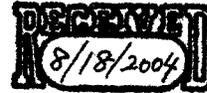


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August 17, 2004

Department of Health and Human Services
Public Health Service
Food and Drug Administration
College Park, MD 20740

RE: 2004Q-0151 Qualified Health Claim (QHC): Soy Protein and
Cancer

Dear Sir or Madam:

This letter responds to the comments submitted by The Westin Price Foundation concerning the Qualified Health Claim (QHC): Soy Protein and Cancer petition submitted by Solae, LLC (hereafter "Solae").

In general, the comment implies that (1) soy protein is not safe for consumption. Soy foods provide and have provided a safe and nutritious protein source for millions of people world wide on a daily basis. The FDA has recognized soy protein as a nutritious, safe food as described in more detail herein. Further, soy-based infant formula has a strong history of safe use and is consumed by 20-25% of formula-fed infants in the United States.

(2) Processing of soy leads to the formation of harmful materials. To the contrary, modern processing techniques often eliminate the potential for production of carcinogens as described herein. In products manufactured by Solae, levels of all regulated compounds are set well below regulatory limits, and in the case of nitrites, are often set to zero.

(3) Solae was remiss in providing the FDA with a thorough review of the scientific literature on soy protein and cancer. To our knowledge, all published epidemiological studies relating soy intake and cancer incidence available in English were reviewed and submitted by Solae to FDA as part of the Soy Protein and Cancer Qualified Health Claim Petition (Petition main body and Appendix III, Other Cancers). Studies using isolated isoflavones as purified compounds were not included in Solae's petition unless there had been particular public interest in individual studies. In the later case, these studies were

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reviewed and included in the petition. Review papers, hypothesis papers, and opinion papers are not listed in the guidance document provided by the FDA (USFDA, 1999) to be included as evidence in health claim petitions, and thus, were not submitted with Solae's health claim petition.

Following are excerpts from Solae's health claim petition, as well as a summary of the scientific literature on soy protein and cancer that was submitted with the petition. Also included are additional supporting materials. With regards to some of the specific comments by The Westin Price Foundation, we have the following remarks:

1. GRAS STATUS OF SOY PROTEIN:

The Westin Price Foundation erroneously cited the GRAS self-affirmation of an isoflavone extract by the Archer Daniel Midland Corporation as the basis for the GRAS status of soy protein. The following was provided in Solae's health claim petition regarding the GRAS status of soy protein.

Use of the Substance is Safe and Lawful

All of the soy protein sources (Table 1), soybean-derived foods (Table 2), and soybean derived food ingredients (Table 3) are generally recognized as safe (GRAS) by self-determination and based on common use in food before January 1, 1958 in conformance with section 201(s) of the Federal Food, Drug and Cosmetic Act (FD & C Act). While soy protein is not listed as GRAS or prior sanctioned in Title 21, CFR, the Agency has stated that these lists "do not include all substances that are generally recognized as safe for their intended use." Further, as noted in §182.1, "It is impractical (for FDA) to list all substances that are GRAS for their intended use."

Table 1. Protein content of soyfoods

Soy Protein Source	Protein (%) (Dry Weight Basis)
Dry Soybeans	40
Soybean Curd	45-50
Soymilk	50
Soy Flour	45-50%
Soy Protein Concentrate	70
Isolated Soy Protein	90
Texturized Soy Protein ¹	45-90

(U.S. Department of Agriculture, 1986)

¹ Texturized soy protein can be made from soy flour, soy protein concentrate, or isolated soy protein.

Table 2. Soybean-derived food products.

Food Product	Derivation
Edamane	Green soybeans
Miso	Fermented soybean paste
Natto	Fermented cooked soybeans
Tempeh	Whole soybeans mixed with other grains, fermented into a cake
Tofu Regular Reduced fat	Dense, solid cake made from hot soy milk, curdled with coagulant same as a regular tofu except derived from reduced fat soy milk
Soy Milk Regular Reduced fat	Finely ground, soaked, strained soybeans with natural oils or with oils removed
Soy Cheese	Coagulated regular or reduced fat soy milk
Soy Yogurt	Fermented regular or reduced fat soy milk
Soy Frozen Desserts	Soy yogurt or soy milk
Soynuts	Whole soybeans soaked in water and baked until brown
Whole Soybeans	Cooked or roasted whole soybeans, green, or dried

Table 3. Soy protein-containing ingredients.

Ingredient	Derivation
Structured vegetable protein	Texturized ISP, SPC, SF or tofu-based mixes
Hydrolyzed vegetable protein	Amino acids from acid hydrolysis of soy protein
Soy grits or meal	Roasted soybeans cracked into coarse pieces
Soy bran	Fibrous material extracted from soybean hulls and refined
Soy isolate fiber	Structured protein fiber or ISP in a fibrous form
Soy sauce	Liquid obtained from fermented soybeans
Okara	Pulp fiber byproduct of soy milk production

In the Proposed Rule, Food Labeling: Health Claims: Soy Protein and Coronary Heart Disease (63 FR 62977), FDA reviewed soy protein in the context of the first soy-related health claim submitted by Solae (then Protein Technologies International) as required in § 101.14(b)(3)(ii). FDA stated:

“Based on the totality of the evidence and, in particular, its common use in food, the agency is not prepared, at this time, to take issue with the petitioner’s view that the use of soy protein is safe and lawful as required in § 101.14(b)(3)(ii). Thus, FDA tentatively concludes that the petitioner has provided evidence that satisfies the requirement in § 101.14(b)(3)(ii) that use of soy protein at the levels necessary to justify the claim is safe and lawful.”
(63 FR 62979)

In the Final Rule, Food Labeling: Health Claims: Soy Protein and Coronary Heart Disease (64 FR 57700), FDA addressed numerous comments on safety of soy protein, including safety of soy protein-based infant formulas, potential effects of lysinoalanine, potential effects of nitrates and subsequent nitrosamine formation, potential effects of trypsin inhibitors, potential effects of phytic acid on mineral balance, potential effects of soy isoflavones (estrogenic effects, fertility effects, developmental effects, and goitrogenic effects), and concerns on allergenicity to soy protein. FDA responded to all these concerns and concluded:

“As stated previously, FDA does not take issue with the petitioner’s self-determination of GRAS status, and the comments, discussed below, have not convinced the agency to change that conclusion.” (64 FR 57702)

“Under the health claim petition process, FDA evaluates whether the substance is ‘safe and lawful’ under the applicable food safety provisions of the act § 101.14(b)(3)(ii). As discussed in greater detail below, FDA did not receive sufficient evidence from comments to challenge the petitioner’s assertion that soy protein ingredients are GRAS by self-determination. The petitioner met the showing required by § 101.14(b)(3)(ii) that the substance be ‘safe and lawful’. (64 FR 57702)

Indeed, a strong administrative record, based on a full and complete review of all relevant science, fully supported FDA’s determination as to the appropriateness of soy as the subject of a health claim.

Solae’s health claim petition also contains the following regarding the GRAS status of soy protein.

A.3. The Substance and Disease Identified in this Petition (Soy Protein and Cancer) Meet the Preliminary Requirements as Outlined in § 101.70.

All of the soy protein sources (Table 1) and soybean-derived food ingredients (Table 3) are generally recognized as safe (GRAS) by self-determination and based on common use in food before January 1, 1958 in conformance with section 201 (s) of the Federal Food, Drug and Cosmetic Act (FD & C Act). Soy protein is not listed as GRAS or prior sanctioned in Title 21, CFR. However, the Agency has stated that these lists "do not include all substances that are generally recognized as safe for their intended use." Further, as noted in 21 CFR §182.1, "It is impractical (for FDA) to list all substances that are GRAS for their intended use."

The fractionation procedures employed to convert vegetable flours to vegetable protein isolates and concentrates were commonplace in various sectors of the grain industry (such as corn processing) well before 1958. Therefore, ISP (and SPC) can be defined as soy flour "subject only to conventional processing as practiced prior to-January 1, 1958." Furthermore, there are no known safety hazards associated with ISP, SPC, SF, or other soy protein foods.

The FDA has recognized soy protein products as having GRAS status at various times throughout the past three decades. The agency proposed to define ISP, as well as SPC and SF, in standards of identity published in 35 FR at 30,489. These products were identified as "safe and suitable edible products." In addition, the USDA, the Association of American Feed Control Officials, the Codex Alimentarius and others have issued over the years various documents that support the GRAS status of products containing soy protein.

2. PROCESSING OF SOY PROTEIN PRODUCTS DO NOT RESULT IN HARMFUL LEVELS OF CARCINOGENIC SUBSTANCES:

The Weston Price Foundation suggests that modern processing techniques produce toxic compounds—nitrosamines, lysinoalanines and nitrite. Nitrosamines are a byproduct of nitrites and nitrates and are not present in soy foods. Modern processing procedures eliminate the potential for lysinoalanine production. Nitrite production during processing is strictly monitored and regulated. Protease inhibitors are found in many plants. Soybeans have the Bowman Birk and Kunitz inhibitors, which are inactivated by heat applied during modern processing techniques. Manufacturing levels for nitrites and the protease inhibitors are set well below regulatory limits, and in the case of nitrites, are often set to zero.

3. REVIEW OF SCIENTIFIC LITERATURE:

(The Totality of Scientific Evidence)

The Weston Price Foundation questioned "the totality of publicly available scientific evidence" that Solae reviewed for the petition. The scientific evidence upon which this petition based is epidemiological studies. Solae stated on page 11 of the Qualified Health Claim Petition: "There have been many epidemiological studies, particularly in recent years, which examined the relationship between consumption of soyfoods and the risk of various cancers in humans. The totality of the publicly available scientific evidence supports the substance/disease relationship that consumption of soy protein-containing

foods is related to a lower risk of certain cancers. The evidence is particularly strong in cancer of breast, prostate, and gastro-intestinal tract.”

(Meta-Analysis)

The Weston Price Foundation provided a discussion regarding the validity of meta-analysis. Solae conducted the scientific review according to *Guidance for Industry: Significant Scientific Agreement in the Review of Health Claims for Conventional Foods and Dietary Supplements* (<http://www.cfsan.fda.gov/~dms/ssaguide.html>). The conclusion that consumption of soy protein-containing foods is related to a reduced risk of breast, prostate, and gastro-intestinal cancer is based on the weight of the totality of scientific evidence from available epidemiological studies that assessed soy protein-containing foods in relation to cancer. Results of meta-analysis are supporting evidence to the conclusion of the scientific review.

Meta-analysis is the statistical analysis of a collection of results from individual studies for the purpose of integrating findings and determining an estimate of an effect. Meta-analysis has been widely accepted by medical research community. There has been a sharp increase in the number of publications in medical journals using meta-analysis as a tool of assessment in recent years (Figure 1). The methods of meta-analysis used for analyzing epidemiological studies for this petition are detailed in *Appendix V. Methods for Meta-Analysis*. An external consultant was engaged to conduct the analysis and provide a final report. The consultant is a professor of statistics and mathematics at Washington University, St. Louis, MO.

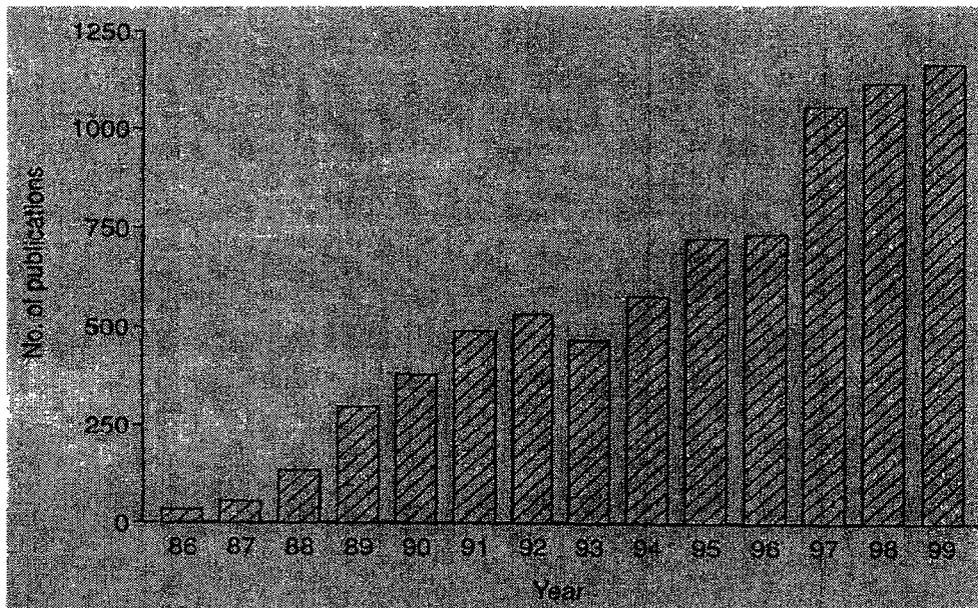


Figure 1. Number of publications concerning meta-analysis, 1986-1999. Results from MEDLINE search using test word and medical subject (MESH) heading “meta-analysis” and test word “systematic review”. (Adapted from *Systematic Reviews in Health Care: Meta-Analysis in Context*, 2nd edition (May 15, 2001), edited by M. Egger, et al. BMJ Publishing Group)

(Inclusion of the Work by Nagata et al (2000) in the Meta-Analysis)

The Weston Price Foundation questioned the ethnicity of not including Nagata's work (2000) in meta-analysis (p.11 and p.32). The inclusion and exclusion criteria of studies for meta-analysis were detailed in *Appendix V. Methods for Meta-Analysis*. Paramount to inclusion, a study must have provided a risk estimate (e.g. odds ratio or relative risk) and 95% confidence interval or data from which a risk estimate and 95% confidence interval can be calculated. Nagata et al (2000) reported a correlation coefficient in their publication. Therefore, it was not possible to include this study in meta-analysis.

(Cell Culture Studies)

The Weston Price Foundation cited *in vitro* studies using purified isoflavone compounds (e.g. genistein) to study tumor cell proliferation or other activities at cellular levels (De Lemos, 2001; Dees et al., 1997; Martin et al., 1978; Strick et al., 2000; Wang & Kurzer, 1997). *In vitro* work does give interesting mechanistic insight. Cell culture work does not accurately depict the impact of dietary soy intake and cancer development in human. Therefore, publications from *in vitro* studies on individual compounds of soybeans such as isoflavones (e.g. genistein) were not reviewed for this health claim petition. We did however provide a detailed discussion on genistein and mammary tumor cell proliferation *in vitro* in *Appendix VI (p. 7). Review of Individual Studies around Concern for Soy – 4. Genistein and Proliferation of Estrogen Dependent Mammary Tumor Cells in Culture*.

Appendix VI. Review of Individual Studies around Concern for Soy 4. Genistein and Proliferation of Estrogen Dependent Mammary Tumor Cells in Culture

Results of in vitro studies show that isoflavone compounds (e.g. genistein) at lower concentrations (10^{-8} M to 10^{-6} M) stimulate the proliferation of estrogen dependent MCF-7 mammary carcinoma cells cultured in estrogen-free medium (Dees et al., 1997; Miodini et al., 1999; Nakagawa et al., 2000; Sathyamoorthy & Wang, 1997; Wang et al., 1996). The MCF-7 cell line, originated from a pleural effusion of a woman with metastatic mammary carcinoma, expresses cytoplasmic estrogen receptors (Brooks et al., 1973) which are capable of transporting the 17 β -estradiol complex into the nucleus. Estrogen stimulates MCF-7 cell proliferation through estrogen receptor-mediated mechanisms. It has been documented that estrogen-like compounds stimulate the proliferation of estrogen dependent cells in vitro in the absence of estrogen. For example, tamoxifen at concentrations of 10^{-9} M stimulates MCF-7 cell proliferation when cells are cultured in an estrogen-free medium (Wakeling et al., 1989). However, in the presence of estrogen tamoxifen inhibits cell proliferation (Wakeling et al., 1989). The inhibition on MCF-7 cell proliferation has also been observed when cells are cultured with genistein in estrogen containing medium (Fioravanti et al., 1998; Miodini et al., 1999). These findings suggest that the inhibition is through a mechanism of competitive binding of these compounds (tamoxifen and genistein) to estrogen receptors. It should be noted while in vitro approaches may be useful

tools in certain laboratory investigations, they do not evaluate the effect of soy protein on cancer development in humans.

(Estrogen-Dependent MCF-7 Tumor Model Studies in Ovariectomized Mice)

The Weston Price Foundation cited studies from the laboratory of Dr. William Helferich investigating effects of genistein on the growth of estrogen dependent MCF-7 mammary tumor in mice (Allred et al., 2004b; Allred et al., 2001a; Allred et al., 2001b; Hsieh et al., 1998; Ju et al., 2001). One of these publications was published after submission of the health claim petition and will be reviewed (Allred et al., 2004b). Pertaining to the remainder of the publications from Dr. Helferich's laboratory (Allred et al., 2001a; Allred et al., 2001b; Hsieh et al., 1998), the most germane to the current petition is the paper by Allred et al (2001a) a on soy protein and MCF-7 tumors. Because the petition deals solely with soy protein containing foods and not with purified genistein, the papers by Allred et al (2001b) and Hsieh et al (1998) were not included in the health claim petition. The following discussion was provided to FDA for review (Allred et al., 2001a):

*Appendix VI (p. 4). Review of Individual Studies around Concern for Soy
2. Genistein and Estrogen Dependent Mammary Tumor in Ovariectomized Mice*

Allred et al (2001a) investigated the effect of ISP on the growth of MCF-7 estrogen-dependent human mammary tumor in ovariectomized, athymic mice. In this study, estrogen-implanted mice were subcutaneously inoculated MCF-7 mammary carcinoma cells. After the establishment of tumor, the estrogen implant was replaced by feeding mice one of the three ISP diets containing different levels of genistein. The endpoint measurement of the experiment was tumor size. Feeding mice an ISP diet containing 15 ppm, 150 ppm, or 300 ppm genistein supports the growth of estrogen-dependent mammary tumor in a dose-dependent manner in ovariectomized athymic mice. The difference between the 150 ppm group or the 300 ppm group and the controls was statistically significant ($P < 0.01$).

MCF-7 mammary tumors are dependent on the presence of estrogen for growth. Tumors developed from MCF-7 cells require exogenous estrogen to support their growth in athymic mice (Brunner et al., 1989; Soule & McGrath, 1980). Providing mice with an estrogen implant supports the growth of MCF-7 tumors, withdrawing the implant stops the growth, and replacing it with an estrogen-like compound maintains the growth of the tumor. For example, when the estrogen implant is replaced with a compound that has a chemical structure similar to that of estrogen, MCF-7 tumor growth is maintained or promoted. This encompasses a wide variety of compounds found in nature and that are produced synthetically. Paradoxically, tamoxifen, a drug widely used for the prevention of breast cancer in women actually promotes growth of MCF-7 tumor growth in this athymic mouse model (Osborne et al., 1995; Osborne et al., 1994).

In addition to the above studies, Dr. Helferich's lab has conducted work on individual isoflavones and growth of MCF-7 estrogen-dependent tumor growth in nude athymic mice (Allred et al., 2001b; Hsieh et al., 1998). Below is a summary of this work. As stated previously, this section was not included in the original health claim petition because the studies did not use soy food as a treatment.

A group of researchers at University of Illinois led by Dr. William Helferich reported that dietary supplementation with genistein or genistin supports the estrogen-stimulated growth of MCF-7 human mammary tumor in ovariectomized athymic mice (Allred et al., 2001b; Hsieh et al., 1998; Ju et al., 2001). Based on the results, the investigators suggested, "There is the potential for dietary genistein to stimulate the growth of estrogen-dependent tumors in human with low circulating endogenous estrogen levels, such as those found in post-menopausal women (Hsieh et al., 1998)."

The MCF-7 mammary carcinoma cells are estrogen dependent cells. Tumors developed from these cells require exogenous estrogen to support their growth in athymic mice (Brunner et al., 1989; Soule & McGrath, 1980). Providing mice with an estrogen implant supports the growth of MCF-7 tumors, withdrawing the implant stops the growth, and replacing it with an estrogen-like compound maintains the growth of the tumor. For example, the chemical structure of tamoxifen is similar to that of estrogen, and replacing the estrogen implant with tamoxifen maintains the growth of MCF-7 tumors (Osborne et al., 1995; Osborne et al., 1994). Tamoxifen is a drug approved by the United States FDA for breast cancer prevention in women who are at high risk of developing breast cancer. The chemical structure of genistein is similar to that of estrogen, and therefore it supports the growth of MCF-7 tumors in mice when it is substituted for exogenous estrogen (Allred et al., 2001b; Hsieh et al., 1998; Ju et al., 2001).

The mechanism of the observed effect of tamoxifen and genistein on MCF-7 tumor growth is that these compounds are capable of competing against estrogen for binding to estrogen receptors, but their estrogenic potencies are significantly weaker than estrogens. The table below lists differences in relative estrogenic potencies of isoflavone compounds compared with estradiol (Markiewicz et al., 1993). In the absence of estrogen (e.g. ovariectomized mice without an estrogen implant), tamoxifen and genistein exert weak estrogenic effect, and therefore support the growth of estrogen-dependent tumors. In the presence of estrogen (e.g. ovary intact mice or ovariectomized mice bearing an estrogen implant), the competitive binding of these compounds to estrogen receptors reduces the number of estrogen molecules bound to the receptors. Therefore these compounds inhibit the stimulatory effect of estrogen on tumor growth. It has demonstrated that tamoxifen (Brunner et al., 1989), genistein (Shao et al., 1998), and fermented soymilk (Chang et al., 2002) inhibit the growth of MCF-7 mammary tumors in athymic mice bearing an estrogen implant.

Relative Estrogenic Potencies of Isoflavone Compounds Compared with Estradiol

Compound	EC ₅₀ ¹ , nM (mean ± SD)	Relative Potencies ²
Estradiol	0.0673 ± 0.03	100
Coumestrol	33.3 ± 3.9	0.202
Genistein	79.8 ± 11	0.084
Equol	111 ± 17.6	0.061
Daidzein	515 ± 41.1	0.013
Biochanin A	>1,000	<0.006
Formononetin	>10,000	<0.0006

¹EC₅₀ = concentrations of test compounds exerting one-half of the maximal increase in alkaline phosphatase activity in Ishikawa-Var I line of human endometrial adenocarcinoma cells. ²Relative potencies = (EC₅₀ (estradiol)/EC₅₀ (isoflavone)) x 100. (Adapted from Markiewicz et al., *J Steroid Biochem Molec Biol* 45(5):399-405, 1993)

It has been documented that the development and growth of MCF-7 mammary tumor in both ovary intact and ovariectomized mice requires exogenous estrogen (Brunner et al., 1989; Soule & McGrath, 1980). The presence of exogenous estrogen makes the circulatory estrogen excessive to the physiological level in both cases. Thus, this athymic mouse model is not relevant to either pre-menopausal or post-menopausal women. Dimethylbenz (a) anthracene (DMBA)-induced mammary tumorigenesis in laboratory animals is estrogen-dependent. Dietary supplementation with soy protein (Appelt & Reicks, 1999; Barnes et al., 1990; Barnes et al., 1994; Hakkak et al., 2000) or genistein (Fritz et al., 1998; Lamartiniere et al., 1995b; Murrill et al., 1996) inhibits DMBA-induced mammary tumor development in female rats. Furthermore, in a most recent publication Wood et al (2004) reported results from a three-year feeding study in postmenopausal monkeys in which isoflavone-depleted soy protein, isoflavone-containing soy protein, and estrogen were compared. Epithelial proliferation and progesterone receptor expression in the breast and uterus are significantly higher in the estrogen group compared with isoflavone-depleted and isoflavone-containing soy protein groups, and there is no significant difference between the isoflavone-depleted and isoflavone-containing groups. Both soy protein treatments result in a significant reduction in serum estrone and estradiol compared with the estrogen group, and the isoflavone-containing soy protein results in a further significant reduction in serum estrone and estradiol compared with the isoflavone-depleted soy protein. The authors concluded, "These findings suggest that high dietary levels of isoflavones do not stimulate breast and uterine proliferation in postmenopausal monkeys and may contribute to an estrogen profile associated with reduced breast cancer risk."

One paper was recently published investigating growth of MCF-7 estrogen-dependent tumor growth in nude athymic mice fed soy foods (Allred et al., 2004b). It published after the submission date and is summarized below.

In a most recent publication, Allred et al (2004b) reported their finding in comparison of soy flour with crude soy extracts, mixed isoflavones, and a pure genistin on the growth of estrogen-dependent MCF-7 tumor in an ovariectomized mouse model. The experimental approach of this study is the same as they used previously (Allred et al., 2001b; Hsieh et al., 1998; Ju et al., 2001). Briefly, ovariectomized mice were implanted with an estrogen pellet to raise blood estrogen level before receiving a subcutaneous inoculation of MCF-7 mammary tumor cells. When tumors reached to a size of 40 mm² in cross-sectional area, the estrogen implant was replaced with one of the dietary treatments discussed above. The primary end point measurement was tumor size. The investigators found that the soy flour is the least estrogenic on MCF-7 tumor growth compared with crude soy extracts, isoflavone mixture, and pure genistin.

The mechanism of action of isoflavone compounds on estrogen-dependent tumor growth is the same as we discussed in the previous section – binding to estrogen receptors without any competition when they are replaced for estrogen implant in ovariectomized mice. The recent observation that soy flour is the least stimulatory with regard to MCF-7 tumor growth is contradictory to earlier findings from this group. Soy flour contains greater amounts of bioactive materials including isoflavones compared to other soy protein ingredients. If isoflavones in fact stimulate MCF-7 estrogen-dependent tumor growth as Dr. Helferich and colleagues have contended, soy flour should possess an enhanced stimulatory effect compared to other soy products because of its relatively high content of isoflavones.

(Genistein and Tamoxifen Interaction)

The Weston Price Foundation provided a study showing that genistein negates the tamoxifen effect on the growth of estrogen-dependent mammary tumors in ovariectomized mice (Ju et al., 2002). However, recent studies show that dietary soy is synergetic with tamoxifen in inhibiting chemically induced mammary tumorigenesis in animals (Gotoh et al 1998, Constantinou et al 2001). Genistein and tamoxifen interaction was discussed in detail in *Appendix VI (p.5-6). Review of Individual Studies around Concern for Soy – 3. Genistein, Tamoxifen, and Estrogen Dependent Mammary Tumors.*

Appendix VI. Review of Individual Studies around Concern for Soy 3. Genistein, Tamoxifen, and Estrogen Dependent Mammary Tumors

Ju et al (2002) reported that genistein attenuates the inhibitory effect of tamoxifen on the growth of estrogen dependent MCF-7 mammary tumor in ovariectomized athymic mice. Mice implanted with estrogen (0.25 mg) and tamoxifen (2.5 mg or 5 mg) were subcutaneously injected MCF-7 cells and assigned to two groups.

One group was fed an AIN93G basal diet, and the other was fed the basal diet supplemented with 1,000 ppm genistein. At the end of the 32-week feeding regimen, tamoxifen at either dose significantly inhibits the estrogen-stimulated tumor growth, whereas dietary supplementation with genistein partially but significantly attenuates the inhibitory effect of tamoxifen.

It has been shown that tamoxifen (Osborne et al., 1985), genistein (Shao et al., 1998), and soy isoflavones (Zhou et al., 2004) inhibit MCF-7 mammary tumor growth in estrogen-implanted athymic mice. The chemical structure of tamoxifen and genistein is similar to that of estrogen, and tamoxifen and genistein each competes against estrogen for binding to estrogen receptors. Thus, these compounds can inhibit, have no effect, or even support the growth of estrogen dependent tumors depending on doses used and the estrogen status of a given model. For example, tamoxifen inhibits estrogen-stimulated MCF-7 tumor growth in ovariectomized athymic mice, however, increasing the dose of estrogen administered to the mice partially reverses the inhibitory effect of tamoxifen (Iino et al., 1991). Currently, there is no data available from animal studies on the competitive binding to estrogen receptors between tamoxifen and genistein in the presence or absence of estrogen. However, recent studies show that dietary soy is synergetic with tamoxifen in inhibiting chemically induced mammary tumorigenesis in animals. Constantinou et al (2001) reported that dietary supplementation with tamoxifen and isolated soy protein (16%) results in a significantly greater inhibition on dimethylbenz (a) anthracene (DMBA)-induced mammary tumor development in female rats than soy protein or tamoxifen alone. Gotoh et al (1998) revealed that miso (a commonly consumed soy-based dish in Japan) is synergetic with tamoxifen in inhibiting N-nitroso-N-methyl-urea (NMU)-induced mammary tumorigenesis in female rats. Both DMBA- and NMU-induced mammary tumors are estrogen dependent for their growth.

The interaction of genistein with tamoxifen has been investigated in vitro. Genistein and tamoxifen are synergetic in inhibiting the proliferation of estrogen dependent (MCF-7) (Tanos et al., 2002) and estrogen independent mammary carcinoma cells (MDA-231, MDA-435) in culture (Shen et al., 1999; Tanos et al., 2002). It has also been shown that genistein reduces the inhibitory effect of tamoxifen on proliferation of estrogen dependent T47D mammary tumor cells in vitro (Jones et al., 2002). In this study, estradiol-treated T47D cells received no treatment (control) or cultured with 1 μ M tamoxifen, 1 μ M genistein, or both. The percentage of proliferative cells after a 48-hour incubation for these treatments is 100%, 93%, 100%, and 97%, respectively. It should be noted while in vitro approaches may be important tools in certain experimental investigations, they do not evaluate cancer development in women consuming soy protein.

(Short-Term Feeding Studies in Women)

The Weston Price Foundation cited three publications on the influence of short-term soy feeding on breast tissues in women (Hargreaves et al., 1999; McMichael-Phillips et al.,

1998; Petrakis et al., 1996). This and related publications were discussed in detail in *Appendix VI (p.2-3). Review of Individual Studies around Concern for Soy – 1. Dietary Soy and Breast Tissue in Women.*

*Appendix VI. Review of Individual Studies around Concern for Soy
1. Dietary Soy and Breast Tissue in Women*

Bundred and co-workers (McMichael-Phillips et al., 1998) (Hargreaves et al., 1999) investigated effects of soy protein intake on human breast tissue in a 14-day feeding study. Eighty-four premenopausal women with benign or malignant breast disease were assigned to two groups. One received a daily intake of 60 g soy protein (containing 45 mg isoflavones), and the other remained on their normal diet. McMichael-Phillips et al (1998) reported preliminary findings of this study with 48 participants. The proliferation rate of breast lobular epithelium significantly increases after 14 days of soy intervention when both the length of menstrual cycle and the age of patients are accounted for. Progesterone receptor expression increases significantly in soy group. However, after the completion of the study, an analysis of data from all participants (n = 84) shows that there are no differences in these measurements between the soy and the control groups (Hargreaves et al., 1999). Hargreaves et al (1999) concluded, "In the early stages of this study, our initial analysis of epithelial proliferation and progesterone receptor expression suggested that both responded to dietary soy supplementation, indicative of an estrogenic response (McMichael-Phillips et al., 1998). However, with observations of double the number of patients, we are now unable to detect any differences in breast epithelial proliferation, apoptosis, hormone receptor status, and Bcl-2 expression in response to soy supplementation."

Petrakis et al (1996) conducted a six-month feeding study with 24 premenopausal and postmenopausal women. The participants consumed no soy during months 1-3 and 10-12, and 38 g soy protein isolate (containing 38 mg genistein) daily during months 4-9. Nipple aspirate fluid, blood, and urine samples were collected before, during, and after soy feeding for biomedical analysis. A six-month soy feeding has a stimulatory effect on breast in premenopausal women, characterized by an increase in breast fluid secretion, appearance of hyperplastic epithelial cells (4 of the 14 premenopausal and 3 of the 10 postmenopausal women), and elevated plasma estradiol. Plasma concentration of estradiol is 81 ± 59 pg/mL, 89 ± 64 pg/mL, 104 ± 70 pg/mL, and 90 ± 61 pg/mL for months 1-3, 4-6, 7-9, and 10-12, respectively. The authors concluded, "This pilot study of nipple aspirate fluid from women consuming soy protein isolate daily for six months revealed apparent estrogenic effect on the breast fluid secretion. In view of the increasing use of soy protein food products in Western populations, more detailed investigations of the effects of soy on the physiology of the female breast appear highly desirable" (Petrakis et al., 1996).

The authors (Petrakis et al., 1996) cautioned that the sample size of the study was small (14 premenopausal and 10 postmenopausal women) and the results were only suggestive of causative effect. In addition, study samples were not obtained at consistent intervals amid menstrual cycles, and the breast fluid secretion remained increased in women even after soy intake is discontinued. The large standard deviation in each of the plasma estradiol measurements (data presented in the above paragraph) indicates that there are no differences among the measurement periods. Results of recently published cross-sectional studies reveal that soy consumption is inversely related with serum estrogen levels in premenopausal (Nagata et al., 1997) and postmenopausal women (Wu et al., 2002a). The effect of soy protein or isoflavone intake on blood estrogens in premenopausal and postmenopausal women has also been investigated in short-term intervention studies. Of 15 available publications, fourteen demonstrate a decrease or no change in blood estrogen concentrations (Lu et al., 2000; Lu et al., 1996; Lu et al., 2001) (Brown et al., 2002; Brzezinski et al., 1997; Duncan et al., 1999a; Duncan et al., 1999b; Martini et al., 1999; Maskarinec et al., 2002; Nagata et al., 1998; Wu et al., 2000) (Baird et al., 1995; Persky et al., 2002; Pino et al., 2000), and one show an increase in follicular estradiol in premenopausal women (Cassidy et al., 1994). Moreover, findings by Petrakis et al (1996) are contrary to epidemiological studies from Asia and soy-consuming populations in the United States. These studies demonstrate that soy consumption is related to a lower risk of breast cancer (Yamamoto et al., 2003) (Shu et al., 2001) (Dai et al., 2001) (Wu et al., 2002b) (Wu et al., 1996) (Lee et al., 1991). There has not been any evidence from available epidemiological studies that soy consumption is related to an increase in breast cancer in women.

(Data Interpretation – Epidemiological Studies)

The Weston Price Foundation, throughout their document, expressed their opinion and interpretation of epidemiological studies that were reviewed and presented by Solae in the Qualified Health Claim Petition. Solae reviewed the existing scientific evidence from epidemiological studies according criteria set by FDA in *Guidance for Industry: Significant Scientific Agreement in the Review of Health Claims for Conventional Foods and Dietary Supplements* (<http://www.cfsan.fda.gov/~dms/ssaguide.html>). The studies reviewed by Solae were presented in a descending order of persuasiveness as per FDA criteria, e.g. cohort studies, case-control studies, cross-sectional analysis, and ecological studies. The design, study populations, methods of assessment, types of soyfoods, intake levels (frequency or quantity), and results of each study were presented in the text and tables. Confounding factors adjusted in each study were presented in tables.

(Inclusion of Animal Studies)

The Weston Price Foundation questioned the total number of animal studies (“39 studies available to date”) Solae reviewed for the petition. Solae reviewed all the animal studies that assessed soy protein as a component of a diet and examined the preventive effect of such a diet in experimentally induced tumorigenesis (Qualified Health Claim Petition,

p.11). Solae presented these studies as supportive evidence to the conclusion from the epidemiological studies. There were 39 studies available at the time of submission of the health claim on the topic of dietary soy protein and cancer development and growth. The findings from these studies demonstrate that soy protein inhibits experimentally-induced tumorigenesis and supports the conclusion from epidemiological studies that consumption of soy protein-containing foods is related to a reduced risk of breast, prostate, and gastro-intestinal cancer. Studies that assessed dietary supplementation with a crude mixture of compounds or a pure compound (e.g. isoflavones, Bowman-Birk inhibitor, or saponins) were not reviewed for this petition. These include studies cited by the Weston Price Foundation on genistein (Allred et al., 2004a; Hilakivi-Clarke et al., 1998; Yang et al., 2000), soy phytochemical concentrate (Zhou et al., 2003), and coumestrol, which is not a soy component (Whitten et al., 1995). Dietary supplementation with a chemical compound in any form does not evaluate the intake of a soy diet in animals and human.

The Weston Price Foundation cited a number of early studies on trypsin inhibitors and/or raw soy flour on biochemical changes or pre-neoplastic lesions in pancreas in rats (Gumbmann et al., 1985; Liener, 1996; Liener et al., 1985; Myers et al., 1991; Rackis et al., 1985; Roebuck, 1987; Spangler et al., 1985). Studies on an individual chemical compound in any form do not evaluate the intake of a soy diet in animals and human, and therefore these studies were not reviewed for this petition.

The Weston Price Foundation cited early studies on lectin and intestine in animals (Ament & Rubin, 1972; Jindal et al., 1984; Poley & Klein, 1983; Torres-Pinedo, 1983). Because studies on an individual chemical compound in any form do not evaluate the intake of a soy diet in animals and human, these studies were not reviewed for this petition.

The Weston Price Foundation claimed that Solae omitted several key studies that link soy protein to prostate cancer (Bylund et al., 2000; Lephart et al., 2001) and the development of intestinal cancers or pre-cancerous damage in laboratory animals (Davies et al., 1999; Govers et al., 1992; McIntosh et al., 1995). Publications by Bylund et al (2000), McIntosh et al (1995), and Davies et al (1999) were reviewed and presented in the petition. The publications by Lephart et al (2001) and Govers et al (1992) did not meet our definition of studies investigating cancer growth and development as an endpoint measurement, and therefore were not reviewed for the petition. The publication by Lephart et al (2001) is a study on brain structure and aromatase activity in rats and that by Govers et al (1992) is a study on colonic epithelial proliferation.

(Prostate Tumor Animal Model Study)

The Weston Price Foundation criticized Solae's interpretation of the study by Pollard and colleagues (2001) that assessed diet and duration of testosterone-dependent prostate cancer in Lobound-Wistar rats. Results indicated a significant reduction in spontaneous P-SV cancer in rats fed a soy protein diet, compared with those fed a soybean meal diet (the control). Pollard et al (2001) stated, "At age 12 months early stage spontaneous

prostate cancer was prevented or reversed by testosterone-deprivation through change of diet from L-485 (soybean meal diet) to soy protein isolate/isoflavone (SPII) diet, thereby preventing the late lethal clinical disease: about 75% of rats at risk of developing testosterone-independent P-SV tumor were free of detectable cancer and about 25% had developed testosterone-independent cancer at age 12 months. The duration of the dependent stage exceeded age 12 months in 75% of the rats at risk. Dietary soybean meal, found in most natural ingredient diets, may promote prostate cancer tumorigenesis, but only in L-W rats.”

(Maternal and Perinatal Genistein Exposure)

The Weston Price Foundation cited studies on maternal and perinatal injection of genistein and experimentally induced mammary tumorigenesis in rats (Hilakivi-Clarke et al., 1998; Yang et al., 2000). However, others have reported that genistein exposure during pregnancy, puberty, and the neonatal period inhibits experimentally induced mammary tumorigenesis in female rats (Fritz et al, 1998; Hilakivi-Clarke et al., 1999c; Murrill et al., 1996; Lamartiniere et al 1995a; Lamartiniere et al, 1995b). Furthermore, Hakkak and associates (2000) have demonstrated that feeding female rats a soy protein over two generations significantly inhibits chemically induced mammary tumor development compared to casein-fed animals. Maternal and perinatal genistein exposure and related studies were also discussed in *Appendix VI (pp. 12-13). Review of Individual Studies around Concern for Soy – 7. Maternal Exposure to Genistein and Mammary Tumorigenesis in Rats.*

Appendix VI. Review of Individual Studies around Concern for Soy 7. Maternal Exposure to Genistein and Mammary Tumorigenesis in Rats

Hilakivi-Clarke et al (1999a) investigated the effect of maternal genistein exposure on mammary carcinogenesis in female offspring. Pregnant rats were subcutaneously injected a vehicle (control) or genistein at 20 µg, 100 µg, or 300 µg daily from gestation days 15 to 20. The female offspring were treated with 7,12-dimethylbenz (a) anthracene (DMBA) when they were 45 or 50 days old. In utero exposure to genistein increases the incidence of DMBA-induced mammary tumorigenesis in a dose dependent manner compared with the controls. Based on these results, Hilakivi-Clarke et al (1999a) suggested, “Thus, where women are consuming soy on an irregular basis, they should consider exercising caution if consumption is likely to be restricted to pregnancy.”

In contrast to in utero treatment, prepubertal genistein treatment results in a significant reduction in DMBA-induced mammary tumorigenesis in female rats (Hilakivi-Clarke et al., 1999c). Prepubertal female rats were subcutaneously injected genistein (~1 mg/kg bw/d) at postnatal days 7, 10, 14, 17, and 20, and then received DMBA treatment when they were 45 days old. Tumor multiplicity and the number of malignant tumors in rats treated with genistein are significantly decreased compared with the controls. Lamartiniere et al (1995a; 1995b) found similar inhibition on DMBA-induced mammary tumorigenesis in

rats neonatally and subcutaneously injected genistein at post-partum days 2, 4, and 6. Furthermore, Fritz et al (1998) reported that dietary supplementation with genistein at 25 mg or 250 mg/kg diet from conception to day 21 post-partum (before puberty) results in a dose-dependent reduction in multiplicity of DMBA-induced mammary tumors in female rats.

Timing of genistein treatment, which may relate to the early differentiation of mammary epithelial cells, may play a critical role in mammary tumorigenesis in animals. Neonatal and prepubertal genistein treatments result in few terminal end buds and more lobules (Hilakivi-Clarke et al., 1999b; Murrill et al., 1996). Terminal end buds are the least differentiated terminal ductal structures which are most susceptible to chemical carcinogens, whereas lobules are highly differentiated structure (Russo & Russo, 1978). A reduction in the number of terminal end buds means fewer susceptible targets for the chemical carcinogen. In utero genistein exposure increases estrogen receptor expression in prepubertal mammary glands, which may increase the susceptibility to carcinogen treatment, and decreases protein kinase C activity (Hilakivi-Clarke et al., 1999a). A low level of protein kinase C may be associated with poorly differentiated mammary epithelial structures (O'Brian & Ward, 1989). It is important to note that while genistein aglycone is used in most genistein studies, it represents a small portion of total isoflavones in soyfoods. For example, it represents only 3.2-5.8% total isoflavones in soy formula (Setchell et al., 1997). Furthermore, the route and quantity of genistein administered to animals in a given model play an important role in determining the outcome of an experiment. These factors should be considered when extrapolating the impact of dietary soy to women's health from these laboratory findings.

To our knowledge, there has not been any publication on maternal dietary soy supplementation and experimentally induced mammary tumorigenesis in animal offspring. However, results of a multi-generation feeding study show that soy protein inhibits DMBA-induced mammary tumorigenesis in rat offspring (Hakkak et al., 2000). In this study, offspring from female rats fed a soy protein diet were mated and maintained on the same diet for two generations before receiving an injection of DMBA. Intake of a 20% soy protein diet for two generations significantly decreases tumor incidence and increases tumor latency period compared with the soy-free casein-based control diet. The soy protein diet contains total isoflavones at 430 mg/kg diet (Hakkak et al., 2000).

(Studies Published After Submission)

A study by Sonoda et al (2004) cited by The Weston Price Foundation was published after Solae's submission of the petition to the FDA. It is a case-control study of diet and prostate cancer in Japan with 140 cases and 140 age-matched hospital controls. The study concluded consumption of all soybean products and tofu (bean curd) is related to a decreased risk of prostate cancer in men. The odds ratio and 95% confidence interval of the 4th and first quartile are 0.53 (0.24-1.14) for all soybean products and 0.47 (0.20-1.08) for tofu. The authors concluded, "Our results provide support to the hypothesis that the

traditional Japanese diet, which is rich in soybean products and fish, might be protective against prostate cancer.”

We updated the meta-analysis (Figure 5 of the petition, p.60) of studies on consumption of soy protein-containing foods and prostate cancer in men by adding work by Sonada et al (2004) to the analysis. The pooled estimate of odds ratio/relative risk is 0.66 (95% CI = 0.54-0.79; $P < 0.001$; Figure 2). Results of this analysis are consistent with our previously analysis (Figure 5 of the petition, p.60) and demonstrate that consumption of soy protein-containing foods is associated with a lower risk of prostate cancer in men.

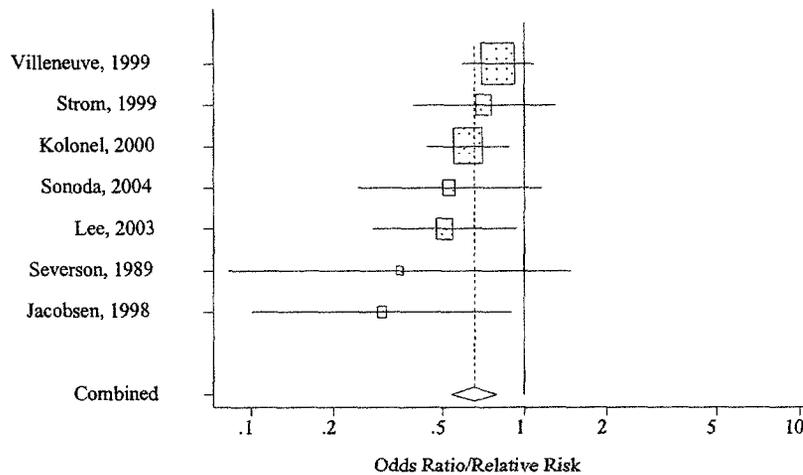


Figure 2. Meta-analysis of studies on consumption of soy protein-containing foods and prostate cancer in men (updated by adding Sonoda 2004 to the analysis). Each study-specific point estimate is plotted as a square box. The size of the box is proportional to the precision of the estimate, and its 95% CI is denoted by a line through the box. The vertical dashed line and the lower vertices of the diamond indicate the pooled estimate of odds ratio/relative risk, and left and right vertices of the diamond represent its 95% CI. The pooled estimate of odds ratio/relative risk is 0.66 (95% CI = 0.54 – 0.79; $P < 0.001$), and no publication bias was detected. Results of this analysis show that consumption of soy protein-containing foods is associated with a lower risk of prostate cancer in man.

(Studies Reporting Data on Bio-Markers)

The Weston Price Foundation cited studies that measured a change in a particular bio-marker (e.g. serum level of prostate specific antigen or insulin-like growth factors) (Adams et al., 2004; Jenkins et al., 2003; Probst-Hensch et al., 2003; Spentzos et al., 2003; Urban et al., 2001). These studies were not reviewed for this petition. The investigation by Probst-Hensch et al (2003) measuring insulin-like growth factor is not a study on soy. The remaining investigations did not report any significant change in prostate specific antigen as a result of soy feeding. FDA clearly stated in their guidance that “In conducting a health claim review, FDA does not rely on a change in a bio-marker as a measurement of the effect of a dietary factor on a disease unless there is evidence

that altering the parameter can affect the risk of developing that disease or health-related condition.” (*Guidance for Industry: Significant Scientific Agreement in the Review of Health Claims for Conventional Foods and Dietary Supplements* (<http://www.cfsan.fda.gov/~dms/ssaguide.html>)).

(Genistein and Uterine Tumor Model)

The Weston Price Foundation cited one study assessing neonatal genistein injection and uterine tumor development in mice (Newbold et al., 2001). It is important to note that neonatal injection of genistein as a pure compound at a high dose and dietary intake of soy foods are two different concepts. Results from a multi-generation feeding study showed that there was no difference in uterine weight in offspring in a study comparing diets based on soy protein or casein, and there was no uterine tumor development in either group (Badger et al, 2001). Furthermore, soy is widely used as a protein source in laboratory animal diets (Thigpen et al, 1999), without concern of widespread tumor development, and has been for a number of years. The study by Newbold et al (2001) and related studies are discussed in detail in *Appendix VI (p. 9-11). Review of Individual Studies around Concern for Soy – 6. Neonatal injection of genistein and uterine tumorigenesis in mice.*

Appendix VI. Review of Individual Studies around Concern for Soy 6. Neonatal Injection of Genistein and Uterine Tumorigenesis in Mice

Newbold et al (2001) reported that neonatal injection of genistein results in uterine tumorigenesis in later life in outbred CD-1 mice. In this experiment, female neonatal mice were subcutaneously injected genistein (50 mg/kg bw/d) for five days (days 1-5 after birth) and terminated at the age of 18 months for uterine examination. Thirty-five percent of mice (6 of 17) in genistein group have uterine adenocarcinoma, and there is no uterine adenocarcinoma found in the controls injected corn oil. Based on these findings, Newbold et al (2001) concluded, “The findings of the present study raise concerns over the amount of phytoestrogens in soy-based infant formula and other soy-based products that are fed to young children (Newbold et al., 2001).”

It has been well documented that treatment of animals in their early stage of development with estrogen or an estrogen-like compound affects the growth and development of the animals. For example, daily injection of diethyl-stilbesterol (a synthetic estrogen compound) (Newbold et al., 1990) or tamoxifen (Newbold et al., 1997) to neonatal female CD-1 mice for five days (days 1-5 after birth) results in uterine tumor development when the mice are examined 14 to 18 months later. The chemical structure of genistein and tamoxifen is similar to that of estrogen. Therefore, these compounds mimic the function of estrogen when neonatally injected, and result in uterine tumor development in this neonatal mouse model (Newbold et al., 2001; Newbold et al., 1997).

The following facts should be considered when extrapolating these results to the consumption of soy-based formula in human infants. First, the route of genistein administration plays an important role in determining the outcome of a study. For example, subcutaneous injection of genistein to immature mice results in a significant uterotrophic effect, whereas oral gavage at the same dose has no such a significant effect on uterus (Ashby, 2000). Second, the dose of genistein injected to neonatal mice (50 mg/kg bw/d) in Newbold study (2001) is far beyond dietary genistein achieved by infants consuming soy-based formula. It is estimated that infants consuming soy formula have an isoflavone intake at approximately 6-11 mg/kg bw/d (Setchell et al., 1998) (Table 1). The U.K. Food Standard Agency reported an isoflavone intake at 4.5 mg/kg bw/d in infants on soy-based formula (MAFF, 1998). It is important to note that the predominant isoflavones in soy-based formulas are β -glycosides (genistin and daidzin) accounting for 79.5% of the total isoflavones, whereas aglycones (genistein and daidzein) represent only 3.2-5.8% of the total isoflavones in soy formula (Setchell et al., 1997). Third, the NIH 31 diet used by Newbold et al (2001) is a soy diet (5% soybean meal or soy protein) containing genistein at 46 μ g/g and daidzein at 48 μ g/g (total 94 μ g/g) (Newbold et al., 2001; Thigpen et al., 1999). For a mouse weighing 25 g with an average food intake of 4 g/d, it would have an approximate intake of total genistein and daidzein at 15 mg/kg bw/d. However, there is no uterine adenocarcinoma observed in control mice maintained on the NIH 31 diet for 18 months (Newbold et al., 2001).

Table 1. Estimated isoflavone intake in infants consuming soy-based formula.

Infant age	Volume (mL/d)	Isoflavone Intake ¹ (mg/d)	Normal Body Wt (kg)	Dosage ² (mg/kg bw/d)
1 wk	500 – 550	23 – 25	2.5 – 3.8	5.7 – 7.3
1 m	700 – 800	32 – 36	2.9 – 5.0	6.0 – 11.9
2 m	800 – 830	36 – 37	3.6 – 5.9	6.1 – 10.0
4 m	800 – 1,000	41 – 45	4.8 – 7.5	6.0 – 9.3

¹Based on isoflavone concentration of 45 mg/L for soy-based infant formula. ²For comparison, the average daily exposure for adults consuming 57-85 g soyfoods containing 50-100 mg isoflavones is 0.7-1.4 mg/kg bw/d. Adapted from Setchell et al (1998).

Badger et al (2001) conducted a multi-generation feeding study in rats aimed at examining the long-term health consequences of early consumption of soy protein. Offspring (F1 generation) from parents on a casein or a soy protein diet (AIN-93G diet with or without 20% isolated soy protein) were weaned to the same diet. At approximately 90 days of age, offspring from different parents within the same dietary group were mated to produce the F2 generation. The F2 offspring were maintained on their respective diets throughout the duration of the study. There is no difference in uterus weight in F2 offspring between the soy and the casein groups, and no uterine tumors reported in any of the dietary groups in this study. Similar results on uterus in F2 offspring have been found in another multi-generation study with a similar feeding regimen aimed at examining the effect of

long-term consumption of soy protein on chemically induced mammary tumorigenesis in F2 offspring (Hakkak et al., 2000).

In a retrospective cohort study, Strom et al (2001) investigated the association of soy formula intake during infancy with endocrinological and reproductive outcomes in young adulthood. Young adults (20 to 34 years of age) who participated in multiple controlled feeding studies when they were infants during 1965 to 1978 were revisited in 1999 (248 were fed soy formula and 563 were fed cow-milk formula). There are no significant differences in cancer, reproductive organ disorders, libido dysfunction, sexual orientation, and birth defect in offspring between the two formula groups. In fact, results of two case-control study conducted in the United States reveal that consumption of soyfoods is associated with a significantly lower risk of endometrial cancer in women (Goodman et al., 1997; Horn-Ross et al., 2003). Furthermore, in a short-term intervention study, Balk et al (2002) found that consumption of soy cereal for six months does not cause any stimulation to endometrium in post-menopausal women

(Prostate Tumor Growth)

The Weston Price Foundation cited one study investigating soy protein and androgen-independent prostate tumor growth in rats (Cohen et al., 2003). There have been many reports on soy protein inhibiting prostate tumor development or growth in laboratory animals using various model systems (Landstrom et al, 1998; Aronson et al, 1999; Bylund et al, 2000; Zhou et al, 1999; Pollard and Luckert, 1997; Pollard et al, 2000; Pollard and Wolter, 2000; and Pollard et al 2001). This study cited by the Weston Price Foundation, as well as related studies, were reviewed in detail in *Appendix VI (p. 8)*. *Review of Individual Studies around Concern for Soy – 5. Dietary soy and the growth of androgen-independent prostate tumor in rats.*

Appendix VI. Review of Individual Studies around Concern for Soy

5. Dietary Soy and the Growth of Androgen-Independent Prostate Tumor in Rats

Cohen et al (2003) investigated the effect of dietary supplementation with soy protein on the growth of androgen independent Dunning R-3327-AT-1 prostate tumor in male Copenhagen rats. Four diets were compared, a soy-free basal diet, or the basal diet containing 5%, 10%, or 20% soy protein isolate. Animals were fed the experimental diet for three days before and six weeks after receiving a subcutaneous injection of tumor cells. Tumor size was an endpoint measurement. There is a 2-fold increase in tumor volume in the 10% and 20% soy groups compared with the control group, and there is no difference between the 5% soy group and the controls. The author indicated “Although there is the possibility that soy protein isolate may inhibit early, androgen-dependent prostate cancer growth, this pre-clinical animal model study casts doubt on the effectiveness of isoflavone-rich soy protein isolates as adjuvant therapy in the treatment of advanced hormone-refractory prostate cancer.”

Explanations for these findings remain speculative. The AT-1 line is an androgen-independent cell line derived from Dunning R3327 prostate adenocarcinoma in rats (Isaacs et al., 1978). It seems unlikely that the observed results can be explained by androgenic effect of isoflavones in the soy diets. The expression of estrogen receptors in prostate tumors and glands in Copenhagen rats was not determined in this study, and therefore no clue on estrogenicity of isoflavones can be drawn from this investigation. Existing publications show that soy protein-containing diets reduce the growth of hormonal sensitive prostate tumors in rats, e.g. Dunning R3327 (Landstrom et al., 1998) and LNCaP tumors (Aronson et al., 1999; Bylund et al., 2000; Zhou et al., 1999). Available studies also reveal that dietary supplementation with soy protein isolate reduces development and growth of methyl-nitroso-urea (MNU)-induced prostate tumor (Pollard & Luckert, 1997; Pollard et al., 2000) as well as spontaneous prostate tumor development in rats (Pollard & Wolter, 2000; Pollard et al., 2001).

(Thyroid Hormones)

The Weston Price Foundation cited studies regarding flavonoids and isoflavones on thyroid *in vitro* and *in vivo* (Divi et al., 1997; Divi & Doerge, 1996; Doerge & Chang, 2002; Doerge & Sheehan, 2002). It is important to note that flavonoids are not constituents of soy and studies using isolated isoflavones in and *in vitro* setting do not reflect dietary effects of soy protein as a food. Results from human intervention studies have indicated no clinically significant changes in any of the thyroid hormone indices (Persky et al, 2002; Ham et al, 1993; Duncan et al, 1999a; Duncan et al, 1999b). Furthermore, recently published epidemiological studies with multiethnic populations in the United States showed that consumption of soyfoods is related to a reduced risk of thyroid cancer in women (Haselkorn et al, 2003; Horn-Ross et al, 2002). Additional information surrounding soy and thyroid status was discussed in *Appendix VI (p.14-16). Review of Individual Studies around Concern for Soy – 8. Thyroid.*

Appendix VI. Review of Individual Studies around Concern for Soy 8. Thyroid

a. Goitrogenicity in animals

Early studies show that soy diet is goitrogenic in iodine deficient animals (McCarrison, 1933; Sharpless et al., 1939). The enlargement of thyroid can be prevented by supplementing iodine to animal diets (Sharpless et al., 1939; Suwa et al., 1979). Heat treatment of soy products partially reduces goitrogenic development in iodine deficient rats (Halverson et al., 1949; Sharpless et al., 1939), and soy protein or peptide-like substances do not seem to be responsible for the goitrogenicity (Suwa et al., 1979). Recent studies show that defatted soybean is not goitrogenic in rats fed an iodine adequate diet (Ikeda et al., 2000; Son et al., 2001).

b. Soy-based formula and goiter in infants

In the 1950s, cases of goiter were reported in infants consuming soy-based formula (Hydovitz, 1960; Shepard et al., 1960; Van Wyk et al., 1959).

Supplementing the formula with adequate iodine since 1960s prevents goiter in infants consuming soy-based formula. Iodine fortification has been recommended by the United States FDA as a precautionary measure against goitrogenic potential of infant food (Fine, 1971). No case of goiter in infants, due to consumption of iodized soy-based formula, has been reported in scientific literature since then, except two cases of infants with congenital hypothyroidism (Chorazy et al., 1995; Jabbar et al., 1997). These cases require about 25% more synthetic thyroid hormone to maintain the normal thyroid function when consuming soy formula. Fort et al (1990) reported that twice as many children with auto-immune thyroid disease have a history of consuming soy-based formula in early infancy (18 out of 59) comparing with their healthy siblings (9 out of 76) or healthy non-related controls (7 out of 54). However, the iodine status of the participants was not presented in this publication, nor a cause-and-effect relationship established.

c. Thyroid function

Divi et al (1997) found that genistein and daidzein inhibit thyroid peroxidase-catalyzed reactions essential to thyroid hormone synthesis using an in vitro enzyme substrate assay. The same research team further reported that dietary supplementation with genistein (5 ppm, 100 ppm, and 500 ppm) from in utero to 20-weeks of age results in a dose dependent inhibition on thyroid peroxidase activity (Chang & Doerge, 2000). Genistein at these doses does not affect serum levels of thyroid hormones and thyroid weights, nor results in any histological change in thyroid gland compared with the controls fed a genistein-free diet. Chang and Doerge (2000) pointed "These results suggest that, even though substantial amounts of thyroid peroxidase activity are lost concomitant to soy isoflavone consumption by normal rats, the remaining enzymatic activity is sufficient to maintain thyroid homeostasis in the absence of additional perturbations." Studies from other laboratories show that soy increases serum levels of thyroid hormones in rodent models. For example, Potter et al (1996) reported that dietary supplementation with 25% isolated soy protein for 35 days significant increases serum thyroxine (T_4) and free T_4 in hamsters compared with a casein-based soy-free control diet. Forsythe (1986) found that consuming a soy protein diet (18%) for four weeks significantly increases plasma T_4 and thyroid stimulation hormone (TSH) in gerbils compared with the controls fed a casein diet.

Results from short-term intervention trials demonstrate that soy does not have any adverse effect on thyroid function in humans. Persky et al (2002) compared the effects of isolated soy protein (ISP) with non-fat dry milk on endogenous hormones in 73 hypercholesterolemic postmenopausal women. Participants had a daily intake of 40 g protein from non-fat dry milk or ISP for six months. Two

types of ISP were compared. One contained isoflavones at 56 mg/40 g protein (ISP-56), and the other 90 mg/40 g protein (ISP-90). Concentrations of T_4 and free T_4 are higher in the ISP-56 group, and the concentration of TSH is higher in the ISP-90 group compared with the milk controls at months three and six. Triiodothyronine (T_3) is higher in the ISP-90 group at month six compared with the controls. In a four-week feeding study with 17 hypercholesterolemic men, Ham et al (1993) found that daily intake of 50 g soy protein for four weeks results in an increase in plasma T_4 , no change in T_3 , and a decrease in TSH compared with the baselines. Duncan and co-workers compared the hormonal effects of soy protein containing different levels of isoflavones in postmenopausal (63 g/d for three months) (Duncan et al., 1999b) and premenopausal women (53 g/d for three months) (Duncan et al., 1999a). Soy consumption does not affect plasma thyroid hormones in either postmenopausal or premenopausal women, except a slight but statistically significant decrease in free T_3 in premenopausal women consuming a high-isoflavone soy diet. The plasma concentration of free T_3 is 3.27 ± 0.11 pmol/L, 3.46 ± 0.13 pmol/L, and 3.46 ± 0.11 pmol/L for the high-isoflavone, the low-isoflavone, and the isoflavone-free group, respectively. Bruce et al (2000) reported that daily intake of 90 mg soy isoflavones for 180 days does not affect thyroid function (no changes in T_4 , T_3 , and TSH) in postmenopausal women compared with those received a placebo for the same length of time.

d. Thyroid cancer

Results of an early study show that feeding female rats an iodine deficient diet containing 40% defatted soybean for 6 to 12 months results in malignant goiter (3 out of 27 rats) (Kimura et al., 1976). The enlargement of thyroid is completely inhibited when a small amount of iodine is added to the diet, and there is no thyroid tumor found in animals given an iodine-containing diet. It is important to note that rats are very sensitive to compounds that perturb the hypothalamic/thyroid axis. Furthermore, in response to compounds that increase demand for T_4 , male rats respond with an increase in production of TSH, thyroid gland hypertrophy, and hyperplasia (Capen, 1997). Recently published case-control studies with multiethnic populations in the United States demonstrate that consumption of soyfoods is related to a lower risk of thyroid cancer in women (Haselkorn et al., 2003; Horn-Ross et al., 2002).

(Spermatogenesis)

The Weston Price Foundation cited a study that studied a variety of compounds, including daidzein and genistein, on lymphocyte and sperm activities *in vitro* (Anderson et al., 1997). It is important to note that *in vitro* work on pure compounds does not accurately depict the impact of dietary soy intake on any physiological development in humans or animals. It has been reported that soy protein (Anthony et al, 1996) and soybean meal (Robertson et al, 2002) have no adverse effect on the reproductive systems in laboratory animals. Furthermore, results from a multigeneration feeding study showed that soy protein does not adversely affect fertility in laboratory animals (Badger et al, 2001). Soy, isoflavones, and spermatogenesis was discussed in detail in *Appendix VI* (p.

17-19). *Review of Individual Studies around Concern for Soy – 9. Spermatogenesis.* Soy and/or isoflavones and fertility was discussed in detail in *Appendix VI (p. 21-23)*. *Review of Individual Studies around Concern for Soy – 11. Infertility.*

Appendix VI. Review of Individual Studies around Concern for Soy
9. Spermatogenesis

a. An in vitro study

Kumi-Diaka and Townsend (2001) investigated the effect of genistein on functional characteristics of spermatozoa isolated from mature breeding rats using in vitro assays. A longer-term incubation with genistein (>6 hours) at high doses ($\geq 50 \mu\text{g/mL}$) interferes with sperm motility, causes detachment of sperm heads, and decreases sperm acrosome reactivity. Genistein has no effect on these variables at a lower dose ($30 \mu\text{g/mL}$). Kumi-Diaka and Townsend (2001) concluded, "In view of the fact that sperm capacitation and acrosome reaction are physiological prerequisites for successful fertilization of oocytes, chronic exposure of spermatozoa to high doses of genistein could be associated with infertility problems through suppression/inhibition of acrosome reaction and sperm motility."

The relevance of doses of genistein directly applied to the cultured cells to the levels of genistein in biological fluids and reproductive organs in humans consuming a genistein-containing diet was not addressed in this publication. Many in vitro studies measure direct interaction of genistein with cultured cells, and concentrations of genistein used are significantly higher than and are not achievable in humans consuming a soy diet. In vitro assays may be useful in evaluating the effect of genistein on a particular event of spermatozoa at cellular levels. However, they do not offer any assessment on spermatogenesis in men consuming a soy protein diet.

b. Animal studies

Sharpe et al (2002) compared soy formula milk with cow formula milk on testis development and blood testosterone levels in neonatal marmoset monkeys. Animals were fed either formula for 30 to 40 days (from ages of 4-5 days to 35-45 days), and then terminated at the end of the feeding. There are no differences in testis weight, the number of testicular Sertoli and germ cells, and pituitary luteinizing hormone β ($\text{LH}\beta$) and follicle-stimulating hormone β ($\text{FSH}\beta$) between the groups. However, plasma testosterone is significantly lower in monkeys fed soy formula than those maintained on cow formula ($1.3 \pm 2.1 \text{ ng/mL}$ vs. $2.8 \pm 3.9 \text{ ng/mL}$), and there is an approximately 74% increase in Leydig cells in the soy group compared with the cow milk group. Sharpe et al (2002) concluded "It is therefore considered likely that similar, or larger, effects to those shown here in marmosets may occur in human male infants fed with soy formula milk." On the contrary, Anthony et al (1996) reported that dietary supplementation with 20% soy protein isolate for six months has no adverse effect on reproductive systems in either sex in peri-pubertal rhesus monkeys compared with the controls fed a soy

protein diet containing a trace amount of isoflavones. The endpoint measurements of this study are reproductive hormone levels and reproductive organ weights at autopsy.

Available data from animal studies show that soy consumption has no adverse effect on male reproductive development. Robertson et al (2002) compared a soybean meal diet (10%) with a soy-free diet on testis function in wild-type and aromatase knockout mice. Maintaining wild-type mice on the soybean meal diet for one year has no effect on testicular morphology and the number of Sertoli and germ cells compared with those fed the soy-free diet. However, the soybean meal diet markedly reduces aromatase knockout induced spermatogenic disruption in aromatase knockout mice compared with the soy-free diet. Kang et al (2002) reported that in utero and neonatal genistein treatments (oral gavage at 0.4 mg/kg bw/d or 4.0 mg/kg bw/d from gestation day 6 to postnatal day 20) does not have any adverse effect on male reproductive organ development in rats. The end point measurements include sperm counts and motility in the caudal epididymis, spermatogenic cells in seminiferous tubule, weight of testis, prostate gland, and seminal vesicle, and histopathological examination of male reproductive organs. Roberts et al (2000) examined the effects of lifelong exposure to dietary genistein on reproductive hormones and spermatogenesis in offspring from dams on a genistein diet (5 mg/kg diet) during gestation. Maintaining offspring on the same diet for 130 days does not have any effect on testis weight, testicular sperm counts, serum testosterone and FSH, and pituitary LH β mRNA and FSH β mRNA, except a slight but significant reduction in epididymis weight and serum LH level, compared with the controls. Furthermore, it is important to note that soy has been widely used as a protein source in laboratory animal diets (Thigpen et al., 1999), and there has not been any evidence of widespread breeding problems in laboratory animals.

c. Findings from epidemiological and intervention studies

Strom et al (2001) investigated the association of soy formula intake during infancy with endocrinological and reproductive outcomes in young adults in a retrospective cohort study. Young adults (20 to 34 years of age) who participated in multiple controlled feeding studies when they were infants during 1965 to 1978 were revisited in 1999 (248 on soy formula, and 563 on cow-milk formula). There are no significant differences between these two formula groups in pubertal maturation in both sexes and pregnancy outcomes in women.

Mitchell et al (2001) conducted an intervention study on soy isoflavones and reproductive health in 14 healthy men (18 to 35 years of age). The subjects consumed a soy extract containing 40 mg isoflavones daily for two months. Blood and semen samples were collected monthly two months before, during, and four months after the intervention. There is no adverse effect on reproductive health found in participants throughout the study. The endpoint measurements include blood levels of testosterone, estrogen, LH, and FSH, testicular and ejaculation volumes, and sperm count, concentration, and motility. The investigators found

an apparent effect on sperm morphology four months after the supplementation, and explained that it is due to adapting changes in the reporting criteria in World Health Organization guidelines in 1999 (World Health Organization, 1999).

Appendix VI. Review of Individual Studies around Concern for Soy

11. Infertility

a. Early studies

Early studies reveal infertility in Australian sheep that graze on Trifolium Subterraneum, a species of clover rich in isoflavone formononetin (Adams, 1981; Bennetts et al., 1946). The syndrome is known as "clover disease." Infertility has also been observed in California quail ingesting leaves of stunted desert annuals which contain isoflavones largely formononetin and genistein (Leopold et al., 1976). These investigations involved plants that are not food sources to humans and isoflavones that are not found in soy. Therefore, findings from these studies are not relevant to human consumption of soy protein. Setchell et al (1987) reported reproductive failure and liver dysfunction in captive cheetahs maintained on a commercial diet containing soy. Substituting the commercial diet with a chicken diet improves liver function in these animals. However, there was no experiment conducted in this study examining the cause effect relationship of soy or isoflavones with the infertility in cheetahs.

b. Laboratory animal studies

Nagao et al (2001) reported that early neonatal exposure to genistein causes dysfunction of postpubertal reproductive performance as well as abnormal development of gonads in female but not male rats. Oral gavage of genistein at 12.5 mg, 25 mg, 50 mg, or 100 mg/kg bw/d during postnatal days 1-5 does not affect male fertility (genistein-treated males cohabited with untreated females). However, genistein at these doses significantly reduces fertility index (number of pregnant rats/number of copulated rats) in female rats (genistein-treated females cohabited with untreated males). Genistein at 100 mg/kg bw/d does not affect male gonads, but results in histopathological changes in ovaries and uterus in females. Nagao et al (2001) pointed "It would be difficult for human beings to consume sufficient amounts of isoflavones from natural soy-foods to reach the toxicological levels that induce the pathologic effects seen in animals. However, with the recent trend toward extracting isoflavones from soy for commercialized over-the-counter soy isoflavone supplements, and because such products are not closely regulated, the potential dangerous effects from self-induced megadosing are a concern."

Gallo et al (1999) examined the reproductive effect of a soy extract (containing 12% isoflavones and 35% saponins) in female rats. Dietary supplementation with 0.7%, 1.2%, or 2.4% soy extract to female rats from weaning (21 days of age) to post-partum day 7 does not have any adverse effect on fertility in female rats compared with the controls fed a standard cereal-based diet. The major endpoint measurements include fertility index (number of pregnant females/number of

females placed with males), gestation index (number of pregnant females with live pups/total number of pregnant females), and litter size, weight, and numbers. There are estrogenic effects (e.g. uterotrophic and an increase in the length of estrus cycle) observed in the group fed 2.4% soy extract. Gallo et al (1999) concluded, "These data suggest that long-term exposure to high doses of phytoestrogens can produce significant agonistic actions in several estrogen-dependent tissues and parameters, even though in this model no clear influence on reproductive processes was observed."

Available data from animal studies show that soy protein does not adversely affect fertility in laboratory animals. Badger et al (2001) conducted a multi-generation feeding study in rats aimed at examining the long-term health consequences of early consumption of soy protein. Offspring (F1 generation) from dams on a casein or a soy protein diet (AIN-93G diet with or without 20% isolated soy protein) were weaned to the same diet. At approximately 90 days of age, offspring from different parents within the same dietary group were mated to produce the F2 generation. There is no difference in breeding success (percentages of males and females that produced offspring after mating) between the groups. The number of offspring, gender ratios, birth weights, birth lengths, health, and general appearance of soy-fed rats are the same as those of casein-fed ones.

It is important to note that soy (e.g. soy protein or soybean meal) has been widely used as a protein source in laboratory animal diets (Thigpen et al., 1999). However, there has not been any widespread breeding problem in laboratory animals reported in medical research community.

c. Epidemiological findings

Strom et al (2001) investigated the association of soy formula intake during infancy with endocrinological and reproductive outcomes in young adulthood in a retrospective cohort study. Young adults (20 to 34 years of age) who participated in multiple controlled feeding studies when they were infants during 1965 to 1978 were revisited in 1999 (248 were fed soy formula and 563 were fed cow-milk formula). There are no significant differences between these two groups in pregnancy outcomes in women.

(Immune Function)

The Weston Price Foundation cited one study on subcutaneous injection of genistein and thymic changes in ovariectomized mice (Yellayi et al., 2002). It is important to note that injection of genistein is not related to dietary exposure to soy. Results from intervention studies in human infants showed that soy formula does not affect the production of immunoglobulin response to oral poliovirus (Businco et al, 1989). Further, in another study in human infants fed soy formula, all vaccine responses were found to be within normal ranges and all immune cell populations, numbers, and percentages were within age-related normal ranges indicating normal immune development (Cordle et al, 2002; Ostrom et al, 2002). Results from available animal studies showed that isoflavones have

no adverse effect on the immune system (Zhang et al, 1997), they enhance immune responses in tumor-bearing animals (Guo et al, 2001), and they enhance anti-inflammatory responses in animals (Regal et al, 2000; Krowicka et al, 1998). Further information on genistein and immune function was discussed in detail in *Appendix VI (p. 31-34). Review of Individual Studies around Concern for Soy – 14. Genistein and Immune Functions.*

*Appendix VI. Review of Individual Studies around Concern for Soy
14. Genistein and Immune Functions*

a. Animal studies

Yellayi et al (2002) investigated the effects of genistein on thymic and immune changes in mice. Ovariectomized and castrated adult mice were used in this study to mimic the endocrine condition in human infants. Subcutaneous injection of genistein to ovariectomized adult female mice (2-200 mg/kg bw/d) for 21 days results in a dose-dependent reduction in thymic weight. There is a similar reduction in thymic weight in castrated male mice injected genistein (200 mg/kg bw/d) for 21 days. A decrease in thymic weight is accompanied with a decrease in thymocyte numbers and an increase in apoptosis. Injection of genistein (8-80 mg/kg bw/d) for 5 weeks produces impairment in humoral immunity in ovariectomized juvenile mice. Dietary supplementation with genistein at 1,000 ppm or 1,500 ppm for 12 days results in a reduction in thymic weight in ovariectomized juvenile mice. In comparing serum genistein in mice injected genistein (≥ 6 -200 mg/kg bw/d) or fed a genistein diet (1,000 ppm or 1,500 ppm) with that in human infants fed soy formula, Yellayi et al (2002) concluded, "These results raise the possibility that serum genistein concentrations found in soy-fed infants may be capable of producing thymic and immune abnormalities."

The relevance of using ovariectomized and castrated adult mice to mimic endocrine conditions of human infants remains to be debated. The route and doses of genistein administration play an important role in determining the outcome of a given study. Furthermore, there is no evidence that genistein aglycone is the same as soy foods of any form including soy-based infant formula.

Results from available studies show that isoflavones have no adverse effect on immune system in laboratory animals. Zhang et al (1997) reported that oral gavage of daidzein (20-40 mg/kg bw/d) for 7 days enhances non-specific immunity in mice, e.g. an increase in thymus weight and phagocytic responses of peritoneal macrophages. Augmentation of spleen IgM producing cells against sheep red blood cells demonstrates an activation of humoral immunity. There are increases in lymphocytes in peripheral blood, suggesting enhanced cell-mediated immunity. These results demonstrate that daidzein affects immune functions in mice by increasing cell-mediated and humoral activities.

Guo et al (2001) reported that oral gavage of genistein (2, 6, and 20 mg/kg bw/d) for 28 days inhibits lung tumor formation in mice intravenously injected B16F10

melanoma cells. There is a dose-related increase in cytotoxic T-cell activity in genistein-treated mice with significant changes at dose levels 6 mg/kg bw/d and 20 mg/kg bw/d. Furthermore, genistein enhances interleukin-2 stimulated natural killer (NK) cell activity in vitro. Guo et al (2001) concluded that genistein-enhanced host resistance to B16F10 tumor development may be related to increases in activities of cytotoxic T-cells and NK cells. In another study, Guo et al (2002) examined the effect of dietary genistein on immunity changes in rats. Genistein-supplemented diets (300 ppm and 800 ppm) were provided to female rats from gestation day one, and animals were terminated with offspring at the postnatal day 21. Genistein does not affect maternal thymus and spleen weights and thymocyte numbers. However, genistein at the high dietary level (800 mg/kg bw/d) reduces thymus weight and the number of lymphocytes. In offspring, genistein does not affect thymic and spleen weight, but reduces CD4⁺CD8⁻ thymocytes. Spleen NK cell activity is increased in male offspring but decreased in female offspring.

Laboratory studies also reveal that isoflavones enhance anti-inflammatory responses in animals. Regal et al (2000) examined the effects of soy isoflavones on ovalbumin-induced respiratory hypersensitivity reaction in ovalbumin-sensitized guinea pigs. Dietary supplementation with isoflavones reduces eosinophilia in lung tissues and bronchoalveolar lavage fluid compared with the controls maintained on an isoflavone-free diet, indicating an anti-inflammatory effect of dietary isoflavones in this guinea pig asthma model. Sadowska-Krowicka et al (1998) reported that genistein (0.1 mg/kg bw/twice a day, subcutaneous injection) has a modest anti-inflammatory effect in trinitrobenzene sulfonic acid- (TNBS) induced chronic ileitis in guinea pigs.

b. Human studies

Results of an early study show that consumption of soy-based infant formula attenuates immunity responses in infants (Zoppi et al., 1983). Zoppi et al (1983) compared antibody responses to poliovirus, diphtheria, pertussis, and tetanus vaccines in infants who consumed human milk (n = 21) or one of the four artificial feeds (n = 7-10) during the first five months of their life. Infants on breast milk or high-protein cow milk have adequate and sustained antibody responses, those on adapted formula have a high but temporary response, and those on low-protein cow milk or the soy-based formula have poor responses.

Businco et al (1989) reported that soy formula does not affect the production of immunoglobulins to oral poliovirus in infants. They investigated antibody responses to oral poliovirus immunization in infants with a positive family history of atopy. Infants were on human milk (n = 34), soy formula (n = 18), or both (n = 55) for the first six months of their life, and received oral poliovirus at ages of 3, 5, and 12 months. Three poliovirus strains were tested. There are no significant differences between geometric mean titres for all three types of feeding and for all three strains of poliovirus at any time. There are also no significant

differences in sero-conversion rates when data are analyzed using feeding groups or types of poliovirus at any time.

Cordle et al (2002; Ostrom et al., 2002) examined the influence of soy-based formula on vaccine responses, morbidity, and immune cell populations in infants. Newborn, term infants were randomly assigned to human milk (n = 81), soy formula (n = 94), or soy formula supplemented with nucleotides (n = 92) for 12 months. Recommended immunizations were administered at 2, 4, and 6 months of age. Immune status was determined from antibody responses to Haemophilus influenzae type b, tetanus, and poliovirus at 6, 7, and 12 months of age. Cellular markers of general pediatric status were assessed, emphasizing on maturation and activation of B, T, and NK lymphocytes. All vaccine responses are within the normal ranges, and all immune cell populations, numbers, and percentages are within age-related normal ranges. Results of morbidity analysis show that only physician-reported diarrhea is different among the groups. Cordle et al (2002; Ostrom et al., 2002) concluded that term infants fed soy protein isolate-based formula have normal immune development, as measured by antibody responses to childhood immunizations, and similar immune cell status compared to human milk-fed infants.

(Equol Production from Daidzein)

The Weston Price Foundation cited three studies on daidzein metabolizers/equol producers in relation to prostate cancer in men (Akaza et al., 2004; Akaza et al., 2002; Miyanaga et al., 2003). The publication by Akaza et al (2002) was reviewed and presented in the petition. The publication by Akaza et al (2004) was published after the submission of the petition to the FDA. This is a case-control study involving Japanese residents in Japan and Korean residents in Korea. It was found that the percentage of equol producers among prostate cancer cases and controls is 29% and 46% in Japan ($P = 0.004$) and 30% and 59% in Korea ($P = 0.001$), respectively. The authors concluded, "These results suggest that the ability of producing equol or equol itself is closely related to the lower incidence of prostate cancer. The results also suggest that a diet based on soybean isoflavones will be useful in preventing prostate cancer." The publication by Miyanaga et al (2003) is a study on green tea. Therefore, this study was not reviewed for this petition.

(Reviews, Editorials, and Letter to Editors)

The Weston Price Foundation cited a number of review articles, editorials, and letters to editors in their document (Abe, 1999; Adlercreutz, 2002; Liener, 1996; Santti et al., 1994; Sheehan, 1998; Strauss et al., 1998; Whitten & Patisaul, 2001). Review articles, editorials, and letters to editors were not reviewed for this petition, because they are not "individual studies" and do not meet the criteria for substantiation for health claims as per *Guidance for Industry: Significant Scientific Agreement in the Review of Health Claims for Conventional Foods and Dietary Supplements* (<http://www.cfsan.fda.gov/~dms/ssaguide.html>)).

(Additional Citations)

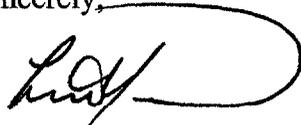
The Weston Price Foundation cited publications that provided data on soybean paste soup and miso soup in relation to prostate cancer (Hirayama, 1979) and gastric cancer (Galanis et al., 1998). Solae stated in section B.3. *Details of the Scientific Review – Objective and Scopes* (pp. 14-15 of the petition) that “Studies included in this review are those focused on high protein-containing soyfoods. Not included are studies focused on soybean paste, soy sauce, and their derivative products. Soybean paste and soy sauce are salted, fermented products that are low in protein and used primarily as condiments.” Therefore, publications by Hirayama et al (1979) and Galanis et al (1998) were not reviewed for the petition.

Some of the publications cited by The Weston Price Foundation are not related to soyfoods and cancer (Velicer et al., 2004); (Nomura et al., 2003); (Chyou et al., 1990); (Fuchs et al., 2002; Giovannucci et al., 1993). Therefore, these studies were not reviewed for this petition and will not be reviewed at this time.

Thank you for this opportunity to comment. Solae is looking forward to the review process. The scientific data overwhelmingly supports that a relationship exists between consumption of soy protein and a reduction in risk of certain cancers. As indicated above, many of the studies cited by the Westin Price Foundation are already addressed in Solae’s petition. To the extent that the studies are not addressed in Solae’s petition, we have concluded that they are not relevant or significant to our petition for a qualified health claim.

We urge FDA to expedite publication of their ruling so food manufacturers can convey this important dietary health information to consumers on food labels.

Sincerely,



Lin Yan, Ph.D.
Director, Cancer Research
Health and Nutrition



Susan M. Potter, Ph.D.
Global Director
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