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June 8, 2004

Dr. Michael D. Shelby
Center for the Evaluation of Risks to
Human Reproduction
P.O. Box 12233
Research Triangle Park, NC 27709

Re: Evaluation of Genistein and Soy Formula

Dear Dr. Shelby:

This letter, submitted by Solae, LLC (a joint-venture between DuPont and Bunge; herein "Solae"), is in response to the National Toxicology Program (NTP) Center for the Evaluation of risks to Human Reproduction (CERHR) request for comments on the future evaluation of Genistein and Soy Formula (Federal Register Docket No. 04-8269).

This letter provides data to demonstrate that soy-based infant formula safely and effectively provides appropriate nutrition for normal growth and development in term infants.

Numerous studies support normal growth and development in term infants consuming soy-based infant formula. In addition, more than 20 million infants have been fed modern soy-based infant formula in the United States alone, and there has been no documentation of adverse health conditions greater than breast-fed or cow's milk formula-fed infants.

We recognize that recent data, generated primarily in animal models using purified genistein derived from soy, has led some groups to question the safety and efficacy of soy-based infant formula. We believe that these groups are misguided for a number of reasons, described below.

We hope the following information is useful. Please contact us if you have any questions or if we may be of any assistance.

Summary of Solae

Solae is a leading manufacturer of high quality soy ingredients, including proteins, lipids, lecithin, and fibers. These ingredients are found in mainstream consumer foods made by major manufacturers worldwide. In addition, our soy protein isolate has been used

globally in the majority of commercially available soy-based infant formulas for more than 40 years.

Solae has a commitment to nutrition research that focuses on both the safety and impact of consuming soy products on human health throughout the life cycle, including infancy. The results of decades of research clearly support the health and safety of soy-based infant formulas for healthy, full-term infants.

Summary of Comments Regarding the Proposed Evaluation of Genistein and Soy Infant Formula

Soy-based infant formula has a long history of safe use by healthy full-term infants. In fact, the American Academy of Pediatrics endorses the use of soy-based infant formula as a safe and effective alternative to provide appropriate nutrition for normal growth and development in term infants (1). In addition, soy-based infant formula meets all the requirements for term infants as set by the Infant Formula Act (2).

In this letter, we discuss why it is inappropriate to extrapolate research results from studies using purified genistein, in some cases very high levels, in variant animal models to the human infants consuming soy-based infant formula that contains natural concentrations and ranges of the three classes of soy isoflavones. Also, results from numerous studies are discussed showing that soy-based infant formula is safe to consume and supports normal growth and development. This includes normal development of the reproductive system, immune function, neurobehavioral development, and thyroid function.

Relevance of Animal Data to Human Health

Particular issues that are important to consider when comparing data from animal models to ingestion of soy-based formulas by infants are:
1) dosage ranges of the injected genistein may be physiologically irrelevant;
2) direct injection of genistein into the bloodstream bypasses the effect the digestive tract has on the isoflavones;
3) typical animal models vary considerably from humans in terms of digestion and absorption of isoflavones; and
4) genistein alone does not elicit the same physiological effects as combinations of soy isoflavones.

Extrapolation of animal data to humans is difficult due to differences in routs of administration, absorption, and metabolism. Injecting genistein bypasses the digestive system and the liver, resulting in a much different exposure in type and intensity of the isoflavone as compared to the effects of consuming soyfoods. In fact, in humans and animals, very little of the free forms of the soy isoflavones enter the blood (3). It is primarily the digestive metabolites of genistein (4-ethylphenol) and daidzein (equol - especially in animals), that are absorbed and thought to be responsible for the physiological effects of ingested isoflavones (4). As such, studies reviewing the effects

of pure isoflavones, such as genistein in the blood, have little relevance to the effects of ingested isoflavones.

In addition, certain animal species can develop reproductive disorders when high levels of isoflavones or genistein are given. However, this is due to differences in isoflavone metabolism in these animals compared to humans. Physiological differences are also important to consider. For example, rats are very sensitive to compounds that perturb the hypothalamic/thyroid axis and are thus a more sensitive species in terms of thyroid tumorogenesis compared to humans.

Importance of Other Isoflavones

Soy-based infant formula contains 12 forms of naturally-occurring isoflavones including the malonyl and acetyl forms and glucoside and aglycone forms of daidzein, genistein, and glycitein. It is important to understand that the physiological effects of genistein administered alone can be different from effects noted when other soy isoflavones are provided concurrently. Administered alone to hamster V79 cells, genistein can cause clastogenicity by interfering with topoisomerase II activity. When genistein is co-administered with daidzein, clastogenicity is not observed (5). Unpublished data from Dr. Neil Shay (personal communication) also demonstrate changes in patterns of gene expression when isoflavones are administered individually versus concurrently.

Given the limitations of animal models and the recent differential findings observed when isoflavone mixtures are administered versus genistein alone, great caution should be exercised when extrapolating results of studies where animals are given genistein alone to infants consuming soy-based formulas containing mixtures of eleven other isoflavones.

Soy-based Infant Formula Supports Normal Growth and Nutritional Status

Numerous studies support normal growth and development in term infants consuming soy-based infant formula (1,6-9). No growth differences in male and female infants consuming similar amounts of energy were detected in either the first month or first four months of consuming a soy formula compared to a cow's milk formula (6). Over a 1-year period, infants fed soy-based formula exhibited similar growth patterns to infants that were initially breast fed then weaned to a cow's milk formula (7). A retrospective study of adults fed either soy-based formulas or cow's milk formulas as part of an infant feeding study in the early 1970s found no differences in height or weight some 30 years later (8). In addition, infants fed soy-based formulas have normal concentrations of serum albumin, hemoglobin (both measures of protein nutriture) and iron (6,7,9,10), and exhibit bone mineralization equal to infants fed cow's milk formula or breast milk (11,12). It is important to recognize that no health advantages have been documented for feeding cow's milk infant formulas over soy-based infant formulas.

Reproductive Development

Critics of soy-based infant formula cite a study in which uterine adenocarcinomas were found at 18 months of age in neonatal mice injected with high levels of genistein (50 mg/kg) on days 1 – 5 after birth (13). Relevance to humans is limited by several factors including: a) differences among animal species in isoflavone metabolism; b) use of genistein alone rather than the ratio of naturally-occurring isoflavones present in soy-based infant formula; c) the use of genistein at levels many times higher than those absorbed by infants fed soy-based formulas; and d) effects of injecting genistein cannot be extrapolated to feeding genistein in the diet.

In contrast, 48 children who were fed exclusively soy-based infant formula for at least 6 months participated in a recent retrospective trial to evaluate potential hormonal influences on development (14). These children were compared to 18 control children who did not consume soy-based infant formula and the results were as follows:

- No signs of precocious puberty in female participants
- No signs of gynecomastia in male participants
- Height and weight, as BMI, were within the normal range as compared with those of children matched for age, sex and race
- Bone age corresponded to the chronological normal range
- Urinary markers of bone metabolism (deoxypyridoline, calcium, phosphate, and creatinine) were all within normal ranges
- Serum levels of bone alkaline phosphatase, osteocalcin, estradiol, and intact parathyroid hormones were all within normal ranges

The authors concluded that long-term feeding with soy-based infant formula in early life does not produce estrogen-like hormonal effects.

Another retrospective study in young adults fed soy-based infant formula for several months as infants, found no evidence of hormonal or other adverse effects for over 30 variables encompassing maturation, menstrual and reproductive history, anthropometrics, and general health (8). Those consuming soy did report a shorter duration of monthly menses by 8 hours and slightly more discomfort with menstruation; however no subjects sought medical attention for either condition. Interestingly, the authors note that if an adjustment is made for the multiple comparisons made in the study, both findings would not be close to reaching statistical significance.

Additional corroboration supporting normal sexual development and maturation in infants fed soy-base infant formula is provided in a plenary review of isoflavone safety by Munro et al. (15). The authors provide an extensive discussion on reproductive and developmental toxicity citing studies on a broad range of species and conclude that "there is no conclusive evidence from animal, adult human, or infant populations that indicates that dietary isoflavones may adversely affect human development or reproduction."

Immune Development

Injections of purified genistein have been associated with impaired immune function in castrated and ovariectomized adult mice (16). It was then suggested that infants consuming soy-based infant formula may therefore develop impaired immune function. As stated previously, injecting genistein causes the compound to bypass the digestive system and the liver, resulting in a much different exposure in type and intensity of the soy compound as compared to the effects of eating soyfoods. In addition, the authors did not have a control group of untreated mice and did not measure immune response; rather they measured the weight and number of cells in the thymus. The thymus naturally becomes smaller with age, and castration and ovary removal may further affect this shrinkage. Researchers have also criticized results because the study design did not account for the age-related thymic involution process, and daily injections for 35 days add an uncontrolled stress known to influence immune function (17).

Two well-designed human infant feeding trials have investigated the effects of soy-based infant formula on immune response and immune cell production (18,19). In these studies, newborn, term infants were assigned randomly to groups fed soy formula with (n = 94) or without (n = 92) added nucleotides for 12 months. Immune status and morbidity were compared to a group of infants (n = 81) fed human milk and weaned to a standard cow's milk formula. Antibody levels to common infant immunizations were similar for all groups. In addition, all vaccine responses were within normal ranges. There were no differences in inflammation of the middle ear or antibiotic use. Parental reports of diarrhea did not differ among groups although physician reports of presence or absence of diarrhea were higher in both soy-based formula groups than the human milk/cow's milk group. When analyzed on the frequency of diarrhea, no statistical differences were found. Breast-feeding has a well-documented anti-diarrheal effect and the differences observed in this study between breast-fed and formula-fed infants are not unusual (20). Leukocyte populations for both the soy-based infant formulas were within reference ranges. Almost no differences existed for lymphocyte subtypes measured at 6, 7, and 12 months of age. No differences existed in T lymphocytes or natural killer cell lymphocytes between the soy formula-fed infants and human milk/cow's milk-fed infants. In addition no differences among groups were observed for naïve helper T cells or memory effector helper T cells. The authors concluded that term infants fed soy protein isolate-based formulas have normal immune development as measured by antibody responses to childhood immunizations and immune cell status. These findings support the earlier observations by Businco's group that soy-based formula-fed infants showed normal immune responses to oral polio vaccines (21).

The animal experiment of Yellayi et al. (16) fails to model the situation of a human infant consuming a soy-based infant formula. Extrapolating data from surgically-altered animals injected with a single, purified isoflavone to human infants consuming soy-based infant formula is a significant stretch in logic and unnecessary when well-controlled clinical data is available that specifically addresses immune function in the population of concern.

Neurobehavioral Development

Again, data from adult rodents suggest that ingestion of soy isoflavones may affect certain aspects of brain structure and function (22). In addition, 71- to 93-year old Japanese American adult tofu consumers were shown to have slightly impaired cognitive function and structural changes in the brain (23,24). These data contrast sharply to a controlled-clinical trial in which memory, cognitive function, and mood of college students were improved after consuming a soy diet high in isoflavones (60 mg/day aglycone) for 10 weeks compared to another group consuming a soy diet low in isoflavones (0.3 mg/day aglycone; 25).

Retrospective studies have evaluated neurobehavioral development measures in adults (8) and in 9- and 10-year old children fed soy-based infant formula as infants (26). In the former study, adults fed soy-based infant formula as infants were found to have no difference in the level of post-high school education compared to a group who had consumed cow's milk formula as infants. In the latter study, intelligence quotient (IQ) was compared in those fed soy-formula as infants to a control group of breast-fed infants after controlling for maternal education level (a known confounder of child IQ). In addition to the lack of any effect on IQ, those who consumed soy-based infant formula as infants exhibited no differences for behavioral problems, learning impairment, or emotional problems. The authors concluded that early feeding of soy-based infant formula has no effect on IQ or other problems that might impair learning.

First and foremost, data from human populations who actually consumed soy-based infant formula as infants should be given priority when evaluating any outcome over other study populations that did not consume soy-based infant formula. The body of literature in this field will be expanding as a long-term study is underway at the U.S. Department of Agriculture Arkansas Children's Nutrition Center. This study will focus on the effect of consuming soy-based infant formula on neurobehavioral and reproductive development. Committee members serving on the expert panel for this review are urged to follow this important study.

Thyroid Function

Early soy-based infant formulas, which were made with soy-flour that was not supplemented with iodine, were associated with an increased incidence of goiter (27). Term infants with normally functioning thyroids exhibit no unfavorable effects from consuming current soy-based infant formulas, which are made with soy protein isolate and supplemented with iodine. A hallmark of hypothyroidism is a slowing of growth. As discussed in the growth section of this comment, soy-based infant formula consumption by healthy term infants clearly supports normal growth and development.

Conclusions

More than 20 million infants have been fed modern soy-based infant formula in the United States alone, and there has been no documentation of adverse health conditions greater than breast-fed or cow's milk formula-fed infants. Numerous studies support normal growth and development in term infants consuming soy-based infant formula. Concerning reproductive and developmental toxicity, Munro et al. (15) in a recent review on isoflavone safety conclude that "there is no conclusive evidence from animal, adult human, or infant populations that indicates that dietary isoflavones may adversely affect human development or reproduction." Controlled clinical trials show no effects of consuming soy-based infant formula on immune development and immune cell status. Retrospective studies on older children or adults fed soy-based formula as infants show no differences in cognitive ability as measured by IQ or scholastic achievement. In addition, available data support normal thyroid function in healthy term infants consuming soy-based formula.

The plethora of data clearly support the viewpoint of the American Academy of Pediatrics – that soy-based infant formula is a safe and effective alternative to provide appropriate nutrition for normal growth and development in term infants. The long history of safe use and the robust body of literature supporting the safety of soy-based formula consumption by human infants should not be overlooked in favor of data from a few ambiguous animal models studied under conditions that in no way mimic how soy formulas are ingested in the real world.

Respectfully submitted,

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