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 Genistein

INTRODUCTION

Genistein is a phytoestrogen found in some legumes and other edible plants, especially soybeans. Phytoestrogens are non-steroidal compounds with estrogen-like activity that occur naturally in some plants. In plants, nearly all genistein is linked to a sugar molecule and this genistein-sugar complex is called genistin. Genistein and genistin are found in many food products, especially soy-based foods such as tofu, soy milk, and soy infant formula, and in some over-the-counter dietary supplements. Soy formula is fed to infants as a supplement or replacement for human milk and as an alternative to cow milk formula. 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 women of reproductive age, and
(3) public concern for effects on infant or child development.

This monograph includes the NTP Brief on Genistein, a list of the expert panel members (Appendix I), the expert panel report on genistein (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 genistein to adversely affect human reproduction or development. Those interested in reading this monograph may include individuals, members of public interest groups, and staff of health and regulatory agencies.

The NTP Brief presents the NTP's interpretation of the potential for exposure to genistein 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 genistein provided in the expert panel report, the public comments on that report, and additional scientific information published following the public meeting of the expert panel.

NTP BRIEF ON GENISTEIN

What is Genistein?

Genistein is a naturally occurring chemical found in some legumes, especially soybeans. The chemical structure is given below **(Figure 1a)**. About 99% of genistein in soybeans is chemically bound to a sugar molecule and, in this form, is called genistin (**Figure 1b**).

Figure 1a.
Chemical structure of genistein

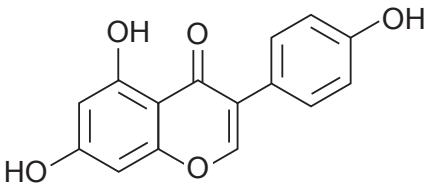
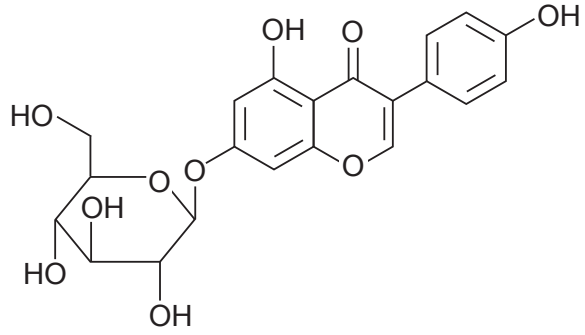


Figure 1b.
Chemical structure of genistin



When soy products or other genistein-containing foods are consumed, the sugar molecule is cleaved to convert genistin to genistein. Genistein is absorbed in the gastrointestinal tract and the body then binds the genistein to another molecule such as glucuronic acid. In human blood, as in the soybean, as much as 99% of absorbed, circulating genistein is bound to another molecule. Unbound genistein is the biologically active form. For the sake of simplicity, this brief will refer to free genistein (not bound to a sugar or other molecule), bound genistein (bound to a sugar or other molecule), and total genistein (the sum of free genistein and bound genistein).

Fermented soy foods, such as miso and tempeh, may contain up to 40% free genistein; non-fermented soy products such as tofu or soy flour may contain up to 4% free genistein. Soy infant formulas provide a source of genistein exposure in infants, primarily in the form of bound genistein.

Although it does not have the chemical structure of an estrogen, free genistein has estrogen-like activity. Because it is a chemical with estrogenic properties derived from plants, it is classified as a phytoestrogen. Genistein is the primary phytoestrogen in soy-derived foods. Other phytoestrogens such as daidzein and glycitein are also present at lower concentrations in soy products.

How Are People Exposed to Genistein?

People are exposed to genistein mainly through consumption of foods derived from legumes, especially soy-based foods such as tofu, soy milk, soy flour, textured soy protein, tempeh, miso, and soy infant formula, and in some over-the-counter dietary supplements and some products marketed for the treatment of menopausal symptoms. Lentils, peas, kidney beans, peanuts, chickpeas, broccoli, cauliflower, and barley meal also contain genistein, but at much lower concentrations than soy products.

Soy products are reported to be present in approximately 60% of processed foods in the United Kingdom. For the United States, no information was located on the production volume of genistein or the percentage of processed foods containing genistein or soy products. In the United States, sales of soy-based products have increased over the years, with the Soyfoods Association of America reporting soy sales increasing from $852 million in 1992 to $4 billion in 2003.

No information is available on the occurrence of genistein in the environment. No information was located on occupational exposures associated with manufacture, packaging, or distribution of genistein or soy products.

*Can Genistein Affect Human Development or Reproduction?**

*Possibly***.** While adverse reproductive and developmental effects have been observed in laboratory rats exposed to free genistein, such effects have not been studied in humans (**see Figures 2a and 2b**). The effects in rodents occurred at free genistein dose levels far above levels consumed by the general population. Humans are exposed to total genistein primarily through consumption of soy-based food products; thus, exposures vary across populations depending on the consumption of such products. Total genistein intakes average 0.014–0.14 mg/kg bw/day in the US

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

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

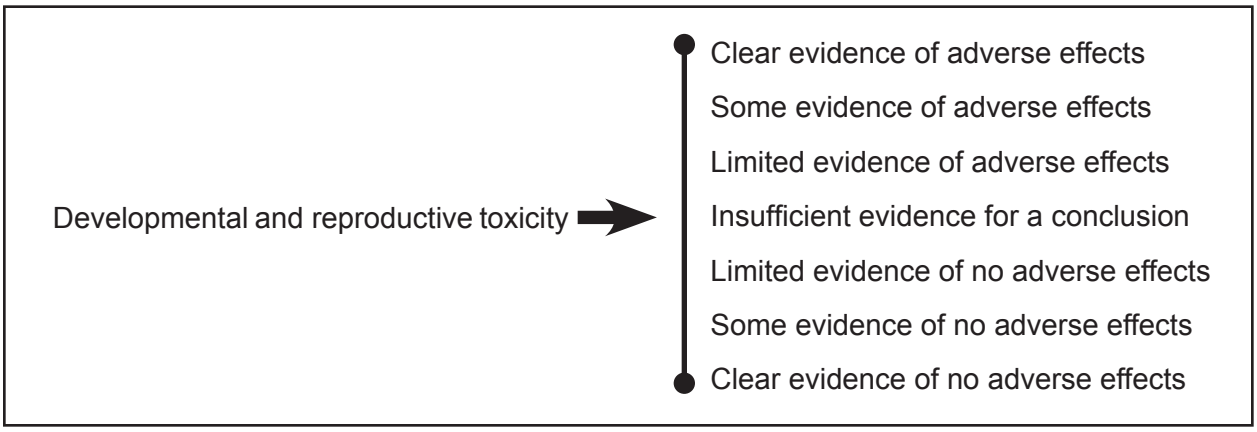
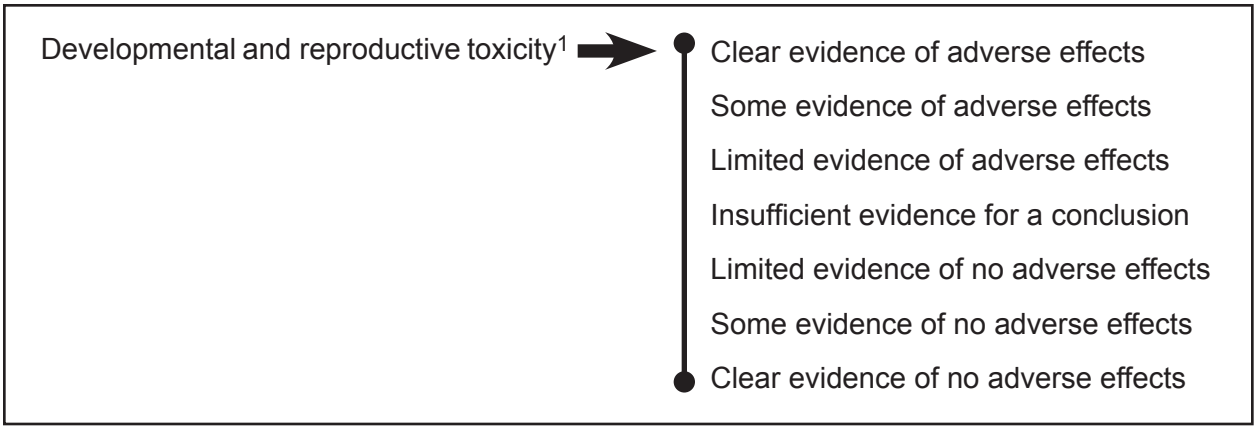


Figure 2b. *The weight of evidence that genistein causes adverse developmental or reproductive effects in laboratory animals*



[1]Manifested as decreased litter size and transient decreased pup weight; decreased anogenital distance in males and females; decreased age at vaginal opening and abnormal estrous cyclicity and increased age at testicular descent in rats.

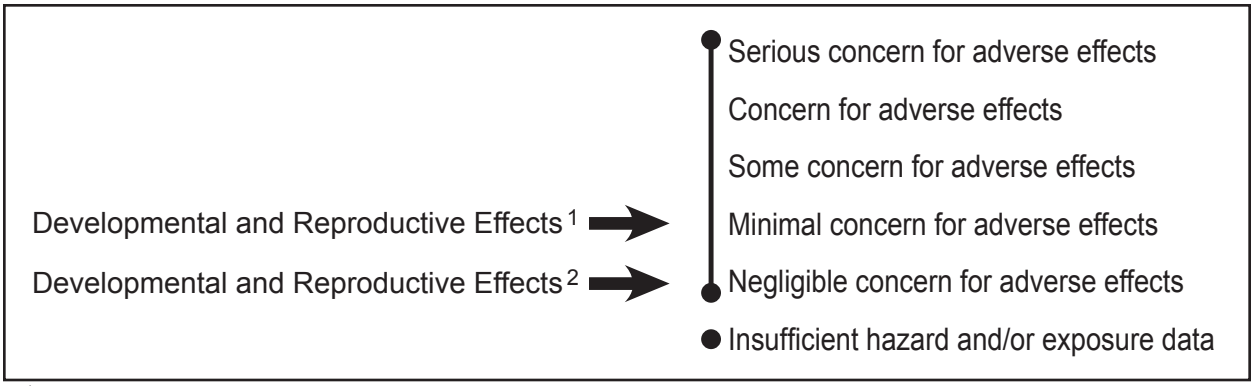
population while in Japan, the general population consumes 0.21–0.43 mg/kg bw/day. Since adverse effects of free genistein in rodent studies were not observed at levels below 35–44 mg/kg bw/day, it is unlikely that the general population would consume sufficient daily amounts of total genistein to cause adverse reproductive and/or developmental effects. The group with the highest exposure to total genistein is infants fed soy formula. They consume about 1–8 mg/kg bw/day. Blood levels of total genistein in infants fed soy formula are very similar to the blood levels observed in rats given doses of free genistein that induce adverse developmental effects. Because of these similarities in blood levels, the possibility that adverse effects might occur in humans cannot be dismissed (**see Figure 3**).

Supporting Evidence

The expert panel report (Appendix II) provides details and literature references regarding human exposures and studies on the possible reproductive and developmental toxicity of genistein. Although there were no human studies evaluating the effects of prenatal and childhood exposures to free genistein, experimental animal data relevant to the assessment of potential human hazard were available for evaluation. The expert panel evaluated reproductive and developmental toxicity studies in laboratory animals following oral exposure during gestation, lactation and into adulthood. Reproductive toxicity was observed in a multigenerational reproductive toxicity study in rats given free genistein in the diet at doses of 35 mg/kg bw/day and 44 mg/kg bw/day in male and female rats, respectively. These findings are described below.

The National Toxicology Program conducted a multigenerational reproductive toxicity study in rats given free genistein in the diet. In that study, rats were given free genistein continuously in the diet for three generations. Animals were bred at 10–12 weeks of age and exposed to free genistein in the feed through gestation and up to postnatal day 140 (F_0, F_1, F_2 generations). The F_3 generation animals were only exposed to free genistein indirectly throughout gestation and lactation. The next generation (F_4 animals) was from parents that had only been exposed to genistein prior to weaning and did not consume free genistein in their diet after they were weaned. Concentrations of free genistein in the feed were 0, 5, 100, or 500 ppm which resulted in ingested doses of approximately 0, 0.3, 7, and 35 mg/kg bw/day for males and 0, 0.4, 9, and 44 mg/kg bw/day for females. Animals were monitored for reproductive endpoints throughout the study. Genistein treatment did not show detectable effects on mating, fertility, or duration of gesta-

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



[1]For infants on soy formula
[2]For adults, children and pregnant or lactating women

tion. Ovarian follicle counts were not affected and there were no detectable effects on sperm parameters. An adverse effect level of 35 mg/kg bw/day (male) and 44 mg/kg bw/day (female) for reproductive/developmental toxicity of free genistein was identified based on decreased anogenital distance in males and females, abnormal estrous cyclicity, decreased age and body weight at vaginal opening (a sign of puberty), increased incidence of undescended testes, reduced litter size, and a transient decrease in body weight in animals continuously exposed.

In a study of 4-month old male infants fed soy formula (see Table 10, Setchell et al., expert panel report), serum levels of total genistein averaged 684±443 ng/ml (±standard deviation). Free genistein was not measured in this study. However, an estimate of the amount of free genistein based on the adult level of 1% of total genistein in the circulation would be 6.8 ng/ml. These concentrations of total and free genistein are approximately the same as serum concentrations at which developmental effects were observed in rat offspring. For example, in the rat multigenerational reproductive toxicity study described above, at the adverse reproductive effect level of approximately 40–50 mg/kg/day free genistein in the feed, the serum concentration of total genistein was determined in the pups at weaning (postnatal day 21). Serum concentrations of total genistein were 564±176 ng/ml for males and 505±81 ng/ml for females (see Table 13, Chang et al., expert panel report). In this study, the amount of free genistein in the blood was not determined. However, other studies have measured total and free 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). These studies reported that approximately 30%, 16%, and 7% of free genistein was in the blood of fetal, post natal day 7, and post natal day 21 rats, respectively. Thus, evidence that total genistein is present at similar blood concentrations in rats exhibiting adverse developmental effects and in infants fed soy formula leads the NTP to conclude that a higher level of concern is warranted than that expressed by the expert panel.[1]

In a paper published after completion of the expert panel report (Dolinoy et al.), effects of dietary genistein on DNA methylation were examined. The mouse strain used expresses a gene that leads to yellow coat color and obesity. The degree of methylation of a DNA region controlling expression of this gene inversely correlates with these phenotypes, i.e., less methylation would yield a yellower, heavier mouse. DNA methylation of this region was measured in the offspring at weaning and coat color and body weight were assessed. The dams and pups had access to a phytoestrogen-free diet supplemented with free genistein at 250 mg/kg of feed during pregnancy and lactation. Food consumption of the dams and/or serum levels of total genistein were not determined. However, this level of exposure probably exceeds that of the general population, even those with a high soy intake. One group that may experience a similar exposure to genistein is infants consuming soy formula.

The authors found that free genistein supplementation shifted the coat color of the mice towards a darker color and increased the proportion of mice with normal body weight. These changes were proportional to the increase in methylation of a promoter region of this gene. These results provide evidence that consumption by mice of high levels of free genistein during pregnancy and lactation affects DNA methylation, gene expression, and development in the offspring.

[1]One member of the expert panel expressed the opinion that the level of concern should be higher than that agreed to by the other panelists.

Should Exposures to Genistein Cause Concern?

Adults

No. In a reproductive toxicity study in rats, the lowest dose level of free genistein in feed that produced adverse effects was 35–44 mg/kg bw/day. A 70 kg adult would need to consume 2450 to 3080 milligrams of genistein per day to achieve this exposure level. The most highly exposed human population studied consumed approximately 30.1 mg/day of total genistein, which is approximately 1% of the level of free genistein that leads to adverse effects in rodents. Although more data comparing the absorption, metabolism, and distribution of total genistein in humans and rats would allow a more direct comparison between species, it is very unlikely that adult humans could consume sufficient amounts of total genistein to cause adverse reproductive and/or developmental effects. Further, when rats were given doses of free genistein that produced blood levels similar to those in adult humans, there were no effects on ovarian follicle counts, sperm parameters, or on the capacity of these rats to reproduce.

Pregnancy and Lactation

Probably not. It is unlikely that adults could consume sufficient amounts of genistein to cause adverse reproductive and/or developmental effects (see **Adults** above). Further, although adverse developmental effects were observed in some rodent studies (**see Figure 2b**), no detectable effect was observed on mating, fertility, or gestation length.

Studies in lactating humans and rodents have shown that the concentration of genistein in milk is approximately one one-thousandth of the concentration of that found in blood. Therefore, in infants, a very low level of genistein would be ingested from breastfeeding.

Children

Probably not. As noted above, the lowest dose of free genistein associated with adverse effects in rats was 35–44 mg/kg bw/day. A 10 kg child would have to consume 350–440 mg/day free genistein to approximate this level, which is about 100 times more than the most highly exposed adult population, i.e., 0.43 mg/kg bw/day total genistein. There were no available data on genistein consumption in children 6 months–6 years of age. However, in the National Health and Examination Survey studies (2001–2002 and 1999–2000), total urinary genistein was measured in children 6–11 and 12–19 years of age. Urinary concentrations in these age groups did not differ from adult concentrations.

Infants

Possibly. Infants fed soy formula ingest 1–8 mg/kg bw/day of total genistein. Of this, approximately 1% is free genistein. Thus, consumption of free genistein is estimated to be 0.01–0.08 mg/kg bw/day. A 5 kg infant would ingest 0.05–0.40 mg/day of free genistein. Although this amount is far less than that resulting in adverse effects in experimental animals, blood levels of total genistein in infants on a soy formula diet are approximately the same as those reported in rats when adverse effects occur. Considering this similarity in blood levels, there are presently not adequate data from human studies to dismiss the possibility of subtle or long-term adverse health effects.

The NTP concurs with the CERHR Genistein and Soy Formula Expert Panel that there is negligible concern for adverse reproductive and developmental effects from exposure of adults in the general population to free genistein.

Results from rodent studies provide evidence that free genistein in the diet causes developmental

and/or reproductive effects at consumption levels above 35 mg/kg bw/day. Because the maximum reported adult human consumption of genistein was approximately one one-hundredth of this amount, it is unlikely that amounts consumed by humans would cause harm.

Results from rodent studies provide evidence that free genistein in the diet causes developmental and/or reproductive effects at consumption levels above 35 mg/kg bw/day. Because the maximum reported adult human consumption of genistein was approximately one one-hundredth of this amount, it is unlikely that amounts consumed by humans would cause harm.

The NTP expresses minimal concern for adverse effects in neonates and infants who may consume up to 1 – 8 mg/kg bw/day of total genistein contained in soy formula.

This level of concern is higher than that expressed by the expert panel and is based on evidence from toxicity studies in rats and from blood level studies in infants consuming soy formula. Because the blood concentrations of total genistein in these infants was approximately the same as the blood levels of total genistein in rats receiving doses of free genistein that produced adverse reproductive or developmental effects, the possibility of subtle or long-term health effects in humans cannot be dismissed. While the possibility of such adverse effects on human reproduction or development has not been adequately studied, no such effects have been reported in humans after more than 40 years of soy infant formula use in the United States.

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

REFERENCES

Dolinoy DC, Weidman, JR, Waterland, RA, and Jirtle RL (2006). Maternal genistein alters coat color and protects the A^{vy} mouse offspring from obesity by modifying the fetal epigenome. Environmental Health Perspectives 114:567–572.