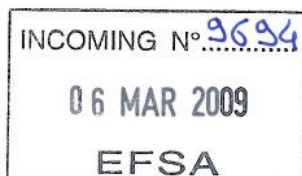


EXECUTIVE DIRECTOR



Parma, 6/31 09  
Ref CGL/DV/MJ/mds (2009)-out 3727397

Note to Hubert Deluyker - Director of Scientific Cooperation and Assistance

**Subject: Mandate proposed by EFSA to the AMU Unit for an internal mandate on the creation of an ESCO Working Group for the hazard characterisation of use of dietary isoflavones and isolated isoflavones from soy or red clover in food and food supplements.**

Herewith I am sending you the internal mandate for the ESCO working group on isoflavones as discussed by the Advisory Forum at its meeting of 18-19 February 2009 and agreed by the Mandate Review Committee of 4<sup>th</sup> March 2009.

I would appreciate to receive the report of this ESCO activity by the year ending 2009.

Should the outcome of the review work of group give indications that a full risk assessment is justified, further work will be recommended.

The EFSA Working Group for the project is currently being constituted; a nomination will be requested from the appropriate Panel, and nominations also from the Advisory Forum will be considered according to the expertise required. Additionally European experts in isoflavone clinical trials, in vivo studies, and thyroid toxicology and cancer epidemiology have been identified

Catherine Geslain-Lanéelle

Annex I: Terms of Reference

Cc: Didier Verloo; Miriam Jacobs

## THE USE OF DIETARY ISOFLAVONES AND ISOLATED ISOFLAVONES FROM SOY OR RED CLOVER IN FOOD AND FOOD SUPPLEMENTS

### Introduction

Isoflavones are bioactive diphenolic plant substances present especially in leguminous plants, but are also ubiquitous in the diet and therefore generally consumed. Highest concentrations occur in soybeans, red clover and kudzu root, and extracts from these are used in the production of nutritional supplements which may contain up to 40% isoflavones.

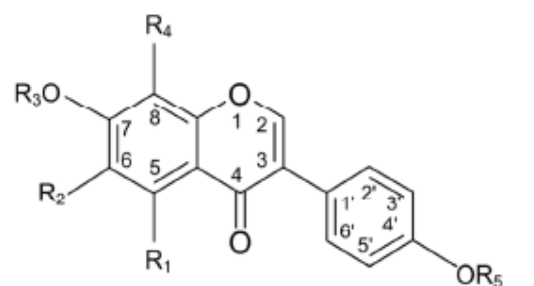
Soybeans contain predominantly genistein and daidzein, also glycitein in lesser amounts; red clover contains predominately formononetin and biochanin A; and the principle isoflavones in kudzu root are puerarin and daizein.

These isoflavones occur naturally in the glucoside form and are shown in Figure 1.

Traditionally the main food sources of isoflavones are soy and other beans and pulses, and also fermented soy foods, where the glucosides have been transformed into aglycones which are absorbed more efficiently than glucosides.

In the past these have been more commonly consumed by Asian populations, but are growing in popularity in Europe. Similarly, in recent decades a new generation of soy products have entered the market (e.g. yogurts, cheeses, soy milk drinks, infant formula's) and commonly consumed food products incorporating soy flour (e.g. bakery products) and protein isolates (e.g. meat products and soy meatless products such as soyburgers). More recently, the development of nutritional supplements rich in isoflavones has targeted niche markets in response to scientific research that suggests a beneficial effect from these food components.

Isoflavones were first discovered in the 1930's as a bioactive agent, following the disruption of oestrogen action and increased infertility in sheep that had been grazing on red clover, thereby earning the often used name 'phytoestrogens'. Subsequently isoflavones have been shown to bind to, or indirectly interact with several key nuclear receptors, including hormonal (oestrogen receptors  $\alpha$  and  $\beta$  [ERs], progesterone and androgen receptors), xenobiotic sensing receptors (Pregnane X receptors [PXR] and Peroxisome proliferator activated receptors [PPARs]), and steroidogenic and hypothalamus-pituitary-thyroid (HPT) axis pathways. Isoflavones are structurally similar to the endogenous oestrogen  $17\beta$  oestradiol, but much less potent on binding to the ERs, although with greater binding affinity for ER $\beta$ .



Compound	R1	R2	R3	R4	R5
Daidzein	H	H	H	H	H
Glycitein	H	OCH <sub>3</sub>	H	H	H
Genistein	OH	H	H	H	H
Formononetin	H	H	H	H	CH <sub>3</sub>
Biochanin A	OH	H	H	H	CH <sub>3</sub>
Puerarin	H	H	H	Glc	H

**Figure 1.** Structures of IF aglycones. Glc: glucose. IF-7-O- $\beta$ -glucosides: R<sub>3</sub> = glucose.

These receptor and cell signalling interactions demonstrate broad metabolic actions and several epidemiological, *in vivo* and *in vitro* studies have correlated or shown evidence of a wide range of health impacts. These range from oestrogenic effects such as lengthening of the menstrual cycle and menopausal impacts, to increased xenobiotic/drug excretion, and multiple beneficial effects on cardiovascular and some breast and prostate cancers, bone density and menopausal symptoms, as well as cholesterol lowering, increase in lean body mass and metabolic syndrome and obesity effects.

While foods rich in isoflavones are considered to be part of a healthy diet, many questions remain surrounding isoflavones with regard to impact upon health, reduction of disease risk and improvement of quality of life. Several promising research outcomes have aroused the interest of the food and pharmaceutical industry in the production of food components, nutritional supplements and pharmaceuticals, and this market is expanding considerably with respect especially to the replacement for hormone replacement therapy, relief of menopausal symptoms, reduction of bone loss, and cholesterol levels, and potential protective impacts upon breast and prostate cancers. Epidemiological evidence of beneficial health effects is based upon communities where exposure to isoflavones starts *in utero* and continues from birth throughout life as consumption of soy and isoflavone rich foods. For a cohort of more than 37,000 British women, the European Prospective Investigation into Cancer and Nutrition has found no evidence for a relationship of risk for breast cancer with dietary isoflavone intake. How an increased dietary exposure to isoflavones later in life affects individuals with traditionally low intake of these food components appears to be a growing cause for concern in relation to breast cancer, for some regulatory authorities, especially in relation to long term self medication use as an alternative to hormone replacement therapy for reducing menopausal symptoms.<sup>1</sup>

<sup>1</sup> Anecdotally reported adverse effects from isolated isoflavone consumption include allergic and flushing symptoms - which are also the symptoms of the menopausal complaints for which treatment is sought.

EFSA has received several functional claims related to soya via the consolidated Member States list of Article 13 health claims under the Health Claims Regulation EC 1924/2006. By July 2009 EFSA will provide scientific opinions on the substantiation of these claims.

Under the framework of Regulation EC 1924/2006 no safety assessment is foreseen. However, some potentially negative impacts have been reported in the scientific literature. For example, recent *in vivo* studies indicate a concentration-dependent mechanistic reduction of efficacy of selective oestrogen receptor modulators (SERMs) such as pharmaceutical ER + agonists (eg. Tamoxifen) used to treat some ER + breast cancers, SERM activity of isoflavones and even promotion of carcinogens, following pre-administration of known carcinogens. Low concentrations (<10µMol/l) of genistein and daidzein have been observed to promote tumour growth, while on the other hand, high doses (>10µMol/l) have been observed to inhibit the growth of breast cancer and augment the tumour inhibiting effect of tamoxifen.

As with several other classes of food constituents, isoflavones are known to interfere with mammalian thyroid<sup>2</sup> metabolism, although this is reversible with iodine supplementation and traditional isoflavone rich Asian diets do also generally contain iodine rich sources. However with the newer food and supplement sources of isoflavones, the need for compensatory additional iodine supplementation may not be so well known, and so not included in the products. Furthermore, the target group of menopausal women at which the isoflavone rich supplements are aimed, may be at greater risk of thyroid metabolism perturbations.

Thus, in an expert opinion from the BfR (No039/2007) it was concluded that isolated isoflavones are not without risk. Through a letter from Prof. Dr. Reiner Wittkowski (reference 25-05-4642008), the BfR requested the EFSA for a scientific opinion on the use of isolated isoflavones in food supplements. The EFSA recognises the potential importance for human health of the issue of isoflavones from food digestion, should the alleged beneficial or detrimental health effects be scientifically proven. In a letter to the BfR the EFSA Executive Director replied that, prior to and in preparation of this hazard assessment, all the relevant scientific information should be available. This includes *inter alia*:

- *To assess the potential of isolated isoflavones to trigger adverse human health effects;*
- *To assess the possible human health benefits of the use of isolated isoflavones for the general population and particularly for women with complaints during and after the menopausal period.*
- *To assess whether there is any scientific basis for differences concerning the hazard assessment of isolated isoflavones from soy and/or red clover in comparison with soy or red clover extracts.*

Following consultation of the Advisory Forum, it was determined that this topic is of interest to several Member States and that therefore an ESCO working group should be set up. This will facilitate the collection and evaluation of more recent relevant information.

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<sup>2</sup> Although rodents are a useful and well characterised risk assessment model for thyroid toxicants, there are significant differences between rodent and human thyroid physiology such that direct mechanistic extrapolation from such models should be interpreted with caution.

## Terms of reference

The working group is to address hazard identification and the potential hazard and benefit characterisation that might be associated with dietary isoflavones in general, isoflavone rich foods, including soya infant formula, as well as isolated isoflavone supplements originating from soy or red clover in particular.

With age and gender considerations, it will assess the available evidence of the possible beneficial or detrimental association, or lack thereof, with non-communicable diseases of

- isoflavones in food, including soy infant formula
- isolated isoflavones and
- extracts from soy/red clover food supplements originating from food.

The reasons for this are that, not only are the sources of isoflavones absorbed similarly, but also similar foods rich in isoflavones are part of a healthy diet, and are available from other vegetable sources, in addition to soy and red clover.

The following will be specifically addressed:

- Isoflavone presence in foods and identification of any key compositional differences between isoflavone rich food sources, infant formula, isolated isoflavones, and soy or red clover extracts;
- Absorption, Distribution, Metabolism and Excretion (ADME) with special focus on bioavailability, intestinal absorption, detailed metabolic pathways and genetic polymorphisms as a potential determinant of the biological response to isoflavones;
- Mechanism of action of isoflavones, including endocrine pathways. Plausibility of such mechanisms and mode of action in humans;
- A critical dose related assessment of *in vivo* dietary isoflavone exposure experimental evidence, particularly with respect to altered endocrine activity, developmental effects, thyroid metabolism, and cancer endpoints;
- A critical assessment of nutritional epidemiological evidence of beneficial and/or adverse health effects in humans;
- Identification of possible specific vulnerability of particular sub-populations such as, but not limited to: infants, children and (post)menopausal women.

The outcome of this will serve to decide on the mandate for a full risk assessment of the consumption of isoflavones and isolated isoflavone supplements, with respect to increasing the risk of non-communicable diseases particularly thyroid dysfunction and hormone related cancers. Should the review identify a lack of key information, the need for further research will be highlighted.

## Proposed approach and Report outline

1. Creation of working group/recruitment of experts (March/April 2009)
2. Systematic collection of data, through a structured search strategy (June 2009):
  - a. The work conducted by the BfR already provides a useful compilation for components of this work
  - b. Voluntary contributions from ESCO working group members, the Advisory Forum and interested parties. It is noted that other reviews, such as the one conducted by the Japan Food Safety Commission, may be very pertinent
  - c. Bibliographic search

3. Organisation of report: proposed categories:
  - a. Presence of isoflavones in food stuffs and dietary supplements
  - b. Mechanism of formation, absorption, bioavailability, pharmacodynamics and kinetics of isoflavones
  - c. Mechanisms of action in the body
  - d. Animal studies
  - e. Key events, i.e. empirically *observable*, precursor steps that are *necessary* elements of the mode of action, or are the markers for such an element), and modes of action of isoflavones
  - f. Short and long term human epidemiological studies and meta-analyses
4. Evaluation and discussion of scientific findings; specifically including an estimation of potential hazard of isolated isoflavone supplements if data available are sufficient to base a reasonable decision
5. Summary of the data
6. Report preparation

#### **Expertise required: Isoflavone specific**

- Occurrence in relation to plant biology and food composition
- Isoflavone chemistry and biochemistry
- Molecular nutrition
- Clinical nutrition
- Endocrine (including thyroid) toxicology
- Hormone related cancer mechanisms
- Developmental nutrition and toxicology
- Nutritional and cancer epidemiology
- Statistics and meta-analyses
- Medical expertise on specific diseases/health conditions (ranging from the menopause to hormone related cancers) and the underlying physiology and biochemistry, particularly with respect to the endocrine system and the thyroid

#### **Timeline**

An EFSA working group will be established during Spring 2009 to undertake the six month review. A first meeting is tentatively scheduled for April 2009. The final report will be presented before the year ending 2009.

#### **Expected deliverables**

The ESCO Working Group is requested to provide a report characterising the potential hazards and health benefits associated with isoflavone consumption to the Executive Director by the end of December 2009.