COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

First draft statement on the potential risks from high levels of soy phytoestrogens and soy products in the infant diet

Introduction

1. The COT has been asked to consider aspects related to the toxicity of chemicals in the infant diet, in support of a review by the Scientific Advisory Committee on Nutrition (SACN) of Government recommendations on complementary and young child feeding. An initial paper (TOX/2012/03), highlighting some of the areas requiring consideration was discussed by the COT in February, 2012, and members agreed that there was a need for more detailed consideration of soy phytoestrogens as a substantial amount of new evidence has become available. A discussion paper on soy phytoestrogens (TOX/2012/39) was presented to Members in December 2012. The minutes of the discussion are included in Annex A.

2. Annex B contains a draft COT statement summarising the available information and the Committee's provisional conclusions on soy phytoestrogens.

3. This first draft statement addresses the points raised by Members during the discussion of the paper in December. Differences in metabolism, including species and developmental differences as well as clarification of the term "absorption" have been included. The key data from experimental animal studies have been tabulated and additional details of epidemiological studies provided as requested.

Questions on which the views of the Committee are sought

4. Members are invited to consider the following questions:

i. Do Members agree with the structure of the draft statement?

ii. Are there some aspects that need further elaboration, or could be shortened?

iii. Do Members agree that the current UK government advice that soy-based infant formula should only be used in exceptional circumstances to ensure adequate nutrition of infants is well supported by the scientific evidence?

iv. Do Members agree that it is not possible to set a health-based guidance value for soy isoflavones, or reference point to be used in risk characterisation?

v. Can Members advise on the conclusions they would like to see in the next draft?

Secretariat
March 2013

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

First draft statement on the potential risks from high levels of soy phytoestrogens and soy products in the infant diet

Section of the minutes of the COT meeting of 11 December 2012

Item 5: Review of potential risks from high levels of soy phytoestrogens in infant diet - TOX/2012/39

22. During the discussion of complementary and young child feeding at the COT meeting in February 2012, Members had requested that a review of soy phytoestrogens in infant diet be prepared, since a substantial new body of evidence had become available since the last COT report on phytoestrogens was published in 2003. Members were provided with a paper TOX/2012/39, summarising the more recent animal and human studies, and were asked to advise on whether there were concerns about the intake of soy-based products, which might indicate a need to revise the current UK Government advice on consumption of soy formula by infants.

23. Members considered that the term “absorption” was not completely accurate for isoflavones, due to the metabolism of glucones by the gut microflora, and suggested that this term needed to be revised or specifically defined for this context. Further clarification was required on observed differences in metabolism and the extent to which these represent species differences, or differences in development. Tabulation of key data would be useful – for example, relating to the potency of different isoflavones at different life stages, and their reproductive effects.

24. Further detail on the epidemiological studies was also required. It was noted that exposures were potentially in the range at which effects might be expected, but it was unclear whether such effects occurred in practice. In humans, the late fetal stage through to infancy would be a critical period for development of reproductive function.

25. It was noted that not all observed effects could be attributed to oestrogenicity – for example, effects of isoflavones on the thyroid, and the allergenicity of soy.

26. The Committee agreed that based on the currently available evidence, it was not possible to establish a health-based guidance value for soy isoflavones or a reference point to be used in risk characterisation. Major differences between species limited the capacity to interpret animal studies. Human studies provided limited exposure data concerning only a few compounds present in soy, and a role of other chemicals could not be excluded.

27. Members noted that soy-based infant formula and soy-based weaning foods were the main sources of isoflavone exposure in infants, and that it was plausible that levels could result in biological effects, although it was unclear whether these would be considered adverse. Exposure from breast milk and cows' milk formula was lower than from soy products and unlikely to be a concern.

28. Members agreed that the current UK Government advice that soy-based infant formula should only be used in exceptional circumstances to ensure adequate nutrition of infants was well supported by scientific evidence. However, those studies looking at allergic reactions and possible long-term effects associated with soy consumption by infants should be explored further.

29. A need was identified for cohort studies comparing infants fed soy-based infant formula and cows' milk formula in populations with a higher prevalence of soy consumption (e.g. Israel, Japan). It was noted that an adequately powered study would be difficult to perform in the UK.

30. A draft statement on soy phytoestrogens in infant feeding would be prepared for discussion at a future meeting. Consideration of species differences, and of the epidemiological data would be important elements.

These minutes are available at:
http://cot.food.gov.uk/pdfs/cotfinalmins11dec2012.pdf

Secretariat
March 2013

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

First draft statement on the potential risks from high levels of soy phytoestrogens and soy products in infant diet

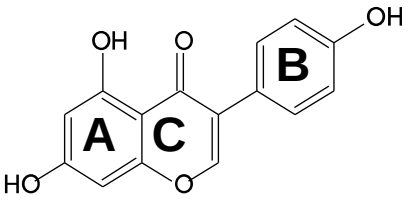
Background

1. Phytoestrogens are chemicals of plant origin that have been shown to influence biological functions mainly due to their structural similarities to oestrogens, and ability to bind to oestrogen receptors (ERs). The largest group of phytoestrogens is formed by flavonoids, which can be further divided into three subclasses, coumestans, prenylated flavonoids and isoflavones. The aim of this statement is to provide an overview of the potential risks from soy phytoestrogens and soy products in the infant diet. Soy-based infant formula and weaning food products containing soy are the main source of phytoestrogen exposure in newborns and infants. Soy formula is produced using soy protein isolate: a particularly rich source of three isoflavones: genistein, daidzein and to a lesser extent glycitein. The content of isoflavones in soy products has been shown to be up to 4 mg of isoflavones per gram fresh weight (Fletcher, 2003). The statement summarises new literature investigating possible health effects resulting from soy isoflavones exposure of infants that has become available since the last COT report (2003)[1] , and considers the implications for the introduction of soy products into the infant diet. This statement focuses on the main phytoestrogens detected in soy: genistein, daidzein and glycitein.

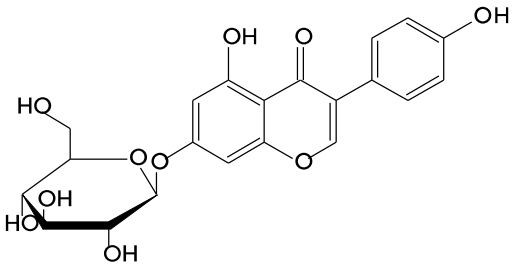
2. The isoflavones, genistein, daidzein and glycitein (Figure 1) share a common structure consisting of two aromatic benzene rings linked through three carbons forming part of an oxygenated heterocyclic ring. The phenolic and hydroxyl moieties (and the distance between them) are key structural similarities between isoflavones and 17β-oestradiol (Figure 2), which allow them to bind to ERs. Numerous studies have indicated that genistein is the most active oestrogenic soy isoflavone (NTP, 2010).

3. Soy isoflavones found in foods exist mainly as carbohydrate conjugates (glycosides), the major group being the glucose conjugates (glucosides), genistin (Figure 1), daidzin and glycitin. When β-glucosidic bonds of glucosides are hydrolysed, the biologically active aglucone forms are produced. Bacterial hydrolysis can significantly increase content of aglucones in fermented soy-based food products such as tofu[2] or tempeh[3]. In soy infant formula aglucones have been reported to constitute 3.2 – 5.8% of the total isoflavones (Chen and Rogan, 2004).

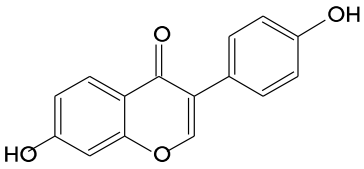
[1] COT Report – Phytoestrogens and Health (2003). Available at: http://cot.food.gov.uk/pdfs/phytoreport0503
[2] Tofu, also called bean curd, is made by coagulation of soy juice and then precipitation of soy curd into blocks.



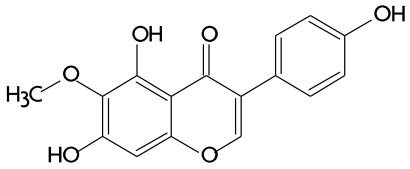
Genistein



Genistin (genistein glucoside)

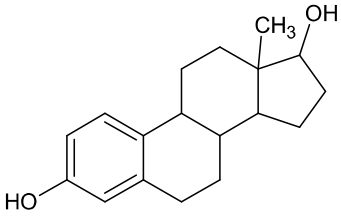


Daidzein

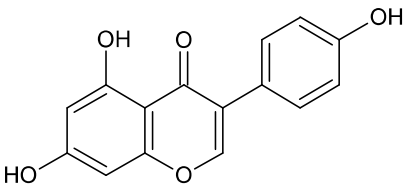


Glycitein

Figure 1. Chemical structures of the isoflavone aglucones genistein, daidzein and glycitein, and the glucoside genistin.
A three-carbon chain forms a closed ring (Ring C) with one of the benzene rings (Ring A and B).



17β-oestradiol



Genistein

Figure 2. The similarity of the structure of 17β-oestradiol and genistein

[3] Tempeh is a fermented soy product made from whole soy beans, having a high content of protein, dietary fibre and vitamins

Current UK Government recommendations in relation to infant diet

4. Based on the COT (2003) report, the SACN concluded that there was no substantive medical need for, nor health benefit arising from, the use of soy-based infant formulas[4]. The Department of Health's Chief Medical Officer in 2004 advised doctors that soy-based infant formulas should not be used as the first choice for the management of infants with proven cows' milk sensitivity, lactose intolerance, galactokinase deficiency and galactosaemia and they should only be used in exceptional circumstances to ensure adequate nutrition (DH, 2004).

Recommendations in other countries

5. The US Food and Drug Administration (FDA) in 1999 reviewed available human studies and gave food manufacturers permission to use a health claim on food labels stating that a daily diet containing 25 g of soy protein may help reduce heart disease risk. There have been no changes in the FDA recommendations since then (personal correspondence with Dr Daniel Doerge, FDA). A minimal concern for possible adverse developmental health effects in infants consuming soy-based infant formula was expressed by the members of the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) expert panel (McCarver *et al.*, 2011).

6. In Israel, France and Germany consumption of soy-based infant formula was recommended only in exceptional medical indications following medical advice, such as galactosaemia, hereditary lactase deficiency and secondary lactose intolerance or preferences for a vegetarian diet (Berger-Achituv *et al*., 2005; AFSSA, 2005; BfR, 2007). The lack of proven health benefits or advantage over breastfeeding and cows' milk-based formula as well as possible health risks were also highlighted by the American Academy of Pediatrics (AAP) (Bhatia and Greer, 2008).

Absorption[5], distribution, metabolism and excretion

7. The 2003 COT report reviewed the absorption, distribution, metabolism and excretion (ADME) human studies carried out and published up to 30th April 2002. The report summarised that "*isoflavones are mainly ingested as glucosides, which undergo hydrolysis most probably in the small intestine through the action of β-glucosidase enzymes associated with the intestinal mucosa and in the lower bowel by the gut microflora. The deglucosylated (aglucone) compounds may be further metabolised by the gut bacteria and/or absorbed, with genistein being converted to the hormonally inert p-ethyl-phenol and daidzein reduced to the oestrogenically active isoflavone equol and the non-oestrogenic O-demethylangolensin (O-DMA). Aglucones are more readily absorbed due to their higher hydrophobicity and lower molecular weight. Once absorbed, these compounds are rapidly and extensively re-conjugated (largely with glucuronic acid or sulphate) and excreted in the bile or urine.*

[4] Scientific Advisory Committee on Nutrition response to the COT Working Group on Phytoestrogens draft report on phytoestrogens and health, 2003. Available at: http://cot.food.gov.uk/pdfs/2003-03.pdf

[5] Absorption occurs primarily after isoflavones are hydrolysed to their aglucones. Therefore, in this statement the term absorption refers to the aglucones and their metabolites rather than the glucones.

Biliary conjugates are hydrolysed by the gut bacteria and/or excreted in the faeces or further metabolised and/or re-absorbed or degraded. There is limited information on how phytoestrogens are handled in the newborn and infants. The pharmacokinetics of absorption in the neonate is unclear but it is likely to differ considerably from that of the adult, particularly as the gut microflora in neonates is not fully developed" (COT, 2003).

8. A few more details concerning isoflavone metabolism were also discussed in the NTP-CERHR report (2010), noting that the principal hydrolysis and Phase I metabolism of glucosides and aglucones within the gut include reduction, deoxygenation, hydroxylation and ring cleavage. Aglucones and their metabolites undergo pre-systemic metabolism by glucuronidation and sulphation, in the intestinal cells and, to a greater extent, in the liver. Glucuronidation is the major route of conjugation. The conjugated compounds are then transported to tissues and excreted in urine or bile (reviewed in NTP, 2010). A simplified schematic representation of isoflavones metabolism is presented in Figure 3.

9. The COT report and the NTP-CERHR report assume that there is no absorption of isoflavone glucosides through the gut wall in either animals or humans. Similar conclusions were also made in studies performed in intestinal gut segments isolated from rats (Steensma *et al.,* 2004). However, some authors claim that there is a partial absorption of isoflavone glucosides in their intact form either by diffusion (Andlauer *et al*., 2000) or by an active transport by a carrier system, e.g. via the sodium dependent glucose transporter (SGLT1) (Gee *et al.*, 1998; Kwon *et al*., 2007).

10. In humans, the maximum concentrations of genistein and daidzein occur in plasma approximately 5.5 and 7.4 hours after administration. Equol, the reductive metabolite of daidzein, is detected in plasma 12 – 36 hours following oral administration of isoflavones (Setchell *et al*., 2003; Setchell and Clerici, 2010). Formation of equol depends exclusively on intestinal bacterial metabolism (Setchell *et al*., 2002) and only 30 to 50% of human adults have been shown to be equol producers (NTP, 2010). Specific bacteria responsible for equol production in humans have been identified e.g. *Lactobacillus sp*., *Enterococcus faecium*, *Bifidobacterium sp*. or *Finegoldia magna* (reviewed by Setchell and Clerici, 2010). Newborns and infants exclusively fed soy infant formula from birth are lacking developed microflora necessary for equol production (Setchell *et al*., 1997 and 1998).

11. Equol, unlike genistein and daidzein, has a chiral centre and can occur as two diastereoisomers S- and R-equol. S-equol was established as the only enantiomer circulating in human plasma and urine. S-equol has a high affinity for ER β (the R form is relatively inactive) and is a more potent oestrogen than oestradiol, therefore has the greatest potential for physiological effects (Setchell *et al*., 2005).

12. The bioavailability of genistein following oral administration of genistin and genistein has been investigated in neonatal mice. The dose-adjusted area under the curve (AUC) for levels of genistein measured in serum after oral exposure to genistin was 83% (total free plus conjugated genistein) and 48% (as free genistein) of that for subcutaneous genistein. Following oral dosing of genistein the bioavailability was lower, at 12 and 15%, respectively. The authors also found that approximately 20%

more oral genistin was needed to result in similar oestrogenic activity in female mouse neonates (increase in uterine wet weight) as that resulting from sc genistein, which is consistent with the bioavailability data (Jefferson *et al*., 2009). Higher oral bioavailability of genistin when compared to genistein was also reported in rats by Kwon *et al.* (2007). The authors explained this by different absorption and metabolic behaviours of both compounds, poor water solubility of genistein and its fast absorption, and the assumption that genistin can be absorbed in both its intact and aglucone forms (Kwon *et al*., 2007).

13. In humans, studies investigating bioavailability of isoflavones consumed as glucosides and aglucones provided conflicting results. Studies reviewed in the COT report suggested that increased bioavailability of isoflavones can be expected when they are ingested as aglucones, as in fermented foods (COT, 2003), In contrast, greater availability of daidzein following ingestion of the glucoside compared to the aglucone was shown by Rufer *et al.* (2008). However, another study showed that isoflavone aglucones were absorbed faster and in larger amounts comparing to their glucosides (Izumi *et al.*, 2000). Lack of differences in bioavailability between both forms has also been reported (Zubik *et al*., 2003).

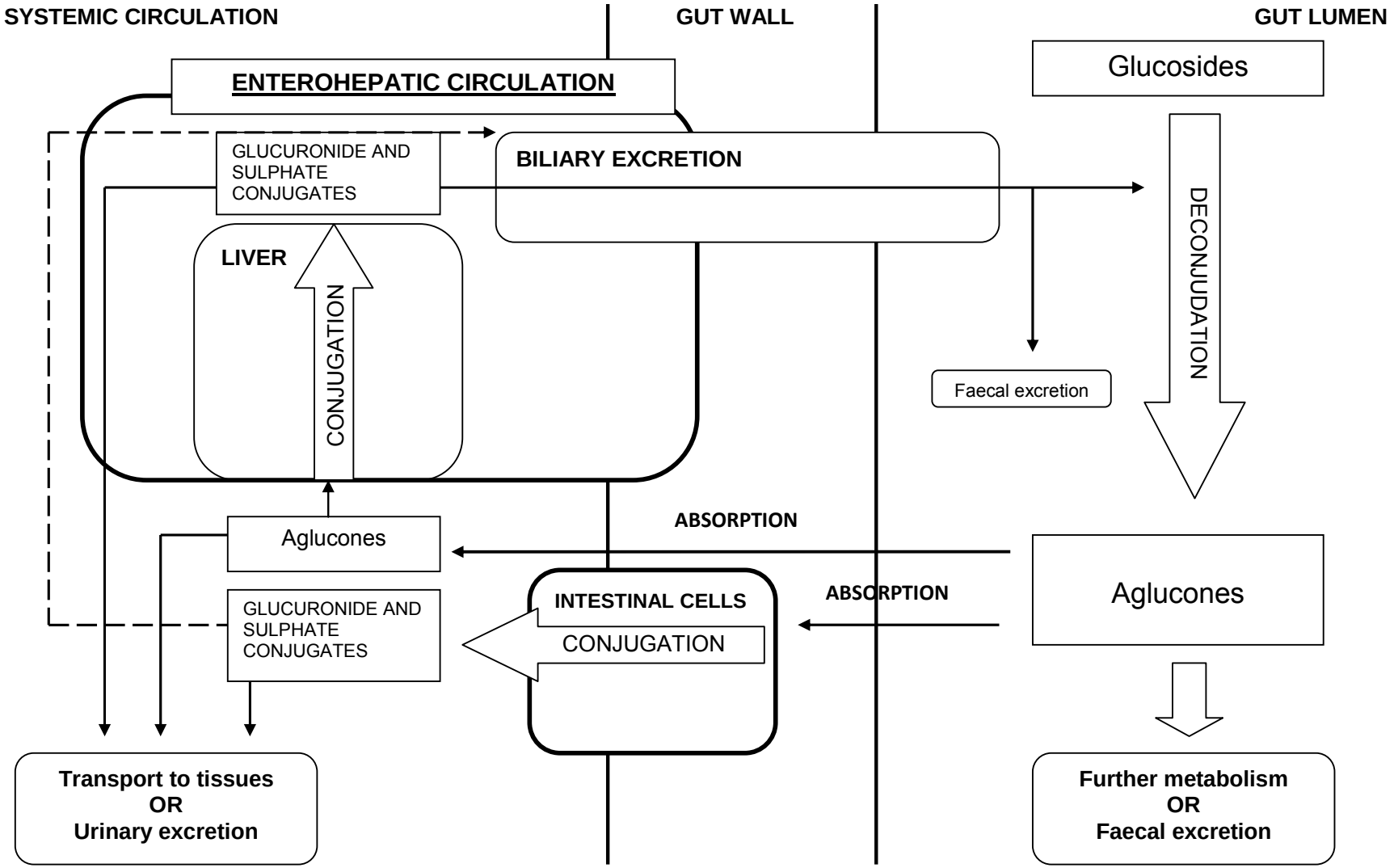


Figure 3. Schematic representation of isoflavone metabolism

Modulation of absorption and metabolism of isoflavones

14. The COT report (2003) stated that "*the gut microflora play a crucial role in determining the absorption, metabolism, re-absorption (enterohepatic circulation), degradation and excretion of ingested isoflavones and their metabolites. Data indicate considerable inter-individual variation in the pharmacokinetic and metabolic handling of ingested phytoestrogens. Such differences may be largely attributed to an individual's unique gut microflora, which is influenced by factors such as diet, particularly fibre content, and intestinal transit time, hygiene, antibiotic use, bowel disease, stress, gut motility, gastric pH, mucin and bile secretion. Gender, age, genetics, food matrix and ethnicity may also be determining factors. An initial colonisation of the gut in infants is especially determined by factors such as the composition of maternal gut flora, the mode of delivery (conventional or caesarean birth), hygiene, environment and genetics. The influence of the diet is greater on the gut microflora of babies who were breast fed than those who were fed infant formula*" (COT, 2003).

Food matrix

15. Cassidy *et al*. (2006) observed that the type of food matrix affects the bioavailability of isoflavones in healthy adults. Three soy foods having different isoflavone composition: soy milk, textured vegetable protein (TVP) and fermented soy product – tempeh (containing a higher proportion of aglucones, approximately 50%) were studied. It was concluded that consumption of tempeh resulted in higher serum peak levels of genistein and daidzein, compared with TVP. However, isoflavones from soy milk were absorbed faster and peak levels were attained earlier than with the other soy foods (Cassidy *et al*., 2006). Another study reported no difference in bioavailability following consumption of miso soup and soy milk (Maskariniec *et al.*, 2008).

Pro- and prebiotics

16. Effects of supplementation of soy milk with probiotics[6], such as *Lactobacillus sp.* and *Bifidobacterium sp*, and prebiotics[7] (e.g. fructooligosaccharides, inulin, pectin or mannitol) were investigated *in vitro* by Yeo and Liong (2010). Prebiotics increased the viability of probiotics and enhanced β-glucosidase activity. As a result an enhanced bioconversion of glucosides to bioactive aglucones, especially genistin and malonyl genistin to genistein, was observed (Yeo and Liong, 2010). Another study showed that administration of *Lactobacillus sp* as a probiotic supplement in the form of capsules decreased urinary isoflavone excretion. The authors suggested that probiotics stimulated isoflavone deconjugation and/or inhibited degradation leading to increased circulating levels of isoflavones (blood levels not measured) (Cohen *et al*., 2007).

[6] Probiotics – live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (WHO/FAO, 2001-2002)
[7] Prebiotics – non-viable food components that confer a health benefit on the host associated with the modulation of the microbiota (FAO, 2001)

Age

17. Metabolism of isoflavones may vary with age. Although several studies investigating isoflavone distribution and metabolism in infants have been published since the previous COT review (COT, 2003), the amount of information on how these substances are handled in infants is still limited. Halm *et al.* (2007) reported that bioavailability of isoflavones was higher in school age children than in adults consuming the same diet (soy nuts). Cassidy *et al.* (2006) found that age and gender did not lead to significantly different results as pre- and postmenopausal women as well as men absorbed isoflavones from a range of different soy-rich foods in a similar manner. Children were not included in this study. Infants can effectively absorb isoflavones from breast milk, soy infant formula and food products containing soy and eventual differences when compared to adults are due to the maturity of the intestinal flora and/or larger intake when adjusted for body weight. They also have decreased ability to glucuronidate isoflavones due to their lower expression of uridine diphosphate (UDP)-glucuronosyltransferases activity (NTP, 2010).

Maturity of gut flora

20. The digestive system of the new born infant is immature and takes several weeks to develop. Initially, the gut is colonised by *Enterobacteria*, *Streptococci* and *Staphylococci* capable of oxidative metabolism. Subsequently, they are replaced by strictly anaerobic bacteria, such as *Bifidobacteria*, *Clostridia* and *Bacteroides*. However, it is unclear at what age infants will develop gut microflora fully capable of metabolism of isoflavones (COT, 2003). The main factors stimulating gut flora maturation process are: decreasing gut permeability (breast milk provides passive immunity - antigen specific IgA), colonisation with mother's bacteria during vaginal birth, maintaining acidic and dominated by *Bifidobacteria* gut flora for the first 6 weeks of life. In contrast, the pH of the gut flora in formula fed infants is higher and even one dose of formula given to the breastfed infant is believed to be able to permanently alter its gut flora (Catassu *et al.*, 1995). Bacterial β-glucosidase activity, associated with bacteria such as Lactobacilli, Bifidobacteria and Bacteroides, necessary for hydrolysis appears to be lower in infants than in adults and shows an age-dependent increase (NTP, 2010; Setchell *et al.*, 1998).

21. Maturity of gut flora related to age and type of food as a determinant of isoflavone uptake ability in infants and children was investigated by Franke *et al.*(2006). In a previous publication the authors had considered isoflavone glucuronides and sulphates (as present in breast milk of mothers eating soy) to be more available to the infant than the glucosides (as in soy food), which require mainly intestinal bacteria for hydrolysis (Franke and Custer, 1996). Although genistein and daidzein were reported to be found in infant urine samples, levels of equol were low or undetectable indicating limited biotransformation beyond the initial hydrolysis (Setchell *et al.*, 1998; COT, 2003). More recently, low isoflavone values were observed in body fluids of breastfed infants, and higher levels, exceeding those observed in adults eating soy products, were reported in weaning infants consuming tofu. The authors commented that this finding was probably due to the very low isoflavone dose, but also to the lower ability of the immature gut flora in breastfed infants to cleave glucuronide and sulphate conjugates for the production of aglucones required for isoflavone uptake relative to adults when adjusted to dose.

Infants consuming tofu were older and it is possible that their gut flora attained ability to hydrolyse β-glucosides efficiently (Franke *et al.*, 2006).

Distribution, metabolism and excretion

22. The COT report summarised that *"isoflavones and their metabolites are widely distributed within body fluids. In general, peak concentrations of daidzein and genistein are achieved within 5-8 hours after ingestion. Plasma concentrations of genistein and daidzein begin to rise within 2 hours of an ingested dose and can occur as early as 15 minutes after ingestion. It has been observed that a number of individuals exhibit more than one plasma peak, which probably reflects enterohepatic circulation of the isoflavones. The plasma half-lives for genistein and daidzein have been estimated at 5-8 hours. There is evidence of transfer of isoflavones and their metabolites to breast milk via the maternal diet and to the foetal compartment as concentrations similar to those in maternal plasma have been detected in umbilical cord plasma and amniotic fluid. However, definitive tissue distribution studies have not been performed in man"* (COT, 2003).

23. Isoflavones present in the mothers' diet can be transferred to babies via breast milk. Levels of isoflavones measured in urine samples of mothers and their babies were similar after the mothers consumed soy protein beverages (Franke *et al.*, 2006).

24. Excretion of isoflavones is rapid and occurs in approximately 24 hours. Hoey *et al*. found that 4-6 month old infants fed soy-based infant formula had significantly higher urinary concentrations of isoflavones compared to controls. Observed excretion of isoflavones and their effective absorption in infants suggested that the ability to hydrolyse glucosides to aglucones develops by or before 4-6 months of age. The majority of older infants, both those who had been fed soy formula in early infancy and those who were not, had similar percentages of isoflavone metabolites in their urine (Hoey *et al.,* 2004). In another study, genistein and daidzein were not detected in most blood or saliva samples obtained from breast milk and cows' milk-fed infants. The median urinary concentration of isoflavones in infants fed soy formula was 500 times higher than in the cows' milk-fed group. Equol was rarely detected (Cao *et al*., 2009), which is consistent with the findings of previous studies (Franke *et al.,* 2006; Setchell *et al.,* 1997).

25. Samples of amniotic fluid and blood, collected during pregnancy and at birth as well as blood samples from umbilical cord were tested for the presence of genistein and daidzein. Amniotic fluid samples from women pregnant with female fetuses, and who reported the use of soy products, were found to have significantly higher concentrations of both isoflavones compared to those with male pregnancies. There were no sex related differences in breast milk, cord serum and serum during pregnancy and at birth. The findings could not be explained by fetal weight as the male and female infants had similar birth weights. The authors suggested that there may be a different metabolic handling of isoflavones during fetal life among boys and girls (Jarrell *et al.*, 2012).

Species differences in isoflavone metabolism

26. Gu *et al*. compared the ability to produce equol from daidzein of women and experimental animals consuming soy protein isolate (SPI). Female monkeys had a serum profile of equol more similar to that of female Sprague-Dawley rats, than to that of women. Rats and monkeys appeared to have intestinal bacterial composition favouring equol biosynthesis, whereas equol was not detected in serum of women or pigs and genistein and daidzein comprised 88 and 91% of summed isoflavones, respectively. Similarly, in urine: the proportion of equol to total isoflavones (including metabolites) was 51 and 69% in monkeys and rats, and only 2 and 0% in pigs and women, respectively. Monkey and rat urine contained high levels of aglucones, whereas pigs and women excreted isoflavone mainly in the form of glucuronides, with <10% as aglucones. Thus pigs may be a better animal model than rats or monkeys for studying the effects of isoflavones in humans (Gu *et al*., 2006).

27. Circulating concentrations of unconjugated isoflavones in rodents and humans were also compared by Setchell *et al.* Based on the steady state percentages of unconjugated isoflavones the authors concluded that the capacity to conjugate isoflavones differs significantly between rats and mice, as well as between rodents and humans and that humans have a much higher capacity to conjugate isoflavones (Setchell *et al.*, 2011). The species differences in metabolism of isoflavones were acknowledged in the past as one of the main factors diminishing the relevance for using animals as research models (COT, 2003). Rats and monkeys have been shown to produce equol more effectively compared to pigs and humans, which show a similar metabolic profile (Gu *et al*., 2006; Setchell *et al*., 2002).

Hazard identification and characterisation

In vitro studies

28. *In vitro* experiments reviewed in the 2003 COT report showed that phytoestrogens could modulate the levels of sex hormone binding globulin (SHBG), inhibit enzymes involved in oestrogen biosynthesis and metabolism to modulate concentrations of endogenous oestrogens, and inhibit thyroid peroxidase activity to reduce the concentrations of thyroid hormones. Genistein was demonstrated to interact with topoisomerase II and protein kinases – enzymes involved in cellular proliferation and differentiation and inhibit human T-cell proliferation and interleukin-2 production (COT, 2003).

Genotoxicity

29. *In vitro* studies reviewed in the COT report indicated some genotoxic effects of various phytoestrogens. Genistein had been shown to induce DNA strand breaks, mutations and micronuclei (MN). It was also weakly mutagenic in bacterial and mammalian mutation assay. It was noted however, that the concentrations used were much higher than would be expected to be achieved *in vivo* following dietary exposure (COT, 2003).

30. Subsequently published studies investigating genetic toxicity for genistein *in vitro* have not detected mutagenicity in bacterial tests (McClain *et al.*, 2006; Yee *et al*., 2008). However, several positive results were observed in mammalian cells. McClain *et al*. (2006) reported mutations in mouse lymphoma cells. Clastogenic (genistein, daidzein) and aneugenic (daidzein, equol) activity was observed in a micronucleus assay with Chinese Hamster V79 cells (Di Virgilio *et al*., 2004). DNA strand breaks were induced by genistein in the Comet assay in cultured human lymphocytes (Ullah *et al*., 2009). There are limited data on genotoxic potential of isoflavones *in vivo*. Studies reviewed in the COT report suggested that genistein at dietary levels was not mutagenic (COT, 2003). This effect was confirmed by McClain *et al*. who observed a lack of mutagenicity *in vivo* in the micronucleus assay in mice and rats (McClain *et al*., 2006).

Animal studies

31. Animal studies performed before 2003 suggested that intake of isoflavones might produce oestrogenic effects, affect thyroid function, alter protein concentrations and structures in the brain (rodents), alter some parameters of immune function (rodents) as well as reproductive health (marmosets) during the neonatal stage. Although some animal studies indicated potential risks to humans overall the animal data provided conflicting results. The COT noted that human data were limited and the majority of scientific information was derived from experimental animal studies, mostly rodents. The extrapolation of such studies to humans was difficult due to inter-species differences in ADME, sexual development and reproduction, unknown oestrogenic responses in rodents; the use of much higher doses or subcutaneous route of administration (unknown influence of gastrointestinal and hepatic metabolism). Although non-human primates were of more relevance, especially when adverse health effects were evaluated, their use in laboratory testing was limited to a small number of studies, *inter alia* due to ethical considerations (COT, 2003).

Effects of phytoestrogens on fertility and development

32. A number of animal studies have been conducted to address potential adverse health effects resulting from exposure to isoflavones in early life. Body weight, onset of puberty, changes in adipose tissue and reproductive organs were among measured endpoints. Observed effects in various studies investigating oestrogenic, reproductive and developmental effects appeared to be inconsistent and are detailed in Table 1. The lowest dose of genistein of 0.42 mg/kg bw/day administered through the diet to pregnant and lactating rats induced increased thymus masses and subpopulations of T cells in the spleen as well as reduced testosterone concentrations in the offspring (Klein *et al*., 2002). The other predominant health effects observed in animal studies included increased uterine weights; increased body weights and dose-related increase in the number of multi-oocyte follicles (MOFs). Health effects observed after s.c. administration of genistein directly to offspring included dose-related increase in MOFs (NOAEL = 5 mg/kg bw/day; LOAEL = 50 mg/kg bw/day) and uterine weights (NOAEL = 12.5 mg/kg bw/day; LOAEL = 20 mg/kg bw/day). Following oral administration there was an

increase in uterine weights (LOAEL = 25 mg/kg bw/day); dose-dependent increase in percentage of MOFs (NOAEL = 6.25; LOAEL = 12.5); delayed vaginal opening (LOAEL = 37.5 mg/kg bw/day) and ovarian cycle abnormalities.

Phytoestrogens and immunosuppression

33. Genistein has been reported to have both inhibitory and stimulatory effects on the immune system in rodents. Several animal studies were reviewed by Cooke et al., who reported following health effects: altered thymic size, decreases in the delayed-type hypersensitivity (DTH) response (decreased cell infiltration; reduced number of $CD4^+$ and $CD8^+$ T cells in popliteal lymph nodes) to sensitising chemical comparing to controls, increased basal splenocyte proliferation and decrease in the production of the cytokine IFN-γ(Cooke *et al.,* 2006).

Phytoestrogens and cancer

34. The National Toxicology Program (NTP) has conducted carcinogenicity studies in which animals were exposed to genistein from the time of conception, through weaning and then for up to two years, with genistein administered in their feed (5, 100, or 500 ppm). There was no carcinogenic activity of genistein in male rats, whereas in female rats the incidences of adenoma or adenocarcinoma of the mammary gland and pituitary gland adenoma and carcinoma were increased (NTP, 2008b).

35. The influence of *in utero* exposure in rats to isoflavones in cows' milk on subsequent susceptibility to induction of mammary tumours by 7,12-dimethylbenz[a]anthracene (DMBA) was investigated by Nielsen *et al*.. Maternal intake of cows' milk containing a low level of isoflavones (101 ± 3.3 ng total phytoestrogens/mL; 1.7 ± 0.6 ng daidzein/mL), resulted in increased circulating oestradiol and IGF-1 levels in the offspring. In contrast, intake of cows' milk containing a high level of isoflavones (429 ± 11.9 ng total phytoestrogens/mL; 5.8 ± 0.3 ng daidzein/mL) had no effect on circulating oestradiol and IGF-1 levels but significantly increased DMBA-DNA adducts in the mammary gland and the number of mammary tumours per animal (Nielsen *et al*., 2011).

Table 1. Health effects reported in animal studies following exposure to isoflavones

Treated population	Dose/ compound [mg/kg bw/day] unless otherwise shown	Route of administration/ duration	Endpoint	Observed health effects in the offspring	Reference
Oestrogenic effects					
Mice					
12-13 week old C57/BL6 female mice	Genistein 20, 80 or 200	Daily sc injections for 21 days (starting a week after ovariectomy)	Effects of injected and dietary genistein on adipose tissue: - body weight - fat pad weight - adipocyte circumference	• ↓ in adipose tissue weight and slightly ↓ body weights at higher doses at 80 and 200 mg/kg bw/day	Naaz *et al*., 2003
25-27 day old C57/BL6 mice	Genistein 8, 20 or 80	Daily sc injections for 28 days (starting a week after ovariectomy)		• ↓ in parametrial fat pad weight and ↓ in adipocyte circumference at 20 and 80 mg/kg bw/day • NOAEL of 8 mg/kg bw/day	
	Genistein 300, 500, 1000 and 1500 ppm	Diet *ad libitum* (starting a week after ovariectomy for 12 days)		• ↓ in parametrial fat pad weight and adipocyte circumference at 500 – 1500 ppm • ↑ uterine weights at all concentrations. Lowest dietary concentration is equivalent to 60 mg/kg bw/day	

Effects on fertility and development					
Mice					
Pups: CD-1 mice, wild type C57BL/6 mice and ERα and ERβ knockout mice; n=16/group	Genistein 0.5, 5 or 50	Daily sc injections (PND 1-5)	Ovaries: ER expression and multi-oocyte follicles (MOFs)	• ↑ in ERα RNA expression on PND 5 at 0.5 mg/kg bw/day and on PND 12 at 5 mg/kg bw/day • ↓ in ERα and ERβ RNA expression on PND 5 at 50 mg/kg bw/day • Dose-related ↑ in MOFs (NOAEL = 5 mg/kg bw/day; LOAEL = 50 mg/kg bw/day)	Jefferson *et al*., 2002; 2006
C57BL/6 mice n≥9	Genistein 0.1, 0.5, 2.5, 10	Daily gavage from GD 12 through PND 21, excluding the day of parturition.	Body weight, AGD (PND 7 and 21), mammary glands (PND 49) in the offspring.	• no detected effects in the offspring (NOAEL = 10 mg/kg bw/day)	Fielden *et al*., 2002
$B6D2F_1$ mice n=10-13	Genistein 0.1, 0.5, 2.5, 10	Daily gavage from GD 12 through PND 21, excluding the day of parturition.	Body weight, AGD (PND 7 and 21), testis and seminal vesicle weight, sperm count and motility, *in vitro* fertilizing ability of sperm in the offspring.	• Small but significant ↓ in AGD (<5%) at 10 mg/kg bw/day on PND 21 • No effects on sperm count, motility, testis, or body weight • Significantly ↑ *in vitro* fertilisation of sperm (17-18%) on PND 105 and 315 (NOAEL = 2.5 mg/kg bw/day)	Fielden *et al*., 2003
Pups: CD-1 female mice	Genistein 12.5, 20 or 25	Daily sc injections (PND 1-5)	Uterotropic response	• ↑ uterine weights (NOAEL = 12.5; LOAEL = 20 mg/kg bw/day)	Jefferson *et al*., 2009

	Genistein 25, 37.5 or 75	Daily oral administration using a pipette (PND 1-5)	Uterotropic response	• No detected effects in uterine weights (slight ↑ at 75 mg/kg/day)	
	Genistin 6.25, 12.5, 25 or 37.5 (as genistein aglucone equivalents)	Daily oral administration using a pipette (PND 1-5)	Uterotropic response, ovarian histology, vaginal opening	• ↑ uterine weights at 25 and 37.5 mg/kg bw/day • Delayed vaginal opening at 37.5 mg/kg/day • Abnormal estrous cycles and • significant ↓ in delivering live pups at 37.5 mg/kg bw/day • Dose dependent ↑ percentage of MOFs (NOAEL = 6.25; LOAEL = 12.5 mg/kg bw/day)	
Pups: C57BL/6 female and male mice n≤8	Genistein 50	Daily oral administration using a pipette (PND 1-5)	Body weight (PND 1-5, 7, 14, 21), vaginal opening, thymic and uterine weight, ovarian histology (PND 5 and 4 mo old).	• Body weights and timing of vaginal opening no different when compared to controls • 28% ↓ in thymic weight • 41% ↑ in uterine weight • ↑number of MOFs • Ovarian cycle abnormalities at 6 months of age LOAEL = 50 mg/kg bw/day	Cimafranca *et al*., 2010
Pups: CD-1 F1 mice n=8-18/group	Genistein 5 and daidzein 2 #	10 or 21 daily sc injections	AGD (PND 21 and 65), vaginal opening, fertility (wk 8), oestrus cycling.	• ↑ body weights (wk 4 to 8) • ↑ AGD and earlier vaginal opening • ↓ fertility • Abnormal oestrus cycles • ↓ number of CL • ↑ incidence of endometrial hyperplasia and atypia	Dinsdale *et al*., 2011

Pups: CD-1 female mice	Genistein and daidzein (5 +2)#	Daily sc injections, PND 1-5.	Body weight, bone mineral content, biochemical strength and microarchitecture of the femur	• ↑ bone development in female mice	Kaludjerovic and Ward, 2009;
		Daily sc injections, PND 1-5 or PND 1-10.	Organ weights and histology of ovaries and uteri were analysed	• ↑ body weights in adulthood after 1-10 day treatment (study 2012) • ↑ percent of hyperplasia in the oviduct • Abnormalities in the uteri and ovaries	Kaludjerovic *et al*., 2012
Rats					
Pregnant and lactating Long-Evans hooded rats	STUDY I: soy milk = isoflavones (genistein:daidzein, 3:2). Single dose level estimated to be between 10-30	STUDY I: daily gavage (PND 1-21)	Body weight, AGDI, age of puberty, oestrus cycling, reproductive organ weights	• Significantly ↑ body weight and ↓ AGDI (female pups) • No detected effects regarding reproductive endpoints, except in male pups - ↓ epididymal weight • ↑ in PR expression in glandular epithelial cells	Hughes *et al*., 2004
	STUDY II: genistein 15	STUDY II: daily gavage (GD 14 through PND 21)[1]		• Significant ↑ in PR expression in glandular epithelial cells (20%) • Earlier onset of puberty in genistein treated males (LOAEL = 15 mg/kg bw/day)	
Pups: Female Wistar rats n=9-10	Genistein or daidzein 1 mg/day (~19 mg/kg bw/day)	Daily sc injections (PND 1-5)	Vaginal opening, ovarian histology, oestrus cycling, lordosis response (LQ)	Effects observed for both, genistein and daidzein: • ↑ vaginal opening • Genistein: prolonged (66%) and	Kouki *et al*., 2003

				persistent (33%) oestrous cycles; ↓ ovarian weights; ↓ mean LQ	
PART I: Pregnant and lactating Long Evans rats n=12 PART II: Male offspring	Genistein 0.42 or 25 5 and 300 ppm)	PART I: Daily oral administration (diet); from 2 weeks before breeding to PND 21 PART II: Weaning – 70th day of age	Body weight, immune organ masses, testosterone levels.	• ↑ thymus masses and ↑ subpopulations of T cells in the spleen at both doses ↓ testosterone concentrations at both doses (LOAEL = 0.42 mg/kg bw/day) • PART II: no additional effect over perinatal exposure	Klein *et al*., 2002
Sprague Dawley rats: n=35/group	Genistein 0.3, 7, 35 (male) and 0.5, 10, 51 (females)	Daily oral administration (diet); from 3 (F_1 and F_2) and 6 (F_0) wk of age, through gestation and lactation up to 140 day of age; F_3: indirectly (*in utero* and lactation); F_4 and F_5 – no exposure	Body weights, vaginal opening, AGD, oestrous cyclicity, litter size	• ↓ body weights and AGD, ↑ vaginal opening, altered oestrous cyclicity at 51 mg/kg bw/d (females) • ↓ litter size in F_1 and F_2 • ↑ rates of mammary gland hyperplasia F_4 and F_5 - no effects (NOAEL = 0.5 mg/kg bw/day)	NTP, 2008a

Marmoset monkeys					
Marmoset monkeys n=7	Isoflavones in soy formula (range: 1.6 – 3.5)	Daily oral administration (syringe) from PND 4-5 until PND 35-45	Body weight, organ weights	• ↑ testicular weight (14%) and numbers of Sertoli (7%) and Leydig cells (32%)	Tan *et al*., 2006

#The mixture of genistein and daidzein comparable to quantity and ratio of each isoflavone in soy protein based infant formula (as reported by Dinsdale *et al*., 2011)
AGD – anogenital distance; AGDI – AGD Index (ratio of AGD/BW); bw – body weight; LQ – number of lordosis behaviours displayed/number of mounts x 100); PR – progesterone receptor; ER – oestrogen receptor; MOFs – multi-oocyte follicle

Human studies

36. Epidemiological and clinical studies aiming to establish the role of phytoestrogens in human health have provided conflicting results. This may be partly due to differences in intake estimation and different analytical methods used to calculate levels of isoflavones in foods (Thompson *et al.*, 2006). Furthermore, in studies on soy formula the reasons for introducing such products into their diet may influence observed health outcomes, but are generally not reported. Soy infant formula is usually introduced by parents due to its perceived health benefits, vegetarian diet or because of intolerance of other types of infant formula. Also the actual feeding regime is not investigated but based on parents/carers reports; the exact amount of soy intake also remains unknown (Bernbaum *et al*., 2008; Zung *et al*., 2008; Gilchrist *et al.*, 2010; Adgent *et al*., 2012).

Oestrogenic potency of phytoestrogens

37. Cellular and molecular mechanisms of oestrogen action as well as estimated oestrogenic potency of phytoestrogens have been extensively described (COT, 2003). Phytoestrogens have been described as substances structurally similar to 17β-oestradiol and having both oestrogen agonist and antagonist activity. The 17β-oestradiol can bind with similar affinities to oestrogen receptors (ER) α and β showing different tissues distributions (COT, 2003; Chen and Rogan, 2004). In contrast, genistein, daidzein and equol have greater affinity to ERβ than ERα (Cooke *et al*., 2006).

38. Genistein and daidzein are the major contributors to the total oestrogenic effect of soy-based products. When infant diet is exclusively based on soy formula, daily genistein intake would be approximately 5 mg/kg bw/day. Isoflavones are considered to have relatively weak oestrogenic properties. Different *in vitro* studies reviewed by Chen and Rogan (2004) provided different estimates of the oestrogenicity of genistein (relative to oetradiol), reporting it to be in a range between 10^{-5} and 0.4 times lower than that of oestradiol. Based on the range of these estimates the authors concluded that genistein intake would be an equivalent intake of 0.05 to 5 µg of oestradiol per kg bw/day. Based on the authors estimate of daily oestrogen intake from contraceptive pills in women as approximately 0.4 - 1 µg/kg bw/day this would equate to infants taking up to five contraceptive pills/day (Chen and Rogan, 2004). However, it appears that authors based their comparisons on oestradiol, whereas the active ingredient in contraceptive pills is ethinyl oestradiol which has 100 times greater oral oestrogen potency than oestradiol (Fritz and Speroff, 2010).

39. Basaria *et al*. evaluated the effects of high-dose isoflavones on self-reported quality of life, cognition, lipoproteins and androgen status in post-menopausal women. A powder containing 20 g of soy protein that consisted of 160 mg of total isoflavones (96 mg aglucones) was mixed with beverages and consumed daily. In this double-blind, randomized, placebo-controlled trial high doses of isoflavones have been shown to reduce testosterone and high-density lipoprotein levels and significantly improve vasomotor, physical and psychosexual aspects of life in post-

menopausal women (n=84). However, isoflavones did not have any significant beneficial effects on cognition or lipids (Basaria *et al.*, 2009).

Effects of phytoestrogens on fertility and development

40. The 2003 COT report summarised that studies on the effects of phytoestrogens on human development and fertility are limited in number and scope and there are no published human studies examining the potential effects of *in utero* exposure to phytoestrogens mainly due to practical and ethical concerns. The human health implications of results obtained in animals are unclear as there are large species differences in sexual development between rodents, non-human primates and humans. Only one human study published before 2003 specifically examined the effects of soy-based formula feeding on development and fertility (Strom *et al*., 2001). No adverse clinical effects were reported with the exception of small increases in the duration and discomfort of menstruation. However, this study was based on recall and did not involve any direct measurements of hormone levels (COT, 2003).

41. Strom *et al.* examined the association between consumption of soy formula during infancy and eventual health and development outcomes observed in adulthood. There was a slightly longer duration of menstrual bleeding (0.37 days) and increased discomfort reported by women who had been fed soy formula (n=128) compared to women who had been fed cows' milk formula as infants (n=563). After adjustment for multiple comparisons, these findings were not statistically significant. There were no differences observed in either men or women in relation to height, weight, body mass index (BMI), self-reported pubertal maturation, reproductive disorders and birth defects in their offspring when compared to participants fed cows' milk formulas (Strom *et al.*, 2001).

42. Studies performed after 2003 investigating the potential oestrogenic impact of exclusive soy formula consumption on infants' development when compared to breastfed or cows' milk formula fed infants are summarised in Table 2. Reported effects include increased vaginal cell maturation (Bernbaum *et al*., 2008), higher prevalence of breast buds and preserving effect on breast tissue expressing itself in slower waning in the 2nd year of life (Zung *et al.*, 2008), higher risk of menarche (Adgent *et al*., 2012). Another study comparing health, growth and development of children found no deleterious effects on reproductive health and no difference in infant weight, length, or body surface area (Gilchrist *et al*., 2010). As a part of the United Kingdom Avon Longitudinal Study of Parents and Children (ALSPAC) sexual dimorphism in gender-role play behaviours was observed. Although authors reported modest increase in masculine typical behaviour among children fed soy in early life, this result did not place them outside the range of normal behaviour (Adgent *et al*., 2011). In Japan, soy intake was reported to be negatively related to oestrone and oestradiol in boys and positively related to testosterone and 5-androstene-3β,17α diol (3β,17α-AED) levels in girls, measured in urine (Wada *et al*., 2011).

Table 2. Human studies of reproductive and developmental outcomes associated with *e*xposure to isoflavones.

Participants	Isoflavone exposure	Endpoint	Observed health outcome	Reference
Children <48 hours to 6 months of age, 37 boys and 35 girls	Exclusively fed soy-based infant formula (as reported by parents)	Measurements of breast adipose tissue, breast buds, and testicular volume; breast and genital development as part of regular physical examinations over a period from birth to 6 months	• ↑ vaginal cell m*atura*tion in 1-6 mo girls • No changes in breast and genital anatomy over the time	Bernbaum *et al*., 2008
Female infants aged 3 to 24 months, n=92	At least 3 months of soy-based formula feeding either exclusively or in co*mbina*tion with breastfeeding or cows' milk formula (as reported by parents)	Measurements of breast buds and breast tissue as part of regular physical examinations over a period of 2 years	• Higher prevalence of breast buds during the 2nd year of life when compared to cows' milk fed infants • Preserving effect on breast tissue and slower waning in the 2nd year of life comparing to children fed breast milk or cows' milk formula	Zung *et al*., 2008
Infants fed soy formula (n=39), cows' milk formula (n=41) and breast milk (n=40)	As reported by parents each group was fed soy formula, cows' milk formula or breastfed either exclusively or for the majority of the	Measurements of body weights, ovaries, number of follicles, testicular volume	• ↓ body weight in boys (comparing to breastfed boys) • ↓ ovarian volume in girls (comparing to cows' formula fed girls) • ↑ number of follicles per cyst per ovary compared	Gilchrist *et al*., 2010

	first 4 months of life		to breastfed girls • ↓ testicular volume compared to breastfed boys • No differences in infant weight, length, or body surface area	
Preschool boys (n=230) and girls (n=198), aged 3-6 years	Soy intake was recorded by parents over 3 days (24.4 g/day for boys and 22.8 g/day for girls)	Sex differences	• Soy intake negatively related to oestrone and oestradiol in boys • Soy intake positively related to testosterone and 5-androstene-3β,17α diol (3β,17α-AED) levels in girls • Levels of oestrone, oestradiol, testosterone, and DHEA in girls were higher than those in boys	Wada *et al.*, 2011
42 month old children (3,664 boys and 3,412 girls)	Early-life feeding plan was reported by parents either as primarily breast, early formula, early soy and late soy	Sexual dimorphism in gender-role play behaviours assessed between infant feeding and scores of the Pre-School Activities Inventory test. Mother's response was scored on a 5-point Likert scale ("never" to "very often"). Higher scores indicated masculine typical behaviour, and lower	• Modest increase in masculine typical behaviour among children fed soy in early life. Authors noticed that those results did not place children outside the range of normal behaviour.	Adgent *et al.*, 2011

		scores indicated feminine typical behaviour.		
Girls (n=2920) for whom at least one puberty questionnaire was available between the ages of 8 and 14.5 years if age	Early-life feeding plan was reported by parents either as primarily breast, early formula, early soy and late soy	Timing of menarche in relation infant feeding	• median age at menarche was earliest for girls receiving an early soy diet (12.4 years), and latest among those who were primarily breastfed (12.8 years) • Early soy-fed girls were at 25% higher risk of menarche throughout the course of follow-up, compared to girls fed non-soy-based or milk formula	Adgent *et al*., 2012

Effects of phytoestrogens on the thyroid gland and thyroid function

43. It has been hypothesised that phytoestrogens may be active in the thyroid due to the chemical structure being similar to that of the thyroid hormones, tri-iodothyronine (T_3) and thyroxine (T_4), with the potential to act through the inhibition of thyroperoxidase (TPO) or possible interactions with thyroid binding globulin (TBG)(COT, 2003).

44. In 2003 the Committee reviewed and discussed the literature published up to 30 April 2002 related to effects of phytoestrogens on the thyroid gland and thyroid function in animals and humans. Available animal studies showed that dietary soy or isoflavones can affect the thyroid function and have a goitrogenic effect in rodents deficient in dietary iodine. In human studies some changes in levels of thyroid hormones were observed but they were described as not physiologically relevant. It was also noted that isoflavones are unlikely to affect thyroid function in normal individuals with adequate iodine intake (COT, 2003).

45. A number of scientific publications evaluated by the Committee in 2003 noted the possibility that soy-based infant formula may be able to affect thyroid function in infants. The increased loss of orally administered thyroxine as well as cases of goitre were reported in 1950s and 60s. As a result changes in processing and formulation of infant formula were made (supplementation with iodine, replacement of soy flour with soy protein isolate) and no further reports of goitre were received since then. No data were found to confirm that maternal ingestion of phytoestrogens during pregnancy can influence the development of thyroid gland. However, it is possible that together with low iodine intake, increased metabolic demands during pregnancy and increased thyroxine need, maternal consumption of soy products could adversely influence the neurological development of the fetus (COT, 2003).

46. Conrad *et al*. performed a retrospective analysis of medical records of infants diagnosed with congenital hypothyroidism and seen at the hospital during their first year of life. Two groups of patients were analysed: the soy diet group consuming exclusively soy infant formula started on treatment at a median age of 15 days (range: 11 – 22) (n=8) and the non-soy diet group, at 17 days (range: 12 – 23) (n=70). There was no significant difference in thyroid stimulating hormone (TSH) and thyroxine (T_4) levels in both groups prior to levothyroxine treatment initiation. Levels of T_4 measured on treatment were similar in both groups: median 153 nmol/L (soy group) and 188 nmol/L (non-soy group). However, after initiation of treatment there was a difference in the first TSH measured at 50 days between the soy and non-soy diet groups: median 42.6 mU/L (range: 30.6 – 63.1) and 6.6 mU/L (range: 1.8 – 20.9), respectively. The soy diet group required a median of 150 days (range: 54 – 229) and non-soy diet group 40 days (range: 19 – 189) for the normalisation of TSH to levels < 10 mU/L. After two and four months of treatment, the percentage of patients with increased TSH was higher in soy diet group: 62.5 %, whereas in non-soy diet group these values were 35.7 and 17%, respectively. Overall, infants consuming soy-based formula had elevated levels of TSH and subsequently increased requirement for T_4. Prolonged increase of TSH was related to malabsorption and increased faecal loss of levothyroxine (Conrad *et al*., 2004).

Allergy

47. The Food Standards Agency (FSA) advises that: "Soy allergy is a common childhood allergy. Most children grow out of it by the age of two, but occasionally adults are allergic to soy. The symptoms of soy allergy are similar to milk allergy and they include rashes, diarrhoea, vomiting, stomach cramps and breathing difficulties. Some people with soy allergy might also react to milk. Very rarely soy can cause anaphylaxis. Infants with other allergic conditions, such as milk allergy, dermatitis etc, are also at higher risk of developing allergy to soy"[8]. The health risks associated with infant feeding and the development of soy allergy will be included in the COT review of risks arising from the infant diet and the development of atopic and autoimmune disease. Details of the proposed scope and approach for the literature review were set in the initial paper (TOX/2012/27) and discussed by the COT in September 2012. Although there are reports of allergy these are likely to be related to the proteins rather than the oestrogenic activity.

Guidance values

48. Soy isoflavones have not been classified as essential nutrients as their absence from the diet does not induce any deficiency syndrome and their presence is not essential in any biological processes. A tolerable daily intake (TDI) value has not been established for soy isoflavones.

49. In 2010 the NTP-CERHR Expert Panel concluded that there was no sufficient evidence to conclude that soy infant formula and soy-based diet posed a developmental toxicity risk in experimental animal studies considered to be relevant to the assessment of human risk. The authors considered evidence to be sufficient to conclude that there was "minimal concern" for adverse effects from consumption of soy infant formula by healthy full-term infants. This evaluation however did not include an assessment on the potential reproductive toxicity of genistein (McCarver *et al.,* 2011). The Expert Panel acknowledged the fact that larger (in terms of sample size) and longer longitudinal, prospective cohort studies were needed, capturing for instance soy exposure from birth through puberty, addressing longer term endpoints such as cancer, bone mineral density or cognitive performance (NTP, 2010).

Occurrence

Levels of isoflavones in human breast milk

50. The 2003 COT report noted that isoflavones are excreted in human milk in low concentrations reflecting maternal diet, with the highest concentrations in the breast milk from mothers following vegetarian or vegan diets. Mean (and ranges in brackets) of total isoflavone concentrations reported in breast milk samples, expressed as a sum of genistein and daidzein as µg aglucone/kg, were as follows:

[8] http://webarchive.nationalarchives.gov.uk/20080910110835;
http://eatwell.gov.uk/healthissues/foodintolerance/foodintolerancetypes/soyaallergy

mothers consuming omnivorous diet (n=14): 1 (0 – 2); vegetarian diet (n=14): 4 (1 – 10) and vegan diet (n=11): 11 (2 – 32) (MAFF, 1998a).

51. Previous studies reported concentrations of total isoflavones to be present in breast milk in a range of 1.6-13.6 µg aglucone/L in women consuming an omnivorous diet (Setchell *et al*., 1997; Setchell *et al*., 1998). Consumption of soy foods such as roasted soybeans has been shown to increase levels of isoflavones in vegans up to 32 µg/L (Franke and Custer, 1996; MAFF, 1998b).

52. In a study conducted in the US, milk samples were collected from breastfeeding mothers before and after consumption of a soy protein beverage (25 g soy protein/36.5 g of beverage containing 55 mg isoflavones: daidzein:genistein:glycitein = 1:1:0.1). The mean levels of isoflavones in breast milk increased from 5.1 ± 2.2 nmol/L to 70.7 ± 19.2 nmol/L after 2 – 4 days of daily consumption. The daidzein to genistein ratio was on average 0.6, therefore it can be estimated that converted to µg/L levels of genistein were increased from 0.55 to 7.64 and daidzein from 0.78 to 10.78 (Franke *et al.*, 2006). In Canada, samples of breast milk were collected from women of late maternal age (at least 35 years of age) after delivery; details on the diet were not provided. The mean concentrations (SD) of isoflavones in breast milk samples were 0.87 ± 2.86 µg/L (genistein) and 0.36 ± 1.41 µg/L (daidzein) from women with male infants and 0.36 ± 1.21 µg/L (genistein) and 0.16 ± 0.49 µg/L (daidzein) from women with female infants (Jarrell *et al*., 2012).

Cows' milk-based infant formula

53. As noted in the 2003 COT report, isoflavones were not detected in three different brands of cows' milk formula purchased in the UK (individual isoflavones were below the limit of detection (LOD) = 0.25 – 0.5 mg/L) (MAFF, 1998b). In another UK study, isoflavones were not detected (LOD = 0.5 mg/kg dry powder) in 6 out of 8 samples of cows' milk infant formula powders. Isoflavones as aglucone equivalents were detected only in two samples, at 1.2 mg/kg total isoflavones (0.7 mg genistein/kg and 0.5 mg glycitein/kg) and 2.1 mg genistein/kg (Hoey *et al*., 2004).

Soy-based infant formula

54. COT (2003) noted that reported isoflavone levels in soy-based formulas were in the range of 18-41 mg aglucone equivalents/L (made up formula ready for consumption) (MAFF, 1998a). Levels of isoflavones in other soy infant formulas obtained in the UK were also measured by other researchers. All soy based infant formulas analysed by Hoey *et al*. contained between 34.0 and 46.7 mg aglucone equivalents/L as fed where genistein comprised 63 ± 5 %, daidzein 27 ± 1% and glycitein 10 ± 5%, of the total (Hoey *et al*., 2004). Kuhnle *et al*. reported the total isoflavone content of the soy infant formula as 1000 times higher than non-soy formula: 25.90 mg aglucone equivalents as fed (Kuhnle *et al*., 2008). Concentrations of isoflavones were higher in powdered soy formula (46 – 47 mg/L) than in liquid formula (32 – 45 mg/L) (Setchell *et al*., 1998). Conjugates of genistein account for >65% of the total isoflavones and only 3.2 – 5.8% exists as genistein and daidzein aglucones (Setchell *et al*., 1998).

55. Total isoflavone concentrations as aglucone equivalents in soy-based infant formulas were also measured in other countries and were reported to be in the range of 81 – 92 mg/kg for genistein and 44 – 55 mg/kg for daidzein (Irvine *et al.,* 1998) and in another study as 89.5 – 155.7 mg/kg for genistein, 52.7 – 101.6 mg/kg for daidzein and 12.8 - 24 mg/kg for glycitein (Franke *et al*., 1998).

Complementary feeding products

Soy–based products

56. Levels of isoflavones in samples of ready-to-eat and instant foods for infants were previously reported by the COT (Table 3). Table 3 also includes data on isoflavones in various foods, such as vegetables, fruit, cheese or meat products, typical of Western diets and possibly consumed by infants (Thompson *et al*., 2006; Kuhnle *et al*., 2008). However, since these sources were based on a very limited number of poorly described samples, and given the uncertainty about the extent of their consumption by infants, the exposure assessment presented here is the data in COT (2003).

Table 3. Isoflavone levels in foods included in infant diet

Food type	Total isoflavone levels as mg/kg of foods as consumed	Source
Ready-to-eat and instant weaning foods	Range: 18 – 78	COT, 2003
Firm tofu	275*	COT, 2003
Other foods with minor levels of isoflavones**	Range: 0.001 – 0.39	Irvine *et al*., 1998 Thompson *et al*., 2006 Kuhnle *et al*., 2008

*No information is provided whether given value is the mean or the mid-point of the range
**Presented range has been reported for following food products: vegetables, fruits, bread, pasta and rice, cheese, meat products, fish, biscuits and cakes. The highest value reported as 0.39 mg total isoflavones/kg was detected in green and white beans (Thompson *et al*., 2006).

Exposure

57. Values of 800 mL and 1200 mL, as reasonable estimates of average and high-level daily consumption of breast milk or infant formula before weaning, have been used in exposure calculations (EFSA, 2012). The mean bodyweights used for calculation of exposures were 5.9 kg, 7.7 kg, 8.9 kg and 9.8 kg for infants aged 0-3, 4-6, 7-9 and 10-12 months old respectively[9].

[9] COT Statement on a survey of metals in infant food (2003). Available at: http://cot.food.gov.uk/pdfs/statement.pdf

Breast milk

58. The data for breast milk samples collected in the UK (see para 53) have been used to estimate exposure of exclusively breastfed infants, based on the reported levels of isoflavones (expressed as a sum of genistein and daidzein) in mothers consuming different diets (Table 4). The estimated exposures range from 0.1-0.2 µg/kg bw/day for infants whose mothers consume an omnivorous diet up to 6.5 µg/kg bw/day for infants whose mothers consume a vegan diet.

Table 4. Estimated total isoflavone exposure (µg/kg bw/day) of exclusively breastfed infants

Isoflavones concentration in breast milk	Age in months (consumption value)			
	0 – 3 (800 mL)	0 – 3 (1200 mL)	4 – 6 (800 mL)	4 – 6 (1200 mL)
Omnivorous diet Mean = 1µg/L	0.1	0.2	0.1	0.2
Vegetarian diet Mean = 4µg/L	0.5	0.8	0.4	0.6
Vegan diet Mean = 11µg/L	1.5	2.2	1.1	1.7
Vegan diet Maximum = 32µg/L	4.3	6.5	3.3	4.9

Cows' milk-based infant formula

59. Infants' exposure was estimated using the maximum level of isoflavone reported for cows' milk formula purchased in the UK (para 55). This level of 2.1 mg genistein/kg in powdered formula was adjusted to 0.28 mg/L to take account of levels following reconstitution. The estimated average and high level exposures from cows' milk-based infant formula are up to 38 and 57 µg/kg bw/day, respectively (Table 5). However, since isoflavones were not detected in most samples of cows' milk-based formula, the exposure in most infants exclusively fed this type of formula, is likely to be lower.

Table 5. Estimated total isoflavone exposure (µg/kg bw/day) of infants exclusively fed cows' milk formula for average and high level consumption, based on the highest reported isoflavone content

Consumption	Infant age (months)			
	0 - 3	4 - 6	7 - 9	10 - 12
Average (800 mL)	38	29	25	23
High level (1200 mL)	57	44	38	34

Soy-based infant formula

60. Based on reported isoflavone levels in reconstituted soy-based infant formulas from the UK (range: 18 – 46.7 mg aglucone equivalents/L) (see para 56) the isoflavone exposure of infants exclusively fed on soy-based infant formula is up to 9500 µg/kg bw/day (Table 6).

Table 6. Estimated total isoflavone exposure (µg/kg bw/day) of infants fed exclusively soy-based infant formula for average and high level consumption, based on the range of reported isoflavone content

Consumption	Infant age (months)			
	0 - 3	4 - 6	7 - 9	10 - 12
Average (800 mL)	2441 – 6332	1870 – 4852	1618 – 4198	1469 – 3812
High level (1200 mL)	3661 – 9498	2805 – 7278	2426 – 6297	2204 – 5718

Complementary feeding products – exposure assessment

63. Infants' exposure to total isoflavones from infant foods (Table 7).has been estimated based on the levels reported for infant foods (Table 3) and consumption data from the 1986 survey of British Infants (Mills and Tyler, 1992).The "ready-to-eat and instant weaning food" group in Table 3 mainly comprised commercial baby foods (including cereal-based foods and desserts).

61. An exposure estimate for tofu, based on a portion size approach (because actual consumption data were not available) was also calculated to allow comparison of exposures between a potentially rich source of isoflavones such as tofu with those estimated for ready to eat and instant complementary foods. A baby food recipe website[10] indicated that 50 g per person per day would be an appropriate portion size. However, the portion size approach is expected to overestimate exposure as it is unlikely that an infant will consume this type of product (e.g. banana and tofu puree) daily over prolonged periods.

Table 7. Estimated exposure of UK infants to isoflavones from complementary foods (in µg/kg bw/day)

Product category	Isoflavone level (µg/g food)	Consumption rate (g/kg bw/day)		Exposure (µg/kg bw/day)	
		Mean	97.5th percentile	Mean	97.5th percentile
Weaning Foods*	Range: 18 – 78	6	22	Range: 108 - 468	Range: 396 - 1716
Tofu**	275	5	n/a	1375	n/a

[10] http://www.annabelkarmel.com/recipes/babies-6-9-months/banana-tofu-puree

* Foods reported in the 1986 British Infants Survey (Mills and Tyler, 1992) such as: instant weaning foods and ready-to-eat foods – most are commercial/retail baby foods (including cereal-based foods). Examples: egg/cheese-based meal dried; rice/semolina/chocolate instant puddings; yoghurt-based dried meals.
**In the absence of consumption data on soy-based food products such as tofu, a portion size of 50 g per infant per day was used to estimate exposure levels, together with an average bodyweight of 9.35 kg for infants aged 6-12 months old[11]

Risk characterisation

62. Based on maximum reported concentrations of isoflavones in breast milk from mothers following a vegan diet, the exposure of exclusively breastfed infants was estimated to be up to 6.5 µg/kg bw/day. Isoflavone exposure from infant formula for exclusively cows' formula-fed infants was up to 57 µg/kg bw/day. The highest exposure was estimated for infants exclusively fed soy-based infant formula reaching values up to nearly 9500 µg/kg bw/day.

63. Limited information is available regarding infants' exposure to isoflavones from complementary foods, but it is potentially in the range of 108 – 1716 µg/kg bw/day.

64. In experimental animals dietary administration of isoflavones has resulted in effects on body weight (observed at 7 x 10^3 µg isoflavones/kg bw/day), mammary gland and oviduct hyperplasia, increased uterine wet weight, altered ovarian differentiation, decreased fertility or delayed parturition (observed at 6.25 – 37.5 x 10^3 µg genistein/kg bw/day), ovarian cycle abnormalities and increased expression of progesterone receptor (observed at 10-30 x 10^3 µg isoflavones/kg bw/day). Additionally increased thymus masses and subpopulations of T cells in the spleen as well as reduced testosterone concentrations in the offspring in rats were observed at 0.42 mg/kg bw/day Although the estimated dietary exposure of infants is in the range of the doses shown to have effects in experimental animals, effects observed in animals cannot be simply extrapolated to humans due to differences in absorption and capacity to conjugate isoflavones.

65. These metabolic differences relate to immaturity of the intestinal microflora, lack of ability to hydrolyse β-glucosides efficiently and/or larger intake when adjusted for body weight. Food matrix, diet, hygiene, stress, genetics and age can also influence metabolism of infants. Furthermore, the species differences in metabolism of isoflavones limit the extrapolation of risk to humans based on studies in animals. It has been noted that the metabolic profile of isoflavones in pigs is closer to that in humans, whereas intestinal bacterial composition in rats is similar to that in monkeys.. An additional complication is that studies performed in animals are often based on exposure to a single compound, such as genistein, whereas in humans the exposure is to the combination of the components of soy, including a mixture of isoflavones and other substances. The estimated exposure of infants is based on total isoflavones, without adjustment for potency or molecular weight.

[11] COT Statement on a survey of metals in infant food (2003). Available at: http://cot.food.gov.uk/pdfs/statement.pdf

66. Observations in children fed soy-based formula were: increased vaginal cell maturation, higher prevalence of breast buds and risk of menarche, increased levels of TSH and requirement for T4, and slightly increased discomfort and duration of menstrual bleeding in adulthood. These studies do not provide detailed information on isoflavone exposure in infants. Only an estimated length of soy formula consumption and possible inclusion of other types of infant feeding were reported by parents. Therefore the available human studies do not support establishment of a health-based guidance value or reference point for use in risk characterisation of soy isoflavones.

67. The oestrogenicity of isoflavones and their influence on reproductive organs have been identified as the main concern in relation to soy formula consumption by infants. Other potential concerns are effects of soy consumption on thyroid function and allergies. The relative oestrogenicity of genistein to oestradiol was estimated to be between 10^{-5} and 10^{-3}, indicating that exposure of 5 mg genistein/kg bw/day could correspond to an intake of 0.05 to 5 µg of oestradiol per kg bw/day. Hoey *et al*. (2004) reported that approximately 63% of total isoflavones content of soy formula, as aglucone equivalents, is formed by genistein. Therefore estimated isoflavone exposure from consumption of soy formula, reaching up to 9.5 mg total isoflavones as aglucone equivalents/kg bw/day (of which approximately 5.7 mg/kg bw/day is genistein), may indicate that infants fed soy formula, can be exposed to levels equivalent to oestrogen exposure of 0.057 – 5.7 µg/kg bw/day, which could be in the range of pharmacologically active levels.

Conclusions

77. [To be drafted after COT discussion]

Secretariat
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References

Adgent MA, Daniels JL, Edwards LJ, Siega-Riz AM and Rogan WJ (2011) Early-life soy exposure and gender-role play behavior in children. *Environ Health Perspect* **119**:1811-1816.

Adgent MA, Daniels JL, Rogan WJ, Adair L, Edwards LJ, Westreich D, Maisonet M and Marcus M (2012) Early-life soy exposure and age at menarche. *Paediatr Perinat Epidemiol* **26**:163-175.

Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency). Report of the working group on phytoestrogens. 2005.

Andlauer W, Kolb J, Furst P (2000) Absorption and metabolism of genistin in the isolated rat small intestine *FEBS Lett* **475**: 127-130

Basaria S, Wisniewski A, Dupree K, Bruno T, Song MY, Yao F, Ojumu A, John M and Dobs AS (2009) Effect of high-dose isoflavones on cognition, quality of life, androgens, and lipoprotein in post-menopausal women. *J Endocrinol Invest* **32**:150-155.

Berger-Achituv S, Shohat T, Romano-Zelekha O, Ophir E, Rachmani S, Malovizky D and Garty BZ (2005) Widespread use of soy-based formula without clinical indications. *J Pediatr Gastroenterol Nutr* **41**:660-666.

Bernbaum JC, Umbach DM, Ragan NB, Ballard JL, Archer JI, Schmidt-Davis H and Rogan WJ (2008) Pilot studies of estrogen-related physical findings in infants. *Environ Health Perspect* **116**:416-420.

Bhatia J and Greer F (2008) Use of soy protein-based formulas in infant feeding. *Pediatrics* **121**:1062-1068.

Bundesinstitut für Risikobewertung (The German Federal Institute for Risk Assessment). Isolated isoflavones are not without risk. 2007.

Cao Y, Calafat AM, Doerge DR, Umbach DM, Bernbaum JC, Twaddle NC, Ye X and Rogan WJ (2009) Isoflavones in urine, saliva, and blood of infants: data from a pilot study on the estrogenic activity of soy formula. *J Expo Sci Environ Epidemiol* **19**:223-234.

Cassidy A, Bingham S and Setchell KD (1994) 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.

Cassidy A, Brown JE, Hawdon A, Faughnan MS, King LJ, Millward J, Zimmer-Nechemias L, Wolfe B and Setchell KD (2006) Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *J Nutr* **136**:45-51.

Catassu C (1995) Intestinal permeability changes during the first month: effects of natural versus artificial feeding. *J Pediatr Gastroenerol Nutr* **21**: 383-386

Chen A and Rogan WJ (2004) Isoflavones in soy infant formula: a review of evidence for endocrine and other activity in infants. *Annu Rev Nutr* **24**:33-54.

Cimafranca MA, Davila J, Ekman GC, Andrews RN, Neese SL, Peretz J, Woodling KA, Helferich WG, Sarkar J, Flaws JA, Schantz SL, Doerge DR and Cooke PS (2010) Acute and chronic effects of oral genistein administration in neonatal mice. *Biol Reprod* **83**:114-121.

Cohen L, Crespin JS, Wolper C, Zang EA, Pittman B, Zhao Z, Holt PR (2007) Soy isoflavone intake and estrogen excretion patterns in young women: effect of probiotic administration. *in vivo* **21**: 507-512

Conrad SC, Chiu H and Silverman BL (2004) Soy formula complicates management of congenital hypothyroidism. *Arch Dis Child* **89**:37-40.

Cooke PS, Selvaraj V and Yellayi S (2006) Genistein, estrogen receptors, and the acquired immune response. *J Nutr* **136**:704-708.

Department of Health's Chief Medical Officer. Advice issued on soya-based infant formulas. CMO Update 37. 2004.

Dinsdale EC, Chen J and Ward WE (2011) Early life exposure to isoflavones adversely affects reproductive health in first but not second generation female CD-1 mice. *J Nutr* **141**:1996-2002.

Di Virgilio AL, Iwami K, Watjen W, Kahl R, Degen GH (2004) Genotoxicity of the isoflavones genistein, daidzein and equol in V79 cells. *Toxicology Letters* **151**: 151-162

EFSA (2012) Scientific opinion on brominated flame retardants (BFRs) in food: brominated phenols and their derivatives. *EFSA Journal* **10**(4): 2634

Fielden MR, Fong CJ, Haslam SZ and Zacharewski TR (2002) Normal mammary gland morphology in pubertal female mice following in utero and lactational exposure to genistein at levels comparable to human dietary exposure. *Toxicol Lett* **133**:181-191.

Fielden MR, Samy SM, Chou KC and Zacharewski TR (2003) Effect of human dietary exposure levels of genistein during gestation and lactation on long-term reproductive development and sperm quality in mice. *Food Chem Toxicol* **41**:447-454.

Fletcher RJ (2003) Food sources of phyto-oestrogens and their precursors in Europe. *Br J Nutr* **89 Suppl 1**:S39-S43.

Franke AA and Custer LJ (1996) Daidzein and genistein concentrations in human milk after soy consumption. *Clin Chem* **42**:955-964.

Franke AA, Custer LJ and Tanaka Y (1998) Isoflavones in human breast milk and other biological fluids. *Am J Clin Nutr* **68**:1466S-1473S.

Franke AA, Halm BM, Custer LJ, Tatsumura Y and Hebshi S (2006) Isoflavones in breastfed infants after mothers consume soy. *Am J Clin Nutr* **84**:406-413.

Fritz MA, Speroff L (2010) Clinical Gynecologic Endocrinology and infertility. Eight edition. Lippincott Williams & Wilkins, p.752

Gee JM, DuPont MS, Rhodes MJC, Johnson IT (1998) Quercetin glucosides interact with the intestinal glucose transport pathway. *Free Radic Biol Med* **25**:19 - 25

Gilchrist JM, Moore MB, Andres A, Estroff JA and Badger TM (2010) Ultrasonographic patterns of reproductive organs in infants fed soy formula: comparisons to infants fed breast milk and milk formula. *J Pediatr* **156**:215-220.

Gu L, House SE, Prior RL, Fang N, Ronis MJ, Clarkson TB, Wilson ME and Badger TM (2006) Metabolic phenotype of isoflavones differ among female rats, pigs, monkeys, and women. *J Nutr* **136**:1215-1221.

Halm BM, Ashburn LA and Franke AA (2007) Isoflavones from soya foods are more bioavailable in children than adults. *Br J Nutr* **98**:998-1005.

Hoey L, Rowland IR, Lloyd AS, Clarke DB and Wiseman H (2004) Influence of soya-based infant formula consumption on isoflavone and gut microflora metabolite concentrations in urine and on faecal microflora composition and metabolic activity in infants and children. *Br J Nutr* **91**:607-616.

Hughes CL, Liu G, Beall S, Foster WG and Davis V (2004) Effects of genistein or soy milk during late gestation and lactation on adult uterine organization in the rat. *Exp Biol Med (Maywood)* **229**:108-117.

Irvine CH, Shand N, Fitzpatrick MG and Alexander SL (1998) Daily intake and urinary excretion of genistein and daidzein by infants fed soy- or dairy-based infant formulas. *Am J Clin Nutr* **68**:1462S-1465S.

Izumi T, Piskula MK, Osawa S, Obata A (2000) Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J Nutr* **130**: 1695-9

Jarrell J, Foster WG and Kinniburgh DW (2012) Phytoestrogens in human pregnancy. *Obstet Gynecol Int* **2012**:850313.

Jefferson WN, Couse JF, Padilla-Banks E, Korach KS and Newbold RR (2002) Neonatal exposure to genistein induces estrogen receptor (ER)alpha expression and multioocyte follicles in the maturing mouse ovary: evidence for ERbeta-mediated and nonestrogenic actions. *Biol Reprod* **67**:1285-1296.

Jefferson WN, Doerge D, Padilla-Banks E, Woodling KA, Kissling GE and Newbold R (2009) Oral exposure to genistin, the glycosylated form of genistein, during neonatal life adversely affects the female reproductive system. *Environ Health Perspect* **117**:1883-1889.

Jefferson WN, Padilla-Banks E and Newbold RR (2006) Studies of the effects of neonatal exposure to genistein on the developing female reproductive system. *J AOAC Int* **89**:1189-1196.

Kaludjerovic J, Chen J and Ward WE (2012) Early life exposure to genistein and daidzein disrupts structural development of reproductive organs in female mice. *J Toxicol Environ Health A* **75**:649-660.

Kaludjerovic J and Ward WE (2009) Neonatal exposure to daidzein, genistein, or the combination modulates bone development in female CD-1 mice. *J Nutr* **139**:467-473.

Klein SL, Wisniewski AB, Marson AL, Glass GE and Gearhart JP (2002) Early exposure to genistein exerts long-lasting effects on the endocrine and immune systems in rats. *Mol Med* **8**:742-749.

Kouki T, Kishitake M, Okamoto M, Oosuka I, Takebe M and Yamanouchi K (2003) Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: estrous cycle and lordosis. *Horm Behav* **44**:140-145.

Kuhnle GG, Dell'Aquila C, Aspinall SM, Runswick SA, Mulligan AA and Bingham SA (2008) Phytoestrogen content of foods of animal origin: dairy products, eggs, meat, fish, and seafood. *J Agric Food Chem* **56**:10099-10104.

Kwon SH, Kang MJ, Huh JS, Ha KW, Lee JR, Lee SK, Lee BS, Han IH, Lee MS, Lee MW, Lee J, Choi YW (2007) Comparison of oral availability of genistein and genistin in rats. *International Journal of Pharmaceutics* **337**: 148-154

Maskariniec G, Watts K, Kagihara J, Hebshi S, Franke AA (2008) Urinary isoflavonoid excretion is similar after consuming soy milk and miso soup in Japanese-American women. *Br J Nutr* **100**: 424-429

McCarver G, Bhatia J, Chambers C, Clarke R, Etzel R, Foster W, Hoyer P, Leeder JS, Peters JM, Rissman E, Rybak M, Sherman C, Toppari J and Turner K (2011) NTP-CERHR expert panel report on the developmental toxicity of soy infant formula. *Birth Defects Res B Dev Reprod Toxicol* **92**:421-468.

McClain RM, Wolz E, Davidovich A, Bausch J (2006) Genetic toxicity studies with genistein. *Food and Chemical Toxicology* **44**: 42-55

Mills A and Tyler H. Food and Nutrient Intakes of British Infants Aged 6-12 months. HMSO. London. 1992.

Ministry of Agriculture Fisheries and Food Report. FS2829 - Levels of oestrogens in the diets of infants and toddlers. University of Reading. 1998a.

Ministry of Agriculture Fisheries and Food Report. Plant oestrogens in soya-based infant formula. Food Surveillance Paper No. 167. London. UK. HMSO. 1998b.

Naaz A, Yellayi S, Zakroczymski MA, Bunick D, Doerge DR, Lubahn DB, Helferich WG and Cooke PS (2003) The soy isoflavone genistein decreases adipose deposition in mice. *Endocrinology* **144**:3315-3320.

Nielsen TS, Purup S, Warri A, Godschalk RW and Hilakivi-Clarke L (2011) Effects of maternal exposure to cow's milk high or low in isoflavones on carcinogen-induced mammary tumorigenesis among rat offspring. *Cancer Prev Res (Phila)* **4**:694-701.

NTP (2008a) Multigenerational reproductive study of genistein (Cas No. 446-72-0) in Sprague-Dawley rats (feed study). *Natl Toxicol Program Tech Rep Ser*1-266.

NTP (2008b) Toxicology and carcinogenesis studies of genistein (Cas No. 446-72-0) in Sprague-Dawley rats (feed study). *Natl Toxicol Program Tech Rep Ser*1-240.

NTP. Final CERHR Expert Panel Report on Soy Infant Formula. 2010.

Rozman KK, Bhatia J, Calafat AM, Chambers C, Culty M, Etzel RA, Flaws JA, Hansen DK, Hoyer PB, Jeffery EH, Kesner JS, Marty S, Thomas JA and Umbach D (2006a) NTP-CERHR expert panel report on the reproductive and developmental toxicity of genistein. *Birth Defects Res B Dev Reprod Toxicol* **77**:485-638.

Rozman KK, Bhatia J, Calafat AM, Chambers C, Culty M, Etzel RA, Flaws JA, Hansen DK, Hoyer PB, Jeffery EH, Kesner JS, Marty S, Thomas JA and Umbach D (2006b) NTP-CERHR expert panel report on the reproductive and developmental toxicity of soy formula. *Birth Defects Res B Dev Reprod Toxicol* **77**:280-397.

Rufer CE, Bub A, Moseneder J, Winterhalter P, Sturtz M, Kulling SE (2008) Pharmacokinetics of the soybean isoflavone daidzein in its aglycone and glucoside form: a randomized, double-blind, crossover study. *J Clin Nutr* **87**: 1314-23

Setchell KD, Brown NM, Zhao X, Lindley SL, Heubi JE, King EC and Messina MJ (2011) Soy isoflavone phase II metabolism differs between rodents and humans: implications for the effect on breast cancer risk. *Am J Clin Nutr* **94**:1284-1294.

Setchell KD, Clerici C, Lephart ED, Cole SJ, Heenan C, Castellani D, Wolfe BE, Nechemias-Zimmer L, Brown NM, Lund TD, Handa RJ and Heubi JE (2005) S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr* **81**:1072-1079.

Setchell KD, Zimmer-Nechemias L, Cai J and Heubi JE (1997) Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet* **350**:23-27.

Setchell KD, Zimmer-Nechemias L, Cai J and Heubi JE (1998) Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. *Am J Clin Nutr* **68**:1453S-1461S.

Setchell KDR, Brown NM, Desai PB, Zimmer-Nechimias L, Wolfe B, Jakate AS, Creutzinger V, Heubi JE (2003) Bioavailability, disposition, and dose response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. *J Nutr* **133**: 1027-35

Setchell KDR, Clerici C (2010) Equol: pharmacokinetics and biological actions. *J Nutr* **140**: 1363S-8S

Setchell KDR, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe BE, Kirschner AS, Heubi JE (2002) Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am J Clin Nutr* **76**: 447-53

Steensma A, Bienenmann-Ploum ME, Noteborn HPJM (2004) Intestinal uptake of genistein and its glycoside in the rat using various isolated perfused gut segments. Environmental Toxicology and Pharmacology **17**: 103-110

Strom BL, Schinnar R, Ziegler EE, Barnhart KT, Sammel MD, Macones GA, Stallings VA, Drulis JM, Nelson SE and Hanson SA (2001) Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. *JAMA* **286**:807-814.

Tan KA, Walker M, Morris K, Greig I, Mason JI and Sharpe RM (2006) Infant feeding with soy formula milk: effects on puberty progression, reproductive function and testicular cell numbers in marmoset monkeys in adulthood. *Hum Reprod* **21**:896-904.

Thompson LU, Boucher BA, Liu Z, Cotterchio M and Kreiger N (2006) Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutr Cancer* **54**:184-201.

Ullah MF, Shamim U, Hanif S (2009) Cellular DNA breakage by soy isoflavone genistein and its methylated structural analogue biochanin A. *Mol Nutr Food Res* **53**: 1676-85

Wada K, Nakamura K, Masue T, Sahashi Y, Ando K and Nagata C (2011) Soy intake and urinary sex hormone levels in preschool Japanese children. *Am J Epidemiol* **173**:998-1003.

Yee S, Burdock GA, Kurata Y, Enomoto Y, Narumi K, Hamada S, Itoh T, Shimomura Y, Ueno T (2008) Acute and subchronic toxicity and genotoxicity of SE5-OH, an equol-rich product produced by Lactococcus garvieae. *Food Chem Toxicol* **46**: 2713-20

Yeo SK and Liong MT (2010) Angiotensin I-converting enzyme inhibitory activity and bioconversion of isoflavones by probiotics in soymilk supplemented with prebiotics. *Int J Food Sci Nutr* **61**:161-181.

Zubik L, Meydani M (2003) Bioavailability of soybean isoflavones from aglycone and glucoside forms in American Women. *Am J Clin Nutr* **77**: 1459-65

Zung A, Glaser T, Kerem Z and Zadik Z (2008) Breast development in the first 2 years of life: an association with soy-based infant formulas. *J Pediatr Gastroenterol Nutr* **46**:191-195.

Abbreviations

AAP	American Academy of Pediatrics
ADME	absorption, distribution, metabolism and excretion
AFSSA	L'Agence française de sécurite sanitarie des aliments (French Food Safety Agency)
AGDI	anogenital distance index
ALSPAC	Avon Longitudinal Study of Parents and Children
AUC	area under the curve
BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
BMI	body mass index
CERHR	Center for the Evaluation of Risks to Human Reproduction
COT	Committee on Toxicity
DH	Department of Health
DMBA	7,12-dimethylbenz[a]anthracene
DTH	delayed-type hypersensitivity
ER	oestrogen receptor
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FDA	Food and Drug Administration
FSA	Food Standards Agency
GD	gestational day
HIM	high isoflavone levels milk
LIM	low isoflavone levels milk
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of Detection
LOQ	Limit of Quantification
LQ	lordosis quotients
MAFF	Ministry of Agriculture, Forestry and Fisheries
MN	micronuclei
MOF	multi-oocyte follicles
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
O-DMA	*O*-demethylangolensin
PND	postnatal day
PR	progesterone receptor
PSAI	Pre-School Activities Inventory
RDI	recommended daily intake
SACN	Scientific Advisory Committee on Nutrition
sc	subcutaneous
SD	standard deviation
SE	standard error
SGLT1	sodium dependent glucose transporter
SHBG	sex hormone binding globulin
SPI	soy protein isolate
SPT	skin prick test
T_3	tri-iodothyronine
T_4	thyroxine
TBG	thyroid binding globulin

TDI	tolerable daily intake
TSH	thyroid stimulating hormone
TVP	textured vegetable protein
UDP	urine diphosphate

Search strategy

General isoflavones/genistein/daidzein exposure search

Databases interrogated –

- EFSA
- COT
- FSA
- JECFA

Scientific publications literature search

Specific search terms:

Isoflavone/genistein/daidzein/soy AND breast milk
Search Dates (From/To) - From May 2002 to present*
*Some papers pre-2002 were included if it felt they added value to the paper, particularly with regards to papers which identified previous cases of chronic and acute isoflavone toxicity and where a dose which lead to toxicity was identifiable.
Exclusion Criteria –

- Supplementation research in undeveloped countries
- Supplementation programs in undeveloped countries
- Deficiency related research

Isoflavone/genistein/daidzein/soy AND infant formula
Search Dates (From/To) - From May 2002 to present*
*Some papers pre-2002 were included if it felt they added value to the paper, particularly with regards to papers which identified previous cases of chronic and acute isoflavone toxicity and where a dose which lead to toxicity was identifiable.
Exclusion Criteria –

- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Infant formulas in non-EU countries

Isoflavone/genistein/daidzein/soy AND infant diet
Search Dates (From/To) - From May 2002 to present*
*Some papers pre-2002 were included if it felt they added value to the paper, particularly with regards to papers which identified levels of isoflavones in foods
Exclusion Criteria –

- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Infant diet in undeveloped countries
- Children's diet (above >2 years) in developed countries

Isoflavone/genistein/daidzein/soy AND weaning
Search Dates (From/To) - From May 2002 to present*
*Some papers pre-2002 were included if it felt they added value to the paper, particularly with regards to papers which identified levels of isoflavones in foods

Exclusion Criteria –

- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Infant weaning in undeveloped countries
- Children's diet (above >2 years) in developed countries

Retinol AND exposure
Search Dates (From/To) - From January 2002 to present*
*Some papers pre-2002 were included if they added value to the paper, particularly with regards to papers which identified isoflavone exposure values

Exclusion Criteria –

- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Deficiency related research

The above mentioned search terms were also used in google. It identified latest government advice and opinions.

Secretariat
March 2013