

GRAS Notification for
Isoflavones
Derived from Soybeans

Provided by: The Archer Daniels Midland Company

An Information Document

Reviewing the

Safety of Soy Isoflavones

used in Specific Dietary Applications

Provided by: The Archer Daniels Midland Company

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Executive Summary

This document is provided in support of ADM's General Recognition of Safety (GRAS) of a micronutrient fraction derived from soybeans called isoflavones. The Archer Daniels Midland Company (ADM) has developed a concentrated isoflavone extract derived from ground, defatted soybeans which retains the isoflavone components in their natural state. These isoflavone components are present in most soy products and soy foods, and as such have been consumed by millions of humans for over two thousand years with no recorded adverse effects. Published epidemiology and feeding studies in both animals and humans indicate no toxic effects at normal dietary levels.

More importantly, epidemiology studies relating disease incidence in relation to Far Eastern and Western diets suggest that soy components provide positive health maintenance benefits. Recently, published direct scientific evidence indicates that soy isoflavones provide positive health benefits for the well being of older men and women. A recent general review article concerning health effects is provided in Reference 53.

On the basis of published safety data, a long safe history of consumption for soy products and soy foods, and that these soy materials typically contain measurable and physiological amounts of isoflavones, we conclude that the supplementation of soy isoflavones in non-soy foods deficient in their isoflavone content is consistent with their use as a GRAS micronutrient. Therefore ADM is providing the following document as a notification to FDA of ADM's determination of GRAS.

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1. Introduction:

The following document provides information to summarize the evidence for general consumption of soy isoflavones and the public literature and scientific data that supports their recognition as safe dietary constituents. A summary is also provided in Section 7 to support the addition of soy isoflavones to food to provide a positive health maintenance benefit, particularly toward postmenopausal women, and men at increased risk of prostate disease. Supplementation of soy isoflavones may occur in the form of an extract or a tablet, in compliance with the conditions of the Dietary Supplement Health and Education Act, or as a directly added GRAS micronutrient in foods.

Epidemiological studies between Western and Far Eastern populations suggest that components of soybeans may contribute to important health effects. Soybeans contain a group of phytoestrogen compounds consisting of derivatives of the isoflavones genistein, daidzein and glycitein. The published scientific literature contains reports to suggest these compounds reduce the risk of certain hormonally related diseases such as osteoporosis, breast and prostate cancer, and cardiovascular disease (1-5).

Soybeans contain about 0.1 to 0.2% isoflavones by weight. These compounds are not removed during crushing and soy oil extraction, but are carried through with the soy fiber, meal and protein products. ADM can now isolate the isoflavones, together with a few other common soy constituents, in their natural state, as a concentrate.

To place human isoflavone consumption in context, it is

necessary to review the consumption of soy products. Soybeans provide one half of the total oilseed crop production in the world.

World production in 1994 amounted to 137.83 million tons of soybeans, while U.S. production was 60.99 million tons. About 50% of the U.S. crop was exported as whole beans and processed products. Soybeans contributed 19.7 million tons of edible vegetable oil and 87.1 million tons of protein meal. Much of this meal is processed into animal food. In 1993, the U.S. generated the following soybean protein products destined for human consumption: 316 thousand tons of flours and grits, 127 thousand tons of protein concentrates, 164 thousand tons of protein isolates, and 5 thousand tons of fiber. Additionally, tofu, soy milk, and other whole soy food forms accounted for 50 thousand tons of soy consumed (5a). Using the U.S. production numbers alone, one may approximate that thousands of pounds of isoflavones have been consumed by humans annually. This figure is much higher when the world production is taken into account.

A. History and Occurrence of Consumption:

i. History:

The first written record of soy is contained in a book authored by Chinese Emperor Shen Nong about 2838 B.C. Later records repeatedly mention soy as one of the five sacred grains, along with rice, barley, wheat and millet. Numerous soy products have been consumed for centuries including natto (developed about 22 A.D.), tofu, koji, tempeh, miso (developed about 300 B.C.), soy sauce (developed about 300 B.C.), soy milk (developed about 100 B.C.) and soy paste (developed 2500 years ago). Many of the soy products and their introduction into other countries are linked to the non-meat eating Buddhists (5b).

Reported introduction into Western culture dates back to the 1700's in both Western Europe and the Americas. However, it was not until the 1920's when the first soybean processing plants were established that soy products came to be more available as Western diet food ingredients. Since the mid-1950's the U.S. has become the world leader in soy product production.

A large body of published literature exists investigating the nutritional aspects of soy products and their contained individual components. These references are too numerous to cite here but may be found in summary form in nutrition text books or in books on soy

(e.g. 5a, 5b, 5c).

On the basis of the long, safe history of use of soybean products and on the basis of the very extensive published and peer reviewed scientific literature studying nutritional and toxicological properties of soy, we conclude that both lines of evidence provide the basis under which soy products are considered Generally Recognized As Safe (GRAS). Soy isoflavones have been components of these soy products and thus are constituents of these GRAS foods.

ii. Occurrence:

Soybean products are the principle source for isoflavones in the human diet. With the common consumption of soybean products in Asian diets, humans have ingested isoflavones daily for thousands of years. Consumption estimates have been made which indicate isoflavones are consumed in Asian diets in amounts ranging from 25 to 200 milligrams per person per day (4,6,7). Western non-soy diets contain about 10 times less isoflavones. Soy isoflavones, as part of a soybean-based diet, are not associated with reports of adverse human health effects.

Some typical soybean products and their isoflavone contents are listed in Table 1. These data indicate isoflavone levels to range from 400 to 3000 micrograms per gram of soy product (as is basis). Studies also show the isoflavone content of the soybeans to vary from 470 to 4216 micrograms per gram, depending on variety, location, growing conditions and stress on the plant (8,9,10). The isoflavone content varies within the anatomy of the bean, with highest concentrations arising in the hypocotyl (9).

Isoflavones are found in a number of bean, legume and other plants. Values for isoflavone content in a number of food sources are presented in Table 2, and this table shows that soy is the major dietary source of isoflavones in humans. Other phytoestrogens are present in certain animal foods, and some of these have been implicated in infertility effects in sheep. These adverse effects were attributed to feeding on subterranean clover and are associated with coumestrol and the isoflavone formononetin.

However, these infertility effects are not general to all animals (11).

Table 1
Total Isoflavone Content of Traditional Soy Foods¹

Product	Content (as is, mg/kg)	Reference
Soybean (Pioneer 9111)	4216	10
Soybean (Hardin variety)	1159	9
Soybean (Strayer 2233)	2344	10
Soybean (Amcor variety)	1498	9
Full Fat Soy Flakes	3309	9
Defatted Flakes	3114	9
Soy Flour	2013, 1338	4, 10
Texturized Vegetable Proteins	2261	10
Soy Isolate	987, 847	4, 10
Tofu	532, 417	4, 10
Tempeh	865, 430	4, 10
Miso	389, 920	4, 10
Fermented Bean Curd	389	10
Soybean Hull	106-200	9
Soybean Hypocotyl	14052-17565	9
Soybean Cotyledon	1585-3192	9

¹ Where two separate values are listed the individual value is documented in the respective reference.

Table 2
Comparison of Isoflavone and Phytoestrogen Contents of
Some Food Items (mg/kg as is) (ref 12)

Food Item	Daidzein	Genistein	Coumestrol ²	Formononetin	Biochanin A
Soybeans 1 (dry)	1001	1023	ND	ND	ND
Soybeans 2 (dry)	701	1082	ND	ND	ND
Soybeans 3 (dry)	1006	1382	ND	ND	ND
Soybeans 3 (roasted)	848	1105	ND	ND	ND
Green beans (fresh)	ND	ND	ND	1.5	tr.
Lima beans (dry)	ND	ND	15	tr.	ND
Red beans (dry)	ND	3	tr.	ND	ND
Garbonzo beans (dry)	ND	ND	ND	ND	15
Pinto beans (dry)	ND	ND	36	tr.	6
Green split peas (dry)	73	ND	ND	tr.	ND
Clover sprouts	ND	4	281	23	4
Alfalfa sprouts	ND	ND	47	tr.	ND
Sesame	ND	ND	ND	ND	ND
Lentils	ND	ND	ND	ND	ND
Barley	ND	ND	ND	ND	ND

² Coumestrol is not an isoflavone but a coumestan

B. U.S. Consumption of Soy Products and Isoflavones

In 1989, the U.S. average per capita daily consumption of soy products, as soybean equivalent, was 4.7 grams, compared with Japan at 32 grams. Typical refined products are soy flour, soy protein concentrates and soy isolates. Most of the soy flour is exported, the isolates are used in infant formulas and nutritional drinks, and the texturized protein products are used in school lunch programs, the military or as meat extenders. These uses make up 90% of the total soy ingested in the U.S. Table 3 provides a comparison of consumption data for significant soy-based products in the U.S. and Japan (in whole soybean equivalents).

Table 3
Consumption of Soy Foods in the U.S. and Japan in 1989 (ref. 34)

Food	U.S. Amount (10 ³ MT)	Japan Amount (10 ³ MT)
Tofu	12.6	607.7
Soy Milk	3.0	3.8
Tempeh	0.6	0.5
Miso	1.2	184.0
Soynuts	2.7	0
Natto	0	125.0
Soy Sauce	12.7	250.0
Soy Protein	394.0	242.0
Other	0	30.0
	426.8	1443.0

Per capita consumption by the typical U.S. resident will be closely related to the total soy food consumption number, since 95% of the soy food is consumed as the protein isolate and as soy sauce. The specialized soy products of tofu, tempeh, miso and soynuts will be consumed by a very limited proportion of the population.

Isoflavone contents of soy protein products are about 1 to 2

mg/g, similar to the whole bean. However, soy sauce contains little isoflavones. Therefore, the principle dietary source of isoflavones for the average U.S. citizen arises from soy proteins.

Soy protein represents 40% of the soybean weight, consequently, the amount of soy protein product consumed is expected to be about 158,000 MT. This means that the isoflavone daily per capita consumption by the average American will be about 2 mg/day. This compares with other estimates of about 2-4 mg/day.

2. Identity:

Soy isoflavones are comprised of 3 aglycone base isoflavone molecules, genistein, daidzein and glycitein. In their natural state in the soybean, these base molecules are glycosylated with one molecule of glucose attached to the 7-position hydroxyl of the "A" isoflavone ring. This glucose molecule is usually further modified in nature with the attachment of an acetyl or a malonyl group to the 6-hydroxyl group of the sugar. Consequently there are typically as many as 3 glycone derivatives each of the 3 aglycone isoflavone species found in natural soy products. These 3 glycosyl forms are collectively referred to as genistin, daidzin and glycitin with respect to the names for the aglycone compounds. The ADM isoflavone product maintains these natural glycosylated and derivative forms.

The Chemical Abstract Numbers and the molecular structures for the aglycone and glycone compounds are given below:

<u>Compound</u>	<u>CAS Number</u>
Genistein	446-72-0
Daidzein	486-66-8
Glycitein	40957-83-3
Genistin	529-59-9
Daidzin	552-66-9
Glycitin	40246-10-4

Figure 1
Chemical Structures

(figure not available in electronic copy)

3. Characteristic Properties

A. Absorption, Distribution, Metabolism & Excretion:

The metabolism of soy isoflavones proceeds via several mechanisms. Initially, isoflavones are metabolized by intestinal

microflora and are converted to the aglycon before adsorption in the gut can occur. Isoflavones are metabolized in the body by, as yet, uncharacterized pathways, converted to glucuronides, and are excreted in the urine. Following soy ingestion, genistein, equol, desmethylangolensin and several intermediates in the pathway to these latter two compounds are found. Uptake may occur within 1 hour and levels may reach up to a microgram/ml in the plasma (7). About 30% of the population metabolizes daidzein to equol, while the majority of the population produces only smaller amounts. The level of urinary equol is reported to be variable between individuals (15). Both the isoflavone metabolites and parent aglycone are absorbed and re-conjugated to glucuronides. They are likely retained in enterohepatic circulation (50, 51, 52). Excretion occurs within 1-2 days.

In pigs, direct absorption of isoflavones also occurs in the stomach, and metabolized compounds are mainly excreted in the urine. Within 3 days after application to the pig, only a trace of the isoflavones remain in the blood plasma (11). Isoflavones do not concentrate in fatty or other tissues.

A number of published studies have shown that soy-consuming populations excrete substantially larger amounts of isoflavones and their metabolites compared to non-soy consumers. Comparisons of urinary excretion between Finnish, American and Japanese premenopausal women and in men show that groups consuming oriental diets or vegetarian diets excrete between 10 to 100 times more isoflavones than Western diet consumers (7, 17, 53). Studies of groups which emigrate from Japan to Hawaii show that their isoflavone excretion levels drop to typical levels of people consuming a Western diet, implying that these emigrants rapidly leave the traditional Eastern diet. Additionally, epidemiological correlations with breast and other cancers indicate that cancer patients have a statistically lower isoflavone and phytoestrogen excretion level compared to non-cancer patients (53). Other published studies clearly demonstrate that, for Far Eastern diet consumers and vegetarians, the excretion levels in the urine and feces, and the circulating plasma levels are all more than 10 times the values compared to Western omnivores (7). These higher isoflavone values can be linked to the higher soy consumption in the diet of these populations (6).

For Eastern diet and vegetarian populations, positive health benefits have been correlated with diet with respect to cancer and heart disease. Additionally, no adverse health effects have been observed for consumers of these diets attributed to the isoflavone consumption. Clearly, daily consumption of isoflavones in amounts of more than 10 times the levels of Western diets does not lead to adverse effects, and may lead to positive health benefits.

These 10 times higher isoflavone values compared to Western diets are normal amounts for Eastern diet consumers, and these

values have likely been typical for hundreds if not thousands of years.

B. Estrogen Potency and Mode of Action:

The estrogenic effects of the soy isoflavones may be mediated through both estrogen receptors and through non-estrogen receptor mechanisms. These mechanisms are not yet clear and depend on the organism and the particular tissue affected.

A comparison of soy isoflavone estrogenic potency, as measured by effect on uterine weight gain, is given in Table 4a and b. In general, the soy isoflavones are about 500 to 1000 times less potent than estradiol, and they are 100,000 times less potent than diethylstilbestrol. The principle soy isoflavone metabolite, equol, is also about 500 times less potent than estradiol (13, 14).

Similar relative potency magnitudes are seen for other biochemical effects.

Genistein is a specific inhibitor of tyrosine kinase enzyme, topoisomerase II and protein histidine kinase. The protein tyrosine kinase activity modulates cellular receptors for epidermal growth factor and mononuclear phagocyte growth factor. Tyrosine kinases are also associated with oncogene products. Putative anticancer effects could be influenced through these inhibitory mechanisms. However, the IC₅₀ values for inhibition in tumor cell lines are an order of magnitude higher than the concentration found in the plasma following ingestion of the isoflavones. Relevance to normal cell lines has not been established in an in vitro assay.

Table 4a
Relative Potency of Estrogenic Substances
Toward Mouse Uterine Weight (ref 13)

Compound	Relative Potency
Diethyl stilbesterol	100,000
Estrone	6,900
Coumestrol	35
Genistein	1
Daidzein	0.75
Biochanin A	0.46
Formononetin	0.26

Isoflavones have been shown to exhibit effects on sex hormone binding globulin (SHBG), which may alter the concentration and metabolism of circulating sex hormones. This influence on the hypothalamic pituitary-gonadotrophic axis has been suggested as a beneficial chemopreventive activity.

Table 4b
 Relative Binding Affinity to Human Estrogen
 Receptor by Phytoestrogens (ref 14)

Compound	Relative Activity
Ethanol (control)	82
Isoflavones	
Genistein	988
Daidzein	469
Formononetin	287
Biochanin A	331
Estrogen Derivatives	
17/s Estradiol (500 times lower concentration)	964
Hexestrol (100 times lower concentration)	800

4. Manufacturing Process:

The process flow diagram for ADM's conventional, food grade soybean fractionation is depicted in Figure 2. Isoflavones are derived by extraction of the defatted soy flakes fraction using 70% ethanol/water. The ethanol is removed by distillation and the isoflavone containing aqueous portion is purified by conventional food production techniques.

Figure 2.

Commercial Soybean Processing Diagram

(figure not available in electronic copy)

5. Product Composition and Food Grade Specifications:

The following chart, Table 5, depicts the expected typical composition of the isoflavone product.

Table 5
Composition of Isoflavone Product

Isoflavones (minimum)	40-50%
Saponins	30-40%
Protein	10%
Fat	0.1%
Ash	2%
Moisture	4%
Phenolic acids	2.5%
Carbohydrate	<1%

Balance (uncharacterized natural soybean components)	1-10%
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Under the current manufacturing method, a significant amount of a second class of phytochemicals co-purifies with the isoflavones. These compounds are triterpene saponins. Almost all of these soy saponins have been identified, and a review of structures can be found in Reference 35. Like the isoflavones, soy saponins have been consumed with soy products for thousands of years and the saponins are also suggested to have positive health benefits, particularly toward active oxygen scavenging. Saponins have low water solubility and are poorly absorbed in the gut. Soy saponins are positive listed as natural food additives in the Japanese Sanitation law. Saponin (in general) use in food and cosmetics, together with safety information was reviewed in 1965 (35a).

Saponins comprise 2 to 5% of the soybean by weight. Chick peas and kidney beans also have similar levels of saponins. It is estimated that typical United Kingdom families (Western diet) consume about 10-15 mg of saponins per person per day while vegetarians, Asians and vegetarian Asians consume between 110 to 214 mg per person per day (35b). Oral toxicity of saponins to warm-blooded animals is relatively low (35c). However, toxicity to fish is high due to permeabilization of respiratory membranes. Data on safety studies is given in the ensuing Safety Section of this document.

To a small extent, phenolic acids will also be found in the preparation (2.5%). There are 5 acids identified: Vanillic acid (6% of total phenolic acids), Syringic acid (38%), p-Coumaric acid (3%), Ferulic acid (31%) and Sinapic acid (22%).

Lignans may also be found but concentrations have not been specifically determined. The scientific literature indicates that soy flour contains 130 µg/100 g of secoisolariciresinol and a trace of matairesinol (36). Based on the ratio of these lignans to total isoflavones in soy flour, the quantity which would co-extract with the isoflavone product would be about 3-4% of the total composition. These two lignans are commonly found in whole grains, fibers and flax seeds, as well as several fruits and vegetables. They may be metabolized by microbial action into enterodiols and enterolactone. All four lignans may be adsorbed from the gut. Lignans are reported to have weak estrogenic effects and they may also exert positive health benefits.

Contaminants have been determined, and pesticide residues have been analyzed. No residues were found for 67 different, commonly used chemicals used in soybean pest management. Aflatoxins B1, B2, G1 and G2 have been specifically analyzed and are absent at a detection limit of <1 ppb.

Table 6 provides analytical values for 3 representative lots of product, demonstrating compliance with food grade standards.

Table 6
Analytical Values for Potential Contaminants

Analyte	Value			Method
	Lot 26A	Lot 28A	Lot 29H	
Heavy Metals as Lead	<5 ppm	<5 ppm	<5 ppm	USP/FCC
Arsenic	<0.1 ppm	<0.1 ppm	<0.1 ppm	hydride generation
Cadmium	<0.04 ppm	<0.04 ppm	<0.04 ppm	AOAC 11.1.26
Lead	0.29 ppm	<0.05 ppm	<0.05 ppm	AOAC 11.1.26, 9.2.17, 9.2.14
Mercury	<0.025 ppm	<0.025 ppm	<0.025 ppm	Analyt. Chem. <u>40</u> , p. 2085
Selenium	0.189 ppm	0.057 ppm	0.057 ppm	AOAC 9.1.01
Aflatoxin B1	<1.0 ppb	<1.0 ppb	<1.0 ppb	(HPLC) FGIS 94-101
B2	<1.0 ppb	<1.0 ppb	<1.0 ppb	(HPLC) FGIS 94-101
G1	<1.0 ppb	<1.0 ppb	<1.0 ppb	(HPLC) FGIS 94-101
G2	<1.0 ppb	<1.0 ppb	<1.0 ppb	(HPLC) FGIS 94-101
Total Plate Count	1700	<10	40	FDA/BAM, Ch. 3
Salmonella	Neg. in 25 gr.	Neg. in 25 gr.	Neg. in 25 gr.	FDA/BAM, Ch. 5
Coliform	240	<3	<3	FDA/BAM
E. Coli	Neg. in 1 gr.	Neg. in 1 gr.	Neg. in 1 gr.	FDA/BAM, Ch. 4
Yeast and Mold	13	10	13	FDA/BAM, Ch. 18

6. Safety:

The soy isoflavones have been widely consumed and are recognized to be non-toxic (13, 16, 49). Almost all human safety information arises from published epidemiology studies and studies of feeding soy products. In 1996, the National Cancer Institute concluded, in a Clinical Development Plan report, that limited safety information indicates that purified genistein is not highly toxic (37). In this study of genistein, a preparation of genistein with daidzein (2:1) was also evaluated with similar results to the purified material.

The NCI protocol review concluded that 90% genistein/7% daidzein and 43% genistein/21% daidzein preparations were suitable to conduct Phase I and limited Phase II clinical trials in humans. NCI results of a 90-day feeding study showed no toxicity in beagle dogs fed up to 63 mg/kg-bw/day genistein or 100 mg/kg-bw/day of genistein/daidzein isoflavone mixture (2:1). A second 90-day feeding study in rats is underway. Genistein has been entered into the National Toxicity Program.

A. Summary of Published Safety Studies:

i. Mutagenicity

Ames test: (ref 38) Genistein and daidzein, or their precursors biochanin A and formononetin, were tested for mutagenic potential in an Ames Salmonella test. None of these four compounds were found to be mutagenic with or without metabolic activation (S-9 mix) using strains TA1538, TA98 and TA100, at levels of test compound from 1 to 100 μg per plate.

Mouse Micronucleus test: (ref. 39) Mouse splenocytes were cultured in vitro in the presence 25 μM , 12.5 μM and

2.5 μM genistein. Compound was administered as a 5% ethanol solution to each of three replicate cultures. For both the 12.5 μM and 2.5 μM concentration, there was no statistically significant increase ($p < 0.05$) in either the number of micronuclei or the number of micronucleated cells per 1000 CB cells. For the 25 μM concentration, a statistically significant increase ($p < 0.05$) in micronuclei and micronucleated cells per 1000 CB cells was observed (approximately a factor of 10 for each). Genistin was also tested at the 25 μM level (only) and was found to produce similar results to the genistein. Other studies show oral gavage at 1.4 g/day produce only 9 μM plasma levels. Therefore, when feeding at 30 times the expected amounts, plasma levels never reach the observed effect level (39).

ii. Oral Feeding:

Sax's Dangerous Properties of Industrial Materials: (ref 40). Genistein is listed as a health risk "D". It is soluble in the usual organic solvents, practically insoluble in water, and soluble in dilute base. Toxicity cited from published reference states an oral dose to mouse yielded a lowest toxic dose of 18600 mg/kg (female 31D pre). There are reproductive toxicity effects.

Registry of Toxic Effects of Chemical Substances: LD_{50} >2 gr/kg intraperitoneal injection daidzein in mouse (40a).

Mouse Acute 5 day: (ref 39) Female C57BL/6J mice (8 week old) were housed in groups of 5, acclimated on a control diet and fed subsequently via gavage (0.5 ml) each day for 5 days with 20 mg genistein per day. Following the fifth day, the animals were sacrificed and splenocyte suspensions were removed from each animal. Micronucleus frequency in cytokinesis-blocked (CB) splenocytes was determined according to criteria defined by Fenech and Morley (1985). There was no statistically significant difference in the micronucleus content between genistein-treated and untreated animals. Plasma levels of genistein-treated animals was 9.2 μM compared with 0.10 μM for untreated mice. The 20 mg/day feeding is equivalent to a human eating roughly 1500 mg/kg-bw/day of isoflavones.

Mouse 4 Week Study: (ref 40b) Male and female Swiss albino mice, 3 weeks old, were divided into groups of uniform weight and the same sex. Genistein was administered in amounts of 5.6 mg and 45.0 mg per mouse for four weeks. Control animals consumed 122 gr of food and attained a final weight of 22.3 gr. Animals on the 5.6 mg diet consumed 101 gr and attained 17.8 gr in weight. Mice on the 45 mg diet lost 0.5 gr in weight, consumed only 69 gr of diet and 2 of 8 animals died.

Genistin was also tested using a 72 mg diet. The mice lost 1.5 gr in weight, consumed only 45 gr of diet and 7 of 8 animals died. Weights of spleen, testes and ovaries were depressed with the highest doses.

Rat 4 Week Study: (ref 41) Young male Sprague-Dawley rats were fed diets containing 0.1% and 0.5% of genistein or genistin for 4 weeks. A total of 6 rats were used for each study dose. Decreases in weight gain were observed for genistein and genistin at the 0.5% test diet level, compared to controls. The effects of genistin and genistein on kidneys and spleen were not significant after adjustment for final body weight. No significant difference in adrenal and testes weights were found.

No apparent effect on hemoglobin was observed, but differences in copper, zinc, calcium, phosphorus and magnesium were evident. Iron levels were elevated in liver and spleen. Zinc in bone and liver was increased. Calcium, phosphorus and magnesium was increased with the test diets and a linear relationship could be demonstrated with dose in the diet. No animal deaths prior to sacrifice were noted.

Rat 5 Week Study + AOM: (ref 42) Seven week old Fischer 344 male rats were fed ad libitum an AIN 76A modified diet with and without genistein. Genistein additions were 75 mg/kg diet and 150 mg/kg diet. Fourteen rats made up each test group and feeding was continued during the succeeding 5 weeks. During the first two weeks,

azoxymethane (AOM) was injected twice to induce aberrant crypt cancerous foci in the colon of the animals.

During the 5 week study, genistein in the diet at 75 or 150 mg/kg had no effect on mean body weight compared to controls. Little effect on food consumption was noted. The average estimated daily intake of genistein was 8 and 17 mg/kg-bw/day during the first week of treatment.

The addition of genistein at 75 and 150 mg/kg diet resulted in a significant reduction in the frequency of aberrant crypts by 29.3% and 34.1%, respectively. A dose response was not statistically justified, and the number of aberrant crypts per focus was not reduced.

Beagle Dog 90 day Feeding: (ref 37) The NCI Chemoprevention Branch evaluated two purified soy isoflavone products containing 90% genistein and 43% genistein (21% daidzein) in rats and dogs. These studies were undertaken preparatory to initiating Phase I clinical trials. Amounts administered were about 0.03 to 0.9 mmole genistein/kg-bw/day to rats and 0.02 to 0.3 mmole genistein/kg-bw/day in beagle dogs. The dog study has been completed and the rat study is in progress.

Genistein preparations were administered in capsules to male and female dogs for 90 days. Doses were 5, 25 and 70 mg/kg-bw/day of the 90% preparation and 10, 50 and 140 mg/kg-bw/day of the 43% genistein/21% daidzein preparation. Upon completion of the study, no clinical or histological signs of toxicity were observed.

Fertility in Mice: (ref 45) Groups of 16 Fawn Farm immature mice were fed 2 mg genistein/mouse/day for 21 days. Times to vaginal opening in weanling females was significantly less than untreated controls. Cornified cells appeared immediately in vaginal smears and persisted through the treatment. One mouse in the control group died.

Three groups of 10 mice were spayed. One group received 5 mg genistein/day diet for 14 days, a second group 10 mg/day and the third was a control. Vaginal smears of the 5 mg group showed no change in leucocytes from the controls. Five of the 10

mice in the higher dose level exhibited cornified smears 1 week after treatment but persisted only 2-5 days.

Inclusion of 15 mg genistein in the diet of 10 intact female mice provoked full cornification within 3 days. Effects were observed on mating with 50% of matings resulting in pseudopregnancy or fetal resorption. All second matings were fertile. Treated male mice showed impaired fertility. No deaths were noted in the study.

iii. Injection:

Rat Prenatal Exposure: (reference 43) Groups of 4 pregnant Charles River CD rats were each injected subcutaneously on day 16 through 20 of gestation with either 25,000 μg genistein, 5000 μg genistein or corn oil. Following birth, the young were developed for 42 days. No effect between genistein and corn oil trials was seen for time of parturition, rates of stillbirth or pup death before day 10 of life. Pup mean birth weights in the 25,000 μg treatment group were lower than for the corn oil or 5000 μg groups. Anogenital distances at birth were lower for the 5000 μg group for both sexes. Administration of GnRH produced no significant concentration differences between groups for luteinizing hormone production. There was only an apparent non-significant decrease in the sexually dimorphic nucleus in the preoptic area of the hypothalamus for the female 5000 μg group. This same group had a later onset of vaginal opening. The 25000 μg group had no significant differences to the control. Estrous cycles of all groups were consistent with one another. (Puberty effect is opposite of previous study results.)

Acute Toxicity to Daidzein: (ref 43a) Groups of 10 male and female each of ICR mice and Sprague-Dawley rats were orally administered 2500 mg/kg, 5000 mg/kg and 10,000 mg/kg of daidzein. A parallel set of tests were run using subcutaneous injections of 1250 mg/kg, 2500 mg/kg and 5000 mg/kg of daidzein. The animals were observed for 14 days. None of the groups showed deaths or toxic symptoms attributable to the test compound.

Mouse Intraperitoneal Injection: Noted no toxic effects of genistein at levels of 500 mg/kg (44).

iv. Human Studies:

Soy isoflavones are generally recognized to be non-toxic when consumed in traditional dietary amounts (13). Published studies have been conducted in humans to evaluate estrogenic effects. A distinction must be made between the ingredient's safety and its observed positive physiological effects. In the works cited below (Table 7), patients were fed between 45 and 177 mg isoflavone per day, which was a 10 to 100 fold elevation over the patient's baseline of a few milligrams per day. In the studies cited, beneficial health effects were observed with no finding of toxicity. Furthermore, the ranges tested were within the amounts consumed routinely by Asians and vegetarians. Details of these feeding studies and other studies which have measured isoflavone levels in soy products consumed are listed below.

Table 7
Comparison of Human Study Isoflavone Consumption

Study	Isoflavone Dose	Number Subjects	Duration	Published Reference
Menstrual Cycle Premenopausal Women	45 mg/day	6	9 months	32
Urinary Excretion	74-177/mg dose	10	single dose	12
Metabolic Fate of Isoflavones	166 mg/day	12	2 days	15

Human Trials (ref 32)

Under controlled conditions, 45 mg of isoflavones per day were administered as 60 g of texturized vegetable protein to 6 premenopausal women. A 1000 fold increase in isoflavone excretion was measured over a complete menstrual cycle. Total urinary isoflavone levels ranged from 1 to 17 µg/day on the control diet

compared to 0.4 to 8 mg/day in the soy diet. Recovery of isoflavones was between 2 and 13% of intake.

Test subjects experienced a prolonged length of menstrual cycle, accompanied by a delay in peak luteal-phase progesterone concentration. Mid-cycle surges in gonadotrophins, luteinizing

hormone and follicle-stimulating hormone were suppressed ($P < 0.05$ and $P < 0.01$ respectively). No adverse effects were noted.

Epidemiology Studies (ref 7)

Literature data was compiled for plasma and excretion concentrations of isoflavones for various populations. Cross sectional studies show urinary excretion of genistein, daidzein and equol are significantly higher in Japanese consuming traditional diets compared to adults in Boston or Helsinki. Plasma levels of isoflavones (free and sulfate) are 10 to 20 times higher in Japanese men compared to Finnish men. Fecal excretion of isoflavones is substantially higher in vegetarians than in omnivores. Japanese populations excrete 20 times more equol (metabolite of daidzein) in urine than Western populations, and they have higher circulating levels of isoflavones compared to the West. The exception noted is that vegetarians and people on macrobiotic diets have the highest levels of isoflavones and phytoestrogens.

Isoflavone Consumption in Soy & Wheat (ref 33)

A randomized double-blind study compared soy flour and wheat flour supplemented diet responses in 58 post menopausal women (47 completed the trial). A significant increase (10-20 fold) in urinary daidzein excretion occurred over 12 weeks of feeding. FSH levels did not decrease for the soy groups and there was no change in vaginal cytology. The soy group reported a rapid decrease in hot flushes over the first 6 weeks of the test with a continued decrease of 40% over the remainder of the 6 weeks of the trial. No differences were found for urinary calcium, hydroxy proline and serum lipids did not change. No adverse effects were noted in the study.

Dietary Intervention (ref 48)

Ninety-seven women were randomly assigned to a soy or control diet (3:1 ratio) after a two week baseline measurement period. For 4 weeks, soy or control foods were consumed, substituting one third of calories. Daily intake of isoflavones was calculated to be about 165 mg/day. Ninety-one post-menopausal women completed the study.

Urinary soy estrogens increased 105 fold for the test group, but wide variations were seen between individuals. Weight gains were stable. Serum FSH and LH tended to decrease slightly in both control and soy groups. Sex hormone binding globulin also decreased for both. Vaginal cytology changed only slightly for the soy group. There was no correlation with degree of change with amount of urinary soy phytoestrogen concentration. No significant effect was found between serum and urine indicators and personal

characteristics, such as time since menopause, age, smoking status, weight, Ovetilet's index and baseline estradiol level. No adverse effects were noted.

Oral Challenge Human Urinary Levels (ref 12)

Overnight urine samples were collected from 10 subjects (6 female, 4 male) and analyzed for baseline amounts of isoflavones. Subjects were fed 40 to 96 grams of roasted soybeans providing 42 to 100 mg genistein and 33 to 77 mg daidzein. Urinary levels of isoflavones measured one night after intake were approximately 100 fold higher and decreased to near pre test levels after the second night. No changes in coumesterol or formononetin phytoestrogen levels were observed during the intervention study. These latter two substances have been linked to an Australian sheep infertility syndrome.

Soy Protein Isolate Intake and Breast Fluid (ref 16)

At monthly intervals for 1 year, 24 pre and post menopausal women underwent nipple aspiration of breast fluid and gave blood and 24 hour urine samples. Soy protein containing a total of 37.4 mg of genistein was fed during months 4 through 9.

Nipple aspirate fluid volumes were increased during the soy administration for premenopausal but not postmenopausal women. No changes were found in plasma prolactin, sex hormone binding globulin, cholesterol, HDL cholesterol or triglyceride concentrations. Plasma estradiol concentrations were elevated erratically through a "composite" menstrual cycle during soy consumption. No significant changes were seen in progesterone levels, and no significant changes were found in plasma estrogen levels in postmenopausal subjects. A decrease in GCDFP-15 glycoprotein levels was found in premenopausal women during soy consumption. However, hyperplastic epithelial cells were found one or more times in 4 of 14 premenopausal and 3 of 10 postmenopausal women in the soy test group. Although the data was statistically significant, the authors cautioned that the population sample size was small and the results are only suggestive of a causative effect.

Oral Challenge (ref 15)

A challenge diet of 40 g of soy flour was fed to 12 volunteers aged 25 to 51 (6 female, 6 male). All women were premenopausal. The soy was analyzed to contain 980 µg genistein, 800 µg daidzein and 30 µg of glycitein per gram. Each individual received 78 mg of genistein, 64 mg daidzein and 24 mg glycitein per challenge over 2 days.

All subjects had substantially higher urinary levels of genistein and daidzein and levels returned to baseline after 2

days. Metabolic products, equol and O-desmethylangolensin (O-DMA), also peaked in urine concentration one day after soy ingestion, but both remained about 5 times higher than prechallenge values after 3 days. A highly variable individual response was noted for the metabolic concentrations of the isoflavone components. No adverse effects were reported.

B. Calculation of Safety Factor:

Example Use foods:

Mature Adult Meal Replacement:	25 mg/meal
Healthy Drinks:	25 mg/12 oz. serving
Performance Bars:	25 mg/bar
Western Dietary Sources (normal):	2-5 mg/day

Assuming consumption of 2 mature adult meals or a combination of 2 drinks and/or performance bars, daily ingestion would amount to about 50 mg isoflavones per person per day. This amount compares similarly to the amount of isoflavones ingested in Far Eastern, soy diets. This amount is 26,000 times lower than the published TDLo toxicity level for genistein, is at least 300 times below the NOAEL found in the NCI 90-day beagle dog feeding study. The 50 mg isoflavones per person per day value is 3 times lower than the no effect single human dose and comparable in amount used in the 9 month human feeding study (Table 7).

The amount of 50 mg is consistent with the minimum amount needed to cause an effect in pre-menopausal women (16,32). But, at this level, minimal elevation will occur in the isoflavone content in breast milk to cause concern (49), should lactating women consume the products.

C. Saponins

As previously mentioned a significant portion of the isoflavone preparation consists of soybean saponins. As with the isoflavones, saponins have been consumed in soy products for thousands of years without note of adverse human health effects. To further underscore the safety of these materials, the following literature information is provided describing various safety studies. It should be noted, however, that the literature is not extensive on this class of soy components, compared with other saponins.

Animal Studies (ref 43b)

A general review of saponins is given in this article in relation to cholesterol metabolism, and the majority of information relates to alfalfa rather than soy. Some soy saponins are a component of alfalfa saponins, but alfalfa also contain a number of aglycone acids. Saponins are implicated in ruminant bloat due to their foaming and rumen gas trapping ability. Studies suggest, but do not prove, that soy and alfalfa saponins reduce serum cholesterol. Evidence suggests that they bind cholesterol and bile acids in the intestine, potentially reducing adsorption and plasma circulation. Saponins can form complexes with minerals, and iron and zinc binding has been demonstrated in rats. There are no direct studies on effects on fat soluble vitamins.

Direct evidence of toxicity on blood or tissue parameters has not been found for alfalfa top saponins in rats or monkeys or for alfalfa seeds in rats or in short term feeding (3 weeks) in humans.

Longer term intakes (6 weeks) of alfalfa seeds in humans produced mild anemia and pancytopenia, which reverted to normal after cessation of intake.

The following are summaries of literature studies directly evaluating soy saponins which are cited in Table 1 of the above Reference 43b article:

- a) A soybean saponin extract was studied in chicks, mice, tadpoles and larvae. Saponins did not impair growth in chicks and mice when intake increased 5 times. Slight growth retention was noted in larvae and a detrimental effect seen on tadpoles. Soy saponin did not form a complex with cholesterol, and saponins are hydrolyzed by caecal micro flora in rat and chick.
- b) Soy saponin extract was fed to rats at a level of 1% of the diet for 3 weeks. An increase in bile acids and neutral sterols excretion was found. Soy saponin did not form complexes with cholesterol.
- c) A human study using 9 subjects compared soy flour (50 g/day) and saponin extracted soy flour in a double blind crossover study. No effect on plasma cholesterol was seen after 4 weeks. An increase in fecal bile acid excretion was recorded.
- d) A human study using 10 hypercholesteremic males compared 50 g/day soy flour containing 4 g or 22 g saponins in a double blind crossover intake study. Neither diet after 4 weeks had any effect on plasma cholesterol or fecal bile acids or neutral sterol excretion.

Ames Test (ref 43c)

Soyasaponin I was tested in the Ames mutation assay with and without S-9 mix activation. Strains TA97, TA98, TA100 and TA102 were used. Soyasaponin I was found to be non-toxic and non-mutagenic at the doses tested.

LD₅₀ Value (ref 43d)

An LD₅₀ value for saponins (general) has been estimated to be in the range of 50 to 1000 mg/kg. This low risk phenomenon is due to the feeble absorption by the body. Few negative effects are observed after continued intake of saponins from edible plants or from ginseng.

I.P. Mouse Injection (ref 43e)

Soybean saponins, extracted and purified from de-oiled beans, were administered by intraperitoneal injection to groups of 5 COF male mice. Adreamycin was also co-administered (to increase lipid peroxides) and the treatments were continued for 5 days. Soy saponins were given in amounts of 1000, 500, 125, 50, 25 and 12.5 mg/kg. Vitamin E and B₂ were also tested. On the sixth day the mice were sacrificed, and heart and livers were analyzed to determine lipid peroxide levels. Both the saponin and Vitamin E groups equally reduced lipid peroxides and were twice as effective as Vitamin B₂. The patent example did not note whether adverse effects on the animals were found.

D. Allergenic Potential of Extract

Certain protein components from soybeans are potentially allergenic to susceptible individuals. Bush and Hefle have reviewed the allergenic proteins contained in soy, and of these, the majority are of molecular weights greater than 20,000 daltons (43f). Since the process uses a purification step which excludes proteins of greater than 20,000 daltons molecular weight, many potential allergenic proteins are removed. This would include a major soy allergen, the Gly 1 protein, whose molecular weight is 30 KDa, several subunits of α , β , γ - conglycinins and 11S globulins.

Several minor allergens such as trypsin inhibitors and a few conglycinin subunits have molecular weights at or below 20 KDa. Hence, allergenic potential cannot be ruled out for the isoflavone composition.

E. Potential Adverse Effects:

Tests of genistein and isoflavones in laboratory animals can

induce decreased food consumption and reduced body weight. Noted genotoxic effects are conflicting, and significant changes in the reproductive system may occur in both ruminant and non-ruminant animals. These effects may be species dependent.

Genistein alters morphologic and physiologic markers of sexual differentiation in utero. In mouse studies, these effects may include precocious vaginal opening, persistent vaginal cornification, uterine abnormalities in females and sterility in males. However, studies are not conclusive and dose levels ranged from 2 to 25 mg per mouse per day administered by sc injection or in the feed (43, 45). In laboratory animals, phytoestrogens have also been shown to cause androgenization, uterine hypertrophy and infertility in females, and in males, feminization, testes atrophy and depressed growth. Animals in many of these studies consumed 0.1 to 0.5% of the isoflavone compound in their diet per day. These amounts are orders of magnitude higher than human ingestion amounts.

In Great Britain, a concern was raised in 1996 about the potential for soy product use in infant formulas to affect infants adversely in later life. This has led the COT to recommend research be undertaken to determine this risk. The COT also noted that Chinese and Japanese populations did not exhibit impaired fertility or altered sexual development. Recently (March, 1997) the New Zealand Nutrition Foundation's Scientific Advisory Committee and Council concluded that there is no credible human data to support the hypothesis that soy infant formula (which contains isoflavones) has adverse effects on the sexual development of the fetus, infants or children.

No direct carcinogenicity studies exist in the literature. Ames tests are negative, but isoflavones have been shown to cause DNA strand breakage in vitro. This strand breakage induced cell maturation in cancer cell lines.

Several dietary supplements used for estrogenic activity by Native Americans contain lignin phytoestrogens. One preparation from chaparral has been linked to hepatic toxicity (46). However, there does not appear to be any connection to the isoflavones and lignans contained in soy.

Estrogenic effects may be manifested in alteration of menstrual cycles, suppression of the mid-cycle gonadotrophin, LH and FSH surges, delay of peak progesterone levels and changes in vaginal cytology. It is not known if adverse estrogen agonist/antagonist effects are observed, similar to tamoxifen induced thromboembolic disease and endometrial proliferation/neoplasia. The presence of insufficient metabolism of nonsteroidal estrogens and/or liver disease could cause accumulation of isoflavones in plasma. In postmenopausal women, dietary soy containing isoflavones induced fewer hormonal effects (47). Estrogenic responses are reported relating to flushing, increased numbers of superficial cells in the vaginal epithelium and suppressed FSH levels (48, 7).

7. Isoflavones Health Maintenance Benefit

A. Chemo-prevention:

Isoflavones are suggested to have a role in reducing cancer risk (Table 8), particularly toward estrogen dependent breast cancer, prostate and colon cancer. Genistein and daidzein have been shown to inhibit human breast cancer and prostate cancer cell lines in culture by mechanisms independent of binding of steroids. The isoflavones may exert their effect through inhibition of tumor growth and induction of cell maturation.

Indirect evidence for a chemo-preventative role is deduced from comparison of risk potential to ingestion of soy products containing isoflavones. From epidemiology studies, a correlation exists between ingestion of soy and breast cancer risk for premenopausal women in China. Soy ingestion has been shown to alter the hormonal characteristics of premenopausal women, which may lead to a reduction in risk factors for breast cancer (4, 16).

Direct studies have not yet been done to determine the contribution of the soy protein and the isoflavone components individually to the risk potential. It is known that soy protein from which the isoflavones have been removed does not exhibit estrogenic effects, while that protein containing isoflavones is estrogenic.

An in-depth clinical and toxicological review of genistein has been prepared by the National Cancer Institute (37). This review thoroughly discusses known mechanisms, effects and metabolism of genistein as a preparatory document to initiate Phase I and II clinical trials as a chemoprotectant. Test materials evaluated included a genistein/daidzein mixture which contained ratios similar to the proposed soy isoflavone product.

B. Bone Health:

Estrogen treatment is used to inhibit bone resorption in post menopausal women. Similarly, the isoflavone 7-isopropoxyisoflavone (ipriflavone), exerts an effect to increase bone mass, but by a different mechanism than estrogen. One of the metabolites of ipriflavone is daidzein and as much as 10% of the metabolic products of ipriflavone is daidzein. This suggests a linkage with soy isoflavones, particularly daidzein, suggesting an effectiveness to maintain bone health without the harmful side effects of estrogen.

Several published studies in rodents and in humans have found greater bone density, greater bone mechanical strength and less calcium excretion in the urine when diets contained soy products or when subjects were supplemented with isoflavones (18, 19, 20).

However, simple dose response relationships with isoflavone content were not demonstrated. A list of relevant works relating to bone health maintenance are given in Table 9.

C. Prostate Health:

Epidemiological studies point to a diet dependent reduction in prostate cancer risk. This reduction has been linked to a diet rich in soy containing foods and low in fat or meat. However, a direct statistical link has not been shown between isoflavones and the soy diet, cancer reduction potential. The soy cancer risk reduction is not due solely to a replacement of fat and meat in the Western diet. In Finland, prostatic cancer rates are lower than in the U.S. even though meat and fat intakes are high. This reduced risk is attributed to a high Finnish consumption of whole grain products which contain other related micronutrient phytoestrogenic compounds, lignans.

Additionally, soy has been shown to have a protective effect on prostatitis in rats (29) and a protective effect toward prostatic dysplasia in mice (30). The observed results were not analyzed to determine the contribution by specific soy product components. Direct evidence for isoflavone chemoprotection is provided by studies showing genistein and its precursor, biochanin A, inhibit the growth of both androgen-dependent and androgen-independent prostate cancer cells, in culture (31).

D. Menstrual Cycles:

Current estrogen replacement therapy treats menopausal symptoms, but treatment can cause unwanted side effects. Isoflavone estrogenic effects suggest these substances may provide a dietary regimen to maintain a healthy menstrual cycle. When added to the diet, soy protein, rich in isoflavones, has been shown to increase the length of the menstrual follicular phase and to alter the onset of menstruations in women age 21 to 29 (32). The increase of isoflavone content in this test diet was about 45 mg per day as aglycone equivalents. More recently, isoflavones have been shown to have a positive effect on reducing post menopausal hot flashes. Significant reductions in the occurrence of hot flashes were observed in women fed soy for 12 weeks (33).

Table 8
Some Studies Related to Possible Anti-Cancer Effects of Isoflavones (ref. 17)

Cancer Type	Source	Organism	Effect
Breast Cancer	Genistein	MCF-7 cells	Compete with estrodiol
Breast Cancer	Diet, phytoestrogen excretion	Women	Low excretion in high risk women
Breast Cancer	Daidzein	ZR-75-1 cells	Inhibition
Breast Cancer	Soy chips	Rat	Inhibit tumor growth
Breast Cancer	Soy food	Singapore women	Inverse relationship
Breast Cancer	Genistein, Biochanin A	MCF-7 & other cells	Inhibition of proliferation
Breast Cancer	Heated soy isolate	Rat	Inhibits tumor progression
Gastric, Esophagus, Colon Cancer	Genistein, Biochanin A	Many types of cells	Inhibit proliferation
Gastric Cancer	Genistein	HGC-27 cells	Growth inhibition
Liver Cancer	Genistein	HepG2 cells	Inhibit proliferation
Leukemia	Genistein	MOLT-4, HL-60 human cells	Inhibit progression
Leukemia	Genistein	Human HL-60, K562 cells	Induce differentiation
Myeloid Leukemia	Daidzein	HL-60 cells	Induce differentiation
Melanoma	Genistein	5 cell lines	Induce differentiation
TPA Mediated Skin Tumor	Genistein	Mouse	Inhibition
Lymphoma	Genistein	Rat Nb2 lymphoma cells	Growth inhibition

Table 9
Effect of Soy Product on Bone Health

Study	Result	Published Reference
Comparison soy/casein diet	Reduced bone loss	21
Comparison soy milk/casein diet (rat)	Greater bone density	18
Urinary calcium excretion (human)	Decreased calcium: creatinine ratio	22
Comparison soy/animal protein diet (human)	Decreased calcium excretion	23
Direct genistein additions (rat)	Positive effect at lower doses	19
Soy protein with and without isoflavone (rat)	Supplemented soy prevent loss	24
Genistein, genistin additions (rat)	Genistein more effective	20
Genistein addition (rat)	Prevent bone loss	25
Comparison of soy/premarin (monkey)	No bone loss prevention	26
Comparison soy/casein (human)	Bone density remained constant in soy diet	27
Comparison soy/wheat (human)	Bone density increase for soy diet	28

8. Conclusion:

1. Soy isoflavones have been consumed by Asian populations for thousands of years. Published epidemiology studies and feeding studies of soy products indicate no toxic effects at dietary levels on animals and humans. A report from the NCI, which included the results of a 90 day feeding study in beagle dogs, further supports this conclusion.
2. Isoflavones are a mixture of derivatives of three well characterized aglycone moieties. The mixture is derived from conventional soybean processing conducted under food GMP conditions. Impurities are also known and characterized

for their safety aspects.

3. The isoflavone product is intended to supplement the diet of older humans who do not sufficiently ingest soy products. Intended dietary uses are consistent with amounts currently ingested by people consuming soy or vegetarian diets.
4. On the basis of this ingredient's extended use and published safety literature, we conclude that soy isoflavones are safe for consumption in food, when prepared according to Good Manufacturing Practice.

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