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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Glycine max* (L.) Merr., semen

Final

Based on Article 10a of Directive 2001/83/EC (well-established use)
Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Glycine max* (L.) Merr., semen
Herbal preparation(s)	Not applicable.
Pharmaceutical form(s)	Not applicable.
Rapporteur(s)	P. Claeson/E. Svedlund
Assessor(s)	E. Svedlund
Peer-reviewer	I. Chinou

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

  Glycine max (L). Merr., semen (soya bean) is an annual herbaceous plant in the family Fabaceae (legume or bean family) that is cultivated. The fruit contains 1-4 ovoid to spherical seeds of variable colour (Bruneton, 1999).

  Constituents *(*Anderson and Wolf, 1995; Bruneton, 1999; Blumenthal *et al*., 2000; Erdman *et al*., 2004; Poluzzi *et al*., 2014; EFSA, 2015)

  - Proteins
    35-40%, including essential amino acids
  - Carbohydrates
    15-35%, mainly as insoluble fibres
  - Lipids
    15-20%, including 2-3% phospholipids. The main phospholipids are phosphatidylcholine (average 76% in lecithin), phosphatidylethanolamine and phosphatidylinositol. The major fatty acids are linoleic (48-58%), oleic (17-30%), palmitic (9-13%), linolenic (5-11%) and stearic (3-5%) acids. The unsaponifable matter contains sterols; β-sitosterol (47-59%), campesterol (19-23%), stigmasterol (17-19%), as well as tocopherols: γ- (44-60%), δ- (30-43%), α- (5-10%), and β (2-3%)
  - Saponins

Isoflavones
the main being genistin, daidzin and glycitin (glycoside form) and their aglycone form (genistein, daidzein and glycitein, respectively). The concentration of individual isoflavones is reported as follows: daidzin, 67–516 µg/g; genistin, 91–1 079 µg/g; glycitin, 12–177 µg/g; malonyldaidzin, 217–768 µg/g; malonylglycitin, 43–158 µg/g, malonylgenistin, 64–2 446 µg/g; genistein, 4.3–265 µg/g.

- Herbal preparation(s)

  - Soya bean oil, refined Soiae oleum raffinatum (see separate EU herbal monograph EMA/HMPC/338914/2016 and assessment report EMA/HMPC/338915/2016)
  - Soya lecithin Lecithinum ex soya (see separate EU herbal monograph EMA/HMPC/220599/2016 and assessment report EMA/HMPC/220598/2016)

  During the assessment of *Glycine max* (L.), Merr., semen, HMPC noted that it would be more appropriate to develop a separate monograph on soya lecithin and a separate monograph on soya oil. Hence, this assessment report excludes these preparations. The herbal preparations from *Glycine max* (L.), Merr., semen, covered in this assessment report is:

  - Dry extract from the soya bean germ (hypocotyl) (DER 100-400:1), extraction solvent ethanol 60%-70% V/V.
    The extract contains 40% isoflavones calculated as the sum of isoflavones (26% isoflavones calculated on genistein).

- Dry extract from the soya bean germ (hypocotyl) (DER 47.61-190.47:1), extraction solvent methanol 80% V/V.
  The extract contains 26% isoflavones calculated as the sum of isoflavones.
- Dry extract from the soya bean germ (hypocotyl) (DER 43-53:1), extraction solvent ethanol 60% V/V.
  The extract contains 10% isoflavones calculated as the sum of isoflavone glucosides.
- Dry extract from the soya bean germ (hypocotyl) (DER 50-70:1), extraction solvent ethanol 60% V/V.
  The extract contains 30% isoflavones calculated as the sum of isoflavone glucosides.

The composition of the isoflavone content differs markedly between products and is affected by processing method. In the scientific litterature it is common to express isoflavone content without specifying whether the amount refers to the aglycone or glycoside value. This distinction is critical, since the amount of the aglycone present is approximately 60% of the total weight of the glycoside. Thus, if the amount is not expressed as the aglycone isoflavone equivalent (AIE) quantity, 100 mg isoflavones may represent anywhere from 60 to 100 mg active isoflavones depending on product (Erdman *et al.,* 2004).

In addition to the herbal preparations reported as constituents of medicinal products, there is a broad range of dietary soya products on the market, including whole soya foods, soya flours, textured soya proteins, soya protein concentrates, soya protein isolates, isoflavone rich soya proteins, isoflavone extracts from the soya seed or soya germ, isolated isoflavone mixtures, pure genistein, lecithin products of varying purity and soya oils. Only references where a medicinal use is described or indicated, and where the extracts have been properly described, are taken into consideration in the assessment report.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable

1.2. Search and assessment methodology

Scientific databases: PubMed, Embase, Cochrane Database of Systematic Reviews

A PubMed search on “soyabean” (MeSH) found 38,193 articles. Database searches combining the following search terms have been made in February 2015: Isoflavones, genistein, soya, soyabeans, *glycine max*, menopause, flushing, sweating, sleep disorders, anxiety, irritable mood, irritability, hypersensitivity, allergy, drug interactions, humans. The abstracts were manually screened and all English articles deemed relevant were accessed and included in the assessment report. Further references found in lists of references were included, if deemed relevant.

Search engines used: Google

Medical databases: Micromedex, HerbMed, MedlinePlus, ESCOP, WHO

Toxicological databases: TOXLINE, HSDB. LactMed

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Table 1: Overview of data on extract of *Glycine max* (L.), Merr., semen obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status**
1. 175 mg dry extract of the germ (hypocotyl) of *Glycine max* (L.), Merr., semen (DER 43-53:1) which corresponds to 17.5 mg isoflavone glucosides. Extraction solvent: ethanol 60% V/V	Herbal medicine used in the treatment of flushing of menopause after any serious pathology has been ruled out.	Capsule, hard, 175 mg/capsule 2 capsules 2 times daily Dosage may be reduced to 2 capsules per day (depending on intensity and frequency of symptoms) Duration of use: No longer than 6 months without medical supervision	2003-2016, BE, WEU
2. 117.85 mg dry extract of the germ (hypocotyl) of *Glycine max* (L.) Merr., semen (DER 50-70:1), which corresponds to 35 mg isoflavone glucosides. Extraction solvent: ethanol 60% V/V	Indicated in adults for the treatment of menopausal flushing after serious pathology has been ruled out.	Capsule, hard, 117.85 mg/capsule 1 capsule 2 times daily Dosage may be reduced to 1 capsules per day (depending on intensity and frequency of symptoms) Duration of use: No longer than 6 months without medical supervision	Since 2014, BE, WEU
3. 175 mg dry extract of the germ (hypocotyl) of *Glycine max* (L.) Merr., semen (DER 43-53:1) which corresponds to 17.5 mg (10%) isoflavone glucosides. Extraction	For the relief of menopausal complaints such as hot flushes and night sweating	Capsule, 175 mg/capsule 2 capsules twice a day during the meal with a big quantity of fluid. Treatment cannot be longer than 4 months, after 4-week break the cure can be repeated. Before starting a new treatment the patients	Since 2003, HU, “healing product”

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
solvent: ethanol 60% (V/V)		should consult with their physicians. If the symptoms persist for more than 8 weeks or worsen during the treatment the patients should consult with their physicians.	
4. 100 mg dry extract of *Glycine max* (L.) Merr., semen (DER 100-400:1), which corresponds to 40 mg of the sum of isoflavones (26 mg of the sum of isoflavones calculated on genistein). Extraction solvent: ethanol 60%-70% V/V	The product is indicated for use in women during menopause to relieve symptoms as: hot flushes, excessive sweating, sleep disturbances, feelings of nervous tension and anxiety.	Coated tablet, 100 mg/tablet 1−2 tablets daily, morning and evening. Not excess of 2 years of using the product.	Since 2005, PL, WEU
5. 230.8 mg dry extract of *Glycine max* (L.) Merr., semen (DER 47.61-190.47:1), which corresponds to 60 mg of the sum of isoflavones. Extraction solvent: methanol 80% V/V	Product is indicated for use in women during menopause to relieve symptoms as: hot flashes, excessive sweating, sleep disturbances, feelings of nervous tension and anxiety.	Tablet, 230.8 mg/tablet 1–2 tablets daily	Since 2008, PL, WEU

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

2.1.2. Information on products on the market outside the EU/EEA

No data available.

2.2. Information on documented medicinal use and historical data from literature

The isoflavones in soya bean, also called phytoestrogens, are non-steroidal, polyphenolic substances belonging to flavonoids type (isoflavones) secondary metabolites, predominantly found in leguminous plants and are especially abundant in soya beans (Messina, 2010; Andres *et al*., 2011).

A lower incidence of menopausal symptoms (e.g. hot flushes) in Asian women who have a diet rich in soya products have been shown in observational studies, generating a scientific interest in the medicinal use of soya isoflavones (An *et al*., 2001). Weak oestrogenic effects of isoflavones were first proposed by in 1992 as a possible explanation for the low reported incidence of hot flushes experienced by women in Japan (Williamson-Hughes *et al*., 2006).

In 1995, the first study to examine soya isoflavones in the reduction of hot flushes when administered as dietary soya flour was published (Murkies *et al*., 1995). The ability to conduct clinical trials was aided by the development of isoflavone food supplements, which first became available in 1996 (Messina and Loprinzi, 2001). Since then a large number of clinical studies evaluating the use of different soya food products in the relief of menopausal symptoms have been conducted.

Health Canada has published a positive monograph on soya bean extracts and isolates. Statements that the product may reduce severe and frequent menopausal symptoms (such as hot flushes and/or night sweats) or helps to attenuate/reduce bone mineral density (BMD) loss in post-menopausal women when used in conjunction with adequate amounts of calcium and vitamin D are accepted. Preparations should be equivalent to 30-100 mg aglycone isoflavone equivalents (AIE) with a minimum of 15 mg AIE from genistein/genistin compounds per day (Health Canada Monographs, 2009).

2.3. Overall conclusions on medicinal use

Ethanolic or methanolic extracts of soya bean containing isoflavones have been used in medicinal products for the relief of menopausal symptoms such as hot flushes and night sweating in the EU/EEA since 2003. There is no information available on products with medicinal use throughout a period of at least 30 years on the market outside EU/EEA. Thus, traditional medicinal use according to Directive 2004/24/EC (i.e. medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA) is not fulfilled. The availability of clinical evidence for ethanolic extracts of soya bean containing isoflavones to establish recognised efficacy and an acceptable level of safety based on Article 10a of Directive 2001/83/EC (well-established use), is evaluated in section 4. “Clinical data”.

3. Non-Clinical Data

There is an enormous amount of non-clinical scientific publications regarding soya isoflavones. Notably, human exposure to soya isoflavones is predominantly through consumption of soya dietary products. The consumption of soya and soya isoflavones have been proposed to have a variety of beneficial effects in animals and humans, but concerns have also been raised in potential adverse effects of isoflavones, particularly with regard to reproductive toxicity and carcinogenicity, due primarily to its proposed weak oestrogenic activity.

The non-clinical data on isoflavones have been reviewed by prominent committees and institutes worldwide, e.g. the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) in 2006, Cochrane in 2013 (Lethaby *et al*., 2013), and European Food Safety Authority (EFSA) in 2015. The Cochrane and EFSA reports cover isoflavones in general and not only soya isoflavones.

In addition, genistein was selected by the National Toxicology Program (NTP) to be examined in a protocol utilizing Sprague-Dawley rats to evaluate the effects of multigenerational and long-term exposures. The results have been presented in two different reports: the toxicology and carcinogenesis 2-year study (NTP TR-545, 2007) and the multigenerational reproductive toxicology study (NTP TR-539, 2008).

The most important parts of these scientific reviews are summarised below.

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

3.1.1. Primary pharmacodynamics

Scientific attention of the biological effects of the isoflavones first came to attention in the 1940s because of breeding problems and infertility in female sheep grazing on isoflavone rich subterranean clover (i.e. *Trifolium subterraneum* L.) (Messina, 2010; Poluzzi *et al.*, 2014).

The oestrogenic activity of soya isoflavones, in particular genistein and daidzein, has been extensively investigated *in vitro* and *in vivo*. The ability of soya isoflavones to interact with oestrogen receptors is ascribed to their structural analogy with 17β-oestradiol (EFSA, 2015).

Soya isoflavones appear to show greater affinity for the oestrogen receptor beta (ERβ) than for the oestrogen receptor alpha (ERα). Unlike 17β-estradiol, which displays relative equivalent potency at both ERα and ERβ, the isoflavone genistein is a 40-fold ERβ ligand *in vitro* (Poluzzi *et al.* 2014). *In vitro*, genistein is more potent than daidzein, however, the binding affinities of genistein to ERα and ERβ are 10 000 and 30 times lower, respectively, than that of 17β-oestradiol (EFSA, 2015).

Although related to pharmacokinetics, it must be emphasised in this section that isoflavones glycosides (e.g. genistin and daidzin) are hydrolysed in the gastrointestinal tract as a result of bacterial β-glucosidase activity (Setchell, 2001). The bioavailable aglycones may be absorbed into the circulation or further metabolised into an array of other metabolites which may exert enhanced or decreased biological activity. In the colonic microflora, daidzein may be metabolised to *O*-demethylangolensin *(O*-DMA) and S-equol, and genistein may be metabolised to 6'hydroxy-*O*-DMA and *p*-ethylphenol (Poluzzi *et al*., 2014). S-equol has been reported to be a 30 times more active metabolite, making it the most potent ERβ isoflavone. The microflora in the intestines of rodents and monkeys are more efficient than in humans with regards to S-equol production, therefore the outcome of studies in rats and mice may not be relevant to humans (Erdman *et al.*, 2004). Monkeys, rats, and mice are described as 100% equol producers, meaning that the microbiotas of these animals are uniformly able to transform daidzein to a considerable extent to S-equol (Gu *et al*., 2006).

In the Cohrane review on phytoestrogens from 2013, the authors conclude that there is no clear explanation for how phytoestrogens might act. It has been suggested that isoflavones may act as selective oestrogen receptor modulators (SERMs), exerting anti-oestrogenic effects in the high-oestrogen environment of peri-menopause and oestrogenic effects in the low-oestrogen environment of postmenopause, where they act as weak agonists by stimulating oestrogen receptors (Lethaby *et al*., 2013).

Importantly, the oestrogenic activity is more complex than receptor binding. Oestrogenic activity requires a consideration of other factors than receptor binding, including the recruitment of co-regulator proteins in the nuclear pathway as well as non-classical membrane oestrogen receptors. Limited information is available on the interaction between isoflavones and these processes (EFSA, 2015).

The available studies in animals on oestrogenic activity of soya isoflavones are discussed in the section on non-clinical toxicity.

3.1.2. Secondary pharmacodynamics

There are also hormone-independent activites of isoflavones reported in non-clinical studies. For example, inhibition of tyrosine kinase activity, inhibition of protein kinase C, inhibition of DNA topoisomerase II, antioxidant activity, anti-angiogenic effects and inhibition of breast cancer resistance

protein (BCRP) have been reported. These effects are obtained with isolated compounds *in vitro* at doses exceeding 10 µM (EFSA, 2015). There are also data from rats fed a high soya-isoflavone diet that revealed improved insulin secretion and better glycemic control (der Marderosian, 2015).

3.1.3. Safety pharmacology

No data found.

3.1.4. Pharmacodynamic interactions

In vitro, isoflavones inhibit thyroid peroxidase, the enzyme that catalyses iodination and coupling of tyrosine residues in thyroglobulin in the synthesis of thyroxine and triiodothyronine (T4 and T3). In the absence of iodine, genistein and daidzein bind covalently to thyroid peroxidase, causing an irreversible inhibition of enzyme activity. *In vivo*, the inhibition of thyroid peroxidase in rats by genistein did not result in any effects on T3/T4 or thyroid-stimulating hormone (TSH) levels (EFSA, 2015).

In experimental studies *in vitro* (MCF-7 cells) and in transgenic mice low doses of genistein has been shown to abolish the inhibitory effect of tamoxifen and show cell proliferation in oestrogen-receptor-positive breast cancer cells (Ju *et al*., 2002; Du *et al*., 2012; van Duursen *et al*., 2013).

3.1.5. Conclusions

Soya isoflavones have been reported to interact with the oestrogen receptors ERα and ERβ in several non-clinical studies. The relevance of these results to humans is not known.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

In the report from the European Food Safety Authority (EFSA) on the risk assessment for peri- and post-menopausal women taking food supplements containing isolated isoflavones, the non-clinical pharmacokinetic data on genistein and daidzein in the scientific literature have been reviewed (EFSA, 2015). The main findings are summarised below.

Absorption

Absorption and absolute bioavailability of genistein and daidzein have been studied in mice and rats. In female BALB/c mice after i.v. and oral administration at doses of 1.2 mg/kg bw genistein and 0.55 mg/kg bw daidzein, the absorption estimated by the comparison of AUCs of the total isoflavones, was complete. The bioavailability amounted to 9–14% for genistein and to 29–34% for daidzein. In another study, after i.v. and oral administration of 20 mg/kg bw genistein in mice, the bioavailability was 23.4% (EFSA, 2015)

After oral and i.v. administration in rats at doses of 4 mg/kg bw, genistein absorption was 56% in males and 111% in females. The bioavailability of genistein was estimated to 7% in male rats and 15% in female rats. In another study, oral genistein doses of 6.25 mg/kg bw, 12.5 mg/kg bw and 50 mg/kg bw and i.v. doses of 12.5 mg/kg bw were administrated and the bioavailability was 21.9%, 33.5% and 19.0%, respectively (EFSA, 2015).

Distribution

In a study in rats, genistein was reported to be distributed to all organs. After 2 and 7 hours, the highest amounts of genistein were measured in the gastrointestinal tract and in the liver and kidney. Genistein was also detected in the reproductive organs testis/ovary, uterus, prostate and vagina. In brain, fat, thymus, spleen, skeletal muscle and bone the concentration of genistein was lower than in the other organs mentioned above (EFSA, 2015).

Metabolism

Monkeys, rats, and mice are described as 100% equol producers, meaning that the microbiotas of these animals are uniformly able to transform daidzein to a considerable extent to S-equol (EFSA, 2015).

In a study in female cynomolgus monkeys (n=15), the metabolite profiles of daidzein and genistein were investigated after feeding a diet formulated with isoflavones for 5 weeks. The isoflavones genistein and daidzein as well as the microbial-derived daidzein metabolite S-equol were present in the serum of the monkeys, predominantly as sulphates (64.9% of total daidzein, 72.8% of total genistein, 64.2% of total S-equol), and to a lower extent as glucuronides (34.5% of total daidzein, 23.8% of total genistein, 29.6% of total S-equol). In the blood serum the proportion of the aglycone was 0.6%, 3.5% and 6.1% for daidzein, genistein and S-equol, respectively (EFSA, 2015).

Elimination

In rats given a single oral dose of a soya extract providing 74 µmol genistein and 77 µmol daidzein/kg bw as conjugates, the urinary excretion of daidzein and genistein was 17% and 11.9%, respectively, of the dose ingested over a 48-hour post-dose period. S-equol excretion was 5% of the daidzein dose and 41.9% of the genistein dose was excreted as 4-ethyl phenol. Faecal daidzein accounted for 2.3±0.5% and faecal genistein for 3.4±0.4% of the respective doses (EFSA, 2015).

Female Sprague–Dawley rats have been reported to excrete a high percentage of daidzein, genistein and S-equol in the urine as aglycones (33–47%). Of the original dose, 2.6% of genistein and 3.3% of daidzein was recovered in the 24-hour urine as aglycones plus phase II conjugates. In addition, 17.3% of the daidzein dose was excreted as S-equol, compared with only 0.3% and 0.2% which were excreted as *O*-desmethylangolensin and dihydrodaidzein, respectively. Total daidzein (aglycone plus phase II conjugates plus microbial metabolites) recovery in the 24-hour urine was 21.2% of the dose ingested (EFSA, 2015).

The excretion of daidzein have been studied in male and female Fischer F344 rats after administration of daidzein (100 mg/kg bw, dissolved in corn oil) by gavage. For both sexes, 86% of the dose was excreted as unchanged daidzein in the faeces within 36 hours after administration, and 8–9% of the dose was excreted in the urine within 24 hours after administration (EFSA, 2015).

In female cynomolgus monkeys, 89-96% of daidzein, genistein and S-equol have been reported in the urine as aglycones (EFSA, 2015).

Pharmacokinetic interactions

Rats pretreated for five days with two different amounts of soya extract (ground, powdered beans, defatted with petroleum ether; 150 mg/kg and 500 mg/kg, respectively), were given a single dose of valproic acid (50 mg/kg i.v.), which resulted in decreases of 57% and 65%, respectively in the C_{max} of valproic acid as compared with the control group. AUC of valproic acid decreased to 83% and 70%, respectively in the soya pretreated groups. A significant induction of the uridine 5'-diphospho-glucuronosyltransferase (UGT) activity was seen, which is in line with other reports on soya foods. It has been reported that valproic acid is metabolised to a large extent by liver UGT enzymes, which might explain the decreased plasma exposure and C_{max} seen in this study (Marahatta *et al*., 2014).

The influence of soya bean administration on the bioavailability of carbamazepine and omeprazole was studied after single dose administration (10 g/kg p.o.) and after chronic administration for 15 days in rats. Carbamazepine was administered orally at a dose of 10 mg/kg and omeprazole at a dose of 20 mg/kg. A decrease in the bioavailability of carbamazepine was seen after both single dose and chronic administration, with significant decreases in C_{max}, T_{max} and AUC. On the contrary, soya bean

administration increased the bioavailability of omeprazole by producing an increase in C_{max} and AUC after single dose and after chronic administration. The half-life of omeprazole was also increased (Singh and Asad, 2010).

Genistein and daidzein have been found to increase the function and expression of P-glycoprotein (P-gp) in human intestinal cells, raising concerns on potential interactions with concomitant drugs binding to P-gp in intestine (Poluzzi *et al.*, 2014). Furthermore, genistein and equol has been found to inhibit CYP1A2 and 2E1 isoenzymes *in vitro* (Helsby *et al.*, 1998).

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

No data found.

3.3.2. Repeat dose toxicity

EFSA risk assessment

In the report from the EFSA on the risk assessment for peri- and post-menopausal women taking food supplements containing isolated isoflavones, the panel assessed the toxicity on the mammary glands, uterus and thyroid gland. Only studies conducted in the most appropriate animal model available which would be considered representative of this population (i.e. ovariectomised animals) have been considered for the risk assessment. A minimum duration of 5 days was used for animal studies, which was an intermediate cut-off point between the minimum recommended duration of 3 days for the test in ovariectomised animals and the minimum recommended duration of 7 days for weak oestrogens in accordance with the uterotrophic bioassay in rodents (OECD TG 440, 2007) (EFSA, 2015). The main findings of evaluated studies are summarised below.

Mammary gland

Ten studies in ovariectomised animals were found investigating cell proliferation in mammary gland (using the Ki-67 prolifiration marker), and 11 animal studies were identified investigating histopathological changes in the mammary gland of animals treated with isoflavones. In the majority of the studies no effect was noted. In two rat studies a stimulating effect on the mammary gland was observed (genistein 5.4 and 54 mg/kg per day and 221 mg/kg bw per day, both studies carried out for 90 days) (EFSA, 2015).

Uterus

Under normal conditions oestradiol secreted from the developing follicle increases the thickness of the endometrium by stimulating the growth of the epithelial cells. This biological response of the uterus to exogenous oestrogens is the basis of the uterotrophic bioassay in rodents, an OECD-validated guideline for screening of oestrogenic properties of chemicals (OECD TG 440, 2007). Either immature female or ovariectomised rats or mice are administered test substances and uterine weight gain is assessed. Isoflavones are known to cause uterotrophic effects in rodents and the guideline includes specifications on maximum levels of genistein in the rodent feed, in order not to reduce the sensitivity of the test (EFSA, 2015).

Thirteen studies in animals investigated uterus cell proliferation, among them three in monkeys, and 22 animal studies examined uterus histopathological changes, among them four studies in monkeys. An effect of isoflavones was not seen in most of the studies. However, a daidzein-rich soya extract

containing daidzein at doses above 40 mg/kg bw per day, caused an increase in cell proliferation of the epithelium and the stroma of the uterus as well as the vaginal epithelium of rats (EFSA, 2015).

Thyroid gland

In vitro, isoflavones inhibit thyroid peroxidase, the enzyme that catalyses iodination and coupling of tyrosine residues in thyroglobulin in the synthesis of thyroxine and triiodothyronine (T4 and T3). In the absence of iodine, genistein and daidzein bind covalently to thyroid peroxidase, causing an irreversible inhibition of enzyme activity. *In vivo*, the inhibition of thyroid peroxidase in rats by genistein did not result in any effects on T3/T4 or thyroid-stimulating hormone (TSH) levels (EFSA, 2015).

Several mechanisms reported from *in vitro* and animal experiments, other than the inhibition of thyroid peroxidase, suggest that isoflavones have an effect on thyroid hormone metabolism. In addition, soya dietary products have been reported to interfere with thyroid hormone absorption (EFSA, 2015).

NTP CERHR risk assessment

The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) has reviewed results of oestrogenicity testing in laboratory animals fed soya-based diets. These studies and the main findings are summarised in table 2 below (NTP CERHR, 2006).

Table 2: Results from oestrogenicity testing in laboratory animals fed soya-based diets summarised by the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) in 2006 (NTP CERHR, 2006)

Animal	Design	Endpoint(s)	Results	Reference
Alpk rat, 21 days old	Rats were fed RM1 (standard) diet or AIN-76A (phytoestrogen-free diet) for 3 days. Some rats fed AIN-76A diet were also administered the anti-estrogen Faslodex.	Uterine weight	Higher in rats fed AIN-76A diet compared to RM1 diet, an effect that was eliminated with Faslodex treatment. The authors had no explanation for the results.	Ashby et al. *(78)*; abstract; Ashby et al. *(77)*
Sprague Dawley rat, > 40 days old, ovariectomized	Rats were fed a soy diet containing 117.8 mg isoflavone/1800 calories or an alcohol-extracted soy diet with 11.6 mg isoflavone/1800 calories for 2 months.	Vaginal cytology, uterine weight, endometrial cell proliferation, lactoferrin expression, luminal epithelial cell height, or apoptosis	No significant effects	Tansey et al. *(79)*
	Rats were fed casein diets or soy diets containing low or high isoflavone levels (as described above) + conjugated equine estrogen at 0.313 or 0.625 mg/1800 calories.	Vaginal cytology, uterine weight, endothelial cell proliferation, and apoptosis.	Estrogen induced changes in all parameters examined; soy isoflavones did not further affect these parameters.	
		Lactoferrin staining	High isoflavones attenuated staining induced by estrogen.	
		Luminal epithelial cell height	High isoflavones attenuated increase induced by estrogen	
Female Sprague Dawley rat, 24 days old	Multigeneration design using casein-based diet or alcohol-washed, isoflavone-poor soy protein diet. A commercial soy extract was added to the isoflavone-poor diet, providing isoflavone levels of 31.7–1046.6 mg/kg feed. Juvenile F_2 females were evaluated on PND 4. Some females were given sc ethinyl estradiol or bisphenol A from PND 21.	Uterine weight, peroxidase, and epithelial height	All 3 estrogenic endpoints were increased by the highest isoflavone diet (1046.6 mg/kg feed). There was no interaction with ethinyl estradiol or bisphenol A except additivity between ethinyl estradiol and isoflavones at the highest dietary level.	Wade et al. *(82)*
Female F344 rat, 3 months old	For 14 weeks, rats (10–14/group) were fed either a casein-based diet, a diet containing 100 g/kg isolated soy	Uterine wet weight and histopathology	No significant effects.	Nakai et al. *(86)*

	protein (2.14 mg aglycones/g isoflavone), a diet containing 200 mg/kg isolated soy protein, a casein-based diet containing 17.2 g/kg isoflavones (11.37 mg aglycones/g isoflavones), or a casein-based diet containing 34.4 g/kg isoflavones.			
Male adult NMRI mouse exposed to diethylstil-bestrol as neonate and castrated in adulthood.	Mice were fed soy-free diets or diets containing 7% roasted soy meal, for up to 10–20 days following castration; 17β-estradiol was given to some mice in each dietary group.	Prostatic metaplastic transformation and expression of c-*fos* oncogene (endpoints of estrogenic action)	Soy diets did not affect either endpoint and did not alter estrogenic effects in mice exposed to 17β-estradiol.	Mäkelä et al. *(87)*
Female Han-NMRI mouse, 16 days old	Mice weaned at 16 days old to soy-free diet or diet containing 7% roasted soy meal. diethylstilbestrol was added to some diets (6 μg/kg **[kg feed assumed]**).	Relative uterine weight after 7 days on diet	Relative uterine weight increased 10–15% by soy diet. Soy diet decreased the diethylstilbestrol-associated increase in relative uterine weight.	Mäkelä et al. *(83)*
Female CD-1 mouse, 15 days old	Mice were weaned at 15 days of age and fed 1 of the following diets for 3, 5, or 7 days: Rodent Chow #5002 (no information on dietary components); Rodent Chow 5001 (reported to have high isoflavone levels and assumed to be soy based); Mouse Chow #5015 (reported to have high isoflavone levels and assumed to be soy based); NIH-07 (12% soybean meal); NIH-31 (5% soybean meal); or AIN-76A (casein based).	Uterine:body weight ratios	Compared to the 5002 diet, uterine weight:body weight ratios were higher with the 5015 diet, NIH 31 diet, and the AIN-76A diet on days 3, 5, and 7; no significant increases in uterine weight were noted for the 5001 or the NIH-07 diets compared to the 5002 diet.	Thigpen et al. 1987 *(84)* and Thigpen et al. 1999 *(85)*
Adult cynomolgus (*Macaca fascicularis*) monkey, ovariectomized	Monkeys (n=12) were fed a soy protein isolate diet providing a dose of 26.6 mg free genistein/monkey/day (the equivalent of a women receiving 99.7 mg genistein/day **[~2 mg/kg bw/day assuming a 58 kg bw]**[b]). A control group (n=13) was given isoflavone-extracted soy diet and a positive control group the extracted soy diet supplemented with estrogen (n = 15). Animals were fed the diets for 6 months.	Vaginal maturation and karyopyknotic indices	Not significantly affected by soy diet	Cline et al. *(80)*
Adult cynomolgus (*Macaca fascicularis*) monkey, ovariectomized	For 36 months, monkeys (n=57–62/group) were fed soy protein isolate that was alcohol treated to remove isoflavones (negative control), untreated soy protein isolate (~91 mg genistein, 31 mg daidzein, and 7 mg glycitein), or alcohol-extracted soy protein isolate containing conjugated equine estrogens (positive control).	Breast and uterine proliferation, sex steroid receptor expression, and serum estrogen level	In the soy protein isolate group, there was no increase in breast or uterine proliferation or steroid receptor expression; mammary gland thickness and serum estrone and 17β-estradiol levels were significantly reduced.	Wood et al. *(81)*.

[a]Since statistical significance was not clearly indicated, only obvious effects are listed.
[b]Assumptions used in dose estimates obtained from *(88)*.

3.3.3. Genotoxicity

Genistein has been reported to be clastogenic in numerous studies conducted *in vitro* (EFSA, 2015). The mechanism of genistein genotoxicity appears to be associated with an inhibition of DNA topoisomerase II (Markovits *et al*., 1989). This genotoxic mechanism is known to lead to the induction of chromosomal aberrations via a thresholded mechanism. Genotoxic positive findings expressed *in vitro* in mammalian cells has also been reported by the two catecholic oxidative metabolites of daidzein 3',4',7-trihydroxyisoflavone and 4',6,7-trihydroxyisoflavone through the same mechanism as genistein. The effect has not been confirmed in studies *in vivo*. EFSA concludes on these bases, that the use of isoflavones in food supplements is not of genotoxic concern (EFSA, 2015).

In the National Toxicology Program (NTP) report of the toxicology and carcinogenesis 2-year study on genstein, the genotoxicity studies on the isoflavone in the scientific literature were reviewed. The ability of genistein to induce chromosomal damage in *in vitro* systems, but not *in vivo* was also

concluded by the NTP. Furthermore, the NTP describes that genistein has been tested for mutagenicity in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535, and TA1538 with and without a rat liver S9 metabolic activation system, and the results were negative (NTP TR-545, 2007).

Misra *et al*. reported a statistically significant but modest (less than twofold) positive mutagenic response in TA100 with S9 activation using an isoflavone mixture containing 40% to 50% genistein, 18% to 25% daidzein, and 1% to 4% glycitein. No statistically significant increases in the number of revertants were seen in TA 1535, TA 1537 and TA 98 (Misra *et al*., 2002).

3.3.4. Carcinogenicity

Questions have been raised concerning the carcinogenicity of isoflavones, due primarily to its proposed weak oestrogenic effects. Hence, genistein was selected by the National Toxicology Program (NTP) to be examined in a 2-year study on Sprague-Dawley rats to evaluate the toxicology and carcinogenesis. The study consisted of three separate study components; in each, animals were exposed to genistein from the time of conception and through weaning through their mothers, who were given genistein in their feed. In one study, feed containing 5, 100, or 500 ppm of genistein were given to groups of 50 male and female rats from conception through two years (see table 3 for approximate ingested doses). In the second study, groups of 50 male and female rats were given the same feed concentrations up to 20 weeks following birth, followed by untreated feed for the remainder of the two years. In the third study, groups of 50 male and female rats were exposed from conception through weaning, and then given untreated feed for the duration of the study. Control animals received the same feed with no chemical added. At the end of the study, tissues from more than 40 sites were examined for every animal (NTP TR-545, 2007). The results are presented in table 4 below.

Table 3: Approximate ingested doses of genistein in rats exposed to 5, 100, or 500 ppm genistein in the 2-year feed study of genistein. The mean ingested dose was calculated for each available week by multiplying the dietary concentration of genistein (ppm) by the mean measured amount of feed ingested weekly and dividing the result by the mean body weight for the week. These values were divided by 7 to give the mean daily dose given in the table. Data are presented as mg genistein/kg body weight per day. PND=postnatal day (NTP TR-545, 2007).

	5 ppm	100 ppm	500 ppm
F_0 Dams, nonlactating period	0.5 ± 0.0 (9)	8.9 ± 0.5 (9)	44.7 ± 2.6 (9)
F_0 Dams, lactating period	0.7 ± 0.1 (3)	14.9 ± 1.3 (3)	74.7 ± 5.2 (3)
F_1 Female pups, continuous dosing, before PND 140	0.4 ± 0.0 (17)	8.4 ± 0.6 (17)	43.7 ± 2.8 (17)
F_1 Female pups, truncated dosing, before PND 140	0.4 ± 0.0 (17)	8.4 ± 0.5 (17)	44.3 ± 2.8 (17)
F_1 Female pups, continuous dosing, after PND 140	0.3 ± 0.0 (21)	5.1 ± 0.1 (21)	28.9 ± 0.7 (21)
F_1 Male pups, continuous dosing, before PND 140	0.4 ± 0.0 (17)	7.2 ± 0.7 (17)	36.8 ± 3.6 (17)
F_1 Male pups, truncated dosing, before PND 140	0.4 ± 0.0 (17)	7.2 ± 0.6 (17)	36.9 ± 3.3 (17)
F_1 Male pups, continuous dosing after PND 140	0.2 ± 0.0 (21)	4.0 ± 0.1 (21)	20.2 ± 0.4 (21)

Table 4: Summary of the 2-year carcinogenesis study of genistein in Sprague-Dawley rats (NTP TR-545, 2007)

	F_1C		F_1T140		F_3T21	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Concentrations in feed	0, 5, 100, or 500 ppm	0, 5, 100, or 500 ppm	0, 5, 100, or 500 ppm	0, 5, 100, or 500 ppm	0, 5, 100, or 500 ppm	0, 5, 100, or 500 ppm
Body weights	Exposed groups generally similar to control group	500 ppm group less than the control group	500 ppm group less than the control group	500 ppm group less than the control group	Exposed groups generally similar to the control group	Exposed groups generally similar to the control group
Survival rates	36/54, 41/50, 43/50, 31/50	26/54, 28/50, 22/50, 21/49	36/54, 34/50, 32/50, 38/50	26/54, 31/50, 32/50, 23/50	33/52, 42/50, 33/50, 36/50	33/53, 30/50, 29/50, 25/50
Early onset of aberrant estrous cycles	N/A	500 ppm	N/A	500 ppm	N/A	500 ppm (also some evidence for effects at 5 and 100 ppm)
Nonneoplastic effects	None	None	None	None	None	None
Neoplastic effects	None	Mammary gland: adenoma or adenocarcinoma (9/54, 4/50, 8/50, 16/49) Pituitary gland: adenoma or carcinoma (38/54, 40/50, 34/50, 46/49)	None	None	None	None
Equivocal findings	None	None	None	Pituitary gland: adenoma or carcinoma (38/54, 32/49, 40/50, 44/50)	None	Mammary gland: adenoma or adenocarcinoma (7/53, 8/49, 11/50, 13/50)
Decreased incidences	None	Mammary gland: fibroadenoma (32/54, 27/50, 28/50, 12/49)	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	Some evidence	No evidence	Equivocal evidence	No evidence	Equivocal evidence

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

The NTP concluded that under the conditions of this study, in none of the three sub studies were any increased rates of cancer in male rats. In female rats exposed to genistein from conception and throughout two years, the NTP concluded that the rates of adenoma or adenocarcinoma of the mammary gland and pituitary gland adenoma or carcinoma were increased. Furthermore, the NTP concluded that in female rats exposed to genistein for 20 weeks following birth, the rates of pituitary gland adenoma or carcinoma were slightly increased, and in female rats exposed to genistein just from conception through weaning, the rates of mammary gland adenoma or adenocarcinoma were slightly increased (NTP TR-545, 2007).

3.3.5. Reproductive and developmental toxicity

The reproductive and developmental toxicity have been evaluated for dietary soya products due to their proposed oestrogenic activity and their role as possible endocrine disruptors have also been discussed (Messina and Loprinzi, 2001).

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) have established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR). The purpose of the Center is to provide scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction and development caused by agents to which humans may be exposed. The NTP CERHR has reviewed results of oestrogenicity testing in laboratory animals fed soya-based diets to evaluate the safety of soya formula. Soya formula contains soya protein isolates and is fed to infants as a supplement to or replacement for human milk or cow milk. The report was published in 2006 and according to the NTP CERHR panel there are only a few studies that have examined the effects of soya product exposure on female reproduction in experimental animals. The panel noted that no reproductive effects were observed in female and male rats or female monkeys at total dietary isoflavone concentrations estimated to be similar or substantially higher (approximately 10 times) than those normally consumed in soya formula by infants. The panel concluded that evidence was insufficient to conclude that soya infant formula would or would not produce reproductive toxicity in men or women and that evidence was insufficient to conclude that soya infant formula would or would not produce reproductive toxicity in experimental animals (NTP CERHR, 2006).

Regarding developmental toxicity, the NTP CERHR panel describes several studies on soya-based diet in rodents. The effects reported in these studies were inconsistent and the planel noted that these differences may reflect different isoflavone contents of the diets used in different studies or other differences in the composition of the feed (NTP CERHR, 2006).

Furthermore, genistein was selected by the National Toxicology Program (NTP) to be examined in a protocol utilizing Sprague-Dawley rats to evaluate the effects of multigenerational and long-term exposures. This study extended over five generations of rats following a parental group of rats that were exposed to genistein in their feed starting at the age of 6 weeks. The first and second generations of offspring were exposed to genistein during conception through their mothers, during weaning through their mothers' milk, and during their lifetimes through feed containing genistein. The third generation was exposed just during gestation and weaning, and the fourth and fifth generations were not exposed directly, to see if any carryover effects resided from exposure of earlier generations. The dosed feed contained 5, 100, or 500 ppm of genistein (see table 5 for approximate ingested doses). The primary measures examined during each generation were body weights, development of reproductive organs, and number of offspring per litter after each cycle of mating (NTP TR-539, 2008).

The NTP concluded from the main findings of the study that exposure to 500 ppm of genistein caused lower body weights and some alterations in the reproductive system of female rats. Exposure to genistein caused lower body weights in one generation of male rats and increases in mammary gland

hyperplasia and renal tubule calcification. Except for lower body weights in pups, there was no evidence for a carryover of genistein effects into unexposed generations (NTP TR-539, 2008).

Table 5: Ingested doses of genistein in rats exposed to 5, 100, or 500 ppm genistein in the multigenerational reproductive toxicology feed study of genistein (NTP TR-539, 2008)

Sex/Dosing Period	Generation	Mean Dose (mg/kg per day) ± Standard Error		
		5 ppm	100 ppm	500 ppm
Male, Entire Feeding Period				
	F_0	0.3 ± 0.03 (12)	5.9 ± 0.5 (12)	28.9 ± 2.5 (12)
	F_1	0.4 ± 0.03 (17)	7.1 ± 0.5 (17)	37.6 ± 2.6 (17)
	F_2	0.3 ± 0.03 (17)	7.4 ± 0.7 (17)	35.7 ± 2.6 (17)
	F_0 - F_2 inclusive	0.3 ± 0.02 (46)	6.9 ± 0.3 (46)	34.6 ± 1.6 (17)
Female, Entire Feeding Period				
	F_0	0.5 ± 0.06 (12)	10.0 ± 1.2 (12)	50.4 ± 6.0 (12)
	F_1	0.5 ± 0.03 (16)	9.8 ± 0.7 (16)	50.6 ± 3.8 (17)
	F_2	0.5 ± 0.04 (17)	10.2 ± 0.7 (17)	50.7 ± 3.7 (17)
	F_0 - F_2 inclusive	0.5 ± 0.02 (45)	10.0 ± 0.5 (45)	50.6 ± 2.4 (46)
Female, Nonlactating				
	F_0	0.4 ± 0.01 (9)	8.0 ± 0.4 (9)	39.8 ± 1.8 (9)
	F_1	0.4 ± 0.02 (13)	9.0 ± 0.7 (13)	45.4 ± 2.7 (14)
	F_2	0.5 ± 0.03 (14)	9.3 ± 0.6 (14)	45.0 ± 2.4 (14)
	F_0 - F_2 inclusive	0.4 ± 0.02 (36)	8.9 ± 0.4 (36)	43.9 ± 1.4 (37)
Female, Lactating				
	F_0	0.8 ± 0.08 (3)	16.1 ± 1.8 (3)	82.2 ± 9.2 (3)
	F_1	0.6 ± 0.08 (3)	13.3 ± 1.7 (3)	74.6 ± 8.5 (3)
	F_2	0.7 ± 0.04 (3)	14.4 ± 1.3 (3)	77.2 ± 3.6 (3)
	F_0 - F_2 inclusive	0.7 ± 0.04 (9)	14.6 ± 0.9 (9)	78.0 ± 3.9 (9)

3.3.6. Local tolerance

The allergic potency of soya has been evaluated and presented in the 'Public statement on the allergenic potency of herbal medicinal products containing soya or peanut protein' (EMA/HMPC/138139/2005) (see section 5.3 Adverse events, serious adverse events and deaths).

3.3.7. Other special studies

Not relevant

3.3.8. Conclusions

In the scientific literature, there are no published results from non-clinical studies on the ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) included in medicinal products on the EU/EEA-market. On the contrary, there is an enormous amount of non-clinical scientific publications regarding dietary soya products and isolated isoflavones. However, the composition of the isoflavone content differs markedly between products and is affected by processing method. Therefore, it is not generally feasible to apply a read-across approach between different preparations.

3.4. Overall conclusions on non-clinical data

Soya isoflavones have been reported to interact with the oestrogen receptors ERα and ERβ in several non-clinincal studies. The relevance of these results to humans is not known. There are also significant inter-species differences in ADME making extrapolation of the effects seen in animals to humans

complex. This is e.g. the case for the pharmacokinetic and pharmacodynamics interactions reported from studies in rats and *in vitro*.

In the scientific litterature, there are no published non-clinical toxicity studies on the ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) included in medicinal products on the EU/EEA-market. On the contrary, there is an enormous amount of non-clinical scientific publications regarding dietary soya products and isolated isoflavones. However, it is common that the isoflavone content is expressed without specifying whether the amount refers to the aglycone or glycoside value. This distinction is critical, since the amount of the aglycone present is approximately 60% of the total weight of the glycoside. In addition, the composition of the isoflavone content differs markedly between products and is affected by processing method. Therefore, it is not generally feasible to apply a read-across approach between different preparations.

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Thermoregulatory disturbances like hot flushes and night sweats, also referred to as vasomotor symptoms, are the most common menopausal symptoms and can occur both during peri- and postmenopause. There is currently no consensus on the physiological pathways involved in the occurrence of menopausal hot flushes. Oestrogen withdrawal, and not just deficiency, plays a key role, but might not solely be responsible, since there appears to be no correlation between oestrogen levels and vasomotor symptoms. Neurotransmitters involved in thermoregulation, such as norepinephrine and serotonin have also been implicated in the onset of hot flushes, as well as β-endorphins (NIH, 2004).

The prevalence of vasomotor symptoms varies with ethnicity. Flushes are less common among East Asian women (median 16%) than among Northamerican and European women (median 55%). Up to 40% of Western women are affected severely enough to seek medical help. Although hot flushes are reported as more prevalent and intense in the perimenopausal and early postmenopausal years, they continue to be important in up to 14.6% of women in their sixties and in 8.6% of women in their seventies (Lethaby *et al*., 2013).

There is no clear explanation on how soya isoflavone mediated effects on menopausal symptoms might work. Isoflavones bind to both oestrogen receptors (ERα and ERβ) *in vitro* and are referred to as phytoestrogens although oestrogenic effects are often not observed *in vivo*. This is not surprising as receptor binding alone is a poor predictor of *in vivo* activity. ER ligands often have very different, and sometimes opposite, physiologic effects depending upon the manner which the ligand-receptor complex interacts with co-activators and co-repressors within the cell (Messina and Redmond, 2006).

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Absorption

The isoflavone glycosides (genistin and daidzin, respectively) are hydrolysed in the gastrointestinal tract as a result of bacterial β-glucosidase activity (Setchell, 2001). The bioavailable aglycones (genistein and daidzein, respectively) may be absorbed into the circulation or further metabolised into an array of other metabolites by the colonic microflora (Poluzzi *et al.*, 2014). Importantly, variability of isoflavone bioavailability can be due to inter-individual differences in metabolism. Only 20-35% of the

adult population is able to convert daidzein into equol (Setchell *et al*., 2001). The prevalence of equol producers ranges from 20–30% in the population of Western countries to 50–60% in Asian populations consuming soya-containing diets (EFSA, 2015). In addition, individual variability in colonic microflora affected by e.g. diet, antibiotic use, surgery or bowel disease, seems to play an important role in determining the preferred pathways of isoflavone metabolism and the bioavailability of isoflavones (Murkies *et al*., 1998; Poluzzi *et al*., 2014).

In humans, the absorption from urinary data was estimated to be 61.3% of the dose for daidzin, 60.4% for glycitin and 35.4% for genistin. After 6.6 mg, 13.2 mg and 26.4 mg of daidzein and of 9.8 mg, 19.6 mg and 39.2 mg of genistein in the glycosylated form, the absorption, calculated from urinary excretion, was found to decline with increasing dose (daidzein: 63.2%, 54.4% and 44.0%; genistein: 25.2%, 13.4% and 15.8%). In another study, the absorption, calculated from urinary excretion data, was 88.5% for daidzein and 44.3% for genistein (EFSA, 2015). However, other authors have reported that urinary isoflavone concentrations are correlated poorly with maximal serum concentrations, limiting the use of urinary measurement as predictor of systemic bioavailability (Setchell *et al*., 2001; Setchell, 2001; Setchell *et al*., 2003; Andres *et al*., 2011).

Peak plasma concentrations of genistein and daidzein are attained in 5 to 6 hours after oral ingestion in adults. Nonlinear increases of AUC for the isoflavones have been seen for doses exceeding 0.5 mg/kg indicating that the absorption capacity may become saturated at high doses (Setchell *et al*., 2001; Setchell, 2001; Setchell *et al*., 2003; Andres *et al*., 2011).

The absorption of isoflavones has been reported to be biphasic indicating an entero-hepatic recirculation (Vergne *et al*., 2007).

Distribution

Absorbed isoflavones circulate primarily in the conjugated form, mostly bound to glucuronic acid (Messina and Loprinzi, 2001).

Metabolism

Daidzein may be metabolised to *O*-demethylangolensin (*O*-DMA) and equol, and genistein to 6'hydroxy-*O*-DMA and hormonally inert p-ethylphenol (Poluzzi *et al*., 2014). Biochanin A is mainly demethylated to genistein and formononetin is demethylated to daidzein (EFSA, 2015).

Concentrations of different phytoestrogen metabolites vary widely between individuals even when controlled quantities of an isoflavone supplement are administered. Enzymes being subject to polymorphism are involved in the endogenous metabolism of phytoestrogens implying the importance of the woman's genotype. Since exogenous metabolism is predominantly determined by the gastrointestinal flora, antibiotic use and bowel disease will possibly modify metabolism. Concurrent dietary intake, in particular high dietary fibre, vegetable and fruit intake and duration of exposure, seems to exert a major influence on isoflavone metabolism as well (de Cremoux *et al*., 2010; Murkies *et al.,* 1998).

Soukup *et al*. investigated the phase II metabolite profile in plasma and urine of 11 German postmenopausal women after ingestion of a bolus dose of a commercial soya extract. The result confirmed the findings of Hosoda *et al*. that sulphoglucuronides are the major metabolites of daidzein and genistein in the plasma and that the 7-O-glucuronides are the predominant metabolites in the urine (Soukup *et al*., 2014; Hosoda *et al*., 2011).

Elimination

Most studies estimate the half-life of isoflavones to be between 4-8 h (Messina and Redmond, 2006). The elimination of isoflavones is suggested to be linear with a $t_{½}$ of daidzein of about 8 h and of

genistein of about 15 h. From 0-24 h, the genistein extraction profile in urine was bell-shaped with a maximal excretion peak at 12 h. The daidzein excretion profile is similar. The authors also conclude that from the data obtained in their study, there is a significant difference in daidzein urine excretion between equol producers and non equol producers, while no difference in plasma pharmacokinetic parameters of isoflavones was revealed between these populations subtypes (Vergne *et al*., 2007).

Reported values for the elimination $t_{½}$ of total genistein are in the range of 5.7–10.8 h and were determined in intervention studies using different soya foods (soya flour, soya beverage, soya nuts, soyabean powder) as isoflavone source. In one study by Setchell *et al*., ^{13}C-labelled genistein and daidzein were used. The $t_{½}$ values of total genistein and total daidzein were determined to be 7.41 ± 0.39 h and 7.18 ± 0.49 h, respectively, for a dose of 0.8 mg genistein and 0.8 mg daidzein/kg bw (Setchell *et al*., 2003; EFSA, 2015).

Isoflavones undergo enterohepatic circulation and may be excreted in the bile, deconjugated by intestinal flora, reabsorbed, reconjugated by the liver, and excreted in the urine, primarily as glucuronide conjugates. Total faecal excretion of isoflavones in humans has been reported to account for less than 5%, and is predominantly in the unconjugated form, with less than 10% being conjugated (Poluzzi *et al*., 2014; EFSA, 2015).

4.2. Clinical efficacy

In addition to the herbal preparations reported as constituents of medicinal products, there is a broad range of dietary soya products on the market. The composition of the bioactive compounds differs markedly between products and is affected by processing method. Therefore, only ethanolic extracts of soya bean containing isoflavones included in medicinal products on the EEA-market are evaluated in this section.

4.2.1. Dose response studies

No relevant clinical study has been found.

4.2.2. Clinical studies (case studies and clinical trials)

For the database search on ethanolic extracts of soya bean containing isoflavones used for the relief of menopausal symptoms, all clinical studies found, whether controlled or not, have been included. However, only studies on products on the EEA market as medicinal products were further evaluated. Studies on products with unknown composition as well as unclear or irrelevant posology were excluded. Also, studies on isolated pure genistein or synthetic genistein have been excluded. The included study is presented in table 6 below.

Menopause is defined as occurring 12 months after last menstrual period. The years after menopause are called ‘postmenopause’, while ‘perimenopause’ refers to the menopausal transition period, and is characterised by changes in menstrual bleeding patterns and dramatically fluctuating hormone levels. This period can last for several years and ends 12 months after the last menstrual period.

The World Health Organization defines ‘perimenopause’ as the period immediately prior to the menopause (when the endocrinological, biological and clinical features of approaching menopause commence) and the first year after menopause and ‘postmenopause’ as the period dating from the final menstrual period. Although women progress through perimenopause to postmenopause it may not be possible to definitively categorise them as peri- or postmenopausal.

Due to lack of guidelines of the evaluation of efficacy of herbal medicinal products in the relief of menopausal symptoms, selected parts of the “Guideline on Clinical Investigation of Medicinal Products

for Hormone Replacement Therapy of Oestrogen Deficiency Symptoms in Postmenopausal Women" (EMEA/CHMP/021/97 Rev. 1) is considered appropriate to use. Since the potential target population for isoflavone treatment may deviate from the population eligible for HRT, it is acknowledged that the proposed endpoints might not be fully applicable. However, in order to achieve reliable results, the study population for isoflavone treatment still needs to be clearly predefined, and have comparable baseline characteristics, such as age, menopausal transition stage (peri- or postmenopausal), hormonal status, time since menopause, as well as frequency and severity of symptoms.

The guideline recommends:

- three months duration of treatment is recommended for symptom efficacy evaluation
- primary efficacy endpoint is frequency of moderate to severe hot flushes
- the Kupperman index (weighted 4-point severity scale of 11 menopausal symptoms) or other fully evaluated scales could be accepted as secondary endpoint

The guideline states that the population of perimenopausal women is not well defined for regulatory purpose. This transitional period is marked by irregular menstrual cycles and of variable "climacteric" symptoms, for a variable duration of time (EMEA/CHMP/021/97 Rev. 1).

In general, clinical studies on the relief of menopausal symptoms, including studies of hormone therapies, are often subject to a marked placebo response. A substantial Hawthorne effect can be seen, with enrollees reporting fewer hot flushes during the run-in period before assignment to treatment arm. Furthermore, the efficacy is often evaluated by subjective means, such as self-reported measures of hot flush frequency and severity, using questionnaires and diaries. In addition, the number of hot flushes is expected to decrease as women move through menopause, even in the absence of treatment (NIH, 2004). This makes adequately blinded, randomised placebo controlled studies vital for the assessment of efficacy. Lack of effect is an important reason for high drop-out rates in placebo groups, which also must be considered when designing trials and recruiting patients.

Table 6: Clinical studies on medicinal products on the EEA market containing ethanolic extracts of soya bean used for the relief of menopausal symptoms

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
To investigate the effect of oral ethanolic extract of soya bean on hot flushes in menopausal women Faure *et al*., 2002	Double-blind, randomised, multicentre, placebo controlled, 2 parallel groups Duration: 4 months	**Treatment:** Two capsules* two times daily corresponding to 70 mg isoflavones per day (n=39) or placebo (n=36) *soya germ isoflavone extract. Total isoflavone content 17.5 mg per capsule; 50% daidzin, 30% glycitin 20% genistin.	75 women, mean age in isoflavone group 53 yrs., placebo group 53.9 yrs. 20 premature study terminations; 6 from active group (15%) and 14 (39%) from placebo group, mainly due to inefficacy (4 from active group, 11 from placebo group).	Healthy French postmenopausal women (6 months since last menstruation, verified with FSH and 17β-estradiol) with minimum seven moderate to severe hot flushes or night sweats/24 hours Main exclusion criteria: other treatment of climacteric symptoms, hormone therapy within six weeks before the study.	61% reduction in daily moderate/severe hot flushes in soya group vs 21% reduction with placebo (p=0.01). Responders (at least 50% reduction at end of treatment): 66% in soya group vs 34% in placebo group (p<0.005). No effect on other menopausal symptoms.	**ITT:** yes (missing data imputed by last observation LOCF). **Power calculation:** 30 patients per arm required to give 90% power to detect a treatment difference of 3 hot flushes per 24 hours, assuming a standard deviation of 3.8 hot flushes per day (p=0.05).	Both peri- and postmenopausal women included. High, disproportionate drop-out rates (39% in placebo group) complicates an unbiased estimation of true treatment effect. Statistical analysis using ITT/LOCF generated a significant treatment effect, but not when using PP/observed data.
To evaluate the efficacy of	Randomised, placebo-	**Treatment:** Two tablets* two times	71 women, mean age in the	Healthy early postmenopausal	After four months of treatment there was a	No information	Inclusion and exclusion

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
ethanolic extract of soya bean on frequency and severity of menopausal symptoms in women in early postmeopausal period Stanosz *et al*., 2006	controlled, three-armed study. Duration: 12 months	daily corresponding to 104 mg isoflavones per day (n=22), 52 mg isoflavones per day (n=26) or placebo (n=23) *extract of soya bean. Total isoflavone content 40 mg (26 mg as genistein) per tablet	low dosage group 50.8 yrs, in the high dosage group 50.8 yrs and on the placebo group 49.5 yrs. Premature study terminations (drop-outs) and statistical analysis of drop-outs not discussed.	women, not further defined. No exlusion criteria stated.	statistically significant decrease in the total score for the low dosage group (9.6±4.9) and for the high dosage group (8.4±5.3) compared to the placebo group (14.8±7.7). At 12 months the menopausal symptoms had almost stopped for the patients treated with low and high dosage of the extract (2.42±1.84 and 1.95±1.40, respectively). In the placebo group the score was 17.52±4.34). After three months of treatment the reduction of weekly number of hot flushes was statistically significant between	given.	criteria not sufficiently described. Double-blinding of the study not adequately described Handling of drop-outs (i.e. the risk of selective reporting bias) not reported.

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
					the high dosage group and placebo (65% versus 34% reduction, $p<0.05$) and between the low dosage group and placebo at five months of treatment (89% versus 41%, $p<0.05$). At 12 months a 100% reduction in the number of hot flushes were reported for both the low dose and high dose group versus 14% in the placebo group.		

Assessor's comments

In the study by Faure et al., the product contains a dry extract from the soya bean germ (hypocotyl) (DER 43-53:1), extraction solvent ethanol 60% V/V. The extract contains 10% isoflavones calculated as the sum of isoflavone glycosides. The product is a medicinal product in Belgium and Hungary.

The study by Faure et al. was a pilot, multicentre, randomised, double-blind, placebo-controlled, parallel-group trial. 75 French women in natural or surgical menopause were studied for four months. The treatment was either 70 mg standardised soya isoflavone per day given as two capsules two times daily (n=39) or placebo (n=36). The patients were seen at a screening visit and were then assessed 2 weeks later, before randomisation, and then again at treatment weeks 4, 8 and 16. For the entire study period, including the prestudy period, each participant filled out a daily evaluation of the number of moderate to severe hot flushes (including night sweats). Adverse events were collected at each visit. The study included women with at least seven moderate or severe flushes per 24 hours during the 2 weeks of the prestudy period. Time since last period should be at least 6 months. No hormone replacement therapy (HRT) or any other drug used for the treatment of climacteric symptoms, such as vitamin E or clonidine, was allowed. Patients previously on HRT had to stop treatment at least 6 weeks before the study period. The mean age was 53.0 (SD±5.6) and 53.9 (SD±4.1) in the soya group and placebo group, respectively. No information on randomisation or treatment allocation is presented. No information on time since last menstrual period is provided. The study size was calculated to give 90% power to detect a treatment difference of three hot flushes per day assuming a standard deviation of 3.8 hot flushes per day (p=0.05). Data were analysed using both intention to treat (ITT) and per protocol (PP). For the ITT analysis, the last recorded treatment value was used in accordance with the "last observation carried forward" (LOCF) principle and included patients who withdrew prematurely from the trial. For the PP analysis, only data observed at the 16-week endpoint were analysed. Responders were classified as patients whose number of hot flushes had been reduced by at least 50% at the end of the treatment period.

In the isoflavone group 61% (SEM 5.9) reduction of frequency of moderate to severe hot flushes, compared with 21% (SEM 16.9) in the placebo group (p=0.01). 65.8% of patients treated with soya extract were classified as responders, compared with 32.4% of patients on placebo. No effect on other menopausal symptoms was seen (data not presented). Three patients presented outlying data (marked increase of hot flush frequency during treatment; one in soya group and two in placebo group).

In the ITT analysis, repeated measures analysis of variance testing differences between treatment groups indicated treatment effect (p= 0.01) on the change in frequency of hot flushes (even after removing outliers), whereas time (p= 0.33) had no significant effect on hot flush frequency.

There was a high, disproportionate drop-out rate with 39% premature study terminations in the placebo group and 15% in the active group, rendering 33 completers in the isoflavone group and 22 completers in the placebo group. The main reason for drop-out was lack of efficacy (67% in the soya group and 79% in the placebo group, respectively). The p values from the PP analyses at the 16-week endpoint were not significant (outliers included and excluded, respectively). Even though ITT/LOCF was carried out, it is difficult to say which statistical analysis is the most appropriate (i.e. including or excluding outliers, using ITT/LOCF data or PP/observed data). Therefore an unbiased estimation of the true treatment effect cannot be achieved. The authors conclude that there is a need for further large studies investigating the areas of clinical effectiveness of isoflavone supplements in the treatment of menopausal symptoms (Faure et al., 2002).

In the study by Stanosz et al., the product contains a dry extract from soya bean (DER 100-400:1), extraction solvent ethanol 60%-70% V/V. The extract contains 40 mg isoflavones (26 mg as genistein). The product is a medicinal product in Poland (WEU herbal medicinal product).

The study by Stanosz et al., was a randomised and placebo-controlled trial. 71 women in "early menopause" were assigned to three groups and studied for 12 months. However, the author's definition of "early postmenopausal women" is missing. The treatment was either 200 mg ethanolic extract (corresponding to 52 mg genistein, n=26) per day given as one capsule of extract and one capsule of placebo two times daily, 400 mg ethanolic extract (corresponding to 104 mg genistein, n=22) per day given as two capsules of extract two times daily or two capsules of placebo two times daily (n=23).

The mean age in the low dosage group was 50.8±3.7, in the high dosage group 50.8±2.5 and in the placebo group 49.5±4.7. The mean menopause duration at the start of the study was in the low dosage group 17.7 months, in the high dosage group 20.6 months and in the placebo group 19.6 months. The standard deviation of mean menopause duration is not presented in the publication. The total score for menopausal symptoms according to Kupperman Index were at baseline 22.7±5.5 for the low dosage group, 21.9±6.5 for the high dosage group and 21.7±6.9 for the placebo group.

The inclusion criteria are poorly described in the study be Stanosz et al. For example, the least number of hot flushes per day is not stated neither is the lowest total score from the Kupperman Index evaluation. Enrolled subjects should have a defined minimum of hot flushes per day or Kupperman Index score at basline. The Kupperman Index calls for self-reported data from women and includes items on 11 menopausal symptoms, each of which is rated for severity on a 4-point scale (none to severe). According to Kupperman Index, a total score of < 20 is considered mild symptoms, a total score of 20-35 is considered moderate symptoms and a total score of >35 is considered severe symptoms. In the study by Stanosz et al., the total score at baseline for all groups were in the lower range of symptom severity, which raises the question whether the claimed findings could be relevant to a wider population of post-menopausal women. Furthermore, the exclusion criteria were not discussed and there was no information on hormone treatment or any other concomitant drugs used during the study period or before the study period.

Other important information missing in the study by Stanosz et al., are the power of the study, the number of drop-outs, and whether ITT or PP were used for data analysis. Also, randomisation is poorly described and the authors only state that the groups were well balanced in terms of age, parity and habits.

The baseline data of the patients were followed up at 1, 2, 3, 4, 6, 9, and 12 months of treatment using the Kupperman Index. There is no information if the patients used a diary to minute vasomotor symptoms such as the number of hot flushes per day. Daily recording decrease and recall errors is preferable to retroperspective measurement. Furthermore, the blinding of the personnel and investigators at follow up and at outcome assessment is not discussed ant it is not clear if the study was a double-blinded study or not.

The authors report that total Kupperman Index score was not different to placebo after three months of treatment of high or low dosage of soya bean extract. However, after four months of treatment there was a statistically significant decrease reported in the total score for the low dosage group (9.6±4.9) and for the high dosage group (8.4±5.3) compared to the placebo group (14.8±7.7). At 12 months the menopausal symptoms were claimed to have almost stopped for the patients treated with low and high dosage of soya bean extract (2.42±1.84 and 1.95±1.40, respectively). In the placebo group the score was 17.52±4.34.

The authors also report that the reduction of weekly number of hot flushes was statistically significant between the high dosage group and placebo at three months of treatment (65% versus 34% reduction, p<0.05) and between the low dosage group and placebo at five months of treatment (89% versus 41%, p<0.05). At 12 months a 100% reduction in the number of hot flushes were reported for both the low dose and high dose group versus 14% in the placebo group. No information regarding the clinical relevance of the treatment could be found, e.g. the reduction of the total number of hot flushes or the fact that there was no difference in the reduction of hot flushes compared to placebo until three months of treatment.

Adverse event occurring at any time during the study were assessed, but there are no adverse events presented in the publication (Stanosz et al., 2006).

To conclude, in the publication by Stanosz et al., several methodological deficiencies have been identified and the quality of the study is considered to be not sufficient to substantiate efficacy. Importantly, inclusion and exclusion criteria were not sufficiently described, the blinding of the study is not adequately described, and the omission of information regarding the handling of drop-outs (i.e. the risk of selective reporting bias) is considered a critical methodological deficiency.

Meta-analysis on isoflavones in the relief of menopausal symptoms

Several reviews have examined the efficacy of phytoestrogen products (e.g. from soya, red clover and flaxseed) in alleviating menopausal symptoms, but most have found no benefit or a very slight reduction in the frequency of daily hot flushes compared with placebo (Lethaby *et al*., 2013).

The Cochrane review by Lethaby *et al.* assessed the efficacy, safety and acceptability of food products, extracts and dietary supplements containing high levels of phytoestrogens (e.g. at least 30 mg per day of isoflavones) when compared with no treatment, placebo or hormone therapy (HT) for the amelioration of vasomotor menopausal symptoms (such as hot flushes and night sweats) in perimenopausal and postmenopausal women (Lethaby *et al*., 2013). Trials that included women who had breast cancer or a history of breast cancer were excluded. The primary outcomes were efficacy (change in vasomotor menopausal scores, change in frequency or severity of individual vasomotor symptom scores and incidence of vasomotor symptoms after treatment). Secondary outcomes were safety (stimulation of endometrium, vaginal stimulation and adverse events) and acceptability (withdrawal due to adverse events or satisfaction rates).

The review found 43 randomised controlled trials (4,364 participants). Many trials were small, of short duration and of poor quality and were determined to be at high risk of bias. The types of phytoestrogen used varied substantially between studies and only five trials with a red clover extract provided data suitable for inclusion in a meta-analysis. Individual results from the remaining trials were compared in broad subgroups such as dietary soya, soya extracts, genistein extracts and other types of phytoestrogens that could not be combined (Lethaby *et al*., 2013). No overall conclusive evidence showed that soya extracts had a positive effect on hot flush frequency or severity, although benefits derived from concentrates of genistein should be further investigated.

4.3. Clinical studies in special populations (e.g. elderly and children)

No relevant study in special populations has been found.

4.4. Overall conclusions on clinical pharmacology and efficacy

The clinical pharmacology and efficacy of ethanolic extracts of soya bean containing isoflavones in the relief of menopausal symptoms are rather complex to overview. As previously described, there is an inherent complexity with regards to the different isoflavones, their metabolites and pharmacokinetic

properties, as well as the lack of information on how different processing methods influence product composition and clinical efficacy. Therefore, the clinical efficacy should be evaluated for each preparation separately.

The results in the study by Faure *et al*., have a high risk of bias, mainly due to high, disproportionate drop-out rates. In addition, both peri-and postmenopausal women were included. Therefore an unbiased estimation of the true treatment effect cannot be achieved.

In the study by Stanosz *et al*., several methodological deficiencies have been identified and the quality of the study is considered to be not sufficient to substantiate efficacy. Importantly, inclusion and exclusion criteria were not sufficiently described, the blinding of the study is not adequately described, and the omission on information regarding the handling of drop-outs (i.e. the risk of selective reporting bias) is considered a critical methodological deficiency.

To conclude, the clinical data available for soya isoflavones for the relief of menopausal symptoms are not considered sufficient for the support a well-established medicinal use according to Article 10a of Directive 2001/83/EC.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

See table 7, clinical safety data from clinical trials in the relief of menopausal symptoms.

Assessor's comment

According to the Guideline on Clinical Investigation of Medicinal Products for Hormone Replacement Therapy of Oestrogen Deficiency Symptoms in Postmenopausal Women (EMEA/CHMP/021/97 Rev. 1), endometrial safety should be assessed with endometrial biopsies conducted at baseline and at end of treatment. Studies of at least 12 months are required. Also breast examinations should be performed.

Table 7: Clinical safety data from clinical trials in the relief of menopausal symptoms

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
To investigate the effect of oral isoflavone extract on hot flushes in menopausal women Faure *et al*., 2002	Double-blind, randomised, multicentre, placebo controlled, 2 parallel groups Duration: 4 months.	**Treatment:** Two soya germ isoflavone capsules* two times daily corresponding to 70 mg isoflavones per day (n=39) or placebo (n=36) *standardised soya germ isoflavone extract. Total isoflavone content 17.5 mg per capsule; 50% daidzin, 30% glycitin 20% genistin.	75 subjects, mean age in isoflavone group 53 years, placebo group 53.9 yrs 20 premature study terminations; 6 from active group (15%) and 14 (39%) from placebo group, mainly due to inefficacy (4 from active group, 11 from placebo group).	Healthy French postmenopausal women (6 months since last menstruation, verified with FSH and 17β-estradiol) with minimum seven moderate to severe hot flushes or night sweats/24 hours. Main exclusion criteria: other treatment of climacteric symptoms, hormone therapy within six weeks before the study.	No significant changes in systolic and diastolic blood pressure were observed in either treatment group. The incidence of medication related adverse events were low in both groups. No patient on the soya group withdrew due to adverse events.	Safety was secondary outcome. No safety signal generated.

Reviews and meta-analysis on the safety of soya and isoflavones

Human exposure to soya isoflavones is predominantly through consumption of soya dietary products (see section 5.2). The consumption of soya isoflavones has been proposed to have a variety of beneficial effects, but concerns have also been raised concerning potential adverse effects of isoflavones. It is questioned whether phytoestrogens, including isoflavone extracts, due to their oestrogen-like properties are associated with oestrogen-related side effects such as breast cancer, endometrial hyperplasia and endometrial cancer (Messina, 2010, Poluzzi *et al*., 2014).

The clinical safety of isoflavones have been reviewed in several publications in the scientific literature and by prominent committees and institutes worldwide, e.g. the German Federal Institute for Risk Assessment (BfR) in 2007, the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) in 2006, Cochrane in 2013 (Lethaby *et al*., 2013), and European Food Safety Authority (EFSA) in 2015. Some of these reports cover isoflavones in general and not only soya isoflavones as active ingredient in medicinal products. The most important parts of these scientific reviews are summarised below.

In a systematic review, 17 studies investigating soya isoflavones were included. The studies were very heterogenous concerning interventions and outcome measures and had numerous quality deficiencies. The products included were soya isoflavones food supplements or isoflavone-enriched food. The duration of the studies varied between 12 weeks up to 1 year. In this review, no proliferative effects on the endometrium, vagina or breast tissue could be detected. In several studies gastrointestinal disorders were reported (Jacobs *et al*., 2009).

The German Federal Institute for Risk Assessment (BfR) advised against long-term consumption of isoflavone-containing products made from soya, when consumed at high doses, such as in the case of menopausal women taking food supplements. The risk concerns possible effects on the mammary gland and the thyroid (BfR, 2007). In accordance with Article 29 (1) of Regulation (EC) No 178/200, BfR asked the European Food Safety Authority (EFSA) to provide a scientific opinion on the possible health risks associated with the intake of isolated isoflavones in food supplements by peri-and postmenopausal women. BfR requested that the opinion should focus on possible harmful effects on mammary gland, uterus and thyroid. The isoflavones considered relevant for the risk assessment were daidzein, genistein, glycitein, biochanin A and formononetin, and their glycosides.

EFSA risk assessment (2015)

EFSA has performed a systematic review to investigate whether an association could be found between intake of isoflavones from food supplements and adverse effects on the three target organs in peri- and postmenopausal women, as was requested by BfR. The human data did not support the hypothesis of an increased risk of breast cancer from observational studies nor of an effect on mammographic density nor on proliferation marker Ki-67 expression in interventional studies. No effect was found on endometrial thickness and histopathological changes in the uterus up to 30 months of supplementation with 150 mgper day of soya isoflavones. After 60 months some non-malignant histopathological changes were reported. Thyroid hormones levels were not changed following intake of isoflavones from food supplements. An overview of doses of isoflavones and duration of intake with no evidence of adverse effects on the three target organs in humans is presented in table 8 below (EFSA, 2015).

Table 8: Overview of doses of isoflavones and duration of intake with no evidence of adverse effects on the three target organs in humans (EFSA, 2015)

Type of preparation	Daily dose without effect (mg/day) Duration of intake (months)			Dose without effect in all three target organs (mg/day)	Duration of intake without effect in all three target organs (months)
	Target organ: mammary gland	Target organ: uterus	Target organ: thyroid		
Soy isoflavones/ soy extract	100 (total) 10	150 (total) 30	200 (aglycones) 24	100 (total isoflavones)	10
Soy protein	99 (aglycones) 12	120 (aglycones) 6	132 (aglycones) 3	99 (aglycone)	3
Daidzein rich isoflavones	120 (total) 24	72 (total) 6	120 (aglycones) 24	72 (total)	6
Genistein	54 36	54 36	54 36	54[(a)]	36
Red clover	43.5 (total) 12	80 (aglycones) 3	120 (aglycones) 12	43.5 (total isoflavones)	3

Mammary gland

Four epidemiological studies investigating breast cancer incidence (involving, in total, 2 216 isoflavone users), eight interventional controlled studies, measuring mammographic density (741 participants), and two interventional controlled studies, investigating histopathological changes (75 participants), did not suggest an association between exposure to isoflavones-containing food supplements and adverse effects in the mammary gland in post-menopausal women at doses and for durations described in table 8 (EFSA, 2015).

Uterus

Endometrial thickness was measured in 25 interventional controlled studies (1 484 participants) and histopathological investigations of endometrium were carried out in nine interventional controlled studies (677 participants). None of the studies reported statistically significant changes in endometrial thickness compared with control. In only two studies were some histopathological effects noted. One study was not properly controlled and had further methodological flaws. In the other study, there were no findings after 2.5 years of intervention, whereas after 5 years of intervention only five cases of simple hyperplasia and one case of complex hyperplasia of the endometrium were observed, but no cases of endometrial carcinoma. The findings could indicate an oestrogenic effect. However, the Panel concluded that that soya isoflavones/soya extract, soya protein, daidzein-rich isoflavones, glycitein-rich isoflavones, genistein and red clover extract have no adverse effects on the uterus in post-menopausal women when is taken in doses and for durations as described in table 8 above (EFSA, 2015).

Thyroid function

Eleven human controlled randomised studies that reported effects of isoflavones on some thyroid-related endpoints were identified. In total, 925 subjects were allocated to isoflavones. In none of the studies was a clinically relevant effect on the thyroid detected. Although the studies have some flaws (thyroid function not the primary endpoint, sample size calculation not given, low power to detect changes) the Panel's conclusion is that administration of food supplements containing isoflavones is not associated with clinically relevant changes in thyroid function (hypo or hyperthyroidism) in post-menopausal women with normal thyroid function (EFSA, 2015).

NTP CERHR risk assessment for soya formulas (2006)

The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) has reviewed the results from seven studies of oestrogenic effects in women receiving soya diets or supplements. Three of the studies did not demonstrate changes in vaginal cytology related to soya product intake. In the more detailed of these studies, women were exposed to up to 1 mg/kg bwper day genistein for 30–93 days. A fourth study that provided very limited detail and included alternating exposure to non-soya-based phytoestrogens reported increased vaginal cell maturation after women received soya flour supplements for 2 weeks (NTP CERHR, 2006).

A fifth study with a longer exposure period (5 years) demonstrated oestrogenic effects on the endometrium. However, in this study baseline and at the 30-month evaluation period 25% of endometrium samples were inaccessible in the treated and placebo groups. It does not appear that women with inaccessible endometrium samples at baseline were excluded for evaluation at future time points. Therefore, it is not known if endometrial hyperplasia was present at baseline in women with inaccessible endometrium samples. It was also noted that no information was provided about endometrial thickness or bleeding patterns (NTP CERHR, 2006).

A sixth study reported increased proliferation of breast lobular epithelium and progesterone receptor expression in women who ate bread rolls containing 60 g soya supplement as textured vegetable protein. The final report with the full cohort of 84 individuals (including 33 added from a tissue bank) showed no differences between the control group and the group eating soya rolls other than plasma isoflavone levels (NTP CERHR, 2006).

5.2. Patient exposure

Aside from market presence and data from two clinical trials (i.e. Faure *et al*., 2002; Stanosz *et al*., 2006), there are no concrete data concerning patient exposure. Most of the available data are from the use of soya bean as food or food supplements. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) of the United Kingdom reports that the Japanese consumer is exposed to 25 to 100 mg isoflavones per day, vegetarian consumer is exposed to 3 mg isoflavones per day, and the average British consumer is exposed to 1 mg isoflavones per day (COT, 2003).

On the basis of published studies and measuring the actual content of isoflavones in a number of food supplements available on the market, it can be estimated according to table 9 and 10 that intake of isoflavones from food supplements is extremely variable(EFSA, 2015).

Table 9: Average daily intake of isoflavones in the European population (EFSA, 2015)

Population group	Number of subjects	Age group (years)	Average intake of isoflavones (mg/day)	Reference
General population, women				
Irish women	662	18–64	0.60	van Erp-Baart et al., 2003
Italian women	827	Up to 94	0.49	van Erp-Baart et al., 2003
Dutch women	2 206	Up to 97	0.83	van Erp-Baart et al., 2003
UK women, n = 168, age 40–64 years	168	40–64	0.66	van Erp-Baart et al., 2003
UK women, non-soy consumers, part of the Norfolk arm in EPIC	708	39–49	0.58	Mulligan et al., 2007
UK women, non-soy consumers, part of the Norfolk arm in EPIC	1 638	50–59	0.50	Mulligan et al., 2007
Spanish women	148	19–69	0.27	Hernandez-Elizondo et al., 2013
European women	23	35–74	1.43	Zamora-Ros et al., 2012
Dutch women, part of EPIC	17	50–69	0.88	Boker et al., 2002
Soy consumers, vegetarians, women and men				
UK women, soy consumers, part of the Norfolk arm in EPIC	41	39–49	11.00	Mulligan et al., 2007
UK women, soy consumers, part of the Norfolk arm in EPIC	83	50–59	8.40	Mulligan et al., 2007
UK vegetarians	10	21–56	7.40	Ritchie et al., 2006
UK adults, vegetarian duplicate diet study	35	39	10.50	Clarke et al., 2003
UK adults, 'health conscious' (including vegetarians)	309	'Adults'	19.40	Zamora-Ros et al., 2012
General population, men and women				
French adults, Total Diet Study TDS2/INCA2	2 624	18–79	0.50	ANSES, 2011
Scottish men, controls in PCANDIET cancer study	197	50–74	1.60	Heald et al., 2006
'Average adult', Total Diet Study, UK 1998	–	'Average adult'	3.00	Clarke and Lloyd, 2004
Finnish adults	2 862	'Adults'	0.79	Valsta et al., 2003
Mediterranean adults, EPIC study	11 285	'Adults'	0.47	Zamora-Ros et al., 2012
Non-Mediterranean, EPIC study	23 469	'Adults'	0.76	Zamora-Ros et al., 2012
UK adults, EPIC study	974	'Adults'	2.34	Zamora-Ros et al., 2012

Table 10: Intake estimates of isoflavones from soya and soya-based products in women above 40 years of age (EFSA, 2015)

Country	Survey	Number of subjects	Food products	Number of consumers	% of consumers	Mean consumption (g/day)	Mean exposure to isoflavones (mg/day)
Austria	ASNS_Adults	133	Soya cheese	1	1 %	27.5	7.2
			Soya drink	2	2 %	187.5	33.8
			Soya yoghurt	1	1 %	150.0	121.5
			Tofu	1	1 %	50.0	24.3
Belgium	Diet_National_2004	916	Soya bread	1	<1 %	69.8	10.3
			Soya drink	10	1 %	147.3	26.5
			Soya yoghurt	4	<1 %	74.9	60.7
			Tofu	2	<1 %	17.3	8.4
Denmark	DANSDA 2005–08	686	Soya beans flour	7	1 %	31.0	55.1
			Soya drink	4	1 %	28.9	5.2
Finland	FINDIET2012	670	Soya beans flour	6	1 %	3.8	6.7
			Soya cheese	7	1 %	9.9	2.6
			Soya drink	11	2 %	188.3	33.9
			Soya yoghurt	10	1 %	127.8	103.5
			Tofu	5	1 %	11.6	5.6
France	INCA2	932	Soya beans flour	7	1 %	3.5	6.3
			Soya drink	28	3 %	115.6	20.8
			Soya yoghurt	57	6 %	56.7	45.9
			Tofu	3	<1 %	36.9	17.9
Germany	National_Nutrition_Survey_II	4946	Soya beans flour	1	<1 %	22.5	40.1
			Soya drink	57	1 %	190.3	34.3
			Soya yoghurt	9	<1 %	93.8	75.9
			Tofu	28	1 %	40.4	19.6
Ireland	NANS_2012	461	Soya cheese	1	<1 %	3.3	0.8
			Soya drink	6	1 %	106.3	19.1
			Soya yoghurt	5	1 %	96.2	77.9
			Tofu	5	1 %	9.4	4.5
Italy	INRAN_SCAI_2005_06	1034	Soya bread	3	<1 %	37.3	5.5
			Soya drink	2	<1 %	175.0	31.5
Netherlands	VCPBasis_AVL2007_2010	547	Soya drink	19	3 %	188.8	34.0
			Soya yoghurt	1	<1 %	75.0	60.8
			Tofu	2	<1 %	39.4	19.1
	VCP-Elderly	366	Soya drink	13	4 %	171.5	30.9
			Soya yoghurt	8	2 %	113.0	91.5
			Tofu	2	1 %	60.4	29.3
Romania	Dieta_Pilot_Adults	440	Soya cheese	2	<1 %	10.7	2.8
			Soya drink	3	1 %	152.4	27.4
Spain	AESAN	91	Soya drink	11	12 %	184.1	33.1
Sweden	Riksmaten 2010	647	Soya drink	9	1 %	163.2	29.4
			Soya yoghurt	6	1 %	95.8	77.6
			Tofu	3	<1 %	34.2	16.6
United Kingdom	NDNS-RollingProgrammeYears1–3	554	Soya cheese	2	<1 %	10.6	2.8
			Soya drink	18	3 %	157.0	28.3
			Soya yoghurt	2	<1 %	46.9	38.0
			Tofu	3	1 %	26.0	12.6

There are some national authorities in Europe that have established maximum limits of soya isoflavones in food supplements (see table 11).

Table 11: Maximum limits set by EU national authorities with respect to the use of isoflavones in food supplements (i.e. not only soya isoflavones). The information presented below may not be exhaustive and other maximum limits may have been set in other EU Member States (EFSA, 2015).

Country	Responsible authority, year	Maximum limit	Additional warning
France	Ministry of the Economy and Finance arrêté plante, 2014	1 mg/kg bw per day of isoflavone (as aglycone), equivalent to 60 mg per day for a 60-kg person	Mandatory warning on the labelling: 'not suitable for women who have a personal or family history of breast cancer'
Belgium	Belgian Health and Social affairs Ministry, 2012	40 mg per day isoflavones (expressed as glycosides of the main component)	–
Italy	Italian Ministry of Health, 2012	80 mg per day total isoflavones	–

5.3. Adverse events, serious adverse events and deaths

Dietary soya products are known to cause allergic reactions including severe anaphylaxis in persons with soya allergy. Patients with known allergy to peanut protein carry an enhanced risk for severe reactions to soya preparations. The allergic potency of soya and peanut has been evaluated in the 'Public statement on the allergenic potency of herbal medicinal products containing soya or peanut protein' (EMA/HMPC/138139/2005).

There are no reports on adverse events from the information obtained from the market overview of medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones).

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

Ethanolic extracts of soya bean containing isoflavones for postmenopausal symptoms are not intended for use in children and adolescents.

5.5.2. Contraindications

Cross-allergy has been reported for patients with known allergies to other legumes. IgE-cross reactions are also reported for patients with birch pollen allergy and associated food allergies (EMA/HMPC/138139/2005).

In the information obtained from the market overview, medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) are contraindicated in case of undiagnosed genital bleeding, endometrial hyperplasia, history of hormone-dependent tumours

including breast cancer, ovarial cancer and endometrial cancer, or predisposition to breast cancer, as indicated by an abnormal mammogram and/or biopsy, or a family member with breast cancer.

Also, the concomitant administration of selective estrogen receptor modulators (SERMs), such as tamoxifen is contraindicated due to drug interaction.

Assessor´s comment

The American Association of Clinical Endocrinologists notes that long-term safety issues, particularly in patients with breast cancer, remain of concern for high-dose menopausal therapy (Goodman et al., 2011). EFSA concludes that the information on women with breast cancer is limited and therefore, the Panel cannot conclude on the risk of isoflavones-based food-supplements in postmenopausal women with a current diagnosis or history of oestrogen-dependent breast cancer. In addition, no information on women with uterine cancer was obtained from this systematic review (EFSA, 2015).

There are no case reports or clinical studies on the interaction between selective estrogen receptor modulators (SERMs) including tamoxifen, in the literature. The contraindication in the product information of medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) is based on non-clinical data (see section 3.1.4). The information is not included in the product information of products containing tamoxifen on the European market.

To conclude, the effects of soya isoflavones on breast cancer and other hormone dependent cancers still remain unclear. Patients with cancer, cancer treatment or symptoms related to cancer should always be under medical supervision. If these patients consider self-medication with medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones), the relevance of this treatment should be discussed with a medical doctor before starting the treatment.

5.5.3. Special warnings and precautions for use

In the information from the market overview of medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones), there is a precaution for use in conjunction with hormone therapy.

If the symptoms worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

Warfarin

One case report describes a decreased INR in a patient taking warfarin after starting using soya milk. A 70-year-old man was stabilized on warfarin 3 mg per day with INR values consistently between 2.3 and 2.5. He started drinking soya milk 480 ml daily. After five days, his INR was 1.9. After four weeks his INR was 1.6. The decreased INR values could not be explained by factors known to reduce the INR such as noncompliance, new medications, other alternative therapies, increased physical activity, changes in medication storage, or increased consumption of vitamin K. INR values returned to therapeutic concentrations within 2 weeks after discontinuation of the soya milk. Repeated coagulation test results during the next 2 months remained within the normal range without changing his warfarin dose (Cambria-Kiely, 2002).

Assessor's comment

The relevance of this single case report for medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) is unknown. As informed in the product information for warfarin products on the European market, soya bean is an abundant vitamin K source.

Levothyroxine

Soya may impair absorption of levothyroxine, resulting in significant fecal levothyroxine loss (Jabbar *et al*., 1997).

There are a few published case reports on persistent hypothyroidism during soya consumption despite thyroid hormone medication in hypothyroid patients. One case involves a 45-year old woman taking a soya protein supplement which had undergone a complete thyroidectomy and required a very high dose of levothyroxine. Temporal separation (approximately 12 hours) of the supplement and the administration of levothyroxine resulted in attainment of serum levels of free T4 and TSH with the use of lower doses of levothyroxine (Bell and Ovalle, 2001). Five cases involve infants taking soya based infant formula. The possible impact of soya on levothyroxine appears to be the result of malabsorption of the medication, not a systemic effect on the thyroid. Ideally, in hypothyroid subjects thyroid function should be reassessed when there is a substantial increase or decrease in soya intake but normal day-to-day variations are unlikely to cause any clinically relevant problems. However, many compounds and dietary consituents, including fiber, also bind levothyroxine (Messina and Redmond, 2006).

Two further cases of children on levothyroxine replacement with continued hypothyroidism, one infant using soya infant formula and one 5-year old child using soya milk, have been reported. The authors recommend that children requiring levothyroxine replacement should avoid the use of soya products unless necessary. If soya products must be used, careful monitoring of thyroid function is needed (Fruzza *et al*., 2012).

Assessor's comment

Soya foods may affect the dose of levothyroxine required by hypothyroid patients. This interaction is included in the product information of oral levothyroxine on the European market. However, the relevance for medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) is unknown. Ideally, the thyroid function is re-assessed in hypothyroid patients when there is a change in dietary habits or medication.

5.5.5. Fertility, pregnancy and lactation

Ethanolic extracts of soya bean are indicated for menopausal women. The menopause is defined as occurring 12 months after last menstrual period. Therefore, medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) are not relevant in fertile, pregnant or lactating women, as well as for adolescents, children and/or men.

5.5.6. Overdose

No case of overdose has been reported.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

Clinical data on the safety of ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) in medicinal products is limited. On the contrary, there is an enormous amount of scientific publications regarding dietary soya products and isolated isoflavones. However, the composition of the isoflavone content differs markedly between products and is affected by processing method. Therefore, it is not generally feasible to apply a read-across approach between different preparations.

Patients with known hypersensitivity to soya bean or peanut to other plants of the Fabaceae (legume) family and to birch pollen should not use soya isoflavone products.

6. Overall conclusions (benefit-risk assessment)

During the assessment of *Glycine max* (L.), Merr., semen, HMPC noted that it would be more appropriate to develop a separate monograph on soya lecithin Lecithinum ex soya (see EU herbal monograph EMA/HMPC/220599/2016 and assessment report EMA/HMPC/220598/2016) and a separate monograph on raffinated soya oil Soiae oleum raffinatum (see EU herbal monograph EMA/HMPC/338914/2016 and assessment report EMA/HMPC/338915/2016). Hence, this assessment report excludes these herbal preparations.

The herbal preparations from *Glycine max* (L.), Merr., semen, covered in this assessment report are:

- Dry extract from the soya bean germ (hypocotyl) (DER 100-400:1), extraction solvent ethanol 60%-70% V/V.
  The extract contains 40% isoflavones calculated as the sum of isoflavones (26% isoflavones calculated on genistein).
- Dry extract from the soya bean germ (hypocotyl) (DER 47.61-190.47:1), extraction solvent methanol 80% V/V.
  The extract contains 26% isoflavones calculated as the sum of isoflavones.
- Dry extract from the soya bean germ (hypocotyl) (DER 43-53:1), extraction solvent ethanol 60% V/V.
  The extract contains 10% isoflavones calculated as the sum of isoflavone glucosides.
- Dry extract from the soya bean germ (hypocotyl) (DER 50-70:1), extraction solvent ethanol 60% V/V.
  The extract contains 30% isoflavones calculated as the sum of isoflavone glucosides.

Medicinal products containing ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) has been used in the EU/EEA since 2003, for the relief of menopausal symptoms such as hot flushes and night sweating. There is no information available on products with medicinal use throughout a period of at least 30 years on the market outside EU/EEA. Thus, traditional medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA according to Directive 2004/24/EC, is not fulfilled.

The clinical data available for ethanolic extracts of soya bean (i.e. extracts of soya bean containing isoflavones) for the relief of menopausal symptoms are not considered sufficient for the support of a well-established medicinal use according to Article 10a of Directive 2001/83/EC.

Based on the above-mentioned information a European Union herbal monograph on *Glycine max* (L.), Merr., semen cannot currently be established.

Annex

List of references