National Toxicology Program
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Center For The Evaluation Of Risks To Human Reproduction

DRAFT
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NTP-CERHR Brief on the Potential Human Reproductive and Developmental Effects of Soy Formula

INTRODUCTION

In January 2004, the CERHR Core Committee, an advisory group composed of representatives from NTP member agencies, recommended genistein and soy formula for expert panel review. Soy formula, also called soy infant formula, is fed to infants as a supplement or replacement for human milk or as an alternative to cow milk formula.

Soy products contain phytoestrogens. Phytoestrogens are plant-derived compounds with biological properties similar to the female hormone estrogen. The presence of these compounds in the diet of infants has raised concerns about possible effects on development. CERHR selected genistein and soy formula for expert panel evaluation because of:

(1) the availability of reproductive and developmental toxicity studies in laboratory animals and humans,
(2) the availability of information on exposures in infants, and
(3) public concern for effects on infant or child development.

This monograph includes the NTP Brief on Soy Formula, a list of the expert panel members (Appendix I), the Expert Panel Report on Soy Formula (Appendix II), and all public comments received on the expert panel report (Appendix III). The NTP-CERHR monograph is intended to serve as a single, collective source of information on the potential for soy formula to adversely affect human reproduction or development.

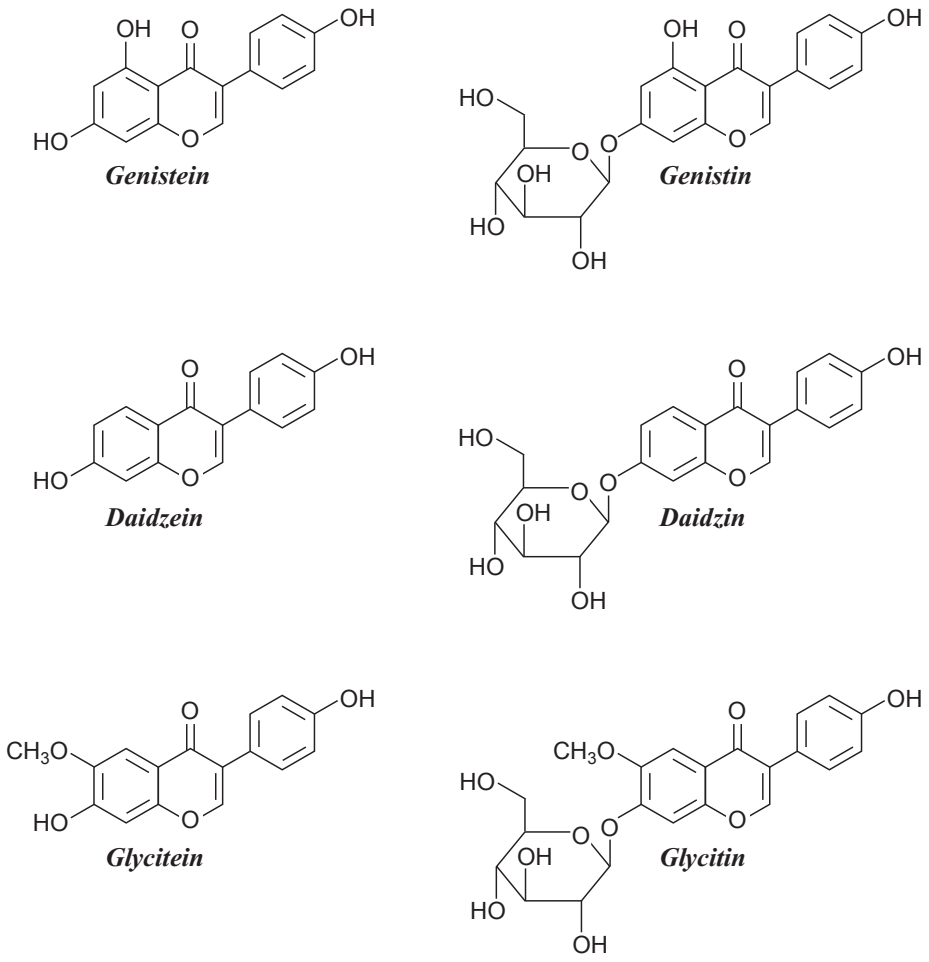
The NTP brief presents the NTP's interpretation of the potential for exposure to soy formula to cause adverse reproductive or developmental effects in people. It is intended to provide clear, balanced, and scientifically sound information. It is based on information about soy formula provided in the expert panel report, public comments on that report, and additional scientific information published following the public meeting of the expert panel.

NTP BRIEF ON SOY FORMULA

What is Soy Formula?

Soy formula is a complete infant diet used as a replacement or supplement for human milk or as an alternative to cow milk formula. It is not the same product as soy milk, which is not a complete infant diet. In the United States, several companies market soy infant formula (see Expert Panel Report, Table 2). The primary ingredients in soy formula include corn syrup, soy protein isolate, vegetable oils, sugar, vitamins, minerals, and other nutrients. Soy protein isolate is made from soybeans and is present in infant formulas at 14–16% by weight. Soy protein isolate contains phytoestrogens, which are plant-derived compounds with biological activity similar to the female hormone estrogen. In soy formula, most phytoestrogens are chemically bound to a sugar molecule. These sugar-bound phytoestrogens are called genistin, daidzin and glycitin. The sugar-free forms of these phytoestrogens are the biologically active forms and are called genistein, daidzein, and glycitein **(Figure 1)**. The relative amounts of phytoestrogens in soy formula are genistin > daidzin > glycitin, which also corresponds to the relative estrogenic potency of the sugar-free forms of these phytoestrogens.

Figure 1.
Chemical structure of phytoestrogens in soy formula



Genistin is the primary phytoestrogen in soy formula and other soy-derived foods. Digestion removes the sugar molecules from the phytoestrogens and the sugar-free forms are absorbed in the gastrointestinal tract. The body then binds the free phytoestrogens to another molecule such as glucuronic acid. As much as 99% of the phytoestrogen is bound to another molecule in human blood. For the sake of simplicity, this brief will refer to the phytoestrogens as free (not bound to a sugar or other molecule), bound (bound to a sugar or other molecule), and total (the sum of free and bound phytoestrogen).

How is Soy Formula Used?

Soy formula is fed to infants as an alternative to cow or human milk. Parents may choose soy formula for their infants to keep a vegan/vegetarian household, or for the perceived benefits of a soy diet. Soy formula is not recommended for preterm infants. Up to 22% of infants in the United States are fed soy formula during the first year of life. Total phytoestrogen intake by infants on a soy formula diet is estimated at 1–12 mg/kg body weight (bw)/day. No information was located on occupational exposures associated with manufacture, packaging, or distribution of soy formula.

*Can Soy Formula Affect Human Development or Reproduction?**

*Possibly***.** Appropriate levels of sex hormones are essential for normal development and function of the reproductive system. Because soy formula contains compounds with estrogen-like activity, concern has been expressed that feeding soy formula might adversely affect development of the reproductive system. There are presently not enough data from studies in humans to confirm or refute this possibility **(Figure 2a)**. Likewise, data from the studies in laboratory rodents and marmosets are not sufficient to permit a firm conclusion regarding the developmental or reproductive toxicity of soy formula **(Figure 2b)**. However, blood levels of total genistein in infants fed soy formula are very similar to the blood levels in rats given doses of free genistein in the diet that induce adverse developmental effects (see NTP Brief on Genistein). Because of this similarity in blood levels and the lack of sufficient studies on the human health effects of soy formula, the possibility that adverse effects might occur cannot be dismissed.

* Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

Figure 2a. *The weight of evidence that soy formula causes adverse developmental or reproductive effects in humans*

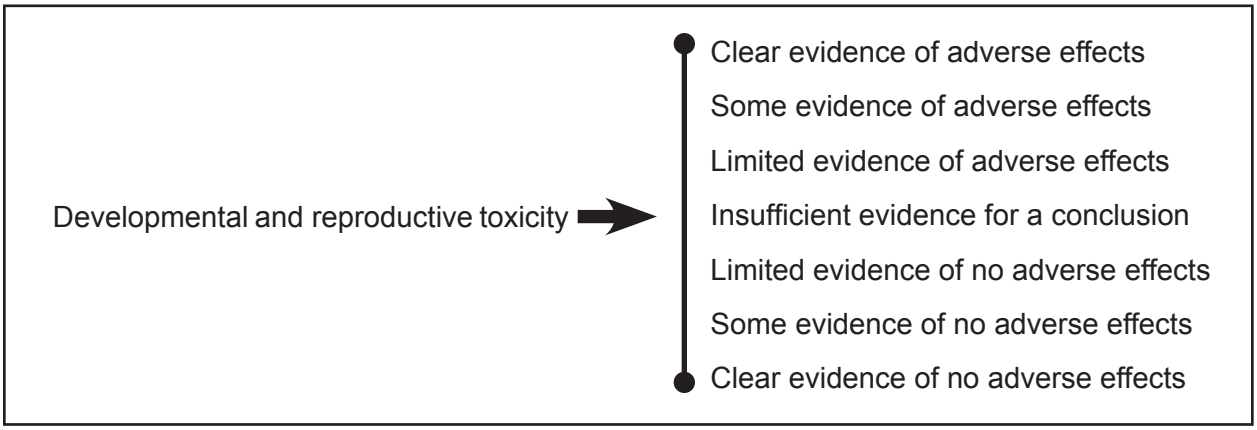
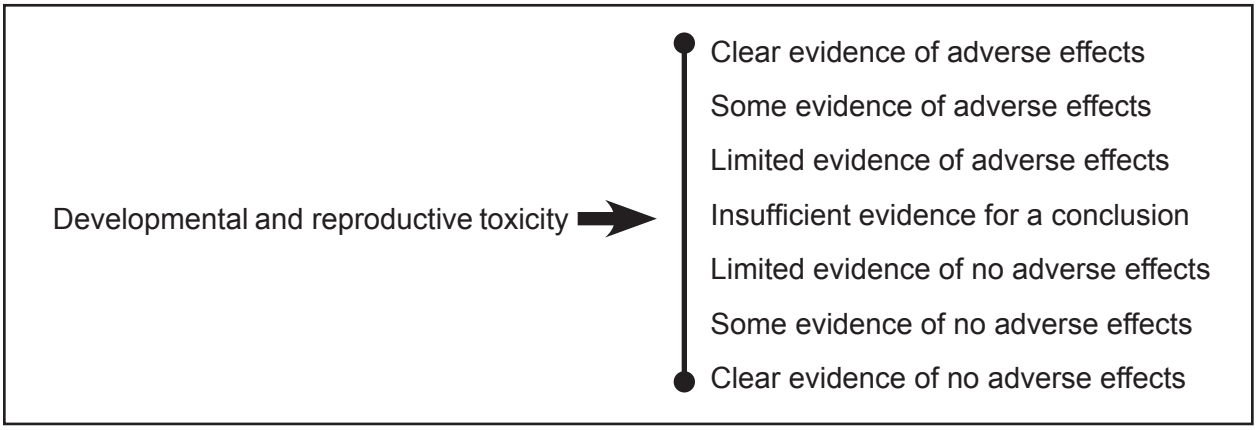


Figure 2b. *The weight of evidence that soy formula causes adverse developmental or reproductive effects in laboratory animals*



Supporting Evidence

The expert panel report (Appendix II) provides details and literature references regarding human exposure and studies on the possible developmental and reproductive toxicity of soy formula.

The expert panel reviewed 38 studies on various effects of soy formula on human health. They concluded that these studies were of limited utility in evaluating possible adverse effects of soy formula on reproduction and development because of poor study design, lack of experimental detail, or small sample size **(Figure 2a)**. In a retrospective epidemiology study by Strom et al., adults (20–34 yrs old) who had participated as infants in controlled feeding studies (248 were fed soy formula and 563 cow milk formula during infancy) were interviewed by telephone. As infants, these adults were enrolled in controlled feeding studies before 9 days of age and fed either soy formula or cow milk formula through 16 weeks of age. Health information and menstrual and reproductive history were self-reported to the interviewer. The authors concluded from the results of these interviews that "exposure to soy formula does not appear to lead to different general health or reproductive outcomes than exposure to cow milk formula."

Infants on soy formula consume approximately 1-12 mg/kg bw/day of total phytoestrogens. The primary phytoestrogen in soy formula, genistein, is consumed at up to 8 mg/kg bw/day. The expert panel noted that no reproductive effects were observed in adult female and male rats or female monkeys at total dietary concentrations or phytoestrogens up to 10 times greater than those normally consumed by infants on soy formula.

In a study of 4-month old human male infants fed soy formula, cow milk, or human milk (see Table 10, Setchell et al., Expert Panel Report on Genistein and Table 7, Setchell et al., Expert Panel Report on Soy Formula), serum levels of total genistein were measured. Blood serum concentrations of total genistein averaged 684±443 ng/ml (± standard deviation), 11.6±2.5 ng/ml, and 10.2±2.7 ng/ml in the groups fed soy formula, cow milk, and human milk, respectively. Free phytoestrogens were not measured in this study. An estimate of the amount of free genistein in soy formula fed infants would be 6.8 ng/ml based on the adult level of 1% of total genistein in the circulation. These concentrations of total and free genistein are similar to those determined in 21-day-old rats fed genistein in the diet that produced adverse developmental effects. Total

serum genistein concentrations were 564±176 ng/ml for male rats and 505±81 ng/ml for female rats (see Table 13, Chang et al., Expert Panel Report on Genistein); the amount of free genistein in the blood was not measured. In the rat study, some adverse effects observed after birth (changes in anogenital distance, decreased birth weight) were most likely due to in utero exposure, as very little genistein is transferred to the pups through the milk. Other adverse postnatal developmental/reproductive effects such as early vaginal opening, delayed testicular descent, and abnormal estrus cycling were noted in older animals, when levels of genistein in the circulation may have been greater than at weaning. Other studies have determined total and free serum genistein in rat pups after dietary exposure of the dams during gestation and lactation (see Table 13, Doerge et al., and Fritz et al. in the Expert Panel Report on Genistein). They determined that the amount of total genistein that was present as free genistein in the blood was approximately 30% in the fetus, 16% on postnatal day 7, and 7% on postnatal day 21.

Ten studies in experimental animals were also judged by the expert panel to have limited utility in their evaluation **(Figure 2b)**. Soy formula is a mixture of ingredients and the addition of individual components such as soy protein isolate, soybean meal, soy milk, or phytoestrogens to the animal diet may not be an appropriate model for assessing the effects of soy infant formula. Further, experimental animals metabolize soy differently than human infants. For example, rats and monkeys metabolize daidzein to equol, which has more potent estrogenic activity than the other phytoestrogens discussed above. Human infants produce little or no equol.

In a paper published after the expert panel meeting, Gu et al. (2006) compared the metabolism of soy protein isolate in female Sprague-Dawley rats, Hampshire/Duroc pigs, cynomolgus monkeys, and humans. Serum and urine phytoestrogen concentrations were determined and significant interspecies differences were reported. Female pigs had a metabolic profile closer to women than to female monkeys or rats. The authors noted that, since metabolism of phytoestrogens determines their estrogenic potency, animal models that metabolize phytoestrogens similarly to humans would be better experimental models. This paper emphasizes the need for studies that utilize an appropriate animal model and/or take into account species differences in metabolism of soy phytoestrogens.

Finally, two studies (see Table 42, Sharpe et al. and Tan et al. in the Soy Formula Expert Panel Report) reported the effects of feeding soy formula to infant marmosets (small, non-human primates) from postnatal day 4 or 5 to postnatal day 35 to 45. The soy formula-fed males had significantly lower plasma testosterone levels at postnatal day 35 to 45 than their cow milk formula-fed counterparts. Male marmosets, which received no soy formula after age 45 days, were subsequently evaluated for reproductive function and testis weight and structure when they were 80 weeks old or older and sexually mature. While the males fed soy formula had significantly heavier testes and greater numbers of Leydig cells and Sertoli cells per testis than the cow milk formula-fed controls, there were no differences in serum testosterone levels or the fertility of the soy formula and cow milk formula-fed animals. The small number of animals studied and the lack of information on normal variability in the endpoints measured limit the utility of these studies for assessing the effects of soy formula. Nonetheless, the reported effects add to the body of evidence that soy formula may affect human development and point to the need for additional studies in humans.

Should Feeding Infants Soy Formula Cause Concern?

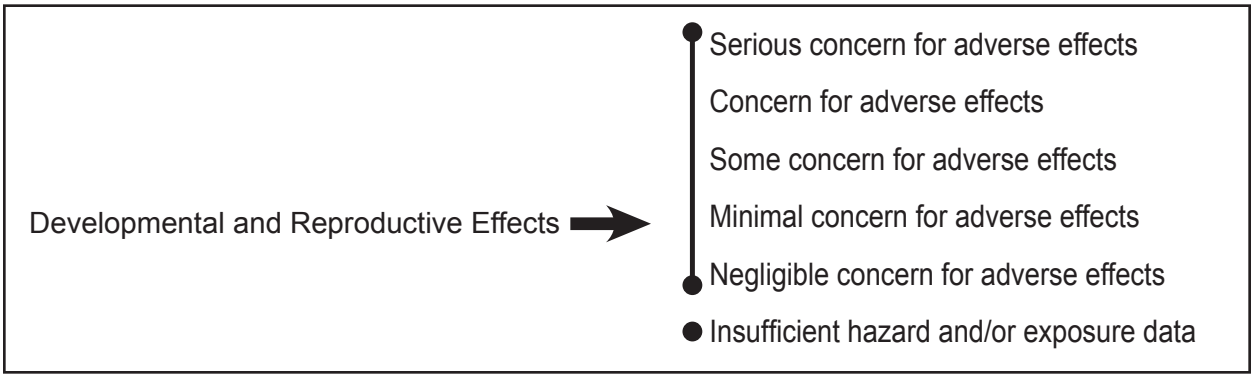
Possibly. Infants fed soy formula consume up to 8 mg/kg bw/day of total genistein. A 5 kg infant would consume up to 40 mg/day of total genistein. Blood levels of total genistein in infants on a soy formula diet are in the same range as those reported in young rats on a genistein-containing diet that produced adverse developmental effects. Considering the similarity in blood levels of total genistein between the rat study in which adverse effects were reported and human infants on soy formula, as well as the effects on the reproductive system of male marmosets fed soy formula as infants, there are presently not sufficient data from human studies to dismiss the possibility of subtle or long-term adverse health effects.

The NTP expresses minimal concern for adverse effects in neonates and infants who may consume up to 8 mg/kg bw/day of total genistein in soy formula.

This level of concern **(Figure 3)** is higher than that expressed by the expert panel and is based on evidence from genistein toxicity studies in rats and a study of total genistein blood levels in infants consuming soy formula. The blood concentration of total genistein in these infants was approximately the same as the blood level in young rats at weaning (21 days old). In the rat study, some adverse effects observed after birth (changes in anogenital distance, low birth weight) were most likely due to *in utero* exposure, as very little genistein is transferred to the pups through the milk. Other adverse postnatal developmental/reproductive effects such as early vaginal opening, delayed testicular descent, and abnormal estrus cycling were noted in older animals, when levels of genistein in the circulation may have been greater than at weaning. In addition, infant marmosets fed soy formula had low testosterone levels at about 40 days of age. As adults, these same animals had normal testosterone levels but higher testis weights and altered testis structure. Based on these findings, the NTP concludes that the possibility of subtle or long-term health effects of soy formula in humans cannot be dismissed. While the possibility of adverse effects of soy formula on human reproduction or development has not been adequately studied, no such effects have been reported after more than 40 years of soy formula use in the United States.

These conclusions are based on information available at the time this brief was prepared. As new information on toxicity and exposure accumulates, it may form the basis for either lowering or raising the levels of concern expressed in the conclusions.

Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to soy formula*



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NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Genistein. *<http://cerhr.niehs.nih.gov/chemicals/genistein-soy/genistein/genistein-eval.html>*