al*., 2002). For example, various research groups have shown that the administration of soy-supplemented feed or the isoflavone genistein in an isolated form to female rats significantly decreased the incidence and growth rate of mammary tumours induced by dimethylbenzanthracene (DMBA) or *N*-methyl-*N*-nitrosourea (MNU), if isoflavone was administered neonatally or before sexual maturation. However if the isoflavone-enriched diet was given at an adult stage of life this protective effect was no longer observed. One possible explanation for this phenomenon is that genistein induces an early or premature differentiation of the mammary gland tissue, rendering this tissue less sensitive to chemical carcinogens (Lamartiniere *et al*., 2002).

Further animal experiments studied the effect of isoflavones on preexisting estrogenreceptor-positive tumour cells. The results showed that the growth of tumour cells can be enhanced by isoflavones. Athymic, ovariectomised nude mice implanted with breast cancer cells (MCF-7 cells) were fed soy protein isolate (SPI) or isolated genistein in an amount equivalent to that in the SPI. The MCF-7 cells started to proliferate more rapidly in these animals than in the control animals given isoflavone-free feed (Ju *et al.*, 2001, 2006). The isoflavone plasma

concentrations of 1-2 μM found in these animal experiments are in a concentration range that can indeed be reached by consuming food supplements. This effect was recently confirmed by another research group (Power *et al*., 2006; Saarinen *et al*., 2006).

Similar results were found in animal experiments aimed to study the effect of isoflavones on mammary tumours induced by MNU in ovariectomised rats (Allred *et al*., 2004). In this case, the genistein-supplemented feed led to a faster growth rate of the tumours when compared to the isoflavone-free feed. As a result of the ovariectomy the endogenous estradiol levels in the rat were comparable to those in post-menopausal women. The genistein levels (3,4 μM) measured in rat plasma lay in a concentration range that can be reached in humans, particularly in those taking highly-dosed isoflavone preparations (dose >1 mg/kg bw) (Tab. 4). There are a few studies cited in the literature that investigated the interaction between isoflavones and tamoxifen. In an experimental animal study, in which MCF-7 cells were implanted into athymic, ovariectomised mice, tamoxifen inhibited estradiol-mediated proliferation of the tumour cells. This effect was abrogated by the simultaneous administration of genistein-enriched feed (Ju *et al*., 2002). In another study with transgenic mice (wild-type erbB-2/neu), treatment with tamoxifen prevented the formation of tumours. This effect was also abrogated by low dose isoflavone-enriched diet (Liu *et al*., 2005).

It has previously been shown that the biological effects of isoflavones in their isolated form are not comparable to the effects of these compounds in a complex food. For example, the estrogenic effect of a soy product (soy flour) with a low degree of processing was compared to a soy extract and to the isolated soy isoflavone genistin (genistein glucoside). The different soy products were adjusted to the same genistein content of 750 ppm and then fed to athymic, ovariectomised nude mice, in which MCF-7 cells had been implanted. The soy flour did not affect the growth of the tumour cells, whereas both, the soy extract as well as the isolated soy isoflavone genistin, increased the proliferation of the MCF-7 cells (Allred *et al*., 2004).

Very few clinical studies have investigated the effect of isoflavones on mammary gland tissue in humans. In a cross-over study with 24 premenopausal women given a 6-month supplementation (genistein dosage: 38 mg/day), the volume of nipple aspirate fluid increased when compared to that of women given an isoflavone-free control diet. Furthermore, a cytological study of epithelial breast cells in the glandular secretion before and after soy supplementation showed hyperplastic cells in 30 % of the women after they had taken the soy product (Petrakis *et al*., 1996). In another study with 19 premenopausal women short-term supplementation with a soy protein preparation (45 mg isoflavones over 14 days) led to

a significant increase in breast cell proliferation rate, measured as the thymidine labelling index, and to an increase in the expression of the progesterone receptor (McMichael-Phillips *et al*., 1998). In a further study an analogous supplementation given to 48 premenopausal women lowered the apolipoprotein D level, whereas the pS2 protein in the mammary fluid aspirate was enhanced. However, in cells cultivated from breast biopsies, parameters such as proliferation rate, degree of differentiation, apoptosis rate and Bcl-2 expression remained unchanged (Hargreaves *et al*., 1999).

In view of the difficulties associated with transferring data from animal experiments to humans and because of the very limited number of clinical studies available, it is not possible at present to make a conclusive assessment of the effect of isoflavones on the female breast, particularly regarding the risk of breast cancer in women at elevated risk or the survival time of breast cancer patients (Messina et al., 2006). Available data from prospective studies on the increased intake of isoflavone-containing foods did not indicate a lowering of breast cancer incidence. However, a conclusion that can be drawn from the available data is that isoflavones, particularly at high dosage, can exert an estrogenic stimulus on the mammary gland tissue in women.

4.2 Effect on the thyroid gland

There is plenty of evidence in the literature to support the correlation between an increased risk of developing goiter and the consumption of soy foods in the presence of an iodine deficiency. This finding has also been confirmed by many experimental animal studies. However, it is not clear whether isoflavones are involved or even responsible for the goitrogenic effect. *In vitro* studies have shown that soy isoflavones themselves can act as a substrate for thyroid peroxidase, i.e. they can be iodinated and therefore can act as competitive substrates (Divi *et al*., 1997). Furthermore, in the case of an iodine deficiency, direct inhibition of the enzyme by covalent binding of the isoflavones has been reported. The half-maximum inhibition (IC_{50}) of thyroid peroxidase is already reached at a genistein concentration of 1 µM. To what extent these *in vitro* data are physiologically relevant is under discussion at present.

Inhibition of sulfotransferases represents a second effect exerted by isoflavones in thyroid hormone metabolism. They are inhibited by isoflavones and contribute to the inactivation and elimination of thyroid hormones and to the reutilization of iodine in the human thyroid gland (Ebmeier and Anderson 2004).

A third target of isoflavones in the thyroid hormone system is transthyretin (TTR, formerly known as thyroxine-binding prealbumin, TBPA). TTR binds up to 20% of the thyroxine (T4) in the serum, and is also involved in distributing T4 throughout the body and preventing T4 excretion by the kidneys. TTR is the most important thyroid hormone-binding protein in cerebrospinal fluid (CSF). Genistein and related (iso)flavones are highly effective in inhibiting binding of TTR to T4 and T3 (K_d = 40 nM, equimolar to T4 binding) in serum and CSF and they may thus alter the distribution of thyroid hormones in the body (Green et al., 2005; Radovic et al., 2006). Particularly during the early embryonic and postnatal development of the brain, for which TTR-bound and -transported thyroid hormones are key regulators, interference with TTR-T4 binding by isoflavonoids e.g. from soy-based infant formulae could lead to adverse effects.

Data from epidemiological and clinical studies suggest that the consumption of soy foods is unlikely to have an adverse effect on the thyroid gland if the iodine intake is adequate. On the other hand, data from animal experiments indicate that if there is an iodine deficiency or hypothyreosis (hypoactivity of the thyroid gland) in combination with an extremely high intake of isoflavones, an adverse effect on the thyroid gland cannot be excluded (Doerge & Sheehan, 2002). An experimental animal study with rats showed that a high intake of soy combined with an iodine deficiency led to a synergistic effect with respect to the development of hyperplasia of the thyroid gland (Ikeda *et al*., 2000). In women, the incidence of subclinical thyroid hypofunction increases with age. About 10 % of all women over the age of 55 suffer from subclinical hypothyroidism (Vanderpump & Tunbridge, 2002). Thus, especially postmenopausal women, representing the main target group for isoflavone-containing food supplements, are considered at risk with regard to potential side-effects in the thyroid gland.

Furthermore, effects of isoflavones in humans with lifelong dependence on thyroid hormone T4 substitution cannot be assessed at present. Such humans include, for example those, born without a functioning thyroid gland (1 in 3500 live births world-wide) or those having had their thyroid gland completely or partially removed due to a thyroid tumour or to a Morbus Basedow-type hyperthyroidism. This group, predominantly female (3:1), have no thyroid hormone reservoir in the gland itself. Therefore, isoflavonoid-induced interferences in the binding of the daily dose of thyroxine to TTR may have an unfavourable effect on the stabilisation of a normal thyroid hormone level and on thyroid hormone bioavailability, particularly if isoflavone concentrates are taken. There is an urgent need for studies regarding the interference of isoflavones with thyroid hormone homeostasis within these populations.

5. Assessment

Isoflavone products based on soy and red clover are currently marketed as food supplements or dietary foods for special medical purposes. However, such preparations should only be consumed (applied as foods) if their safety has been proved. In traditional Asian soy products that have been fermented, such as tempeh and miso, most of the isoflavones are present as their aglycons, exhibiting different bioavailability and pharmacokinetics when compared to the corresponding glycosides.

Based on data available in the literature, the safety of such products cannot be derived. There are two main reasons for this:

1. The potential of isoflavones to trigger adverse health effects and the dose-dependency of such effects have not been adequately studied up to the present time. For example, red clover preparations contain isoflavone compounds that have not yet been studied with respect to their biological effects and metabolism.

2. Presently marketed isoflavone preparations from soy and red clover not only differ quantitatively with respect to their total isoflavone content, but also qualitatively with respect to their isoflavone spectrum (ratios between individual isoflavones, ratio of free isoflavones to glycosidically bound isoflavones) and with respect to the associated matrix. Safety assessment requires, however, accurate knowledge of the composition of the preparations. A safety assessment based on scientific criteria, as already recommended by the SKLM in previous opinions on polyphenols/flavonoids (SKLM, 2005), functional foods (SKLM, 2005) and food supplements with constituents other than vitamins and minerals (SKLM, 2006), thus requires a case-by-case evaluation.

Postmenopausal women are to be regarded as a particular risk group for undesirable effects associated with the intake of isoflavone preparations:

1. An increased cancer risk associated with hormone replacement therapy in postmenopausal women has been observed in large-scale clinical studies (Women's Health Initiative Study WHI, 2002; Women's Health Initiative Memory Study, 2003; Million Women Study, 2003). Cancer statistics show that breast cancer is the most common type of cancer in women. The probability of developing breast cancer increases with age: 75% of all breast cancer patients are postmenopausal women over the age of 50 (Working Group on Population-based Cancer Registries in

Germany, 2004). The results summarised here do not allow to exclude, for example, an estrogenic stimulus, also encompassing a growth stimulus on premalignant or malignant cells as a consequence of an increased intake of isoflavone preparations.

2. The risk of developing subclinical hypothyroidism in women increases with age. On the basis of currently available data, it cannot be excluded that this risk is increased by the consumption of isoflavone preparations.

6. Research needs and gaps of knowledge

In the opinion of the SKLM there is a need for scientific research to improve the database for risk assessment concerning isoflavones in food supplements and dietary foods for special medical purposes. This applies in particular to the following topics:

- Survey of reliable and current data on the consumption of such products and on the associated isoflavone intake
- Study of the influence of matrix components and formulation on the bioavailability of isoflavones
- Measurement of the isoflavone concentration in plasma and other compartments, particularly of concentrations in human tissues
- Analytical characterisation of red clover products (systematic studies)
- Study of red clover isoflavones, including the minor components, with respect to biotransformation and toxic potential.
- Development of biomarkers to study the effect of isoflavones on breast tissue in women with an increased risk of breast cancer
- Elucidation of the role played by isoflavones in mediating goitrogenic effects, particularly in combination with iodine deficiency (postmenopausal women), and elucidation of the mechanisms involved in the potential adverse effects of isoflavones on the thyroid hormone axis
- Research adressing potential adverse health effects of isoflavones in patients without a functioning thyroid gland and in patients dependent on thyroid hormone substitution; elucidation of the underlying mechanisms
- Identification of further potential risk groups

7. Conclusion/Recommendation

A round table meeting „Phytoestrogens in food supplements and dietary foods for special medical purposes“ was held on 20th / 21st November 2008. The following experts participated:
Gerhard Eisenbrand (chair), Hildegard Bauer-Aymanns, Verónique Coxam, Patrick Diel, Martina Dören, Jan-Ake Gustafsson, William G. Helferich, Hans-Georg Joost, Josef Köhrle, Sabine Kulling, Alfonso Lampen, Leane Lehmann, Tonghua Liu, Sari Mäkelä, Doris Marko, Doris Mayer, Yuanjiang Pan, Andrea Renggli, Klaus Richter, Christian Steffen, Günter Vollmer, Wolfram Wuttke, Ming-Yong Xie Piwen Zhao
The SKLM conclusions and recommendations from 2006 were discussed and, at the plenary meeting on 20th February 2009, confirmed in the following version:

Biological effects connected with the intake of isoflavones in an isolated, highly dosed or enriched form are not comparable to those associated with the intake of isoflavones in complex foods, as is the case for the consumption of soy-based foods in Asian countries. The safety of isoflavone preparations based on soy and red clover as food supplements or as dietary foods for special medical purposes cannot be derived from the traditional use of soy-based foods. The potential of isoflavones in such products to cause adverse health effects and the dose-dependency of such effects have not yet been adequately studied. Moreover, the exact composition of the preparations (total isoflavone content, isoflavone spectrum, associated matrix) is usually not known or standardised, so that each individual case requires a separate safety assessment based on scientific criteria. Such criteria have already been formulated by the SKLM for functional foods and for food supplements containing constituents other than vitamins and minerals (SKLM, 2005 and 2006). The SKLM expresses the view that particular problems might be associated with the intake of isoflavones by postmenopausal women, since they represent the main target group for these preparations, but also are considered at particular risk with regard to undesirable side-effects. Therefore, the currently available data do not allow a conclusive assessment of the effect of isoflavones on the breast tissue of women with an increased risk of breast cancer. It is possible that isoflavones may negatively impact existing or as yet undetected breast cancer. Furthermore, isoflavones may enhance the risk to develop a subclinical hypothyroidism and may contribute to goiter development/formation.

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ANNEX

Tab. 3: Maximum isoflavone concentrations in plasma (C_{max}) after consumption of isoflavones in the form of soy or red clover extracts by healthy test subjects. The total concentration (free form and phase-II conjugate) of the individual isoflavones or metabolites is given as the calculated aglycon equivalent.

No.	Isoflavone source, additional information	No. of test subjects n	Consumed isoflavone quantity/dose	C_{max} (ng/mL)	C_{max} nmol/L	Reference
1	Soy extract Retard formulation Single dose 90% of the isoflavones as glucosides	n=10 (f) postmenopausal women	daidzein: 7.5 mg genistein: 22.3 mg glycitein: 2.0 mg **∑ isoflavones: 31.8 mg**	39 ± 3.7 46 ± 4.4 18 ± 1 **103**	143 ± 14 179 ± 16 63 ± 4 **385**	Setchell *et al*., 2005
2	Soy extract (capsules) Single dose Isoflavones as glucosides	n=12 (f) postmenopausal women	daidzein 4.8 mg genistein 14.1 mg **∑ isoflavones: 18.9 mg**	96 262 **358**	378 969 **1347**	Anupongsanugool *et al*., 2005
3	Red clover extract (tablets) Daily dose for 14 days ; Isoflavones as aglycons	n=14 (6 m; 8 f)	biochanin A: 52.4 mg formononetine: 31.4 mg daidzein: 0.9 mg genistein: 2.6 mg **∑ isoflavones: 87.2 mg**	48 ± 5 11 ± 2 63 ± 9 114 ± 30 **236**	168 ± 18 42 ± 7 248 ± 35 422 ± 111 **880**	Howes *et al*., 2002
4	Soy extract (tablets) Single dose Isoflavones as aglycons	n=15 (f) 46 ± 6 years old	daidzein: 15.8 mg genistein: 14.2 mg glycitein 2.2 mg **∑ isoflavones: 32.2 mg**	135 ± 52 144 ± 90 n.i. **279**	530 ± 205 534 ± 333 n.i. **1064**	Zubik & Meydani, 2003
5	Soy extract (tablets) Single dose Isoflavones as glucosides	n=15 (f) 46 ± 6 years old	daidzein: 12.0 mg genistein 17.2 mg glycitein 2.0 mg **∑ isoflavones: 31.2 mg**	101 ± 26 154 ± 79 n.i. **255**	396 ± 104 569 ± 294 n.i. **965**	Zubik & Meydani, 2003

n.i. = no information; f = female, m = male.

Tab. 4: Maximum isoflavone concentrations in plasma (C_{max}) after consumption of isoflavones in a highly purified form (Nos. 1-4) and in an isolated form as the pure substance (5 and 6) by healthy test subjects. The total concentration (free form and phase-II conjugate) of the individual isoflavones or metabolites is given as the calculated aglycon equivalent.

No.	Preparation, isoflavone source, additional information	No. of test subjects (n)	Consumed quantity of isoflavone	C_{max} (ng/mL)	C_{max} nmol/L	Reference
1	Formulation of isolated isoflavones as ≥ 97% isoflavone aglycons (90 ± 5 % GEN, 9-10% DAI)	men (40-69 years) n=3	1.0 mg/kg bw genistein 2.0 mg/kg bw genistein 4.0 mg/kg bw genistein 8.0 mg/kg bw genistein 16.0 mg/kg bw genistein	**251** ± 24 **566** ± 122 **1194** ± 676 **2172** ± 595 **2053** ± 374	**929** ± 88 **2095** ± 451 **4418** ± 2502 **8037** ± 2203 **7595** ± 1384	Busby *et al*., 2002
2	Formulation of isolated isoflavones as 100% isoflavone aglycons (87% GEN, 12% DAI, 1% GLY)	post-menopausal women (46-68 years) n=3	2.0 mg/kg bw genistein 4.0 mg/kg bw genistein 8.0 mg/kg bw genistein 16.0 mg/kg bw genistein	**929** ± 385 **2310** ± 168 **3831** ± 1214 **7611** ± 4312	**3440** ± 1425 **8545** ± 621 **14172** ± 4492 **28158** ± 15954	Bloedon *et al*., 2002
3	Formulation of isolated isoflavones as 100 % isoflavone aglycons (43% GEN, 21% DAI, 3% Gly; other accompanying substances are not known)	men (40-69 years) n=3	1.0 mg/kg bw genistein 2.0 mg/kg bw genistein 4.0 mg/kg bw genistein 8.0 mg/kg bw genistein 16.0 mg/kg bw genistein 0.49 mg/kg bw daidzein 0.98 mg/kg bw daidzein 2.0 mg/kg bw daidzein 3.9 mg/kg bw daidzein 7.8 mg/kg bw daidzein	**738** ± 462 **1484** ± 410 **2562** ± 558 **4830** ± 656 **7422** ± 4157 **363** ± 195 **564** ± 163 **1234** ± 97 **1950** ± 498 **4303** ± 2212	**2729** ± 1710 **5492** ± 1516 **9479** ± 2053 **17870** ± 2426 **27460** ± 15380 **1429** ± 767 **2221** ± 640 **4857** ± 384 **7678** ± 1961 **16940** ± 8711	Busby *et al*., 2002

Continuation of Table 4:

No.	Preparation, isoflavone source, additional information	No. of test subjects (n)	Consumed quantity of isoflavone	C_{max} (ng/mL)	C_{max} nmol/L	Reference
4	Formulation of isolated isoflavones as 100 % isoflavone aglycons 43% GEN, 21% DAI, 3% Gly; other accompanying substances are not known	post-menopausal women (46-68 years) n=3	2.0 mg/kg bw genistein 4.0 mg/kg bw genistein 8.0 mg/kg bw genistein 16.0 mg/kg bw genistein 1.0 mg/kg bw daidzein 2.1 mg/kg bw daidzein 4.2 mg/kg bw daidzein 8.4 mg/kg bw daidzein	**1524** ± 640 **2344** ± 505 **4118** ± 450 **6869** ± 2361 **640** ± 216 **914** ± 122 **1648** ± 199 **2279** ± 749	**5638** ± 2369 **8672** ± 1869 **15235** ± 1665 **25413** ± 8733 **2521** ± 849 **3600** ± 482 **6488** ± 785 **8973** ± 2950	Bloedon *et al*., 2002
5	Pure substance, single dose, aglycon form	n=6 premenopausal women	daidzein: **50.0 mg**	**194** ± 31	**760** ± 120	Setchell *et al*. , 2001
	Pure substance, single dose, glucoside form	n=4 premenopausal women	daidzein: **30.5 mg**	**394** ± 61	**1550** ± 240	
	Pure substance, single dose, aglycon form	n=6 premenopausal women	genistein: **50.0 mg**	**341** ± 74	**1260** ± 270	
	Pure substance, single dose, glucoside form	n=3 premenopausal women	genistein: **31.3 mg**	**341** ± 127	**1220** ± 470	

Continuation of Table 4:

No.	Preparation, isoflavone source, additional information	No. of test subjects (n)	Consumed quantity of isoflavone	C_{max} (ng/mL)	C_{max} nmol/L	Reference
6	Pure substance, single dose, type of study: cross-over aglycon form	n=7 (m)	**1.0 mg/kg bw daidzein**	**109** (48-142) **38** (3-109) **9** (2-22) **11**	**daidzein: 430** (190-560)[1] **DHD: 148** (10-427)[1] **ODMA: 34** (6-86)[1] **OH-DAI[2]: 39** **∑ 651**	(Rüfer, 2005; Rüfer *et al*. 2005)
	Pure substance, single dose, type of study: cross-over glucoside form	n=7 (m)	**1.0 mg/kg bw daidzein**	**646** (491-976) **192** (5-436) **29** (8-62) **23**	**daidzein: 2540** (1930-3840)[1] **DHD: 750** (20-1700)[1] **ODMA: 111** (32-239)[1] **OH-DAI[2] : 85** **∑ 3486**	

[1] Given range of values; [2] Sum of 3-hydroxy, 8-hydroxy- and 6-hydroxy-DAI; m = male; DAI= daidzein, GEN = genistein, DHD=dihydroxydaidzein, ODMA = *O*-demethylangolensin, OH-DAI = monohydroxylated daidzein derivatives;
n.i. = no information; f = female, m = male.