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June 14, 2004

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

Dear Sir or Madam:

This letter responds to the comments by Richard James, dated 4/27/04, concerning the Qualified Health Claim (QHC): Soy Protein and Cancer submitted by Solae, LLC (hereafter "Solae").

In general, Mr. James implies that Solae was remiss in providing the FDA with a thorough review of the scientific literature on soy protein and cancer. We vigorously disagree. Following is a summary of the scientific literature on soy protein and cancer that was submitted with the petition.

To the best of our knowledge, all epidemiological studies relating soy intake and cancer incidence available in English were reviewed and submitted to FDA as part of the Soy Protein and Cancer Qualified Health Claim Petition. Studies using isolated isoflavones as purified compounds were not included in the documentation unless there had been particular public interest in individual studies. In this case, these studies were 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 the health claim petition.

With regards to the specific comments, we have the following remarks (the following paragraph numbers correspond to the paragraphs in Mr. James' letter):

1. Mr. James cites a study by Sun et al (2002) on soy and urinary bladder incidence. Solae submitted this study to the FDA with our petition as well as a study which concluded that intake of soy juice was related to a decrease in risk of urinary bladder cancer. The following summary of the two epidemiological studies available in English on soy intake and urinary bladder cancer appears in the health claim petition.

Results of a cohort study show that consumption of soyfoods is related to the risk of urinary bladder cancer (adjusted RR = 2.34, 95% CI = 1.07 – 5.09) (Sun et al., 2002). Findings from a case-control study show that intake of soy juice is not related to urinary bladder cancer (adjusted RR = 0.95, 95% CI = 0.43 – 2.12) (Lu et al., 1999). Appendix III Original Health Claim Petition—Solae, LLC

Solae also provided FDA with a review of scientific evidence from epidemiological studies that assessed the relationship between soy consumption and the risk of: endometrium/ovary, thyroid, lung, pancreas, liver, nasopharyngeal, and urinary bladder cancer. We also included three case-control studies on endometrial/ovarian cancer, three case-control studies on thyroid cancer, seven case-control and one ecological study on lung cancer, one case-control study on pancreatic cancer, one case-control study on hepatic cancer, and one cohort and one case-control study each on nasopharyngeal cancer and urinary bladder cancer. Results from these studies reflect a trend that consumption of soy protein-containing foods is related to a lower risk of cancer. Appendix III Original Health Claim Petition—Solae, LLC

Results from certain studies that have garnered lay press attention were also provided to FDA for review. These included studies of the classification that that alone would not support a health claim decision according to FDA (USFDA, 1999). These types of studies are used for insight into mechanism (*in vitro* and animal studies). However, in order to provide FDA with a thorough understanding of the scope of the literature, Solae did provide reviews of a variety of studies that have generated interest.

2. Mr. James cites an *in vitro* study with purified genistein Dees et al, (1997). Solae submitted a review of this study to the FDA with our petition. Solae also provided additional analysis of the following *in vitro* studies in order to supply a more thorough assessment of the literature.

Results of in vitro studies show that purified 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. Appendix VI Original Health Claim Petition—Solae, LLC

3. Mr. James cites a study by Petrakis et al, 1996, a small, pilot study in which pre- and postmenopausal women were fed soy products for a period of time and breast aspirates were taken. No control group was present to account for the stimulatory effect of taking breast nipple aspirates measurements in itself. This study has never been repeated nor does it stand up to the literature indicating consumption of soy protein containing foods is associated with a reduced risk of breast cancer in both pre- and postmenopausal women (see 2004Q-0151 Qualified Health Claim Petition: Soy Protein and Cancer).
4. Mr. James cites a study by Wrensch et al, 2001. This study is not relevant as it is **not** related to soy consumption.
5. Mr. James cites *in vitro* work on the interaction of purified plant compounds, including genistein at high concentrations, with estrogen receptors and cell growth (Martin et al, 1978). This was not included in Solae's health claim petition, as much progress has been made in definition of types of estrogen receptors (ER α and ER β), and thus, more current literature is more applicable and was included in Solae's petition as was described in item #2.
- 6-8 Mr. James cites experiments done with isolated compounds found in soybeans (Allred, 2001b; Hsieh et al, 1998) conducted by Dr. William Helferich and colleagues. Of the work published by Dr. Helferich's laboratory (Allred, 2001a;

Allred, 2001b; Hsieh et al, 1998), the most germane to the current petition is the paper by Allred et al, 2001a 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, were not included. The following discussion was provided to FDA for review (Allred et al, 2001a):

Allred et al (2001) 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).

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9. Mr. James cites a preliminary report from a study assessing a variety of estrogenic indices in women was published as part of the proceedings from a soy symposium (McMichael-Phillips et al, 1998). This report is incomplete. Hargreaves and associates subsequently published the full study in 1999. Mr. James cited only the preliminary report from McMichael-Phillips et al, 1998. The findings from the complete study showed no differences in breast epithelial proliferation, apoptosis, hormone receptor status, and Bcl-2 expression in response to soy supplementation (Hargreaves et al, 1999)

10. Mr. James cites a study by Ju and colleagues (2002) that was provided to FDA for review in Solae's petition. In addition, Solae submitted the following information on tamoxifen, genistein, and soy:

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 in vitro studies. 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. Appendix VI Original Health Claim Petition—Solae, LLC

11. Mr. James cites a study by Divi et al (1997). This study was submitted to FDA in Solae's petition. In addition, the following was provided to demonstrate that soy does not have any adverse effects on thyroid function in humans.

Results from 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 were higher in the ISP-56 group, and the concentration of TSH was higher in the ISP-90 group compared with the milk controls at months three and six. Triiodothyronine (T_3) was higher in the ISP-90 group at month six compared with the controls. However, none of these concentrations were outside normal ranges and did not have any clinical significance.

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 resulted 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 did 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 did 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. Again, no changes represent shifts outside normal ranges and do not support the hypothesis by Divi et al, (1997). Appendix VI Original Health Claim Petition—Solae, LLC

- 12-13. These items cited by Mr. James represent review or hypothesis papers (Foster et al, 2001; Ardies and Dees 1998), and thus were not provided to FDA as part of the health claim petition. There does exist dozens of review or opinion papers on the mechanism by which soy and/or isoflavones prevents the development of cancer (for example: Klein and Thompson, 2004; Sarker and Li, 2003; Kris-

Etherton et al, 2002; Kucuk, 2002; Ganry, 2002). Again these were not included with health claim materials for previously stated reasons.

14. Mr. James cites research by Newbold et al (2001) that was reviewed in the health claim petition Solae presented to FDA. In this study, neonatal mice were injected with genistein at remarkably high levels. This study is not useful in assessing the relationship between the incidence of certain cancer in humans and soy consumption.

Neonatal mice were injected of genistein at remarkably high levels. 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). 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). Appendix VI Original Health Claim Petition—Solae, LLC

15. Mr. James cites results from a study by Thigpen et al in 2001 on isoflavones and vulvar tumors in mice that was not provided to FDA as it had not garnered public interest. However, other work from this laboratory has been pivotal in the understanding that soy isoflavones are widely present in laboratory animal diets from the soybean meal (which is extremely high in isoflavones) that is used as the dietary protein source (Thigpen et al, 1999). To date there has not been widespread breeding or developmental problems in laboratory animals reported in medical research community.

- 16-17. Mr. James cites research conducted by Hilakivi-Clarke and associates (1998, 1999a) in which pregnant mice were injected with genistein and mammary gland development was assessed. A review of the 1999a reference was provided to FDA in the health claim materials because it had obtained public interest. Again, it is unclear how these two studies using genistein injection can be related to human soy consumption and cancer incidence. In addition to these two publications, reviews of Dr. Hilakivi-Clarke's publications related to breast cancer prevention in the rodent model were also provided (Hilakivi-Clarke et al, 1999c; Hilakivi-Clarke et al, 1999b).
18. Mr. James cites an opinion or hypothesis paper (Abe, 1999) which was not provided to FDA as this is not appropriate for consideration in the health claim process (USFDA, 1999). The opinion paper is related to infantile leukemia and soybeans. Soy-based infant formula has been used by over 20 million infants in the United States for more than 40 years and has a strong history of safe and nutritious utilization.
19. Mr. James cites a paper that was published after submission of our materials to FDA in which pregnant rats were injected with genistein or diethylstilbesterol (DES; Davies et al, 2004). In this same study, another group of suckling newborns was also fed from dams who were provided either rice milk or soy milk. A variety of endpoints were measured. As expected DES had significant effects on the uterus, as did injected genistein. Soy milk was associated with increased growth of the rat pups, progesterone receptors, and uterine glandular tissue. It is unclear how these results relate to both human reproduction development and animal husbandry and development as noted in items #15 and #18.

In addition to the above mentioned scientific citations, Mr. James lists compounds potentially present in soy foods as a result of processing—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. Manufacturing levels for nitrites are set well below regulatory limits and are often set to zero.

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 Mr. James 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 that food manufacturers can convey this important dietary health information to consumers on food labels.

Sincerely,

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C: Gregory Paul, Ph.D.
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