1. Introduction

In response to a submission by Protein Technologies International (PTI), the FDA has tentatively concluded that, based on the totality of publicly available scientific evidence, soy protein included in a diet low in saturated fat and cholesterol may reduce the risk of coronary heart disease.

Should PTI's health claim petition be granted it would pave the way for labelling such as "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease".

My submission does not address the issue of the health claim that soy protein reduces the risk of coronary heart disease *per se*. Rather, it opposes the claims by PTI that soy protein is a GRAS foodstuff and that there are no risks associated with the daily consumption of between 25 g and 100g of soy protein.

In fact, the FDA has never granted soy protein GRAS status. I would submit that the proof required to make such a claim is lacking. Also, there are very real risks associated with consuming soy protein. These risks appear to have been ignored by PTI and it is essential that the FDA give their full attention to the exposures to isoflavones and nitrosamines that will occur on daily exposure of up to 100g of soy protein.

In this regard, FDA must fully disclose to consumers the possible risks of soy protein as well as the possible benefits.

2. Safety for human use

PTI describe soy protein as a safe and lawful food and claim GRAS status by self-determination based on common use in food before January 1 1958. PTI also claim that the "FDA has recognised soy protein products as having GRAS status" and argue that the reason soy protein is not listed as GRAS is because "it is impractical for FDA to list all substances that are GRAS for their intended use".

Firstly, one must note that the self-determination of GRAS status of soy protein by the soy industry is meaningless and such self determinations should not be given any credence. In order to protect consumers only independent bodies, or those with recognised legal status, can perform legitimate determinations on the safety of consumer products.

Secondly, PTI's claim that products such as soy protein isolate were in common use in food before January 1 1958 is incorrect. The Select Committee of GRAS Substances (SCOGS) provided an independent evaluation of soy protein in the form of isolated soy protein in its 'Evaluation of the Health Aspects of Soy Protein Isolates as Food Ingredients' (1). The SCOGS committee found that at the time of their review (some 20 years after 1958) the use of soy protein was uncommon. In their determination of the likely average dietary exposure to soy protein

isolate, the SCOGS committee reported a maximum per capita daily intake of soy protein isolate of about 150 mg from food items and a negligible amount due to migration from packaging.

Thirdly, and quite simply, soy protein is not legally a GRAS foodstuff, as the FDA has never codified it as such. In their review of soy protein isolates the SCOGS committee noted that such products were initially developed as binders in paper coatings. Soy protein isolates were GRAS under the provisions of the Code of Federal Regulations as substances migrating from paper and paperboard products used in food packaging because it was assumed that only very small amounts would be subject to human ingestion.

The SCOGS committee concluded that there was no evidence that soy protein isolates were a hazard when consumed at levels typical at that time (or levels that might reasonably be expected to be in future use) provided acceptable levels of lysinoalanine, nitrite and nitrosamines were established. To this end the committee recommended that food-grade soy protein isolates have specified acceptable levels of lysinoalanine, nitrite and nitrosamines.

To date, acceptable levels of lysinoalanine, nitrite and nitrosamines have not been established for food-grade soy protein isolates and one might conclude that soy protein isolates have not been granted GRAS status for this reason.

To claim that the FDA has not codified soy protein isolate as GRAS because it is impractical to list all substances that are GRAS for their intended is inconsistent with the fact that other food items that the SCOGS committee found were GRAS have subsequently been codified as such. Had the SCOGS committee been satisfied that soy protein isolate was safe for intended use, there is little reason to suggest that GRAS status would not have been codified. Quite simply, soy protein was not granted GRAS status because the SCOGS committee was not convinced it was GRAS.

The findings of the SCOGS committee still have particular relevance to the PTI health claim petition. The PTI petition recommends a daily intake of 25 to 100 g of soy protein and it is quite evident that at this level of intake is far greater than the SCOGS committee ever anticipated when they were conducting their GRAS review. Hence, the concerns expressed by the SCOGS committee relating to exposure to lysinoalanine, nitrite and nitrosamines take on additional significance. For example, a daily consumption of 25 to 100 g of isolated soy protein may result in nitrosamine exposures that exceed established No Significant Risk Levels (NSRL's). This is discussed further in Section 3.2.1.

There are also several other issues which were not examined by the SCOGS committee during their review that are further reasons why soy protein isolates should not be granted GRAS status. These issues relate to the isoflavone content of isolated soy proteins and are discussed in Section 3.2.2.

Until the safety issues discussed in Sections 3.2.1 and 3.2.2 are adequately addressed soy protein still cannot, and should not, be viewed as GRAS.

3. Risks associated with soy consumption

3.1 Recommended daily intake

PTI state that the “effective daily level of intake of soy protein associated with a significant effect on blood lipids and lipoproteins is 25 grams”. According to PTI, this amount of soy protein could be obtained from 46 to 48 g of steam treated soy flour (52 to 54% protein) or 27 to 28 g of isolated soy protein (90 to 92% protein).

PTI also state that the “upper level of soy protein intake” should be “100 g of protein on a daily basis” although they imply that a diet of up to 155 g of soy protein per day is unlikely to cause harmful effects. In their submission of support of PTI health claim, the American Soybean Association (ASA) recommends a maximum daily level of daily intake of 100 g of soy protein. According to PTI this amount of soy protein could be obtained from 185 to 192 g of steam treated soy flour or 109 to 111 g of isolated soy protein.

However, there appears to have been little thought given to the level of exposure to potentially toxic factors, such as isoflavones, that would result from such diets. It behoves PTI and the ASA to fully determine the risks associated with the exposure to isoflavones that could occur when soy protein is consumed at the levels they have determined as appropriate to result in a reduction in the risk of coronary heart disease. However, neither PTI nor the ASA have performed any analysis of this kind and by not doing so have neglected their duty to consumers and cast doubt on their ability to act as a responsible producers of food commodities. This oversight also makes a mockery of the claims by PTI and the ASA that their petitions are “representative and balanced”

The manner in which PTI dismiss the very real risks that factors such as isoflavones pose is of considerable concern. In stating that “most domesticated animals and fowl are fed soy based rations and fertility is not a reported problem” PTI enter a new realm of deceitfulness.

3.2 Recommended daily exposure level and exposures to harmful compounds

3.2.1 Isoflavones

The potential for biological effects in humans due to soy isoflavones has been clearly identified and includes changes in the function of sex glands, the central nervous system, the thyroid, and behavioural patterns (2-7).

Soy flour may contain up to 3080 μg/g total isoflavones (8) and isolated soy protein may contain up to 2500 μg/g total isoflavones (9). Therefore, daily ingestion of the qualifying amount of soy protein (25 g) could result in a daily intake of up to 148 mg isoflavones if the soy protein were derived from soy flour or up to 70 mg isoflavones if the soy protein were derived from isolated soy protein.

The maximum recommended level of daily intake of soy protein (100 g) could result in a daily intake of up to 591 mg isoflavones if the soy protein were derived from soy flour or 278 mg isoflavones if the soy protein were derived from isolated soy protein.

By recommending at least 25 g, but not more than 100 g, per day of soy protein to achieve a reduction in the risk of coronary heart disease PTI and the ASA are effectively sanctioning the ingestion of up to approximately 600 mg of isoflavones per day.

The effects of large acute and/or chronic doses of isoflavones in humans have not been established. However, even at modest doses, isoflavones are biologically active in humans. For example, a study of dietary intake of 45 mg total isoflavones (measured as total genistein and total daidzein) per day for 30 days in pre-menopausal women resulted in significant biological effects (10). These effects were a reduction in mean mid-cycle levels of LH and FSH to 33% and 53% respectively of the levels observed when the women were fed control diets that did not contain soy. Some individuals responded to the isoflavones less than others, however, in one individual LH and FSH levels were reduced to 17% and 32% of normal levels respectively.

In the Cassidy study all of the women still ovulated but the effects of the isoflavones continued for three months after the diet ceased. Clearly there is potential for women who are exposed to dietary isoflavones to suffer sufficient reduction in LH and FSH levels that they might become anovulatory.

PTI also cite the Cassidy study and note that “the changes in menstrual cycle length and hormone levels observed in these women were similar to those reported in response to treatment with Tamoxifen”. One must assume that PTI have little understanding of the likely effects of exposing Tamoxifen *en masse* to the general population at more than ten times a biological dose. Although isoflavones are typically thought of as having the potential to combat hormone related disease, there will be doses at which they are acutely and chronically toxic.

Prominent isoflavones researchers have warned about the dangers of “mega-dosing” on these compounds (11) although to date a “mega-dose” has not been defined. A daily intake of 600 mg of isoflavones is more than one order of magnitude greater than the level that produced a biological effect during the Cassidy study and represents a dose that could readily result in adverse effects. To be certain to maintain a diet containing no more than a biological dose of isoflavones (45 mg) it is

advisable that women consume no more than approximately 15 g of soy flour or 18 g of isolated soy protein per day.
Other effects on the sex steroid hormone status of women and men are quire possible. *In vitro* the soy isoflavones are potent inhibitors of 17-β-hydroxysteroid oxidoreductase (12-13) and, therefore, can modulate the synthesis and metabolism of oestradiol and other steroid hormones (14).

There have also been reports of soy isoflavones increasing nipple fluid secretion (15) and, although the study was not conclusive, consumption of soy was identified as a significant positive association in an increased occurrence of premature thelarche in Puerto Rico (16).

Contrary to PTI's claim, the reproductive and developmental toxicity of isoflavones has been demonstrated in several species of animals including domestic animals and fowl (17-21). In fact it was the toxicity of dietary levels of isoflavones to animals that first raised the awareness of the scientific community to the fact that soy isoflavones were endocrine disruptors (22). Reproductive effects, infertility, thyroid disease or liver disease due to dietary intake of isoflavones had been observed for several animals including mice (20), cheetah (21), quail (23), pigs (24), rats (25), sturgeon (26) and sheep (27).

As well as direct reproductive system effects, there are other biological effects of soy isoflavones. Like many endocrine disruptors, the soy isoflavones cause thyroid dysfunction in humans. Several papers from the 1960s reported that infants fed soy-based formulas developed goitre although the goitrogenic factors were not identified at that time (28-32). More recent reports have identified the actual and potential toxicity of soy on the thyroid (33-36) and the active factor in soy has been identified as the isoflavones. *In vitro* these compounds are potent inhibitors of thyroid peroxidase; more potent, in fact, than common anti-thyroid drugs (37).

If FDA approve the PTI health claim they will effectively endorse the consumption of up to 600 mg isoflavones per day. Such exposures to very large quantities of anti-thyroid compounds should be avoided at all costs. Chronic exposures to other anti-thyroid flavonoids in millet have resulted in endemic goitre in certain regions of Africa. Such exposures to anti-thyroid agents, regardless of whether or not iodine intake is sufficient, have the potential to induce thyroid disease (38).

3.2.2 Nitrosamines

The potential for soy products to contain nitrosamines was discussed at length during a GRAS evaluation of isolated soy protein (1). A discussion of the toxicity and mechanism of formation of nitrosamines is outside the scope of this submission; it is sufficient to note that:

- nitrosamines are among the most carcinogenic compounds in existence.

- nitrosamines are not naturally-occurring in soybeans but form during processing of soy products by reaction of nitrite with amines.

There is little information on the levels of nitrosamines in soy products although given the manner in which products such as isolated soy protein (which includes acid washing and spray drying steps) or steam treated soy flour are prepared it is likely these compounds will be present. A single report found that a diet comprised of 11.9% isolated soy protein contained 1.5 ng/g of N-nitrosodimethylamine (39). This data suggests that consumers that ingest the maximum recommended amount of isolated soy protein (111 g) could be exposed to up to 1.4 μg of N-nitrosodimethylamine per day.

The California Environmental Protection Agency Office of Environmental Health Hazard Assessment (OEHHA) has established NSRL's for nitrosamines (40). These levels range from 40 ng per day for N-nitrosodimethylamine to 80 μg per day for the least potent nitrosamine listed (N-nitrosodiphenylamine).

As discussed in Section 3.1 the maximum daily intake of soy protein recommended by Protein Technologies (100 g) is equivalent to 109 to 111 g of isolated soy protein. Therefore, consumers of soy protein could be exposed daily to levels of N-nitrosodimethylamine that are 35 times greater than the NSRL.

For the level of intake of soy protein recommended by PTI, the NSRL's determined for N-nitrosodimethylamine will be exceeded if it is present at a level in excess of 0.20 ng/g (parts per billion) in steam treated soy flour or 0.36 ng/g in isolated soy protein. The NSRL's determined for the least potent OEHHA listed nitrosamines will be exceeded if they are present at levels in excess of 0.42 μg/g (parts per million) in steam treated soy flour or 0.72 μg/g in isolated soy protein.

In accordance with the SCOGS committee review, PTI must establish that their products contain acceptable levels of nitrosamines before they recommend daily intake levels for soy protein. It must also be understood that the NSRL's discussed in this submission are those defined by OEHHA for a 70 kg adult male. Lower NSRL's are defined, for example, for adult women and teenagers.

3.3 Disclosure of risk

The risks associated with consumption of up to 100 g of soy protein per day have been discussed in Section 3.2. In the event that FDA approve the PTI heath claim petition regarding the possible benefits of soy protein, FDA are duty bound to also fully disclose these risks.

4. Summary

PTI have claimed that a diet of soy protein may reduce the risk of heart disease. PTI also claim that soy protein is a GRAS foodstuff and that there are no risks associated with the consumption of up to 100g of soy protein.

In response, my submission is that soy protein is not a GRAS foodstuff and that the evidence required in order to make such a claim is still lacking.

My submission has noted that there are very real risks associated with consuming soy protein at the levels PTI have recommended (up to 100g of soy protein per day). These risks relate to the potential exposure to high levels of isoflavones (up to approximately 600 mg per day) and to nitrosamines.

The FDA must be willing to disclose to consumers the possible risks as well as the possible benefits of a diet of up to 100 g of soy protein per day.

5. References

1 Life Sciences Research Office. Evaluation of the Health Aspects of Soy Protein Isolates as Food Ingredients. 1979.

2 Clarkson TB *et al*. Estrogenic soybean isoflavones and chronic disease. Risks and benefits. Trends Endocrinol Metab 6: 11-16 (1995).

3 Chapin *et al*. Endocrine modulation of reproduction. Fund Appl Tox 29: 1-17 (1996).

4 Santi R *et al*. Phytoestrogens: potential endocrine disruptors in males. Tox Ind Health 14: 223-237 (1998).

5 Sheehan DM. Herbal medicines, phytoestrogens and toxicity: risk: benefit considerations. PSEBM 217:379-385 (1998).

6 Tönz O and Zimmerli B. Phytoöstrogene in säuglingsnahrung auf sojaproteinbasis. Paediatrica 8: 14-15 (1997).

7 Theo Colborn, Dianne Dumanoski and John Peterson Myers. Our Stolen Future, Little Brown and Company, London, 1996.

8 Taylor GJ and Burlingame BA. Phytoestrogens in selected New Zealand Foods, Crop and Food Research Report No. 241, April 1998.

9 Hendrich *et al*. Defining food components as new nutrients. J Nutr, 124: 1789S-1792S (1994).

10 Cassidy A *et al*. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 60: 333-340 (1994).

11 Setchell KDR *et al*. Exposure of infants to phytoestrogens from soy-based infant formula. Lancet 350: 23-27 (1997).

12 Keung W-M. Dietary estrogenic isoflavones are potent inhibitors of β-hydroxysteroid dehydrogenase of *P.Testosteronii*. Biochem Biophys Res Comm 215: 1137-1144 (1995).

13 Makela SI *et al*., Estrogen specific 17β-hydroxysteroid oxidoreductase type I (E.C.1.1.1.62) as a possible target for the action of phytoestrogens. PSEBM 208: 51-59 (1995).

14 Santti R *et al*. Phytoestrogens: potential endocrine disruptors in males. Toxicol Ind Health 14:1-2 223-37 (1998).

15 Petrakis NL *et al*. Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. Cancer Epid Bio Prev 5: 785-794 (1996).

16 Freni-Titulaer *et al*. Premature thelarche in Puerto Rico. AJDC 140: 1263-1267 (1986).

17 Carter AW *et al*. Effect of genistin on reproduction of the mouse. J Nutr 55: 639 (1955).

18 Matrone G *et al*. Effect of genistin on growth and development of the male mouse. J Nutr 59: 235 (1956).

19 Leopald AS. Phytoestrogens: Adverse effects on reproduction in California Quail. Science 191: 98-100 (1976).

20 Drane HM *et al*. Oestrogenic activity of soya-bean products. Fd Cosmet Technol 18: 425-427 (1980).

21 Setchell KDR *et al*. Dietary estrogens - a probable cause of infertility and liver disease in captive cheetahs. Gastroenterology 93: 225-233 (1987).

22 Pope GS and Wright HG. Oestrogenic isoflavones in red clover and subterranean clover. Chem Ind 1019-1020 (1954).

23 Leopald AS. Phytoestrogens: Adverse effects on reproduction in California Quail. Science 191: 98-100 (1976).

24 Drane HM *et al*. Possible oestrogenic effects of feeding soyameal to prepuberal gilts. Br Vet J 137: 283-288 (1981).

25 Kimura S *et al*. Development of malignant goiter by defatted soybean with iodine-free diet in rats. Gann 67: 763-765 (1976).

26 Pelissero C *et al*. Estrogenic effect of dietary soy bean meal on vitellogenesis in cultured Siberian Sturgeon *Acipenser baeri*. Gen Comp End 83: 447-457 (1991).

27 Braden *et al*. The oestrogenic activity and metabolism of certain isoflavones in sheep. Aust J Agr Res 18:335-348 (1967).

28 Van Wyk *et al*., The effects of a soybean product on thyroid function in humans. Pediatrics 24: 752-760 (1959)

29 Hydovitz JD. Occurrence of goiter in an infants on a soy diet. New Eng J Med 262: 351-353 (1960).

30 Shepard TH. Soybean goiter. New Eng J Med 262: 1099-1103 (1960).

31 Ripp JA. Soybean induced goiter. Am J Dis Child 102: 136-139 (1961).

32 Pinchera A *et al*. Thyroid refractoriness in an athyreotic cretin fed soybean formula. New Eng J Med 273: 83-87 (1965).

33 Fort P *et al*. Breast and soy-formula feeding feedings in early infancy and the prevalence of autoimmune thyroid disease in children. J Am Coll Nutr 9: 164-167 (1990).

34 Ishizuki Y *et al*. The effects on the thyroid gland of soybeans administered experimentally in healthy subjects. Nippon Naibunpi gakkai Zasshi 67: 622-629 (1991).

35 Chorazy PA *et al*. Persistent hypothyroidism in an infant receiving a soy formula: case report and review of the literature. Pediatrics 148-150 (1995).

36 Jabbar MA *et al*. Abnormal thyroid function tests in infants with congenital hypothyroidism: the influence of soy-based formula. J Am Coll Nutr 16: 280-282 (1997).

37 Divi RL *et al*. Anti-thyroid isoflavones from the soybean. Biochem Pharmacol 54: 1087-1096 (1997).

38 Hill RN *et al*. Thyroid follicular cell carcinogenesis. Fund Appl Tox 12: 629-697 (1989).

39 Rackis JJ *et al*. The USDA trypsin inhibitor study: Background, objectives and procedural details. Qual Plant Foods Hum Nutr 35: 213-244 (1985).

40 The California Environmental Protection Agency Office of Environmental Health Hazard Assessment. Status report: no significant risk levels for carcinogens and acceptable intake levels for reproductive toxins, January 1994.