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ORIGINAL SUBMISSION

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By Hand Delivery

Dr. Nega Beru
Consumer Safety Officer (HFS-206)
Office of Premarket Approval
Center for Food Safety & Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notification for Isoflavones Derived from Soybeans

Dear Dr. Beru:

Enclosed please find an original and two copies of the Generally Recognized **As** Safe ("GRAS") Notification being submitted by the Archer Daniels Midland ("ADM") Company for isoflavones derived from soybeans. While the enclosed submission is officially addressed to Dr. Alan Rulis, because of the earlier meeting we are directing the Notification to you.

As noted in the ADM cover letter, ADM has determined that the use of soy isoflavones as a micronutrient in food is GRAS. In this regard, ADM is also enclosing a review article from the Annals of Medicine entitled, "Phyto-oestrogensand Western Diseases," 29 Ann Med., 95-120 (1997).

Thank you for your assistance. Please do not hesitate to contact me if you have any questions.

Very truly yours,

Gary L. Yingling

SO/kkv
Enclosure(s)
cc: Archer Daniels Midland Company

Processors of agricultural products

ADM

ARCHER DANIELS MIDLAND COMPANY BOX 1470 DECATUR, ILLINOIS 62525 TEL 217/424-5200

GRN # 000001

February 4, 1998

By Hand Delivery

Alan M. Rulis, Ph.D., Director
Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

Re: GRAS Notification for Isoflavones Derived from Soy Beans

Dear Dr. Rulis:

The Archer Daniels Midland Company ("ADM"), by this letter and enclosed documents, is providing the Food and Drug Administration ("FDA") notice in accordance with the FDA proposed regulation at 62 Fed. Reg. 18938, 18960 (April 17, 1997), that it has determined, based on a review of the data referenced in the enclosed summary, that the substance, soy isoflavone, is generally recognized as safe ("GRAS") for use as a micronutrient in food. This substance is a naturally occurring constituent of soybean products and has been a part of the Asian diet for thousands of years. Published epidemiology and feeding studies referenced in the summary in both animals and humans indicate no toxic effects at dietary levels.

ADM's soy isoflavones are a mixture of various forms of three well characterized glycosyl moieties naturally found in soy products. The mixture is derived from conventional soybean processing conducted under food Good Manufacturing Practices. Published literature and scientific data supports the safety of these isoflavone dietary constituents.

The summary includes published literature that suggests that soy isoflavones provide positive health maintenance benefits, particularly for postmenopausal women and men at increased risk of prostate disease. For this reason, ADM anticipates that soy isoflavones will not only be added to the diet by direct addition to conventional foods, but also as dietary supplements.

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This GRAS notification is based on published, generally available and accepted scientific data, information, methods, and principles as well as experience based on common use in food. ADM has determined that the use of soy isoflavones as a micronutrient in food is GRAS, and therefore, it is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. The data and information supporting ADM's GRAS determination are available to the Food and Drug Administration,, upon request.

Very truly yours,

Mark W. Empie
Director, Regulatory Affairs
Archer Daniels Midland Company

MWE/jm

Enclosures

GRAS Notification for

Isoflavones

Derived from Soybeans

Provided;by: The Archer Daniels Midland 'Company

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 Biteratare 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 .conditionsof 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. Historv:

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 Foods[1]

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, a47	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, spy protein concentrates and soy isolates. Most of the soy flour is exported, the isolates are used in infant formulas and nutritional drink's, 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 (10^3MT)	Japan Amount (10^3MT)
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 otber estimates of about 2-4 mg/day.

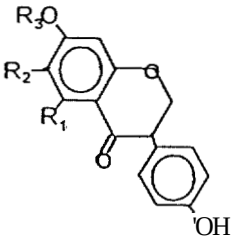
2. Identity:

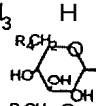
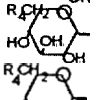
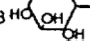
Soy isoflavones are comprised of 3 aglycone base isoflavone molecules, genistein, daidzein and glycitein. In their natural state in the soybean, these base molecules are glycosylated with one molecule of glucose attached to the 7-position hydroxyl of the "A" isoflavone ring. This glucose molecule is usually further 'modified in nature with the attachment of an acetyl or a malonyl group to the 6-hydroxy1 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:

Compound	CAS Number
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



R_1	R_2	R_3	R_4	Compound
H	H	H	–	Daidzein
OH	H	H	–	Genistein
H	OCH_3	H	–	Glycitein
H	H		OH	Daidzin
OH	H		OH	Genistin
H	OCH_3		OH	Glycitin

Other forms: $R_4 = CH_2COO-$ 6-O"-acetyl glucoside
$R_4 = HOCOCH_2COO-$ 6-O"-malonyl glucoside

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 reconjugated 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 IC,, values for inhibition in tumor cell fines'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 hypothalmic pftuitary-gonadotrophic axis has been suggested as a beneficial chemopreventive activity.

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

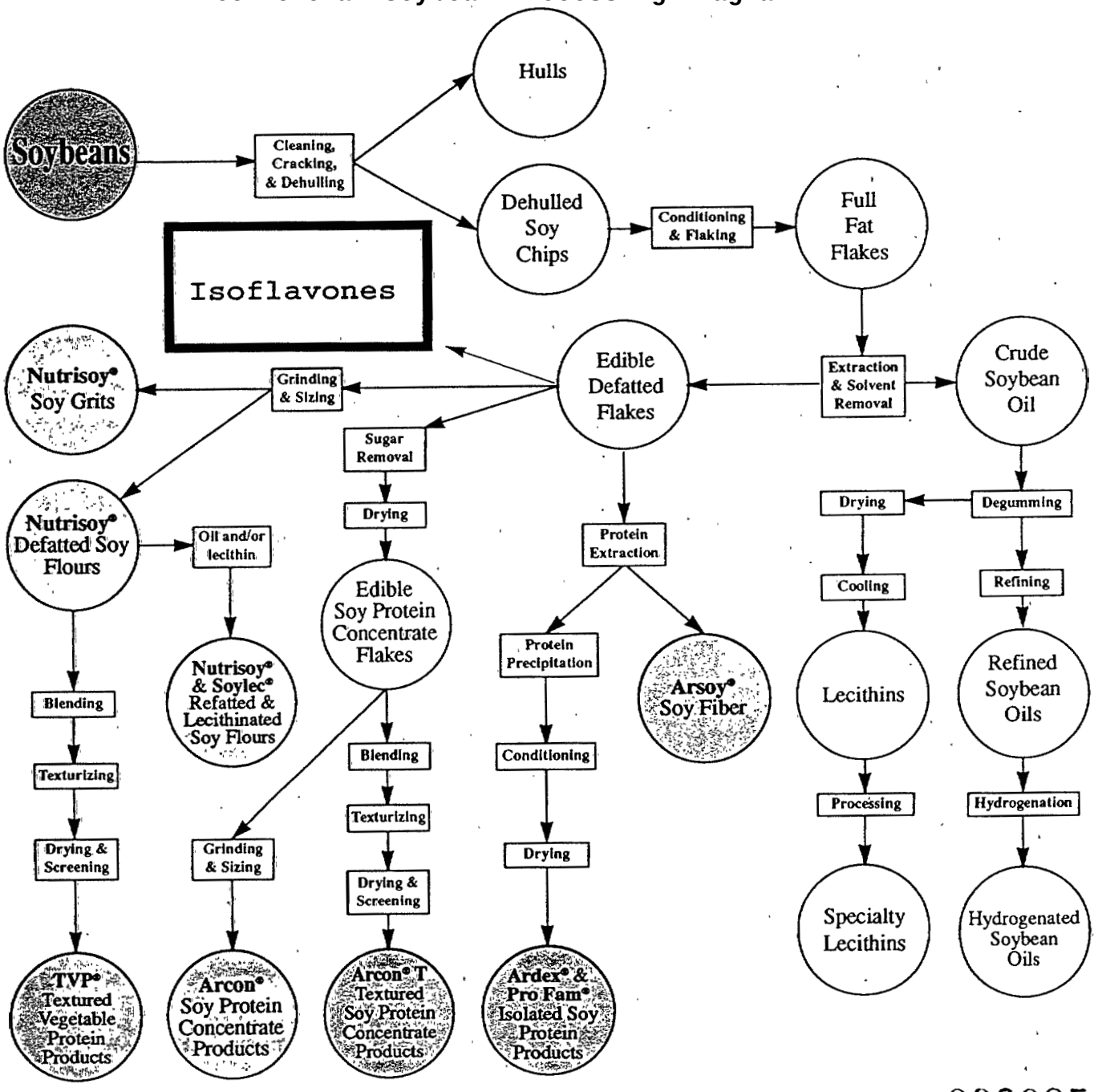
Compound	Relative Activity
Ethanol (control)	82
Isoflavones	
Genistein	988
Daidzein	469
Formononetin	287
Biochanin A	331
Estrogen Derivatives	
17/s Estradiol (500 times lower concentration)	964
Hexestrol (100 times lower concentration)	800

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



5. Product 'Compositionand 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%
Carbohvdrate	<1%
Balance (uncharacterized natural soybean components)	1-10%

Under the current manufacturing method, a significant amount of a second class of phytocompounds 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 enterodiol 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	c0.1 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. 40, 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 25μM, 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).

9i. 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,, >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 Eower 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. 'Therewas 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 Studv Isoflavone Consumption

Study	Isoflavone Dose	Number Subjects	Duration	Published Reference
Menstrual Cycle Premenopausal Wbmen	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 randomize6 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-desmethylangolension (O-DMa), also peaked in urine concentration one day after soy ingestion, but both remained about 5 times higher than prechallenge valves 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 blend 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.

LD_{50} Value (ref 43d)

An LD_{50} 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 Iniection (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 B_2 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 B. 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 afso 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 Pignans 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), particulaxly 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.

El. 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 'cancerrisk. 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, 1ignans.

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 iso'flavones,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 lumphoma cells	Growth inhibition

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Table 9
Effect of Sov Product on Bone Health

studv	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 sov diet	27
Comparison soy/wheat (human)	Bone density increase for soy diet	28

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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.

9. Published References

The references listed below have been compiled by ADM and are available for FDA review and/or copying.

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FACSIMILE TRANSMISSION INFORMATION

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FACSIMILE #:	(202) 418-3131
TELEPHONE #:	(202) 418-3101
FROM:	Gary L. Yingling
TELEPHONE #	202-496-7645
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CLIENT/MATTER #:	10779-004
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See attached.

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By Facsimile/Confirmation By Mail

Linda S. Kahl, Ph.D.
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
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Washington, D.C. 20204

Re: ADM GRAS Notification for Soy fsoflavones

Dear Dr. Kahl:

As discussed in our telephone conference on May 6,1998, we are enclosing a copy of a letter addressed to me from the Archer Daniels Midland Company ("ADM"), that lists the proposed food uses for soy isoflavoncs. Thc listed applications make use of directly added, known amounts of isoflavones, derived from a purified soy extract preparation.

We also enclose. for your information, the article referenced as number 24 in the ADM GRAS notification, Arjmandi, B.H., et al., "Dietary Soybean Protein Prevents Bone Loss in an Ovariectomized Rat Model of Osteoporosis," 1996: J. Nutr., 126, p. 161-167.

Please let me know if you require further information or clarification.

Very truly yours

Gary L. Yingling

SO/so
Enclosure(s)
cc: Mark Empie (ADM)
Suzan Onel

Processors of agricultural products

ADM

ARCHER DANIELS MIDLAND COMPANY BOX 1470 DECATUR, ILLINOIS 62525 TEL: 217/424-5200

May 20, 1998

Mr. Gary Yingling, Esq.
McKenna & Cuneo LLP
1900 K Street N.W.
Washington, D.C. 20006-1108

Dear Gary:

In your letter of May 13, you requested that we provide you with a list of proposed food uses for soy isoflavones, as intended by ADM's GRAS Notification. The intended uses, as suggested in the notification, are listed as follows:

Type	Isoflavone Amount
Adult Meal Replacement (Ensure™-Type Produat)	25 mg/8 oz meal
Flavored Health Drink	25 mg/12 oz
Nutritional Power Bar or Wafer	25 mg/65 gr bar

These applications make use of directly added, known amounts of isoflavones, derived from a purified soy extract preparation. The adult diet will also contain sources of isoflavones derived from inclusion of soy protein products. However, soy protein products are not as commonly utilized as other proteins by the food industry due to flavor and functional properties in the final foods. A concentrated isoflavone product provides a more convenient material for the food product formulator to introduce isoflavones into the food.

We wish to note that the inclusion of isoflavones in food products is to supplement the diet, not to replace an existing functional food ingredient or to be a source of macronutrients. Consequently, the foregoing uses are provided as those immediately anticipated.

If you have any further needs for information or clarification, please do not hesitate to contact me.

Sincerely,

Mark W. Empié, Ph.D.
Director, Regulatory Affairs

MWE/jm

Pages 000706 - 000712 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Received June 1, 1998

McKenna & Cuneo, L.L.P.

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May 22, 1998

Gary L. Yingling
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By Facsimile/Confirmation By Mail

Linda S. Kahl, Ph.D.
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W. (HFS-206)
Washington, D.C. 20204

Re: ADM GRAS Notification for Soy Isoflavones

Dear Dr. Kahl:

As discussed in our telephone conference on May 6, 1998, we are enclosing a copy of a letter addressed to me from the Archer Daniels Midland Company ("ADM"), that lists the proposed food uses for soy isoflavones. The listed applications make use of directly added, known amounts of isoflavones, derived from a purified soy extract preparation.

We also enclose, for your information, the article referenced as number 24 in the ADM GRAS notification, Arjmandi, B.H., et al., "Dietary Soybean Protein Prevents Bone Loss in an Ovariectomized Rat Model of Osteoporosis," 1996: J. Nutr., 126, p. 161-167.

Please let me know if you require further information or clarification.

Very truly yours,

Gary L. Yingling

SO/so
Enclosure(s)
cc: Mark Empie (ADM)
Suzan Onel

Processors of agricultural products

ADM

ARCHER DANIELS MIDLAND COMPANY BOX 1470 DECATUR, ILLINOIS 62525 TEL: 217/424-5200

May 20, 1998

Mr. Gary Yingling, Esq.
McKenna & Cuneo LLP
1900 K Street N.W.
Washington, D.C. 20006-1108

Dear Gary:

In your letter of May 13, you requested that we provide you with a list of proposed food uses for soy isoflavones, as intended by ADM's GRAS Notification. The intended uses, as suggested in the notification, are listed as follows:

Type	~~Isoflavone Amount~~
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If you have any further needs for information or clarification, please do not hesitate to contact me.

Sincerely,

Mark W. Empié, Ph.D.
Director, Regulatory Affairs

MWE/jm

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August 24, 1998

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Dr. Linda S. Kahl (HFS-206)
Office of Premarket Approval
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: Meeting on Isoflavones

Dear Dr. Kahl:

As you are aware, my client, Archer Daniels Midland Company, submitted a GRAS notification for isoflavones on February 9, 1998. We are aware that others have submitted information commenting on the notification.

My client has now reviewed the information provided pursuant to an FOI request and would like to request a meeting with FDA to discuss the information submitted and the status of the notification.

A convenient time for my client and their two consultants would be September 9. We will of course submit a meeting agenda a week prior to the meeting. I will call you in three or four days to determine if such a schedule is feasible.

Very truly yours,

Gary L. Yingling

GLY/lh

cc: Dr. Laura M. Tarantino, FDA Biotechnology Policy Branch
Dr. George Pauli, FDA OPA
Archer Daniels Midland Company
Dr. Charles Capen
Dr. Joseph Borzelleca

AM

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September 3, 1998

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Via Facsimile

Dr. Linda S. Kahl (HFS-206)
Office of Premarket Approval
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: Meeting on Isoflavones

Dear Dr. Kahl:

Enclosed is our proposed Agenda for the September 9, 1998 meeting.

Very truly yours,

Gary L. Yingling

GLY:cem

Enclosure (1)

1998 SEP -8 P 11: 38

Meeting Agenda
Center for Food Safety And Applied Nutrition
Office of Premarket Approval
1110 Vermont Avenue, N.W.
September 9,1998 11:00 A.M.

I. AGENDA SOY ISOFLAVONES

1. Introduction

2. Dietary Consumption of Soy Isoflavones

   a. Animal Safety Data
   b. Human Safety Data

      i. Notification
      ii. Health Claim Petition
      iii. Other

3. End Point Studies
   a. Gonadal
   b. Thyroid

4. Status of Notification

5. Conclusion

II. ARCHER DANIELS MIDLAND COMPANY REPRESENTATIVES

1. Mark Empie, Ph.D., Director of Regulatory Affairs, ADM

2. Eric Gugger, Ph.D., Nutrition Research Scientist, ADM

3. Joseph Borzelleca, Ph.D., Pharmacology & Toxicology, Inc.

4. Charles Capen, Ph.D., Ohio State University, Professor, Department of Veterinary Biosciences

5. Gary L. Yingling, Esq., McKenna & Cuneo, L.L.P.

Processors of agricultural products

ADM

ARCHER DANIELS MIDLAND COMPANY BOX 1470 DECATUR. ILLINOIS 62525 TEL 217/424-5200

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1998 NOV -3 P 3:08

November 2, 1998

Linda Kahl, Ph.D.
Office of Premarket Approval
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notice #000001

Dear Dr. Kahl:

The Archer Daniels Midland Company ("ADM"), by this letter, requests that the Food and Drug Administration ("FDA") commit no further resources to the review of ADM's GRAS Notification for isoflavones derived from soy beans while ADM is in the process of incorporating additional information to update the file.

Please contact me at (217)424-2463 or Gary L. Yingling at (202)496-7645 if you would like to discuss this further.

Sincerely

Mark W. Empie
Director, Regulatory Affairs

MWE/jm

cc: Gary L. Yingling

000834

SU

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July 16, 1999

Privileged and Confidential

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Alan M. Rulis, Ph.D.
Director, Office of Premarket Approval (HFS-206)
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

Re: Draft Expert Panel Report on Isoflavones for Pre-Meeting

Dear Dr. Rulis:

Enclosed you will find an original and one copy of the draft Opinion of an Expert Panel on the Generally Recognized as Safe (GRAS) Status of Isoflavones Derived from Soybeans for use a Microingredient in Foods. The draft report is being sent to FDA as background information prior to the scheduled meeting on July 21 at 2:30 p.m.

Please note that we have marked the document Firm Confidential because this is a pre-meeting and my client still has under consideration how and where this opinion will be used. It is marked draft because the original is being sent to the committee members to be signed.

We will come to the July 21 meeting with a signed copy, but wanted the agency to have an opportunity to review this before the meeting.

1999 JUL 16 P 2:45

If you have any questions, please contact me.

Very truly yours,

Gary L. Yingling

GLY/lh
Enclosure(s)
cc: Dr. Linda Kahl, FDA OPA
Dr. George Pauli, FDA OPA
Dr. Laura Tarantino, FDA OPA

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ISOFLAVONES DERIVED FROM SOYBEANS FOR USE AS A MICROINGREDIENT IN FOODS

(Supplement to GRASN 000001)

PANEL MEMBERS:

Joseph F. Borzelleca, Ph.D.
Charles C. Capen, D.V.M., Ph.D.
Walter H Glinsmann, M.D.
John A. Thomas, Ph.D.

Date: July 15,1999

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OPINION OF *AN* EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ISOFLAVONES DERIVED FROM SOY BEANS FOR USE AS A MICROINGREDIENT IN FOODS

1. INTRODUCTION

The undersigned, an independent panel of recognized experts (the Expert Panel), qualified by their scientific and/or medical training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by the Archer Daniels Midland Company to reevaluate the Generally Recognized as Safe (GRAS) status of Novasoy™ isoflavones derived from soy beans ("Novasoy") for use as an ingredient in foods. In February 1998, the Archer Daniels Midland Company submitted a GRAS Notification (GRN 000001) to the U.S. Food and Drug Administration (FDA) for the use of a mixture of soy isoflavones with a composition of isoflavones that is proportional to that found in soy foods such as tofu. Comments on the 1998 Notification were provided by the Division of Genetic and Reproductive Toxicology and the Division of Chemistry at the FDA's National Center for Toxicological Research (NCTR). Within the last year, considerable additional literature has been published in the areas of dietary soy isoflavone intake, metabolism, mechanisms of action, and health effects. This literature includes critical reviews, symposia proceedings, and a Federal Register Health Claims proposal. Thus a further review of safety of use is being provided in addition to an assessment of issues raised by NCTR.

A comprehensive search of the scientific literature for safety and toxicity information and for health effects of soy isoflavones was conducted through March 1999 by Archer Daniels Midland Company (Dr. Mark Empie) and was made available to the Expert Panel. Reprints, abstracts, summaries, laboratory data and annotated bibliographies were also provided. The Expert Panel independently and critically evaluated materials submitted by Archer Daniels Midland Company and other materials deemed appropriate or necessary. The Expert Panel conferred several times and unanimously agreed ,to the conclusion described herein.

1.1 Recent reviews, symposia publications, and Health Claim proposal for soy protein

Extensively referenced recent reviews and symposia proceedings and a Federal Register document discuss fundamental issues related to a safety evaluation. These include:

Aldercreutz, H. and Mazur, W., 1997: "Phyto-oestrogens and Western Diseases".
Aldercreutz, H., 1998: "Human Health and Phytoestrogens".
Bingham, SA, Atkinson, C, Liggins, J, Bluck, L, and Coward, C., 1998: "Phyto-oestrogens: where are we now?'.

Davis, SR, Murkies, AL, Wilcox, G., 1998" "Phytoestrogens in Clinical Practice".
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1.2 Orientation to literature review

The above documents reflect both the complexity of assessing soy isoflavones as they relate to human physiology and safety of use as well as the potentially beneficial health effects of increased isoflavone consumption to levels equivalent to those in certain vegetarian and Asian populations. They review evidence which supports that there can be considerable variation in the metabolism of isoflavones between species and within individuals, particularly with respect to reproductive physiology. Species variation may be due to fundamental differences in metabolism or mode of action but, as noted in the Health Claims Proposal, "...studies of soy isoflavones in primates showed no effects on male or female reproductive tissue or ability", based on studies by Anthony et al. (1996), Honore et al. (1997), and Cline et al. (1996). Individual human variation is associated with equol forming ability and by factors, such as antibiotic use and dietary fiber intake, which influence the metabolism of gut microflora that convert conjugated dietary isoflavones into bioactive compounds.

Considering that species variation appears to be considerable and that common factors are known to produce individual variation, it becomes more difficult to document from animal experiments, using a typically large safety factor, that isoflavones are safe at common use levels or that adverse health effects are not to be anticipated in the general population. Thus human and related non-human primate data become increasingly important in an assessment of safety of use. Nonetheless, the Panel considered that the existing animal toxicology data, the absence of demonstrated adverse effects in human populations after extensive historical use, the absence of effects in higher non-human primates, and the results of existing clinical trials were sufficient to render an opinion on the General Recognition of Safety of the Archer Daniels Midland "Novasoy™" soy isoflavone mixture at the projected levels of use.

1.3 General background

Soy products containing high quality protein and isoflavones have an extensive history of use before the 1958 Food Additive Amendments to the U. S. Federal Food, Drug & Cosmetic Act. Interest in the potentially beneficial health effects of both soy protein and soy isoflavones has increased progressively during the past two decades and there has been increasing use of soy products in the U. S. food supply. Early interest focused on the observations that soy protein lowered plasma cholesterol and reduced risk of coronary heart disease. The recent FDA proposal for a Food Label Health Claim relates a daily intake of 25 g of soy protein to decreased coronary heart disease. The isoflavones are often retained in isolated soy protein products, e.g., textured soy protein contains up to or greater than 2 mg of isoflavones per gram. Thus, daily intakes of isoflavones approximating 50 mg can be achieved by following the recommendations of the soy protein Health, Claim. A randomized trial with soy protein containing varying amounts of isoflavones strongly supports that isoflavones contribute to the cholesterol lowering effect (Crouse et al., 1999).

This estimated level of use is within the range that is consumed by regular users of soy products, including many Asian cultures that consume 20-80 mg per day (Tham et al., 1998) and as much as 200 mg per day. Relative to the U.S. population, high levels of soy isoflavones and their metabolite "phytoestrogens" are present in maternal plasma and' plasma from infants born to Japanese women consuming what would be considered a normal Japanese diet (Aldercreutz et al., 1999). Infants raised on soy formulas also have increased plasma levels.

Adverse effects of isoflavones in certain animal species have, in part, led to the consideration that increased soy isoflavonoid exposures have the potential in humans for causing longer term effects such as altered neurobehavioral development. The panel found no substantial evidence in humans to support such a contention. For instance, children born to mothers consuming higher levels of soy protein products or infants raised on soy protein-based formulas appear to grow and develop normally. Soy infant formulas support normal growth and development and have a long history of safe use (Klein, 1998).

Increasingly, data also has been generated on the influence of soy isoflavones on estrogen receptors and other biochemical processes that are associated with decreased risk of breast and prostate cancers and potentially beneficial effects on cardiovascular health and bone integrity. There is considerable scientific and public health interest in the possibility that increasing U.S. dietary intake of soy isoflavones and other dietary "phytoestrogens" will lessen risk of a number of chronic diseases, a view clearly expressed by Aldercreutz and Mazur (1997).

A technical issue that may confuse discussions of dose-effects of soy isoflavones is that there is a considerable difference in molecular weight between aglycone and glycone derivatives of soy isoflavones. The Panel favors expressing standardized dosage based on aglycone forms.

2. PROPOSED AND CURRENT USES

Soy and soy products have been consumed for centuries; some uses have been recorded as far back as 2800 B.C. in China. Soy was introduced into Western Europe and the Americas in the eighteenth century. Soybean products enjoy a long history of safe use.

Soybeans traditionally contain between 0.1 to 0.2% isoflavones by weight. The isoflavones are not removed during crushing and soy oil extraction but remain with the fiber, meal and protein products. The isoflavones are extracted with food grade ethanol from edible defatted flakes. Isoflavones are consumed in Asian diets in amounts from 25 to 200 mg/person/day. Western non-soy diets contain about 10 times less isoflavones.

U.S. average per capita daily consumption of soy products, as soybean equivalents, was 4.7 grams, compared with 32 grams in Japan in 1989. The per capita American daily consumption of isoflavones currently is approximately 2-4 milligrams (Coward, et al, 1993; Messina, 1995).

The Code of Federal Regulations, Title 21, lists a number of approved uses of soy products. These products include soy bean oil fatty acids, hydroxylated; soy fatty acid amine, ethoxylated; soybean oil, hydrogenated; soybean oil, unhydrogenated; soybeans; soy concentrate, enzyme activated; and soy protein; and protein isolates.

Isoflavones derived from soybeans are being proposed for use as ingredients in adult meal replacements, beverages, and snack foods to a combined total daily level of 50 milligrams. Exposure calculations are presented in Appendix A.

3. IDENTITY AND DESCRIPTION

The isoflavones extracted from soy consist of various modifications of 3 discrete isoflavone molecules. These 3 isoflavones are genistein, daidzein and glycitein. Each of these isoflavones may be further modified by attachment of a glucose molecule on the 7-position hydroxyl of the "A" isoflavone ring. This glucose moiety may be further derivatized with an acetyl or malonyl attachment to the 6-hydroxyl of the sugar. As naturally found in soybean, the extract product contains the majority of the isoflavones in the derivatized and glucoside forms. Chemical structures and CAS numbers are available for the aglycone and glucosyl forms. The extract contains other natural soybean components such as protein, triterpene saponins and phenolic acids. Most of the sugars and salts are removed.

The proportional composition of individual isoflavones in the ADM Novasoy isoflavone extract is almost identical to that of soy foods such as tofu (cf. following table).

Table 1. Proportional Composition of Novasoy Isoflavones

	Novasoy	Tofu
Daidzein	38%	40%
Genistein	50%	50%
Glycitein	12%	10%

4. MANUFACTURING PROCESS

The manufacturing process utilizes technology commonly practiced by the soy industry. Soybeans are cleaned, cracked and dehulled. The soy meats are flaked and extracted with solvent (hexane) or by pressing to remove the oil. The resultant de-fatted flakes are subjected to an alcohol (ethanol) water extraction to remove sugars and isoflavones. This alcoholic extract is further purified by ultrafiltration and column chromatography to derive the concentrated isoflavone extract. The final product is spray or drum dried. All materials used are food grade.

5. SPECIFICATIONS AND BATCH ANALYSES

The ADM isoflavone extract (Novasoy) product is produced according to Good Manufacturing Practice (GMP) regulations governing the manufacture of safe and suitable food ingredients. The product has been produced on both pilot and fill-production scale. The following table depicts the specification of the product.

Table 2. Novasoy Specifications

Isoflavones	40% minimum
Protein (N x 6.25)	7-12%
Moisture	6% maximum
Fat	1%
Ash	4%
Saponins and other soy components	by difference
Heavy metals	$\leq$ 1 ppm
Aflatoxin	< 1 ppb
Total Plate Count	< 5000/g
Salmonella	negative in 25 g
E. Coli	negative in 5 g

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Table 3A. Typical Batch Analyses of Novasoy

	Lot		
	9903191	9902071	9903101
Isoflavones	41.3	40.1	43.0
Protein (N x 6.25)	10.4	11.3	8.8
Moisture	3.2	2.8	3.0
Fat	0.2	0.2	0.3
Ash	2.3	4.1	3.3
Specific plate count/g	20	300	50
Salmonella/25 g	Neg	Neg	Neg
Coliform/g	<3	<3	<3
Yeast & mold	<3	10	3
Staph	<10	<10	<10

Table 3B. Potential Metal Contaminants

	Lot	
Heaw metals	09302	10191
Total as lead	<5 ppm	<5 ppm
Arsenic	<0.10 ppm	<0.10 ppm
Cadmium	<0.04 ppm	<0.04 ppm
Lead	<0.03 ppm	<0.03 ppm
Mercury	<0.03 ppm	<0.03 ppm
Selenium	0.11 ppm	0.16 ppm

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6. SAFETY

6.1 Absorption, Distribution, Excretion

In humans, the soy isoflavones are ingested from food sources, deglycosylated and metabolized by intestinal bacteria, absorbed into the blood stream, and transported to the liver. Western diets produce plasma isoflavone levels ranging from the nanomolar to less than 2 micromolar range. Excretion values for humans consuming Western diets are 3 to 10 times lower than for Far Eastern soy consumers. This is true for genistein and daidzein, but not always for equol, the breakdown product of daidzein. Daidzein is metabolized to equol by only 30 to 40% of the population (Adlercreutz, 1997)

Plasma and urinary concentrations of isoflavones increase proportionately to the amount ingested. Responses from baseline can result in as high as a 100-fold increase in urinary excretion (Baird, 1995). For humans, plasma levels can reach concentrations of 1 to 4 micromolar. In animals, intake can result in plasma concentrations above 10 micromolar.

Absorption and elimination half-lives have been determined. Generally, absorption from the intestine occurs with a peak time of 1 to 4 hours. Maximal plasma levels occur after about 7 to 8 hours. Daidzein reaches maximal plasma concentrations slightly faster than genistein by about one hour. Elimination occurs with a half-life of between 3 and 9 hours. Serum analysis shows equol is produced several hours after daidzein uptake, is not concentrated by the body, and is eliminated in the feces and urine in 1-2 days (King, 1998).

In humans, soy isoflavone concentrations have been measured in a number of biological fluids following their ingestion. Concentrations in prostate fluid, milk, and saliva are found in the range of less than 40 nanomolar for Western diets. Far Eastern diet consumers may exhibit prostate fluid equol concentrations of up to 700 nanomolar (Morton, 1997).

Rats that received 68 mg soy isoflavone/kg-bw orally may increase uterine and prostate concentrations ten-fold from baseline (Dalu, 1998). Isoflavones were also detected in other tissues, including kidney, liver, lung, spleen, testis, and brain.: There does not appear to be an accumulation in these tissues.

6.2 Biotransformation

Soy isoflavone metabolism has been characterized. Circulating isoflavones are found predominantly in the glucuronidated and sulfated forms, while free forms account for only 1 to 10%. The isoflavones are metabolized in the liver to a number of breakdown products, including equol, O-desmethylangolensin, and para-phenethyl phenol. A portion enters into enterohepatic circulation. Complete excretion occurs in the bile, the urine and the feces within 24 to 36 hours after ingestion (Adlercreutz, 1997).

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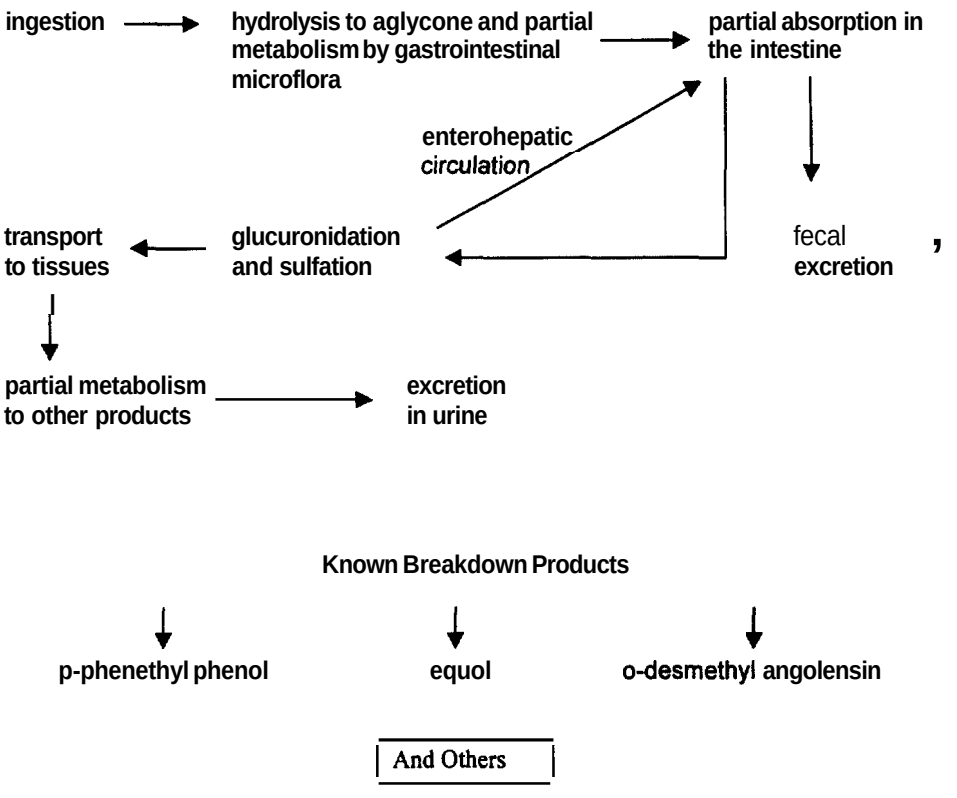
Figure 1 below depicts the current consensus understanding of ingested soy isoflavone absorption, biotransformation and excretion in humans and in many. animal species.

Figure 1. Soy Isoflavone Metabolism



6.3 Biochemical/Physiological Effects

Soy isoflavones bind weakly to alpha and beta estrogen receptors. Binding is **2** to **3** orders of magnitude weaker than estradiol. Of the isoflavones, genistein and the daidzein metabolite equol are the most estrogenic, being about 5 times more potent that daidzein. The soy isoflavones act as weak estrogens, anti-estrogens and non-estrogens depending on the concentration and the tissue involved. They appear to act' independently, or exert additive effects with estrogen, environmental chemical estrogens or drug anti-estrogens (Bickoff, **1962;** Kuiper, **1997, 1998).**

Genistein and other soy isoflavones or their metabolites exert a number of effects on biochemical reactions that appear to be independent of binding to a receptor (Aldercreutz, **1998;** Hughes, **1998;** Hughes and Tansey, **1998).** Most **of** these actions,

described only in ***in vitro*** or in isolated enzyme assay systems, may be responsible for a number of the beneficial health effects attributed to soy products. They include modification (inhibition) of cellular proliferation, tyrosine protein kinases, protein histidine kinase, **DNA** topoisomerases I and 11, aromatase, **17** β-hydroxysteroid dehydrogenase, ribosomal **S6** kinase, **5** ∀-reductase, and human mitochondrial alcohol dehydrogenase. The 7-0-glucosyl derivatives also appear to inhibit aldehyde dehydrogenase.

Genistein *in vivo* also inhibits the formation of reactive oxygen species in LDL-cholesterol. It can stimulate apoptosis and prostaglandin H synthase.

6.4 Toxicological/Safety Studies - Non-human

6.4.1 Acute toxicity studies (ADM Unpublished)

ADM isoflavone extract was administered by gavage to five male and five female Crl:CD (SD) BR strain rats at a single dose of 3000 mgkg bw. There were no signs sf toxicity and no mortality. All animals gained weight during the 14-day observation period. The only finding at necropsy was fluid-filled uterine horns and this may be assumed to be due to the estrogenic activity of the test material. The acute oral LD„ of the ADM isoflavone extract exceeds 3000 mg/kg bw.

A comprehensive search of the scientific literature failed to identify studies of the acute oral toxicity of soy, soy proteins or soy isoflavones.

6.4.2 Short-term toxicity studies

ADM 90-Day Feeding Study (Unpublished). ADM isoflavone extract was administered to male and female Crl:CD(SD) BR strain rats, 10/sex/level, by gavage at doses of 0, 2.8, **8,** 30, and 60 mg/kg bw/day, for at least 13 weeks., A second group of rats, same strain, six/sex/level, received the ADM isoflavone extract by gavage at doses of **2.8, 8,** and 60 mg/kg bw/day for at least 13 weeks. These animals were bled predose and at four, eight, and 16 hours postdosing on day one and during week 13 for toxicokinetic evaluation. The plasma was analyzed for isoflavones (genistein, daidzein and glycitein) and their metabolites. There were no consistent treatment-related or dose-dependent signs of toxicity in either **group.** One male in the **8** mg/kg bw/day group was found dead during week **4** and the cause of death could not be determined. Females that received ***60*** mg/kg bw/day had statistically significant lower body weight gains and food consumption. Males that received **30** or ***60*** mg/kg bw/day **had** statistically significantly.lower blood cholesterol levels. There were no apparent test material-related effects on TSH or **T4** (thyroxine) (cf. table below).

Table 4. Effect of Novasoy on Thyroid Parameters

Novasoy	Week 1		Week 13	
	TSH ng/ml	T4 µg/dl	TSH ng/ml	T4 µg/dl
		Males		
0 mgkg bw	1.55 ± 0.424	6.93 ± 1.810	1.41 ± 1.150	5.00 ± 0.588
15 mgkg bw	1.06 ± 0.056	7.20 ± 1.485	1.37 ± 0.290	5.01 ± 0.523
60 mg/kg bw	0.93 ± 0.872	5.70 ± 1.017	1.63 ± 1.060	4.45 ± 0.330
		Females		
0 mgkg bw	0.42 ± 0.134	5.05 ± 0.359	**0.82** ± 0.318	**4.27** ± 0.810
15 mgkg bw	0.29 ± 0.159	3.89 ± 0.998	0.44 ± 0.150	4.08 ± 0.906
60 mgkg bw	0.62 ± 0.502	3.71 ± 0.999	1.07 ± 0.154	4.36 ± 0.049

Marginal differences in the incidence and/or seventy of subacute inflammation and hepatocellular vacuolation in the liver, tubular mineralization in the kidney, and alveolar macrophage infiltrate and interstitial inflammation in the lung were observed in various groups. These changes are common lesions for rats **of** this age and are not considered treatment-related (cf. incidence/severity table below).

Table 5. Incidence of Inflammatory Change in Treatment Groups

	Males					**Females**				
	Group					Group				
Incidence (Severity)	1	2	3	4	5	1	2	3	4	5
Liver (sub-acute inflammation)	**10** **(1.8)**	3 (1.3)	**2** (2.5)	**2** (2.0)	**10** (2.2)	**9** **(1.8)**	1 **(1.0)**	1 **(1.0)**	**0** **(0.0)**	10 (1.3)
Hepatocellular vacuolation	**8** (1.5)	3 *(2.0)*	**2** **(2.5)**	2 **(3.0)**	**9** **(1.9)**	**0** **(0.0)**	**0** **(0.0)**	**1** (3.0)	0 (0.0)	3 **(0.4)**
Kidney: mineralization	**5** **(0.7**	0 **(0.0)**	0 **(0.0**	**1** **(1.0)**	**5** **(1.2)**	**10** (3.2)	**0** **(0.0)**	**0** **(0.0)**	**0** (0.0)	**10** **(2.9)**
Lung: alveolar macrophage infiltrate	9 (1.3	**0** (0.0	**0** (0.0)	**0** (0.0)	**5** *(0.5)*	**5** **(0.7)**	**0** **(0.0)**	**0** (0.0)	**0** (0.0)	3 **(0.4)**
Interstitial	***6***	0	**1**	**0**	**3**	**1**	**0**	**0**	**0**	**1**

inflammation	(0.6)	(0.0)	(1.0)	(0.0)	(0.3)	(0.1)	(0.0)	(0.0)	(0.0)	(0.1)

Severity – mean of n animals graded on a scale from 0 (none) to 5 (severe)

The NOAEL for this study is 30 mgkg bw/day based on a significantly lower body weight gain in females at the next level, 60 mgkg bw/day, the highest dose tested. It should be noted that these females also consumed less food. Males were not affected.

Genistein. The Chemoprevention Branch and Agent Development Committee of the Division of Cancer Prevention and Control, National Cancer Institute (NCI) has undertaken a clinical development program for genistein. As part of this program, 90-day preclinical toxicity studies were undertaken in rats and dogs.

Rats. Twenty male and twenty female Sprague-Dawley (CD) rats' per level received 0, 10, 50, or 250 mg/kg bw/day of a semipurified product containing approximately 90% genistein or 20, 100, or 500 mg/kg bw/day of a natural or crude product containing approximately 43% genistein for 97 days. There were no deaths and no signs of treatment-related toxicity. Females at the highest doses, 500 mg/kg bw/day of the 43% genistein or 250 mgkg bw/day of the 90% genistein, gained less body weight (7.-10%) than the controls. There were no treatment-related effects on food consumption, ophthalmological findings, hematology and gross necropsy findings. Mild hypertriglyceridemia was observed only in male rats receiving the highest doses (250 mg/kg bw/day of the 90% genistein and 500 mgkg bw/day of the 43% genistein) during weeks 4 and 12 of the study and was statistically significant during week 4. Cholesterol levels were lower in males receiving the 500 mg/kg bw/day dose. Calcium levels were increased in male and female rats but were dose-dependent in female rats only. Increases in phosphorus levels were mild and not dose-dependent.

There were increases in adrenal/body weight and adrenal/brain weight ratios in male rats at the highest levels (250 and 500 mgkg bw/day; + 16% and + 15% respectively above control group), in females at all doses of the 43% genistein (+11%, + 13.9%, and + 35.8% above controls respectively) and in females at the 250 mgkg bw/day receiving 90% genistein (+ 16.5% increase above controls). This effect appears to be treatment-related. Adrenal cortical hyperplasia was reported in female rats only and was considered to be treatment-related. It consisted of diffuse enlargement of the zona fasciculata of the cortex and was usually of minimal or moderate severity. The incidence of adrenal cortical hyperplasia was 0/20, 1/20, 1/20, 8/20 for female rats receiving 0, 20, 100 or 500 mg/kg bw/day of the 43% genistein. The incidence in female rats receiving the 90% genistein was 0/20, 2/20, 0/20, 3/20, respectively, in the animals receiving 0, 10, 50 or 250 mg/kg bw/day levels. The cause of adrenal cortical hyperplasia is unknown but would be consistent with a stress induced reponse. Plasma genistein concentrations (total and free; unconjugated and 'glucuronide conjugate) were determined during weeks 7 and 13. Levels were dose-dependent and were higher in females than males. "The low plasma genistein (unconjugated) concentrations obtained in rats during weeks 7 and 13 correlated with the lack of toxicity observed in this study. Most of the genistein in the plasma compartment

was conjugated (glucuronide and/or sulfate)." (SRI, 1997). The low levels could be the result of limited absorption.

Although effects on adrenals were noted, the study monitors concluded that the NOAELs for these studies are 500 mgkg bw/day of the natural product (43% genistein) and 250 mg/kg bw/day of the semipurified product (90% genistein), the highest levels tested (SRI, 1997).

Dogs. Four male and four female Beagle dogs per level received 0, 5, 25, or 70 mgkg bw/day of a semipurified product containing 90% genistein or 10, 50, or 140 mgkg bw/day of a natural product containing 43% genistein in gelatin capsules daily for 98 days. There were no deaths and no signs of treatment-related toxicity. Females that received the highest dose of the natural product (43% genistein) had a 9% lower body weight gain than controls. There were no biologically or statistically significant changes in body weight gains in the study. There were no treatment-related effects on food consumption, electrocardiogram, ophthalmological findings, hematology and clinical chemistry parameters, urinalysis parameters, gross necropsy findings, absolute and relative organ weights or ratios and histopathological findings. The authors of the report state: "The low plasma genistein (unconjugated) concentrations obtained in dogs 3 hours after dosing at weeks 4 and 13 correlated with the lack of toxicity observed in this study. The lack of drug-induced toxicity may have been due to limited absorption of the drug and/or rapid conjugation of the parent compound, as shown by the higher levels of the conjugated drug. However, since the plasma drug level samples were obtained only at one time point after dosing at weeks 4 and 13, the extent of the drug absorption or the degree of conjugation of the parent compound are unknown."

The NOAELs for these studies are 140 mg/kg bw/day of the natural product (43% genistein) and 70 mg/kg bw/day of the semipurified product (90% genistein), the highest levels tested (SRI, 1997).

Mice. Male and female Swiss albino mice, eight/group, received 5.6 or 45.0 mg of genistein per animal for four weeks. The animals were three weeks old at the onset of the study. The treated mice gained less weight and consumed less food than controls. Two of the eight mice that received the 45 mg dose died (Carter, et al., 1960).

Rats. Young male Sprague-Dawley rats, six per dose level, received genistein or genistin as a dietary admixture at levels of 0.1 and 0.5% for four weeks. There were no deaths and no treatment-related effects on hemoglobin. Iron levels were increased in the livers and spleens but not in the kidneys with both materials; copper content of the kidneys, livers and spleens receiving either material were not significantly different from controls; calcium, phosphorus and magnesium levels in bone increased with treatment of either material (dose-dependent). Values for the control animals are presented but it cannot be determined whether these values are within the normal range for rats of this strain and age. The author concluded that "The results of *this* study indicate that genistin and genistein

enhance the deposition of calcium, phosphorus and magnesium in the bones of young rats. Presumably, these plant estrogens could be influencing the absorption and utilization of calcium, phosphorus and magnesium or they could be affecting the mechanism(s) which controls the deposition of these minerals in the bones.'' (Magee, 1963).

Rhesus monkeys. Fourteen male and 13 female prepubertal monkeys on moderately atherogenic diets were tested for an effect of 9.41 mg/kg bw/day of soy isoflavones to lower blood cholesterol. Intact or alcohol-extracted soy protein were compared. A crossover design was used with each period lasting 6 months. Cholesterol fractions were lowered by the isoflavone-containing diet but no effects were seen in free thyroxine, sex hormone-binding globulin, dehydroepiandrosterone sulfate, estradiol, testosterone, or organ weights of the reproduction system (Anthony et al., 1996).

6.4.3 Chronic toxicity/carcinogenicity studies

There are no carcinogenicity studies on the ADM isoflavone extract nor on genistein. Carcinogenicity studies on genistein are planned by the NCI. However, soy meal is commonly used in the production of animal feed, including that used for laboratory animals, to raise protein quality; approximately 40-50% of the soy meal is protein. The. isoflavone content of animal diets and the approximate time the animals consume the feed" are presented in the following table.

Table 6. Isoflavone Content of Diets for Animals

Animal	Typical Time on Feed	Typical Soy Meal Content*	Typical Daily Consumption	Daily Isoflavone Dose mg/kg-bw
Pigs (100 kg)	120 days	15-30%	3 kg/day	9-18
Sheep (50 kg lamb)	60-90 da	0-5%	1.5 kg/da	0-3
(70 kg gestating ewe)	several yrs	0-5%	1.7 kg/da	0-2
Cattle (600 kg)	8-9 mo	5-10%	20 kglda	3-6
Chickens (2 kg broiler)	7 wk	20-30%	0.15 kg/da	30-45
Chicken (1.5 kg layer)	1-2 yr	15-20%	0.1 kg/da	20-27
Dog (23 kg)	many yrs	(260 ppm isoflavones) direct measurement	0.35 kg/da (recommended)	4
Cat (4 kg)	many yrs	(250 ppm isoflavones) direct measurement	0.065 kg/da (recommended)	4

*assume soy meal contains –2 g/kg isoflavones

6.4.4 Genotoxicity studies- in vitro, in vivo

Genistein, daidzein, biochanin **A** and coumestrol were evaluated for the induction of micronuclei in Chinese hamster V79 cells, characterized for presence of centromere, interference of microtubule complex and mitotic spindles of V79 cells, inhibition of microtubule polymerization under cell-free conditions, induction of mitotic arrest in V79 cells, chromosomal aberrations of cultured human lymphocytes and mutation of the HPRT locus in V79 cells. "Genistein and coumestrol did not interfere with microtubules but did cause DNA strand breaks, structural chromosomal aberrations, CREST-negative nuclei and HPRT mutations. Biochanin A inhibited aneuploidogenic potential in V79 cells, but did not inhibit polymerization of cell-free microtubules, Daidzein was devoid of genotoxicity at the studied endpoints.'' (Metzler, 1998)

Genistein, daidzein, biochanin A and formononetin were evaluated in Ames strains **TA 1538, TA** 98 and TA 100 with and without metabolic activation (S-9) and none were mutagenic (Bartholomew and Ryan, 1980).

Genistein and genistin were evaluated in mouse splenocytes cultured ***in vitro.*** Only at the highest concentration tested, 25 μM (but not at **2.5** μM or **12.5** μM) did both agents cause a statistically significant increase in micronuclei and micronucleated cells (Record, et al., 1995).

6.4.5 Reproduction/developmental toxicity studies

Isoflavones have known estrogenic activity and these effects are pharmacological and not toxicological, unless there is specific species sensitivity. **As** noted above, significant species sensitivity exists.

Virgin female Sprague-Dawley rats were fed genistein **as** a dietary admixture at levels of 0, 25 and 250 mg/kg of diet equivalent to 0, 1.25 and 12.5 mgkg bw/day of genistein "from **2** wkeks prior to and after the start of breeding with proven **studs"** (a one-generation study). The number of litters produced were **35/40** (88%), 25/29 (86%) **and** 44/57 (77%) in the control, 1.25 and 12.5 mg genistein per kg bw per day. There were no statistically significant, treatment-related adverse effects on fertility, male/female ratio of pups, body weight, uterine weights, mammary gland size of perinatal fed pups at 21 or 50 days, male or female anogenital distances, testes descent, time to vaginal opening, follicles or corpora lutea and no histopathological changes in vaginal, uterine or ovarian tissue at 100 days. There were no differences in F^1 female estrous or follicle development (Fritz et al., 1998). The NOAEL for this study is **12.5** mg/kg body weight/day, the highest level fed.

In another one-generation study, Awoniyi, et al. (1998) fed pregnant Sprague-Dawley rats either a control diet **(8** rats) or a diet containing genistein at a level of **5** mgkg feed (approximately 50 micrograms/day) (12 rats) beginning on gestation day 17 and

continuing until postpartum day 21 (end of lactation). On day 21, the genistein-treated females were divided into two groups: one continued to receive the genistein diet (G-70) and the other group returned to control diet **(Ex-G).** Animals were necropsied on days 21 and 70. Daily mean consumption of genistein per rat was **52.8** ± 1.0 microgram during the first week after weaning and 53.0 ± **3.0** micrograms during the second week. There were no reported adverse effects on gestation, parturition litter size or pup survival. The authors reported that "the weight of the ovaries and uterus and serum levels of E_2 and progesterone in genistein-exposed rats on day 21 *(G2*1) were significantly reduced compared with control rats. On day 70, serum levels of E„ progesterone, LH and **FSH** were similar in all groups. Atretic follicals and secondary interstitial glands were more common in G70 and **Ex-G** rats compared with control rats. Cystic rete ovarii was observed in some G70 and Ex-G rats." They further concluded that "In *summary,* our results indicate that in utero and neonatal exposure to genistein may adversely affect reproductive processes in adult female rats." However, the functional significance of these findings is unclear since their data indicate that there were no reported adverse effects .on any of the reproductive parameters evaluated in a single mating. In view of the fact the offspring were not mated to produce a second generation, the functional significance of the finding is unknown. The authors recommend "further studies investigating the impact of prolonged fetal, neonatal, or adult exposure to estrogens on reproductive function."

There were no reported multigeneration or developmental studies on ADM isoflavone extract although there are a number of studies involving reproductive performance/outcome in various species using various isoflavone preparations. For example, Setchell (1987) reported that the consumption of 50 mg of isoflavones/day by cheetahs (<0.5 mg/kg body weight/day) resulted in impaired liver function, venocclusive disease and impaired fertility (cystic endometrium, myometrial fibrosis and endometrial fibrosis).

Infertility has been reported in sheep and cattle grazing on red and subterranean clovers, lucerne and other legumes. This effect has been attributed to the isoflavones present in clover, predominantly formononetin and biochanin **A** and **5-10%** genistein and daidzein. Fodders and silage of these pasture legumes could also contain zearalenone and coumestrol (which is more estrogenic than genistein). Commercially produced soy products do not contain either zearalenone or coumestrol. The estrogenic activity of isoflavones and not the isoflavone content ***per*** *se* has been reported to cause permanent estrous, ovarian cysts and infertility in sheep and cattle. The isoflavone content **of** cattle and sheep pasture feed is five to ten times higher than for soy products ***(5%*** vs 0.25%). Daily isoflavone consumption is about 500 mg/kg bw for cows and **364** mg/kg bw for sheep compared with **<1 mg/kg** bw for humans. Gastrointestinal microflora convert formononetin and biochanin **A** into daidzein, equol or genistein and the effects observed may be due to the parent compounds or their biotransformed products (Lundh, 1995).

An infertility syndrome in ruminants and other animals is positively correlated with high amounts **of** isoflavones in feed. It is noted that soy meal is commonly used in food for

dogs, cats, cattle, pigs and chickens. Isoflavone consumption in these species can approach **20** times that of normal human consumption. No adverse effects have been reported in' these animals. In contrast, cheetahs fed feline chow diets containing soy isoflavones exhibit a uniquely sensitive response, by exhibiting infertility and liver disease. This may in **part** be due to the limited capacity of certain cats to conjugate isoflavones with glucuronic acid. Thus, it appears that very significant species variation exists in response to dietary isoflavones.

The significance of the animal infertility syndrome relative to humans is limited because there are differences in the isoflavones consumed, significant differences in the quantities consumed, differences in the anatomy and physiology of the gastrointestinal tracts of ruminant and non-ruminant animals and humans, and species differences in sensitivity (e.g., the cheetah) to the effects of these substances.

The relative potency of forage estrogens vs. diethylstilbestrol and estrone were evaluated using a standardized mouse uterine weight bioassay procedure (Bickoff et al., **1962).** The results are summarized below.

Table 7. Relative Potency of Forage-containing Estrogens (Bickoff, **1962)**

Compound	Relative Potency* (based on setting **DES** equal to 10^5)
Diethylstilbestrol	**100,000**
Estrone	**6,900**
Coumestrol	**35**
Genistein	**1**
Daidzein	0.75
Biochanin **A**	**0.46**
Formonometin	**0.26**

*measured **as** the dosage required to produce a **25** mg uterus **in a mouse**

Kuiper, et al **(1997)** compared the ligand binding specificity of estrogen receptors alpha (uterus) and beta (prostate) and reported that genistein and coumestrol have greater binding affinity for the beta receptor than the alpha (five times greater for genistein and two times for coumestrol). The binding affinities are lower than those for DES and estrone. For DES, the RBAs (relative binding affinities) for alpha and beta were **468** and **295;** for estrone, *60* and 37; for coumestrol, **94** and **185** and for genistein, 5 and **36.** In a subsequent paper by Kuiper, et **al. (1998),** the following were reported: binding affinities for ER alpha and ER beta expressed **as RBA:** 17-beta estradiol, **100** and 100; zearalenone, **7** and **5;**

coumestrol, 20 and 140; genistein, **4** and 87; daidzein, 0.1 and **0.5;** formononetin, <0.01 and < 0.01; biochanin A, <0.01 and < 0.01.

6.4.6 Special studies

Thyroid Function. In 1997, Divi published a paper demonstrating that a purified, cell-free extract of porcine thyroperoxidase enzyme (TPO) could be inhibited by isoflavones. He hypothesized that inhibition of this enzyme would impair thyroid function by reducing T, and T_4 levels while increasing TSH levels. The higher TSH values were suggested to lead to overstimulation of the thyroid, producing goiter, antithyroid (auto) antibodies, and possibly thyroid cancer. Thyroperoxidase inhibition was observed for genistein at an IC„ value of **7.6** μM and for genistin glycone at an **IC„** value of 38 μM. The 5'-deioodinase enzyme also can be inhibited by isoflavones.

A number of literature reviews concerning the effect of **soy** and the development of thyroid goiter have recently been published. Hard (1998) has discussed the current understanding of thyroid mechanisms, particularly in relation to **risk** assessment of insult by environmental chemicals. At present, the only verified cause **of** thyroid cancer in humans is ionizing radiation. The TPO enzyme may be a useful marker for **risk** assessment. However, there are significant inter-species differences between susceptibilities for TPO inhibition by chemicals and for the development of thyroid **tumors** (e.g. rats and mice are very susceptible whereas non-human primates and humans are resistant).

The New Zealand Ministry of Health (1998) has written a position paper focusing on soy infant formula intake and thyroid function. Twelve individuals were identified with documented thyroid abnormalities associated with ingestion of soy infant formula. Another report indicated that an association between autoimmune thyroid disease (ATD) and soy infant formula intake may exist (Fort, et al, 1990). The review points out that these analyses may contain a significant flaw since the reason for using soy formula **was** not investigated. It is possible that the infants were switched to the soy infant formula because of ATD. Williams (1998) also points out that circulating thyroid autoimmune antibodies are common in the general population.

The New Zealand Ministry of Health review (1998) further considered the mechanism proposed by Divi (1997). They noted that added iodine completely abolished inactivation of TPO, and that the Divi work has not been replicated ***in vivo.*** .Modulations of T„ T_4 and TSH that have been reported have been within physiological ranges and variations in TSH could be accounted for by estrogen-mediated increases in TBG. The review also points out that Divi did not address uptake into the cells of the thyroid gland. In particular, it was pointed out that the free non-bound, non-glycosylated 'fraction of isoflavones in serum will be significantly less than the total plasma levels which are in the range of IC„ values found by Divi (1997).

The number of studies correlating the effect of soy on thyroid endpoints is not as extensive as for the reproductive organs. However, results do not indicate that soy isoflavones decrease T, and T, while consistently increasing TSH as would be expected if soy isoflavones inhibited TPO. Such an effect is observed with other TPO inhibitors such as sulfamethazine, methimazole, and propylthiouracil. A recent study in humans (Duncan, **1999)** has shown isoflavone consumption of **1** or **2** mg/kg bw/d, during **3** menstrual cycles plus **9** days, does not change T,, free T_4, T,, TBG or TSH in pre-menopausal women. In this study, free T, values were slightly reduced in the **2** mgkg group. In this respect, it is important to note that a number of non-thyroidal factors can result in decreased circulating levels of T, (total and free), including a number of common diseases such as chronic renal' disease, diabetes mellitus, protein starvation, and febrile illnesses. Feeding studies **(9.4** mg soy isoflavonesikg bw/d) in Rhesus monkeys for **6** months produced no differences in free thyroxine values (Anthony, **1996).** In rats, feeding isoflavones for **28** days produced no differences in T, and elevated values of T,, FT, (Balmir, **1996),** or no changes in the thyroid and thyroxine values (Webb, **1992),** in dietary amounts up to **25** mgkg bw/d fed for **84** days. These findings are not consistent with an inhibition of TPO by isoflavones.

In hamsters, feeding soy protein isolate with isoflavone levels of **35** mg/kg bw/d for **56** days produced no differences in T, and FT, index or percent T_3. Serum T, and FT, index values were lower with casein, casein plus isoflavone, and soy isolate diets (Balmir et al, **1996).** In a second hamster study, Potter et **al(1996)** incorporated **3** and **37** mg/kg bw/day isoflavones from soy protein in the diet for **35** days. No significant differences were found between control and high isoflavone diets for serum T_4 and FT_4. The low doses elevated T_4, FT,. The T, values were lower for the high dose but not the low. In another rodent species, gerbils fed 50 mgkg bw/d exhibited no difference in T_3 and an increase in T_4, TSH (Forsythe, **1986)** after **28** days. Consequently, rodent studies show a lack of correlation between isoflavone intake and changes in circulating levels of thyroid hormones and TSH.

Akiba **(1982,1983)** investigated the effect of housing temperature on chickens fed a corn-soy diet for **22** days compared to either fish meal or yeast diets. Temperature effects were noted in T, and T, levels between diets. At **13-24** °C coop temperatures, no differences were found in T,, T_4 between feeds. At **24-35** °C, the soy diets increased T_3 and T, relative to the fish and yeast diets. Thyroxine also was increased. Therefore, soy diets do not appear to lower thyroid hormone levels. These findings are inconsistent with the Divi **(1997)** hypothesis that isoflavones significantly inhibit TPO ***in vivo.***

In a **6-7** week cross-over study relative to casein, pigs exposed to soy isoflavonoids for **45** days showed no differences in T_3, while an increase in thyroxine was found (Barth, **1988).** In cattle no differences in **T,,** T, or TSH were seen after estradiol was administered. Differences in T,, T, were observed between a roasted and non-roasted soy meal (Rumsey, **1996, 1997).** The 5'-deiodinase activity was reduced by roasting. The activity reduction was reversed by E, addition. Because roasting should have little effect on isoflavones, other heat sensitive factors most likely contributed to this effect.

In related ***in vitro*** studies, Holting (1989) demonstrated that genistein could reverse TGF and EGF stimulation of thyroid tumors at 0.4 μM concentration. At **4** μM, genistein inhibits thyroid tumor invasion and growth.

Four 90-day sub-chronic feeding studies with genistein conducted by NCI in rats and dogs made no note of any thyroid findings. Dose levels in these studies reached **70** and 250 mg/kg bw/d, respectively, or up to 140 and 500 **mg/kg** bw/d of an impure genistein extract, respectively. In the **ADM** feeding study using the Novasoy extract, no differences were noted in thyroid function.

In the limited number of clinical cases of thyroid abnormalities associated with soy diets reported in the literature, the isoflavone levels of the formula or the diet were never measured. In contrast, the widespread use of soy infant formulas and soy foods, and the lack of reports of goiter supports the view that this does not occur with any regularity in the general population. Klein (1998) has recently reviewed the soy-infant formula area and concluded: "The literature offers no evidence of endocrine effects in humans from infant consumption of modem soy-based formulas. Growth is normal and no changes in the timing of puberty or fertility rates have been reported in humans who consumed soy formulas **as** infants.".

6.5 Human studies

In spite of the long history of safe use of soy products, the Panel is not aware of any substantial evidence supporting the contention that human populations consuming higher levels of soy isoflavones are at increased risk of endometrial cancer; however, there is some evidence that incidence rates in women consuming soy products may actually be lower (Parkin et al., 1992; Goodman et al., 1996). There also is no evidence that these populations have decreased fertility, birth abnormalities, or are at increased risk of having progeny with impaired neuroendocrine or developmental health conditions. High levels of soy isoflavones are found in healthy Japanese infants and in their associated amniotic fluid, indicating that isoflavones cross the placenta (Aldercreutz et al., 1999). There are effects on menstrual cycle length associated with changes in gonadotrophin and steroid hormone. metabolism or action, which are generally considered to be beneficial. These latter effects and other issues related to safety of use are reviewed below.

6.5.1 Estrogenic effects on female menstrual cycle and reproductive physiology

Cassidy et al. (1994) reported that, in **6** healthy non-vegetarian young women consuming soy protein containing 45 mg of isoflavones for **30** days, there were significant delays in follicular phase length, a suppression of mid-cycle luteinizing hormone and follical stimulating hormone release, and increases in menstrual cycle length. These responses are potentially beneficial with respect to risk factors for breast cancer. Lu et al. (1996) also reported in six healthy premenopausal females that high daily isoflavone

intakes (with approximately 100 mg of genistein and 100 mg of daidzein) for one month decreased the luteal phase and increased menstrual cycle length. There was also a decrease in the levels of the ovarian steroid 17 beta-estradiol and the adrenal androgen dehydroepiandrosterone sulfate. Xu et al.(1998) studied 12 healthy premenopausal women, in a randomized cross-over study with 3 diet periods consisting of 3 cycles each. Effects of diets containing daily isoflavone intakes of 1 or 2 mg per kg body weight were compared with a control diet. Isoflavone consumption decreased the urinary excretion of estradiol, estrone, and total estrogens as well as the potentially genotoxic metabolites, 16 alpha-hydroxyestrone, 4-hydroxyestrone, and 4-hydroxyestradiol.

Nagata et al. (1998) tested 31 premenopausal Japanese women on a daily diet containing 400 ml of soymilk (109 mg isoflavones) and also noted an increase in menstrual cycle length. Duncan et al. (1999) also provided evidence that soy isoflavones exert a modest effect on the mentrual cycle. Graded doses of dietary isoflavones - 10, 64, and 128 mg per day were given to 14 premenopausal women. The 64 mg diet decreased LH and FSH levels during the pre-ovulatory phase. The 128 mg diet decreased free dehydroepiandrosterone sulfate levels during the early follicular phase and estrone during the midfollicular phase. Therefore, the effects of soy isoflavones on the menstrual cycle are well documented and do not raise significant issues with respect to safety.

Postmenopausal women consuming diets containing 165 mg/day of soy isoflavones experience a minor stimulation of superficial vaginal cells but little change in other estrogenic responses (Baird et al., 1995). In a study in both pre- and postmenopausal women by Petrakis et al. (1996), 38 grams of a soy isolate containing 38 mg of genistein was ingested daily for 3 months. In premenopausal women, this resulted in an increase in nipple fluid aspirate volume and a moderate decrease in gross cystic disease fluid protein. Some epithelial hyperplasia in cells from breast fluid and transiently elevated estradiol levels were noted in some women. No changes occurred in plasma prolactin, progesterone, sex hormone binding globulin, cholesterol, HDL-cholesterol, or triglycerides. Plasma estradiol concentrations were not altered in postmenopausal women and little or no increase occurred in nipple aspirate volume. Some evidence of a stimulus to epithelial hyperplasia in premenopausal females was not anticipated. In a report by McMichael-Phillips et al. (1998), 29 women were given soybeans containing 45 mg of isoflavones for 14 days and breast cell proliferation in breast biopsies was assessed using tritiated thymidine and the antigen Ki67. An increase in the proliferation rate was noted. The significance of the proliferative changes is not clear. A decrease in gross cystic disease fluid protein in Asian women relative to white and African-American women 'is known, and a similar decreased response was noted in the Petrackis (1996) study cited above.

There is some evidence that soy phytoestrogens may assist in decreasing hot flashes at time of menopause (Murkies et al., 1995); however, this area has not been studied in a systematic manner, and it is an area of testing where there can be a significant placebo effect that is difficult to control.

6.5.2 Infant formulas

An infant consuming a soy based infant formula may be exposed to relatively high soy isoflavone levels of 5-8 mg/kg body weight per day. This exposure level has led to a general recommendation in New Zealand (Ministry of Health, New Zealand, 1998) that soy infant formula should be used under the care of a health professional when there are such conditions as cow's milk protein allergy. In the United Kingdom, cows' milk formulas are preferred over soy-based formula (MAFF UK, November 1998). No such distinction is made within the United States. Klein (1998) has reviewed this area and provided evidence for the safety of using soy infant formulas. Setchell (1997) estimated that breast milk may contribute up to 0.1 mg (0.002 mg/kg-bw) to an infant's consumption of isoflavones. Irvine et al. (1998) studied daily isoflavone excretion rates and concluded that "young infants are able to digest, absorb, and excrete genistein and daidzein from soy-based infant formulas as efficiently as do adults consuming soy products."

Medlock et al. (1995, a, b) compared the effects of equol in neonatal rats with untreated controls. They administered for 5 postnatal days 0.5, 5, and 50 mg equol/kg-bw subcutaneously and followed uterine weight, luminal epithelial cell hypertrophy, and estrogen receptor binding. They estimated equol potency for uterine growth to be about 10^{-5}-fold less than diethylstilbestrol. Equol unexpectedly produced a lowering of uterine weight without an effect on estrogen receptor levels. This led the authors to conclude: "The data presented in this study would suggest that there are no long-term adverse effects following neonatal exposure to the concentrations of phytoestrogens as are seen for 10 µg DES.". Further, it is known that uptake from the diet results in glucuronidation of isoflavones. Glucuronides exhibit substantially lower binding affinities to estrogen receptors. Subcutaneous administration of equol, as opposed to oral consumption, would lead to higher equol levels in plasma, and presumably lead to a greater response per dose administered. The mechanism whereby equol lowered uterine weights is unknown and the relevance of the levels used in the Medlock papers is uncertain with respect to health effects of soy isoflavone consumption from soy infant formulas.

6.5.3 Soy and thyroid gland activity

This subject has been reviewed earlier under special studies (6.4.6).

6.5.4 Developmental change

The possibility that the increased maternal ingestion of soy products may result in adverse neuroendocrine changes in the fetus remains a theoretical concern triggered by studies in experimental animals. As noted above, there is no evidence that the Panel is aware of to support the contention that human populations who routinely consume high

levels of isoflavones in soy products demonstrate an increase in neuroendocrine-related abnormalities in their progeny.

6.5.5 Vascular dementia

A relationship between isoflavone intake and vascular dementia is an issue raised in the commentary on the original GRAS notification (GRN 000001) from the National Center for Toxicological Research. This concern is based on an analysis of an epidemiological study in Hawaii termed the Honolulu-Asia Aging Study (White, 1996). In this study, **3734** Japanese-American men were followed. It was concluded that "Prevalence of Alzheimer Disease in older Japanese American men in Hawaii appears to be higher than in Japan but similar to European-ancestry populations. Prevalence of vascular dementia appears to be only slightly lower than in Japan, but higher than in European-ancestry populations." Thus, there well may be some effect of migration on the type of dementia that was prevalent. ***An*** abstract by White et al. (1996b), apparently never published as a full report, comments on retrospective food frequency interviews in the population studied and concludes that mid-life consumption of tofu may have been causally related to the increased prevalence in vascular dementia in Japanese-American men. The Panel is unable to evaluate this comment on this apparent ecological relationship without additional supporting data. It finds no reason to consider such a relationship potentially valid at the present time.

6.5.6 Beneficial effects

Soy isoflavones have been studied for their potentially beneficial health benefits, and many studies form the basis for evaluating isoflavone effects in humans. General' health benefit reviews can be found in the bibliographic references found in Section 1.1, and an abbreviated synopsis is provided below.

Soy isoflavones have been shown to exhibit estrogenic, anti-estrogenic and non-estrogenic effects both ***in vitro*** and ***in vivo.*** Genistein inhibits several processes related to cancer development. ***In vitro*** studies support that genistein induces cell cycle arrest and causes apoptosis at greater than 10 μM concentrations in MCF **7** breast cancer cells, BPH cells, and prostate cancer cells. These results, together with ***in vivo*** data and epidemiology data showing Far Eastern soy-consuming populations have lower incidences of certain cancers, have generated considerable research interest in the role of isoflavones in decreasing the risk of breast and prostate cancer.

The estrogenic character of the soy isoflavones supports the possibility that they may have a positive effect on maintaining bone integrity during aging. Studies have shown that the consumption of isoflavones increases calcium retention.

Soy isoflavones appear to have a positive effect on cardiovascular health. There is growing evidence to suggest that isoflavones alone or in conjunction with soy protein exert

a cholesterol-lowering effect, particularly with respect to LDL cholesterol. Data also suggests that isoflavones exert positive benefits toward vascular reactivity, i.e., contractile response to effectors such as acetylcholine that can be used as a measure of blood vessel elasticity.

Soy isoflavones are being evaluated for their ability to maintain prostate health and to help maintain menopausal health as women experience their transitional stages later in life.

CONCLUSIONS

Based upon an independent and critical evaluation and analysis of the information summarized above, the Expert Panel determined Novasoy isoflavones, a mixture of isoflavones with the natural proportion of isoflavones found in soy beans, meeting appropriate food grade specifications and produced by current good manufacturing practice (21 CFR 182.1(b)), to be generally recognized as safe (GRAS), for use as an ingredient in adult meal replacements, beverages, and snack bars to a level of 50 mg per day.

Summary and Conclusions

We, the Expert Panel, partially in response to concerns raised by the National Center for Toxicological Research, independently and critically evaluated a great deal of information on the safety, toxicity and health effects of soy isoflavones. The results of toxicity studies, including oral feeding studies in rats and dogs, did not indicate consistent compound-related and dose-dependent adverse effects for soy isoflavones. Soy isoflavones have been a part of the diet in the Far East for centuries and have been consumed in infant formula since the 1960's without indications of untoward effects on fertility, growth and development, or endocrine function. The Panel notes that significant differences in isoflavone physiological effects exist between different animal species. These differences were taken into account when extrapolating the results to human physiological expectations.

In a number of human studies involving both pre- and post-menopausal women, modest to no changes were noted in circulating sex hormone levels, depending on the hormone examined. Slight elongation of the menstrual cycle was noted which is considered to be beneficial, as was a decrease in cystic changes in the breast. In two reports, a slight stimulation of the breast (epithelial cell hyperplasia) was noted. The significance of this effect is not clear in view of the known epidemiological evidence supporting a decreased risk of breast cancer associated with soy isoflavone intake.

Data on thyroid function in both sensitive animal species and in humans did not support the hypothesis that isoflavones impair the status of thyroid hormones (T3, T_4, TBG, TSH). The lack of thyrotropic effects of soy isoflavones is supported by the lack of evidence for increased prevalence of thyroid disease in soy-consuming Far Eastern peoples and in infants consuming soy formulas. The available data for soy isoflavones was critically evaluated by the Expert Panel in relation to the incidence of vascular dementia or neuroendocrine related abnormalities. The Panel did not find scientific support for a linkage with either vascular dementia or neuroendocrine disorders.

The Expert Panel notes that extensive research is being conducted on the potentially beneficial effects of soy isoflavones on decreasing the risk of breast cancer and prostate disease, maintenance of bone calcium and plasma cholesterol levels consistent with good health, and protection against cardiovascular disease:

In summary, we, the Expert Panel, have critically evaluated the information summarized in this report and conclude that Novasoy™ isoflavones, a mixture of soy isoflavones with the natural proportion found in soy beans, meeting appropriate food grade specifications and produced by current good manufacturing practice (21 CFR 182.1(b)), to be generally recognized as safe (GRAS), using scientific procedures, for use as an ingredient in adult meal replacements, beverages, and snack bars to a level of 50 mg per day.

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Dr. George S. Pauli
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U.S. Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: Submission of Information to GRASN 000001

Dear Dr. Pauli:

Enclosed please find a copy of my letter to Dr. Rulis submitting to the file for GRAS Notification No. 00001 a copy of the summary from the Opinion of an Expert Panel on the Generally Recognized as Safe Status of Isoflavones Derived from Soybeans for Use as a Microingredient in Foods (Supplement to GRASN 000001), on behalf of the Archer Daniels Midland Company.

If you have any questions, please do not hesitate to contact me.

Very truly yours,

Gary L. Yingling

GLY/lh
Enclosure(s)

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Re: Submission of Information to GRAS Notification No. 000001

Dear Dr. Rulis:

As you are aware, my client filed a GRAS notification for soy isoflavones (Novasoy™ isoflavones). In an effort to make sure that FDA currently has in its files information that it or a party filing an FOI request may find useful, on behalf of my client, the Archer Daniels Midland Company (ADM), I am enclosing a copy of the Summary from the expert GRAS panel report, "Opinion of an Expert Panel on the Generally Recognized as Safe (GRAS) Status of Isoflavones Derived from Soybeans for use as a Microingredient in Foods (Supplement to GRASN 000001)," that has been prepared for ADM.

Should the agency have any questions concerning this letter, please do not hesitate to contact me.

Very truly yours,

Gary L. Yingling

GLY/lh
Enclosure(s)
cc: Archer Daniels Midland Company

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Summary and Conclusions

We, the Expert Panel, partially in response to concerns raised by the National Center for Toxicological Research, independently and critically evaluated a great deal of information on the safety, toxicity and health effects of soy isoflavones. The results of toxicity studies, including oral feeding studies in rats and dogs, did not indicate consistent compound-related and dose-dependent adverse effects for soy isoflavones. Soy isoflavones have been a part of the diet in the Far East for centuries and have been consumed in infant formula since the 1960's without indications of untoward effects on fertility, growth and development, or endocrine function. The Panel notes that significant differences in isoflavone physiological effects exist between different animal species. These differences were taken into account when extrapolating the results to human physiological expectations.

In a number of human studies involving both pre- and post-mencpausal women, modest to no changes were noted in circulating sex hormone levels, depending on the hormone examined. Slight elongation of the menstrual cycle was noted which is considered to be beneficial, as was a decrease in cystic changes in the breast. In two reports, a slight stimulation of the breast (epithelial cell hyperplasia) was noted. The significance of this effect is not clear in view of the known epidemiological evidence supporting a decreased risk of breast cancer associated with soy isoflavone intake.

Data on thyroid function in both sensitive animal species and in humans did not support the hypothesis that isoflavones impair the status of thyroid hormones (T_3, T_4, TBG, TSH). The lack of thyrotropic effects of soy isoflavones is supported by the lack of evidence for increased prevalence of thyroid disease in soy-consuming Far Eastern peoples and in infants consuming soy formulas. The available data for soy isoflavones was critically evaluated by the Expert Panel in relation to the incidence of vascular dementia or neuroendocrine related abnormalities. The Panel did not find scientific support for a linkage with either vascular dementia or neuroendocrine disorders.

The Expert Panel notes that extensive research is being conducted on the potentially beneficial effects of soy isoflavones on decreasing the risk of breast cancer and prostate disease, maintenance of bone calcium and plasma cholesterol levels consistent with good health, and protection against., cardiovascular disease.

In summary, we, the Expert Panel, have critically evaluated the information summarized in this report and conclude that Novasoy™ isoflavones, a mixture of soy isoflavones with the natural proportion found in soy beans, meeting appropriate food grade specifications and produced by current good manufacturing practice (21 CFR 182.1(b)), to be generally recognized as safe (GRAS), using scientific procedures, for use as an ingredient in adult meal replacements, beverages, and snack bars to a level of 50 mg per day.

Dr. Joseph F. Borzelleca, Ph.D. Dr. Charles C. Capen, D.V.M., Ph.D.

Dr. Walter H. Glinsmann, M.D. Dr. John A. Thomas, Ph.D.

Reference List for Industry Submission, GRN 000001

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NA- Not applicable